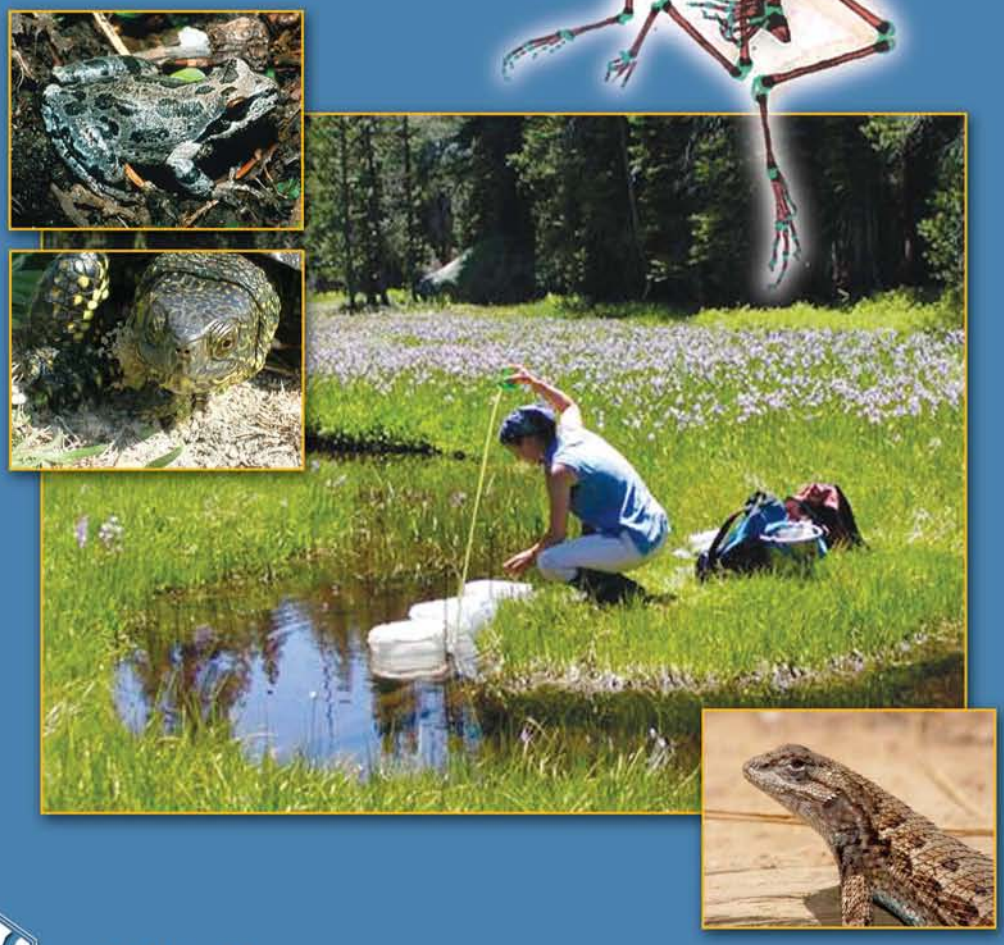
Ecotoxicology of Amphibians and Reptiles

Second Edition



Edited by
Donald W. Sparling, Greg Linder,
Christine A. Bishop, Sherry K. Krest

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Second Edition

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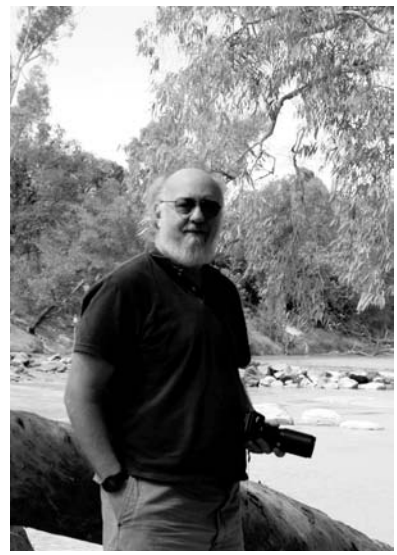
Contents

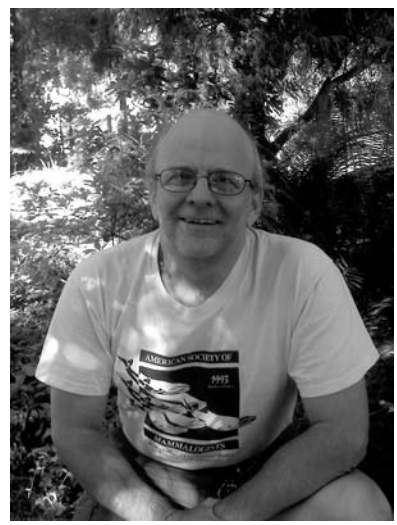
List of Figures

List of Tables

About the Editors



Donald W. Sparling received his bachelor's and master's degrees in Zoology at Southern Illinois University, and his Ph.D. in biology from the University of North Dakota. After a postdoc and a brief stint in academia he began a career with the US Department of Interior, starting with the US Fish and Wildlife Service (USFWS) in 1982 and ending with early retirement from the US Geological Survey (USGS) in 2003. For most of that time, he was a research wildlife biologist at Patuxent Wildlife Research Center and conducted research on a variety of contaminants and their effects on birds and amphibians. In 2004 he returned to Southern Illinois University as associate director of the Cooperative Wildlife Research Laboratory, where he has continued contaminant research and supervised research on upland game birds. Don has approximately 100 publications in the scientific literature and has coedited 4 books, including being lead editor on the 1st edition of *Ecotoxicology of Amphibians and Reptiles.*



Greg Linder coauthored a chapter in and helped edit the 1st edition of *Ecotoxicology of Amphibians and Reptiles.* During that same period, he worked with Don Sparling and Sherry Krest to convene the SETAC/Johnson Foundation workshop on multiple stressors and declining amphibian populations. For more than 30 years, Greg has worked for local, state, or federal regulatory and natural resource agencies as an applied ecologist and environmental toxicologist. Greg has worked with a wide range of plants, animals, and microorganisms, contributing to the development of toxicity test methods for fishes and aquatic invertebrates, wild mammals, amphibians, vascular plants and mosses, and a number of soil invertebrates, bacteria, and fungi. Greg works for the USGS, Columbia Environmental Research Center from his HeronWorks field office in Oregon's Willamette Valley, where he designs and implements integrated field and laboratory studies across a wide spectrum of habitats, most often in support of ecological risk assessments and habitat restoration projects.

Christine A. Bishop is a research scientist with the Canadian Federal Department of the Environment and an adjunct professor at Simon Fraser University and at the University of British Columbia. She studies the effects of habitat loss and habitat quality on wildlife. Her research focuses on the effects of multiple stressors on amphibian, reptilian, and avian populations and the recovery of populations of Species at Risk. Her doctoral studies at McMaster University examined the effects of pesticide use on birds nesting in apple orchards. Her master's degree research at York University was the study of the effects of organochlorine contaminants on common snapping turtles (*Chelydra serpentina serpentina*). She received her bachelor's degree in Agricultural Science (Honors) at the University of Guelph. During the past 26 years, she has combined her research interests with many on-the-ground conservation projects involving habitat restoration and preservation in Ontario and British Columbia, Canada. She cofounded the Canadian Amphibian and Reptile Conservation Network, has published more than 70 peer-reviewed scientific articles, and has coedited books including *Ecology, Conservation, and Status of Reptiles in Canada* and the 1st edition of *Ecotoxicology of Amphibians and Reptiles.*

Sherry Krest is the Environmental Contaminants Team Leader for the Chesapeake Bay Field Office in Annapolis, Maryland, USA. Sherry obtained a B.S. in Secondary Education from Bloomsburg University and an M.S. in Environmental Biology from Hood College. She has worked for the US Army Biomedical Research and Developmental Laboratory, where she conducted her master's study on the effects of lead on developing amphibians using the Frog Embryo Teratogensis Assay — *Xenopus* (FETAX). She began her career for the USFWS as an assistant course leader at the National Conservation Training Center, and then as a staff biologist in the Division of Environmental Quality in Arlington, Virginia. Today, she concentrates her efforts on addressing the use of frogs as a biomonitoring tool following the remediation of trap and skeet ranges. She is also the Natural Resource Damage Assessment and Restoration biologist and is actively involved in several cases in Maryland and Delaware.

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Preface for Second Edition

Ten years ago in the preface of the first edition of this book, we declared that the amount of information available on the ecotoxicology of amphibians paled in comparison to that available on birds, mammals and especially fish. Over the past 10 years, great advances have been made in understanding the effects of contaminants on amphibians. There have been more scientific, peer-reviewed papers on this topic written since 2000 than in the 30 years preceding that time. In addition to developing a better understanding of the dose-response relationships to contaminants in all the familiar chemical classes (metals, non-halogenated pesticides, organochlorinated pesticides, other halogenated organics and polyaromatic hydrocarbons), the number of species studied has increased and research is extending into emerging chemicals such as surfactants and pharmaceuticals. Perhaps the most exciting research, however, is occurring in the study of chemical interactions with other ecological stressors such as competition, predation and diseases. Another area of growth is the use of mesocosms to study the effects of contaminants on manmade communities; this type of research is revealing some surprising results compared to single organism, laboratory studies. The most critical concern at this time is maintaining the interest and momentum of amphibian-based ecotoxicological studies.

In the preface of the first edition, we also lamented the paucity of studies on reptiles. Since then, there have been several studies published. In particular, prior to 2000 the vast majority of research focused on body burdens of metals and persistent organic pollutants in a few species of reptiles such as turtles. Over the past decade, there has been a shift of interest to documenting the effects of contaminants, including lethality and sublethal maladies, on this vertebrate class. However, the overall production of new information in reptilian ecotoxicology continues to be well behind that of other vertebrates. Reptiles are more than "featherless birds" — they live in different habitats, have major physiological differences and process chemicals in ways that other vertebrates do not. Thus there is a clear need to increase scientific focus on the ecotoxicology of reptiles.

In 2000, we also stated that amphibian population declines were of substantial concern to the conservation community. Various causes for their declines including "fungal disease, habitat degradation, introduced predators and competitors, ultraviolet radiation, and contaminants" (p. xiv). Declining amphibian populations are still of concern although it seems that the public perception of these declines has waned. The same litany of possible causes is cited with perhaps a greater emphasis on fungal diseases, especially *Batrachochytrium dendrobatidis,* the cause of chytridiomycosis. We are not aware of any "smoking guns" involving contaminants as a clear and solitary cause of amphibian declines but their potential influence, especially through debilitating sublethal mechanisms remain just as real as they did 10 years ago. In fact, in light of the multitude of sublethal effects caused by contaminants — as espoused in the chapters of this book — support for contaminants as hidden and insidious causes of amphibian declines has increased substantially. In 2000 very little was known about the status of reptile species around the world. Since then, the IUCN undertook a serious examination of reptiles and concluded that a great many species are in dire straits. Undoubtedly scientists will find that contaminants have played and continue to affect declining species of reptiles.

This book is intended to provide a current synthesis of the scientific state of amphibian and reptile ecotoxicology. We have updated many of the chapters in the first edition, dropped a few along the way, and included a few more to present topical issues. In preparing this book, we sought

out many of the same authors as in the first edition and included several others that have made important contributions to our understanding of amphibian and reptile ecotoxicology over the past decade. The choice of authors is always a difficult one because for each one that is invited, there are several others whom you cannot invite. As with the first edition, all praise should go to the authors who have contributed to this book and any complaints can be directed to the editors.

– Donald W. Sparling

– Greg Linder

– Christine A. Bishop

– Sherry K. Krest

Preface from the First Edition

An international concern for the status and welfare of amphibians and their populations has been building since the late 1980s, reaching a turning point in the mid-1990s when two independent sets of events occurred. One set was several conferences that were held across the globe to discuss the status of amphibian populations. For several years prior to these conferences, similar discussions were stymied from reaching definitive conclusions by a lack of long-term population monitoring and historical data. At that time, however, scientists generally came to agree that the apparent declines were genuine and that numerous populations and species were at risk or even extirpated. Several reasons for these declines were espoused, including fungal disease, habitat degradation, introduced predators and competitors, ultraviolet B radiation (with and without interaction by chemicals), and contaminants. At the present time, none of these hypotheses has been universally supported.

The other significant event in the mid-1990s was the observation of numerous malformed frogs in Minnesota. Again, a lack of readily available historical data resulted in more questions than answers on the significance of this observation. As a result, several extensive surveys have been implemented in the US and Canada to determine the extent of these malformations and to identify possible causes. Proposed causes include ultraviolet B radiation, parasitism, and contaminants.

In an attempt to further understand the possible role of contaminants in amphibian population declines and malformations and in order to develop research that would help address these problems, each of us independently began a review of the existing literature. As we did so, we quickly became aware that information on contaminant effects and burdens in amphibians was extremely scarce and dispersed compared to that of other vertebrates, especially fishes, birds, and mammals. Even more apparent was an almost total lack of knowledge about contaminant effects on reptiles. Some body-burden data could be found, but hardly anything at all was found on the effects of these contaminants on the health or survival of reptiles. What information existed was heavily skewed towards a few species of turtles and clearly was not representative of the class.

In response to the absence of a concerted effort to evaluate the effects of contaminants on amphibians and reptiles, we endeavored to enlist the efforts of other researchers and develop a current state of science and synthesis of what is known with the hopes that such a compilation would spur additional inquiry and research. The results of these efforts follow.

In developing the book, we realized that several audiences might have an interest in its content. However, two general groups were foremost in our minds. First were the herpetologists, ecologists, and zoologists who might be interested in amphibians and reptiles in their own right but who might not have a strong background in ecotoxicology. The other major group was ecotoxicologists, resource managers, and policymakers who are versed in contaminant ecology and want to know more about amphibians and reptiles and how they compare to the better known classes of vertebrates. To meet the needs of both groups we have arranged for a variety of chapters covering 1) basic ecology, distribution, and physiology of these vertebrates; 2) syntheses of the existing information on specific groups of contaminants and herpetofauna; and 3) issues of risk assessment and study designs for those wishing to conduct additional research. Through this whole process and praise should be given to the authors, and any complaints can be directed toward us.

– D. Sparling

– G. Linder

– C. Bishop

1 Recent Advancements in Amphibian and Reptile Ecotoxicology

Donald W. Sparling, Greg Linder, Christine A. Bishop, and Sherry K. Krest

CONTENTS

When the first edition of *Ecotoxicology of Amphibians and Reptiles* was published in 2000, I reviewed the state of the literature from 1972 through 1998 (Sparling et al. 2000). That review covered 11 271 contaminant citations listed in Wildlife Review and Sports Fisheries Abstracts published by the US Fish and Wildlife Service. Among its findings, only 2.7% of the cited papers were on amphibians and 1.4% on reptiles. This equated to an average annual rate of 11.5 citations for amphibians and 6 for reptiles, although the distribution of citations was not homogeneous through the years. In contrast, 61.8%, or 280, contaminant citations per year were on fish. Among the amphibian citations, most focused on effects, 23% dealt with metals, 22% with acid precipitation, and 19% with nonchlorinated pesticides. The remaining 38% covered all of the other contaminants of interest at that time. Almost all of the citations on reptiles dealt exclusively with residues, and turtles (Chelonia) were overrepresented compared to the percent of reptilian species comprised by this order. The most important categories of contaminants included metals (24%), organochlorine pesticides (23%), and polychlorinated biphenyls (PCBs) (19%), all persistent pollutants.

At that time we raised the question why these 2 classes of vertebrates should be so underrepresented in the contaminant literature. Amphibians make up approximately 20% and reptiles 28% of known vertebrate species, so the number of contaminant citations was far fewer than would be predicted by species richness. In addition, both classes are extremely important ecologically in their respective habitats (Stebbins and Cohen 1995). More recently, a series of papers (Ranvestal et al. 2004; Regester et al. 2006; Regester and Whiles 2006; Whiles et al. 2006) have documented that amphibian declines may very seriously and negatively affect the nutrient balance, species diversity and richness, and energy flow of ponds, streams, and associated uplands.

TABLE 1.1
Status of At-Risk Amphibians and Reptiles

	Amphibians	Reptiles
Number of species evaluated	5918	664
Critically rare or endangered	442	73
Endangered	738	100
Vulnerable	620	125
Total (percent of evaluated)	1800 (30.4)	298 (44.9)

Source: IUCN (2007).

Many species of amphibians and reptiles around the world are endangered or threatened with endangerment. Of 5915 amphibian species evaluated, the IUCN (2007) lists 1808 (30%) at risk (Table 1.1). This contrasts with only 127 similarly listed species in 1996 (IUCN 1996). Even more telling, of approximately 6000 species of reptiles, the IUCN (2007) has evaluated the status of only 1385 (ca. 23%). Among these, 549 (39.6%) are considered at risk. To the extent that contaminants have a role in the imperiled status of amphibians and reptiles, further research is demanded (Gibbons et al. 2000; Hopkins 2000).

Since the 2000 review there has been a dramatic increase in the number of research studies and papers focusing on the effects and burdens of contaminants on these 2 important classes of vertebrates. While we would like to think that the publication of the first edition of *Ecotoxicology of Amphibians and Reptiles* had a major role in this increased interest, other factors such as symposia (e.g., Midwest Declining Amphibian Conference, Milwaukee, Wisconsin, 1998; Ecological Society of America, Memphis, Tennessee, 2006), a temporary increase in federal funding on problems associated with amphibian declines and monitoring (e.g., US Geological Survey's Amphibian Research and Monitoring Initiative and North American Reporting Center for Amphibian Malformations, US Fish and Wildlife Service's investigation for malformed frogs on national wildlife refuges), other, nonfederal organizations (e.g., Partners in Amphibian and Reptile Conservation [PARC], Declining Amphibian Population Task Force [DAPTF], now united with International Union for the Conservation of Nature), and publications (e.g., Lannoo 1998; Linder et al. 2003a, 2003b; Gardner and Oberdörster 2006) were very instrumental in this surge of interest. This chapter provides an overview of the increased research efforts since the publication of the first edition of *Ecotoxicology of Amphibians and Reptiles*. We finish with a description of what readers may expect to find in the subsequent chapters of this book.

1.1 CURRENT STATUS OF ECOTOXICOLOGICAL RESEARCH ON AMPHIBIANS AND REPTILES

1.1.1 Source of Publication Information

Because Wildlife Review and Sports Fisheries Abstracts are no longer published, we had to rely on a different source of citations. The increased technology in literature review services since 2000 greatly expanded the opportunity to examine a large number of citations in a comparatively short period of time. However, differences in search methodology and the extensive databases that are now available affect meaningful comparisons between the first edition and this chapter. To help mitigate this problem and to ensure continuity with the 2000 edition of this book, we extended our search back to 1996 through 2008 to develop a more historical perspective on changes in amphibian and reptile studies. We used the ISI Web of Knowledge, specifically Web of Science, as our data source.

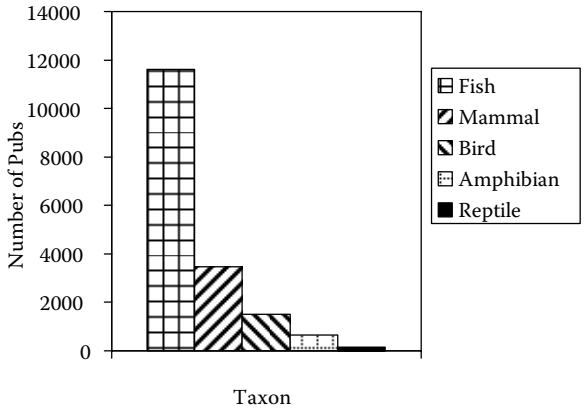

FIGURE 1.1 Total number of contaminant-related papers published between 1996 and 2008 by vertebrate class.

1.1.2 Comparisons among Vertebrates

Initially, we ran a search on each of the vertebrate classes to derive a relative measure of emphasis. In this search we did not examine specific titles. We matched the keywords *metal**, *organochlorine*, *pesticide*, *PCB**, *PAH*, *contaminant*, and *toxic**, joined by the Boolean operator *or*, with each of the taxa and determined the number of citations. The asterisk (*) is a wild card or generic expression allowing any subsequent characters through if the preceding characters are matched. For mammals, we made the additional choice of excluding citations with keywords *human*, *mouse*, and *rat* to reduce the number of citations that were primarily or exclusively oriented to human health. The choice of keywords in literature searches is a subtle science, and we acknowledge that our search was not exhaustive. However, we feel that this method provided a reasonable comparison of research productivity by vertebrate class.

By far, the number of citations for fish remained much higher than those of other classes (Figure 1.1). Of the 17 375 citations examined, 11 601 (66.7%) were for fish, 3457 (19.9%) for mammals, 1520 (8.8 %) for birds, 645 (3.8%) for amphibians, and 152 (0.8%) for reptiles. This distribution is very similar to the one found in 2000, except that the relative position of birds and mammals is switched. Only a very small fraction of the literature on vertebrate ecotoxicology pertains to amphibians or reptiles.

1.1.3 Productivity within Amphibians and Reptiles

In a more refined search, we examined each year (1996 to 2008) and entered the keywords *amphibian**, *salamander*, *frog*, *toad*, *newt*, *Bufo*, *Rana*, *Hyla*, *Acris*, *Pseudacris*, and *Ambystoma* for amphibians and *reptile**, *alligator*, *snake*, *turtle*, *lizard*, *Chelydra*, and *anole* for reptiles. In addition, for reptiles we excluded *venom**, *grass*, and *river* because of frequent noncontaminant hits on these keywords. Each title was reviewed to determine if the citation was contaminant related and to which class of compounds and taxonomic group it could be attributed. In scoring, a given citation may have had 1 or more "hits," with a hit consisting of a specific class of contaminant, taxon, or analysis. For example, a citation with the hypothetical title "Effects of Heavy Metals and PCBs on *Ambystoma gracile* and *Bufo cognatus*" would have received 1 hit each for effects, heavy metals, PCBs, *Ambystoma*, and *Bufo*. Abstracts and publications were searched when titles alone were insufficient to identify types of contaminants examined. In all, 25 998 amphibian and 15 057 reptile citations were searched. We recognized the following categories or classes of contaminants:

1) pesticides — including all nonhalogenated pesticides;
2) metals — particularly heavy metals such as lead, arsenic, cadmium, zinc, copper, and mercury, but could also include metalloids and other metals, such as aluminum, that can be toxic under certain conditions;
3) ultraviolet radiation — including UVA and UVB, whose environmental levels have been accentuated in recent times;
4) simazines such as atrazine;
5) nitrogenous compounds, particularly nitrates, nitrites, and ammonia, that are used as fertilizers;
6) PCBs, dioxins, and furans;
7) polycyclic aromatic hydrocarbons (PAHs);
8) organochlorinated pesticides such as endosulfan, dichlorodiphenyltrichloroethane (DDT), and dichlorodiphenyltrichloroethylene (DDE); and
9) other — including polyhalogenated diphenyl ethers, ingested plastics in the case of sea turtles, radioactive molecules, pharmaceuticals, and various biocides and antiseptics.

There was a significantly increasing trend in the total number of contaminant and noncontaminant citations by year for reptiles ($r = 0.9240$, $p < 0.0001$) but not for amphibians ($r = 0.2059$, $p = 0.4997$) (Figure 1.2), suggesting that there has been an overall increase in productivity in reptile biology. However, amphibians are used extensively in a variety of different research contexts, including embryology, physiology, genetics, and medical and human health studies that vary independently of ecological or contaminant-related interests; thus, large volumes of research are continually being produced for this class. Reptiles are used less in these types of studies, and their increase in the number of citations through time may more closely mirror greater interest in reptile behavior, ecology, and conservation status.

Annual numbers of contaminant-related amphibian papers, although showing year-to-year variation, significantly increased from 1996 to 2008 ($r = 0.8566$, $p = 0.0002$) (Figure 1.3), as did their relative contribution to the total number of amphibian papers published ($r = 0.7507$, $p = 0.0031$) (Figure 1.4). However, the percent of total publications that are contaminant-related has remained relatively stable since 2002 (Figure 1.4). The total number of contaminant-related publications between 2000 and 2005 exceeded that for the combined 22 years prior to 2000 for amphibians. In contrast, the number of contaminant-related papers for reptiles has been more erratic than for amphibians but has shown a significant increasing trend through the years ($r = 0.7098$, $p = 0.0066$) (Figure 1.3). Similarly, the percent of total publications that are contaminant related has not significantly increased since 1997 ($r = 0.3873$, $p = 0.1910$) (Figure 1.4). These data indicate

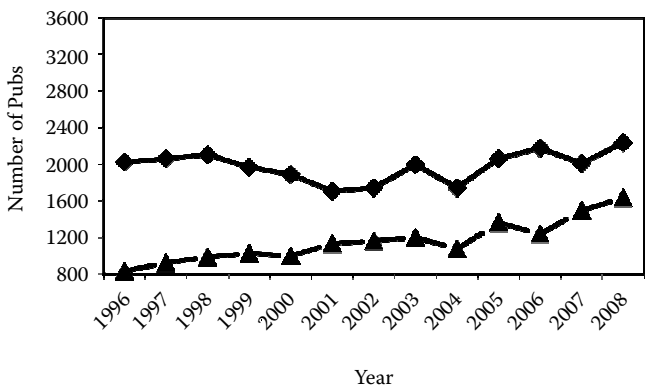

FIGURE 1.2 Annual numbers of scientific publications for amphibians and reptiles between 1996 and 2008 as listed in ISI Web of Science.

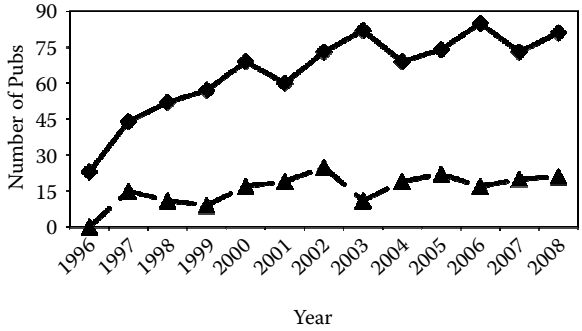

FIGURE 1.3 Number of contaminant-related papers published for amphibians and reptiles between 1996 and 2008. (Data from ISI Web of Science.)

that increased interest in amphibian ecology since worldwide population declines were generally accepted has resulted in elevated scientific productivity. By comparison, reptile ecotoxicology remains relatively unexplored and unknown. From 2006 through 2008 only 17, 20, and 21 citations, respectively, could be found for any contaminant study in reptiles, whether it focused on effects or residues. As Hopkins (2000) pointed out, this dearth of knowledge should be of great concern.

1.1.3.1 Amphibians

Among amphibians, the most intensely studied class of contaminants has been nonchlorinated pesticides, including organophosphorus, carbamate, and pyrethroid compounds (Figure 1.5). This class is followed in order by metals; ultraviolet radiation; endocrine disruptors (which include several classes of contaminants); PCBs and related molecules; simazines (mostly atrazine, of course); nitrogenous compounds, including nitrates, nitrites, and ammonia; acidity; polycyclic aromatic hydrocarbons; and organochlorine pesticides. Changes in emphasis from pre-2000 include a sharp decrease in publications on acid precipitation and an increased focus on pesticides, ultraviolet radiation, endocrine disruption, and atrazine. Slightly less than 6% of the contaminant papers published between 1996 and 2008 dealt extensively with residue data without providing new information on the effects of those residues.

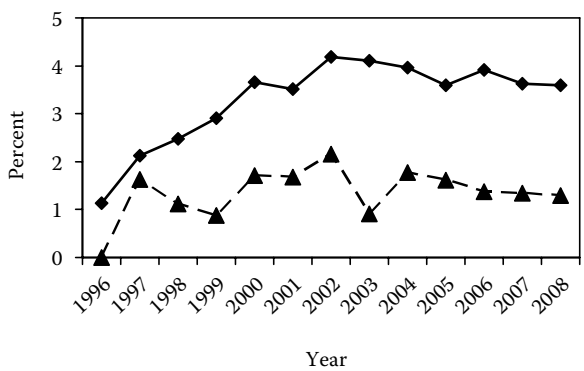

FIGURE 1.4 Percent of all scientific publications for amphibians and reptiles between 1996 and 2008 that were contaminant related. (Data from ISI Web of Science.)

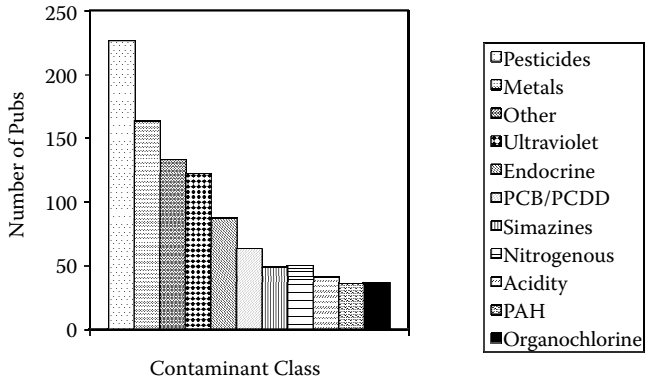

FIGURE 1.5 Contaminant-related papers published on amphibians between 1996 and 2008 by chemical class. (Data from ISI Web of Science.)

Ranids have been the most studied family of amphibians (Figure 1.6), accounting for more studies than Bufonidae, Hylidae, Ambystomatidae, and other families combined. For *Xenopus,* we only counted studies that had direct ecotoxicological relevance. Even with that constraint, *Xenopus laevis* remains the most commonly studied amphibian species.

In comparison to other issues related to amphibian population declines, contaminant ecology remained the leading topic of study up to 2007. From 2005 there has been a surge in disease-related papers, no doubt stimulated by findings that chytrid fungus (*Batrachochytrium dendrobatidis*) and ranaviruses have been linked to population declines (Longcore et al. 1999; Daszak et al. 1999) (Figure 1.7). In 2007 the number of disease-associated papers exceeded the number of contaminant-related papers for the first time in at least a decade, and the number of disease papers continued to surpass the number of contaminant papers in 2008. Extremely few papers were published on malformations prior to 1996, but the topic became important after the identification of several malformed tadpoles in Minnesota in 1995; this increase lasted about 10 years and then dissipated. Climate change, which has been cited as a possible mechanism of amphibian population decline (e.g., Davidson et al. 2001; Pounds 2001; Carey and Alexander 2003; Daszak et al. 2005), has not received rigorous investigation.

The scientific community is just beginning to explore possible interactions between contaminants in the broad sense and other stressors. We identified 36 research papers published since 1996 with clearly specified objectives of testing the interaction between one or more contaminants (including

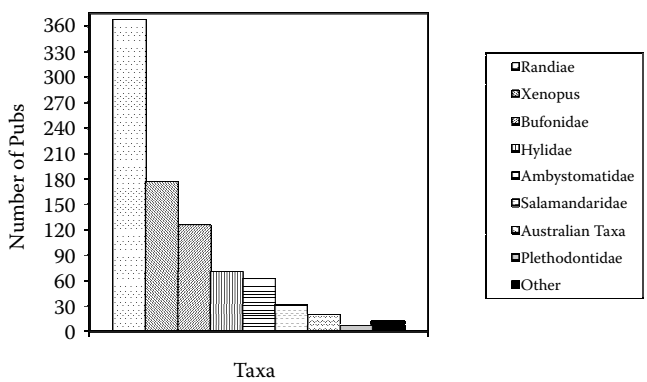

FIGURE 1.6 Contaminant-related papers for amphibians that were published between 1996 and 2008 by taxonomic group. (Data from ISI Web of Science.)

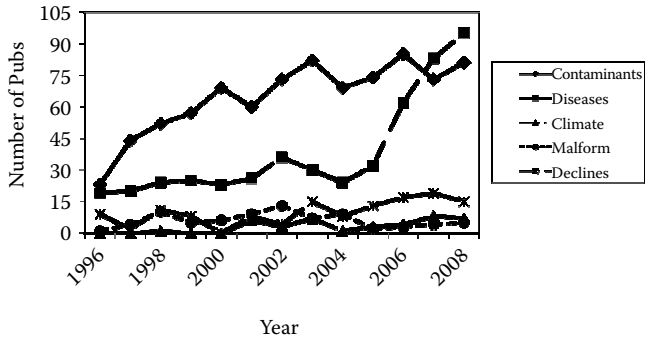

FIGURE 1.7 A comparison of the numbers of papers published for amphibians between 1996 and 2008 by associated stressor. (Data from ISI Web of Science.)

ultraviolet radiation) and other stressors. Of these, 19 (53%) investigated the interactive effects of predation, 12 (33%) examined disease, and 5 (14%) looked at interspecific competition or other factors. Relyea's chapter in this book (Chapter 14) comprehensively examines the interaction between contaminants and other stressors.

1.1.3.2 Reptiles

In reptiles, the most studied class of contaminants is heavy metals, followed by endocrine disrupting chemicals, organochlorine pesticides, and nonchlorinated pesticides (Figure 1.8). Simazines, ultraviolet radiation, acidification, and nitrates/nitrites were lightly studied. However, compared to the years prior to 2000, even a few papers marks increased interest in these topics. Metals and organochlorine pesticides were frequently cited throughout both time periods, but more papers were published on metals and pesticides in the years between 2000 and 2008 than in the combined 28 years preceding 2000. Whereas the vast majority of papers written prior to 2000 were based on residue information with little data on effects, 64% of the papers published after 2000 focused on effects. Thus, we are in the beginning stages of understanding how contaminants affect reptiles.

By far, the most intensively studied reptilian species in ecotoxicology is the American alligator (*Alligator mississippiensis*); (Figure 1.9). Largely due to the research of Guillette and others

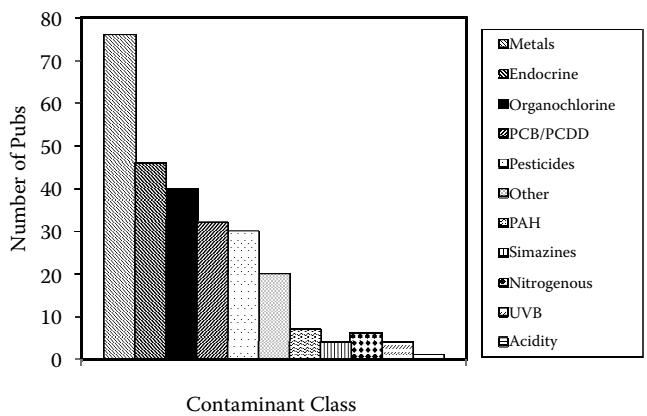

FIGURE 1.8 Number of contaminant-related papers published between 1996 and 2008 on reptiles by type of contaminant. (Data from ISI Web of Science.)

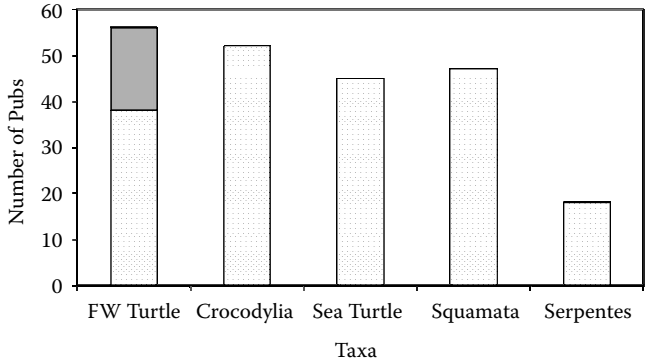

FIGURE 1.9 Number of reptile contaminant-related papers published between 1996 and 2008 by taxonomic group. (Data from ISI Web of Science.)

(Jagoe et al. 1998; Guillette et al. 1999, 2000), alligators contribute the majority of studies under Crocodylia, and many of these papers have been on endocrine disruption and persistent organic pollutants. Freshwater turtles, especially common snapping turtles (*Chelydra serpentina*), would be next in importance, followed by sea turtles and Squamata (lizards). Contaminant papers on Serpentes (snakes) are uncommon. The preference for snapping turtles is probably due to their large size, relatively high visibility and capture rates, widespread distribution, long lifespan, and bottom dwelling; hence, they have ample opportunity to assimilate and accumulate persistent organic pollutants and metals and are easily studied.

When compared to amphibians, existing literature on other factors affecting reptile declines (Gibbons et al. 2000) remains sparse. Whereas a variety of papers deal with habitat needs and use, behavior, and general ecology, only disease-related studies were specifically related to population declines, and many of these were related to captive animals. This category includes data on zoonoses, parasitism, and incidence or diagnosis of disease under laboratory, zoological, and ecological conditions. More than twice the number of papers was published on disease issues than on contaminants from 2000 through 2008 (Figure 1.10). Only a scattering of papers were found for reptile declines or climate change.

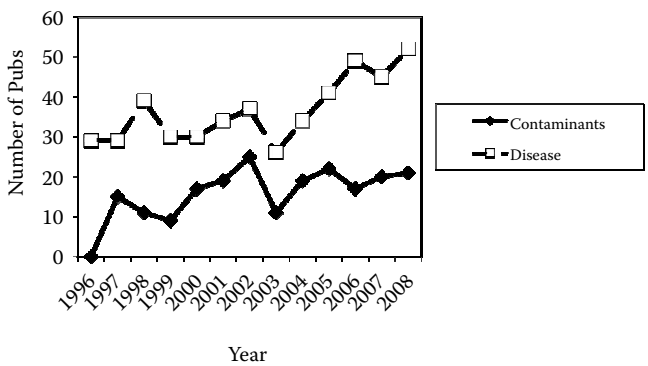

FIGURE 1.10 A comparison of the number of contaminant- and disease-related papers published annually for reptiles from 1996 to 2008. (Data from ISI Web of Science.)

1.1.4 Conclusions

Considerable progress has been made in developing new information on the ecotoxicology of amphibians and reptiles over the past few years compared to the nearly 3 decades preceding 2000. Since 2000, total publications in both vertebrate classes have exceeded those appearing from 1972 to 2000 combined. The increased interest and productivity in ecotoxicology has been most notable in amphibians. Significant increases have occurred in contaminant ecology of amphibians in both the number of papers and the percent of all amphibian papers published. No doubt, these increases are related to the upsurge in interest due to widely accepted phenomena of population declines and species extinctions. Among reptiles, however, the increased productivity has not been as obvious, even though many species appear to be suffering population losses as great as those in amphibians (Hopkins 2000; Chapters 2 and 3, this book). Whereas the total number of contaminant-related papers increased for this class since 2000, so did the total number of papers, and there was no noticeable surge in ecotoxicology of reptiles. Moreover, the actual number of papers that are published annually on contaminants and reptiles is very meager.

Using the number of citations as a guide, specific gains have been made in certain areas of amphibian and reptile ecotoxicology during the last few years. Most notably, research is being conducted and published on the effects of contaminants on reptiles, not just on body burdens. Whereas these effects papers derived from a very few species, mostly alligators, turtles, and a couple of lizards, new information is being obtained. Another significant trend is an increase in the number of amphibian species that have been examined. Whereas there are a few more intensively widely studied species, notably *Xenopus laevis*, *Rana pipiens*, *R. sphenocephala*, *Bufo fowlerii*, *B. cognatus*, *B. bufo*, *Hyla versicolor*, and various Ambystomids, more than 12 families have been investigated at least once. No studies were found on the poorly known order Gymnophiona (caecillians).

Much more work needs to be done for a fuller understanding of contaminant effects in amphibians and reptiles. The first goal should be to obtain more information on the effects of contaminants on reptiles. For many years the only contaminant data available for this class were on body burdens or residues of persistent organics. In addition to looking forward to new scenarios, it may be useful for some laboratory studies to take a retrospective look and determine if the reported burdens could have been detrimental to individuals or populations. Additional research on the interaction between chemicals with other stressors is of nearly equal importance. Contaminants will most often negatively influence populations in subtle ways (Sparling 2003; Chapter 14, this volume) in consort with disease, predation, competition, and food availability. Bioindicator development is another area that needs to be developed further in both vertebrate classes. Temperature-dependent sex determination in many reptiles and some amphibians offers many opportunities to examine sex-related hormone disruption, and metamorphosis in anuran amphibians provides an excellent background for thyroid-disrupting chemicals. Analyses of how contaminant effects vary during entire amphibian life cycles can provide new understanding on the effects of these contaminants on population declines. It is our hope that readers of this volume will be inspired in many ways to develop new research thrusts in the ecotoxicology of amphibians and reptiles.

1.2 WHAT'S IN THIS BOOK?

As with the first edition of *Ecotoxicology of Amphibians and Reptiles* (Sparling et al. 2000), the second edition can be divided into a few parts based on the general content. Whereas readers of the first edition will recognize the names of some authors, the editors for the second edition make a point of inviting some new authors so as to obtain a different perspective on some of the major contaminant issues affecting amphibians and reptiles. In all cases we encouraged authors to use similar chapters in the first edition as jumping off places. Authors were encouraged to

emphasize new information published after the first edition came out but to draw from that source when appropriate.

The first portion of the second edition may be thought of as background material. In it readers may find current thoughts on the status of amphibians and reptiles (Chapters 2 and 3), an overview of contaminant ecology in these 2 classes (Chapter 4), and a thoughtful perspective on amphibian and reptile physiology from a contaminants exposure and effects perspective (Chapter 5). The authors for Chapter 2 hope that their presentation will whet the appetites of readers for the rest of the book.

The second portion of the book focuses on the effects and residues of specific contaminant groups on amphibians and reptiles. Chapters 6 and 7 deal with nonchlorinated pesticides such as organophosphorus, carbamate, and pyrethroid compounds. In the last few years the herbicide atrazine has been extensively studied with regard to amphibians because of its widespread and common use and because there has been a great deal of controversy over whether it interferes with normal development of gonads at very low concentrations. Christine Bishop, one of the editors of this volume, brings the reader up to date (as of late 2008) on this important chemical (Chapter 8). Organic contaminants, including chlorinated pesticides, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and their related dioxins and furans, are the focus of Chapters 9 and 10. Metals and metalloids are discussed in Chapters 11 and 12.

Section 3 consists of chapters that might be called "contaminants plus." Chapter 13 reviews work conducted on amphibians (mostly) and ultraviolet radiation (UV). Although it can be argued that UV is not a contaminant in the usual sense, we have included this chapter because UV radiation has increased in recent decades, in part due to the thinning of the ozone layer, which is affected by contaminants, and because UV affects the toxicity of some contaminants, such as PAHs. The multiple stressors chapter (Chapter 14) focuses on the increasing body of knowledge on how contaminants interact with other stressors, such as disease, competition, and predation, to potentially exert greater effects than the same contaminants by themselves. The implications of these interactions for making exposure tests increasingly realistic are obvious. While we have a working knowledge of the effects of many contaminants that have been around for awhile, there is a whole new cadre of chemicals that have recently or are about to come on the market for which we have very little or no information. Some of these are potentially alarming. Chapter 15 examines some of the major chemicals in this arena. As presented in the first part of this chapter, amphibian malformations have received considerable attention since 1995. While the first edition reviewed the current literature up to 2000, Chapter 16 in this volume reports on a major study conducted since that time and reviews the work of many scientists. Over the past few years efforts led by the US Geological Survey's Amphibian Research and Monitoring Initiative have greatly improved methods for monitoring and enumerating amphibian populations. Because many contaminant-related field studies rely on some form of surveying, Chapter 17 quickly encapsulates the most important advances for surveying and monitoring. The final chapter, or epilogue, is the editors' almost random thoughts on what we learned during the development of this book and where we believe the science of ecotoxicology of amphibians and reptiles should be heading.

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2 Declines and the Global Status of Amphibians

Ross A. Alford

CONTENTS

The global diversity and status of both amphibians and reptiles at the end of the 20th century were described in thorough detail in the previous edition of the current volume (McDiarmid and Mitchell 2000). During the 1990s, however, it became clear that the diversity and status of amphibians were changing rapidly, with substantial declines in amphibian populations in many regions (Alford and Richards 1999). There has also been widespread concern regarding possible increases in the prevalence of developmental abnormalities and malformations in amphibians (Johnson et al. 2003). Great advances have been made in understanding these problems since 1999. This chapter concentrates on defining the problems, illustrating and interpreting our understanding of their causes, and developing ideas regarding how the problems can be managed.

2.1 AMPHIBIAN DECLINES: DEFINING THE PROBLEM

It has been almost 20 years since the problem of global amphibian declines became widely recognized (Barinaga 1990; Beebee et al. 1990; Blaustein and Wake 1990; Vitt et al. 1990). Early investigation of the problem was impeded by a lack of data on the normal behavior and population dynamics of amphibians. The extremely dynamic nature of amphibian populations (Pechmann et al. 1991; Sjogren-Gulve 1991) made it difficult to determine whether observed declines might be part of normal population processes, leading to an initial debate over whether there really was a widespread problem (Wake 1991; Crump et al. 1992; Blaustein 1994; Pechmann and Wilbur 1994; Travis 1994). Herpetologists quickly realized, however, that the great extent and precipitous manner of at

least some declines (e.g., Fellers and Drost 1993; Richards et al. 1993; Vial and Saylor 1993) were not consistent with even the most extreme predictions of models of population or metapopulation dynamics. A formal test of models by Pounds et al. (1997) showed that at least some declines were inconsistent with normal population fluctuations. Many herpetologists adopted the position advocated by McCoy (1994), affirming that unusually severe declines had recently occurred, but that additional data were necessary to clarify the extent and nature of the problem.

Although the phenomenon of amphibian declines was first recognized and publicized in the early 1990s, they appear to have commenced earlier. Precipitous declines of regional faunas occurred in the 1970s and 1980s in North America (Corn and Fogleman 1984; Carey 1993), Central America (Pounds and Crump 1994), South America (Heyer et al. 1988; Eterovick et al. 2005), and Australia (Czechura and Ingram 1990). Widespread declines may have commenced in the 1960s (Houlahan et al. 2000), but a reanalysis of the same data (Alford et al. 2001) suggested that widespread declines primarily commenced in the 1990s. Regardless of when they commenced, there is presently a consensus among amphibian biologists that the group as a whole has suffered and continues to suffer calamitous declines in most regions that have sufficient data available to detect them.

The response to the amphibian decline crisis commenced in earnest in February 1990, when a meeting sponsored by the US National Academy of Sciences concluded that there appeared to be a genuine problem, and that it should be addressed by an international working group of scientists (Heyer and Murphy 2005). This led to the formation of the International Union for Conservation of Nature/Species Survival Commission (IUCN/SSC) Declining Amphibian Populations Task Force (DAPTF) in December 1990 (Heyer and Murphy 2005). Throughout the 1990s, until its merger with the Amphibian Specialist Group of IUCN/SSC in 2007, the DAPTF played a major role in the global coordination of efforts to understand and address the problem of amphibian declines. It established scientific working groups by discipline and by region, organized meetings and symposia to facilitate exchange of data on the problem, and facilitated the creation of the first book of standardized protocols for surveying amphibian populations (Heyer et al. 1994). It also established a seed grant program to fund start-up projects, particularly targeting projects and areas of the world that might have difficulty in obtaining funding from other bodies; as of 2002, 95 projects had been awarded almost US$210 000, resulting in numerous publications and allowing many awardees to obtain additional larger-scale funding based on their preliminary data (Heyer and Murphy 2005). Regional and national working groups associated with the DAPTF produced published reports on the status of amphibians in the Lesser Antilles, the Commonwealth of Independent States, Canada, Australia, and the United States (Campbell 1999; Heyer and Murphy 2005). These gave a clearer focus to the problem and began to explore the nature of its causes and possible solutions.

A series of review papers published in the 1990s presented the issues and revealed the rapid increase in knowledge of the status of amphibians and of factors affecting that status. Early efforts (Blaustein et al. 1994b; Kuzmin 1994; Pechmann and Wilbur 1994) emphasized the lack of knowledge of, and need for more extensive data on, the normal behavior of amphibian populations. Populations tended to be thought of as defined by census units, which for amphibians are often breeding aggregations. A commonly held idea was that amphibians are highly philopatric as adults, returning to the same breeding site annually, and that this is often their natal site, making dispersal relatively uncommon (Sinsch 1990, 2006). Blaustein et al. (1994b, p 60) specifically mentioned that "due to the physiological constraints, relatively low mobility, and site fidelity of amphibians we suggest that many amphibian populations may be unable to recolonize areas after local extinction." In parallel with work directed at the phenomenon of amphibian declines, more recent work on the movements and population dynamics of amphibians has indicated that many species may have much more complex spatial dynamics, occurring in metapopulations or even in unified populations using a variety of reproductive sites spread over a relatively large area (Sjogren-Gulve 1991, 1994; Skelly et al. 1999; Marsh 2001; Marsh and Trenham 2001; Trenham et al. 2001, 2003).

By 1999, a substantial body of work had accumulated on the problem. A review by Alford and Richards (1999) cited 206 references, most published between 1980 and 1999, that addressed relevant topics. They separated nominated threats to amphibian diversity into 7 categories, and

pointed out that most studies, while concentrating on single factors, acknowledged that interactions among factors were likely to be important. The complex nature of potential interactions has become increasingly evident in more recent literature, as will be discussed below and elsewhere in this volume. Alford and Richards also emphasized the need for more targeted studies at the population and metapopulation levels, and pointed out the difficulties inherent in interpreting the simple count data at breeding sites on which most amphibian monitoring had been based.

The clear need for a better understanding of the global status of amphibians led the IUCN to organize and conduct a large-scale project, the Global Amphibian Assessment (GAA), to provide the best possible snapshot of the status of all amphibians (Stuart et al. 2004b). The world was separated into geographical regions, with a regional coordinator appointed to oversee the assessment process in each. Experts were involved in consultation processes in which every known species of amphibian was assessed against the IUCN criteria for species status (Stuart et al. 2004b). The results of the GAA indicated that a real crisis is occurring. The GAA assessed the status of 5711 species of amphibians. Almost one-third of the extant species (32.5%) were considered to be of conservation concern (IUCN categories "vulnerable" or higher; Stuart et al. 2004a). This is a substantially higher proportion than for either birds or mammals (Stuart et al. 2004a), although the status of 22.5% of species could not be assessed due to insufficient data. Stuart et al. (2004a) also pointed out that, unlike birds and mammals, the situation of amphibians appeared to be deteriorating rapidly, with the majority of known extinctions occurring late in the 20th century. Stuart et al. (2004a) attempted to examine trends by extrapolating the status of amphibians back to 1980. They used a conservative approach, incorporating any data that were available on population trends, changes in habitat, and conservation actions, with the default being that species had not changed since 1980 (Stuart et al. 2004b). Additional data accumulated when the GAA was partially updated in 2007 (IUCN 2008).

A comparison of the overall numbers of species in each IUCN Red List category estimated for 1980 and observed in 2004 and 2007 appears in Table 2.1. The deterioration of the overall status of amphibians is most clearly illustrated by the dramatic increase in the numbers of species in the

TABLE 2.1
Summary of the Status of Amphibians from the Global Amphibian Assessment (Stuart et al. 2004a, 2004b) and Its Recent Partial Update

	Number of Species			Percent Change	
Category	**1980**	**2004**	**2007**	**1980–2004**	**2004–2007**
Extinct	25	34	34	36.0	0.0
Extinct in the wild	0	1	1	—	0.0
Total extant species	5718	5709	5881	–0.2	3.0
Critically endangered	231	427	441	84.8	3.3
Endangered	807	761	737	–5.7	–3.2
Vulnerable (VU)	734	668	630	–9.0	–5.7
Total VU and above	2094	2215	2277	4.7	–2.6
Near threatened (NT)	322	359	369	11.5	2.8
Least concern	2322	2199	2277	–5.3	3.5
Data deficient	1302	1294	1426	–0.6	10.2

Source: IUCN (2008).

Note: The status of amphibians deteriorated markedly between 1980 and 2007. Although the numbers of species in some of the less threatened categories decreased, the table shows that this was due primarily to species moving upwards into the critically endangered and extinct categories. The small decrease in total NT and above in 2007 reflects a few species changing from vulnerable to lower threat categories based on improved information. The large increase in data-deficient species in 2007 largely reflects an increase in the rate of description of new species, particularly from tropical areas.

"critically endangered" category in the interval between 1980 and 2004. Much of this occurred as species moved up from less threatened categories, causing the numbers in the categories of intermediate threat to decrease. The numbers in the "near threatened" (NT) category also increased, as species moved from "least concern" (LC) to NT. Even in the short interval from 2004 to the next partial update of the GAA in 2007, an additional 14 species were added to the "critically endangered" category. A few species were moved downward as additional data became available, and a substantial number of new species were described, mostly from poorly known areas in the tropics, causing an increase in the number of species regarded as "data deficient."

Stuart et al. (2004a) discussed the changes in status of amphibians between 1980 and 2004 in much greater detail, pointing out that a total of 435 species moved to higher categories of threat during this period. They also examined the sources of threats to these "rapidly declining" species, and found that the primary threat to 50 species was overexploitation, to 183 was habitat loss, and to 207 was "enigmatic declines," which they defined as declining, even when suitable habitat remains, for reasons that are not fully understood. The distributions of these threats are geographically clustered, with overexploitation most common in the Asian region, habitat loss in North America, Europe, and Africa, and enigmatic declines predominant in Central and South America and Australia.

The problem of amphibian declines is therefore clear; a substantially higher proportion of extant amphibians are vulnerable to extinction than are either of the 2 other terrestrial vertebrate groups for which a full assessment has been made. The best assessment to date suggested that approximately 8% of species had undergone rapid declines during the period 1980 to 2004. Many species are threatened by the clear anthropogenic factors of habitat loss and overexploitation, but well-documented rapid declines to local extinction occurred during the 1980s and 1990s, at protected sites where habitat loss was not occurring — the "enigmatic declines" of Stuart et al. (2004a). Fortunately, during the period 2000 to present, a great deal of progress has been made toward understanding the causes of declines, including those labeled by Stuart et al. as enigmatic.

2.2 POTENTIAL CAUSES OF THE AMPHIBIAN DECLINE CRISIS

Most of the potential causes of amphibian declines were suggested early in the history of research into the problem. One of the earliest published summaries (Blaustein and Wake 1990) nominated habitat destruction, exotic predators, pollution, and utilization as food and pets as possible causes. Three more potential causes were quickly added to the list: effects of climate change (Herman and Scott 1992; Pounds and Crump 1994), disease, in combination with environmental factors (Carey 1993; Blaustein et al. 1994a), and increased levels of ultraviolet radiation (Blaustein et al. 1995). Disease acting alone, in the form of an invasive exotic pathogen, was suggested as a potential cause of widespread declines in eastern Australia by Laurance et al. (1996). Most recently, Harris et al. (2006) suggested that changes in the cutaneous bacterial assemblage of amphibians might increase their vulnerability to disease. From the beginning (Blaustein and Wake 1990), most authors acknowledged that causes were likely to be complex, involving interactions of more than a single factor. This was particularly true for climate change, which usually is suggested to act through changes in other factors.

The process of linking specific threats to the species they affect is incomplete and often based largely on opinion. However, for most species under greater threat that have been more intensively investigated, there is now at least some understanding of the source of threats. Table 2.2 summarizes threats nominated for all species in the current update of the GAA (IUCN 2008). The proportion of species in each threat category subject to each nominated type of threat obviously differs across threat categories. This might be expected for the categories near threatened (NT), least concern (LC), and data deficient (DD), which have been studied to different degrees and for which nominated threats may reflect opinions or extrapolations. However, even when only the 3 most threatened categories, vunerable (VU), endangered (E), and critically endangered (CE) are considered, the source of threats differs significantly among categories (chi-squared contingency test, $X^2 = 121.4$, 10 d.f., $p < 0.0001$). For easier visualization, these data are presented as percentages in Figure 2.1. This shows

TABLE 2.2
Summary of Numbers of Species in Each IUCN Category Thought to Be Subject to Threats of Different Types

	Number of Affected Species in IUCN Category					
Threat	**CE**	**E**	**VU**	**NT**	**LC**	**DD**
Habitat loss	145	308	211	130	457	194
Invasive species	62	91	68	55	149	57
Utilization	30	43	48	35	104	21
Pollution	168	213	197	110	401	110
Disease	200	125	79	32	54	50
Unknown threats	3	7	18	25	867	96
Total number of species in category	441	737	630	369	2277	1426

Source: IUCN (2008).

Note: Numbers within categories sum to more than the number of species in that category as some species are subject to multiple threats. CE = critically endangered, E = endangered, VU = vulnerable, NT = near threatened, LC = least concern, DD = data deficient.

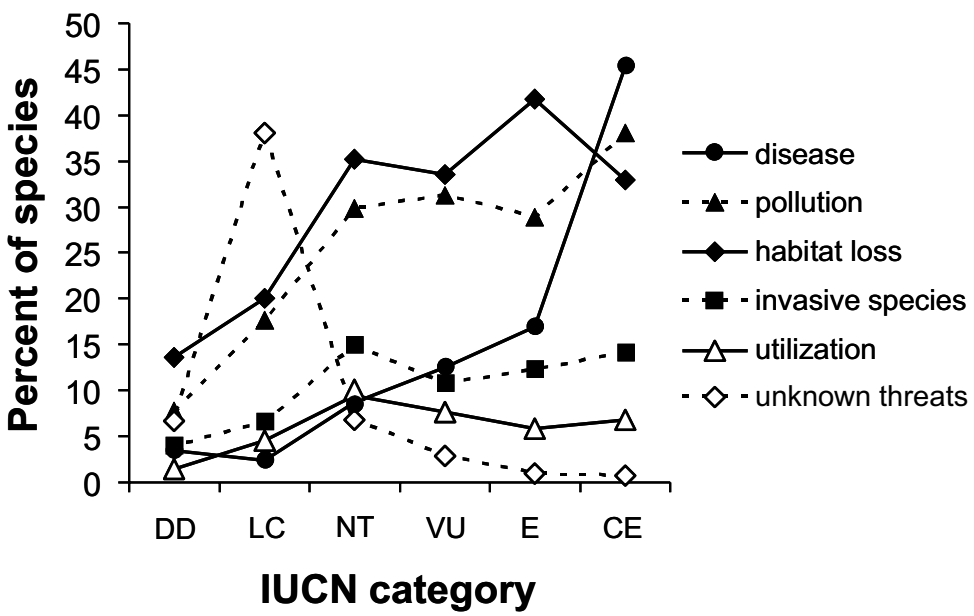

FIGURE 2.1 Percentage of amphibian species with extant populations in nature in each IUCN threat category for which 6 categories of threats are believed to be operating. Threat categories are arranged in order of increasing severity. From the left they are DD = data deficient, LC = least concern, NT = near threatened, VU = vulnerable, E = endangered, CE = critically endangered. Connecting lines are included to aid in visualizing how percentages change across categories. The total proportion for which at least one known threat has been nominated differs among categories, probably because greater effort has been focused on species under greater threat. The proportion of species known to be threatened by disease increases dramatically as the threat category increases. This is due mostly to the large number of species in the American tropics and Australia that have declined in association with outbreaks of the disease chytridiomycosis. (Data from IUCN 2008.)

that the most common threat to species in the CE category is disease, threatening 200 species; most of these are threatened by the global emergence of the disease chytridiomycosis (Skerratt et al. 2007), which has repeatedly caused dramatic declines to local extinction of entire regional faunas (e.g., Lips et al. 2006). Figure 2.1 makes it clear that although this disease has led to dramatic declines and may be responsible for many of the transitions of species into the CE category that occurred between 1980 and 2007 (Table 2.1), other factors also represent major, ongoing threats to amphibian biodiversity. Recent work on each major threat category is discussed under separate headings.

2.2.1 Habitat Destruction

The effects of habitat destruction are clear; when the major part of the natural habitat for a species is harvested or is converted to agricultural production or human habitation, species are negatively affected (Ash 1988; Semlitsch and Bodie 1998; Azevedo-Ramos and Galatti 2002). These effects can be felt even when remaining natural areas are set aside as reserves; populations in remaining fragments can be subject to extinction due to demographic stochasticity (Shaffer 1981; Lande 1993; Green 2000, 2003), and species living in metapopulations (Hanski 1994), or in integrated populations that shift their spatial distributions with changing habitat quality (Marsh and Trenham 2001; Bradford et al. 2003; Skelly et al. 2003; Smith and Green 2005), may be subject to extinction even when 20% or more of the original habitat is intact (Hanski 1994). More subtly, habitat destruction can have strong effects when it disrupts the ability of amphibians to undergo habitat shifts, as many species do either seasonally or at life history transitions (Becker et al. 2007).

In the current GAA database, habitat destruction is the threat most commonly identified for species in all categories except CE (Table 2.2). However, clear examples of large-scale declines of amphibians caused by habitat destruction are rare in the literature. This may be because most broad-scale clearance of native habitat is presently occurring in regions where the amphibian fauna is relatively poorly known. For example, Crump (1971, 1974) documented an extraordinarily diverse amphibian fauna in the vicinity of Santa Cecilia, Ecuador, in an area that was subsequently heavily modified for agriculture (M. Crump, personal communication); most species disappeared locally from the modified habitat, but whether this substantially affected the total population of any species is not known. Habitat disturbance caused by mining in the Western Ghats of India reduced amphibian species richness by 50% (Krishnamurthy and Hussain 2004). Woinarski et al. (2006) showed that broad-scale clearance of native vegetation in Queensland, Australia, led to declines in many native vertebrates, including frogs, and an increase in the abundance of exotic invaders. Diniz et al. (2006) examined an area of the Brazilian Cerrado for possible compromises between preserving amphibian diversity and allowing development for human use, and found that although there is a positive correlation between the desirability of areas for human use and conservation, it might be possible to design a relatively effective set of reserves by conserving approximately 10% of the original habitat. When intensive habitat modification occurs in relatively small patches, amphibians that vacate those patches can often quickly recolonize them (Ash 1988, 1997; Alford and Richards 1999; Aubry 2000; Lehtinen and Galatowitsch 2001; Ash et al. 2003). On the other hand, even relatively minor changes in remnant patches in landscapes that are already heavily modified may tip the balance between persistence and extinction (Semlitsch and Bodie 1998; Babbitt et al. 2006).

In addition to outright habitat destruction, amphibians can be affected by changes in habitat quality. Probably because amphibian populations have often been defined as the individuals using a particular breeding site (Marsh and Trenham 2001), many studies have focused on the quality of aquatic habitats and in some cases a narrow band of terrestrial habitat surrounding them.

One of the simplest aspects of quality for the pool and pond habitats used by many amphibians is hydroperiod, which can strongly affect the composition of larval and breeding assemblages (Adams 1999; Eason and Fauth 2001; Baldwin et al. 2006; Seigel et al. 2006; Werner et al. 2007). Changes in hydroperiod have been suggested as causes of regional declines in some areas (Daszak et al. 2005; Palis et al. 2006). Changes in land use of surrounding terrestrial habitat can alter hydroperiods

(Gray and Smith 2005), as can groundwater extraction (Guzy et al. 2006), and shifts in the hydroperiod of pools and ponds throughout entire regions due to global climate change may be a major threat to amphibians in the near future. These may be exacerbated by or interact with changes in the timing of seasonal reproduction caused by changes in temperature regimes (Blaustein et al. 2001; Chadwick et al. 2006). The quality of the aquatic habitat can also be modified biologically. Exotic animals and diseases will be considered separately, but 1 often overlooked factor is invasive aquatic vegetation. For example, Brown et al. (2006) showed that the invasive weed purple loosestrife (*Lythrum salicaria*) can negatively affect *Bufo americanus* tadpoles, probably through a combination of toxicity through ingestion of the leaves and alterations in the phytoplankton assemblage of pools infested with the weed.

Many other aspects of water quality can affect the suitability of breeding habitats. Environmental contaminants can have drastic and interactive effects, as discussed more fully below and elsewhere in this volume. Other factors that are commonly found to affect the suitability of habitat include pH and aluminum (e.g., Anderson et al. 1999), pH conductivity, and depth (e.g., Babbitt et al. 2006), and combinations of these with multiple other factors (Brodman et al. 2003).

In addition to water quality, the suitability of water bodies as reproductive sites is affected by the surrounding terrestrial habitat. These effects can act by altering the quality or quantity of water available, or changing the hydroperiod (Jansen and Healey 2003; Dayton and Fitzgerald 2006), but can also be caused by the suitability of the surrounding habitat for terrestrial juvenile and adult amphibians (Skelly 2001; Bradford et al. 2003; Knapp et al. 2003; Van Buskirk 2005; Baldwin et al. 2006; Otto et al. 2007). Many amphibians can occupy nonbreeding habitats that are not immediately adjacent to reproductive sites; when this occurs, connectivity between breeding sites and nonbreeding habitat is important (deMaynadier and Hunter 1999). Some disruption of habitat, for instance, by roads, can be tolerated by some species while adversely affecting others (deMaynadier and Hunter 2000). Becker et al. (2007) used the term "habitat split" to describe disruption of the connectivity between habitats used by differing life history stages, and showed a negative correlation between increasing habitat split and the species richness of amphibians with aquatic larvae in the Brazilian Atlantic Forest. They suggested that habitat split may be 1 reason why species with aquatic larvae are often suggested to be more subject to declines than are terrestrial breeders.

Pond-breeding amphibian species occur in nested subsets at various spatial scales; those with greater specificity in terrestrial or aquatic habitat are more nested, as are poorer dispersers (Hecnar and M'Closkey 1997), a pattern suggesting that both selective extinction and selective colonization are responsible for patterns of occurrence at a regional scale. Habitat quality at multiple scales, within breeding sites and terrestrial sites, affects population density of European palmate newts (*Triturus helveticus*; Denoel and Lehmann 2006), and at least some amphibian species show distinct thresholds for habitat variables or combinations of variables at which density or occupancy changes abruptly (Denoel and Ficetola 2007). Homan et al. (2004) showed that threshold densities of forest cover vary depending on distance from breeding site for a frog (*Rana sylvatica*) and a salamander (*Ambystoma maculatum*); both species required substantial cover at relatively great distances from breeding sites (1 km). Houlahan and Findlay (2003) showed similar effects for a multispecies assemblage. Habitat loss and degradation are often continuous processes, so that slow loss of populations, and increasing exposure to exotics and invaders from modified habitats, can lead species to these thresholds (Hobbs and Mooney 1998).

Cushman (2006) found that connectivity is an important determinant of the viability of regional amphibian faunas. He pointed out that the impacts of loss and fragmentation are likely to be felt first by more dispersive species, but that over longer timescales equal effects are likely on less dispersive species.

Hazell (2003) suggested that conservation research in Australia should focus on how animals cope with modified habitats, and Johansson et al. (2005) demonstrated that effects can be complex and depend on factors that vary regionally. Gardner et al. (2007) pointed out that habitat change is associated with the greatest number of amphibian population declines, and that most knowledge of its effects comes from the relatively depauperate faunas and heavily modified habitats of North America and Europe. They suggested that more research needs to focus on its effects, particularly in areas of the world where habitat change is more rapid and extreme. Understanding the factors affecting the

distribution and abundance of amphibians, and planning for their conservation, requires a detailed understanding of how they use habitat across a wide range of scales, from individual behavior, food, and microhabitat requirements (Altig et al. 2007) to regional vegetation structure and connectivity.

2.2.2 Exotic Predators

Amphibians and their larvae are vulnerable to a wide array of predators (Hecnar and M'Closkey 1996; Alford 1999; Gunzburger and Travis 2005), many of which are likely to affect population dynamics and ultimately persistence (Kats and Ferrer 2003). Given the wide range of potential predators of amphibians, and the widespread introductions of predators that have occurred globally in both terrestrial and aquatic habitats (Kats and Ferrer 2003), the literature on possible effects of exotic predators on amphibian populations may be overly concentrated on the effects of fishes and exotic amphibians in aquatic systems. There appears to be very little information on possible effects of other aquatic predators, or on the effects of exotic predators on the terrestrial stages of amphibians (Ahola et al. 2006; Anthony et al. 2007).

Predatory fishes have been widely introduced for sportfishing in many countries, and have strongly affected many of the systems they have occupied. They appear to be a major factor in declines of frogs in the California Sierra (Bradford 1989; Bradford et al. 1993; Fellers and Drost 1993; Drost and Fellers 1996; Knapp and Matthews 2000; Vredenburg 2004; Knapp et al. 2007). They are also implicated in declines of amphibians in other regions of North America (Monello and Wright 1999; Pilliod and Peterson 2001), and in southeastern Australia (Gillespie 2001; Hamer et al. 2002), Europe (Meyer et al. 1998; Martinez-Solano et al. 2003; Denoel et al. 2005; Orizaola and Brana 2006), and South America (Ortubay et al. 2006).

The North American bullfrog, *Rana catesbeiana*, has been introduced to many areas outside its native range by the aquaculture industry. It is a voracious predator of larvae and often adults of other amphibians, and has been implicated in a number of declines (Hayes and Jennings 1986; Lawler et al. 1999; Monello and Wright 1999; Adams 2000; Doubledee et al. 2003; Bradford et al. 2004; Pearl et al. 2004). Other predators that may have caused declines include mink (*Neovison vison*; Ahola et al. 2006) and crayfish (Gamradt and Kats 1996; Cruz and Rebelo 2005; Cruz et al. 2006).

In addition to their direct effects via the consumption of eggs, larvae, or adults, exotic predators can have a range of indirect effects. They may serve as vectors for parasites or pathogens (Daszak et al. 2004), and can increase the costs of antipredator behavior, reducing opportunities for growth and foraging (Chivers et al. 2001; Teplitsky et al. 2003; Cruz and Rebelo 2005). Their presence increases the frequency and intensity of stress responses, and may increase the effects of environmental contaminants (Relyea 2003, 2004, 2005; Teplitsky et al. 2005). Conversely, the effects of contaminants may alter antipredator behavior, making some species more vulnerable to predation (Bridges 1999).

The effects of exotic predators may increase or decrease with broad-scale environmental changes associated with global climate change. Adams (1999, 2000) suggested that the spread of exotic predators (*R. catesbeiana* and sunfish) was facilitated by changes in hydroperiod toward greater permanence. Broomhall (2004) demonstrated that the thermal regime experienced by frog eggs can affect their sensitivity to environmental contaminants, and Rohr and Madison (2003) demonstrated that environmental moisture levels reduced the responses of newt efts to conspecific predator avoidance cues, suggesting that climate change might increase their vulnerability to predation.

2.2.3 Environmental Contamination

Environmental contaminants are frequently suggested as potential primary factors or cofactors in amphibian declines (e.g., Alford and Richards 1999; Blaustein et al. 2003; Sih et al. 2004). There is strong correlational evidence from landscape-scale data that windborne pesticides have contributed to declines of *Rana muscosa* in the California Sierra Nevada (Davidson and Knapp 2007). Correlational evidence also suggests that declines, for example, those of *Acris crepitans* (Reeder

et al. 2005) and *Desmognathus fuscus* (Bank et al. 2006), have been caused by surface-borne pollutants. The prevalence of such effects may well be underestimated; Schiesari et al. (2007) showed that tropical fauna, and species with limited geographic ranges, are understudied; if broad ranges are correlated with broad tolerances, this may mean that the literature is biased against both tropical species and species with narrower environmental tolerances. Effects of contaminants are discussed at length elsewhere in this volume, so the treatment here is brief, considering mostly studies that highlight the complex, interactive nature of the effects of environmental contamination.

Many recent experimental studies have revealed that the effects of environmental contaminants are not well characterized by standard laboratory assays. This has 3 general causes. First, and simplest, the physical environment in the field differs from that in the laboratory; temperature, humidity, and the background chemistry of water do not mimic standard laboratory conditions, and any of these may alter the effects of contaminants. Second, many species are exposed to more than one, and often many, contaminants simultaneously, and their effects can interact in complex and unpredictable ways. Third, the fitness of animals in natural populations is determined by both their physical and their biological environments, and the effects of contaminants as mediated by the biological environment can be unpredictable and sometimes paradoxical.

Temperature regime can determine how contaminants affect larval amphibians (e.g., Boone and Bridges 1999; Broomhall 2004). This implies both that more realistic toxicological studies should examine the effects of contaminants at a wider range of temperatures (Boone and Bridges 1999) and that the effects of contaminants may be altered by climate change (Broomhall 2004), which could lead to the emergence of impacts on species that have historically coexisted with contaminants.

The effects of mixtures of contaminants can be highly unpredictable. For example, relatively low concentrations of carbaryl and nitrate, when either was applied alone, increased the rate of development and mass at metamorphosis of larval *Rana clamitans* (Boone et al. 2005). However, this positive effect disappeared when the contaminants occurred together. Hayes et al. (2006) examined 9 pesticides alone and in a variety of mixtures, and found that more complex mixtures generally had more negative impacts, both on life history characteristics such as growth rate and on the development of the endocrine system.

The effects of environmental contaminants can interact with those of predation. Relyea (2003) demonstrated that the effects of carbaryl on larvae of several species increased, sometimes greatly, when they were also exposed to the stress caused by the presence of predatory newts. Predator cues also amplified the effects of the herbicide Roundup on larval *Rana sylvatica* (Relyea 2005). Conversely, exposure to the herbicide amitrole decreased the response of larval *Bufo bufo* to the chemical cues released as a predator fed on conspecifics (Mandrillon and Saglio 2007); this could increase the negative effects of predators on frog populations. A similar effect was suggested by Bridges (1999) and Teplitsky et al. (2005). Other interactions may reduce the effects of contaminants; for example, Relyea et al. (2005) showed that in mesocosms, the insecticide malathion, which can be toxic to tadpoles at high dosages, can increase their survival at low dosages by removing predatory insects. Boone and Semlitsch (2003) found a similar effect of carbaryl, which decreased the effect of predatory crayfish on larval *Rana catesbeiana*. However, higher concentrations of carbaryl negatively affected tadpole survival, outweighing the positive indirect effects of predator removal. Carbaryl had very similar effects in a study by Mills and Semlitsch (2004) on larval *Rana sphenocephala*. Davidson et al. (2007) convincingly demonstrated using correlational data that both windborne pesticides and introduced fishes have contributed to declines in *Rana muscosa*.

As well as affecting the interactions of amphibians with predators, contaminants may also modify their interactions with diseases and parasites. This can occur via direct effects of contaminants on the amphibian immune system (Christin et al. 2003; Linzey et al. 2003; Houck and Sessions 2006). Interactions of contaminants with specific parasites and diseases have been documented in a variety of systems and are discussed in the following sections dealing with parasites and diseases. Contaminants can also interact with the effects of intraspecific and interspecific competition. Rohr et al. (2006) found that the immediate negative effects of exposure of larval *Ambystoma barbouri* to atrazine were partially ameliorated by reductions in intraspecific competition, although in the longer

term, postexposure decreases in survival of exposed animals reduced this effect. Interspecific competition can also be reduced by contaminants; for example, carbaryl can decrease the competitive effects of zooplankton on tadpoles (Boone and Semlitsch 2001).

Few experimental studies have set out to examine the effects of competition, predation, and contaminants simultaneously. Several studies by Boone et al. (Boone and Semlitsch 2001; Boone et al. 2004, 2007) have used experiments in mesocosms and larger systems to examine contaminant effects in complex assemblages. The outcomes of these experiments are complex and almost certainly specific to the systems examined, but they clearly support the general conclusion that the effects of environmental contaminants in natural systems are likely to be different in unpredictable ways from their effects in simple standardized assays.

2.2.4 Human Utilization

The GAA database identifies utilization as a threat to between 5 and 10% of species in each threat category (Table 2.2, Figure 2.1). Most utilization is as food for human consumption or in the pet trade, although there is substantial use of amphibians for other purposes, such as in traditional medical preparations, in some regions.

Frogs are commonly used as food in many parts of the world. There is a large international trade in fresh and frozen frogs and frogs' legs. Many of the species used as food are large ranids (Dash and Mahanta 1993; Kusrini and Alford 2006; Tyler et al. 2007). Some of these species appear to be capable of sustained harvesting; Kusrini and Alford (2006) estimated that the annual frog harvest for human consumption in Indonesia is on the order of 100 million frogs, but suggested that the harvest currently appears to be operating within the bounds of sustainability. However, trade at a similar level (Pandian and Marian 1986) led India to ban the export of frogs' legs in the 1980s as a factor threatening frogs and reducing their provision of ecosystem services in the form of insect control. The international trade in live frogs and frog parts for human consumption also poses a threat via the likely transport of pathogens (Berger et al. 1999; Daszak et al. 2003; Mazzoni et al. 2003; Rowley et al. 2007; Skerratt et al. 2007).

Amphibians are also common in the pet trade. Documentation of the extent of this trade and its potential impact on amphibian populations is surprisingly difficult to come by. It is clearly massive, and much of it is so poorly documented that impacts may be impossible to assess (Schlaepfer et al. 2005). Schlaepfer et al. (2005) found that more than 2.5 million wild-caught amphibians and reptiles imported into the United States annually between 1998 and 2002 were not identified in official trade records to the species level. Harvesting for the pet trade was suggested by Andreone et al. (2005, 2006) to be a major threat to the endangered frogs of Madagascar. The harlequin frogs of the genus *Atelopus*, which have been heavily affected by epidemic outbreaks of chytridiomycosis, have also been heavily collected for export as pets (La Marca et al. 2005), as have many species of dendrobatids (Gorzula 1996). The pet trade may well pose an even greater risk for transporting exotic pathogens than the food trade does; although the number of animals transported is lower, the conditions in which the pet trade occurs probably make it likely that diseases and parasites can be transmitted to naïve hosts, and it is also more likely that pets may be accidentally or deliberately released into habitats to which they are exotic.

Another form of utilization, with particularly negative indirect consequences, is the use of amphibians as live bait for fishing in some regions. This practice occurs in many areas of the United States, supplied by a large, and largely unregulated, interstate trade (Riley et al. 2003; Jancovich et al. 2005; Storfer et al. 2007).

2.2.5 Disease

Some of the most rapid progress in understanding the causes of amphibian declines in the past decade has been made in the area of threats due to disease. Diseases and parasites are very unlikely

to drive hosts with which they have long histories of coexistence to extinction (deCastro and Bolker 2005). However, the emergence of new infectious diseases, facilitated by rapid global transport and rapidly changing climatic conditions and ecological relationships, has recently been recognized as a major threat to the health of wildlife and humans (Daszak et al. 2001, 2003; Harvell et al. 2002). Emerging infectious diseases (EIDs) are defined as "diseases that are newly recognized, newly appeared in the population, or are rapidly increasing in incidence or geographic range" (Daszak et al. 2000, p 446). Even EIDs are likely to drive host species to local or global extinction only under certain conditions, including small host population sizes, the presence of pathogen reservoirs outside the affected host species, and possibly the specific behavior or social system of the affected host (deCastro and Bolker 2005). Disease is presently listed as a threat to 540 amphibian species (Table 2.2), and threatens the greatest proportion of species in the CE category (Figure 2.1). When amphibian declines are linked to EIDs, it is important to understand the causes of emergence (Rachowicz et al. 2005), since those are the ultimate causes of those declines. When diseases cause local extinctions, understanding how this can happen (deCastro and Bolker 2005) can suggest strategies for managing those diseases in other populations or species.

Early suggestions relating amphibian declines to disease focused on known pathogens. Many local disease outbreaks recorded in North America and Europe prior to 1990 were attributed, often entirely on the basis of symptoms, to the disease red-leg. This can be caused by a variety of pathogenic bacteria, but was commonly attributed to *Aeromonas hydrophila* (Cunningham et al. 1996; Green et al. 2002). Because the symptoms of red-leg are very general, and are consistent with a variety of diseases, it seems likely that many of these outbreaks were caused by other diseases, such as ranaviral infections or chytridiomycosis (Green et al. 2002). Carey (1993) suggested that environmental stresses might compromise the immune function of amphibians, causing disease emergence due to increases in susceptibility to infections. The fungus *Saprolegnia ferax*, which attacks aquatic egg masses, experimentally decreased survival rates in frogs of the northwestern United States (Blaustein et al. 1994a; Kiesecker and Blaustein 1995), and has been suggested to possibly affect amphibians in other regions. Ranaviruses were isolated and identified from outbreaks in amphibian populations in several areas in the mid 1990s (Cunningham et al. 1996; Jancovich et al. 1997). Laurance et al. (1996) suggested that widespread outbreaks of an emerging pathogen might have been the proximate cause of local and global extinctions in a regional amphibian fauna.

Although increased research on amphibian diseases has rapidly increased the number known (Carey 2000; Essbauer and Ahne 2001; Kiesecker et al. 2004), substantial research has been concentrated on 3 categories of disease that can produce mass mortality: ranaviruses and their relatives; saprolegniosis, caused by fungi in the genus *Saprolegnia*; and chytridiomycosis, caused by the amphibian chytrid fungus *Batrachochytrium dendrobatidis.*

2.2.5.1 Ranaviruses

Ranaviruses appear to be widely endemic. Several ranaviruses have been described from wild and captive amphibians, including *Ambystoma tigrinum* virus (ATV; Jancovich et al. 1997) and Bohle iridovirus (BIV; Speare and Smith 1992). They are all closely related to fish viruses, for example, epizootic haematopoietic necrosis virus (EHNV) (Hengstberger et al. 1993; Yu et al. 1999; Cullen and Owens 2002). Some of the viruses in this group can be transmitted between amphibians and fishes (Ahne et al. 1997); however, at least ATV appears to be specific to salamanders (Jancovich et al. 2001). The species that can cross-infect both classes may have major potential for outbreaks, since there are many possible reservoirs and modes of transport (Ahne et al. 1997).

Ranaviruses can cause large-scale die-offs in captive or laboratory populations (Cullen and Owens 2002). They have also caused die-offs in natural populations of salamanders (Jancovich et al. 2001; Brunner et al. 2004; Collins et al. 2004; Storfer et al. 2007) and frogs (Pearman and Garner 2005; Fox et al. 2006; Harp and Petranka 2006). They seem to cycle through local populations (Brunner et al. 2004), causing occasional epidemic outbreaks and mass mortality, but do not appear to be major threats to the persistence of any known amphibian species. This may change in

the presence of environmental contaminants; Forson and Storfer (2006) demonstrated that larval *Ambystoma tigrinum* that were exposed to atrazine were more susceptible to ATV infection than unexposed larvae, suggesting that contaminants might alter the dynamics of the disease to favor outbreaks. There is also a danger of larger-scale outbreaks caused by transport of animals or contaminated water. Harp and Petranka (2006) showed that placing sediments contaminated with ranavirus in mesocosms transmitted the infection to tadpoles. This suggests that uncontrolled disposal of water used to transport amphibians or tropical fishes may pose a serious risk of long-distance transport and introduction of exotic ranaviruses, which could have serious effects on naïve species or populations of amphibians.

2.2.5.2 *Saprolegnia* and Other Fungal Pathogens

Fungi of the genus *Saprolegnia* have long been known to attack fishes (Blaustein et al. 1994a). They can attack larval amphibians (Bragg and Bragg 1958; Walls and Jaeger 1987), but the greatest concern has been raised over their effects on eggs. Blaustein et al. (1994a) documented an outbreak of the fungus *Saprolegnia ferax* that killed an estimated 95% of the eggs deposited by a large population of *Bufo boreas* in Oregon. Kiesecker and Blaustein (1995) reported strong effects of the fungus on eggs of both *B. boreas* and *Rana cascadae*; these appeared to be stronger in eggs exposed to higher levels of UV-B radiation. Some negative effects of the fungus may be compensated by decreased density dependence in the larval stage; Kiesecker and Blaustein (1999) found that in artificial pond experiments, *Rana cascadae* that were exposed to *S. ferax* as eggs but survived to hatching grew and developed faster than individuals that were not so exposed, and therefore experienced higher larval densities. Kiesecker et al. (2001) suggested that *Saprolegnia* might have an increasing effect on amphibians as decreased water depths at oviposition sites caused increased exposure of eggs to UV-B radiation. The effects of *Saprolegnia* may be modified by predation on the fungus; Gomez-Mestre et al. (2007) found that larval *Rana sylvatica* grazed on and removed fungus from the eggs of *Bufo americanus*, increasing their survival.

Other fungi, including *Aphanomyces* sp., also infect tadpoles (Berger et al. 2001). The fungus *Mucor amphibiorum* has produced epidemic outbreaks in captive populations of adult frogs (Creeper et al. 1998); however, only isolated cases have been observed in the wild (Speare et al. 1994; Berger et al. 1997).

2.2.5.3 Amphibian Chytrid Fungus

Berger et al. (1998) showed that chytridiomycosis was the proximate cause of population crashes in Australia and Central America. The pathogen was described by Longcore et al. (1999) as *Batrachochytrium dendrobatidis*. It has become widely accepted (Rachowicz et al. 2005) that chytridiomycosis is the proximate cause of many of the amphibian declines categorized by Stuart et al. (2004a) as "enigmatic."

2.2.5.3.1 Characteristics of the Pathogen

Chytridiomycosis has caused population declines and extinctions across most of an entire class of vertebrates (Daszak et al. 1999). The very broad host range of *B. dendrobatidis*, including larvae and adults of many species that are resistant to chytridiomycosis as well as those of many species that are vulnerable to the disease, may explain its ability to drive populations of many species to extinction, since it can persist in less affected reservoir hosts (Hanselmann et al. 2004; deCastro and Bolker 2005; Woodhams and Alford 2005; Smith et al. 2007). The thalli of *B. dendrobatidis* live in the epidermis, which becomes infected by contact with waterborne flagellated zoospores. Propagation occurs by producing zoosporangia that release zoospores to the external environment. The development of chytridiomycosis thus requires multiple generations of successful colonization and recolonization of the host by propagules (Carey et al. 2006).

In vitro, the fungus usually exhibits endogenous development (James et al. 2000), with zoospores encysting on a substrate, developing a network of rhizoids, and subsequently developing

a zoosporangium, or sometimes several zoosporangia. On living amphibians, the zoosporangia begin developing within cells in the deeper layers of the epidermis and complete their development as those cells migrate toward the skin surface. The details of the process of encystment and initial infection on living amphibians have not been documented. However, to reach the interior of cells deep in the epidermis, it appears that *B. dendrobatidis* must switch to an alternate developmental pathway, exogenous development (James et al. 2000), in which the zoospore encysts on the host surface, then develops a germ tube through which the nucleus migrates into the interior of a host cell or tissue (James et al. 2000). In culture, encysted zoospores can develop apparent germ tubes extending toward isolated pieces of frog skin (J. Longcore, personal communication).

The ability to facultatively switch developmental mode appears to be unusual for fungi of the phylum Chytridiomycota (Powell and Koch 1977). The flexibility exhibited by *B. dendrobatidis* may suggest that it regularly exploits a variety of substrates in nature. Johnson and Speare (2005) showed that *B. dendrobatidis* can survive for extended periods in the laboratory on a variety of potential environmental substrates, and Lips et al. (2006) found *B. dendrobatidis* DNA on 1 of 9 haphazardly chosen stream boulders. More work is needed on alternative substrates, to increase understanding of how *B. dendrobatidis* persists in the environment and how it may be dispersed. This may be aided by techniques recently developed for sampling environmental water for the presence of *B. dendrobatidis* DNA (Kirshtein et al. 2007; Walker et al. 2007).

2.2.5.3.2 Emergence and Persistence of Chytridiomycosis

At present, *B. dendrobatidis* is widely distributed in Africa (Weldon et al. 2004; Goldberg et al. 2007), Europe (Mutschmann et al. 2000; Bosch and Martinez-Solano 2006), North America (Green and Muths 2005; Ouellet et al. 2005; Longcore et al. 2007; Pearl et al. 2007), Central and South America (Ron 2005; Carnaval et al. 2006; Lips et al. 2006; Puschendorf et al. 2006b), and Australia (Drew et al. 2006). It also occurs on many islands, both in the Atlantic and Carribbean and in the Pacific (Bell et al. 2004; Burrowes et al. 2004; Beard and O'Neill 2005; Diaz et al. 2007). It has not yet been detected in continental Asia; however, very few surveys have been done there (Rowley et al. 2007).

Debate regarding the reasons for the emergence of chytridiomycosis has centered on 2 competing hypotheses, which Rachowicz et al. (2005) described as the novel and endemic pathogen hypotheses. The novel pathogen hypothesis postulates that *B. dendrobatidis* has recently greatly increased its geographic range, encountering naïve hosts that have no defenses against it. The endemic pathogen hypothesis in its simplest form suggests that *B. dendrobatidis* has historically had a wide geographic distribution, and has emerged as a major pathogen of amphibians due to widespread ecological changes, possibly associated with climate change, that have "tipped the balance" in the host-pathogen relationship (e.g., the climate-linked epidemic hypothesis of Pounds et al. [2006]). Various authors have suggested that the true causes may be a combination of local or global range expansion with environmental changes that favor the pathogen (Rachowicz et al. 2005; Blaustein and Dobson 2006).

Genetic studies might provide a definitive answer to the question of the pathogen's origin. However, to date, they have not been conclusive. Morehouse et al. (2003) used multilocus sequence typing to examine levels of nuclear genetic diversity among 35 strains of *B. dendrobatidis* from North America, Africa, and Australia. They found very low levels of variation, nearly constant frequencies of heterozygous genotypes, and little geographic patterning, suggesting that most reproduction is clonal and that the pathogen has recently dispersed globally. Morgan et al. (2007), however, examined 15 variable nuclear loci and found evidence for both recent dispersal and endemism in the Sierra Nevada of California. Berger et al. (2005a) showed that the virulence of *B. dendrobatidis* differed significantly among 3 strains, and Piotrowski et al. (2004) demonstrated differences in growth rate and sensitivity to pH among strains grown in vitro. These strain-specific differences might reflect genetic differentiation among strains.

Two studies have examined large samples of museum specimens to look at the historical occurrence of *B. dendrobatidis*. Ouellet et al. (2005) examined specimens from 25 countries collected between 1895 and 2001, the great majority of which were collected after 1960 from North America. The earliest specimens they found infected with *B. dendrobatidis* were *Rana clamitans* collected in Quebec in 1961. They found that the prevalence of *B. dendrobatidis* infections in Quebec had not changed significantly between the decades 1960 to 1969 and 1990 to 1999, and suggested that the organism had been widespread in North America since at least the early 1960s. They noted that the number of specimens collected prior to 1960 that they had examined was not sufficient to establish with any certainty that the pathogen was absent before that time.

Weldon et al. (2004) examined 697 specimens of *Xenopus* spp. collected in southern Africa between 1879 and 1999. The overall prevalence of *B. dendrobatidis* in the sample was 2.7%; there was no statistically significant trend in prevalence over time. They suggested that this established that the fungus is endemic to South Africa, in a stable association with *Xenopus* species, and that the disease might have been spread worldwide by the extensive trade in *Xenopus*. This untested hypothesis has frequently been cited as an established fact in the public media, and may be contradicted by the lack of genetic variation among African isolates (Morehouse et al. 2003) and the recent occurrence of epidemic outbreaks of chytridiomycosis in southern Africa (Hopkins and Channing 2003).

Far more work needs to be done before any hypothesis is accepted; for example, a strong case could also be made for the hypothesis that the fungus originated in North America. It is widespread there, occurs in apparently stable associations with nondeclining species (Daszak et al. 2005), has been found in relatively early museum records, and shows some genetic differentiation on a local scale (Morgan et al. 2007). It could have been disseminated globally via the trade in bullfrogs (Garner et al. 2006).

Laurance et al. (1996) examined the spatiotemporal pattern of declines in Queensland, Australia, and suggested that an epidemic disease was traveling northward at approximately 100 km·year^{-1}. Their analysis was criticized by Alford and Richards (1997), who pointed out that their conclusions were based on 3, or at best 4, clusters of decline sites that could not be regarded as independent within clusters, and that within the largest cluster, spanning approximately 380 km, there was no evidence of a wavelike progression of outbreaks. The wavelike nature of outbreaks has also been emphasized in a series of publications by Lips (Lips 1999; Lips et al. 2006, 2008), in which the timing of outbreaks of chytridiomycosis leading to declines of montane amphibians in Central and South America was suggested as most consistent with a hypothesis of at least 4 separate introductions of *B. dendrobatidis* in Central and South America, followed by spread of the pathogen across the landscape. The pathogen presently occurs in almost all suitable habitats in Australia, Europe, and North, Central, and South America. Many of the sites at which it now occurs are remote, and have no introduced amphibian populations within at least hundreds of kilometers. If it originated in southern Africa (Weldon et al. 2004), *B. dendrobatidis* is capable of extremely rapid and efficient dispersal through a wide variety of relatively undisturbed habitats. This makes it surprising that it remained confined to certain frog species in southern Africa for its entire preceding evolutionary history.

The endemic pathogen hypothesis also is supported by some lines of reasoning and evidence. Epidemic outbreaks do not always occur within weeks or months of the organism first appearing at a site. DiRosa et al. (2007) reported that *B. dendrobatidis* was present at a site in Italy for several years before an epidemic outbreak occurred. Richards et al. (1993) documented declines at Kirrama, Queensland, in the highly susceptible species *Litoria rheocola* approximately 1 year after the first known date of occurrence of *B. dendrobatidis* in that area (Berger et al. 1999). Puschendorf et al. (2006a) showed that *B. dendrobatidis* was widely distributed in Costa Rica as early as 1986, at least a year before the first known declines associated with chytridiomycosis in Costa Rica (Pounds and Crump 1994). Despite the widespread occurrence of *B. dendrobatidis* in many species in North America (e.g., Ouellet et al. 2005; Longcore et al. 2007; Pearl et al. 2007), only localized epidemic outbreaks have been reported, and amphibian populations are known to have coexisted with the pathogen for many years (Daszak et al. 2005).

In regions where the pathogen is now endemic, the environment strongly affects its prevalence and the probability of epidemic outbreaks. In northeastern Queensland, Australia, for example, where all known populations above approximately 400 m elevation of 7 susceptible species were extirpated by epidemic outbreaks of chytridiomycosis during the late 1980s and early 1990s, no populations below that elevational cutoff were affected (McDonald and Alford 1999). The prevalence of *B. dendrobatidis* in Queensland frogs now fluctuates seasonally in a manner consistent with those elevational effects (McDonald et al. 2005; Woodhams and Alford 2005); it is higher in the cool, dry winter months and lower in the warm, wet summer months. Similar seasonal and elevational effects have been reported in other areas (Ron 2005; Longcore et al. 2007).

Environmental temperature is probably 1 cause of these patterns. Piotrowski et al. (2004) examined the growth rates of *B. dendrobatidis* populations in vitro and showed that the pathogen grew between 4 and 25 °C and reproduced most rapidly between 17 and 25 °C. Temperatures above 30 °C killed the fungus. Woodhams et al. (2008) found that although development of the fungus slowed at temperatures below 17 °C, the numbers of zoospores produced by each thallus increased, so that rapid population growth on the host can probably be maintained over a wide range of temperatures between approximately 10 and 25 °C. They also found that sudden temperature decreases stimulated the release of zoospores; in nature, synchronous release of zoospores by many zoosporangia on many infected individuals could result in large peaks in the rate of acquisition of new infections during the cooler months.

Several studies have produced correlational evidence that the timing of epidemic outbreaks of chytridiomycosis is controlled by weather, which may be changing with the global climate. Pounds et al. (2006) demonstrated a relatively strong relationship between increased air temperatures and the last year observed for species of *Atelopus*. They suggested that the apparent effects of higher temperatures might be caused by increases in cloud cover, which retains heat at night, causing amphibians to experience temperatures within the thermal range optimal for growth of *B. dendrobatidis* over longer periods of time. Bosch et al. (2007) showed a correlation between increasing temperatures and outbreaks of chytridiomycosis in Spain, and Laurance (2008) found that frog declines in upland Australian rainforests tended to occur during warmer periods. Outbreaks of chytridiomycosis in *Atelopus* species were linked by Lampo et al. (2006) to a severe dry season.

Studies at the individual level provide some insight into the possible mechanisms of the effects of weather and climate. Woodhams et al. (2003) found that 16 hours of exposure to temperatures of 37 °C cured all infected *Litoria chloris,* while 16 hours at 8 °C caused infections to progress more slowly than they did in frogs housed at a constant 20 °C. Rowley (Rowley 2006; Rowley and Alford 2007b) tracked frogs of several sympatric species in the Australian Wet Tropics, and found they used the environment in ways that exposed them to very different moisture and temperature microenvironments. Both across species and within species, individuals that attained body temperatures above 25 °C had lower probabilities of infection by *B. dendrobatidis*. Rowley and Alford (2007a) demonstrated that the behavior patterns of tracked frogs were also likely to affect the probability of transmission of *B. dendrobatidis*.

Taken together, individual level studies suggest that in the field, infections on individuals of many species may be maintained at relatively low prevalence and intensity by various combinations of dry environmental conditions, which inhibit the release of zoospores and thus reduce the rate of reproduction of the fungus; and higher body temperatures, which slow the growth rate of the fungus, may increase the effectiveness of immune responses, and may clear infections at temperatures above 30 °C. If this is the case, periods of overcast weather and high humidity may increase the growth rates of *B. dendrobatidis* populations on infected individuals, releasing large numbers of infective zoospores into the environment and producing epidemic outbreaks.

Whatever the history of dispersal of *B. dendrobatidis* has been, it may presently be absent from some areas (Rowley et al. 2007), and it is clear that strong precautions should be taken to avoid aiding its dispersal by anthropogenic means (Skerratt et al. 2007).

2.2.5.3.3 Determinants of Vulnerability to Chytridiomycosis

In epidemic outbreaks and when it is present as an endemic, vulnerability to chytridiomycosis caused by *B. dendrobatidis* varies widely among species. Initial epidemic outbreaks often drive populations of some species to local extinction, cause others to decline but not to extinction, and leave others apparently unaffected (Richards et al. 1993; Lips 1999; Fellers et al. 2001; Bell et al. 2004; Bosch and Martinez-Solano 2006; Lips et al. 2006).

Several studies have looked for correlations between the ecological and life history characters of amphibians and vulnerability to declines caused by chytridiomycosis. At a national Australian workshop in late 1997, McDonald and Alford (1999) presented an analysis of patterns of frog declines in eastern Australia showing that species more tightly associated with streams were more likely to suffer declines. This pattern has repeatedly emerged in subsequent analyses of tropical rainforest amphibians (Williams and Hero 1998; Lips et al. 2003; Hero et al. 2005). Several studies have also found correlations between the severity of the effects of chytridiomycosis and other characteristics, including geographic range size, body size, and fecundity (Williams and Hero 1998; Lips et al. 2003; Hero et al. 2005). These relationships are difficult to interpret, since when local extinctions associated with chytridiomycosis have been documented, they typically involve rapid and complete mortality of all terrestrial members of populations (McDonald and Alford 1999; Lips et al. 2006), rather than the slow spiraling toward extinction that might be expected from low fecundity and limited geographic range. Hamer and Mahony (2007) pointed out that *Litoria aurea*, which has suffered severe declines in association with chytridiomycosis, has life history characteristics more usually associated with invasive species than declining ones. Only Murray and Hose (2005) have examined correlates of decline in frogs using phylogenetically independent contrasts. They found that only geographic range size was correlated with the probability of decline across a large number of clades.

Immune function can affect vulnerability to *B. dendrobatidis* and other diseases. Carey (2000) suggested that disruption of amphibian immune function by environmental stressors might be a factor in the emergence of disease. It appears that the adaptive and cellular immune systems of amphibians do not show strong responses to *B. dendrobatidis*, even in advanced stages of chytridiomycosis (Pessier et al. 1999; Berger et al. 2005b). Amphibians also possess innate immune defenses, in the form of antimicrobial peptides (AMPs) that are secreted by the granular glands onto the skin surface. Rollins-Smith et al. (2002a, 2002b) demonstrated the effectiveness of a variety of AMPs against *B. dendrobatidis*. Further studies (Rollins-Smith et al. 2003, 2005; Rollins-Smith and Conlon 2005) showed that many amphibian species produce one or more AMPs that can strongly inhibit the fungus. Woodhams and his co-workers (2006a, 2006b, 2007a) have produced strong correlational evidence that vulnerability of amphibian species to population declines caused by *B. dendrobatidis* is related to the effectiveness of their AMPs against the fungus. Davidson et al. (2007) showed that levels of AMP production in *Rana boylii* were decreased by exposure to the insecticide carbaryl. Much more work is needed, for example, to understand whether AMP production responds to infection or is purely constitutive, whether the chemical composition of AMP secretions varies among individuals and populations and whether it is affected by environmental conditions, and how this relates to susceptibility to chytridiomycosis.

Another factor that can affect vulnerability to chytridiomycosis is interactions between *B. dendrobatidis* and other microbes that inhabit amphibian skin. The skin of many amphibians supports a complex microbial assemblage, with which the zoospores of *B. dendrobatidis* must interact during the infection process (Belden and Harris 2007; Culp et al. 2007). Changes in the composition of this assemblage are likely to alter these interactions; some assemblages may exclude the pathogen while others may be readily invaded by it (Belden and Harris 2007). Harris et al. (2006) demonstrated that several genera of bacteria commonly isolated from salamanders of 2 species inhibited the growth of *B. dendrobatidis* in culture. The composition of the bacterial assemblages on *Plethodon cinereus* and *Hemidactylium scutatum* and the nature of their interactions with fungi were explored in greater detail by Lauer et al. (2007, 2008). One of the bacterial metabolites that can inhibit *B. dendrobatidis*

was identified and characterized by Brucker et al. (2008). Two other bacterial metabolites occur on the skin of *P. cinereus* at concentrations sufficient to completely inhibit the growth of *B. dendrobatidis* in vitro (R. N. Harris, personal communication). Cutaneous bacteria can also contribute to the defenses of frogs against *B. dendrobatidis*; Woodhams et al. (2007c) found that a significantly greater proportion of individuals of the threatened species *Rana muscosa* carried bacteria with activity against *B. dendrobatidis* in a population that had coexisted with the pathogen for 6 years than in a population that was declining due to chytridiomycosis. Harris et al. (2009) experimentally demonstrated that *Plethodon cinereus* that had been exposed to the bacterium *Pseudomonas reactans* suffered fewer negative effects after exposure to *B. dendrobatidis* than salamanders that had not been exposed to the bacterium. It is likely that the AMPs of amphibians interact with and modify their skin microbiota (Woodhams et al. 2007b). Understanding the complex ecology of the microbiota of amphibian skin is at a very early stage, but may lead to an ability to manipulate interactions, for example, through probiotic applications or introductions of bacteria that tip the balance against *B. dendrobatidis* (Woodhams et al. 2007b; Harris et al. 2009).

2.2.6 Amphibian Limb Malformations and Their Relation to Parasitic Disease

Reports of possible increases in the incidence of developmental abnormalities, particularly limb malformations, in amphibians have attracted wide popular attention (Johnson et al. 2001, 2003; Loeffler et al. 2001; Chapter 16, this volume). Several factors can cause limb abnormalities in amphibians (Loeffler et al. 2001). These include environmental contaminants (La Clair et al. 1998; Natale et al. 2000; Qin et al. 2005; Taylor et al. 2005; Papis et al. 2006; Piha et al. 2006; Webb and Crain 2006), injuries to developing limb buds (Loeffler et al. 2001; Johnson et al. 2006), the direct effects of UV-B radiation (Starnes et al. 2000), and encystment of parasitic trematodes (Johnson et al. 1999, 2002; Kiesecker 2002). Johnson et al. (1999) demonstrated that cercariae of the trematode *Ribeirioa* sp. (later assigned to *Ribeiroia ondatrae*; Johnson et al. 2004) attacked larvae of the frog *Hyla regilla*, encysting near the developing hindlimb buds and producing hindlimb malformations similar to those found at field sites. Several subsequent studies by Johnson et al. (2001, 2002, 2003) demonstrated that *Ribeiroia* infections are a common, though not universal, cause of limb malformation in amphibians, and that it is likely that the prevalence of *Ribeiroia*-induced malformations increased in the late 20th century.

Johnson et al. (2003) suggested that amphibian limb deformities caused by parasitic infection can be regarded as an emerging infectious disease. Johnson and Chase (2004) suggested that the emergence of infection by *Ribeiroia* spp. might be due to changes to aquatic food webs, in which nutrient runoff causes eutrophication, which causes a shift in the composition of the snail assemblage in ponds toward the genus *Planorbella*, which are intermediate hosts of *Ribeiroia*, leading to higher levels of infective cercariae in the water column and higher rates of limb malformations in frogs emerging from eutrophic water bodies. This hypothesis was experimentally demonstrated to be feasible by Johnson et al. (2007), who also showed that infection by *Ribeiroia* decreased survival of larval amphibians, as well as increasing rates of limb malformation. In addition to the effects of eutrophication, environmental contaminants present in agricultural runoff may affect the prevalence and intensity of trematode infections in larval amphibians, possibly by affecting immunocompetency (Kiesecker 2002). Although infection by *Ribeiroia* spp. is clearly not the only cause of amphibian malformations (Skelly et al. 2007), it appears to be a common one, with a pattern of emergence that is strongly linked to anthropogenic effects on the environment via eutrophication.

2.2.7 Ultraviolet Radiation

One of the early and obvious signs of global-scale human influences on the climate and environment was increases in levels of incident ultraviolet radiation caused by changes in the outer layers

of the atmosphere (Kerr and McElroy 1993). Ultraviolet-B (UV-B) radiation can directly reduce the survival of eggs and embryos of amphibians that rely on egg masses that remain near the surface of shallow water bodies (Ovaska et al. 1997; Lizana and Pedraza 1998; Broomhall et al. 2000; Hakkinen et al. 2001). In many systems, current evidence suggests that ambient UV-B levels are not high enough to cause such damage (Langhelle et al. 1999; Merila et al. 2000; Starnes et al. 2000; Calfee et al. 2006). However, evaluating direct effects on egg and embryo survival may not be sufficient; Pahkala et al. (2001) found that effects of higher levels of UV-B exposure only appeared in the larval stage, where they found a higher rate of developmental abnormalities and slower rates of growth and development in *Rana temporaria* that had been exposed to enhanced UV-B as embryos. Belden and Blaustein (2002) found similar effects on *Rana aurora* exposed to full ambient levels of UV-B in the field.

A great deal of protection from environmental UV-B can be provided by the low transparency of many natural water bodies at these wavelengths (Palen et al. 2002) and by the optical properties of the jelly capsules of many amphibian eggs (Licht 2003). The direct effects of UV-B on eggs and embryos are likely to be mediated by the activity of repair enzymes such as photolyase (Blaustein et al. 1996, 1999; van de Mortel et al. 1998), which can change in response to changes in UV-B exposure (Smith et al. 2000). Experimental work on Patagonian frogs, which are exposed to increased levels of UV-B radiation due to ozone depletion (Perotti and Diegeuz 2006), suggested that melanin levels in eggs and embryos increase in response to increased UV-B exposure, but these increases may be insufficient to eliminate increases in malformation rates when UV-B exposure is high.

In addition to its effects on aquatic stages of amphibians, UV-B could directly or indirectly affect terrestrial individuals. High levels of UV-B may damage the immune system (Carey et al. 1999), and might increase the susceptibility of individuals to disease (Kiesecker et al. 2001). Kiesecker et al. (2001) showed, using correlative and experimental data, that increases in exposure to UV-B increase the vulnerability of frog embryos to the pathogenic fungus *Saprolegnia ferax*. However, Garcia et al. (2006) found no evidence for any interaction between the effects of UV-B and chytridiomycosis on 3 species of western North American frogs.

Evidence is also accumulating that damage caused by UV-B may interact, sometimes multiplicatively, with the effects of environmental toxins and other threats (Blaustein et al. 2003). The effects of high levels of nitrate fertilizers on the growth and development of larval amphibians can depend in a complex way on levels of exposure to ambient UV-B (Hatch and Blaustein 2003). Fite et al. (1998) showed that high ambient levels of UV-B can cause retinal damage in adult frogs, and Blaustein et al. (2000) demonstrated that exposure to UV-B changed the overall activity levels of newts (*Taricha granulosa*). Increasing levels of UV-B exposure decreased the expression of antipredator behavior by juvenile toads (*Bufo boreas*) and frog tadpoles (*Rana cascadae,* Kats et al. 2000); in nature, this should lead to decreases in survival and recruitment. Some species may be capable of responding evolutionarily to increases in ambient UV-B; Weyrauch and Grubb (2006) found that populations of *Rana sylvatica* with lower genetic diversity experienced higher larval mortality rates when exposed to UV-B than did populations with higher levels of genetic diversity.

Although it appears that present levels of ambient UV-B are not a major cause of amphibian declines, it may have a role in some systems. That UV-B exposure can interact in unpredictable ways with other threats indicates that a much fuller understanding of its effects would be useful in the future management of threatened amphibian populations.

2.2.8 Climate Change

Most of any effects of global climate change on amphibians that may have occurred to date are likely to have arisen through interactions of changed environmental conditions with other factors, many of which are discussed above. Most herpetologists appear to agree with Carey and Alexander (2003), who pointed out that it is unlikely that the simple thermal or other effects of recent climate change have been sufficient to explain recent declines in amphibian populations. However, effects of

climate change can certainly cause changes in the abundance of amphibians in particular localities or habitats. Whitfield et al. (2007), for example, documented a long-term decrease in the abundance of amphibians at LaSelva, Costa Rica, and linked this change to climate-driven decreases in the quantity of leaf litter. There is evidence (Seimon et al. 2007) that some species of amphibians have extended their ranges to higher elevations due to deglaciation. Climate change within the ranges predicted for the near future may have severe impacts on many amphibian populations without the need for interactions with other factors. Williams et al. (2003), for example, suggested that an increase of 3.5 °C in average temperatures could cause the extinction of most of the vertebrates endemic to the Australian Wet Tropics, including many amphibians.

There may be statistical problems with some of these predictions (Pearson and Dawson 2003; Dormann 2007). In addition, they assume that species' ranges are presently limited only by environmental factors, and that the nature of these limits will not change as the environment changes. They thus ignore the well-established effects of history, evolution, and biotic factors (e.g., Williams and Pearson 1997; Graham et al. 2006; Dormann 2007; Heikkinen et al. 2007) on species' ranges. It is also clear (Hauselberger and Alford 2005; Rowley and Alford 2007a) that sympatric amphibian species can experience very different environmental regimes due to microhabitat selection and other behavioral differences. Taken together, these suggest that accurate predictions of responses to climate change are not possible at present. However, it is very clear that if the environment changes sufficiently, there will ultimately be large effects on amphibians and on all other elements of the planet's biota. More detailed knowledge of how amphibians experience and interact with the physical environment in the field should aid in refining the predictions of climate-based models, and in focusing conservation efforts to minimize as much as possible the effects of climate change on amphibians.

2.3 MANAGEMENT OF AMPHIBIAN POPULATIONS

Managing and conserving amphibian populations has been greatly hindered by the relative scarcity of information on their ecology at the individual and population levels. Early regional and national plans (e.g., Tyler 1997) included large components of research; proposed actions focused on habitat conservation and, in some cases, preliminary work toward the ability to carry out management in captivity. Substantial progress has been made toward a fuller understanding of many of the threats to amphibians, including environmental contaminants, UV-B, disease, and habitat modification, since these early plans were adopted, and it is likely that many of them need to be redrafted in the light of current knowledge. The Global Amphibian Conservation Action Plan (Gascon et al. 2007) may serve as a useful template, since it incorporates very current input from many conservation specialists and researchers. The "Summary of Action Steps" on pages 6 to 11 of that document presents most of the considerations necessary for a well-thought-out conservation plan. The remainder of the action plan provides a very useful review of the conservation biology of amphibians.

At present, when species are imminently threatened, either by unknown causes or by outbreaks of chytridiomycosis, management options are limited. Mendelson et al. (2006) argued strongly in favor of short-term captive management as a tool to "buy time" for critically threatened species, and this idea has been adopted for some species. There is still a need for a much greater understanding of the basic behavior and ecology of many species (Biek et al. 2002), particularly those in the more poorly studied regions and faunas (Schiesari et al. 2007). The rapidly growing field of microbial interactions on amphibian skin offers some hope of eventually managing populations threatened by the amphibian chytrid fungus in the field (Woodhams et al. 2007b). It is very clear that habitat conservation must remain an important part of efforts to conserve amphibians (Gardner et al. 2007), and that it is essential to control human-assisted movements of organisms of all sorts (Kats and Ferrer 2003; Skerratt et al. 2007; Tyler et al. 2007). Failing to do that is difficult to understand, because unlike many other problems, solutions exist and are technically achievable.

It is also crucial to continue to document the diversity of amphibians. Parra et al. (2007) point out that the number of known amphibian species has increased by approximately 40% since 1987, and

that as many as half of the world's species may currently be undescribed. This is a far higher rate of discovery and proportion of undescribed taxa than occurs in any of the other groups of terrestrial vertebrates. The process of status assessment also needs to be streamlined and made ongoing, rather than episodic (Stuart 2007).

2.4 SUMMARY AND CONCLUSIONS

In less than 20 years, the phenomenon of global amphibian declines has moved from initial recognition to be one of the better-studied aspects of what is clearly a global crisis for biological diversity of all sorts (Gascon et al. 2007). Amphibians appear to be the most threatened class of vertebrates (Stuart et al. 2004a). The picture is not entirely gloomy, however. Research carried out since the problem was first identified has enormously deepened our understanding of the biology of amphibians and the nature of the factors threatening them. Two or more generations of planning and responses have begun to converge on action plans that should be possible to implement and should aid greatly in the conservation of many species. Entire fields of research that were largely or entirely unknown in 1990 have opened up and begun to be applied to solving the problem of amphibian declines. Much more study is still needed, but as the public has become aware of the extent of the problem, and the challenges it poses, the resources available to apply to the problem have begun to increase. The total budgets suggested in all chapters of the global amphibian conservation action plan (Gascon et al. 2007) amount to less than the costs of 2 large commercial airliners. It is to be hoped that the global community will perceive that preserving several thousand species of amphibians is worth that much.

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3 The Global Status of Reptiles and Causes of Their Decline

Brian D. Todd, John D. Willson, and J. Whitfield Gibbons

CONTENTS

Reptiles have been considered by some to be of "minor importance," and their disappearance has been suggested to "not make much difference one way or the other" (Zim and Smith 1953). Linnaeus himself described reptiles in his 1758 Systema Naturae as "foul and loathsome animals … abhorrent because of their cold body … fierce aspect … and squalid habitation." Thankfully, such sentiments are increasingly outdated as scientists reveal the significant roles that reptiles play in many ecosystems.

Although reptiles remain among the least studied vertebrate groups and are still frequently considered of less general interest than other fauna (Gibbons 1988; Bonnet et al. 2002), interest in the preservation of biodiversity, and consequently interest in reptile conservation, is growing. Declines of reptile populations, whether unnoticed or widely documented, are troubling not just because of the ecological relevance of reptiles to many habitats, but also because they portend a general decay of environmental health similar to declines of other species. Regardless of the motivation, the desire to conserve reptiles and to better understand their ecology requires knowledge of their status, distribution, and the factors that contribute to their decline.

Our goals in this chapter are to describe the major anthropogenic threats faced by reptiles and to summarize the perceived global conservation status of the traditionally recognized major taxonomic groups of reptiles. Specifically, we discuss the Crocodilia, Squamata, and Testudines, all historically included in the class Reptilia. Unfortunately, because so little is known about the basic ecology, distribution, and status of amphisbaenids, we exclude discussion of them from this chapter. Likewise,

given the low diversity (2 species) and extreme geographical restriction of tuataras (Sphenodontia), we do not separately review this group. Our specific objectives in this chapter are to

1) call attention to the lack of data on the status of reptile populations and describe strategies for documenting range-wide and localized declines,
2) describe the major threats facing reptile populations globally, and
3) describe current patterns of imperilment in reptile populations.

Two major reviews have recently described the main threats to reptile populations, and we expand on these using recent examples from an ever-growing body of literature (Gibbons et al. 2000; Irwin and Irwin 2005).

3.1 DETERMINING THE STATUS OF REPTILE SPECIES AND POPULATIONS

As with many fauna in recent years, conservation biologists have raised concerns over reported reptile declines, some of which have garnered widespread recognition (e.g., Asian turtle crisis; Buhlmann et al. 2002). Nevertheless, the considerable and notable lack of information regarding the status of reptiles in most regions has hampered full understanding and appreciation of their current plight. Without question, some reptile populations have been extirpated and numbers of some species have declined with little indication of the underlying cause. In some cases, concerns about declines are based wholly on anecdotal observations or on a growing perception of a species' rarity without accompanying quantitative data. Clearly, a primary goal of herpetologists and wildlife biologists should be to clarify the global status and distribution of reptile populations.

The World Conservation Union (IUCN) has been a global leader in assessing the status of many floral and faunal species as part of its ongoing Red List program (Baillie et al. 2004). Although the IUCN has comprehensively assessed birds, mammals, and amphibians to date, a global reptile assessment was only recently begun. Currently, reptiles remain one of the least known vertebrate taxa, and the conservation status of only about 6% of species has been assessed (Baillie et al. 2004). Some impediments to determining the status of reptiles derive from difficulties in studying or monitoring their populations. For example, many reptiles are characterized by cryptic coloration or behavior, which can impede observation or capture (Zug et al. 2001; Dorcas and Willson 2009). Detectability of reptiles also depends to some degree on the survey technique used, the seasonal or daily timing of surveys, and the environmental conditions during which surveys are conducted (Todd et al. 2007). Additionally, the status of reptiles can be assessed on at least 2 scales: local populations and regional distributions.

Concerns over declines of some reptiles arise from general impressions of range-wide or regional contractions in species' distributions. Obviously, such declines are cause for alarm because they may point to wide-ranging or systemic threats to species. However, adequate quantitative documentation of range-wide or regional declines in reptile species is infrequent, possibly due to the time and collaboration required to collect sufficient data at such large spatial scales. Nevertheless, successful models of range-wide investigations do exist and demonstrate the usefulness of constructing large-scale overviews of changes in species' distributions. In 1 example, investigators compiled distributional information from published scientific records, unpublished reptile surveys, museum databases, state natural heritage programs, and contributions by individual researchers to compare the historical vs. currently known distribution of the southern hognose snake, *Heterodon simus* (Tuberville et al. 2000). This collaborative effort revealed substantial declines of the species in portions of its range and provides a powerful example for other studies of distributional change in reptiles. In some cases, resurveying habitats across a species' range can also reveal that populations may be more widespread than previously suspected, as was the case for the sharp-tailed snake, *Contia tenuis*, in Oregon (Hoyer et al. 2006).

At a finer resolution, studies of individual reptile populations are also useful for revealing the plight of species, albeit at a more limited scope. As pointed out by other authors, studies of individual populations should use standardized methods to monitor changes in abundance or density over long periods of time (Gibbons et al. 2000). As an example, a long-term study of the abundance of eastern kingsnakes, *Lampropeltis getula*, revealed an alarming disappearance of the species from 1 site over 30 years (Winne et al. 2007). Although the decline could not be attributed to any single cause in this case, the examination of local populations is often more likely to reveal specific causes for a species' decline than are range-wide studies. Ultimately, a combination of studies at multiple scales will provide the most comprehensive assessment of the status and distribution of reptiles, as has been the case with the Texas horned lizard, *Phrynosoma cornutum* (Donaldson et al. 1994).

Lastly, as reptile populations fall under increasing scrutiny by scientists and conservation managers, it will be important to distinguish between natural declines and anthropogenic ones, and even to determine whether fluctuations in distribution or abundance represent "declines" per se (Alford and Richards 1999; Pechmann 2003). All animal populations presumably experience some level of normal fluctuation in abundance that will vary depending on the species or population in question. Thus, short-term monitoring that provides limited snapshots of population size may reveal current status but will not expose longer-term population trends or their causes (Gibbons et al. 2000). For this reason, the value of long-term studies and the data they generate cannot be overstated. Nevertheless, accumulation of numerous accounts from short-term studies may reveal a declining trajectory that can lend credence to conservation concerns for a given species or population (Gibbons et al. 2000). The scientific community would do well to take notice of the incredible mobilization of inventory and monitoring programs and other research activities that have followed recognition of the acute imperilment of many amphibians. Proactive recognition of the need to closely monitor reptiles may be instrumental in preventing or mediating their declines and minimizing the economic cost of reactive, and sometimes belated, conservation efforts.

3.2 FACTORS CONTRIBUTING TO REPTILE DECLINES

Establishing a causal link between any specific factor and declines of reptile populations can be difficult but is of foremost concern for effective conservation. Although in some cases 1 factor may weigh heavily on a population, multiple interacting factors nearly always affect a species' abundance and distribution. Several factors have been identified as threats to reptile populations and are implicated in declines of at least some reptiles, including habitat loss and fragmentation, unsustainable removal, anthropogenic environmental contamination, climate change, invasive species, disease and parasitism, and trophic cascades, and we discuss these in detail in the following sections. Two other seldom mentioned but nonetheless important factors bearing on the status of reptile populations are social apathy and special or political interests. Indeed, social apathy can be a major obstacle to reptile conservation because many reptiles are subjects of personal derision, a problem that must be overcome before appropriate motivation can spur conservation interest (Gibbons 1988). Similarly, the willingness of nongovernmental organizations and state, provincial, or national governments to recognize the plight of declining species and the need for conservation effort often depends on special or political interests and will undeniably have considerable impact on the persistence of many reptiles.

3.2.1 Habitat Loss

Habitat loss, including degradation, fragmentation, or conversion for other use, is typically regarded as the single greatest cause of faunal declines globally (Wilcove et al. 1998; Sala et al. 2000). Thus, the fact that habitat loss is considered to be the leading cause of reptile declines is not surprising (Mittermeier et al. 1992; Gardner et al. 2007). Habitat loss due to conversion of land for human use typically occurs for agriculture, housing or infrastructure development, commercial forestry, and to support recreation, including constructing golf courses or dredging lakes and other aquatic habitats.

Habitat loss from conversion can result in direct animal mortality that is often difficult to quantify. Nevertheless, mortality of turtles and aquatic snakes during lake dredging has been documented (Aresco and Gunzburger 2004), and entombment of live gopher tortoises (*Gopherus polyphemus*) during land development was widespread in Florida prior to a ban on the practice (Cox 2007). Ultimately, however, immediate mortality from habitat alteration likely poses less threat than the subsequent long-term, indirect effects of habitat loss and degradation on survival and reproduction.

Habitat loss can affect reptiles indirectly by limiting their ability to meet ecological needs for survival and reproduction. For example, many reptiles decline in abundance over time following the clearing of primary forest or conversion to plantation forest (Glor et al. 2001; Kanowski et al. 2006). In fact, the decline of multiple reptile species in the southeastern United States has followed widespread and nearly complete loss of native longleaf pine habitat (Ware et al. 1993; Gibbons et al. 2000). At a finer scale, 1 study from the southeastern United States demonstrated that planted pine forests and recent clear-cuts supported reduced abundances of small snakes compared with open-canopied partially harvested forests (Todd and Andrews 2008). The precise mechanisms of decline remain unknown but are presumably related to a general degradation of habitat quality from anthropogenic land conversion. Additional examples of the effects of habitat degradation include the loss of foraging and refuge due to bush-rock collection, which has contributed to the decline of the Australian broad-headed snake (*Hoplocephalus bungaroides*; Shine et al. 1998), and reductions in lizard abundance due to human-induced bush encroachment in Africa (Meik et al. 2002).

Loss and degradation of aquatic habitats also pose a serious threat to reptiles. Notable examples include declines of the crocodilian fauna of the Ganges and Yangtze Rivers (IUCN 2009), which have become increasingly imperiled following damming, flow modification, and general degradation of river habitat (Dudgeon et al. 2006). Similarly, channelization and dam building have been implicated in declines of river-dwelling North American map turtles (*Graptemys* spp.; Kofron 1991). The vulnerability of sea turtles to coastal development that degrades or eliminates nesting habitat has been appreciated for decades (Lutcavage et al. 1997; Spotila 2004). Likewise, the effects of terrestrial habitat alteration that disturbs or eliminates nesting and refuge sites of freshwater turtles can also be severe (Buhlmann 1995; Burke and Gibbons 1995). Many semiaquatic snake species that use wetland habitats share the plight of turtles if wetlands are lost or terrestrial habitat around sensitive aquatic resources is altered (Roe et al. 2003, 2004; Willson et al. 2006).

Habitat fragmentation is the emergence of discontinuities in an organism's preferred environment. Habitat fragmentation may occur due to natural processes but increasingly results from anthropogenic habitat loss or land conversion that isolates remaining patches of suitable habitat. The degree to which habitat fragmentation threatens a species depends on how greatly a species' movements are affected by the interspersed barriers that separate remaining usable habitat. Some lizard, snake, and turtle species are vulnerable to habitat fragmentation, with general declines in abundance being reported (Dodd 1990; Kjoss and Litvaitis 2001; Driscoll 2004), whereas others are not (Driscoll 2004). In other cases, habitat fragmentation affects the demography of remaining reptile populations. For example, patch size was positively correlated with abundance, survivorship, and recruitment of Florida scrub lizards, *Sceloporus woodi* (Hokit and Branch 2003). Of particular concern is the possible role that roadways play in fragmenting habitat. Because road mortality of turtles and snakes is often high (Aresco 2003; Gibbs and Steen 2005; Andrews and Gibbons 2008; see the next section), roads effectively become barriers that separate and isolate habitat (Roe et al. 2006). Again, however, species will differ in the extent to which roads act as barriers to movement (Andrews and Gibbons 2005), and therefore fragment populations.

3.2.2 Unsustainable Removal

Removal of reptiles from wild populations occurs both commercially and noncommercially for food, "traditional" medicine, curios, and the pet trade, as well as unintentionally as by-catch in

other harvesting activities and, increasingly, as a result of road mortality. Although removal per se is not necessarily harmful to population persistence — many reptile populations could presumably sustain some low level of harvest — removal at unsustainable rates is a serious threat that places many reptile populations and species in peril. To date, few studies have demonstrated sustainability of removal activities on reptile populations. In contrast, studies documenting intense levels of reptile harvesting and subsequent declines in wild populations are common.

Perhaps the mostly widely recognized removal-driven peril results from the ongoing use of reptiles for food, skins, or "traditional" medicines. Imperilment of Asian turtles due to unsustainable removal has reached crisis levels and has grim consequences for the persistence of many freshwater and terrestrial turtles if not remedied (Buhlmann et al. 2002). The exploitation of turtles, however, is not restricted to Asia; many Central and South American cultures relish turtles and their eggs, resulting in continued threats to both freshwater and marine turtle populations (Lagueux 1991; Thorbjarnarson et al. 1997). Moreover, the consumptive use of reptiles is not limited to turtles. In Asia, snakes face rapidly growing pressure from exploitive use, with as many as 1 million snakes being harvested in northeast China and nearly 8 million kg traded each year across the country (Zhou and Jiang 2004, 2005). Likewise, in Cambodia, an estimated 6.9 million aquatic snakes are removed annually from Tonle Sap Lake to feed the growing crocodile farms in that region (Brooks et al. 2007). Subsequently, hunters have reported a 74% to 84% decline in snake catch from 2000 to 2005 (Brooks et al. 2007). Pythons (*Python* spp.), too, face significant harvest pressure in many parts of Indonesia (Shine et al. 1999). Monitors (*Varanus* spp.) and tegus (*Tupinambis* spp.) are heavily harvested for their skins at rates of as much as 1 million animals per year in the case of South American tegus (Pianka and Vitt 2003; Mieres and Fitzgerald 2006). In Africa, bushmeat consumption often extends to highly endangered species such as the dwarf crocodile, *Osteolaemus tetraspis* (Willcox and Nambu 2007). The complete list of affected species is lengthy (see also reviews in Gibbons et al. 2000; Irwin and Irwin 2005) and a precautionary policy of preventing massive exploitation until sustainable removal limits are identified appears to be the best method of ensuring the persistence of reptile populations.

The commercial removal of reptiles from wild populations for use as pets is another consumptive use affecting reptiles globally. In many cases, evidence linking collection of animals for the pet trade to declines of wild reptile populations is lacking because long-term studies of the status of reptile populations following targeted collecting have not been conducted. Nevertheless, collection of reptiles for the pet trade may endanger wild populations due to the large scale at which some reptiles are collected. Many species of turtles, snakes, chameleons, and other lizards are under increasing demand, and collection in wild populations continues at high levels (Reed and Gibbons 2003; Carpenter et al. 2004; Schlaepfer et al. 2005) that can threaten population persistence (Webb et al. 2002).

Removal of individual reptiles also occurs as by-catch in fisheries and from intentional and unintentional road mortality. Mortality of long-lived sea turtles in longline fisheries and shrimp nets is likely unsustainable and contributes to the rapid and ongoing declines of many sea turtle species (Lewison et al. 2003, 2004). Similarly, both commercial and recreational crab trapping have been implicated in declines of the North American diamondback terrapin, *Malaclemys terrapin* (Bishop 1983; Dorcas et al. 2007), a species already heavily depleted from commercial exploitation for food in the 1800s to early 1900s (Carr 1952). In developed countries, roads represent an additional, substantial, and often ignored source of mortality in many reptile populations. Snakes and turtles are probably hardest hit by road mortality because they are large, often move slowly, and are sometimes direct targets for persecution by motorists (Andrews and Gibbons 2005; Ashley et al. 2007). Increasingly male-biased sex ratios in turtle populations attest to the potential long-term effects of road mortality on reptiles; female turtles are more likely to cross roads during nesting forays and are enticed to oviposit on road shoulders due to the presence of sunny nesting habitat, leading to disproportionately higher female mortality (Aresco 2005; Steen et al. 2006).

3.2.3 Environmental Contamination

Release of contaminants — including pesticides, herbicides, heavy metals, and radioactive waste — into the environment has been listed as 1 of the 6 major contributors to the global decline of reptiles (Gibbons et al. 2000). Reptiles exhibit a suite of ecological and life history characteristics that make them particularly vulnerable to contaminants (Hopkins 2000). With the exception of a few lizard and turtle species, reptiles are strictly carnivorous, and many occupy high trophic positions within food webs. Thus, reptiles are at risk from biomagnification of contaminants. Additionally, many reptiles are long-lived and have small home ranges compared to similar-sized endotherms, making them susceptible to long-term contaminant exposure and subsequent bioaccumulation (Hopkins 2000; Shelby and Mendonca 2001; Bergeron et al. 2007). Although reptiles may be at particularly high risk from contaminants, they are currently the least studied vertebrate group in ecotoxicology (Hopkins 2000; Chapter 1, this volume). Within reptiles, ecotoxicological research has primarily been restricted to turtles and crocodilians, and our knowledge of the effects of toxins on squamates is still limited (Campbell and Campbell 2001).

The most overt measurable effect that contaminants can have on reptiles is direct mortality of individuals resulting from exposure. Several studies have reported mortality of reptiles in association with intentional (e.g., pesticide application) or accidental (e.g., spills or contaminant leakage) introduction of toxins into the environment (reviewed in Campbell and Campbell 2000, 2001), but few authors have related such acute mortality events to population declines. However, Romero and Wilkelski (2002) noted population declines of Galapagos marine iguanas (*Amblyrhynchus cristatus*) following a low-level oil spill. Declines were not observed on islands that remained unaffected by the spill. Romero and Wilkelski (2002) speculated that iguanas died of starvation after the digestive bacteria in their guts were killed by oil residues found in their diet of marine algae. In a similar example, Ernst et al. (1994) noted the disappearance of yellow-blotched map turtles (*Graptemys flavimaculata*) from sections of river immediately downstream from a paper mill. However, it is unclear if changes in turtle abundance represented population declines or movement of turtles out of contaminated areas due to lack of prey (aquatic invertebrates) or other factors. To date, reports of population declines associated with environmental contamination are largely restricted to turtles and large lizards. Cryptic behavior and/or low activity levels (which result in low capture rates during surveys; Dorcas and Willson in press) of many snakes and smaller lizards would conceal mortality events and hamper detection of population declines.

Although less obvious than direct mortality, sublethal effects of contaminants may be more detrimental to the long-term persistence of reptile populations. High tissue loads of various contaminants have been documented from reptiles in the field (e.g., lizards and snakes; reviewed in Campbell and Campbell 2000, 2001; Bergeron et al. 2007; this volume), and sublethal effects of contaminants on reptile locomotor performance (e.g., Hopkins et al. 2005; Holem et al. 2006; Hopkins and Winne 2006; DuRant et al. 2007a) and metabolic energy consumption (e.g., Hopkins et al. 2005; DuRant et al. 2007b) have been demonstrated in the laboratory. Although these studies provide insight on the mechanisms linking sublethal contaminant exposure to population dynamics, few studies have attributed declining reptile populations to sublethal contaminate exposure. A well-cited exception is the decline of American alligators (*Alligator mississippiensis*) in a Florida lake contaminated with estrogenic compounds (Guillette et al. 1994; Semenza et al. 1997). Alligator declines were attributed to reproductive failure resulting from reduced testosterone levels and gonadal malformations. Likewise, Shelby and Mendonca (2001) found reduced testosterone levels in some male yellow-blotched map turtles (*Graptemys flavimaculata*) from polluted habitats, suggesting that effects of pollutants on reproduction may have been responsible for population declines previously observed at that site. Although much of the investigation of indirect effects of contaminants on reptiles has focused on the effects of endocrine disrupters on reproduction, exposure to contaminants may also affect energy acquisition and expenditure. For example, Hopkins et al. (1999) found that banded watersnakes (*Nerodia fasciata*) collected from a wetland polluted with coal combustion waste had

elevated tissue concentrations of trace metals and standard metabolic rates that were 32% higher than those of snakes from an unpolluted site. Elevated metabolic rates presumably limit energy availability for growth and reproduction in snakes from contaminated sites.

Finally, consider possible synergistic effects of contaminants with other threats facing reptiles. For example, a plausible scenario is one in which sublethal exposure to contaminants compromises immunocompetence, resulting in outbreaks of opportunistic pathogens that might otherwise be benign or manageable under normal circumstances. Although these types of questions have not been addressed in reptiles, consideration of the effects of multiple stressors on reptiles will undoubtedly become increasingly important as human populations continue to grow and expand around the globe.

3.2.4 Climate Change

Global climate change has been an ongoing process throughout the evolutionary history of reptiles and has been nearly continuous in the past 65 million years (Zachos et al. 2001). However, given indications that recent global climate warming is occurring at a pace unprecedented in recent history (IPCC 2007), rapid climate change is particularly relevant in our consideration of threats to reptile populations. Possible effects from climate change fall broadly into categories of direct and indirect effects, both of which have either caused or are expected to cause changes in reptile populations.

Because reptiles are ectothermic, they are highly dependent on suitable external temperatures to regulate their own body temperatures and support metabolic and other functions. Subsequently, direct effects of climate change may manifest as changes in growth rates or the age at onset of reproductive maturity, as shown in painted turtles, *Chrysemys picta* (Frazer et al. 1993). Theoretical models further suggest that changes in global climate can have profound effects on reptile populations. Dunham (1993) used individual-based models to estimate physiological responses of Big Bend Canyon Lizards (*Sceloporus merriami*) to climate change and predicted that increases in air temperature of 2 °C to 5 °C could constrict activity sufficiently to drive populations to extinction. Others have expressed concern that climate change may have a considerable impact on reptiles with temperature-dependent sex determination, such as some turtles, lizards, and crocodilians, by altering sex ratios within populations (Janzen 1994).

Other obvious effects that global climate change may have on reptile populations include direct and indirect influences on habitat suitability. For example, changes in temperature and precipitation may directly affect the habitability of a reptile's environment and could cause shifts in reptile distributions at a large scale. Araújo et al. (2006) explored possible scenarios of habitat change in Europe and concluded that, although suitable habitat for European reptiles is likely to expand under most circumstances, limited dispersal abilities of reptiles may increase their vulnerability to climate change. Dispersal capability may be further constrained by the ever-increasing habitat fragmentation that accompanies human population growth. Furthermore, regional changes in precipitation and temperature regimes are likely to broadly affect community composition, and some landscapes may change dramatically (Guertin et al 1997; Still et al. 1999). Whitfield et al. (2007) suggested 1 instance of indirect effects of climate change when they documented a steady decline in several lizard species at La Selva, Costa Rica, over a 35-year period. They attributed declines to climate-driven reductions in the quantity of leaf litter. Also, severe climatic events are expected to become more frequent due to the destabilization of regional weather patterns under many global warming scenarios (IPCC 2007). Subsequently, droughts or other meteorological events such as cyclones may negatively affect reptile populations (Seigel et al. 1995; Willson et al. 2006).

3.2.5 Invasive Species

Recent expansion of human populations and increases in global transportation and trade have resulted in introduction and establishment of many species in areas outside of their native geographic range. Many introduced species have subsequently proliferated, resulting in severe ecological and

economic damage (Pimentel et al. 2000). Consequently, invasive exotic species are currently recognized as one of the foremost threats to global biodiversity (Park 2004), including reptiles (Gibbons et al. 2000).

Invasive exotic species affect native species in a variety of ways. One of the most obvious ways introduced animals can affect reptile populations is by directly preying upon them. Predator introductions that have resulted in the decline or extirpation of many species are especially obvious on island ecosystems that were previously bereft of predators. For example, introduced predators (mongoose, *Herpestes javanicus*, and rats, *Rattus rattus*) have been identified as the single greatest threat to snakes on the Lesser Antilles and have been implicated in at least 6 historical snake extirpations and at least 1 historical extinction in that region (Henderson 2004). Similarly, introduction of brown treesnakes (*Boiga irregularis*) to the island nation of Guam has devastated the vertebrate fauna of that island, including populations of several native lizards (Fritz and Rodda 1998). A final example demonstrates that introduced predators need not be large vertebrates. Since their accidental introduction to Mobile, Alabama, in the 1930s, imported red fire ants (*Solenopsis invicta*) have spread throughout much of the southeastern United States (Wojcik et al. 2001). Mount (1981) expressed concern about the potential impacts of predation by *S. invicta* on vertebrates in the Southeast, noting observations of fire ant predation on eggs and hatchlings of several reptile species. He also made anecdotal observations of declines of many litter-dwelling snakes and lizards, and large terrestrial oviparous snakes in the Alabama coastal plain in conjunction with *S. invicta* invasion. Although quantitative evidence of the effects of *S. invicta* on native reptiles has been slow to emerge, fire ants are documented predators of turtle nests (Buhlmann and Coffman 2001) and have been implicated in the decline of southern hognose snakes (*Heterodon simus*; Tuberville et al. 2000), eastern kingsnakes (*Lampropeltis getula*; Wojcik et al. 2001, Winne et al. 2007), and Texas horned lizards (*Phrynosoma cornutum*; Goin 1992). Importantly, the effects of *S. invicta* on reptiles may be exacerbated by habitat disturbance, possibly leading to synergistic effects of habitat alteration and predation (Todd et al. 2008).

Introduction of exotic prey can also have profound effects on reptile populations. In some cases, exotic prey may possess defenses (e.g., poisons, morphological defenses) to which native predators are unaccustomed, resulting in direct mortality of reptiles that attempt to consume the exotics. For example, exotic cane toads (*Bufo marinus*) possess potent parotoid secretions and have become abundant in many areas of tropical Australia since their introduction in 1929 (Lampo and de Leo 1998). Covacevich and Archer (1975) noted several instances of direct mortality of snakes and monitor lizards (*Varanus* spp.) that attempted to ingest the toads. Other authors have observed declines of several snake and lizard species following arrival of *B. marinus* (Phillips et al. 2003), as well as mortality of Australian freshwater crocodiles. Moreover, laboratory studies have shown that many Australian snake species are sufficiently vulnerable to toad toxins to die after ingesting a single toad, prompting Phillips et al. (2003) to suggest that the toads threaten as much as 30% of Australia's terrestrial snake species. In other cases, exotic prey may be palatable, but of poorer nutritional quality than native prey taxa. For example, exotic Argentine ants (*Linepithema humile*) have been introduced worldwide (Suarez et al. 2001) and eliminate nearly all native ground-dwelling ants when they invade new habitats (Suarez et al. 1998). Suarez and Case (2002) demonstrated that Argentine ants represent an inferior prey resource for coastal horned lizards (*Phrynosoma coronatum*), a species that has declined dramatically in California. They found that hatchling *P. coronatum* fed a diet of introduced *L. humile* exhibited zero or negative growth, but resumed normal growth when switched back to a diet of native ants.

Introduced exotic prey taxa may sometimes be beneficial to native reptiles. For example, round gobies (*Neogobius melanostomus*) introduced into the Great Lakes region of North America have become favored prey of the federally listed Lake Erie watersnake (*Nerodia sipedon insularum*), resulting in increased growth and body sizes of snakes (King et al. 2006a). Introduction of gobies has been implicated as a partial cause for the recovery of this snake in recent years (King et al. 2006a) to levels that warrant delisting under the US Endangered Species Act (King et al. 2006b).

Introduced species may also exert substantial indirect effects on native reptile populations through competition for resources. For example, introduced geckos (*Hemidactylus frenatus*) compete with and

have displaced native geckos (*Lepidodactylus lugubris*) throughout the tropical Pacific (Case et al. 1994). Likewise, competition with *H. frenatus* has caused declines and extirpations of several species of night geckos (*Nactus* spp.) on the Mascarene Islands. Introduced slider turtles (*Trachemys scripta*) compete with native European pond turtles (*Emys orbicularis*) for basking sites (Cadi and Joly 2003), resulting in weight loss and reduced survival of *E. orbicularis* in experimental mixed populations compared with controls (Cadi and Joly 2004). Importantly, in some cases, introduced species may gain a competitive advantage because of release from their native pathogens or parasites (Reed 2005).

Another indirect means by which invasive species can affect native reptiles is through habitat modification. The most obvious examples of this phenomenon occur in cases where invasive plant species displace native vegetation, rendering habitat unsuitable for native species. For example, lush growth of invasive annual plants in the Mojave Desert of the American Southwest negatively affects desert tortoises (*Gopherus agassizii*), primarily through increased fire frequency (Brooks and Pike 2001). Likewise, habitats dominated by exotic rubber vine (*Cryptostegia grandiflora*) are avoided by native Australian lizards (Valentine 2006).

Finally, introduced species may be important vectors for disease and parasites (see Section 3.2.6). Unlike other ways in which exotic species may affect reptiles, exotic species do not need to become established in the wild to serve as disease vectors. In fact, release of a single infected individual, or human contact with a wild reptile after handling an infected captive reptile, may be sufficient to introduce a pathogen to native reptile populations. Reed (2005) cautioned that release of captive boas and pythons could be an important source of disease to native snakes such as the federally threatened eastern indigo snake (*Drymarchon couperi*) or boid species native to the United States (rubber boa, *Charina bottae*, and rosy boa, *Lichanura trivirgata*).

3.2.6 Disease and Parasitism

Pathogens and parasites have long been recognized as potentially important factors regulating natural populations (Anderson and May 1978; Dobson and Hudson 1986). Virtually every species hosts a multitude of parasites and pathogens, some of which can cause dramatic population fluctuations (e.g., Hudson et al. 1998). However, when human activities alter rates of disease transmission or reduce resistance of animals to disease, the results can be catastrophic (Daszak et al. 2000). For example, outbreaks of pathogenic chytrid fungus (*Batrachochytrium dendrobatidis*) have devastated amphibian populations worldwide (Daszak et al. 2003). Moreover, the spread of chytrid appears to have been facilitated by global climate change (Pounds et al. 2006), introduction of exotic species (Mazzoni et al. 2003), and direct spread by humans (Weldon et al. 2004).

Disease outbreaks have been implicated in declines of several reptile taxa and are apparently of particular concern for turtles. For example, incidence of upper respiratory tract disease (URTD) infections of gopher tortoises (*G. polyphemus*) and desert tortoises (*G. agassizii*) has increased in recent years, and URTD has been implicated in declines of some populations (Dodd and Seigel 1991; Seigel et al. 2003). Upper respiratory tract disease may have been introduced into natural populations through release of infected captive individuals (Dodd and Seigel 1991), and authors have expressed concern that spread of this disease could be exacerbated through translocation of infected animals for conservation purposes (Dodd and Seigel 1991). Disease outbreaks have been noted in other protected turtles, such as the flattened musk turtle (*Sternotherus depressus*; Dodd 1988; Fonnesbeck and Dodd 2003) and green sea turtle (*Chelonia mydas*; Chaloupka and Balazs 2005); however, factors underlying these outbreaks are poorly understood.

3.2.7 Cascading Declines

An additional consideration seldom explicitly addressed is how reptiles respond to declines in other taxa (but see Irwin and Irwin 2005). Although ecologists have yet to form a consensus about the role of biodiversity in maintaining ecosystem function (Thompson and Starzomski 2007), loss of

important species could result in subsequent extinction of previously unaffected taxa. Living in a time when species extinctions are increasing at an alarming rate, we are probably only beginning to see the long-term effects of extinctions that have already occurred.

An obvious concern regarding cascading declines is the possible response of reptiles to the recent catastrophic declines of many amphibian populations. Many snake species are specialists that feed exclusively on amphibians (Toledo et al. 2007); we might therefore expect that these species would experience declines concomitant with those of amphibians. Whiles et al. (2006) reported the disappearance of several common frog-eating riparian snakes soon after amphibian declines related to chytridiomycosis in Panama. Similarly, in the Sierra Nevada Mountains of the American West, the presence of mountain garter snakes (*Thamnophis elegans*) is strongly associated with the presence of anurans (Matthews et al. 2002). Matthews et al. (2002) suggest that amphibian declines associated with stocking of predatory trout may have a strong adverse effect on populations of *T. elegans.*

Cascading declines may be most frequently associated with the disappearance of keystone species, those species that play a role disproportionate to their abundance in maintaining community composition and ecosystem function (Power et al. 1996). For example, the gopher tortoise (*G. polyphemus*) is considered a keystone species (Eisenberg 1983) because its burrows provide critical refugia for a variety of upland species in the southeastern United States. Gopher tortoises have declined across their range (McCoy et al. 2006), and loss of tortoise burrows is considered a serious threat to persistence of the federally threatened eastern indigo snake (*Drymarchon couperi*; Stevenson et al. 2003) and eastern diamondback rattlesnake (*Crotalus adamanteus*; Timmerman and Martin 2003), among other species. Although such cascading declines affect all species, reptiles, which often have highly specialized food and habitat requirements, may be less able than more generalist taxa to withstand sequential removal of individual species from ecosystems.

3.3 GLOBAL STATUS OF REPTILE POPULATIONS

As discussed previously, the status of many reptiles remains unknown and is the subject of ongoing global assessment by the IUCN (Figure 3.1). Here, we describe the current perceived status of reptile populations and provide overviews of reptile declines among turtles, crocodilians, lizards, and snakes. Our reports on the current status of reptile populations represent our best understanding of the available scientific literature and current information from the 2008 IUCN Red List (IUCN 2009).

3.3.1 Testudines

A greater proportion of turtles are recognized as imperiled and in categories of conservation concern than any other group of reptiles excluding the 2 tuatara species, Sphenodontia (Figure 3.1). Overall, 42% of turtle species included in the IUCN 2004 global assessment were classified as threatened (including all IUCN categories of imperilment; Baillie et al. 2004). However, at that time only a portion of all described species were evaluated. Consequently, the actual rate of imperilment of evaluated species is closer to 62% (Baillie et al. 2004). Perhaps most alarming is that the IUCN currently lists a total of 8 turtle species that have gone extinct in the wild in modern times (IUCN 2009). The Turtle Conservation Fund even compiled a list of 25 additional turtle species on "death row," that is, the most endangered species of tortoises and freshwater turtles in the world (Buhlmann et al. 2002). Although not included in the list, all 7 species of sea turtles (families Cheloniidae and Dermochelyidae) are considered imperiled. The taxonomic distribution of endangered species (critically endangered [CE] and endangered [EN]) includes species from each of the 11 families of freshwater turtles, the single family of tortoises, and both families of sea turtles (Baillie et al. 2004).

Turtles are long-lived (Gibbons 1987), and commercial harvesting of wild populations of most species is not sustainable (Reed and Gibbons 2003). Nonetheless, turtles differ from other reptile groups in that human consumption is the documented cause for the majority of declines on a global scale (Buhlmann et al. 2002). Commercial harvesting in Southeast Asia is a major cause

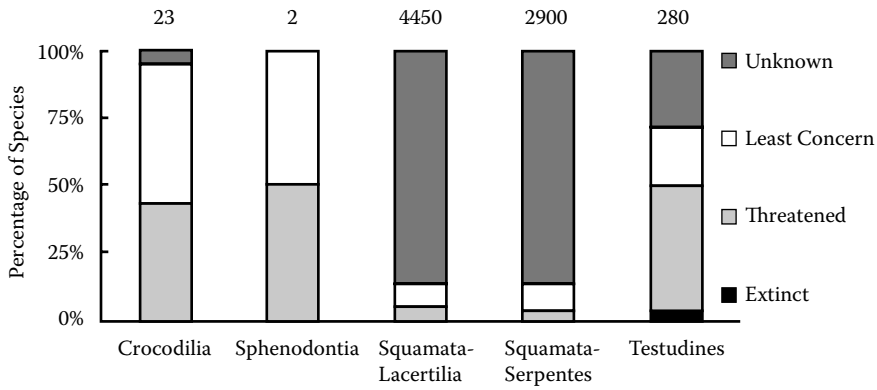

FIGURE 3.1 Status of the major lineages of reptiles according to the World Conservation Union (IUCN) Red List in 2009. Status categories include "extinct" (including species extinct in the wild but extant in captivity), "threatened" (including IUCN categories "critically endangered," "endangered," and "vulnerable"), "least concern" (including IUCN categories "lower risk" and "near threatened"), and "unknown" (including species that have not been evaluated by the IUCN and those that have been evaluated but were deemed "data deficient"). Numbers above bars indicate the approximate number of species within each lineage according to Zug et al. (2001). Data were accessed from the IUCN database on March 18, 2009 (IUCN 2009). Note that although bars are difficult to see, 11 species of lizards (0.25%) and 3 species of snakes (0.10%) are listed as extinct by the IUCN as of 2009.

for concern, with some turtle species clearly on a trajectory toward extinction at current rates of removal from the wild (Baillie et al. 2004). Indeed, of 73 species of tortoises and freshwater turtles classified as endangered and critically endangered in 2002, more than half were from Asia, with the remaining species being distributed geographically among North America, Mesoamerica, South America, the Mediterranean, Africa, and Australasia (Buhlmann et al. 2002). Outside of Asia, terrestrial terrapins and tortoises are imperiled by excessive harvesting combined with loss of suitable habitat (Baillie et al. 2004). Collection and removal of turtles from North America, mostly for the export trade, has also been significant (Franke and Telecky 2001; Ceballos and Fitzgerald 2004) and could understandably be implicated in the decline of some species in the wild. Sea turtles, all of which are classified as imperiled, are subject to unique threats, with declines attributed to mortality from incidental by-catch, harvesting of turtles and eggs for consumption, and degradation of nesting and foraging habitat (Lutcavage et al. 1997; Spotila 2004).

3.3.2 Crocodilians

A quarter of a century ago, every crocodilian species in the world was categorized as endangered or threatened. Ironically, because the fate of only 7 of the 23 species remains uncertain at the beginning of the 21st century, the group is considered by many conservationists to be a major success story. Conservation efforts by the Crocodile Specialist Group of the Species Survival Commission–IUCN are generally viewed as the cause for an upturn in the status of two-thirds of the crocodilian species that have traditionally suffered from the pressures of harvesting and habitat loss (IUCN 2009).

Despite the noted conservation successes in reducing declines and extinction threats for some crocodilian species, the status of 7 species, including the Chinese alligator (*Alligator sinensis*), the black caiman (*Melanosuchus niger*), the Indian gharial (*Gavialis gangeticus*), and 4 species of crocodile, remains one of teetering on the brink of extinction. In mainland Asia, only about 150 Chinese alligators are estimated to be present in their native habitat, and the Siamese crocodile (*Crocodylus siamensis*) is effectively extirpated from its native home of Thailand (formerly Siam), with few populations persisting in other parts of Southeast Asia. Only a single established population of the

Philippine crocodile (*Crocodylus mindorensis*) is known to exist at this time, with the species now occupying less than 20% of its former range of most of the Philippines Archipelago. Likewise, the Indian gharial is considered to be critically endangered despite strong and effective conservation programs in some parts of the country. In fact, nearly 80 gharials, representing 6% of the known population, died from unknown causes in an Indian forest preserve from December 2007 to January 2008 (Mahmood 2008). Initial reports identifying liver cirrhosis in the dead animals suggested that parasites or environmental contaminants may have played a role in the deaths.

In the western hemisphere, no fewer than 3 crocodilian species remain on the list of special concern, although some seem to be recovering through regional conservation efforts. In tropical South America, the most endangered species are the black caiman and the Orinoco crocodile (*Crocodylus intermedius*), a species whose decline has been attributed in part to the unrestricted use of pesticides for agricultural purposes. The Cuban crocodile (*Crocodylus rhombifer*), whose geographic range once included several islands in the West Indies, is now represented in the wild only from highly localized areas in Cuba. Nevertheless, many conservationists point to the American alligator (*Alligator mississippiensis*) as one of the greatest models of successful recovery for any threatened vertebrate.

Most of the remaining 16 species of crocodilians — including 3 native to Africa, 2 found in Australia, and others at scattered locations in tropical America and the Pacific — are considered to be vulnerable only if the strict conservation programs in place are discontinued. Also, although many of these remaining species are not considered to be threatened with extinction throughout their geographic ranges, some still have critically endangered regional populations.

3.3.3 Squamates: Lacertilians

Like many other reptiles, the status of most lizard species and populations is largely unknown (Figure 3.1). However, based on current information, lizards appear to have a small proportion of imperiled species (IUCN 2009). This is due in large part to life history attributes that make many lizards less susceptible to decline from anthropogenic factors. Notably, many lizards occur at high population densities, have short generation times, high fecundity, and are not as long-lived as other reptiles. Consequently, lizards may sometimes adjust rapidly to environmental change or rebound quickly from short-term population reductions. In fact, some lizard species fare well in human-modified or early successional habitats (e.g., *Anolis* spp., *Hemidactylus* spp.). In several cases, these life history attributes have contributed to the successful establishment of exotic lizards introduced into areas outside of their native ranges. For example, there are more than 30 species of nonnative lizards in Florida, representing over two-thirds of the total lizard fauna in that state (Meshaka et al. 2005). Ultimately, however, several lizard species are declining and have been classified as threatened or worse under the IUCN Red List system (IUCN 2009). Moreover, the lack of data on the status of many lizard populations may further jeopardize their long-term persistence.

Causes for the endangerment of lizards vary widely, but life history characteristics greatly influence the degree to which different factors threaten species. Imperiled species are those that typically have attributes such as endemism, restricted geographic ranges, large body size, long lives, late maturity, or low fecundity, which make them susceptible to population declines from anthropogenic factors. For instance, the slow-maturing, long-lived, and often endemic giant land iguanas of the Caribbean (*Cyclura* and *Brachylophus* spp.) are among the most threatened lizards globally (Pianka and Vitt 2003). In some cases, populations of Caribbean iguanas have dwindled to as few as 100 individuals. Much of their decline has been attributed to the historical harvest of these lizards for food and the introduction of nonnative pests such as mongooses, rats, goats, and pigs onto their island homes. The fate of these lizards now depends almost entirely on human intervention to preserve habitat, control introduced predators, and increase recruitment to avoid permanent extinction. Many varanoid lizards are also slow maturing and long-lived, and several of them are currently protected in parts of their ranges due to population declines (Pianka and Vitt 2003; Pianka et al. 2004). Although phylogenetically distinct from lizards, tuatara (*Sphenodon* spp.) share these "slow"

life history characteristics and have long incubation periods that place them in similar jeopardy (McIntyre 1997).

At least a few smaller, "typical" lizards are also threatened or critically endangered despite having high fecundity and early sexual maturity. The largest contributor to the endangerment of these species is often their endemism, restricted ranges, or highly specialized habitat requirements. High fecundity and early sexual maturity may safeguard them from declines associated with harvesting but greatly increase their risk of decline from habitat loss. In fact, habitat loss is listed as a contributing factor in the imperilment of more than half of squamates currently recognized as near threatened or worse on the IUCN Red List (IUCN 2009). For instance, the Coachella Valley fringe-toed lizard (*Uma inornata*), the island night lizard (*Xantusia riversiana*), and several island geckos from Madagascar and the Caribbean (*Phelsuma* spp. and *Sphaerodactylus* spp.) are threatened or critically endangered due to combinations of restricted geographic ranges and habitat loss. Nevertheless, some species, such as Madagascar's Antsingy leaf chameleon (*Brookesia perarmata*), also presumably suffer from heavy collection, which has led to restrictions on their international trade (CITES 2003).

3.3.4 Squamates: Serpents

Despite recent reports of reptile declines, the global status of snake populations has received relatively little attention. Indeed, along with lizards, snakes have yet to receive comprehensive review by the IUCN. According to the 2004 IUCN Global Species Assessment, only 3.4% of squamate species had been evaluated, compared with 67% of turtles, 90% of mammals, 100% of birds, and 100% of amphibians (Baillie et al. 2004). Five years later, the online IUCN Red List still demonstrates that the status of most squamates is unknown (Figure 3.1; IUCN 2009). Snakes are notorious for their cryptic behavior and low or sporadic activity, which seriously complicates efforts to assess population status (Parker and Plummer 1987). Thus, even for relatively well-studied species, population size or density often remains unknown (Dorcas and Willson 2009). As in lizards, risk of imperilment in snakes generally correlates more closely with life history attributes and geography than with taxonomy. Threatened species are most often those with specialized habitat requirements, small geographic ranges, or life history characteristics such as large body size, delayed sexual maturity, and/or low reproductive rates. Additionally, there is regional variation in the relative importance of threats faced by snakes such that major threats and taxa at risk vary among regions or continents.

Many snakes have specialized habitat requirements, making them particularly susceptible to habitat loss or degradation. For example, many of the most threatened snake species in the eastern United States, including the eastern indigo snake (*Drymarchon couperi*), eastern diamondback rattlesnake (*Crotalus adamanteus*), pine snake (*Pituophis melanoleucus*), and southern hognose snake (*Heterodon simus*), are those associated with the nearly eliminated longleaf pine ecosystem (Todd and Andrews 2008). Likewise, loss or degradation of wetland habitats has prompted federal listing of several North American snakes, including the wetland-associated eastern massasauga (*Sistrurus catenatus catenatus*), San Francisco garter snake (*Thamnophis sirtalis tetrataenia*), and copperbelly watersnake (*Nerodia erythrogaster neglecta*). Small geographic range, combined with specialized habitat requirements, puts species at risk from a variety of threats to their habitat. For example, the broad-headed snake (*Hoplocephalus bungaroides*), considered Australia's most endangered snake species, is restricted to small regions of rock outcrop habitat in eastern Australia and has suffered extensively from habitat degradation due to rock removal, collection for the pet trade, and canopy closure from fire suppression (Shine et al. 1998; Webb et al. 2002, 2005). Many of the most dramatic cases of imperilment due to small geographic range occur among snake species endemic to islands such as the Caribbean Lesser Antilles. The Lesser Antilles harbor 25 snake species, 87.5% of which are endemic, and are home to some of the rarest snakes in the world (e.g., the Antiguan racer, *Alsophis antiguae*; Daltry et al. 2001). The region has suffered between

6 and 11 historical extirpations and at least one historical extinction, primarily due to predation by introduced mongooses (Henderson 2004). Although data are lacking, similar declines may also be occurring in other island archipelagos across the globe.

Across all animal taxa, life history attributes such as large body size, delayed sexual maturity, and low reproductive output contribute to imperilment, and many of the most threatened snake species also share these characteristics (Meffe and Carroll 1997). Among snakes, body size correlates strongly with home range size (Reed and Shine 2002; Reed 2003). Thus, larger species typically need larger tracts of suitable habitat and move more extensively than smaller species, presumably putting them at greater risk from road mortality or other threats (Andrews and Gibbons 2008). Many taxonomic groups of snakes have intrinsically slow growth and low reproductive rates, making them particularly susceptible to overharvesting and less able to recover from short-term population declines. For example, many species of European and Asian vipers (*Vipera* spp.) are considered threatened (IUCN 2009), in part because their "slow" life history characteristics put them at risk from persecution, collection, and habitat loss. Among Australian elapid snakes, Reed and Shine (2002) found that the characteristics that correlated most strongly with species endangerment were foraging strategy (ambush foragers were most imperiled) and mating system (species with female-biased sexual size dimorphism and lacking male-male combat were frequently threatened). They postulated that snakes employing ambush foraging had more specific habitat requirements and exhibited "risky" life history attributes such as low reproductive rates and slow growth rates. Likewise, large female body size potentially increased vulnerability of females to anthropogenic sources of mortality. Their results suggest that factors contributing to endangerment in snakes may differ substantially from other taxa (especially endotherms) and are not always intuitive (Reed and Shine 2002). Unfortunately, similar macroecological analyses have not been performed for other snake groups or geographic regions.

Substantial regional differences exist in the threats affecting snake populations and, consequently, in the status of snake populations. Throughout most temperate regions of Europe and North America, the paramount threat to snake populations is apparently habitat loss and degradation. However, road mortality, persecution, and collection for the pet trade have been implicated in the decline of some species, and causes of apparent declines in others remain enigmatic. Little data exist on the status of snake populations in tropical regions of the world; however, as with other taxa, snakes are undoubtedly suffering as a result of rampant habitat destruction occurring in tropical regions. A relatively novel, but poorly understood threat to snakes in these regions is the phenomenon of cascading declines discussed previously. In Asia, snakes face greater pressure from exploitative use than in other regions of the world, with estimated millions of snakes harvested annually from China and other regions of Southeast Asia (Zhou and Jiang 2004, 2005; Brooks et al. 2007). Finally, the myriad introduced exotic species in Australia (e.g., foxes, feral cats, cane toads) pose serious threats to the fragile ecosystems of that island continent. Indeed, as noted previously, cane toads alone have been suggested to threaten as much as 30% of Australia's terrestrial snake species (Phillips et al. 2003).

Although our knowledge of the status of global snake populations remains woefully inadequate, increasing awareness of the importance of snakes as top predators in many ecosystems (e.g., Ineich 2007) and advances in methodology for studying snake populations (Dorcas and Willson in press) will undoubtedly increase our ability to effectively conserve snake populations in future decades.

3.4 CONCLUSION

Continuing to determine the status, distribution, and basic ecology of many reptiles is of paramount importance. Although low detectability of reptiles, and subsequently poor awareness of declines in their populations, may hamper research and conservation efforts, ongoing advances in field methodology, mark-recapture analyses, and our understanding of reptile life histories and behaviors should

continue to improve our knowledge of the status and distribution of many reptiles. As recognition of declining reptile populations increases, determining causes of those declines should also become a primary goal. Some populations and species may be affected by one or a few factors, but multiple interacting stressors or threats likely affect many reptile populations. Numerous studies have demonstrated direct effects that environmental contaminants have on reptiles. But the many ways that environmental contaminants could exacerbate ongoing declines from other threats, such as disease and parasitism, habitat loss, and introduced invasive species, remain underappreciated. The hope that reptile declines will tail off or that highly imperiled species will be able to claw their way back from near extinction rests on a full understanding of the plight of reptiles, threats to their populations, causes for their declines, and effective mobilization of conservation resources.

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4 Ecotoxicology of Amphibians and Reptiles in a Nutshell

Greg Linder, Christine M. Lehman, and Joseph R. Bidwell

CONTENTS

Concern over the localized reduction of amphibian populations and the potential role that chemical stressors were playing in these declines was expressed nearly 30 years ago (Gibbs et al. 1971; Birge et al. 1980; Bury 1999), an observation recently revisited for reptiles (Gibbons et al. 2000). However, it has only been over the last decade that amphibians, and to a lesser extent reptiles, have been recognized as vertebrates truly unique from other terrestrial wildlife and the fishes. The burgeoning interest in the ecotoxicology of the herpetofauna has been driven in part by their continued population declines at a global scale and a growing appreciation that terrestrial wildlife such as birds and mammals and aquatic vertebrates such as fish may not adequately represent contaminant exposure experienced by amphibians and reptiles during the various phases of their life cycles. For most amphibians, this includes laying permeable eggs in water, an aquatic larval stage, a physiologically demanding period of metamorphosis from larva to juvenile, and an adult stage that occurs in both aquatic and terrestrial habitats. Even more recently, the importance of reptiles as ecological receptors in both aquatic and terrestrial habitats has been recognized. While lacking the distinctly bimodal life cycle seen in most amphibians, the long lives, philopatric tendencies, and for some species, amphibious lifestyles exhibited by reptiles may expose them to a variety of contaminants over very long time periods (Sparling et al. 2000a). The continuing increase in ecotoxicological research activities with herpetofauna has supported synthesis publications such as Sparling et al. (2000b), Linder et al. (2003a, 2003b), Campbell and Campbell (2000, 2001), and Gardner and

Oberdörster (2006), and compilations of ecotoxicological data (Devillers and Exbrayant 1992; Pauli et al. 2000).

In this chapter, we provide a snapshot of the existing literature pertaining to the ecotoxicology of amphibians and reptiles, particularly for species reliant to varying extents on aquatic environments. We will briefly consider the historic context of these organisms as players in the discipline of ecotoxicology. Then, we will discuss their current roles in guiding ecotoxicologists toward a future where these previously undervalued "orphan groups" are more fully appreciated as critical components of a wide range of aquatic, wetland, and terrestrial habitats.

4.1 HISTORIC DEVELOPMENTS: AMPHIBIANS AND REPTILES IN ECOTOXICOLOGY

4.1.1 Amphibians

The eggs and larvae of anuran amphibians have a long history of use as models for the study of early vertebrate development, including the effects of materials that disrupt developmental patterns (e.g., Chang et al. 1954; also see Callery 2006 for review). In the 1970s and early 1980s, an increasing number of studies began evaluating the effects of organic and inorganic chemicals on fish and invertebrates (e.g., Mount and Brungs 1967; Cairns and Dickson 1973; Committee on Methods for Acute Toxicity Tests with Aquatic Organisms 1975; Birge and Black 1977; Birge 1978; Peltier 1978) and amphibians (e.g., Sanders 1970; Cooke 1972; Johnson 1976; Birge et al. 1980) for the sake of determining the risk these compounds might pose to organisms in the field. ASTM standards (e.g., E-729, *Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians*) initially published in 1980 were instrumental in setting the stage for developing tools for toxicity assessment. Also, as reviewed by Birge et al. (2000), some of this work ultimately led to methods that were adopted by the USEPA to support biomonitoring programs promulgated as part of the Clean Water Act (although amphibians were not ultimately included as species commonly used in these tests). At this same time, Dumont and colleagues (Dumont et al. 1979, 1983; Bantle 1995) were undertaking work with *Xenopus laevis* to develop screening tests that could be used to indicate effects of process waters and complex mixtures associated with oil and gas extraction. These methods development efforts ultimately lead to the protocol for the Frog Embryo Teratogenesis Assay–Xenopus (FETAX).

Beyond these simple laboratory-based tools used to evaluate chemical effects on amphibians, Semlitsch and Bridges (2005) have advocated incorporating realism into experiments (e.g., utilizing native species in toxicity tests), focusing on differences in life modes and rates of development (e.g., direct vs. indirect developers), incorporating greater genetic variation of test organisms into studies, increasing the spatial scale of studies, and examining direct vs. indirect effects of contaminants. They also encourage exploring the biological links between ecotoxicological studies and conservation, links that are potentially disrupted when challenged, yielding adversely affected regulation of species populations and community structure. Contemporary studies are steering away from single-species, single-contaminant approaches that dominated early investigations focused on the ecotoxicology of herpetofauna, and go beyond observations that populations of some amphibians were highly reliant on aquatic habitats for their early developmental stages. While chemicals entering aquatic habitats remain stressors critical to long-term sustainability of amphibian populations, opting to a multiple stressors approach for managing amphibian populations may be more beneficial to their long-term sustainability.

4.1.2 Reptiles

In contrast to the nearly 40-year history of amphibians being used in aquatic toxicology, the role of reptiles has unfortunately been relatively minor and more episodic (Hopkins 2000), despite the

fact that reptiles have been undergoing population declines much the same as amphibians (Gibbons et al. 2000). Compilations of toxicity data are available (see Sparling et al. 2000b; Pauli et al. 2000), but relatively underdeveloped, given the sparse literature to support such reviews. Until recently, the paucity of toxicity data stemmed from the lack of standard laboratory test procedures for evaluating toxic effects of chemicals on this taxon. While more standard methods for conducting toxicity tests with a reptile model have recently become available (Brasfield et al. 2004), the focus is most relevant to terrestrial habitats.

While studies with amphibians have consistently outpaced those with reptiles, especially with respect to characterizing toxicity data, the evaluation of bioaccumulation appears better developed for reptiles than for amphibians. As noted in Sparling et al. (2000a), published works focused on bioaccumulation of chemicals demonstrate the significant role that monitoring of reptiles such as turtles has played in the evaluation of aquatic contaminants. For example, early-exposure assessments using wildlife frequently documented tissue residues in field-collected turtles (e.g., Bishop et al. 1998; Golet and Haines 2001), and studies of alligators (*Alligator mississippiensis*) in Florida were important for characterizing uptake and accumulation of mercury (e.g., Heaton-Jones et al. 1997) and endocrine-disrupting chemicals (EDCs; e.g., Guillette et al. 1994, 1995). While chemical effects and exposure data for herpetofauna will undoubtedly continue to increase in the future, the paucity of data underscores data gaps clearly apparent for reptiles. Work focused on amphibians should also increase, or at the very least, continue at its current pace.

4.2 CONTAMINANT EXPOSURE PATHWAYS FOR AMPHIBIANS AND REPTILES

The complex life cycle of most amphibians leads to diverse ways in which they are exposed to environmental contaminants. As discussed by Birge et al. (2000), uptake of chemicals can begin shortly after egg deposition, as water moves into the egg capsule. There is actually some indication that the eggs themselves may receive a residue load from maternal transfer (Birge et al. 2000; Kadokami et al. 2002, 2004; Hopkins et al. 2006), a phenomenon reported for reptiles (see next paragraph), but much less so for amphibians. As larvae and tadpoles, uptake of waterborne chemicals across the permeable skin is an important source of exposure. The skin continues to play a role in chemical uptake by most adult amphibians as a result of its continued function as a respiratory surface, with the lungs also providing possible routes for volatile compounds. Uptake of chemicals through food may be important throughout the life cycle, although the importance of this route may vary. For example, within their lifetime, many anurans exist on 2 trophic levels, eating algae as tadpoles and invertebrates as adults. Adult amphibians can burrow into sediment and soils during hibernation and aestivation, making uptake of contaminants from these solid phases (across their permeable skin) a potential avenue of exposure as well (see Chapter 5, this volume; Boutilier et al. 1992; Larsen 1992; Shoemaker et al. 1992).

As noted earlier, maternal transfer of both organic and inorganic contaminants has been described for reptiles, with the eggs often used to indicate maternal contaminant burdens (Pagano et al. 1999; Nagle et al. 2001). This may lead to significant impacts on early life stages as demonstrated by Rauschenberger et al. (2004), who found that maternal transfer of organochlorine pesticides was associated with reduced egg and embryo viability in American alligators. Although not singly dependent on aquatic habitats to complete early developmental stages of their life cycle, many reptiles nest in terrestrial and wetland environments in close association with surface waters, and the eggs, when buried in soils, may accumulate chemicals from the surrounding matrix in association with water that is imbibed during the course of development. Moeller (2004) reported significant accumulation of lead, cadmium, and zinc in embryos of red-eared slider turtles (*Trachemys scripta*) derived from eggs that had been incubated on contaminated substrates. Since the skin of most reptiles is relatively impermeable, dermal uptake of chemicals may not be a particularly important route of exposure for these organisms. However, some aquatic turtles rely on water held in their

buccal cavity for oxygen uptake (Withers 1992), and this may also provide a pathway for entry of dissolved chemicals.

Whereas terrestrial exposure may seem less important for some herpetofauna, McDiarmid and Mitchell (2000) comment on the relatively long distances that species of both amphibians and reptiles may move daily, seasonally, and annually, a feature that can significantly influence the potential to be exposed to contaminants outside the aquatic environment. In adults, bioaccumulation may be dominated by dietary exposures, yet chemicals of the exposure matrix (e.g., metals) may be readily absorbed across dermal epithelia or volatilize from a solid or liquid phase into air, providing another means of exposure (see, for example, Noble 1931; Boutilier et al. 1992; Shoemaker et al. 1992; Duellman and Trueb 1994; Stebbins and Cohen 1995 regarding cutaneous respiratory surfaces and potential routes of metal uptake). Many variables affect the magnitude of bioaccumulation in terrestrial exposures to adults or early developmental stages (e.g., in reptile eggs), and the transfer of chemicals within food chains may be conveniently described by transfer coefficients or functions that characterize the relationships among trophic levels (Pastorok et al. 1996; Pascoe et al. 1996; Linder et al. 1998; Linder and Joermann 1999). These factors may be abiotic, as reflected by physicochemical characteristics of a chemical and an exposure matrix (sediment or soil), or biological in character, as captured by life-history-dependent attributes related to gastrointestinal or nutritional physiology, foraging, or feed preference (see Young 1981; Larsen 1992; Hamelink et al. 1994; Langston and Spence 1995; Linder et al. 2002).

4.3 TOXICITY ASSESSMENT: LABORATORY STUDIES

4.3.1 Toxicity Tests Using Amphibians

A key focus of applied toxicology is to assess the risk that potential chemical stressors may pose to natural populations. Laboratory studies may be used in an a priori sense to help predict the effects of a chemical before it is released into the environment, or to reassess the risk of materials as additional data regarding their environmental effects become available. For example, basic laboratory tests with a common herbicide formulation indicated the potential for adverse effects on tadpole stages in Australian anurans and resulted in revised labeling guidelines and restrictions on its use (Mann et al. 2003). Laboratory toxicity tests are also used extensively for regulatory assessments of wastewater discharges, and Cooney (1995) reviewed some of the basic methods, test species, and statistical endpoints relevant to freshwater tests.

The list of response parameters that can be evaluated in a laboratory setting is extensive, and those used in a particular study are obviously influenced by study objectives. Mortality and growth of test organisms are common endpoints that may be used with most test species (ASTM 2005a, 2005b), while endpoints more specific to amphibians include the length of the larval period and size at metamorphosis. Examples of laboratory tests using native North American amphibian species and associated endpoints are included in Table 4.1. As further described later. FETAX can be used to evaluate the incidence of malformation in addition to growth and mortality (ASTM 2005c). Other endpoints that have been used in amphibian tests include measures of performance and/or behavior after exposure to chemical stressors, such as swim speed, the ability to avoid predators, general activity levels, and time spent feeding (Bridges 1997; Savage et al. 2002; Richards and Kendall 2003; Broomhall 2004; Widder and Bidwell 2006, 2008). Recent laboratory work using high-speed video to investigate the subtleties of the escape response (C-start response) in fish and aquatic amphibian stages (e.g., Azizi and Landberg 2002) should also prove useful for investigating sublethal contaminant effects on escape responses. Finally, various biochemical and physiological "biomarkers" can and have been investigated in the laboratory setting, as discussed further later.

Laboratory toxicity tests using amphibians have been conducted with embryo and larval stages (collectively considered early life stage [ELS] tests), metamorphs, juveniles, and sexually mature

TABLE 4.1
Examples of Recent Laboratory, Mesocosm, and Field Studies Using Native North American Amphibian Species

Study Type	Stressor	Endpoint	Life Stage	Species	Reference
Laboratory	Atrazine Iridovirus	• Development • Growth • Survival	Larvae	*Ambystoma macrodactylum*	Forson and Storfer 2006
Laboratory	Multiple agricultural chemicals	• Development • Growth • Gonadal development • Immune function	Tadpole to metamorph	*Rana pipiens* *Xenopus laevis*	Hayes et al. 2006
Laboratory	Atrazine Nitrate	• MMM, LLP • Gonadal differentiation • Sex ratio	Tadpole to metamorph	*Rana pipiens*	Orton et al. 2006
Laboratory	Atrazine Density	• Postexposure survival	Embryos Larvae	*Ambystoma barbouri*	Rohr et al. 2006
Laboratory	Glyphosate	• MMM, LLP • Activity • Survival • Abnormalities	Tadpoles to metamorph	*Rana cascadae*	Cauble and Wagner 2005
Laboratory	Fungicide	• % metamorph • Behavior • MMM, LLP	Tadpoles to metamorph	*Rana temporaria*	Teplitsky et al. 2005
Laboratory	Atrazine	• MMM, LLP • Gonad development	Embryo to metamorph	*Rana clamitans*	Coady et al. 2004
Laboratory	Cadmium	• Hibernation success	Juveniles	*Bufo americanus*	James et al. 2004b
Laboratory	Malathion	• Acute survival	Tadpoles	*Bufo americanus*	Relyea 2004
	Predation			*Hyla versicolor*	Relyea 2003
	Carbaryl			*Rana catesbeiana*	
	Predation			*R. clamitans* *R. pipiens* *R. sylvatica*	

(*continued*)

TABLE 4.1 (CONTINUED)
Examples of Recent Laboratory, Mesocosm, and Field Studies Using Native North American Amphibian Species

Study Type	Stressor	Endpoint	Life Stage	Species	Reference
Laboratory	Endosulfan Temperature	• Predator avoidance • Survival	Tadpoles	*Litoria citropa*	Broomhall 2002
Mesocosm	Carbaryl Nitrates	• MMM, LLP • Survival	Tadpoles to metamorphs	*Rana clamitans*	Boone et al. 2005
Mesocosm	Cadmium Tissue residue	• MMM, LLP • Survival	Tadpole to metamorph	*Bufo americanus* *Rana sphenocephala*	James et al. 2005
Mesocosm	Carbaryl Competition	• MMM, LLP • Lipid reserves • % metamorphs	Larva to metamorph	*Ambystoma maculatum* *A. opacum*	Metts et al. 2005
Mesocosm	Roundup®	• Survival	Larvae Metamorphs	*Bufo americanus* *Hyla versicolor* *Pseudacris crucifer* *Rana pipiens* *Rana sylvatica*	Relyea 2005
Mesocosm	Atrazine Endosulfan Competition Predation	• MMM, LLP • Activity • Predator avoidance	Tadpole to metamorph	*Rana sylvatica*	Rohr and Crumrine 2005
Mesocosm	Carbaryl Competition Predation	• MMM, LLP • Survival	Tadpole to metamorph	*Rana sphenocephala*	Mills and Semlitsch 2004
Mesocosm	Copper Pathogen	• MMM, LLP • Survival	Tadpole to metamorph	*Hyla chrysoscelis*	Parris and Baud 2004
Mesocosm	Copper Pathogen	• Fluctuating asymmetry • MMM, LLP • Survival	Tadpole to metamorph	*Bufo fowleri* *Hyla chrysoscelis*	Parris and Cornelius 2004
Mesocosm	Atrazine Limited food Hydroperiod	• Behavior • MMM, LLP • Survival	Egg to metamorph	*Ambystoma barbouri*	Rohr et al. 2004

Mesocosm	Mercury	• MMM, LLP • Malformity • Survival	Tadpole to metamorph	*Rana sphenocephala*	Unrine et al. 2004
Mesocosm	Carbaryl	• MMM, LLP • Survival	Tadpoles to metamorphs	*Rana clamitans*	Boone and Bridges 2003
Mesocosm	Atrazine Carbaryl Competition Hydroperiod	• MMM, LLP • Survival	Tadpoles to metamorphs	*Ambystoma maculatum* *A. texanum* *Bufo americanus* *Rana sphenocephala*	Boone and James 2003
Mesocosm	UV radiation Carbaryl	• MMM, LLP • Survival	Tadpoles to metamorphs	*Rana sphenocephala*	Bridges and Boone 2003
Mesocosm	Atrazine Carbaryl Endosulfan Octylphenol Limited food	• Activity • Growth rate • Hatching • Survival	Egg to metamorph	*Ambystoma barbouri*	Rohr et al. 2003
Mesocosm	Carbaryl Competition Predation Hydroperiod	• MMM, LLP • Survival	Tadpoles to metamorphs	*Rana blairi* *R. clamitansi* *R. sphenocephala* *B. woodhousei* *Notophthalmus viridescens*	Boone and Semlitsch 2002
Mesocosm	Carbaryl	• MMM, LLP • Survival	Tadpoles to metamorphs	*Rana clamitans*	Boone et al. 2001
Field survey	Atrazine	• Aromatase activity • Hormones	Frogs	*Rana catesbieana* *R. clamitans* *R. pipiens*	Murphy et al. 2006a
Field survey	Atrazine	• Gonad histology	Juveniles Adults	*Rana catesbieana* *R. clamitans* *R. pipiens*	Murphy et al. 2006b
Field survey	Retinoids	• Weight • Tissue residues	Frogs	*R. catesbieana*	Berube et al. 2005
Field survey	OC pesticides	• Tissue residues	Tadpoles	*Rana lessonae* *R. esculenta*	Fagotti et al. 2005

(*continued*)

TABLE 4.1 (CONTINUED)
Examples of Recent Laboratory, Mesocosm, and Field Studies Using Native North American Amphibian Species

Study Type	Stressor	Endpoint	Life Stage	Species	Reference
Field survey	Environmental exposure	• Tissue PCB residues	Tadpoles	*Rana temporaria*	Hofer et al. 2005
Field survey	Coal combustion waste	• Tissue residues	Tadpoles Metamorphs Frogs	*Bufo terrestris* *Rana sphenocephala*	Roe et al. 2005
Field survey	Environmental exposure	• Residues • Population status	Frogs	*Rana muscosa*	Fellers et al. 2004
Field survey	Environmental exposure	• Species richness • Reproductive success	Frogs	Various species	Knutson et al. 2004
Field survey	Environmental exposure	• Species richness • Tissue PCB residues	Frogs	Various species	DeGarady and Halbrook 2003
Field survey	PCBs Toxaphene	• Tissue residues	Tadpoles	*Hyla regilla*	Angerman et al. 2002
Field survey	Environmental exposure	• Population persistence	Frogs	Various species	Davidson et al. 2002
Field survey	OCs, PCBs Metals	• Residues • Deformities	Frogs	*Rana clamitans*	Gillilland et al. 2001
Field manipulation	Environmental exposure	• Hatching • Growth • Species richness • Survival • Tissue residues	Eggs Embryos Tadpoles	*Rana clamitans* *R. pipiens*	Karasov et al. 2005
Field manipulation	Release®	• Avoidance • Growth • Survival	Tadpoles	*Rana clamitans* *R. pipiens*	Wojtaszek et al. 2005
Field manipulation	Environmental exposure	• Cytochrome p450 activity	Tadpoles	*Rana clamitans melanota*	Jung et al. 2004
Field manipulation	Glyphosate	• Avoidance response • Growth • Survival	Tadpoles	*Rana clamitans* *R. pipiens*	Wojtaszek et al. 2004
Field manipulation	UV Nitrates	• Mass • Length • Survival	Tadpoles Larvae	*Ambystoma macrodactylum* *Hyla regilla*	Hatch and Blaustein 2003

Integrated study	Coal combustion waste	• Abnormalities • Behavior • Hatching • Tissue residue	Eggs Tadpoles	*Gastrophryne carolinensis*	Hopkins et al. 2006
Integrated study	Environmental extracts	• MMM, LLP • Survival • Abnormalities	Tadpoles to metamorphs	*Hyla arenicolor* *Pseudacris crucifer* *P. regilla*	Bridges and Little 2005
Integrated study	Environmental extracts	• MMM, LLP • Survival • Abnormalities	Tadpoles to metamorphs	*Rana pipiens*	Bridges et al. 2004
Integrated study	Agricultural chemicals	• Population status	Frogs	*Bufo canorus* *Rana aurora draytonii* *R. boylii* *R. cascadae* *R. muscosa*	Davidson 2004
Integrated study	Atrazine	• Gonadal morphology	Frogs	*Rana pipiens*	Hayes et al. 2003
Integrated study	Environmental and in-lab exposure	• Hatching • Deformity • Survival	Eggs to tadpoles	*Ambystoma gracile* *Rana aurora*	de Solla et al. 2002
Integrated study	PCBs	• MMM, LLP • Survival • Species richness • Tissue residue	Eggs to frogs	*Rana pipiens* *R. utricularia*	Glennemeier and Begnoche 2002
Integrated study	Agricultural runoff Parasite	• Immune function • Deformities	Tadpole Metamorph	*Rana sylvatica*	Kiesecker 2002
Integrated study	Carbaryl	• MMM, LLP • Survival	Eggs Hatchlings Tadpoles	*Hyla versicolor*	Saura-Mas et al. 2002
Integrated study	PCB-laden sediment	• Behavior • Survival	Tadpoles	*Rana sylvatica*	Savage et al. 2002

Note: FETAX studies are not included, although the number of publications using the protocol is extensive. See text for further discussion of FETAX. MMM = mass at metamorphosis, LLP = length of the larval period.

adults. Of these stages, embryos and larvae have been used most commonly to evaluate the effects of aquatic contaminants. Part of the reason for this is the common assumption in aquatic toxicology regarding greater sensitivity of early life stages, and there are a number of studies that have indicated that tadpoles are more sensitive to contaminants than eggs and postmetamorphic adults (Ralph and Petras 1998; Mann and Bidwell 1999). However, studies that compared the sensitivity of different life stages of amphibians with a finer degree of resolution have indicated enhanced effects during metamorphosis, which may be due to the physiological demands associated with the "reorganization" that organisms experience through developmental time (Howe et al. 1998; Natale et al. 2000; Fort et al. 2004a). Another factor leading to the widespread use of aquatic stages (in particular, tadpoles) is their greater availability (particularly in the case of field-collected organisms) and use for waterborne exposures. While an early approach by Birge and Black (1977) placed fish and amphibian embryos in contact with contaminated sediments, the majority of laboratory tests with amphibian larvae have focused on exposure via the water column (for example, see the methods described in ASTM E-729 [2005a]). Since postmetamorphs and adults of many species drown if held in water constantly, a key challenge in evaluating contaminant effects in these stages is the development of an appropriate exposure system. Some particularly novel work has considered uptake through the diet and dermal exposure in active and hibernating adults (James 2003; James et al. 2004a, 2004b).

Bantle (1995) discussed the importance of standardization of test methods, since it facilitates comparison of species sensitivity to the same contaminant. In this regard, FETAX and its derivatives are probably the most popular of the laboratory test methods that have used amphibians to assess single chemicals and complex mixtures. Bantle (1995), Dumont et al. (2003), and Fort et al. (2003) have summarized the development of FETAX, which became available as a standard aquatic toxicity test as ASTM E-1439 in 1991 (ASTM 2005c). Interestingly, the method was originally designed in the 1970s and early 1980s as an outcome of methods development intended to screen chemicals that were potential human developmental hazards (Bantle 1995). FETAX, however, exceeded these intentions and contributed to heightening focus on amphibians as receptors incorporated into the ecological risk assessment. As a test species, *X. laevis* and the smaller *X. tropicalis* present life history attributes amenable to laboratory testing, since they can be easily maintained in the laboratory, can be hormonally induced to reproduce throughout the year (unlike North American amphibians, which are seasonally reproductive and are usually not as amenable to laboratory culture), and can provide large numbers of embryos for testing. Exposures are initiated at early blastula and continue through primary organogenesis to ensure a baseline assessment of early life stage effects. Endpoints include mortality (which is evaluated over the course of the exposure), embryo growth (as length and mass), number of abnormal embryos, and type of abnormailities observed at the end of the test. These data provide LC50 and EC50 data (median lethal concentration and median effective concentration for terata based on number of abnormal embryos among the survivors, respectively). *X tropicalis* completes its life cycle in a shorter amount of time than *X. laevis*, which has stimulated interest in its use in life cycle toxicity tests (Fort et al. 2004b). These tests can be important for understanding the reproductive effects that contaminants have on amphibians. For example, Lienesch et al. (2000) observed adverse effects of sublethal cadmium exposures in female *X. laevis* oocytes at all stages of oogenesis, including greatly increased numbers of atretic oocytes and other indicators that oocytes would be significantly reduced in their viability. Christensen et al. (2004) describe an amphibian sperm inhibition toxicological test (ASITT) that examines the effects of contaminants on *Xenopus* sperm motility. Other genera besides *Xenopus* may also be tested with the FETAX protocol. For example, *Rana catesbiena*, *R. pipiens*, *Bufo fowleri*, and *B. americanus* are alternative test organisms identified in ASTM E-1439. When applying the protocol to other species, recall that development is highly temperature dependent and varies greatly among species, so if developmental stage serves as the endpoint for test termination (e.g., for FETAX, Stage 46 as characterized by Nieuwkoop and Faber [1956]), exposure times may vary among taxa.

Another test system that utilizes early amphibian life stages in a fashion similar to FETAX is the AMPHITOX method described by Herkovits and Pérez-Coll (2003). This procedure actually comprises a group of different types of bioassays that evaluate acute (AMPHIACUT), short-term chronic (AMPHISHORT), and chronic (AMPIRCHRO) exposures. By plotting toxicity endpoints derived from these tests, a family of toxicity curves can be generated from exposure data collected for 24 hours to 14 days. Conventional measures of toxicity, such as no observed effect concentrations (NOECs), lowest observed effect concentrations (LOECs), and median effect concentrations (LC50s), may also be calculated. By employing an early life stage test (AMPHIEMB), developmental effects may also be evaluated. Results of AMPHITOX suggest that the toxicity of a wide range of environmental samples may be evaluated by selecting the most appropriate toxicity test included in the test suite, with the test selection determined in part by an initial screening level evaluation of toxicity of the sample and the endpoint of concern.

The role of laboratory toxicity studies with amphibians can and should extend beyond the assessment of waterborne stressors. For example, while sediment studies are relatively uncommon in amphibian ecotoxicology, tadpoles of many species spend much of their time in the sediment and even forage on decomposing organic matter found there. Thus, contaminants that are bound to sediment (and absent or in low concentrations in the water column) can serve as an important source of exposure via both dermal and dietary routes of exposure. For example, Lehman et al. (personal communication) found that tadpoles reared with sediment from contaminated sites in the Alaskan Kenai Peninsula were smaller upon metamorphosis than tadpoles reared under control conditions. Furthermore, because amphibians have permeable skin, contact with sediments may represent an important route of uptake via dermal exposure. This is the case with developing tadpoles as well as hibernating adults and juveniles that burrow into sediments. Bleiler et al. (2004) have published methods that address the current deficit in sediment testing procedures using amphibians. Studies incorporating sediment exposures should be designed to allow comparisons of toxicity similar to those of invertebrate test methods for assessing contaminated sediments (ASTM 2005d).

Standard laboratory studies following the FETAX protocol have been used to evaluate extracts from soils and sediments collected from contaminated areas (Fort et al. 2001). Tests have also been conducted to test soils, soil eluates, and sediments directly by suspending embryos over a sediment sample in an exposure chamber (Birge and Black 1977; Fort et al. 1999). Outcomes of these tests indicated that toxic constituents may not be tightly bound to the solid-phase material. This type of work can play an important role in evaluating the presence of an existing contaminant load in wetland habitats and help direct management or remedial decisions (e.g., Hutchins et al. 1998).

Some species of amphibians are not amenable to laboratory testing due to factors such as low survival in a laboratory setting, difficulty in capturing sufficient numbers of individuals to conduct statistically defensible tests, or in the case of threatened or endangered populations, legal and ethical considerations associated with continued depletion of natural populations. As such, surrogate test organisms are often used to generate response data, with *Xenopus* often the species of choice for many of those wishing to examine the effects of contaminants on amphibians. The key advantage of these organisms is that they are easily kept in the laboratory and can yield eggs for tests year-round. However, Birge et al. (2000) stated that the higher tolerance of *Xenopus* to chemical stressors, as compared to other amphibian species, makes it less suitable for use in routine testing for aquatic risk assessment. In their evaluation of FETAX for use in ecological risk assessments, Hoke and Ankley (2005) concluded that risk assessments using acute hazard data with traditional laboratory species were more protective of native amphibians than assessments based on hazard data from FETAX. On the basis of the available comparative toxicity data, *X. laevis* sensitivity appears to lie mid-range, tending toward being less sensitive than many North American species tested (Birge et al. 2000). These observations inevitably vary as a function of toxicant and endpoint. For example, Mann and Bidwell (2000) found that *Xenopus* was the most sensitive of the organisms they evaluated in FETAX and modified FETAX assays of an agricultural surfactant, and Hoke and

Ankley (2005) observed that growth was a more sensitive endpoint in the assay than malformation or survival. Although the species has invaded areas of Florida and southern California, *Xenopus* is not native to North America and may be limited in use as a surrogate for North American anurans. While native species are often only seasonally available, the ecological relevance of native forms may outweigh this issue, particularly in cases of site-specific risk assessments. A key objective in this regard is to extend the existing comparative toxicity data for native species, since the use of surrogates (native forms) is likely to remain an important approach.

One of the most significant criticisms of laboratory toxicity tests is their potential to inadequately predict the effect chemical stressors might have on organisms in the field (Burkhart et al. 2003). Certainly, the "real world" situation imposes a suite of stressors that the often simplistic exposure environment of the laboratory fails to address. Still, laboratory toxicity tests continue to play an important role in evaluating chemical effects. A significant advantage of these procedures is that they allow control of potentially confounding physical (e.g., temperature) and chemical (e.g., pH and hardness when using formulated diluents) variables, making them critical for establishing cause-and-effect relationships between the presence of contaminants and the response of organisms. Laboratory toxicity tests have also played a valuable role in characterizing the sensitivity of different amphibian groups to chemical stressors (Bridges and Semlitsch 2005). Although standardization of test methods is important in this regard, within the scope of amphibian declines most effects on populations may not be attributable to lethal concentrations of contaminants present in a habitat; hence, some laboratory-generated toxicity estimates (e.g., median lethal concentrations) may be relatively limited in applications far removed from evaluation of field settings. However, examining alternative endpoints related to adverse effects on life history traits may be more applicable to characterizing ecologically acceptable levels of a contaminant rather than relying only on traditional lethality-based endpoints. For instance, the length of the larval period and mass at metamorphosis are traits critical in determining an individual's fitness (i.e., survival and future reproductive success). Amphibians that metamorphose at larger sizes and earlier in the season have a greater chance of surviving over winter and will reproduce at younger ages (Smith 1987; Semlitsch et al. 1988). Further, a short larval period is especially important to amphibian species breeding in temporary ponds, where any factor that lengthens the larval period, such as the presence of an environmental contaminant, can lead to mortality due to desiccation or prolonged exposure to predators. Many contaminants alter life history traits at concentrations well below those that express themselves by decreased survival. The use of novel exposure scenarios in the laboratory has also indicated the complexity of the organismal response to chemicals, as demonstrated by Relyea and Mills (2001), who found the presence of a predator enhanced the toxicity of a pesticide to gray treefrog (*Hyla versicolor*) tadpoles.

4.3.2 Toxicity Tests Using Reptiles

With regard to ecotoxicology studies focused on herpetofauna, amphibians have outpaced reptiles, although the latter have accounted for an increased number of citations over the past 10 to 15 years. As part of their effort to initially characterize the state of the science of ecotoxicology for amphibians and reptiles, Sparling et al. (2000a) completed a literature search that spanned more than 25 years between 1972 and 1998, and found that only 1% to 2% of the publications focused on vertebrates concerned reptiles and their role in evaluating or monitoring chemicals in the environment. As shown in Chapter 1 of this book, this relative standing of reptiles in ecotoxicological research has not changed appreciably. This is likely attributable to amphibians, in general, being more amenable to toxicity tests than reptiles, especially during the formative years of aquatic toxicology. Many anurans lay thousands of eggs at a time, providing ample numbers of tadpoles (i.e., test animals and adequate replication) for experiments. Urodeles commonly have lower reproductive output than anurans, but can still produce offspring in sufficient numbers for aquatic toxicity

tests. Reptiles, by comparison, are more difficult to collect and produce relatively fewer offspring per clutch than most amphibian species.

At present, toxicity test procedures for reptiles are largely focused on snakes and lizards (see Table 4.2 for examples). Toxicity testing with selected terrestrial species of lizard (primarily eastern and western fence lizards, *Scaphiopus undulatus* and *S. occidentalis*, respectively) is increasingly reported in the literature, yet requires standardization for its widespread application as a tool to inform risk management. No test systems are available to evaluate reptiles that are predominantly aquatic species. In general, slow growth rates, long life cycles, and complex relationships between size and age at sexual maturity are some of the challenges in establishing captive breeding populations of aquatic and semiaquatic reptiles for developing standard test methods comparable to those available for fence lizards or the common aquatic vertebrate test species.

Although Crews et al. (2003) have suggested that red-eared sliders (*Trachemys scripta elegans*) be applied in screening tests for endocrine-disrupting chemicals, no standardized test has been developed. Turtle eggs, however, have also been used to study maternal transfer of contaminants, uptake of contaminants from nest substrate, and effects of those contaminants on hatchlings (Table 4.2). From a practical perspective, tests using fence lizard eggs may be preferred over those of turtles, since fence lizards will grow and reproduce in the laboratory, and their eggs would be more readily available. In contrast, turtle eggs are usually only seasonally available from field collections, where they may have been exposed to chemicals while lying in the nest or maternally transferred. For studies focused on endpoints related to endocrine-disrupting chemicals, external differences in the sex of fence lizards are more easily determined early in the animal's development; in turtles, dissection is usually required to determine sex, particularly in juveniles. Fence lizards also appear to be good candidates for use in a reptilian reproductive toxicity test because populations mature quickly and reproduce well in captivity.

Within ecological contexts, the herpetofauna include many species that potentially link aquatic habitats and immediately adjacent wetland or terrestrial habitats. For example, semiaquatic reptiles, in particular their early life stages developing in ovo, have been used to evaluate chemicals in seasonally hydric soils. Although saturated soils may not be appropriate for egg incubation, within ecological contexts toxicity evaluations framed within the life history patterns of semiaquatic reptiles better serve the ecological risk assessment process than alternatives simply focused on chemical analysis of substrates that yield modeled extrapolations based on other terrestrial vertebrates. Despite advances since Sparling et al. (2000b) initially summarized the state of the science for the ecotoxicology of amphibians and reptiles, development of comparative toxicity data for reptile orders continues to lag and remains one of the long-term objectives identified by Hopkins (2000) to advance reptilian toxicology.

4.4 TOXICITY ASSESSMENT: MESOCOSM STUDIES

Interpretation of laboratory data within the context of ecological risk requires validation and confirmation of laboratory toxicity results under field conditions, with the goal being to minimize laboratory-to-field extrapolation errors. Effects of many chemical contaminants may be altered by field conditions that vary with habitat, making it important to evaluate toxicity while accounting for environmental factors potentially influencing exposure or effects. For example, UV radiation can cause some environmental chemicals to be more toxic and, in other instances, it may promote degradation of contaminants to less toxic forms (Bridges et al. 2004). In aquatic habitats, hydroperiod and larval density may influence the effects of chemical contaminants on amphibian larvae (e.g., carbaryl; Boone and James 2003) by lengthening the larval period and causing animals to be smaller at metamorphosis. Predation may also influence toxic effects linked to chemical exposure, potentially yielding increased toxicity for some chemicals under field conditions (see, for example, Relyea and Mills 2001 as briefly noted later).

TABLE 4.2
Examples of Recent Laboratory and Field Studies with Aquatic (Freshwater) Reptiles

Study Type	Stressor	Endpoint	Life Stage	Species	Reference
Laboratory	Carbaryl	Swimming performance	Neonate	*Seminatrix pygaea* *Nerodia fasciata* *N. rhombifer* *N. taxispilota*	Hopkins and Winne 2006
Laboratory	Carbaryl	Swimming performance	Neonate	*Seminatrix pygaea* *Nerodia rhombifer*	Hopkins et al. 2005
Laboratory	Toxaphene	Morphological Physiological	Eggs Hatchlings	*Alligator mississippiensis*	Milnes et al. 2004
Laboratory	Trace elements	Tissue residues Behavior Growth Body condition	Juveniles	*Nerodia fasciata*	Hopkins et al. 2002
Laboratory	Metals	Egg residues, hatchling mass	Eggs	*Trachemys scripta*	Moeller 2004
Field survey	Metals	Egg residues	Eggs	*Trachemys scripta*	Tryfonas et al. 2006
Field survey	Metals	Tissue residues	Adults	*Akistrodon piscivorous* *Nerodia fasciata* *N. taxispilota*	Burger et al. 2006
Field survey	Metals	Tissue residues	Adults	*Nerodia sipedon*	Burger et al. 2005 Campbell et al. 2005
Field survey	OCs Mercury	Tissue residues	Adults	*Agkistrodon piscivorus*	Rainwater et al. 2005
Field survey	Selenium	Tissue residues	Adults	*Nerodia fasciata*	Hopkins et al. 2005
Field survey	OCs	Tissue residues	Eggs	*Crocodylus moreletii*	Pepper et al. 2004
Field survey	Environmental exposure	Tissue residues	Eggs	*Chelydra s. serpentina*	Ashpole et al. 2004

Field survey	Selenium	Tissue residues	Eggs Hatchling	*Alligator mississippiensis*	Roe et al. 2004
Field survey	Environmental exposure	Bone composition	Adults	*Alligator mississippiensis*	Lind et al. 2004
Field survey	OCs	Tissue residues	Adults	*Lepiochelys kempii* *Caretta caretta*	Keller et al. 2004a, 2004b
Field survey	Metals	Tissue residues	Adults	*Malaclemys terrapin*	Burger 2002
Field survey	Mercury	Tissue residues	Eggs	*Crocodylits moreletii*	Rainwater et al. 2002
Field survey	Environmental exposure	Blood	Adults	*Agkistrodon piscivorus* *Nerodia erythrogaster* *N. rhombifer* *Trachemys scripta*	Clark et al. 2000
Field survey	OCs PCBs	Plasma residues	Adults	*Nerodia sipedon insularium* *Nerodia s. sipedon*	Bishop and Rouse 2000
Field study	OCs	Egg residues	Eggs	*Crocodylits moreletii*	Rainwater et al. 2000
Integrated study	Trace elements	Tissue residues	Adults	*Nerodia fasciata*	Hopkins et al. 2001

More often than not, field exposures to environmental chemicals that occur at less than highly acute, but more than no observable effect concentrations, contribute to poor predictions of chemical effects in the natural environment. Mesocosm studies afford an intermediate between field studies and laboratory studies, wherein a few factors considered influential to exposures in field settings are examined. In these intermediate test systems, interactions among environmental factors and chemical contaminants may be studied under controlled situations that are more complex than what can be reproduced in a laboratory. Indeed, exposures more closely approach those observed in open field settings. While field studies more directly relate to the realities of field settings, these studies may not be feasible, and mesocosm studies may provide solutions otherwise dismissed for the sake of more convenient, yet reality-limited laboratory studies. Experiments reliant on mesocosms offer the control of a laboratory study (e.g., allowing examination of a few known factors) but may capture exposures and associated effects under more natural influences (e.g., ambient temperature fluctuations, UV radiation), potentially adding a measure of ecological relevance lacking in laboratory studies. Following a call for an increase in their applications in ecotoxicology (Rowe and Dunson 1994), mesocosm studies have been increasingly used to examine the impacts of chemical contaminants on amphibians (Table 4.1). For example, in their review of the recent ecotoxicological literature focused on amphibians, Boone and James (2005) found 28% of the reviewed studies involved work outside the laboratory, which represents an enormous increase over previous years. Much of this recent work spanned the range of herpetofauna and involved evaluations of chemical effects considered within the context of species life history and endpoints viewed within a multiple stressors framework at various levels of biological organization (e.g., larval amphibian communities experiencing natural stresses of competition for resources, predation, and pond drying).

Mesocosms may shed light on how contaminants affect the dynamics of amphibian communities better than laboratory studies, and may better characterize whether contaminants influence amphibians directly or indirectly. For example, in the laboratory environmentally relevant concentrations of carbaryl can negatively impact tadpole behavior (Bridges 1999), yet using mesocosms, adverse effects of carbaryl observed in the laboratory were not necessarily played out in the field. In some studies, carbaryl even appeared beneficial to anurans, since increased body size at metamorphosis was observed (Boone and Semlitch 2002). For salamanders, however, similar exposure conditions were associated with decreased body size at metamorphosis and reduced survival (Boone and James 2003). These contrasting effects appeared to be largely due to carbaryl's effects on the zooplankton community under the study's test conditions. Simply stated, carbaryl killed zooplankton. Zooplankton, however, are a food source for salamander larvae, and with the absence or reduced numbers of zooplankton, salamander larvae competed with tadpoles for food resources, for example, algae (Boone and Semlitsch 2002; Mills and Semlitsch 2004). Impacts of carbaryl on tadpoles may also be mediated by indirect effects on the predators rather than directly affecting the tadpoles (Boone and Semlitsch 2003). In laboratory studies, Relyea and Mills (2001) had observed that the presence of a predator was associated with increased carbaryl toxicity. However, in mesocosm studies subsequently focused on the interactions of multiple stressors — pH, predators, and carbaryl — these factors were not associated with outcomes that suggested that interactions would dominate the effects signature displayed in laboratory studies (Relyea 2006).

4.5 TOXICITY ASSESSMENT: FIELD STUDIES

Increased awareness of declining amphibian and reptile populations has contributed to their oftentimes being considered "sentinels" of environmental change (e.g., Sparling et al. 2001; Kiesecker et al. 2004). For example, given their dependence on wetlands, some species of amphibians and semiaquatic reptiles may be directly affected by habitat alteration or destruction, by non-point-source runoff, and by the accumulation of sediments and sediment-bound chemicals that are associated with soil erosion. Physical and chemical alterations of habitat may also be associated directly or indirectly with pathogens (see Carey and Bryant 1995; Crawshaw 2000). Anthropogenic impacts

on aquatic habitats, and adverse biological effects associated with these habitat alterations have undoubtedly contributed to the retraction of species distributions and decline of herpetofauna populations (Barinaga 1990; Corn 2000). Studies of field populations of amphibians and reptiles may therefore be undertaken to evaluate conditions before the start of some activity that may result in habitat effects (for example, the release of a wastewater discharge, physical disturbance in the riparian zone, flow alterations), or to provide retrospective analyses that indicate the extent of an existing impact, or to evaluate the success of remedial or restoration efforts. For amphibians, a variety of field survey methods have been developed to characterize basic population parameters such as taxa richness and/or diversity (e.g., Heyer et al. 1994; see Table 4.1 for examples). Methods to specifically incorporate herpetofauna into wetland monitoring programs are also available (USEPA 2002). Studies evaluating population level effects of contaminants on reptiles in the field remain limited, with most focused on residue monitoring (Table 4.2).

Observational studies and experimental manipulations may also be completed in field settings following various study designs. For example, exclosures or semipermeable enclosures may be used in situ to exclude various influences such as predators or competitors, while allowing most interacting abiotic factors to occur (see Bishop and Martinovic 2000; Linder 2003). While early-stage embryos can be collected from reference sites and placed in areas of concern for evaluating adverse effects in the field, alternative methods may be equally amenable to field studies or integrated field-laboratory investigations. While there are drawbacks to field studies (reviewed in Boone and James 2005), many biotic and abiotic influences readily present in the field, but not accounted for in laboratory or mesocosm studies, can be examined. Whole pond manipulations are even less common (but see Boone et al. 2004), as they can be rife with external influences impossible to control or account for statistically. Overall, there appears to be greater focus on monitoring activities in field settings rather than actual experimental manipulations (see Table 4.1). Boone and James (2005) cited only 19 ecotoxicological field studies using amphibians.

Most field-only investigations are correlative and require a combination of field and laboratory studies in order to more clearly understand these relationships. Bringing field-collected water into the laboratory for static-renewal exposures lends itself to more controlled experimentation than allowed in the field, but can be cumbersome when large volumes of water are required to satisfy test conditions. Another option is the use of semipermeable membrane devices (SPMDs) to sequester certain contaminant types from an environment, which are then evaluated under more controlled laboratory settings (Bridges and Little 2003). These studies have been a useful first step in situations where it is desirable to determine whether contaminants are impacting amphibian populations without having to undergo costly chemical analyses on water samples. For example, Davidson et al. (2001, 2002) observed that a number of amphibian species with declining populations occurred upwind from agricultural lands in the central valley in California. Using SPMDs, Bridges and Little (2005) were able to determine the presence of chemical contaminants within these areas, and that reduced growth and development of native tadpoles occurred when SPMDs were evaluated in laboratory toxicity tests. Sparling et al. (2001) had noted inhibition of cholinesterase in field-caught frogs collected from ponds similarly situated to those observed by Davidson et al. (2001, 2002).

A range of integrated field and laboratory studies have focused on various environmental chemicals and their potential risks for herpetofauna in various managed landscapes, as illustrated by Hayes et al. (2003). In this study, leopard frogs were sampled at various locations across the United States, and the incidence of intersex individuals was correlated with concentrations of the herbicide atrazine measured at these sampling locations. These findings from field-collected samples were further addressed in controlled laboratory investigations that indicated that low-concentration exposures to atrazine can generate intersex individuals (Hayes et al. 2002). Similarly, Fort et al. (1999) had earlier applied similar approaches for investigating the role of environmental chemicals in explaining observations of hind limb deformities in anurans collected from ponds in Minnesota and Vermont. In these studies, Fort et al. (1999) used a combination of laboratory FETAX assays of water and sediments to characterize causal relationships between chemical exposure and the

high incidence of amphibian malformations. While the role of integrated field and laboratory studies may involve any organism amenable to such studies, the herpetofauna (especially, amphibians) provide witness that the integration of field and laboratory studies combines the strengths of both approaches in assessing risk of chemical stressors and reducing uncertainties potentially influencing management actions.

4.6 BIOMARKERS OF EXPOSURE AND EFFECT IN AMPHIBIANS AND REPTILES

Biomarkers have been defined as biochemical, physiological, and histological endpoints that can be used to evaluate exposure to or effects of chemical stressors (Huggett et al. 1992). Some authors apply a more general definition that includes morphological alterations, genetic effects, behavioral parameters, and tissue residue levels (Walker et al. 2001). Venturino et al. (2003) provided an extensive review of biomarkers that have been used to indicate contaminant effects in anuran amphibians, and additional examples for both amphibians and reptiles are presented in Table 4.3.

An expected advantage of evaluating biochemical and physiological parameters in organisms exposed to chemical contaminants is that these endpoints may be influenced by a stressor before responses are seen at the whole organism or population level. As such, biomarkers may provide an early indication of contaminant exposure and/or effects (Newman and Unger 2003). It may also be possible to compare values of parameters from a contaminant-exposed population with reference values to indicate the influence of chemical exposure on the general health status of the organisms. While a lack of suitable reference data may limit this application for herpetofauna (Henry 2000), this issue exists for other wildlife species and may be addressed by comparison to a suitable reference population. When interpreting the results of such evaluations, it is important to consider the influence of potentially confounding factors such as sex, developmental stage, and season (Rie et al. 2000; Venturino et al. 2003). In addition, the influence of temperature on physiological processes in poikilotherms like amphibians and reptiles is an important consideration when comparing biomarkers between different populations. For example, Johnson et al. (2005) found a significant difference in acetylcholinesterase activity (a common biomarker of exposure to carbamate and organophosphorous pesticides) between 2 groups of Pacific tree frogs (*Hyla regilla*) that had been raised from tadpoles under different temperature regimes.

Basic differences in biochemical and physiological characteristics between animal groups can also affect the utility of some variables for indicating contaminant exposure. For example, induction of liver enzymes that are part of the mixed-function oxidase (MFO) system has been used to indicate exposure to a range of organic chemicals (including pesticides) in a number of vertebrates, but reduced activity of these enzymes in amphibians may limit the use of this parameter as a biomarker for this group (DeGarady and Halbrook 2003; Venturino et al. 2003).

Biomarkers have been evaluated in herpetofauna in both laboratory and field settings (e.g., Overmann and Krajicek 1995; Vogiatzis and Loumbourdis 1999; Rie et al. 2001; Keller et al. 2004a, 2004b), and may be useful for bridging studies that compare the exposure scenarios. However, a potential weakness regarding the use of these variables for risk assessment is the often poor understanding of the ecological relevance of observed responses in biomarkers. For example, Widder and Bidwell (2006, 2008) observed up to a 43% reduction in cholinesterase activity in southern leopard frogs (*Rana sphenocephala*) exposed to the organophosphate pesticide chlorpyrifos, but did not observe any effect on survival, growth, or swim speed. Studies such as those by Sparling et al. (2001) that evaluated possible links between cholinesterase enzyme activity, pesticide residue levels, and population status of anuran amphibians in the Sierra Nevada Mountains of California, and DeGarady and Halbrook (2003), which evaluated a biomarker of organic contamination along with abundance and richness of amphibian populations from sites subject to long-term PCB contamination, are important for indicating physiological and biochemical variables that may best be linked to responses at higher levels of organization. However, there remains a need for studies that more clearly demonstrate how a response to chemical exposure that originates at the subcellular level will

TABLE 4.3
Examples of Recent Biomarker Studies Conducted with Amphibians or Aquatic (Freshwater) Reptiles

General Biomarker Category	Specific Marker	Initiating Chemical Stressor(S)	Species	Reference
Histological effects	Inclusion bodies in kidney	Lead	*Rana ridibunda*	Loumbourdis 2003
Enzyme inhibition	Esterases, reductases, e.g., acetylcholinesterase	Organophosphate pesticides	*Hyla regilla*	Sparling et al. 2001
			Rana sphenocephala	Widder and Bidwell 2006, 2008
			Rana spp.	Van den Brink et al. 2003
			Nerodia rhombifer	Clark et al. 2000
			Agkistrodon piscivorus	
			Trachemys scripta	
Indicators of synthesis disruption	Intermediate metabolites or degradation products of heme synthesis, e.g., porphyrins and aminolevulinic acid dehydratase (ALAD)	Lead	*Bufo arenarum*	Arrieta et al. 2004
Xenobiotic metabolism	Mixed-function oxidases and associated enzymes	Polynuclear aromatic hydrocarbons, other organochlorine compounds	*Bufo arenarum*	Venturino et al. 2001
			Rana ridibunda	Kostaropoulos et al. 2005
				Gunderson et al. 2004
Other liver enzymes and carbohydrate stores	Aminotranferases and glycogen	Copper	*Rana ribunda*	Papadimitriou and Loumbourdis 2005
Endocrine	Effects on sex hormones — gonad morphology, reproductive hormone levels, vitellogenin levels	Various chemicals	*Xenopus laevis*	Hayes et al. 2002
			Rana pipiens	Hayes et al. 2003
	Effects on thyroid hormones — thyroxine (T4) and triiodothyronine (T3) levels		*R. nigromaculata*	Yang et al. 2005
			Chinemys reevesii	Tada et al. 2004
			Rana esculenta	Mosconi et al. 2005
			Alligator mississippiensis	Gunderson et al. 2002
Immunologic markers	Numerous, including nonspecific cytotoxic cells (NCCs) and macrophages	Low pH	*Rana pipiens*	Vatnick et al. 2006
Genetic markers	DNA strain breakage sister chromatid exchange	Various chemicals	*Rana catesbiana*	Wirz et al. 2005
			Bufo boreas	Tverdy et al. 2005

ultimately extend to the population and community, and also how environmental variables such as temperature influence these relationships.

Several studies have examined the influence of contaminants on selected suborganismal parameters in reptiles, with most focus on aquatic species (e.g., Lamb et al. 1995; Overmann and Krajicek 1995; Ulsh et al. 2000; Willingham and Crews 2000; Sanchez-Hernandez 2003; Keller et al. 2004a, 2004b; Tada et al. 2004). In their review of the ecotoxicology of metals in amphibians and reptiles, Linder and Grillitsch (2000) included a discussion of the available literature that examined biochemical effects on reptiles, and Portelli and Bishop (2000) provided a similar overview for reptiles (mostly turtles) exposed to organic contaminants. Meyers-Schöne and Walton (1994) also discussed biochemical and histopathological responses to stress in their review on the use of turtles for monitoring chemical contaminants. Due to their generally larger body size, certain aquatic to semiaquatic reptiles may be more amenable to studies of biomarkers than amphibians, since reptiles generally offer greater tissue mass and/or blood volume for analyses. There may also be a greater possibility of extracting blood or tissue samples from reptiles without having to kill the organisms, an important consideration when working with low-density populations or threatened or endangered species.

If tissue residues accumulated through bioconcentration and bioaccumulation are considered biomarkers of exposure, studies with reptiles are probably more common than those with amphibians (Table 4.2). Furthermore, aquatic species are more frequently reported in the literature when our focus resolves on tissue residue studies. Sparling (2000) stated that, when considering the taxonomic diversity of reptiles, a disproportionate amount of research had been conducted on turtles and tortoises, with much of this work focused on metals, chlorinated pesticides, and polychlorinated biphenyls (PCBs). Since that publication, there has been an increase in the number of papers that studied the effects of these contaminants (Chapter 1, this book). Reviews of tissue residue studies for both amphibians and reptiles can be found in Sparling et al. (2000 and various chapters of this book), and Meyers-Schöne and Walton (1994) have discussed residue studies with turtles. Campbell (2003) assembled, reviewed, and summarized available organic, inorganic, and radionuclide contaminant accumulation and effects studies for crocodilians and characterized data gaps in order to promote their future inclusion in environmental contamination studies and ecological risk assessments. Reptile eggs can accumulate contaminants from both maternal transfer and soil during the incubation period and may serve as indicators of chemicals that are bioavailable. For example, Pepper et al. (2004) examined the exposure of crocodiles to organochlorine (OC) pesticides using the chorioallantoic membrane (CAM) of reptile eggs. CAM is critical to embryonic development when it functions in gas exchange, nutrient transport, and waste storage for the developing embryo. As a nonlethal biomarker, CAM serves as a noninvasive indicator of exposure, since it remains with the eggshell after hatching, and has been successfully used to examine contaminant exposure and predict chemical concentrations in multiple species of birds and egg-laying reptiles (see, e.g., Pastor et al. 1996; Cobb et al. 1997). In their study, Pepper et al. (2004) found OC burdens in crocodile CAMs confirmed contamination of eggs, which suggested that exposure to females and embryos in ovo had occurred. Recent work by Unrine and Jagoe (2004), Fagotti et al. (2005), Keller et al. (2005), Gardner et al. (2006), and Tryfonas et al. (2006) further illustrates the continued interest in studying contaminant residue levels in tissues of amphibians and reptiles in both controlled laboratory experiments and field monitoring studies.

As reviewed by Hayes (2000) and Guillette (2000), amphibians and reptiles have played an important role as indicators of endocrine-disrupting chemicals in aquatic systems. Most work to date has focused on effects related to sex determination, with biomarkers including gonadal morphology and/or circulating levels of plasma hormones or specific proteins (e.g., Noriega and Hayes 2000; Shelby and Mendonca 2001; Hayes et al. 2003). Many egg-laying reptiles may be particularly suited for studies of chemicals that affect sex hormones, since they display temperature-dependent sex determination that may facilitate manipulation of sex ratios in experimental animals to more clearly evaluate chemical effects (Crews et al. 1995; Newman and Unger 2003). Recent studies have

also indicated a significant role for herpetofauna in the evaluation of chemicals that disrupt the thyroid axis, since thyroid hormones are important for initiating metamorphosis in amphibians or egg hatching in reptiles (Brasfield et al. 2004; Furlow and Neff 2006; Tata 2006).

The study of physiological energetics is another tool that deserves mention under the general heading of biomarkers. A clear advantage of studying variables related to energy balance is their direct ecological links to individual, population, and community levels of biological organization (Congdon et al. 2001). Rowe et al. (2003) discuss energetics as it relates to larval, juvenile, and adult stages of anuran amphibians and the role chemical stressors may play in increasing maintenance costs and decreasing energy available for growth. Amphibians may be particularly sensitive to factors that deplete energy reserves during metamorphosis, since feeding may cease during this time due to reorganization of the mouthparts and digestive system (Rowe et al. 2003). Reptilian eggs may also serve as valuable models to study the energetic effects of chemical stressors, since development of the embryo relies entirely on internal yolk stores. As such, this dependency likely increases exposure in ovo, since contaminants may pass across the eggshell in association with imbibed water (Moeller 2004).

4.7 RECURRING AND EMERGING ISSUES: FUTURE CHALLENGES FOR TOXICOLOGISTS STUDYING AMPHIBIANS AND REPTILES

4.7.1 Recurring Issues in the Ecotoxicology of Amphibians and Reptiles

Surrogate species are widely used in the field of ecotoxicology, particularly when the species of primary concern is threatened, endangered, or simply hard to come by. The herpetofauna are rife with members that are characterized by sparse to nonexistent ecotoxicological information, and often species are relatively poorly characterized with respect to their life histories (e.g., caecelians, amphisbaenids). As demonstrated in Chapter 1 of this book, most ecotoxicological studies have focused on 4 genera of amphibians, including *Rana*, *Bufo*, *Ambystoma*, and *Xenopus*. These data gaps present problems regarding species sensitivity to environmental chemicals and assessing threats that contaminants may pose.

Because many species of amphibians and reptiles continue to display declines in their populations, the use of surrogate species has a particularly high demand within this group. Yet, many factors must be considered when using surrogates, as the herpetofauna present outward appearances that belie the diversity of amphibians and reptiles in terms of life history strategies. For example, most ecotoxicological studies focused on amphibians rely on the aquatic stage of the biphasic life cycle that is typical of many North American species. But, amphibians include members that are strictly fossorial (e.g., caecelians), some are solely aquatic (e.g., sirens, hellbenders), and others bypass the process of metamorphosis in favor of direct development (e.g., some plethodontid salamanders). Amphibian eggs can develop in water (e.g., ranging from waters of lotic or lentic habitats to pools lying within the folds of a bromeliad leaf), under logs, and even within the vocal sacs or along the backs of parent frogs. Consequently, data collected from species exhibiting typical biphasic life cycles may not be as relevant for these alternative life history strategies, especially when long-term exposure to less than acutely lethal concentrations is considered.

Similarly, within those species presenting a biphasic life cycle, a range of life history strategies has been observed. Aquatic amphibian species differ from one another in the length of their larval period; for example, some desert-dwelling species of toad complete metamorphosis in 8 days, while bullfrogs in North American may take 3 years to go undergo the process. Similarly, neotenic salamanders display facultative metamorphosis, and depending on environmental conditions, these species oftentimes remain aquatic throughout their lives. The longer the larval period, exposures to waterborne contaminants will be increased, and even relatively resistant species may express adverse effects associated with prolonged dependencies on aquatic habitats. Other life history attributes similarly affect exposure and remain a recurring source of uncertainty when reliance on

surrogate species data is incorporated into the risk assessment process for amphibians and reptiles. For example, dietary exposures differ among herpetofauna, as exemplified by anuran larvae generally being filter feeders, while most urodeles are carnivorous. These differences in early life stages undoubtedly affect exposure and consequent effects.

Reptiles present similar challenges with respect to life history strategies, especially regarding their poorly developed reproductive toxicity data for any species. Reproductive strategies are widely divergent among the reptiles, which contributes to the problem of developing suitable surrogates for assessment purposes. Reptiles can be oviparous, viviparous, or ovoviviparous, which necessarily complicates development of test systems focused on critical life stages involving in ovo and in utero exposures, as well as maternal transfer of chemicals; for example, in viviparous species, maternal transfer may be the only route of exposure during development, but in oviparous reptiles maternal transfer may be supplemented by uptake of environmental chemicals from the surrounding soil or matrix of nest materials. As noted by Linder et al. (Chapter 5, this book) in their overview of the physiological ecology of herpetofauna, amphibians and reptiles differ markedly in their adaptations to the environment, differences that inevitably influence exposure in these animals. For example, in contrast to amphibians, reptiles have keratinous skin that tends to limit dermal uptake of water and water-soluble environmental contaminants. Life history attributes linked to reproduction also influence exposure, as exemplified by differences in potential for maternal transfer of chemicals between mother and offspring. Reptiles also tend to produce fewer offspring per reproductive effort and are longer-lived than amphibians, which are life history attributes that complicate development of standardized toxicity tests. Indeed, the diversity of the herpetofauna is no less, and likely exceeds, that of the charismatic megafauna that frequently dominate the ecological risk assessment process. As with any surrogate species, if species of amphibians and reptiles amenable to routine toxicity assessments become available in the future, caution must be applied to minimize uncertainties associated with broad generalizations developed from these select few whose life history attributes ensure their being amenable to laboratory manipulation but inevitably may mean they are far from representative. Indeed, the good of the few, or the one, may not outweigh the good of the many.

4.7.2 Emerging Chemical Contaminants

Perhaps foreshadowed by observations of endocrine-disrupting chemicals in the environment (see Hayes 2000; Guillette 2000), the past 5 to 10 years have been characterized by the growing recognition that a number of chemicals not previously considered as contaminants are present in the environment on a global scale (USGS 2006), especially in surface waters, sediments, and water treatment residuals (e.g., sewer sludge and biosolids). Future investigations will undoubtedly lead to expanded discovery of these "emerging chemical contaminants" in terrestrial and wetland habitats. These emerging contaminants include hormones, pharmaceuticals, personal care products, and other organic compounds that are frequently derived from municipal, industrial, and agricultural sources. See Kolpin et al. (2002), Barnes et al. (2002), and Chapter 15, this book, for comprehensive lists. Yet undiscovered are environmental derivatives associated with the developing nanotechnology industry and intentional, coincidental, or accidental release of these materials to the environment. Regardless of their form, the discovery of these contaminants, largely facilitated by the development of analytical techniques that allow their detection, has met with increased concern for their effects on humans and ecological receptors in the environment. Sanderson et al. (2004) characterized 4 broad classes of pharmaceuticals found in freshwater (antibiotics, antineoplastics, cardiovascular drugs, and reproductive hormones) and used quantitative structure-activity relationships to predict that nearly one-third of all drugs could be very toxic to aquatic organisms. Not surprisingly, however, empirical toxicological data for these chemicals are largely absent, although recent studies anticipate future efforts, particularly for the herpetofauna (primarily, amphibians). Smith and Burgett (2005) reported no effects on growth and variable effects on other measures of biological activity levels in *Bufo americanus* tadpoles exposed to individual

treatments of 3 common organic wastewater contaminants (acetominaphen, an antipyretic; triclosan, an antimicrobial agent; and caffeine, a stimulant). Studies with fish have indicated that wastewater contaminants with low estrogenic activity could have a combined potency that leads to observable biological effects, and that longer-term chronic tests are most appropriate to elucidate these effects (Rodgers-Gray et al. 2000; Thorpe et al. 2003; Sumpter and Johnson 2005). Needless to say, where amphibians and semiaquatic reptiles fit into the range of responses must be further characterized with test systems that provide exposure to realistic test concentrations and exposure durations.

Beyond the pharmaceuticals, other chemicals have recently emerged as contaminants of ecological concern, in part because of improved analytical techniques that have allowed for their detection at environmental concentrations. For example, perfluorooctanesulfonate (PFOS) and related perfluorinated compounds were historically used in numerous industrial and consumer products because of their capacity to repel both water and oil. While no longer manufactured in the United States, PFOS and associated compounds are increasingly being considered chemicals of concern because of their persistence and widespread distribution. In toxicity studies with northern leopard frogs, Ankley et al. (2004) observed bioaccumulation of waterborne PFOS and effects on growth and time to metamorphosis. However, based on the few studies completed, these workers posited that anurans do not appear to be exceptionally sensitive to PFOS in terms of either direct toxicity or bioconcentration potential. Before their early dismissal, however, the contaminant's comparative toxicity and its bioconcentration and bioaccumulation potential among aquatic test species must be more adequately addressed within the context of risks linked to multiple stressor exposures commonly encountered in field settings.

4.7.3 Ecotoxicology and Multiple Stressors

As Burkhart et al. (2003, p 111) observed, "Contaminants typically occur in aquatic and terrestrial environments as complex mixtures of natural and anthropogenic origin, yet the evaluation of the effects of chemical contaminants on amphibians is still primarily based on exposure to single compounds under highly controlled conditions." While chemical-by-chemical evaluation supports a relatively uncluttered understanding of individual compounds within the context of species, life stage, dose, and mode of action, it falters within an ecological context, stemming from our inability to adequately address chemical interactions, either among chemicals of the mixture or between chemicals of the mixture and other components of the environmental matrix in which it occurs. In contrast to the laboratory environment, field settings are highly dynamic and reflect exposures to numerous chemical and nonchemical stressors. There are many scenarios for potential acute, chronic, and pulsed exposures in amphibians across various life stages and in reptiles challenged by environmental chemicals across their range of habitats. Data are beginning to accumulate suggesting that the detrimental effects of aquatic contaminants on amphibians and reptiles are underestimated using the approaches commonly applied in ecotoxicology investigations (Fort et al. 1999; Mann and Bidwell 1999; Sparling et al. 2000a; Relyea 2004, 2005). For example, Johnson and Sutherland (2003) discussed the importance of considering multiple environmental stressors, including contaminants, when investigating the interrelationships between the causative agents of trematode infections that might induce limb deformities in amphibians and co-occurring chemical stressors.

Clearly, multiple stressor considerations are not unique to herpetofauna, but could influence chemical response in any ecological (or human) receptor. However, the biphasic nature of the common amphibian life cycle and the occurrence of semiaquatic reptiles at the land-water interface inevitably suggest that nonchemical stressors may be particularly important components of exposure-response scenarios, especially when spatial heterogeneity of habitat yields a gradient of exposure conditions. Physical habitat alteration such as sedimentation associated with soil erosion in areas of disturbance, and interactions between those physical stressors and environmental chemicals have frequently been identified as major factors in multiple stressor exposures (e.g.,

Linder et al. 2002). Also, temporal components have frequently been identified as major factors influencing exposure. For example, seasonal changes in wetlands may be management-critical factors in reducing or mitigating risk associated with environmental chemicals. Spring runoff and autumn dry-down will affect environmental concentrations of agrichemicals in wetlands and seasonal ponds. At least 2 synthesis publications focusing on multiple stressor effects in amphibians (Linder et al. 2003a, 2003b) highlight the importance of these issues.

4.8 SUMMARY

From the earlier overview of the ecotoxicology of amphibians and reptiles by Sparling et al. (2000), it became apparent that the available literature through 1998 was relatively sparse compared to publications focusing on wild mammals, birds, and fishes. The chemical contaminant literature available for amphibians had been haphazardly developed over the preceding 25 to 30 years and had focused on metals and chlorinated organic chemicals to yield data sufficient for speculative analyses of risks for these environmental chemicals. In contrast, much of the literature for reptiles focused on compiling tissue residue values for free-ranging animals. The past 10 to 15 years have yielded an increase in research on herpetofauna and the effects of environmental chemicals on these taxa. An updated search focused on herpetofauna as key elements in aquatic toxicology studies yielded over 700 publications since 1998 (see Chapter 1, this book), a number far outpacing annualized counts reported in that earlier publication (Sparling et al. 2000). Today, amphibians are more commonly considered in toxicity assessments, whether implemented as detailed for FETAX in ASTM E-1439 or as any of various alternatives. Still, much work remains to be initiated and completed, if the herpetofauna are going to be sufficiently represented in the discipline of ecotoxicology.

There is little argument that amphibians and reptiles are sensitive to environmental chemicals (Birge and Black 1977; Cowman and Mazanti 2000; Sparling 2000; Hayes 2000; Ouellet 2000). Fortunately, the available cross-species data from single-chemical toxicity tests do not suggest that the herpetofauna are a priori more sensitive or more resistant than any other species (McCrary and Heagler 1997), but the data available for such comparisons are not sufficient to conclude that existing "safe levels" for single chemicals are protective across the range of species and habitats in which the herpetofauna occur (see Vaal et al. 1997). At present, uncertainties relative to risks associated with exposures in aquatic, wetland, and terrestrial systems vary from adequate, for example, for simple evaluations of the toxicity of some metals, to absent for the vast majority of chemicals that herpetofauna encounter in the field. Work with reptiles still lags behind that of amphibians, especially with respect to questions focused on habitats, interactions, and life history. Their roles in exposures of reptiles to environmental chemicals remain largely undiscovered. In addition, environmental contamination studies are relatively limited and are not available for many reptiles. For example, more than half of the 23 crocodilians remain unstudied, and when these reptiles have been considered in field studies, efforts focused on accumulation and effects of mercury and endocrine-disrupting chemicals (EDCs) on American alligator in Florida (see, e.g., Guillette et al. 1994, 1995; Heaton-Jones et al. 1997). Campbell (2003) indicated that the effects of EDCs on crocodilians are not confined regionally and probably occur in many parts of the world, especially in developing tropical areas where organochlorine pesticides are more widely used. Similarly, effects associated with inorganic contaminants such as mercury and other metals are poorly characterized in these species. At present, any aquatic, sediment, or soil benchmark values are not supported by the scant empirical data that are available for these organisms.

Although long overlooked and consistently undervalued, amphibians and reptiles have recently gained increased recognition as critical components within many ecosystems. That heightened awareness among resource managers and members of the research community, however, must be matched by increased efforts to address data gaps in our existing knowledge of chemical toxicity to the wide range of species in these vertebrate groups. More importantly, the interrelationships of these animals with other ecosystem attributes and other physical and biological stressors must be characterized to

enable amphibians and reptiles to better serve as indicators of habitat quality and ecosystems at risk. Indeed, for aquatic habitats such as wetlands of various types, indigenous amphibians may be more important to evaluating system sustainability than presently appreciated. Similar observations apply to reptiles where their unique roles and habitat dependencies (e.g., deserts throughout their various categories) often outpace those of wild mammals and birds. The herpetofauna must receive increased attention in future environmental research, particularly as that relates to enhancing our understanding of their ecotoxicology and the role that long-term, low-level chemical exposures play in their future. Failure to do so may ultimately serve as a harbinger of our shared loss.

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5 Physiological Ecology of Amphibians and Reptiles

Natural History and Life History Attributes Framing Chemical Exposure in the Field

Greg Linder, Brent D. Palmer, Edward E. Little, Christopher L. Rowe, and Paula F.P. Henry

If we are what we eat, then we are fast, cheap, and easy.

— Anonomyous

CONTENTS

With publication of the first edition of *Ecotoxicology of Amphibians and Reptiles* (Sparling et al. 2000a), the environmental toxicology community was introduced to historically undervalued animal classes whose contaminant-related literature was highly diffuse and relatively sparse. In contrast to mammals and birds, throughout the short history of ecotoxicology, "one-stop" information resources for herpetofauna were few in number and frequently difficult to obtain. More frequently, these data and information resources were nonexistent. Sparling et al. (2000a, 2000b), then, achieved the goals of making the science and resource management communities aware of the role that amphibians and reptiles should play in ecotoxicology, and identified research necessary to address the shortfall in ecotoxicological information critical to resource managers working in conservation programs focused on these animals.

Now early in the 21st century, the science of ecotoxicology has entered its adolescence, and an update to that first edition is amply warranted. However, for much of the original edition's coverage, a simple update would entail additional materials that would preclude a thrifty second edition. Hence, the present chapter does not simply replace the works of Henry (2000) and Palmer (2000) on the physiology of amphibians and reptiles, respectively, but focuses on physiological traits of amphibians and reptiles that place constraints on fitness when exposed to contaminants in the field. In short, our primary focus resolves on the physiological ecology of amphibians and reptiles, particularly with reference to the interactions between animals and their environment as they affect exposure. Whereas our primary focus resolves on the nexus of physiological ecology and ecotoxicology of the herpetofauna, it would have been presumptuous of us had we thought that characterizing a fully developed state of the science was easily attainable in our single chapter, particularly given the scant literature available for the herpetofauna. Rather, we present a few examples of how studies regarding the ecotoxicology of the herpetofauna could benefit from the mind-set of a physiological

ecologist when characterizing exposure, and we look toward traditional physiological literature to shape the context of our interpretations. Indeed, Henry (2000), Palmer (2000), Feder and Burggren (1992), Duellman and Trueb (1994), and the classic references of Noble (1931) and Gans (Gans and Pough 1982a, 1982b; see Dudley et al. 2006) should not be off-loaded to secondhand book stores or lost to the obscure bowels of reference libraries. And, references such as McNabb (2002), Prosser (1991a, 1991b and earlier editions), Schmidt-Nielson (1997 and earlier editions), and Kasarov and Martínez del Rio (2007) should become common companions to these contemporary classics in the libraries of current and future generations of ecotoxicologists. Indeed, these references remain vital source materials for research ecotoxicologists and the wide spectrum of biologists studying amphibians and reptiles.

5.1 PHYSIOLOGICAL ECOLOGY AND EXPOSURE TO CHEMICALS IN THE ENVIRONMENT

Simply characterized, physiological ecology concerns the biophysical, biochemical, and physiological traits that have evolved in response to chemical and physical factors in the environment. In concert with an individual's behavioral and morphological adaptations, these processes mediate spatiotemporal interactions with other organisms in shared habitat. Frequently, an energetics-based approach is applied to the study of physiological ecology, which potentially affords the ecotoxicologist a wide variety of tools for analysis of exposure and effects linked to chemical stressors. Energetics concerns the flow of energy and material within a system at any level of biological organization — molecular, cellular, organismal, population, community, or ecosystem. Applying an energetics-based framework for evaluating exposure and effects enables the use of a "common currency" readily applicable to evaluations of multiple stressors and capable of easing the analysis of chemical stressor effects within and among levels of organization. This framework potentially reduces uncertainty associated with extrapolations traditionally made from individual to population levels of organization, and further to landscapes and ecosystems.

As suggested by Linder, Lehman, and Bidwell (Chapter 4, this volume), physiological ecology, and more specifically physiological energetics, deserves greater attention in the evaluation of exposure and effects of chemical stressors in amphibians and reptiles. Congdon et al. (2001) and Rowe et al. (2003) clearly identified that a physiological energetics framework provides a foundation for evaluating chemical stressors, since the focus on variables related to energy flow directly links ecological attributes of individuals, populations, and communities. Depending on the level of biological organization and the spatiotemporal setting for the analysis, opting for a physiological ecologist's view of energy and material flows provides for rigorous evaluation of the effects of multiple stressors on amphibians and reptiles. For example, Rowe et al. (2003) discussed physiological energetics as it relates to larval, juvenile, and adult stages of amphibians and the influences of chemical stressors on maintenance costs and, subsequently, growth. Similarly, in reptiles early developmental stages may serve as valuable models to study energetic effects associated with exposures to chemical stressors (e.g., embryos may be exposed to chemicals due to maternal transfer during vitellogenesis and from uptake of water from contaminated soils in the nest; Moeller 2004).

Nutritional, behavioral, and energetic interactions influence exposure to environmental chemicals and may dramatically affect risks to wildlife. Behavioral interactions influencing exposure were observed early in ecotoxicological investigations (e.g., Steele et al. 1989; Strickler-Shaw and Taylor 1990, 1991; Taylor et al. 1990; Steele et al. 1991 on amphibians), but nutritional and energetic interactions that modify exposure remain relatively poorly characterized in most ecotoxicological contexts, as evidenced by these topics being absent or practically so in references such as Hoffman et al. (2003), Newman and Unger (2003), Newman and Clements (2007), Schüürmann and Markert (1997), and Walker (2001). For discussions focused on amphibians and reptiles, the technical aspects of these interactions are even more scant than for other vertebrates. Indeed, few

studies have characterized their roles in influencing exposure and mediating biological effects that may dramatically affect the risk to herpetofauna. The ecological consequences of these biological processes may be associated with responses that are as significant as toxicity linked directly to chemical exposure. Our understanding of exposures linked to whole animal interactions with environmental substrates (e.g., dermal uptake of chemicals from soils) is also limited, although an increasing number of studies are addressing chronic exposures in sediments, soils, and other matrices (James 2003; James et al. 2004a, 2004b). Routine consideration of nutritional, behavioral, and energetic processes would provide a biological and ecological context to ecological risk assessment; however, these factors have often been neglected in the analysis and interpretation of chemical risks to wildlife, especially amphibians and reptiles.

5.1.1 Brief Overview of Physiological Energetics

The flow of energy and material in an animal is illustrated in Figure 5.1. The dominant practice today for evaluating hazards and risks focuses on materials, or more specifically chemicals within and transfers between a system's compartments. Food consumption is routinely measured or estimated in the traditional exposure model for terrestrial and semiaquatic vertebrates. Yet, the evaluation process could easily be extended in research studies undertaken beyond a screening level application. Combined with measures of tissue residues and material inputs, characterization of intake energy (IE) would provide the basis for more comprehensive studies of energy allocation and material partitioning among "loss compartments" (urinary and fecal production) and "storage compartments" (tissues). At present, however, little thought is given to energy-materials interrelationships or differential expenditure of energy when challenged by multiple factors, be they chemical, physical, or biological.

While the process summarized in Figure 5.1 and detailed elsewhere tracks energy and materials (for example, see Karasov and Martínez del Rio 2007), the compartments characteristic of the biological process are conveniently identified by their various forms of "energy," since the physiological ecologist's focus often resides in organismal "currency" (e.g., kilocalories) and its relationship to materials. If we follow conventional leads and focus on food chain as our tool to evaluate exposure in wildlife, the role of energy flow in material transfers becomes an integral component of the analysis. Here, IE (generally measured in calories or joules determined by complete oxidation of

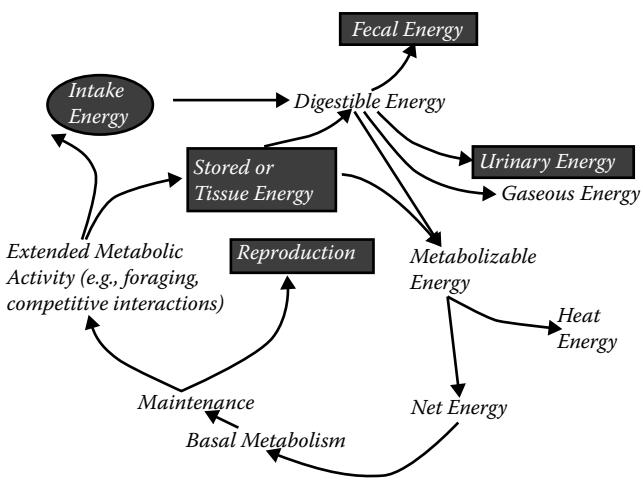

FIGURE 5.1 The conceptual model of energy and material flow provides a physiological energetics framework for evaluating exposure with traditional food chain models.

material in a bomb calorimeter) represents the total energy in materials consumed (e.g., forage items plus substrates such as soil and sediment). Most often, this would be estimated as a function of gross energy (energy per unit mass) and foraging rate (mass consumed per unit time). Not surprisingly, gross energy and nutritional content of feed materials vary widely from season to season and from year to year; hence, IE will likely vary as well. Additionally, thermodynamic theory dictates that individuals cannot be 100% efficient in material and energy transfer and utilization. Therefore, the amount of food and energy not used by the animal must be determined, if a realistic accounting of food constituents — be they nonnutritive constituents, nutrients, or chemical toxicants — is desired. Fecal energy (FE) characterizes the material and energy content of feces that can be measured in samples collected in field or controlled laboratory feeding studies. As such, digestible energy (DE) is relatively straightforward in its derivation, being the difference between ingested energy and fecal energy (IE – FE). This estimate is more accurately referred to as "apparent digestible energy," since contributions to fecal energy are derived from multiple sources, including the microbial flora of the gut, intestinal secretions from the gastrointestinal tract, and sloughed intestinal lining. Determination of true DE will likely remain a research question of nutritional ecologists, yet opting for a physiological ecologist's mind-set seems a preferred basis for research efforts targeted on improving the ecological risk assessment process.

Beyond a simple food chain exposure, other sources of energy and material "loss" must also be accounted for to comprehensively assess effects of stressors on an energetics basis. For example, gaseous products of digestion (gaseous energy [GE]) resulting from microbial activity in the large bowel (and, depending on species, their associated diverticula) may be critical to a complete accounting of energy, particularly if gastrointestinal function is compromised consequent to exposure. Although limited in their contribution to material and energy balance focused on many chemicals of concern, the energy and materials in these gases are unavailable to the animal, as is energy associated with elimination of nitrogenous waste (e.g., ammonia, uric acid, and urea in amphibians and reptiles, depending on species), or urinary energy (UE). Both UE and FE have associated material loss critical to the analysis of a chemical's disposition in food chain exposures.

As a consequence of these losses, DE can be further refined and called metabolizable energy (ME), which is

$$ME = IE - (FE + GE + UE) \tag{5.1}$$

Foods associated with ME yield energy available for metabolism, although part of the energy is inevitably lost to heat production or heat energy (HE). Physiologically, even in ecothermic vertebrates, heat is produced during basal metabolism, digestion, fermentation by enteric microbes, formation of waste products, and other biological processes. Although an animal's thermoregulatory activities, endothermic, ectothermic, homeothermic, or poikilothermic, are critical to its survival and long-term success as a species, energy lost as heat is not available for processes such as growth, reproduction, foraging, and other metabolicactivities. As such, the net energy (NE) of a biological system is ME less HE, with energy and materials potentially allocated to physiological and biochemical functions such as basal metabolism, maintenance, growth, reproduction, stored and tissue energy production, and detoxification). These physiological and biochemical functions are responsive to various environmental stressors, including chemicals presenting ecological risk. As suggested by Figure 5.1, the flow of material and energy characteristic of a given parcel of food is relatively easy to summarize conceptually. Food chain analyses can be accomplished beyond that captured by simple screening level. The allocation of material and energy can be further quantified within an exposure assessment focused on food chain analysis (e.g. measuring food intake, urinary and fecal outputs, and reproductive parameters) relative to tissue residues and observed adverse effects.

However, routes of exposure critical for amphibians and reptiles are not adequately characterized by simple food chain analyses. From an ecological and biological perspective, once a screening

level analysis of exposure is completed, exposure should no longer simply be a question of knowing a chemical's concentration in various substrates, foraging items (feed or coincidentally ingested soil or sediment), and a foraging rate. Rather than a regulatory toxicological perspective, an ecological perspective seems more appropriate to the evaluation of chemical risks once the screening level analysis is complete, especially for animals such as reptiles and amphibians whose life histories include critical life stages that are incompletely modeled by a simple food chain analysis.

5.1.2 Physiological Ecology, Exposure Models, and Food Chains

Despite their veneer of arithmetic sophistication (see Pilkey and Pilkey-Jarvis 2007 for general discussion of models applied to environmental science), exposure models (Equation 5.2) for terrestrial and semiaquatic vertebrates that focus solely on materials transfer are relatively primitive for reptiles, and even more so for amphibians.

$$E = \frac{1}{T}\sum C_{ijk}\, t_k \tag{5.2}$$

where

E = exposure concentration or exposed dose,
T = total time and space over which the concentrations in various microenvironments or habitats are to be averaged,
C_{ijk} = concentration in microenvironment k that is linked to environmental matrix i by pathway j, and
t_k = time and space that accounts for a receptor's contact with specific microenvironment or habitat k.

Most often, Equation 5.2 is decomposed and simplified, and as a unitless narrative equation yields

$$ED = \sum Der_{ed} + Inh_{ed} + f(Inh_{ed}) + Ing_{ed} + f(Ing_{ed}) + DW_{ed} + f(DW_{ed}) \tag{5.3}$$

where

ED = exposed dose,
Der_{ed} = dermal or cutaneous exposed dose,
Inh_{ed} = inhalation exposed dose,
$f(Inh_{ed})$ = exposed dose coincidental to inhalation,
Ing_{ed} = ingestion exposed dose,
$f(Ing_{ed})$ = exposed dose coincidental to ingestion,
DW_{ed} = drinking water consumption exposed dose, and
$f(DW_{ed})$ = exposed dose coincidental to drinking water consumption.

Ultimately, given practical matters and all too frequently, the relatively sparse to nonexistent data available, risk analysts further simplify, which yields a food-chain-dominated exposure model:

$$ED = \sum Ing_{ed} + f(Ing_{ed}) + DW_{ed} \tag{5.4}$$

where exposed dose contributed by dermal and inhalation routes is considered negligible to the derivation of exposed dose (USEPA 2003, 2005). Subsequently, the fraction of exposed dose that is absorbed is frequently assumed to be 100% or estimated using available literature values to account for efficiencies in uptake of material across surfaces such as the walls of the alimentary canal or other serosal-mucosal barriers between external and internal environments or across cell membranes. Beyond screening level evaluations, these estimates are most often derived in the absence of energy and nutritional considerations for foods in their diet, and the variability linked to, for

example, seasonal patterns characteristic of an animal's dietary intake (Robbins 1993; Barboza et al. 2009). Rather, food chain models consider "whole animal" functions of water consumption, foraging rate, food intake, and food processing through lumped exposure parameters (e.g., site use factors, assimilation values). In short, you are what you eat and drink.

Within an ecological risk context, food chain modeling has become a primary tool for evaluating exposure in terrestrial and semiaquatic wildlife (USEPA 2003, 2005). Yet depending on the animal at risk and the life history stages most sensitive to exposure (often the early developmental stages), the tool may be relatively ineffective in evaluating potential adverse effects and risks linked to exposures in field settings. For example, discounting dermal exposures and percutaneous uptake to nil is highly problematic, given the natural history and life history characteristics of members of the Amphibia (e.g., their semipermeable skin plays a critical role in respiration, such as cutaneous respiration in the lungless plethodontids, and uptake of materials from the environment and may be a critical route of exposure for some environmental contaminants).

5.2 PATHWAYS OF CONTAMINANT EXPOSURE FOR AMPHIBIANS AND REPTILES

If we adopt a physiological ecologist's frame of reference in the analysis of exposure and effects, we might appreciate more the value of energetic costs associated with chemical exposure. For example, a more complete accounting of time and energy expended on preying or foraging for food potentially contaminated with environmental chemicals would be gained, and effects linked to reduced nutritive value and altered energetic costs could be characterized. Chemically contaminated food resources may have increased metabolic costs associated with their disposition, for example, detoxification and elimination, or transport to storage compartments. The variety of physiological, morphological, and behavioral adaptations characteristic of biota potentially exposed to environmental chemicals precludes a comprehensive accounting of energetic costs associated with contaminant exposures. Indeed, as Rowe et al. (2003) observed, biological diversity is realized in part because a wide variety of adaptive strategies have evolved toward optimization of reproductive fitness. When biological, ecological, and abiotic conditions in the environment diverge significantly from optimal fitness, population level changes may result. In the following sections, our overview of physiological systems key to exposure will be based on the exposure equation (Equation 5.3) that reflects field settings commonly encountered by amphibians and reptiles. While this field-based exposure equation moves beyond a simple food chain model of exposure and is characterized by sparse data sufficient for analysis, uncertainties in evaluation of ecological risks will only be reduced when life history strategies and an animal's natural history are considered in total. Inconvenience should not foster complacency in the risk assessment community. Rather, developing models based upon numerous life history traits can aid in identifying research that will benefit the screening level risk assessment process, while enhancing our understanding of the herpetofauna and their adaptions to the environment.

5.3 PHYSIOLOGICAL ECOLOGY OF AMPHIBIANS AND REPTILES: NATURAL HISTORY AND LIFE HISTORY ATTRIBUTES INFLUENCING EXPOSURE

Exposure of herpetofauna occupying aquatic and terrestrial habitats is no less significant than that for other wildlife. As McDiarmid and Mitchell (2000) observed, an animal's daily, seasonal, and annual movements undoubtedly affect exposure in the herpetofauna, given the relatively long distances some species move. Indeed, exposures may be as complex as any envisioned for other species at risk, and for many herpetofauna, these movements may encompass a wide range of

aquatic, wetland, and terrestrial habitats in their sojourns across the landscape. Movement across heterogeneous habitats will likely be overlain with a similarly heterogeneous range of types and concentrations of environmental chemicals and other stressors, which will complicate forecasting models. For example, in typical exposure models bioaccumulation is often dominated by dietary exposures (food and water consumption) in terrestrial vertebrates, yet chemicals occurring in the exposure matrix may be readily absorbed across dermal epithelia or volatilized from a solid or liquid phase into air, providing other means of exposure (see, for example, Noble 1931; Boutilier et al. 1992; Shoemaker et al. 1992; Duellman and Trueb 1994; and Stebbins and Cohen 1995 regarding cutaneous respiratory surfaces, which may serve as potential routes of uptake). Many variables affect the magnitude of bioaccumulation in terrestrial exposures in adults or early developmental stages (e.g., in reptile eggs). While the transfer of chemicals within food chains may conveniently be described by transfer coefficients or functions that characterize the relationships among trophic levels (Pastorok et al. 1996; Pascoe et al. 1996; Linder et al. 1998, Linder and Joermann 1999), we have only a limited understanding of the interrelationships among other exposure matrices and critical life stages in the ontogeny of amphibians and reptiles. These environmental factors may be abiotic, such as physicochemical characteristics of chemical or exposure matrix (sediment or soil), or biological in character, such as life-history-dependent attributes related to gastrointestinal or nutritional physiology, foraging, or food preference (see Larsen 1992; Hamelink et al. 1994; Langston and Spence 1995; Linder et al. 2002). Needless to say, beyond the convenience of screening level exposure models, the perspective of a physiological ecologist would undoubtedly contribute to developing improved models by investing research into the basic physiology of the herpetofauna.

The following sections will focus on the physiology of amphibians and reptiles that bear directly on exposure models such as Equations 5.3 and 5.4 that are called on by risk analysts as part of their evaluation of ecological risks.

5.3.1 Dietary Exposures, Gastrointestinal, and Digestive Physiology

The significance of gastrointestinal and digestive physiology in the evaluation of dietary exposures to environmental chemicals has received little, if any, study by ecotoxicologists working with herpetofauna. Physiologists of various persuasions, however, have devoted much effort toward characterizing the anatomical, morphological, and physiological responses of the vertebrate gut to a wide range of food sources and environmental stressors (see, e.g., Secor 2005a, 2005b). Indeed, researchers and regulators would benefit from an increased knowledge of these organismal responses to the range of stressors that inevitably affect responses of herpetofauna to environmental chemicals in the field.

For example, Secor (2005b, p 282) observed that "vertebrate intestinal tracts possess an array of structural and functional adaptations to the wide diversity of food and feeding habits," which inevitably begs the question of what role this variability plays in evaluating dietary exposures to environmental chemicals in herpetofauna. Furthermore, anatomical, morphological, and physiological differences associated with diet (e.g., differences between alimentary canals of herbivores and carnivores), and the adaptive plasticity of the gut clearly point to sources of uncertainty that currently are not captured by regulatory applications common to the risk assessment process. The capacity to which intestinal performance responds to changes in digestive demands is a product of evolutionary and cellular mechanisms (Secor 2005b). Inevitably, exposure of herpetofauna to chemical stressors in the field cannot help but be complicated by feedbacks that control function and structure of the gut in unchallenged systems. The issues associated with this dietary route of exposure become even more prominent when static "snapshots" focused on threshold values, such as toxicity reference values (TRVs), are applied to the ecological risk assessment process. While consensus TRVs for amphibians and reptiles are lacking, the paucity of data encourages that their future development be by design rather than limited by the convenience of existing data. Indeed, life history attributes of

the herpetofauna that influence exposure must not be undervalued in developing threshold values. The current lack of TRVs for amphibians and reptiles gives us time to develop regulatory benchmarks that are ecological in nature and more adequately estimate risks to herpetofauna.

Uptake of environmental chemicals by herpetofauna resulting directly from dietary exposures may be simply viewed as suggested in exposure models currently in vogue. Yet, beyond a screening level analysis, the simple exposure model exemplified in Equation 5.3 or 5.4 undervalues our current understanding of some dietary constituents such as metals and some organics that have nutritive roles, or for those whose uptake is mediated by gastrointestinal processes characteristic of the herpetofauna. For example, the regulation of intestinal performance (i.e., the total small intestinal capacity to absorb nutrients estimated as a product of small intestinal mass and mass-specific rates of nutrient uptake [Secor 2005a]) varies with type of food consumed and level of feeding activity. From a physiological ecologist's perspective, intestinal performance may be conveniently categorized, based in part on life history attributes that capture the range of exposures potentially associated with amphibians and reptiles. It is not a "fits-all-sizes" world when dietary routes of exposure are considered for the herpetofauna, particularly within the context of exposure to chemical stressors in the field. For example, "sit-and-wait foragers" (e.g., common to some snakes) rely on ingesting a single, frequently large meal followed by an extended nonforaging, resting state. This life history strategy is also represented by other estivating or hibernating herpetofauna, which experience long episodes of fasting accompanied by downregulation of intestinal morphology and function. Not surprisingly, fasting reduces energy expenditure during these extended periods. In contrast to these sit-and-wait foragers, "frequently feeding foragers" regulate intestinal performance by alternately fasting and feeding to earn energy savings that offset costs associated with upregulating the gut during feeding episodes. In the herpetofauna the regulation of intestinal performance varies widely, in part as a function of the degree to which mass-specific rates of nutrient transport are depressed due to loss of intestinal mass during fasts common to these vertebrates.

While models focused on dietary routes of exposure may be simple implementations of food chain analysis, these analyses for wildlife do little beyond providing a simple, screening level effort potentially disconnected from reality. Simply stated, the physiology of the intestinal tract drives the relationship between consumption and assimilation. The diversity of vertebrate food and feeding habits is matched by an array of adaptive intestinal morphologies and physiologies (Stevens and Hume 1995; Karasov and Hume 1997), which potentially could be incorporated into refined exposure models. For example, compared to carnivores, the intestinal tracts of herbivores are longer, more complex, and generally include fermentation chambers (e.g., intestinal diverticula). The longer herbivore gut is necessary because plant material is more difficult to digest than animal material (Stevens and Hume 1995). Physiologically and biochemically, intestinal tracts of herbivores hydrolyze and transport simple sugars at greater rates relative to amino acids. In contrast, the intestinal tracts of carnivores generally favor transport of amino acids (Karasov and Diamond 1988; Stevens and Hume 1995). Differences in substrate breakdown and transport between herbivores and carnivores need to be considered when developing more comprehensive and realistic models of exposure, particularly in relationship to the effects and the metabolic fate of the chemical once ingested.

Among the many species of vertebrate, the gut — physiologically and morphologically — is highly malleable and adaptive to changes in digestive demand. Environmental factors such as seasonal changes in feeding regimens (primarily, changes in diet or in feeding behaviors) influence these physiological and morphological changes and their associated changes in energy requirements and ingested nutrients (Piersma and Lindström 1997). The most frequently noted responses to changes in digestive demand are morphological, for example, increased or decreased intestinal mass (Karasov and Diamond 1983) and physiological, as exemplified by altered modulation of intestinal function (e.g., changes in enzyme activity and nutrient dynamics; see Karasov and Hume 1997). Not surprisingly, much of the work characterizing gastrointestinal responses to environmental stressors and changes in nutritional physiology and ecology was initially completed in birds

and mammals, but regulation of intestinal performance among amphibians and reptiles has gained increased research interest and has been recently reviewed (see, e.g., Secor 2005a, 2005b).

The gastrointestinal (GI) tract, like other major organ systems, can experience a wide range of demands. However, unlike renal, pulmonary, or cardiovascular systems, which must provide at least a minimum level of performance under nominal environmental conditions, GI tracts of many organisms are routinely quiescent during periods in an animal's lifespan. The duration of quiescence will vary with species, ranging from little, if any (e.g., common to endotherms classically considered as having high metabolic demands), to extended fasts with associated gut quiescence ranging from a few hours (e.g., common to organisms having daily feeding patterns) to several months (e.g., commonly associated with periods of hibernation, estivation). As a shared attribute throughout the vertebrates, feeding or foraging strategies of the herpetofauna vary widely, depending on species and habitat. In some vertebrates, extended, nearly yearlong fasts may be a common species attribute. For example, female rattlesnakes (*Crotalus viridis*) may not feed during breeding and perinatal periods, which means individuals will fast for 2 hibernating cycles and the intervening summer (Macartney and Gregory 1988).

Amphibians and reptiles that feed frequently generally possess a relatively limited range in digestive performance with neither feeding nor fasting occurring as dominant features in their natural history. At the other end of the spectrum, those animals having life histories characterized by long episodes of fasting tend to regulate digestive performance much more widely with feeding and fasting. As Secor (2001) observed, these bounding patterns of digestive regulation are primarily distinguished by the ability to "upregulate" or "downregulate" digestive performance, depending on species characteristics and food availability. Downregulation of digestive performance in ecotherms such as amphibians and reptiles is linked with fasting that commonly occurs as aphagia when the gut is quiescent (Gregory 1982; Pinder et al. 1992). During these fasts, animals depend on stored energy to meet metabolic needs, and adaptive responses reduce energy expenditures to ensure survival (see Secor 2005a, 2005b). Depending on species, differential capacity to regulate digestive performance will be linked to time-dependent factors that influence the gut's response, such as duration of the fast, which inevitably influences chemical exposure through the diet. The wide range of life histories characteristic of amphibians and reptiles captures a similarly wide range of feeding habits and digestive performances. Secor (2005a, 2005b) noted that a wide regulation of intestinal performance is exhibited within the herpetofauna, where fasts may occur for months and yield 5- to 30-fold changes in nutrient uptake capacity. Frequently feeding species having limited fasting periods vary with respect to nutrient uptake capacity; many display only slight increases, while others (e.g., *Ambystoma tigrinum* larva, *Bufo speciosus*, and *Rana pipiens*) present significant increases in nutrient uptake capacity with feeding. Even a cursory review of Stevens (1988) or Stevens and Hume (1995, 2004) clearly suggests potential research opportunities for physiological ecologists who expand their research horizons to include ecotoxicological issues related to exposure modeling.

The vertebrate gut, then, potentially displays variability in its morphology and physiology as a function of digestive demand, and in its function within the context of natural history strategy (e.g., feeding habits and food preferences). The capacity to regulate digestive performance in response to variation in digestive demand is a common adaptation associated with a range of adaptive strategies, and generally reflects a product of changes in size, type, or an interaction of meal size and type. Developmental changes (e.g., larvae to adult) are also commonly observed pressures that influence the function and structure of the vertebrate gut, which necessarily affects exposure to environmental chemicals as constituents in the diet. Adaptive strategies seen across the spectrum of vertebrate life histories may be shaped relatively little in those species displaying little variability in meal quality or quantity, but in those species having a widely varying diet, digestive capacity may be similarly variable, which dramatically influences adaptive responses observed in gut structure and function.

Digestion and nutrient uptake involves components of the gut working in concert with other visceral organs in an integrative response initiated by the feeding event, an event that has become a dominant focus in contaminant exposure modeling. An integrative approach incorporating chemical

stressor exposure and uptake of nutrients could not help but improve our understanding relationships between effects and "exposed dose–chemical assimilation–absorbed dose." Interactions potentially in play for the regulation of tissue performance vary with digestive demands, which likely means the greatest metabolic and functional demands on an organism occur during digestion or during other highly integrated functions, such as reproduction. Hence, maximum digestive demand may set the upper limits to the performance of supportive physiological systems considered next.

5.3.2 Thermoregulatory, Osmoregulatory, and Excretory Physiology

Within the context of exposures in the field, an initial consideration of whole animal responses to environmental factors focused on temperature and water is critical to any stressor evaluation. Physiologically, amphibians and reptiles are similar, yet sufficiently different to warrant a brief overview of their physiological attributes related to interrelationships in functions managing temperature and water homeostasis.

Amphibians and reptiles are ectothermic; hence, ambient temperatures affect molecular and cellular processes (e.g., enzyme activities and protein synthesis), which are commonly observed as integrated organ system responses in the animal, such as digestion, and whole-animal regulatory functions related to sensory and behavioral interactions between organism and the environment (Wieser 1973). In amphibians, for example, temperature coupled with humidity will influence reproductive activities such as emergence (Bellis 1962; Cree 1989), vocalization, egg deposition (Blair 1961), embryonic development (Herreid and Kinney 1967), growth (Bellis 1962; Berven et al. 1979), metamorphosis, and the immune response (Maniero and Carey 1997; Jozkowicz and Plytyez 1998; Taylor 1998). For amphibians, temperature and moisture affect physiological and behavioral responses to contaminant exposure, particularly given these factors' roles in up- or downregulation of metabolic processes that influence chemical uptake, metabolite production, and clearance from the system. Within species and among populations, thermal limits vary geographically, seasonally, and diurnally. An individual's previous experience with temperature extremes will also influence exposure (Berven 1982). Thermal tolerance and preferences can be altered by exposure to chemicals such as organophosphorus compounds (Johnson 1976; Johnson and Prine 1976) and other chemicals that could interfere with endocrine and neuroendocrine regulation (Hutchinson and Dupre 1992).

Many amphibian life history attributes and environmental cues keyed to homeostatic adaptations and attributed to temperature and thermoregulation are ultimately linked to osmoregulation and water conservation or repiratory gas exchange (Bellis 1962). Amphibians generally tolerate temperatures below their preferred temperature better than above their preferred range of temperatures. Temperatures greater than their preferred upper limit are linked to excessive water loss and are accompanied by increased risks of desiccation. Short-term responses to increased temperature depend on the duration of the thermal or osmotic stress, and may elicit integrated responses to that challenge. For example, amphibians may respond physiologically and modify behaviors in response to temperature challenge (Londos and Brooks 1990; Rome et al. 1992). Behavioral changes include basking, adjusting their posture to minimize or maximize surface-to-volume ratio for both thermal and water regulation, and aggregating to areas in which temperatures may be cooler, more stable, and less affected by solar radiation fluctuations (e.g., under forest substratum or at the bottom of ponds). Prolonged physiological adjustments include developmental adaptations, modifications in ventilation, metabolism, glomerular filtration, and hormonal feedback (Kim et al. 1998). During seasonal or otherwise prolonged changes in environmental temperature and humidity, amphibians can become dormant and enter hibernation or estivation. In the dormant animal, nonessential behaviors are reduced and metabolism is lowered to minimize depletion of energy stores and maximize survival.

Hibernation and estivation are adaptations to environmental stressors such as temperature extremes or reduced food resources. If resources are not sufficient to maintain their lower bounds of metabolic activity required for day-to-day activities, attendant outcomes are starvation and energy

depletion (e.g., Scott 1994). Hibernation is a common response to the cold winter of temperate climates and continues to be a research topic of keen interest to physiological ecologists. Indeed, hibernation represents a topic that could warrant review by ecotoxicologists, since the interrelationships between temperature and reduced water resources would likely influence exposures to chemical stressors in field settings. Temperate amphibians, ranging from those dominantly aquatic in their habitat requirements to those strongly terrestrial in their preferred habitats, overwinter in hibernacula that range from below-frost-line burrows in soil to shallow digs in near-surface materials (e.g., forest duff) to sediments in aquatic habitats. For example, many temperate frogs overwinter buried to varying depths in surface litter and soils, where the animals are likely to be exposed to dehydrating conditions and subzero temperatures, which they can survive by virtue of their profound tolerances to dehydration (Hillyard 1999) and freezing (Schmid 1982). Wood frogs (*Rana sylvatica*) range further north than other anurans, and recover from severe dehydration (Churchill and Storey 1993) and survive the freezing of up to 70% of their body water at temperatures between –4 and –6 °C (Storey and Storey 2004). Various molecular, biochemical, and physiological adaptations provide freeze-thaw tolerance of tissues, since preventing deep freeze and internal fluid crystallization is critical for survival (see Tattersall and Ultsch 2008).

Estivation occurs during prolonged heat or drought conditions. Desert spadefoot toads (*Scaphiopus couchii* and *S. hammondii*), for example, inhabit areas in which it may not rain for up to 1 year. As in hibernation, gluconeogenesis is initiated prior to estivation to accumulate energy reserves, then metabolism is reduced, although aerobic respiration is maintained. If the animal becomes too dehydrated, ventilation rate is lowered, increasing the risk that toxic levels of CO_2 will accumulate in the blood (Pinder et al. 1992). Examples of estivating behaviors to decrease water loss include burrowing further into moist, cool soils and forming cocoons. Cocoons can be formed by wrapping a layer of mud obtained from the bottom of a pond or by shedding dead epidermal layers of squamous epithelial cells to completely encase the estivating individual. The oral cavity is left open and pulmonary gas exchange predominates, effectively reducing water loss by 90 to 95%. Conserving water is the prominent determinant contributing to onset of hibernation or estivation. Again, depending on the habitats occupied during estivation, environmental chemicals may contribute to the multiple stressor exposure experienced by the animal.

In amphibians, most water, ion, and gas exchange occurs through the skin; however, dermal uptake and osmotic regulation requirements differ between aquatic and terrestrial systems. Hence, dermal exposure and consequently response to chemicals may be different (Cree 1989). For example, larval epidermis in amphibians is ciliated and composed of several cell layers overlying a thin basement membrane (Burggren and Just 1992; Duellman and Trueb 1994). Throughout development, the dermis and epidermis are restructured, thickened, or keratinized; dermal glands are developed and pigmentation is changed. Adjustments in skin permeability are under neural and hormonal control (Reboreda et al. 1991). Whereas drought conditions increase water uptake and could place amphibians at a higher risk to waterborne chemicals, adaptations such as cocoons may protect individuals temporarily from direct exposure to chemicals in soil, water, or air (Stiffler 1988). Some amphibians may be at greater risk of chemical exposure during the early breeding season. Osmotic permeability fluctuates seasonally, accounting for the spring water drive during which terrestrial amphibians migrate to the breeding ponds (Duvall and Norris 1980; Boutilier et al. 1992). Plethodontid adults that rely more than 90% on transepithelial respiration may also be at higher risk of exposure.

Water imbition occurs through percutaneous routes in amphibians and is primarily mediated through epithelia that have a long history of studies focused on ion transport and osmotic water uptake from the organism's environs (Jørgensen 1997; Larsen 1991). Uptake of water and ionic solutes is followed by internal processing, with final disposition occurring, in part, through the excretory system. Amphibians are relatively intolerant of salt challenge; hence, most amphibians are limited to freshwater environments. To maintain homeostasis, Na^+ ions and K^+ are transported into the intestines, where Cl^- efflux is coupled to Na^+ influx. Na-K ATPase is present throughout extracellular compartments. As with many cellular processes, changes in environmental pH affect ion

transport (e.g., acidic pH inhibits Na^+ uptake and jeopardizes the integrity of the outer membrane's tight junctions, yielding intracellular ionic depletion; Freda and Dunson 1984; Freda 1986). Ionic regulatory systems involving Na^+ and acid-base coupling also are active in the amphibian renal and bladder systems (Rohrbach and Stiffler 1987; Stiffler 1988; Toews and Stiffler 1989). Despite slight differences in the rate of glomerular filtration between salamanders and anurans, the kidneys of both groups produce large volumes of dilute urine when the animal is in freshwater. When water is unavailable, filtration rates drop and no urine is produced; hence, water is conserved. The bladder and lymph sacs continue to resorb and store water during dehydration, but the tight epithelium becomes selectively permeable to ions and water, so that urine formation and glomerular filtration can be controlled (Tufts and Toews 1985). Frogs can excrete urate salts to further rid the dehydrated system of excessive Na^+ and K^+. Depending on environmental conditions, amphibians have proven adaptive to a variety of dehydrating habitats. For example, spadefoot toads store urea in plasma and muscle as a mechanism to offset hydrostatic pressure, and amphibians living in brackish waters (e.g., *Rana cancrivora* and *Bufo marinus*) can retain protein by-products (e.g., urea and amino acids) to maintain osmotic equilibrium.

Unlike amphibians, many reptiles maintain body temperatures as high as or higher than those of endotherms (birds and mammals). However, in contrast to birds and mammals that rely on metabolic heat to maintain homeothermy, reptiles generally rely on external heat sources. While some sea turtles and pythons generate significant metabolic heat, most reptiles are poorly insulated by prominent layers of subcutaneous fat (except for leatherback sea turtles [*Dermochelys coriacea*]), fur, or feathers. Without insulation, metabolic heat is quickly lost. As do poikilotherms in general, reptiles characteristically display body temperatures that fluctuate rather than being controlled by physiological means. Yet, reptiles do exhibit a range of thermoregulatory ability, and control of heat gain and loss from external sources and maintenance of body temperature are achieved primarily through behavioral mechansisms. Thermoconformers regulate their body temperature to a limited extent, and generally track environmental temperatures in the classic definition of a poikilotherm. Such species generally live in relatively invariant thermal environments such as those living in water, caves, or burrows or under thick forest canopy. On the other hand, thermoregulators control their temperature very precisely — they make for a very good homeotherm or, more specifically, an ecothermic homeotherm — often through a combination of behavioral and physiological means. Body temperature is regulated to optimize physiological processes, and if environmental conditions are sufficient, the range in which a reptile regulates its body temperature is commonly the species' activity-temperature range. Within the activity-temperature range is the selected (preferred) temperature range, which is more narrowly regulated if possible.

Behavior is critical to thermoregulation in reptiles, and without access to a range of environmental temperatures or heat sources, most reptiles will have a body temperature close to ambient. However, if variations in environmental temperatures do exist in their habitat, many reptiles can significantly regulate their own body temperature. This behavioral temperature regulation, especially in large-bodied species, can result in a near homeothermic body temperature. As a heat transfer medium, blood and its flow within vascular networks is key to regulating body temperature. Reptiles have a variety of anatomical and physiological means to alter their thermal conductivity by varying blood flow, including cardiac and vascular shunts, altered heart rate, vasoconstriction, vasodilation, and countercurrent heat exchange mechanisms (Espinoza and Tracy 1997).

Adaptations for water conservation and osmoregulation differ between amphibians and reptiles. Reptiles have several major adaptations for terrestrial life, such as more intricately structured, yet less permeable skin, a more advanced urinary system, and shelled amniotic eggs. These adaptations permit reptiles to survive in terrestrial habitats in the absence of sources of freshwater critical to the reproductive biology of nearly all amphibians. Although 1 species of turtle (northern long-necked turtle, *Chelodina rugosa*) lays eggs underwater in sediments or submerged soils (Kennett et al. 1998), reptiles generally rely on terrestrial habitats for egg laying. Even oviparous aquatic species such as sea turtles return to land to lay their eggs.

Structurally, reptilian skin is a relatively complex, layered organ in contrast to the skin of amphibians. In reptiles the upper layers of skin are highly keratinized, with keratin being produced by keratinocytes in the basal layers (*stratum germinativum*) of the skin. Keratin represents a major proportion of the skin's proteins in the form of scales. Although the keratin helps reduce water loss and protects the skin, it is not completely impermeable to water. The major barrier to water imbition is a layer of lipids within the skin, which varies significantly among species. Percutaneous and dermal routes of exposure should not be dismissed as insignificant, especially in those species that have a reduced underlying lipid layer that, in its absence or reduced state, may increase likelihood of uptake of polar, waterborne compounds. Alternately, those with relatively impermeable skin, due to cutaneous layers of lipid, may be more prone to cutaneous absorption of lipophilic compounds.

From a comparative perspective, the excretory system of amphibians and reptiles represents a snapshot in the evolution of the kidney as an osmoregulatory organ. In adult amphibians, the kidney is a modified mesonephros and includes development of some features characteristic of the metanephric kidney, for example, the absence of nephrostomes and the joining of nephric tubules to collecting ducts that have developed as outgrowths of the mesonephric ducts that occur in the kidney's metanephric zone (Clothier et al. 1978; Meseguer et al. 1978; Hinton et al. 1982; Sakai and Kawahara 1983; Uchiyama et al. 1990; Møbjerg et al. 1998). Due to its unique structure and associated function, the amphibian kidney is often regarded as the opisthonephros (Kardong 2005). In contrast, the mesonephros in reptiles forms the functional excretory organ through early development (in ovo); then at hatching, the metanephric kidney supplants that embryonic structure in excretory functions and water-solute conservation. Although all reptiles possess metanephric kidneys, they are highly variable in size and shape. For example, in turtles and most lizards, the urinary bladder arises from the ventral wall of the cloaca and is connected to the kidneys via the ureters. In contrast, a urinary bladder is absent in snakes and crocodilians, and the kidneys empty directly into the cloaca (Zug et al. 2001).

The disposition of nitrogenous wastes differs between the classes of herpetofauna. From an energetics perspective, ammonia is relatively inexpensive to metabolize, but is highly toxic in all but the most dilute solution. Hence, aquatic organisms have a relatively great advantage in handling nitrogenous wastes as ammonia, and avoid ammonia toxicity by its dissolution in large quantities of water and its excretion in dilute solution. In adult amphibians and reptiles, ammonia is commonly converted into urea or uric acid, as an adaptation responsive to ammonia's high toxicity in wetland and terrestrial environments. At the same time, both urea and uric acid require less water in their synthesis; hence, water is conserved, which is also a driving force behind adaptations to habitats characteristic of many herpetofauna. As with ammonia, urea is highly soluble in water, but it is much less toxic. Urea can also be accumulated to relatively greater concentrations in tissues without undo adverse physiological effects and can be excreted in a concentrated form. Although relatively costly to metabolize, the synthesis of urea from ammonia and carbon dioxide (Grisolia et al. 1976) benefits the water conservation processes necessary for survival in terrestrial habitats. In contrast to ammonia and urea, uric acid is nearly insoluble in water, but nonetheless, is primarily excreted by the kidney. In reptiles, uric acid passes to the cloaca via the ureters. Water is highly conserved in the disposition of nitrogenous wastes as uric acid, but incurs high energetic cost in its production.

Most adult amphibians are ureotelic, although larval tadpoles or highly aquatic adults rely to varying degrees on ammonia as the dominant form of nitrogenous waste. Reptiles tend toward reliance on uric acid as their nitrogenous waste, although the class is far from exclusively uricotelic. In reptiles inhabiting freshwater habitats (e.g., crocodilians), elimination of nitrogenous wastes while maintaining osmotic balance is not a problem, since the influx of water into the body and the osmotic loss of ions occurs as a consequence of their environs. The flux of water and ions is reduced in part by the keratinized skin of reptiles, and the kidneys produce dilute urine; hence, ions are conserved, water in excess is eliminated, and nitrogenous wastes occur as ammonia (Minnich 1982). In terrestrial and marine environments, additional osmotic stress is experienced by reptiles.

Although some terrestrial reptiles still eliminate a significant proportion of their nitrogenous wastes as ammonia, others reduce urinary output to conserve water and concentrate nitrogenous wastes as either urea or uric acid (Minnich 1982). Tortoises and turtles occupying terrestrial habitats tend to be uricotelic, as do some anurans. Marine environs pose similar challenges with the added hazard of an influx of salts. Because their metanephric kidneys cannot produce hypertonic urine, marine reptiles have adapted various glands for elimination of excess salt. For example, in estuarine crocodiles, special glands on the tongue excrete salt (Taplin and Grigg 1981), and in marine iguanas, nasal glands produce concentrated brine that is expelled from the nose (Dunson 1969). Similarly in sea turtles, lacrimal glands produce a constant efflux of salt (Schmidt-Nielsen and Fänge 1958). The driving force for metabolic adaptation and the nitrogenous wastes characteristic of a species are osmotic stresses placed on the reptile by its environment. In part, these stresses reflect inputs from various routes of exposure, including dermal and percutaneous pathways, and those linked with respiration and gas exchange.

5.3.3 Dermal and Percutaneous Exposure, Respiratory Physiology, and Gas Exchange

From the perspective of exposures to multiple environmental stressors — be they anthropogenic or natural in origin — integrated responses are likely due to functional overlaps among the broadly characterized physiological categories considered in Sections 5.2.1.1 and 5.2.1.2. Simply stated, exposures dominated by dietary routes may be mediated by gastrointestinal and digestive systems of exposed biota, yet those responses may inevitably be tied to responses linked to thermoregulatory, osmoregulatory, or excretory functions. Similarly, interrelationships between functions considered under the auspices of thermoregulatory, osmoregulatory, and excretory physiology, and those linked to respiratory physiology and gas exchange share a route of exposure reflected in exposure models such as that presented in Equation 5.3, wherein dermal and percutaneous routes of exposure variously contribute to total exposed dose realized in field settings.

Mechanisms of gas exchange reflect differences in selective pressures characteristic of aquatic and terrestrial environments. In aquatic habitats, oxygen is less available and more difficult to extract from water, while in terrestrial systems, oxygen is readily available for transcutaneous diffusion across respiratory membranes. Carbon dioxide, however, is not as readily released to the atmosphere unless respiratory systems are functionally and structurally adapted to promote release of CO_2. Hence, respiratory systems in amphibians vary with life stage; for example, the primary respiratory organ in many amphibian embryos and larvae is the epithelia of the skin (Seymour and Bradford 1995). Within each life stage, species-specific functional and structural adaptations to the environment reflect the wide range of responses associated with constraints on gas exchange in aquatic and terrestrial habitats (e.g., the presence or absence of ventilating systems such as lungs, availability of a surface for diffusion and gas exchange, and the concentration of oxygen in the surrounding medium).

For amphibians, life stage and abiotic and biotic characteristics of the environment determine the mode and effectiveness of gas exchange. Within the range of environmental conditions that limit an animal's distribution across the landscape, respiratory functions provide means that ensure gas exchange and contribute to acid/base homeostasis within the animal. Feedback systems serve to control exposures to environmental stressors, such as reduced dissolved oxygen in surface water, and responses to these exposures that are potentially linked to adverse effects (e.g., hypoxia or hypercapnia). At the same time, these respiratory responses, and accompanying morphological and behavioral changes potentially linked to these physiological responses, likely influence exposures to environmental chemicals.

Although the primary site for gas exchange in amphibians is the skin, differences among species and life stages reflect conditions in their environment. These differences in part are due to morphological adaptations for gas exchange throughout the range of habitats where the herpetofauna occur. For example, in the aquatic larvae of amphibians, branchial uptake is linked to the ability to filter feed through the use of a buccal pump mechanism. Filtered water entering the mouth and nostrils is

pumped through a pharyngeal cavity over gills into an opercular chamber and out through spiracles. Aquatic salamanders frequently display neotenic traits, most notably the retention of external gills that allow water entering the mouth to pass over gill slits for gas exchange. To some extent, gill growth is oxygen dependent, with elevated O_2/CO_2 ratios suppressing growth and elevated CO_2/O_2 ratios promoting growth and branching (in *Ambystoma maculatum*; see Branch and Taylor 1977; Duellman and Trueb 1994). Respiration in amphibians that inhabit well-oxygenated mountain streams is primarily transdermal; therefore, gills and lungs (if present) are reduced. Gills in species occupying lentic habitats are more developed and have increased surface area on the villi for oxygen uptake. Amphibians inhabiting lentic conditions may also undulate in the water to further increase convection and gaseous exchange. Under anoxic conditions, gill ventilation ceases and transepithelial loss of oxygen predominates. Aquatic animals can reduce oxygen demand either by decreasing activity (thereby increasing the risk of anaerobic accumulation of lactic acid) or by rising to the water's surface to gulp air (thereby increasing risks of detection by predators; Kramer 1988; Boutilier et al. 1992). Anurans and salamanders with developed lungs typically respond to oxygen stress by swimming up to the water's surface to gulp air, yet this characteristic stress response would not be observed in some *Bufo* spp. tadpoles that lack lungs. However, *Bufo* spp. will swim to the surface of the water to absorb oxygen through cutaneous respiration (Duellman and Trueb 1994). Gas exchange through the gills is diffusion rate limited, but transcutaneous transfer can be up- or downregulated to some extent by changes in dermal folding and glandular/mucous secretions. Gills also have a role in ion exchange; hence, species-specific anatomy and physiology of gills may be important in explaining differential sensitivity to some chemical exposure (Honrubia et al. 1993; Lajmanovich et al. 1998).

When larval gills are resorbed, gill slits are closed and lungs develop. In urodeles, specialized vessels and noradrenergic inputs shunt blood from the gills to the lungs (Malvin 1989). With the exception of both neotenic salamanders, which retain their gills, and members of the lungless Plethodontidae, adult caecilians, salamanders, and anurans use transepithelial, pulmonary, and buccopharyngeal respiration (see Duellman and Trueb 1994). In Plethodontidae, 90% of respiration is dermal, and 10% of the total respiratory vasculature is in the epithelial lining of the mouth.

The capacity for diffusion across pulmonary tissue is greater than that of the skin surface, but under conditions commonly encountered in humid, terrestrial habitats, transepithelial diffusion of oxygen from very dense air into the tissues is the predominant respiratory process. Effective ventilation in adults reliant on respiratory surfaces of the lung will depend on lung characteristics such as pulmonary surface area, tidal volume, ventilation rate, capillary density, diffusion distance, and partial pressures. Amphibian lung volumes vary with environmental conditions. Among cold-water inhabitants, lung volumes are relatively small when compared to the more substantial structures characteristic of terrestrial and some neotenic salamanders. The size of the lung also reflects environmental conditions. For example, in *Rana catesbeiana* lung size increases in response to hypoxia; arterial partial pressure of oxygen, capillary distribution, and rate of blood flow can also be altered in response to changes in surrounding gases (Burggren and Mwalukoma 1983; Duellman and Trueb 1994).

Chemoreceptors in the brain alter ventilation rates and other behaviors in response to decreased environmental O_2, or increased oxygen demands, and to elevated CO_2 concentrations (Shoemaker et al. 1992). During hypercapnia, some species such as adult *Bufo* spp. increase pulmonary ventilation to reduce CO_2 and acidosis. In contrast, aquatic amphibians actively transport HCO_3 and H^+ and *Ambystoma* spp. to increase ion excretion. Metabolic or respiratory acidosis linked to increased activity can stimulate the renin-angiotensin system, increasing aldosterone circulation to promote cutaneous excretion of acid equivalents (Eskandari and Stiffler 1997). Dehydration, on the other hand, tends to decrease transepithelial oxygen uptake to reduce water loss that would be experienced with increased ventilation. In such cases, ambystomids compensate by moving to more hydric soils and by increasing glandular secretions that help in water conservation, while enhancing gaseous exchange at the skin surface.

Efficient oxygen distribution throughout the body is dependent on blood flow, hematocrit, and blood-oxygen carrying capacity (Taketa and Nickerson 1973a, 1973b; Boutilier et al. 1992). Erythrocytes are nucleated in all known amphibians except Plethodontidae. Aquatic species typically have higher blood volumes than do their terrestrial counterparts and also have elevated oxygen carrying capacities, which help in diving and during periods of prolonged submersion (Boutilier and Shelton 1986; Burggren and Just 1992).

As a class, reptiles occupy a variety of habitats that have driven development of a wide range of adaptations to ensure adequate gas exchange. Because reptiles occupy habitats ranging from fully aquatic to completely terrestrial, a variety of morphological and anatomic adaptations have evolved that enable body surfaces as effective gas exchange surfaces. In some reptiles, multiple respiratory surfaces may be used at any particular time, depending on habitat or fluctuating environmental conditions. Throughout the reptiles, lungs are the principal respiratory organs for adults. Structually, the reptilian lung is markedly underdeveloped in comparison to birds and mammals. Their lungs have bronchi connected to a relatively simple, sac-like strucuture having a limited number of alveoli for increasing surface area. The alveoli are vascularized and serve for gas exchange.

Some reptiles (e.g., varanids, crocodilians, turtles, agamids, iguanids, and chameleons) have a unicameral lung in which the sac-like center of the lung is surrounded by a few septa (Bickler et al. 1985; Powell and Gray 1989). The chambers formed by these septa are each supplied by a bronchiole and are partitioned further by alveoli. Because of their elongated body form, snakes have special adaptations in their respiratory structures, and typically have an enlarged right lung and a rudimentary left lung. The elongated lungs of snakes may be divided into an anterior bronchial lung and a posterior air sac (Stinner 1982). Many snakes also possess a tracheal lung, which is structurally similar to the functional lung but branches from the trachea from where the tracheal rings are incomplete dorsally.

The mechanics of inhalation and exhalation are accomplished through a variety of adaptations. Air is tidally exchanged through the lungs by thoracic aspiration. While crocodilians possess a diaphragm that contracts for inhalation and abdominal muscles that contract for exhalation, most reptiles achieve inhalation by contraction of the intercostal muscles; exhalation is passive, resulting from elastic recoil of the thoracic cavity and the weight of the internal organs upon the lungs. Turtles present unique adaptations for respiration because the thoracic cavity is enclosed within a rigid shell. In turtles, the posterior abdominal muscles and pectoral girdle muscles can expand the body for inhalation. Exhalation is accomplished either passively or by retraction of the limbs into the shell, compressing the internal organs and lungs.

Other structures also can provide important surfaces for gas exchange. Oxygen and CO_2 exchange can take place across the skin, buccopharyngeal, or cloacal surfaces (Dunson 1960; Girgis 1961). To increase gas diffusion, filamentous projections occur on the pharyngeal surfaces of some softshell turtles (Girgis 1961; Winokur 1973). More than a single mechanism may be used for respiration at any time, and methods can change, depending upon circumstances. Although aquatic turtles normally breathe air, during prolonged submergence such as hibernation, buccopharyngeal respiration may dominate gas exchange. Softshell turtles, for example, can obtain as much as 50% of their oxygen requirements from cutaneous and buccopharyngeal respiration (Zug et al. 2001). Similarly, Sabenau and Vietti (2003) considered respiratory and metabolic acidosis and the importance of recovery periods to loggerhead turtle (*Caretta caretta*) confronted by repeated submergence challenge. In turtles, as well as other diving semiaquatic vertebrates, recovery from any physiological acid-base disturbance is accomplished, in part, by immediately surfacing after the dive, hyperventilating, and resumption of normal voluntary diving behavior, following a partial to complete recovery from the acid-base disturbance.

As previously noted, under conditions commonly encountered in the environment of amphibians and reptiles, oxygen content of water is less than that of air at the same temperature. Water's O_2 carrying capacity is temperature dependent; cold water contains more oxygen than warm water under the same atmospheric pressure. Water and air, however, generally have environmental CO_2 concentrations

that are much lower than those levels commonly found in the blood of an animal occupying that environment. These concentration differences lead to O_2 absorption and CO_2 release following a typical vertebrate pattern involving lung epithelia. However, CO_2 release frequently occurs through supplementary pathways; some CO_2 exchange occurs across the surface of the skin. Although reptilian skin is more impermeable because of the scales, significant CO_2 exchange can occur across the scale hinge-interscalar spaces. As much as 20 to 30% of CO_2 exchange can occur across the skin in this fashion (Zug et al. 2001). Although more predominant in some aquatic reptiles, some terrestrial reptiles (e.g., *Lacerta* spp.) consistently rely on CO_2 loss from the skin (Zug et al. 2001).

Respiratory surfaces may represent a significant route of contaminant absorption and uptake, particularly for aquatic organisms. The diversity of respiratory surfaces in reptiles has considerable implications for the absorption of toxic compounds. Depending on the developmental stage or phase of annual activity cycle, reptiles may use different respiratory surfaces, and the differences in membrane surfaces will affect exposure and dose. For example, chemical absortion across skin versus buccopharyngeal mucosa versus cloaca mucosa will affect the extent of chemical uptake from the environment. As noted for observations focused on dietary exposures and their links to gastrointestinal and digestive physiology, and for observations focused on integrative processes related to thermoregulatory, osmoregulatory, and excretory physiology, the range of functional and morphological adaptations associated with gas exchange and respiratory systems in reptiles ensures that general statements regarding exposures in the field must be developed with caution. Beyond the relatively simple appearance of exposure equations (Equations 5.3 and 5.4) and their regulatory applications, there are ample research opportunities for physiological ecologists that would undoubtedly improve our analysis of exposure in herpetofauna.

5.4 ENDPOINTS COMMONLY LINKED TO CHEMICAL EXPOSURES TO AMPHIBIANS AND REPTILES IN LABORATORY AND FIELD

Consistent wth our cursory overview of exposure as viewed through the eyes of a physiological ecologist, in the following sections we will briefly focus on effects as outcomes of exposure. Again, the physiological ecologist has long considered outcomes as adaptations linked to selective pressures associated with exposures to environmental stressors such as challenging osmotic environments and extreme temperatures. Similarly, outcomes through the eyes of the ecotoxicologist are commonly called endpoints. While not generally considered within an evolutionary context, outcomes as endpoints share technical foundations with outcomes as adaptations. Indeed, early in its development, ecotoxicology's relatively close ties to physiological ecology are apparent given the role of chemical stressors such as early-generation chlorinated hydrocarbons as "selective pressures" for development of pesticide resistance in target species and observations of metal resistance in plant species long exposed to soils rich in metallic ores.

Research on amphibian ecotoxicology has continued to expand, as indicated by Sparling et al. (Chapter 1, this volume), including an increased number of endpoints that can be measured and interpreted within the context of effects linked to chemical stressor exposure. Although recent additions to the collection of endpoints have been noted (see, e.g., Chapter 14, this volume), common endpoints for evaluating effects in amphibians remain focused on growth and development (including stage-specific and time-specific endpoints related to metamorphosis), behavioral alterations, and biomarkers of exposure (e.g., changes in nucleic acids, enzyme activity, mRNA, protein synthesis) and biomarkers of effects (e.g., limb and skeletal deformities). Similarly, research focused on the ecotoxicology of reptiles has yielded similar lists of outcomes related to exposure and effects.

5.4.1 Growth

For both amphibians and reptiles, ecological parameters such as fecundity, juvenile dispersal, adult fitness, and survivability are often dependent on growth and size reached during development and maturation. Although growth (e.g., length as snout-to-vent length [SVL] and body weight) and other

growth-related endpoints may be evaluated from organismal data collected from the field or from laboratory exposures, baseline values for measurements such as SVL, developmental rate, and size at metamorphosis tend to be more variable under field conditions. Thus, age-specific body-size-related endpoints may not always be sensitive measures to evaluate chemical effects on herpetofauna. Physiological ecologists ply their energetics-based tools (Rice 1990; Widdows and Donkin 1991) to ecotoxicological problems to evaluate growth and reproductive potential in amphibians and reptiles (Rowe et al. 1998). For example, food will be consumed, and energy and materials assimilated, then allocated by priority to maintenance costs supporting basic physiological processes. Energy and materials remaining after maintenance costs are satisfied are then available for growth, reproduction, or storage. Exposure to pollutants can increase energy requirments (e.g., increased metabolic rate or protein synthesis) or can decrease assimilation efficiency (e.g., decrease in foraging efficiency) and create a deficit in the energy balance model (Calow 1989, 1991; Rice 1990; Rowe et al. 1998). The difference in maintenance costs can readily serve as an endpoint. Physiological costs associated with a chemical's direct effects as a toxicant, or indirect effects as a chemical stressor, can be applied to these bioenergetics models to examine the potential effects that they may ultimately have on growth or reproduction. Depending on the toxicants to which the animal is exposed, mechanisms of toxicity acting at the cellular level will be translated into bioenergetic responses that may then be measured as an integrated effect on the individual. Energy available to offset costs linked to growth, reproduction, and survival may subsequently be derived for populations and communities, if models sufficient to these estimations are available or developed (Rice 1990; Widdows and Donkin 1991).

Interpreting growth measurements in amphibians and reptiles is not always straightforward (Petranka 1989; Pfennig et al. 1991). Contaminant research may report both decreased growth (Lefcort et al. 1997) and increased growth depending on the chemical effect and test conditions (Rowe et al. 1998). For example, a chemical may either stimulate growth or stimulate early metamorphosis at a smaller size. Alternatively, a chemical that is lethal to tadpoles in a tank may optimize conditions for density-dependent growth in survivors, or chemicals and test conditions that are stressful to tadpoles may increase body size through edema. Although there is a genetic component to amphibian development through its various life stages — development in ovo, hatchling and development of larvae to juvenile status, then attainment of sexually mature adults — growth is also associated with, and responsive to, environmental factors such as temperature, abiotic conditions of microhabitats, and biological interactions such as predation and competition (Bellis 1962; Jung and Walker 1997; Kiesecker and Blaustein 1998).

As in the amphibians, temperature effects on growth and development are similarly expressed in reptiles (see, e.g., Booth 2006). For example, ambient nest temperatures experienced during incubation influence size, shape, color, behavior, locomotor performance, and sex determination in many reptiles. In considering growth as an endpoint in ecotoxicological investigations in the field, one should remember the range of selective pressures that influence the life history of animals, particularly as a function of their unique natural history (Peters 1983), as well as proximate factors that constrain body sizes (e.g., Van Valen 1973; Schmidt-Nielsen 1984; Maurer et al. 1993; Brown et al. 1993). Indeed, in the absence of both laboratory and field studies, if competing factors that influence growth, such as temperature (Huey and Berrigan 1991; Sinervo and Adolph 1994), are not given sufficient consideration in study design, a solitary focus on dietary exposures as dominant factors influencing chemical exposures may lead investigators astray. For example, while measuring growth (e.g., as length in terms of SVL or weight) in the laboratory may be relatively straightforward, in translating those growth endpoints with those same endpoints observed under field settings, investigators must be wary of the wide array of selection forces and mechanisms that influence growth. Body size may be difficult to quantify (Dunham 1978; Gaston and Lawton 1988) relative to exposure conditions related to predation pressure (Owen-Smith 1993) or interspecific competition for food (Illius and Gordon 1987). In the field, body size in reptiles is often limited by food intake, which in turn depends on available forage or prey resources. Differences in supply

and quality of foods, food intake, and their implications for phenotypic differences in body size may be compared energetically with, for example, field metabolic rates from the literature (Nagy 1982; Nagy and Shoemaker 1984), and with experimental outcomes of feeding trials completed under controlled conditions wherein chemical contaminants are incorporated into diets. By opting to integrate field-laboratory studies, distinguishing between effects of "environmental chemicals" and "other stressors" may be achieved by tracking differences in growth and net energy gains (or losses) between variously challenged animals. From companion field studies, competing factors influencing growth and body size (e.g., predation and interspecific food competition) can be more adequately considered in the interpretation of potential differences in body size based on food availability alone or reduced growth linked to diminished net energy available for growth associated with chemical exposures.

Field-oriented physiological ecologists have long appreciated the heterogeneous world in which animals live and have learned to embrace variance. In fact, changes in the variance of physiological responses within populations may be an effect of environmental change. Hochachka and Somero (2002) amply summarized mechanisms for short- and long-term responses to environmental shifts such as temperature, yet studies of responses to changes in food supply (both quantity and quality) remain challenging given the integrative systems (e.g., digestive, hepatic, renal, circulatory, and neurobehavioral) and tissues (e.g., adipose, muscle, and skeletal) that may be involved. Likewise, depending on the level of organization, temperature-related studies focused on these response components may vary as a function of spatiotemporal scales (from local to global, and from seconds to months to years) that strongly influence exposure.

5.4.2 Reproduction and Endocrinology

Reproduction is a crucial event in the life history of every organism that ensures continuation of the species. Anything that interferes with reproduction or subsequent embryonic development ultimately may lead to extinction. Because chemical signals (often hormonal or pheromonal) direct reproduction and development, these processes are sensitive to chemical perturbations from the environment. In fully formed adults, disruptions due to environmental chemicals may lead only to activational effects, which are often temporary imbalances. However, for developing embryos, chemical signals direct the formation of anatomical systems and establish physiological set points, and signal disruption due to environmental contaminants during development may lead to permanent organizational level effects, such as anatomical defects or physiological imbalances.

Toxicological effects on amphibian and reptilian reproduction can range from prezygotic effects to postzygotic effects. For example, impaired gametogenesis in adults exemplifies a prezygotic effect that reduces fertilization and subsequently the production of hatchlings. Postzygotic effects may contribute to reduced offspring survivorship (e.g., through maternal transfer of contaminants) or through disruption of normal development patterns. Adverse behavioral effects may diminish reproductive success through prezygotic (e.g., by impaired mating displays and reduced abilities to attract mates, or more generally, by impaired timing and type of breeding behavior) or postzygotic (e.g., egg attendance, hiding, guarding, carrying, and feeding offspring [Gross and Shine 1981]) mechanisms. As simply measured by the number of offspring entering the next generation, reproductive success depends on unaffected embryonic development, gender determination, and hatchling growth, all of which commonly vary across a range of species within a genus or family. Within a species, these factors may be highly variable from breeding season to breeding season, depending on environmental conditions potentially affecting reproductive fitness (Highton 1956; Blair 1961; Corn and Livo 1989). Given the variability in various life history traits linked to reproduction, it is best if such information is characterized for the species of concern to the evaluation. This information increasingly is being provided as part of amphibian and reptilian toxicological research (Berrill et al. 1995; Blaustein et al. 1996; Gardner and Oberdörster, 2006), yet remains insufficient to most implementations of the risk assessment process focused on the herpetofauna.

5.4.2.1 Reproduction and the Environment

Reptiles and amphibians have evolved to survive in many biomes, ranging from the tropics to above the Arctic Circle, and from the oceans to the deserts. Within these biomes, they have further adapted for many microhabitats, including terrestrial, fossorial, arboreal, freshwater, and marine. To maximize reproductive output across members of each class, reproductive characteristics vary among species. In most species, reproduction is seasonally timed against extrinsic factors such as temperature, photoperiod, and precipitation in order to optimize survival of the offspring (Karsch et al. 1984; Wingfield and Kenagy 1986).

In temperate zones, amphibian breeding is cyclic, gametogenesis is seasonal, and adult gametes mature uniformly. Breeding activity is controlled by endogenous neuroendocrine cycling coupled with extrinsic, seasonally derived triggers such as temperature, photoperiod, and precipitation (Blair 1961). Timing varies geographically and latitudinally, although within a species or population, the cue is constant. In tropical zones and regions of prolonged conditions, such as desert droughts, gametogenesis is continuous; gametes at various stages of development enable some part of the population to be ready to breed at all times (Jacobson 1989). In amphibians, activity may be initiated at any time but typically occurs around rainstorm events. Desert-dwelling *Scaphiopus* spp. are opportunistic breeders and are physiologically ready to lay their eggs in rain pools at the first major rain event.

In temperate reptilian species, reproductive seasonality is typically dependent upon photoperiod and temperature, whereas in tropical species wet and dry cycles may also play a significant role in regulating reproduction (see Palmer et al. 1997 for review). Reproductive cycles range from very short cycles in northern species to nearly continuous reproduction in some tropical species (Fitch 1970, 1982; Moll 1979; Duvall et al. 1982). Species with wide geographic ranges are subject to local environmental variation in conditions. Consequently, these species exhibit within-species variation in reproductive patterns. For instance, some sea turtles (*Chlonia mydas*, *C. depressa*) nest throughout the year in tropical portions of their range, but seasonally in temperate regions (Grace 1997).

Our understanding of the reproductive endocrinology of reptiles and amphibians continues to advance, yet does not match that of endotherms. Only a few species have been intensively studied. For the vast majority of reptilian and amphibian species, little or no information is available regarding reproductive endocrinology or physiology (Palmer 2000).

5.4.2.2 Pineal Gland

The pineal gland (epiphysial gland) is the principal organ for detecting environmental cues and translating them into endocrine signals for regulating reproductive cycles. The pineal gland is absent in crocodiles (Roth et al. 1980), yet occurs in most fishes, amphibians, and reptiles as a sac-like diverticulum of the third ventricle of the brain. In anamniotes and lizards, the basal portion of the pineal gland is photosensory, while in other reptiles the gland connects to the suprachiasmatic nucleus from which it receives photoperiod information from the eyes (Quay 1979; Collin and Oksche 1981). During periods of darkness, the pineal gland utilizes N-acetyltransferase and hydroxyindole-O-methyltransferase to synthesize serotonin and then melatonin from tryptophan (Quay 1974). Melatonin also may be produced by the retina in amphibians and reptiles (Ralph 1980; Pang and Allen 1986), and concentrations of plasma melatonin vary diurnally, having greater concentrations during the dark phase (scotophase) than during the light phase (photophase). The cyclical level of melatonin has several functions, including regulating circadian rhythms, influencing body coloration by changing melanophore size, and regulating annual reproductive cycles by influencing gonadotropin release (Underwood 1992). By linking reproductive events with environmental factors, offspring are more likely to emerge during favorable conditions (Marion 1982).

5.4.2.3 Hypothalamus and Pituitary

The pineal gland responds to the external environment, functioning as a transducer of the physical environment linked to endocrine signals (melatonin levels). Melatonin, in turn, influences the

hypothalamus and subsequently the pituitary, which together regulate the reproductive organs, the ovaries or testes. The hypothalamus secretes gonadotropin-releasing hormone, which in turn stimulates the adenohypophysis of the pituitary to produce the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Although well characterized in endotherms such as mammals, the functions of endogenous LH and FSH in reptiles and amphibians remain obscure. In turtles and crocodilians, both FSH-like and LH-like molecules have been detected (Licht and Papkoff 1974; Ishii 1991), but squamates appear to rely on a single FSH-like gonadotropin, which may have both LH and FSH activities (Licht 1979). In hypophysectomized *Anolis* spp., exogenous FSH maintains testicular weight, whereas both exogenous FSH and LH stimulate increased androgen levels (Licht and Pearson 1969). Until conspecific gonadotropins are regularly available for the study of reproduction in reptiles, hormonal function and its role in regulation will have to rely on administration of gonadotropins from other vertebrates.

5.4.3 Female Reproduction

5.4.3.1 Vitellogenin

Amphibians and reptiles produce macrolecithal eggs, in which a large yolk serves as an energy reserve for the developing embryo. In nonmammalian vertebrates, yolk production is initiated in the liver of adult females with the production of vitellogenin (Ho 1987). Once synthesized and released by the liver, vitellogenin enters systemic circulation, and is subsequently taken up by developing oocytes and converted to egg yolk (lipovitellins and phosvitins). Structurally, vitelloginin is a phospholipoglycoprotein precursor of egg yolk, and across a range of species its molecular weight commonly ranges from 200 to 250 kDa. In the systemic circulation, vitellogenin usually occurs as a dimer of 400 to 500 kDa (Callard and Ho 1987). Multiple forms of vitellogenin are present in the plasma of several species, including *Xenopus*, chicken, and several fish species, but each is glycosylated and contains about 1.4% carbohydrate by weight (Ho 1987; Lazier and MacKay 1993).

Estrogen is the primary stimulus for vitellogenin production. Estrogen promotes vitellogenesis in members of all nonmammalian vertebrate classes (Ho 1987). In each of these groups, the time of vitellogenin production in adult females corresponds to the period of elevated estrogen levels. Vitellogenesis can be induced in males and in nonvitellogenic females by administration of estrogen. The production of vitellogenin in response to estrogenic compounds is rapid, sensitive, and dose dependent (Ho et al. 1981). Vitellogenin can be utilized as a biomarker for exposure to xenobiotic estrogens (Palmer and Palmer 1995; Palmer et al. 1998; for review, see Palmer and Selcer 1996). Estrogen or estrogen mimics are the sole inducers of vitellogenin (Tata and Smith 1979), although other factors have a role in modulating the vitellogenic response (Ho et al. 1981; Wangh 1982). The strength of the vitellogenic response indicates the relative ability of xenobiotic compounds to stimulate estrogenic pathways. Additionally, through vitellogenesis, transfer of lipophilic contaminants from female fat stores or her proximate diet can expose developing embryos to potentially hazardous concentrations, leading to developmental and other effects (see Rowe 2008).

5.4.3.2 Ovarian Structure and Function

The amphibian ovary is a hollow, sac-like structure. The ovaries of reptiles are saccular or membranous structures in which enlarged follicles are prominent. Among crocodilians and chelonians, the membranous ovaries are symmetrically positioned ventral to the kidneys. Among lizards, the ovaries may be either symmetrically or asymmetrically positioned, with one ovary more anterior than the other. In snakes, asymmetrically placed ovaries are the rule, with the right ovary usually larger and more anterior than the left, which corresponds to the length of the adjacent oviduct. The left ovaries of snakes may be reduced or undeveloped.

The female ovary consists of oocytes surrounded by both granulosal and thecal layers, with the granulosa layer serving as the primary site of estrogen synthesis (Callard and Ho 1980; Callard and

Kleis 1987). Estrogen stimulates development of the oviduct in reptiles and amphibians, although outcomes of that development vary across species. In squamates, for example, pyriform cells have been identified in the granulosa, which may be involved in early oocyte development (Uribe et al. 1995). Once vitellogenesis begins, the pyriform cells degenerate, and the theca surrounds the granulosa layer. During development, the thecal and granulosal layers become separated by the acellular *membrana propria* (Uribe et al. 1995); the *theca interna* remains glandular, whereas the *theca externa* becomes fibrous. Rising levels of gonadotropins stimulate follicular recruitment (Palmer et al. 1997), and in reptiles, interstitial glands may form from atretic follicles and exhibit an endocrine function. While ovulation in amphibia is under the control of LH and progesterone, the endogenous stimulus for ovulation in reptiles is unknown. However, administration of exogenous FSH can induce ovulation in *Anolis* (Jones et al. 1988).

In oviparous amphibians, ova are stored until released. Ova released during ovulation subsequently enter the oviducts where egg jellies are deposited. Following ovulation, the granulosal and thecal cell layers of the follicle are transformed into *corpora lutea*, which secrete progesterone. In amphibians, progesterone induces responsiveness of the oviducts to arginine vasotocin (AVT), with AVT acting to stimulate oviductal contractions during oviposition. Once eggs are fertilized and deposited, the perivitelline chamber increases in volume, as a result of the uptake of surrounding water and accumulation of waste products. The outer membrane is water permeable, but is sensitive to pH outside the neutral range (Dunson and Connell 1982).

In reptiles, *corpora lutea* have been shown to exhibit 3β-hydroxysteroid dehydrogenase activity and synthesize progesterone (Klicka and Mahmoud 1972, 1973, 1977; Licht and Crews 1976). Following ovulation, egg yolks enter the paired oviducts where fertilization occurs. Fertilization is internal in all reptiles and presumably occurs in the anterior oviducts prior to deposition of any egg coats (Palmer and Guillette 1988). In oviparous species, albumen and eggshell layers are deposited within the oviducts. Albumen is a complex mixture of water-soluble proteins that may influence embryonic development (Palmer and Guillette 1991), and is deposited by the anterior glandular portion of the oviduct (Palmer and Guillette 1988, 1991). Subsequently, the eggshell is deposited by the uterus (Guillette et al. 1989; Palmer and Guillette 1990; Palmer et al. 1993), and consists of an inner fibrous portion and an outer calcareous layer (for review, see Schleich and Kästle 1988; Packard and DeMarco 1991). In turtles and squamates, the uterus is homogeneous and produces both the fibrous and calcareous eggshell layers from endometrial glands along its entire length (Palmer and Guillette 1988; Guillette et al. 1989; Palmer et al. 1993). In crocodilians, the uterus is divided into morphologically distinct fiber-producing anterior and calcium-secreting posterior regions (Palmer and Guillette 1992). Oviductal function is regulated primarily by estrogen and progesterone, although androgens such as testosterone and dihydrotestosterone (DHT) also may play minor roles (for review, see Palmer et al. 1997; Selcer and Clemens 1998; Selcer et al., 2005). The oviducts also have been shown to possess specific androgen receptors and aromatase (Smith et al. 1995). Progesterone in reptiles inhibits muscular contractions of the uterine walls by blocking formation of arginine vasotocin (AVT) receptors, preventing early parturition or oviposition (Guillette et al. 1991a,b; Guillette et al. 1992). *Corpora lutea* typically persist until oviposition or parturition. Declining progesterone levels associated with the involution of the *corpora lutea* release the inhibition on parturition and oviposition (Guillette et al. 1991b). AVT induces smooth muscle contraction by stimulating local synthesis and release of prostaglandins (PGs) from the uterine wall. The combination of AVT and PGs leads to peristaltic waves of muscular contraction that expels the eggs or embryos (Guillette et al. 1990, 1991a, 1991b, 1992).

5.4.3.3 Reproductive Strategies

Amphibians and reptiles show extremely diverse reproductive strategies, including oviparity, viviparity, ovoviviparity, and various degrees of parental care (Guillette 1987, 1989, 1991; Hanken 1989; Wake 1993). Caecilians, salamanders, and frogs display both oviparity and viviparity. All turtles and crocodilians are oviparous, but viviparity has evolved numerous times

among the squamata (Blackburn 1982, 1985; Shine 1985; Shine and Guillette 1988). In viviparous reptilian species, the embryo implants into the uterine lining, and the oviductal glands that secrete the eggshell membranes degenerate prior to implantation. The thin uterine lining becomes highly vascularized and strongly co-mingled with embryonic tissues to form a placenta (Weekes 1935; Yaron 1985; Stewart and Blackburn 1988; Stewart 1992). Most nutrients are supplied to the embryo in the form of yolk provided by the ovary. However, significant gas exchange occurs across the placenta.

Parthenogenesis has been reported in some lizard and snake species. Examples are found among species of *Sceloporus*, *Cnemidophorus*, *Ramphotyphlops*, *Elaphe*, *Agkistrodon*, *Gymnophthalmus*, and some geckos (Burgin et al. 1997). Their ova initiate development without the presence of males, and the unfertilized ova develop normally into exact copies of the mother. Some salamanders of the genus *Ambystoma* are gynogenetic. Gynogenesis is similar to parthenogenesis, with the exception that the eggs must be stimulated by the presence of sperm in order to start development. Both parthenogenesis and gynogenesis reduce genetic variation within the population and thus limit the population's ability to adapt to new environmental challenges, such as the introduction of emerging contaminants.

Annual fecundity in amphibians can range from 1 to more than 80,000 viable hatchlings per clutch (Jørgensen 1992; Baker 1992). Larger species and those with generalized modes of egg production deposit larger clutches. Generally, less than 5% of eggs will survive to metamorphosis. Among reptiles, the fecundity can range from 1 to over 100 eggs per clutch for sea turtles.

5.4.4 Male Reproduction

5.4.4.1 Testis Structure and Function

The testis of urodeles is of the cystic type, similar in structure and function to those of fishes. The uredele testis consists of 1 or more lobes, each containing several ampullae, which in turn are composed of several germinal cysts. Germ cells within a cyst divide synchronously, so that all sperm mature within a cyst at the same time. The testis may have cysts in various stages of development, representing differing reproductive episodes, such as temporally separated breeding events. The urodele testis exhibits Sertoli cells and lobule boundary cells, which both express steroidogenic activity. The anuran testis is structurally more similar to that of amniotes, consisting of seminiferous tubules with a permanent germinal epithelium and conspicuous interstitial tissues. The interstitial cells are steroidogenic and produce androgens. The seminiferous tubules contain Sertoli cells, which regulate sperm production and are also steroidogenic.

In male reptiles, the seminiferous tubules of the testis are the functional units of reproduction, and testicular recrudescence is stimulated by rising levels of gonadotropins (Licht et al. 1977; Licht 1979; Ishii 1991). Sertoli cells are present within the seminiferous tubules among the developing sperm cells, whereas interstitial Leydig cells are found between the tubules. Both Sertoli and Leydig cells secrete androgens (Mahmoud et al. 1985). Following completion of spermatogenesis, sperm migrate into the epididymis where they are stored until subsequent release.

In contrast to other orders in the class, male squamates have an accessory reproductive organ known as the renal sex segment, which is part of the kidneys, developing from the uriniferous tubules (Prasad and Reddy 1972). Under the influence of androgens, the tubules of the renal sex segment hypertrophy and become engorged with secretory granules. These secretions become mixed with the semen during ejaculation and may function in maintaining sperm viability, although their specific function remains unknown. Some authors have suggested that the sex segment is homologous to the seminal vesicles of mammals (Norris 1997).

5.4.4.2 Fertilization and Copulatory Organs

Fertilization may be either internal or external, depending upon species. Anuran mating generally occurs by way of cloacal apposition, wherein eggs are laid in water, and fertilization is external.

Though there are salamanders that exhibit external fertilization, 90% of the species fertilize eggs internally. Some terrestrial salamanders breed in the fall and store sperm internally in spermatheca or dorsal diverticulum of the female cloaca and in urogenital pouches (Massey 1990), and eggs are deposited and fertilized the following spring. Ambystomid males deposit spermatophores (cloacal gland secretions of sperm packets encased in a jelly-like substance) in ponds (Bishop 1941), where females subsequently pick up the spermatophores and deposit eggs. Caecilian species reproduce biennially, and exhibit intromission and internal fertilization (Jørgensen 1992; Wake 1993). Caecilians possess an elaborate intromittent organ, the phallodeum, associated with the posterior portion of the cloaca. Fertilization in reptiles is always internal, due to production of shelled amniotic eggs or live young. Male reptiles possess copulatory organs used to transfer sperm to the female. In turtles and crocodilians, the copulatory organ is a single penis, and in squamates, paired hemipenes are present. These organs are housed within the cloaca until intercourse, at which time they become engorged with blood and extend through the cloacal opening.

5.5 REPRODUCTIVE ECOLOGY

5.5.1 Parental Care

Parental care is rare among caecilians, and is found in some anurans (Townsend et al. 1991) and in many, if not the majority of, urodeles (Tilley 1972). Parental care includes attending eggs, transporting eggs or young, and feeding young. Depending on species, duration of parental care can extend to more than 150 days (Duellman and Trueb 1994) and can involve either the male (e.g., in Hynobiidae) or female (e.g., in Plethodontidae, *Desmognathus fuscus* and *Aneides lugubris*). For review and species references, the reader is referred to Duellman and Trueb (1994).

Parental care is absent among turtles and tortoises, which all bury their eggs, but is found in some lizards, snakes, and the crocodilians. Some skinks guard their eggs and presumably aid in maintaining adequate moisture levels during incubation. Several pythons not only guard their eggs, but actively increase incubation temperature by active shivering thermogenesis, thereby shortening incubation time. Crocodilians exhibit extensive care of both offspring and eggs by guarding them aggressively from predators and assisting the young to emerge for the nest mound.

Studies indicate that, in many cases, parental care improves rate of hatching and survival. The energetic cost of care depends on the extent to which parents continue feeding and the frequency of egg deposition. Underlying physiological and endocrine mechanisms associated with these diverse strategies need additional study (Townsend et al. 1991), and contaminant effects, direct with respect to care or indirect with respect to the energetic costs of care, have yet to be investigated.

5.5.2 Offspring Survival

To a large extent, growth and posthatch activities of offspring are environmentally determined. For example, many studies indicate that large-bodied offspring exhibit increased survival and are more fit than their small-bodied cohorts (John-Alder and Morin 1990; Platz and Lathrop 1993); hence, some anurans (e.g., *Rana catesbeiana* and *R. clamitans*) overwinter as tadpoles to metamorphose in the spring when their body mass would be greater. If not overwintering, these species would be more susceptible to predation as tadpoles, and the risk of their ponds evaporating prior to their metamorphosis would be increased (Anderson et al. 1971; Cooke 1973; Bishop 1992; Hota 1994; Bridges 1997). In amphibians, overall ecological and physiological costs and benefits of remaining aquatic or metamorphosing to a terrestrial adult depend on the relative quality of the pond and surrounding terrestrial habitat. Further, studies using allometric engineering in reptiles indicate that alterations in the quantity of yolk may alter offspring size (Sinervo et al. 1992). Production of vitellogenin is also susceptible to environmental endocrine disruptors (Palmer and Palmer 1995; Palmer et al. 1998), which may alter egg or clutch size (Irwin et al. 2001). Ecotoxicological implications

of altered offspring size or delayed or reduced growth resulting from contaminant exposure need to be considered at the population level and within the field environment (Smith 1987; Figiel and Semlitsch 1990; Pfennig et al. 1991; Whiteman et al. 1996).

5.5.3 Lifespan and Exposure

Very little information is known about basic life history and reproductive potential of most amphibians and reptiles — even for the more commonly studied groups (Matson 1998). The lifespan for amphibians ranges from less than 1 year to more than 30 years (*Bufo americanus*; Carey and Judge 2000), and that of reptiles to well over 100 years (Cary and Judge 2000). Information on reproductive potential and duration (e.g., age at sexual maturation, average lifespan) is vital to evaluating contaminant-induced die-offs and other catastrophic events on population recovery over time. For those organisms that deposit many thousands of eggs, information on recruitment and reproductive strategies may be far more important than the clutch size or SVL when evaluating ecotoxicological effects (Larson 1998).

5.6 DEVELOPMENT

Within field settings, development of amphibians and reptiles from early embryo to adult occurs as a series of events sensitive to environmental cues. As key factors contributing to the normal developmental process, these environmental cues have acted through evolutionary time and have influenced development to yield the wide range of phenotypes manifested as varying expressions of the genotypes characteristic of the herpetofauna. The environment can affect development in several ways, ranging from cued events normally experienced by organisms during their ontogeny to those interactions with environmental stressors that are newly encountered or encountered under conditions that were not previously experienced in the animal's phylogenetic history. For example, through evolutionary time seasonal cues such as photoperiod, temperature, or hydration may alter an organism's development to increase its fitness, yet newly encountered environmental stressors may contribute to disruption of the normal developmental process for a species. As such, both physiological ecologists and ecotoxicologists view developmental events characteristic of the herpetofauna as outcomes potentially linked to exposures to environmental stressors encountered in the field. However, there may be time frame differences between the natural stressors and anthropogenic stressors, which typically appear much more quickly in the environment.

Embryonic development is one of the more sensitive stages in the life of amphibians and reptiles. Yet, the role of exposures to environmental chemicals is not completely understood and continues to be the subject of study. Since the anatomical and physiological systems developing in embryonic reptiles are controlled by numerous, and oftentimes interacting, chemical signals, adverse effects potentially linked to exogenous chemicals should be more sufficiently characterized without undo reliance on comparative analyses focused on endotherms or a few species of amphibians or reptiles that are regarded as representative of their class. Chemicals in the environment can alter either the signals themselves or the animals' ability to recognize them.

5.6.1 Sex Determination

Sex determination is linked to several different developmental mechanisms. Genotypic sex determination (GSD) results from the genetic makeup of the embryo, frequently manifested by differences in sex chromosomes. The homogametic sex will have 2 identical sex chromosomes, and the heterogametic sex will have 2 different sex chromosomes. In mammals and many anuran amphibians, the female is the homogametic sex designated by 2 X chromosomes (XX), and the male is the heterogametic sex designated by an X and a Y chromosome (XY). Birds and many urodele

amphibians present males that are homogametic (designated ZZ), while females are heterogametic (designated ZW; see Norris 1997).

Temperature-dependent sex determination (TSD) is encountered in all crocodilians, many turtles, some lizards (geckos and lacertids), and perhaps some amphibians (Norris 1997; Zug et al. 2001). TSD is characterized by a relatively narrow range of temperatures that affect sex determination. Temperature sensitivity usually occurs in a relatively limited time frame, generally in the first to middle third of the incubation period (Vogt and Bull 1982; Bull et al. 1990; Haig 1991; Desvages et al. 1993; Spotila et al. 1994). There is considerable variation among reptiles regarding the temperature that determines a particular sex, and the critical temperature at which both sexes are produced. In most species, there is an "all or none" effect in which only males or only females are produced on either side of the critical temperature range. There are 3 general patterns of TSD in reptiles (Bull 1980). In some turtles, males are produced at low temperatures (generally less than 25 to 28 °C), and females are produced at high incubation temperatures (usually greater than 31 to 33 °C). Intermediate temperatures produce a gradation of males and females (Vogt and Bull 1982; Ewert and Nelson 1991). In contrast, in some lizards lower temperatures produce females and higher temperatures produce males (Bull 1987). Different yet are the crocodilians, some turtles (*Chelydra* spp.), and some geckos (e.g., *Eublepharis macularius* and *Hemitheconyx caudicinctus*) in which males are produced at intermediate temperatures and females at both high and low incubation temperatures (Webb and Cooper-Preston 1989; Ewert and Nelson 1991; Viets et al. 1993; Lang and Andrews 1994). In the wild, significant temperature variation can occur among nesting localities, resulting in sex ratio variation of recruits (Vogt and Flores-Villela 1992). Over the long term and considering large numbers, these deviations tend to balance out and produce roughly equal numbers of males and females (see reviews in Bull 1980; Janzen and Paukstis 1991; Lang and Andrews 1994; Viets et al. 1994).

Sex steroids and the metabolism of sex steroids play a role in TSD. Administration of estrogen or exogenous estrogens at male-inducing temperatures in reptiles can reverse the production of males and lead to a higher percentage of females than normal or even completely sex reverse the embryos, producing all females (Raynaud and Pieau 1985; Wibbels et al. 1994). Compounds that interfere with estrogen synthesis or action may disrupt ovarian development or even induce testis formation at female-producing temperatures (Lance and Bogart 1991, 1992; Wibbels and Crews 1992, 1994; Crews et al. 1994; Pieau et al. 1994a; Richard-Mercier et al. 1995). This indicates that estrogens play a major role in sexual differentiation in reptiles with TSD. Temperature also influences the synthesis of estrogen by influencing the activity of aromatase, the enzyme responsible for the formation of estrogen (Crews 1994; Jeyasuria et al. 1994; Jeyasuria and Place 1997). In turtles (Desvages and Pieau 1992; Pieau et al. 1994b) and alligators (Smith 1997), aromatase is active only at female-producing temperatures, which suggests that aromatase activity and the production of estrogen may stimulate female development in reptiles with TSD.

Temperature effects on androgen synthesis and activity may also contribute to TSD in reptiles. For example, administration of testosterone has little effect in inducing males in species with TSD, but DHT can induce predominantly male hatchlings at temperatures that would normally produce both sexes in *Trachemys scripta* (Crews et al. 1994). DHT is produced from testosterone via the action of 5-alpha-reductase, and inhibitors of 5-alpha-reductase demonstrated that the enzyme plays a role in testis differentiation and ultimately in production of males (Crews and Bergeron 1994). Although additional study must be completed, species-specific differences in the synthesis and activities of estrogens and androgens in species with TSD may determine an embryo's sexual differentiation. The ability of environmental factors to alter reptilian sex ratios may be critical to population level responses to chemical stressors, and altered outcomes linked to TSD-chemical stressor interactions may have significant implications for ecotoxicology (Crain and Guillette 1998). Despite increased knowledge gained since Palmer's original overview (Palmer 2000), questions regarding the mechanisms of TSD in reptiles remain an active area of physiological research. The role that

chemical stressors — singly or in concert with other environmental stressors — play in exposures to reptiles in the field requires additional study.

In anurans, initial sexual differentiation and development of secondary sex characteristics during maturation are at some level dependent on aromatase activity and exposure to steroids (estrogen or androgen) and thyroid hormones (see Hayes 1997 for review; Ertl and Winston 1998). In amphibians, metabolism generally is temperature dependent (Hayes and Licht 1995), and since aromatase activity is influenced by temperature, alterations in estrogen and testosterone levels may also be induced by temperature. Gonadal reversal experiments conducted with *Pleurodeles*, similar to those conducted with reptiles (Bergeron et al. 1994; Crews et al. 1996), successfully demonstrate a thermosensitive window; however, test conditions were not considered environmentally relevant (Pieau et al. 1994b; Chardard et al. 1995). Although effects on sex characteristics observed under field conditions could be related to endocrine disrupting chemicals (EDC) exposure that results in altered endogenous androgenic or estrogenic receptor binding or function, observed effects could also result from the activity of the temperature-sensitive steroidal enzyme aromatase (Pieau et al. 1994b; Sheffield et al. 1998; Ertl and Winston 1998).

Hormonal and reproductive measures under baseline or controlled conditions are potential endpoints for evaluating EDC effects in animals tested during laboratory and microcosm studies, and in those collected from contaminated field conditions (Gendron et al. 1997). Hormone disruptors, such as estrogenic or androgenic EDCs, can affect critical life stages, for example, the organization of gender determination of the gonads and brain during initial development and the activation of endocrine and behavioral responses during sexual maturation (Noriega et al. 1997). For example, brain neurons associated with the male frog larynx are sexually dimorphic; treating females with androgens can masculinize their larynx (Burggren and Just 1992). Some EDCs, such as dioxins, also target the thyroid system, and may therefore have effects on sexual differentiation and other developmental processes, including metamorphosis. Research on the role of environmental factors in sex determination and on the activation and timing of the interaction between steroid and thyroid hormones continues.

5.6.2 Metamorphosis

Most amphibians follow a developmental process unique to vertebrates — metamorphosis. Metamorphosis consists of a series of postembryonic biochemical, morphological, and physiological changes that transform larvae into adults (Dent 1988; Galton 1988; Eales 1990; Hayes and Licht 1995; Kaltenbach 1996; Wright et al. 1997; Denver 1998). Not all amphibians metamorphose, however. Most Plethodontidae salamanders and some anurans deposit eggs on land where embryos undergo direct development, effectively bypassing the larval stage. Neotenic mudpuppies (*Necturus* spp.) and hellbenders (*Cryptobranchus* spp.) lay their eggs in water and remain aquatic throughout their life. When amphibians do metamorphose, the changes that occur are significant (Frieden 1963; Kaltenbach 1996). Stages during metamorphosis are defined by hormonal and anatomical events such as tail resorption or skin keratinization.

The thyroid plays a critical role in regulating metamorphosis. During premetamorphosis, follicular cells of the paired thyroid glands grow and become secretory (Gancedo et al. 1997). Tetraiodothyronine (T4, thyroxine) and triiodothyronine (T3) are released into the bloodstream, stimulating an increase in the peripheral thyroid receptors (Wright et al. 1997). Subsequent to this stage, T4 and T3 levels increase and the hypothalamic-pituitary-thyroid axis is activated (Norris and Gern 1976). Thyroid-stimulating hormone (TSH) from the anterior pituitary acts on the thyroid to control gland activity (Norman and Norris 1987; Miranda et al. 1996). During metamorphic climax, thyroid hormones (primarily T3) induce the biochemical, morphological, and functional changes associated with the transition to adulthood (Frieden 1963; Dent 1988; Galton 1988). Direct and indirect endocrine functions include the thickening and keratinizing of the thin, multilayer larval skin, which helps to conserve water and reduce the potential for serious mechanical injury in

the terrestrial adult. Mucus produced by dermal glands helps in thermoregulation and osmoregulation, forming a barrier to epithelial water loss (Shoemaker et al. 1992; Duellman and Trueb 1994). Thyroid hormones influence ossification of cartilage and myosin/tropomyosin synthesis for muscle (review in McNabb and King 1993). Thyroid activates lipogenesis, resulting in lipid storage in the liver and fat bodies to meet increased energy needs. During metamorphosis, the tail is resorbed, gill arches degenerate, and gills regress (Duellman and Trueb 1994). Following tail loss, thyroid hormone levels decrease (Boutilier et al. 1992).

Other hormones involved in metamorphosis include prolactin, growth hormone, insulin, and adrenal corticoids (Brown et al. 1991; Kobayashi and Kikuyama 1991; Hayes and Wu 1995; Hayes 1995a, 1995b; Kloas et al. 1997). Anterior pituitary prolactin stimulates tissue growth (e.g., tail and gut in tadpoles and gills in salamander), regulates water and electrolytes, stimulates intestinal absorption of amino acids and glucose, and decreases hydrolytic enzyme activity involved with tissue regression (Dent 1988). Lipolytic prolactin levels run counter to thyroid hormone concentrations, possibly regulating at the level of the brain nerve terminals and monoaminergic system rather than at the receptor level (Burggren and Just 1992). Adrenal steroids accelerate thyroid-induced metamorphosis, whereas growth hormone promotes tissue growth. Pancreatic insulin stimulates cutaneous ion transport (Boutilier et al. 1992) and helps control blood glucose levels. Insulin levels increase in the pancreas and serum in the larvae until metamorphic climax.

Each of the transition states of metamorphosis — egg to larva to adult — presents different interactions with the environment; hence, exposure across life history stages potentially varies significantly. The disposition of chemicals accumulated in an earlier stage of development may affect stages yet to come; for example, chemicals stored in the larval tail may be redistributed and become available for metabolism, and epithelial restructuring can modify rate and transport during chemical uptake. Historically, both laboratory and field studies have indicated varying sensitivities to chemical exposure across stages of development (Sanders 1970; Saber and Dunson 1978; Dial and Bauer 1984; Dial and Dial 1987; Berrill et al. 1993). Factors contributing to differential sensitivity include individual tolerance (Dial 1976; Dial and Bauer 1984; Dial and Dial 1987; Rowe et al. 1998), development of resistance (Browne and Dumont 1979), time of exposure relative to organogenesis and metabolic state of the embryo (Honrubia et al. 1993), differential development of immune or other physiological resistance response mechanisms (Dial and Dial 1987; Sheffield et al. 1998; Van Der Kraak et al. 1998), and the ability to modify chemical uptake, metabolism, or clearance due to temperature regulation, degree of hydration, or protein and enzyme synthesis and induction (Suzuki and Akitomi 1983; Rosenbaum et al. 1988; Herkovits and Oerez-Coll 1993; Lizana and Pedraza 1998). The extent and permanence of adverse effects depends on the timing of exposure during cellular development (Honrubia et al. 1993) or on the stage-dependent ability to synthesize effective isoforms of proteins such as metallothionein (Herkovits and Oerez-Coll 1993; Vogiatzis and Loumbourdis 1998). Many of the more persistent lipophilic chemicals may be sequestered in lipid-storing premetamorphs, but they may be redistributed in the carbohydrate-storing postmetamorphs (Honrubia et al. 1993). The egg stage cannot completely avoid chemicals that may occur in their aquatic environment; however, depending on the chemical, envelope and jelly coatings may confer some protective barrier to the developing embryo (Berrill et al. 1997; Jung and Walker 1997). The extent of this protection may depend on the number and type of envelopes and on the distribution of eggs and egg mass design (Dunson and Connell 1982; Seymour and Bradford 1995; Carey and Bryant 1995; Ovaska 1997). Risks may differ depending on exposure; for example, aquatic amphibians cannot completely avoid dissolved chemicals, whereas terrestrial ones may be able to avoid contaminated microhabitats behaviorally (e.g., by burrowing). For example, postmetamorphic juvenile *Scaphiopus couchii* are more susceptible to herbicide toxicity than are their adult counterparts, possibly because their smaller size and larger surface-to-volume ratio lead to increased chemical uptake (Judd 1977). On the other hand, late-stage larvae of both *Rana pipiens* and *Bufo americanus* are more sensitive to herbicides than are those tested at an earlier stage (Howe et al. 1998). Interspecific sensitivity may

be related to size of eggs and developmental stage (Berrill et al. 1997). Because of differences in limb development, chemicals such as tributyltin are effective teratogens on hindlimb development but not on limb regeneration (e.g., in *Ambystoma mexicanum* [Chang et al. 1976; Scadding 1990]). EDCs can alter the timing and rate of metamorphosis, the development of immune and stress responses, and the overall fitness of the transformed population (Bishop 1992; Bonin et al. 1997; Larson 1998; Rohr et al. 2003, 2004, 2006). In addition, exposure of larvae to EDCs can have insidious and persistent effects on adults (Rohr and Palmer 2005).

In some instances, species may become reproductively mature without completing metamorphosis. Pedomorphic larvae are sexually mature as a result of accelerated gonadal development (Pough 1989; Whiteman 1994; Whiteman et al. 1996). Neotenic larvae are able to reproduce as a result of delayed somatic development. Under normal conditions, neoteny does not occur in anurans, and obligate neoteny in species of *Necturus*, *Proteus*, and *Amphiuma* is related to tissue insensitivity to thyroid hormones (Norris et al. 1977; Hayes 1997; Larson 1998). Facultative neotenic populations such as *Ambystoma tigrinum* occur under certain environmental conditions. One neotenic morph remains aquatic throughout its life cycle and lives in a permanent pond environment; a smaller aquatic morph inhabits more ephemeral ponds and metamorphoses to a reproducing adult; and a third morph remains aquatic but morphologically develops a larger head, wider mouth, and longer teeth to become a more significant carnivorous predator (Collins 1981; Whiteman and Howard 1997).

5.6.3 Endocrine-Disrupting Compounds

As demonstrated previously, reproduction and development are strongly regulated by the endocrine system and susceptible to the effects of EDCs (see Hayes 2000; Guillette 2000). Recent studies with wildlife indicate that other endocrine systems can be critically impaired by exposure to EDCs, independent of direct receptor-binding interference and outside of initial development and metamorphosis. Additional systems and hormones potentially affected in amphibians and reptiles include, but are not limited to, the following:

- neuroreceptors associated with the pineal gland, pheromones, and sensory organs used for intraspecific communication;
- nonsteroid hormones such as GnRH and arginine vasotocin related to reproduction and mating behavior;
- pituitary melanophore-stimulating hormone and its control of skin pigmentation;
- parathyroid hormones, prolactin, and vitamin D related to Ca regulation;
- pancreatic insulin and glucagon regulation of glucose and fat deposition associated with seasonal activities; and
- catecholamines that stimulate glycogenolysis and control the cardiovascular system and adenocorticotrophin and glucocorticoids (e.g., corticosterone) associated with quick response and long-term adjustments to stress or environmental change (see discussion in Honrubia et al. 1993; Hayes et al. 1997).

Any disruption to normal endocrine activity needs to be understood within the context of life stage and potential intrinsic interactions. Reference hormonal levels and their influence may be different in larval and adult animals (Duvall and Norris 1980; Kwon et al. 1991, 1993; Hayes and Licht 1995; Hayes and Wu 1995; Hayes 1995a, 1995b; Hopkins et al. 1997; Kloas et al. 1997) and under different environmental conditions (Johnson 1976). Differences may also be expressed among species. For example, T4 is involved in molting in salamanders but not in anurans (Herman 1992), and as a result, sensitivity and response to EDCs will likely differ. The endocrine interactions among species, developmental stage, reproductive condition, and the environment need further research in order to accurately interpret both laboratory and field experimental data.

5.7 BEHAVIOR

Changes in behavior are often the first indication of exposure to environmental stressors, including chemical contaminants. For example, a common observation used in laboratory studies with amphibians is avoidance response to gentle prodding; for example, unexposed tadpoles will move directly away from prodding (Rosenbaum et al. 1988; Walker et al. 1996; Sparling et al. 1997). Observed swimming patterns can be indicative of central-neural, peripheral-neural, or neuromuscular system effects. Observations can be quantified; swimming speed for individuals in *Bufo americanus* and *Rana clamitans* has been a useful measure of contaminant effects (Jung and Walker 1997; Raimondo et al. 1998). Although there are some recent data on indirect toxic effects on predation (Jung and Walker 1997; Raimondo et al. 1998; Relyea and Mills 2001; Relyea 2003, 2004, 2005; Rohr and Crumrine 2005), to date very little data have been recorded on effects of contaminants on activities such as amplexus, calling, brooding and parental behavior, ability to catch prey, or level of seasonal migratory drive. Similarly, although the behavior of reptiles, particularly those activities linked to integrative responses associated with hormonal control of reproduction, continues to be a research area of keen interest to herpetologists and evolutionary biologists, very little work has focused on the role that environmental chemicals may have in modulating behavior with the exception of work related to EDCs in the environment.

5.7.1 Sensory Organs

Linking external environmental stimuli to responses observed in herpetofauna in field or laboratory settings hinges, in part, on sensory organs characteristic of amphibians and reptiles (Gans and Crews 1992). As Kardong (2005) suggests, the sensory systems of amphibians and reptiles typically present structures and functions similar to other vertebrates, although species-specific differences across the range of animals contribute to much variation on the basic vertebrate motif. Sense receptors may be simply categorized as somatic receptors (e.g., neuromast organs, the membranous labyrinth of the inner ear, light receptors, proprioreceptors, and capsulated and uncapsulated cutaneous receptors) and visceral receptors. Modifications of these basic types occur in some of the herpetofauna, such as infrared receptors of snakes. As in vertebrates across all classes, visceral receptors include olfactory organs, taste buds, and the vomeronasal organ, as well as naked nerve endings in the viscera that serve as stretch receptors, chemoreceptors, baroreceptors, and osmoreceptors. Sensory organs may be characterized as being relatively widespread, serving a general function such as sensation of temperature or proprioreceptors. On the other hand, specialized sensory organs are limited in their distribution and function; for example, chemoreceptors of the nasal or vomernasal organs display a range of specialized structures in the herpetofauna that may capture different modalities, such as infrared receptors of snakes like the pit vipers, than commonly presented by "higher vertebrates." Given the typical vertebrate layout of sensory receptors and sensory organs, it is not surprising that we know relatively little regarding the effects of chemical exposure on these structures or their functions.

Aquatic amphibians, like the fishes, have lateral lines to help them navigate and maintain balance. These sensory organs consist of mechanoreceptors and electroreceptors that are located within canals on the surface of the head and body. Lateral lines and their receptors help aquatic amphibians navigate, particularly when visual orientation is difficult because of murky water, and detect wave or pressure changes created by a predator's or prey's movement (Burggren and Just 1992; Butler and Hodos 1996). Anurans and caecilians lose their lateral line organs at metamorphic climax, while urodeles retain them (Lannoo and Smith 1989).

Pheromones and specialized olfactory sensory organs are active under fossorial or other conditions of low light (e.g., in *Hydromantes* spp., *Plethodon* spp., and caecilians); auditory stimuli are used to locate calling frogs (e.g., *Bufo* spp.). The primary system used for detecting predator or prey in adult anurans and salamanders is visual (Brooks 1981). During the transition from water

to air, adjustments are made in the visual system (for review of specific morphological changes, see Duellman and Trueb 1994). Focus is adjusted for changes in the fluid medium, and structural modifications are made in the cornea and lens. For example, numbers and types of photoreceptor cells increase, and there is a shift from primarily cones to primarily rods. Photopigments change from primarily 3-dihydroretinal-based porphyropsin (blue) to retinal-based rhodopsin (red), with larval pigment absorptions being at the longer wavelengths (for review, see Burggren and Just 1992; Wilczynski 1992). Eyelids are developed for life on land. As the adult anuran becomes carnivorous and its foraging mechanism changes, there is a corresponding shift to accommodate the ipsilateral optic projections, brain connections, and binocular vision needed to capture moving prey (Burggren and Just 1992). Such changes in the visual system are not limited to metamorphosis, however. *Notophthalmus* newts are first terrestrial, then aquatic, then terrestrial again, and pigmentation enzymes are converted accordingly. In adult anurans, random isomerization of visual receptors is temperature sensitive. Despite the relatively low body temperature of the adult frog, the threshold for receptor isomerization has an even lower set point to ensure that vision-dependent foraging and other activities are negatively affected at ambient temperatures commonly encountered during evening forays (Rand 1988; Wilczynski 1992). Very little information about chemical effects on amphibians' vision and auditory systems has been published, but given the importance of these systems to survival, additional research is warranted.

While the early literature focused on the sensory biology of reptiles remains valuable to our understanding of the interactions between organisms and their environments, there is scant work in the peer-reviewed literature regarding effects of chemical exposure on altered sensory functions, especially as relates to changes in behavior due to exposure or that might alter exposure (e.g., through contaminant avoidance). Although the interrelationships between sensory systems and behavioral responses linked to exposures to environmental chemicals have been repeatedly recognized as critical to our understanding events occurring in the field (see Grue et al. 1997; Burger 2006), responses of integrated systems such as the sensory-behavior system of the herpetofauna and their responses to environmental stressors must receive more focused study in the future.

5.7.2 Locomotion and Foraging

Foraging strategies and feeding behaviors are limited by locomotion and its underlying biomechanics supported by physiology and biochemical processes. Locomotion and foraging involve a range of integrated processes reliant on interactions among gastrointestinal and nutritional physiology, neurophysiology, and skeletomuscular and behavioral mechanisms. Each of these processes may be considered at various levels of biological organization ranging from organismal to molecular, yet all reflect responses to various types of environmental stressors. Variability linked to these environmental stressors and the resulting organismal responses may provide insight into patterns of adaptation that influence exposure to environmental chemicals, since "whole animal" responses (e.g., behavioral strategies linked to foraging) to environmental conditions influence foraging, and are subsequently linked to digestive efficiency. All are intricate links to chemical exposures in the field, and outcomes of exposure may subsequently influence developmental patterns and growth, as well as other endpoints identified by ecotoxicologists.

5.7.3 Amphibians and Reptiles: Entanglements of Chemical Exposures, Foraging, and Feeding Habits

If exposure to chemical stressors in the herpetofauna were dominated by dietary routes, then screening level evaluations of effects in amphibians and reptiles linked to chemical stressors would be guardedly developed under the best of circumstances. And, beyond a screening level analysis, the sparingly available existing data and previously published information clearly suggest additional

study be considered, if herpetofauna are central to the evaluation process. While existing data suggest that amphibians sense and subsequently avoid toxins from conspecifics or predators, little, if any, information is available related to their ability to detect the presence of chemical contaminants (Steele et al. 1991). Depending on the species' natural history and preferred habitats, chemical exposure may be reduced if avoidance behaviors are sufficient and habitat heterogeneity provides refugia to reduce or avoid chemical exposures. For example, aquatic amphibians may not be able to avoid chemical exposure if chemicals released to their local environment are widespread or their eggs or early developmental stages are limited with respect to their avoidance capabilities. Similarly, if contaminated soils are widely distributed throughout preferred habitats, reptiles and terrestrial life stages of amphibians may be chronically exposed to contaminated soils as a consequence of their high fidelity to sites within these contaminated areas (Matson 1998). Indeed, depending on the matrix involved in chemical exposure, habitats linked to critical life stages, or critical periods in a species' life history (e.g., breeding, egg development), chemical exposures will inevitably be entangled with the integrated processes characteristic of foraging and feeding habits, as well as physiological events predicated on these starting points intended to acquire essential energy resources.

Entanglements among chemical exposures, foraging, and feeding habits may ultimately yield adverse effects on integrated neurological and musculoskeletal mechansisms that diminish locomotor activity, which inevitably affects foraging. For example, in amphibians each stage of development generally has different locomotor adaptations (e.g., larval anurans have spinal cord segmentations for localized locomotor control, but these segmentations are condensed during metamorphosis), and these in part may determine differential behavioral responses consequent to exposure. After metamorphosis, locomotion in both anurans and urodeles is shifted from swimming or walking in water to traveling on land. In aquatic habitats, adult frogs swim by propelling water between their hind limbs and pushing off with the webbing between their toes, and hop by using a series of short leaps that bring both legs into the air simultaneously. In contrast, aquatic salamanders swim by undulating their tails. Most of the rhythmic activity in the anuran (i.e., tail undulation, kicking, or jumping) is due to a central pattern generator, which is an interneuronal network coordinating and synchronizing motor neurons (Burggren and Just 1992; Butler and Hodos 1996) that may be adversedly effected in response to a wide range of environmental chemicals. Hence, exposures in the field may disrupt neurological function and control of locomotor activities, which leads to deficits in foraging and predator avoidance behaviors (Rohr et al. 2003).

As with any foraging or prey-predator system, feeding activities present increased risks of predation to herpetofauna. But, unless adversely affected from chemical exposure, nominal predator avoidance reduces those predation risks, as a result of integrated neurobehavioral and musculoskeletal actions yielding a range of escape behaviors. Energy reserves are required to physically avoid predators. For example, high aerobic–low anaerobic metabolism and aerobic citrate synthase are associated with slow-moving amphibians (e.g., toads), whereas high anaerobic–low aerobic metabolic adjustments and glycolytic enzyme activity are associated with quick-moving amphibians (e.g., tree frogs). As in other vertebrates, if anaerobic demands are too high, lactic acid in tissues increases, resulting in exhaustion (Pough et al. 1992). Some amphibians escape predation by secreting tetrodotoxins or by colorfully advertising their toxin production, but scant information on effects of chemical exposure related to color changes or toxin secretions has been reported. Similarly, little, if any, characterization of chemical effects on integrated functions critical to foraging and acquisition of energy stores is available for reptiles.

Comparative physiologists and physiological ecologists strive to synthesize generalizations from the diversity of traits observed in animals, particularly as those generalizations relate to integrated functions such as locomotion and derivative activities such as foraging. Variations due to size, temperature, locomotor mechanisms, gaits, differential influence of Reynolds number and lotic habitats on body size, and capacity to store mechanical strain energy all contribute to this variation, and energy resources serving these functions display a similar range in diversity. Regardless of

their preferred foods and the time course for acquisition, foraging incurs a major energetic expense across the wide range of species of herpetofauna (reviewed in Pough et al. 1992; Bridges 1997). These energetic costs depend on environmental factors such as distribution of prey and predators, temperature, habitat quality, pH, and humidity, each of which may interact with chemicals in the environment. Environmental chemicals directly and indirectly affect an animal's foraging and feeding activities, or the assimilation of foods once consumed, for example, by reducing or eliminating forage or prey items available in the field, by incorporation of tissue residues into the food base and rendering it toxic, and by altering the animal's ability to consume and assimilate the food (Hall 1990; Walker et al. 1996).

Risk of exposure to environmental chemicals and their effects will depend, in part, on the animal's foraging behaviors and feeding strategies. As noted in Section 5.3.1 some amphibians and reptiles can survive without food for extended periods, ranging from weeks to months to years. In other species, risks linked to foraging behaviors and feeding strategies will also vary as a function of developmental stage. For example, during metamorphosis in amphibians, feeding is reduced, growth becomes arrested, and developmental events characteristic of metamorphosis rely on available energy stores, including those derived from tail resorption. During metamorphosis, changes in diet are associated with changes in the digestive structures. The foregut-midgut of herbivorous anuran larva stores food and associated glands help in extracellular digestion. Absorption is maximized by the characteristic gut coiling and extensive production of pancreatic and hepatic enzymes. As the gut regresses, the coiling is lost, and pepsin-secreting cells form the functioning stomach, while larval laminar cilia are replaced by functional microvilli (Duellman and Trueb 1994). Parallel to these changes, the pancreas is restructured as a functional component of the endocrine system, and kidney and liver enzyme functions shift from excreting water to conserving fluids, and from producing ammonia to producing urea waste (Duellman and Trueb 1994). With the change in available oxygen, hemoglobin production and control by the spleen and liver are increased. The integrated outcome yields postmetamorphosic anurans as carnivores. Salamanders do not undergo such extreme morphological transitions and are therefore able to feed indiscriminately on aquatic invertebrates and algae and, as they grow, other larval amphibians.

Throughout the herpetofauna, foraging tactics vary across species. Within a species, foraging tactics may differ from habitat to habitat, and within-habitat differences in predatory and foraging behaviors will depend on prey or forage items available (e.g., foraging in preferred habitat may differ from that displayed in marginal habitats). In both amphibians and reptiles, selection of forage and prey items may change with increased animal size (Leff and Bachmann 1986); for example, maximum prey size may increase as a function of gape size, which in turn may reflect the changing energetic demands linked to growth and reproduction. Effects of chemicals occurring in food items may affect growth of early life stages, and rates of development may be critical in determining whether the organism will meet its nutritional requirement as it matures. Growth and associated gape size also influence consumption rates, which inevitably influence ingestion of environmental chemicals contained in food sources.

Regardless of the role that chemical stressors play as components in an animal's interaction with the environment, a general metric for success of individuals or a population is the balance between energetic costs and benefits directly or indirectly related to these costs (see Cohen 1978 and Stephens and Krebs 1986). For example, foraging by terrestrial herpetofauna reflects a dynamic balance involving a wide range of behaviors and "hunting" techniques (regardless of their being carnivores or herbivores) that vary in duration, locomotor costs, and energetic rewards. Various feeding strategies are reflected in the life histories of amphibians and reptiles, and may conveniently be categorized based on feeding habits. The adaptive interplay between foraging and feeding behaviors, and the capacity to regulate digestive performance among amphibians and reptiles (as well as vertebrates in general) clearly have implications for evaluating exposures to environmental chemicals. While recent activity indicates an increased awareness of research needs to characterize endpoints pertinent to amphibians and reptiles, much additional work must be

completed to achieve parity with other vertebrates when topics related to chemical exposure and effects are considered.

5.8 BIOMARKERS, METABOLISM, AND DEVELOPMENT OF ENERGETICS-BASED TOOLS

Biomarkers have increasingly been applied to field and laboratory studies focused on chemical exposures and effects in herpetofauna. For example, Venturino et al. (2003) identified biomarkers commonly applied to studies focused on amphibians, and Linder, Lehman, and Bidwell (Chapter 4, this volume) noted that biomarkers have increasingly been applied to evaluating exposure and effects in herpetofauna exposed to environmental chemicals in aquatic habitats. Walker et al. (2001) characterized biomarkers as morphological alterations, genetic effects, behavioral parameters and tissue residue levels, which extended Huggett et al. (1992), who had identified biomarkers as biochemical, physiological, and histological endpoints used to evaluate exposures and effects of chemical stressors. Biomarkers used in ecotoxicological investigations focused on amphibians and reptiles are similar, if not identical, to those applied to studies of other vertebrates. Ideally, by using biochemical and physiological endpoints to evaluate exposure, adverse effects linked to chemical exposures may be anticipated before responses are observed at organismal or population levels of organization (Newman and Unger 2003; Mitchelmore et al. 2006). Although data may not be available for evaluating the status of herpetofauna across their current range of habitats (e.g., comparing populations presumptively exposed in the field to similarly collected data for populations at reference areas; see Henry 2000), long-term acquisition of such data will undoubtedly contribute to future characterizations of chemical exposure and the general health status of the herpetofauna existing under a wide range of environmental conditions in the field (Rie et al. 2000; Venturino et al. 2003).

While larger-bodied herpetofauna (e.g., turtles) have historically found wide use in studies concerned with measurement of tissue residues, small body size and limited blood volume characteristic of many species of amphibians and reptiles may account for the lack of reference or baseline data for biochemical and hematological attributes. These measures vary with factors such as species, developmental stage, gender, reproductive status, season, and nonspecific stressors (Zhukova 1987). In contrast to data available for fishes, few reference data for biochemical and physiological markers (e.g., hepatic oxidized/reduced glutathione ratios, lipid peroxidation, other tissue-specific indicators, and routinely measured serum or plasma chemistries) have been compiled for herpetofauna. Although designed laboratory studies characterizing baseline conditions are encouraged to offset data deficiencies, opportunistically recording these data may help us to accumulate baseline measures and decrease the variance for future reference (Henry 2000).

Biochemical and physiological biomarkers for the herpetofauna include endpoints shared with other vertebrates. For example, in studying exposure and effects of lead in amphibians, Loumbourdis (2003) considered histological effects — the development of kidney inclusion bodies — in *Rana ridibunda*, while Arrieta et al. (2004) focused on the disruption of heme synthesis by measuring intermediate metabolites or degradation products (e.g., porphyrins) and altered aminolevulinic acid dehydratase (ALAD) activities in lead-exposed *Bufo arenarum*. Other studies applying biomarkers to the analysis of exposure and effects of environmental chemicals in herpetofauna have focused on enzymes frequently used in studies of other vertebrates exposed to chemicals in the field (e.g., Sparling et al. 2001, van den Brink et al. 2003 on amphibians, and Clark et al. 2000 on reptiles). Evaluations of enzyme activities characteristic of xenobiotic metabolism of polynuclear aromatic hydrocarbons and organochlorine compounds in amphibians and reptiles (e.g., measurements of mixed-function oxidases and associated enzymes) have been reported (Venturino et al. 2001; Gunderson et al. 2004; Kostaropoulos et al. 2005). For example, induction of liver enzymes that are part of the mixed-function oxidase (MFO) system has been used to indicate exposure to a range of organic chemicals (including pesticides) in a number of vertebrates, but reduced activity of these

enzymes in amphibians may limit their use as a biomarker for this group (DeGarady and Halbrook 2003; Venturino et al. 2003). While components of the MFO system are present in amphibians and reptiles, their activities occur at levels lower than those measured in mammalian systems (Schwen and Mannering 1982a, 1982b, 1982c; Ertl and Winston 1998). Desulfuration, hydroxylation, epoxidation, conjugation, reduction, and hydrolsis reactions are readily measured in tissues (e.g., liver), but induction of cytochrome P450 is consistently lower in the herpetofauna than in birds and mammals (Ertl and Winston 1998; Huang et al. 1998), which may reduce its effectiveness as a biomarker of exposure. The capacity to detoxify chemicals via MFOs will vary from substrate to substrate and will likely differ for aquatic and terrestrial amphibians. Similarly, MFO activity will vary as a function of developmental stage. For example, *Rana catesbeiana* tadpoles collected from chemically polluted sites exhibited higher metabolic rates than did those collected from reference sites (Beatty et al. 1976; Rowe et al. 1998), yet developmental time may influence the capacity for enzyme induction across a range of species. In general, species of herpetofauna may have developed mechanisms of resistance to environmental chemicals as a result of natural selection (Boyd et al. 1963; Hall and Kolbe 1980).

Studies focused on herpetofauna and the role that environmental chemicals have in disrupting endocrine function illustrate recent efforts to measure biomarkers of exposure and effects in field and laboratory studies. For example, numerous studies have considered exposure to endocrine-disrupting chemicals and subsequent effects on gonad morphology, sex hormones, and the levels of reproductive hormones in various amphibians and reptiles (see, e.g., Hayes et al. 2002, 2003; Yang et al. 2005), which reflect increased research beyond Hayes (2000) and Guilette (2000). Indeed, amphibians and reptiles have played an important role as indicators of EDCs in aquatic systems. Most work to date has focused on effects related to sex determination, with biomarkers including gonadal morphology or circulating levels of plasma hormones or specific proteins (e.g., Noriega and Hayes 2000; Shelby and Mendonca 2001; Hayes et al. 2003). Many egg-laying reptiles may be particularly suited for studies of chemicals that affect sex hormone balance, since they normally have TSD, which facilitates manipulation of sex ratios to more clearly evaluate chemical effects (Crews et al. 1995; Newman and Unger 2003).

Another biochemical marker frequently applied to studies focused on herpetofauna exposed to EDCs is vitellogenin (see Section 5.4.3.1). Although males and females are both genetically capable of synthesizing vitellogenin, production is induced by estrogenic compounds; hence, vitellogenin is a relatively sensitive biomarker of estrogenic chemical exposure to males (Palmer et al. 1998). Similarly, recent studies also indicate a significant role for herpetofauna in the evaluation of chemicals that disrupt the thyroid axis. Effects on thyroid hormones — thyroxine (T4) and triiodothyronine (T3) — linked to exposures to environmental chemicals have been observed in a range of amphibians and reptiles over the past 10 to 15 years (Gunderson et al. 2002; Tada et al. 2004; Mosconi et al. 2005; Yang et al. 2005). The role of thyroid hormones for initiating hatching and the onset of metamorphosis was well studied in herpetofauna prior to the ecotoxicologists' focus on endocrine-disrupting chemicals released to the environment (Brasfield et al. 2004; Furlow and Neff 2006; Tata 2006). Given the heightened awareness of emerging contaminants that occur in treated effluents and water treatment residuals such as biosolids, future work focused on effects linked to altered endocrine function will inevitably increase.

As in birds and mammals, metallothionein[1] (MT) has been used as a biomarker for exposure to metals, such as copper, zinc, and cadium, which induce MT synthesis in herpetofauna as they do in other vertebrates (Suzuki and Akitomi 1983; Vogiatzis and Loumbourdis 1998). Similarly, as a diagnostic tool for exposure to organophosphorus (OP) and carbamate pesticides, cholinesterase

[1] Metallothioneins (MTs) are low-molecular-weight proteins found in all eukaryotes (often in multiple copies) as well as some prokaryotes. MTs are unusually rich in cysteine residues that coordinate multiple zinc and copper atoms under physiological conditions. Cadmium-induced synthesis of MTs has been observed in herpetofauna, as was previously described in fishes and wildlife.

inhibition measured in serum, plasma, red blood cells, or other tissues (Sparling et al. 2001) has been increasingly applied in ecotoxicological studies focused on amphibians and reptiles. Additional studies relying on measurements of cholinesterase activity as a tool to evaluate OP and carbamate exposure should be encouraged to ensure the tool's value being comparable to that seen in other vertebrate classes (Baker 1985; Rosenbaum et al. 1988; Bonin et al. 1997). Research on amphibian response to OP and carbamate chemical effects indicates a wide range of species sensitivity as measured by various endpoints (Hall and Kolbe 1980; Rosenbaum et al. 1988; Snawder and Chambers 1993; Honrubia et al. 1993; Sparling et al. 1997; Taylor et al. 1999a, 1999b, 1999c, 1999d). As with other vertebrates, field and laboratory investigators have also employed a range of diagnostic tools to evaluate exposure and effects, including clinical chemistry analyses on serum and tissue samples (see, e.g., Papadimitriou and Loumbourdis 2005).

Relatively new to ecotoxicology and herpetofauna research are immunological markers, including tools that evaluate cellular immune function through nonspecific cytotoxic cells and macrophages. These tools have been advanced by researchers evaluating a variety of chemical stressors (e.g., *R. pipiens* exposed to low pH; see Vatnick et al. 2006). At present, relatively little is known about the immune system in most of the species of amphibians (Carey and Bryant 1995; Taylor 1998), and based on the differential susceptibilities to infections such as red leg caused by *Aeromonas hydrophilla*, there are potentially significant interspecific differences. Amphibians possess the major tissues associated with immune response, such as thymus, spleen, kidney, bone marrow, and lymphoid cells (Carey and Bryant 1995), as well as the ability to induce antibody response to initial and subsequent antigen exposure. There are differences in immune response depending on stage of development, and the immune system seems to undergo near-complete change during metamorphosis (Carey and Bryant 1995). Immune cell function, however, is also temperature and season dependent (for review, see Taylor 1998). Although comparative immunologists have considered the herpetofauna over many years, ecotoxicologists have yet to benefit from that experience, in many respects tracking the history of the discipline's experience with fishes, birds, and mammals.

Matching the increased interest in immunological markers are tools focused on measurement of genetic markers. Expanded suites of tools focused on genetic markers have been developed in the recent past focused primarily on vertebrates, but these tools are relatively underemployed in studies targeted on herpetofauna. Measures related to DNA strain breakage and sister chromatid exchange have been applied to studies of herpetofauna (see, e.g., Wirz et al. 2005; Tverdy et al. 2005), but the area is potentially rich for development by ecotoxicologists.

Various tools are available in the physiological ecologist's tool box that would be amenable to application to ecotoxicological studies, including energetics-based biomarkers that could provide a common currency — energy and materials — that directly links individual, population, and community levels of organization (Congdon et al. 2001). Rowe et al. (2003) discussed energetics as it relates to larval, juvenile, and adult stages of anuran amphibians, and clearly identified the role that chemical stressors may play in increasing maintenance costs and decreasing energy available for growth. Along similar lines of discussion, reptilian eggs may also serve as valuable models to study the energetic effects of chemical stressors, since development of the embryo relies entirely on internal yolk stores, and contaminants may pass across the eggshell in association with imbibed water (Moeller 2004). Application of energetics analysis has been advocated for studies focused on amphibians and reptiles (Rowe et al. 2003), and a wide range of tools are potentially available to the ecotoxicologist. For example, 1 tool commonly deployed to study energetics in vertebrates — measurement of specific dynamic action — has received only limited use by ecotoxicologists in their study of herpetofauna.

5.8.1 Specific Dynamic Action

Specific dynamic action (SDA) represents the summed energy expended on ingestion, digestion, and assimilation of food (Brody 1945; Kleiber 1975). Given likely directions of regulatory applications

of food chain analysis as a tool to evaluate risks, deploying SDA in conjunction with integrated field and laboratory studies focused on dietary exposures to environmental chemicals seems pertinent to developing our tool box for evaluations of herpetofauna. SDA directly relates to the presumptive role of diet as a critical link between environmental chemicals and receptors. Before an animal can allocate ingested energy to growth, maintenance costs supporting daily functions and metabolism must be attained (Angilletta 2001). Physiological processes that contribute to SDA include gastrointestinal motility, production of digestive enzymes and nitrogenous wastes, protein catabolism and synthesis, and intestinal nutrient transport (Jobling 1981; Hailey 1998; Secor 2003; McCue 2006). Variations in the relationship between SDA and nutritient content of prey can influence growth, which can in turn impact survivorship, reproductive success, and ultimately fitness (Brodmann et al. 1997; Rosen and Trites 2000; Babu 2001). SDA reflects maintenance costs associated with food processing and, depending on species, has a varying impact on the net assimilated energy available for growth and reproduction, endpoints commonly measured in ecotoxicological studies.

In contrast to endotherms (see Costa and Kooyman 1984 and Hawkins et al. 1997), for amphibians and reptiles, the process of ingestion, digestion, and assimilation of food accounts for a much greater increase in metabolic responses (e.g., increased metabolic rates; see Coulson and Hernandez 1979; Secor and Diamond 1997a, 1997b; Secor and Phillips 1997; Powell et al. 1999; Secor 2001). Furthermore, these marked increases in metabolism are captured by an immedidate postprandial metabolic response and an extended period beyond the postprandial response in which metabolic rates are increased during the digestion and assimilation process (e.g., at least 2 weeks; see Secor and Diamond 1997a, 1997b; Secor 2005a, 2005b). As a consequence, from the perspective of developing an energy budget for a given animal, SDA contributes significantly to an ectotherm's energy budget (Secor and Nagy 1994; Peterson et al. 1998). While work with SDA has considered a wide range of metabolic responses experienced by amphibians and reptiles during digestion and assimilation, only limited consideration has been given to toxicant interactions with quality and quantity of meal, feeding frequency, body temperature, or body size as that tracks age or availability of food sources (see Secor 2005a, 2005b; McCue 2006; Secor and Boehm 2006). SDA as a "measure of effects" would enable ecotoxicologists to tap into existing literature in the physiological ecology of amphibians and reptiles. As such, using SDA as a measurement endpoint might benefit the evaluation of chemical effects linked to multiple stressor exposures in the field. Studies focused on measurement of SDA have relied heavily on amphibians and reptiles as experimental models, which clearly suggest that SDA and other tools of the physiological ecologist may be applied by ecotoxicologists to address toxicant effects in animals presumptively exposed predominantly via diet.

Overall, the role of biomarkers in evaluating herpetofauna exposure and effects and differences in biochemical and physiological characteristics between animal groups are presently incompletely understood, which may initially affect the utility of some variables for indicating contaminant exposure in regulatory applications. Nonetheless, our current implementation of biomarkers for the study of herpetofauna exposed to chemicals in the field is better developed than 10 years ago. Research over the next 10 years should refine those tools to a greater extent and allow time to more adequately develop tools potentially beneficial to the evaluation process for amphibians and reptiles.

5.9 INTERACTIONS OF CHEMICALS WITH PHYSIOLOGICAL AND ENVIRONMENTAL FACTORS

Exposures in the field and multiple stressors are commonly linked, oftentimes intractably, which contributes to confounded interpretation of effects associated with exposure to chemical stressors. Interactions between a wide range of variably responsive receptors and environmental factors, including chemical stressors, define the common ground of an ecotoxicologist and a physiological ecologist (see Relyea, Chapter 14, this volume). There are innumerable ways in which environmental factors can interact with the physiology of the herpetofauna. Here we illustrate 2 commonly

encountered environmental factors — ultraviolet-B (UV-B) radiation and stress — that are repeatedly encountered in characterizing effects associated with chemical exposures.

5.9.1 Ultraviolet Radiation

Ultraviolet (UV) radiation is that portion of the electromagnetic spectrum occurring between x-rays and visible light, having wavelengths between 40 and 400 nm. Its spectrum has been divided into vacuum UV (40 to 190 nm), far UV (190 to 220 nm), UV-C (220 to 290 nm), UV-B (290 to 320 nm), and UV-A (320 to 400 nm; see ISO/DIS 21348 [ISO 2005] for additional specifications on characterization of UV radiation). From an ecotoxicologist perspective, however, work focused on UV radiation relates primarily to observations of adverse effects linked to UV-B exposures, particularly those associated with elevated fluxes of UV-B radiation linked to ozone depletion. In applications to human and veterinary health, UV-B has long been studied in animal models because of its role in the synthesis of vitamin D_3, since UV-B at wavelengths between 270 and 300 nm (peak synthesis occurs between 295 and 297 nm) initiates conversion of 7-dehydrocholesterol to vitamin D_3. Historically, pathological and toxicological studies on UV-B have focused on adverse effects linked to prolonged exposures to nominal atmospheric fluxes, yet recent findings of diminished atmospheric ozone and the resulting increase in UV-B point toward singly or jointly acting effects linked to UV-B exposure.

In terrestrial and aquatic systems, UV-B is the wavelength of UV radiation of primary concern. In aquatic systems, UV-B penetration into freshwaters is strongly influenced by altitude, by the extent of plant canopy adjacent to the habitat, and by the concentration of dissolved organic material (DOM) in the water. DOM in surface waters results from heterogeneous inputs of decomposing plant, microbial, and animal materials that act as the primary absorber of UV-B. Interactions between DOM and UV-B may lead to photodegradation of these organic materials (Häder et al. 1998; Häder 2006), which in turn could promote positive feedback wherein UV-B exposure leads to greater UV-B flux and less DOM protection. In field settings, plant canopy characteristic of adjacent terrestrial and wetland habitats influences input of UV-B and other wavelengths of radiation to aquatic systems by absorbing and reflecting UV-B. Depending on the vegetation and the incidence of radiation, plant canopy may remove up to 90% of the incident light (Xenopoulos and Schlinder 2001).

Herpetofauna cannot help but be affected by increased incidence of UV radiation across the habitats upon which they depend. Little and Calfee (Chapter 13, this volume) extend previous reviews focused on UV radiation and its effects on freshwater vertebrates (Little and Fabacher 2003). In part, these reviews coincidentally followed from observations of Henry (2000) that depletion of atmospheric ozone significantly influenced the increase of UV-B globally and that increased incidence of UV-B inevitably played directly as a physical stressor for a wide range of receptors, including members of the herpetofauna. Interactions between UV-B radiation and chemical stressors have been increasingly reported as jointly acting physical-chemical stressors that serve reactive chemical species in the exposure mileau of a wide range of receptors (Little and Calfee, Chapter 13, this volume; Little and Fabacher 2003). While photodegradation contributes to fate processes for chemicals in the environment, UV-B photoactivates chemicals such as polynuclear aromatic hydrocarbons (PAHs) to yield hydroxyl radicals and oxides (see Sparling, Chapter 9, this volume). In part, the complexity of exposure in the field and the "thrust and parry" exchanges between stressors and receptors are captured by entangled systems that involve UV-B photoactivation, chemical stressors, and biota, since an animal's susceptibility to the UV-B effects may depend on its metabolic ability to bind and clear these highly reactive compounds (Ovaska 1997; Walker et al. 1998). Species-specific sensitivity has been correlated to photolyase activities and to specific life history strategies. Photolyase provides a repair mechanism for the DNA molecule damaged by radiation (Ovaska 1997). The enzyme is present and active in the less chemically

sensitive species (e.g., *Rana aurora*, *Hyla regilla*) and is less active, if present at all, in the more sensitive species (e.g., *Rana cascadae*, *Bufo boreas*, *Ambystoma gracile*; Blaustein et al. 1996).

Another major factor determining sensitivity to UV-B is the individual's likelihood of being exposed to UV-B. Radiation effects are cumulative; therefore, the thickness and moisture of the individual's skin surface and the burrowing habits and daily and seasonal activity of the individual are important considerations. DNA damage is particularly deleterious during embryonic development or metamorphosis, and factors such as egg pigmentation, distribution and arrangement of eggs and egg masses (laid singly, as sheets, or in masses), and the depth and clarity of the water in which eggs are deposited should also be considered. For example, under reference conditions, *Bufo bufo* eggs deposited in deep water are less exposed to sunlight than are the eggs of *B. calamita*, which are deposited surfically. If environmental conditions change, such as during global warming or drought, the *B. bufo* embryos with less photolyase could become more susceptible to UV-B effects than embryos developed from *B. calamita* (Lizana and Pedraza 1998). These species-specific sensitivities may be linked to population level effects, given studies on anurans and salamanders completed in the recent past that suggested that ambient levels of UV-B (290 to 320 nm) can affect individuals and populations (Blaustein et al. 1994b, 1996; Lizana and Pedraza 1998).

Direct UV-B effects observed include embryonic mortality and failure to hatch (Blaustein et al. 1994a, 1996), abnormal larval development, increased limb and musculature deformities, neurological damage, immunosuppression, and increased cellular damage in the eyes and skin surface (see Ovaska 1997 for review). Indirect effects include increased susceptibility to fungal infestations (Lizana and Pedraza 1998). A biochemical assay to measure photolyase activity in oocytes is available (Blaustein et al. 1994c) and helps to evaluate the ability of a species to repair DNA following UV-photoinduced damage. Such a marker provides a measure of whether a species may be at increased risk; additional information on the exposure to UV-B based on behaviors helps to complete the evaluation.

In terrestrial systems, amphibians and reptiles are exposed to altered fluxes of UV-B and other atmospheric gases, and responses to these fluxes will range widely in a dose-responsive manner, ranging from inconsequential to beneficial effects through extinction level events linked to elevated fluxes of UV and other deleterious electromagnetic radiation (see Cockell 1999). In herpetofauna, UV-B plays roles similar to those observed in typical mammalian and avian models, wherein UV-B stimulates synthesis of vitamin D_3, which ultimately follows various species-specific metabolic pathways characterized by a range of biological activities. For example, fish, amphibians, reptiles, and birds rely on vitamin D_3, while many mammals benefit from vitamin D_3 or vitamin D_2.

Although the literature detailing UV-B effects on reptiles is increasing relative to the literature available a decade ago, it remains sparse. However, there are no reasons to discount exposure of reptiles to elevated UV-B more than other vertebrates, given the predisposing behaviors of many reptile species. For example, terrestrial saurian reptiles frequently depend upon sun basking, sun-shade shuttling, and other heliothermic behaviors for regulation of core body temperature; these behaviors will ensure that these animals experience potentially significant exposure to solar UV radiation (UVR). These exposures may place them at increased risk of deleterious UV-B effects, especially given the absence of protective feathers or pelage common to other terrestrial vertebrates. Although the keratinized skin and scales of reptiles may confer protective benefits that offset increased incidence of UVR, given the range of effects that have been exhibited by other terrestrial vertebrates subjected to increased UV-B exposures, reptiles are likely to present similar responses when exposed. For example, cutaneous UV-B exposure alters immune function in rodents, for example, inhibition of delayed-type hypersensitivity reactions (Kim et al. 1998) and splenic and peritoneal macrophage functions (Jeevan et al. 1995). In fish, effects have been observed in laboratory exposures involving single acute low-dose UVR exposures of non-UVR-adapted fish. UVR exposures yielded stimulation of whole blood phagocyte respiration, but demonstrated a decreased activity of head kidney granulocytes (Salo et al. 2000). Observations have also been recorded in fish

that suggest species tolerant of UV-B radiation contain a methanol-extractable nonmelanic photoprotective substance in the skin (Blazer et al. 1997; Fabacher and Little 1995, 1998). In contrast to these findings for fish, the heliothermic green anole appears resistant to UVR inhibitory effects on cutaneous cell-mediated immune responses and splenic phagocytic function. These observations in reptiles may result from a combination of epidermal-dermal factors and are not linked to extractable photoprotective substances synthesized in the skin (Cope et al. 2001). Currently, mechanisms associated with UVR resistance are incompletely characterized in reptiles, and development of solar UVR-induced immunosuppression in green anoles and other members of the class Reptilia should not be underestimated. Our understanding of humoral and cellular immune responses to cutaneous UVR exposure is largely unknown, and given their phylogenetic distance, UVR-induced immunosuppression in reptiles may be markedly different from those mechanisms characterized for mammals. Given observations that amphibians and reptiles may be expressing an increased susceptibility to disease in the field, immune responses such as delayed-type hypersensitivity reactions and systemic macrophage functions may be useful markers of UVR effects on the reptilian immune system.

5.9.2 Stress

Whether acting singly or interactively, changes in environmental conditions shape responses in systems at various levels of biological organization. These environmental stresses (e.g., temperature, ambient radiation and other physical stressors, and chemical and biological stressors) acting jointly with environmental chemicals can potentially work in various ways to stress an animal. For example, from an energetics perspective, chemical stressors may directly or indirectly disrupt energy balance either by decreasing the resources available or by increasing the energy required for maintenance (e.g., eliminating insect prey through pesticide use will decrease energy sources). Chemical stressors acting singly or jointly with UV-B may physically damage epithelium and predispose the animal to diseases, bacterial infection (Faeh et al. 1998), pathogenic fungi (Taylor et al. 1999a, 1999c, 1999d), or water mold (Lefcort et al. 1997). Environmental chemicals also act as nondistinct stressors and become part of the sublethal environmental changes (e.g., EDCs or other chemically induced problems in steroid feedback, metabolic activity, sensory organ function, gaseous exchange, or liver function). As a stressor, toxicants may activate an organism's normal stress response (corticosterone release) and eventually compromise their ability to respond to stress (Gendron et al. 1997; Hayes et al. 1997; Hopkins et al. 1997). Despite their many adaptations, amphibians and reptiles are susceptible to synergistic or additive effects of multiple stressors.

From the perspective of the physiological ecologist, generalized responses to stress are readily apparent and well characterized, but mechanisms linked to these organismal responses continue to be objects of research across a wide range of animals. Generalized responses to stress, oftentimes linked to extremes in environmental temperature, resource availability (e.g., seasonal variations in prey or vegetation), or limited water, may serve as existing physiological adaptations to offset exposures to environmental chemicals. Dormancy is a commonly observed response to unfavorable environmental conditions, conditions potentially characterized by the occurrence of stressors that exceed species-specific preferences. For example, dependence upon external conditions for regulating metabolic rate limits the distribution of amphibians and reptiles, and when prevailing conditions exceed species-specific tolerances, animals will reduce activity and enter dormancy until acceptable environmental conditions return. In most temperate species, periods of dormancy are a normal feature of their yearly cycle of activity, and are most often adaptations to avoid or minimize exposures to seasonal extremes in temperature or moisture levels. For some species, dormancy may account for a significant portion of their yearly cycle. Dormancy takes 2 major forms: hibernation for the avoidance of cold and estivation for avoidance of other environmental factors, such as drought (Gregory 1982).

5.9.2.1 Hibernation

Anticipatory or seasonal prehibernation adjustments in response to cold prepare the animal for the depletion of energy stores and restriction of caloric intake (Herman 1992; Pinder et al. 1992). Homeoviscous acclimation refers to biochemical and structural changes made at the level of the membranes in response to colder conditions. Such modifications may result in a greater percentage of unsaturated fatty acids, an increase in permeability, and control of transport mechanisms (Pinder et al. 1992; Crockett 1998). Food is converted to glycogen and lipids, is stored in the liver and fat bodies, and metabolism is depressed. The extent to which stores are used or conserved depends on whether the amphibian is terrestrial or aquatic (e.g., toads depend on lipids, frogs on glycogen), their prior thermal acclimation (e.g., species living at higher altitudes or in colder climates can avert starvation longer), and the degree to which they may be freeze tolerant. Submerged aquatic amphibians risk anoxia and osmotic stress but are well hydrated. Laboratory studies indicate that, within limits, anoxic, cold submerged frogs can maintain cellular adenosine triphosphate (ATP) by increasing their carbohydrate metabolism, by using muscle, liver, and heart glycogen stores, and by depressing metabolism (Donohoe and Boutilier 1998). Terrestrial amphibians in hibernacula risk dehydrating, freezing, and accumulating toxic nitrogenous wastes, but they are safe from predators and generally do not lack oxygen. They hibernate below the frost line to avoid sudden freeze, whereas aquatic amphibians will move deeper into the ponds to reduce the risk of surface freeze.

In temperate species, winter presents a significant physiological challenge. This challenge is met by both behavioral and physiological means. Hibernation can be divided into 4 stages: fasting, entering the hibernaculum, dormancy, and metabolic depression (Gregory 1982). Decreasing temperature and light are generally regarded as stimuli for entering hibernation. Declining temperatures may also suppress appetite (Gatten 1974) and initiate the fasting associated with hibernation. As temperatures fall, many species will seek refugia, or hibernacula, that will not freeze during the coming winter months.

During hibernation, reptiles depress many physiological processes because food and even air may be inaccessible. To survive for several months, metabolic functions are slowed during hibernation, even more than predicted by the decreased body temperature (Zug et al. 2001). This indicates that some physiological processes have been curtailed. This reduced physiological state can conserve valuable energy supplies for significant periods of time. When metabolism is slowed, breathing and heart rate are reduced, but the supply of blood and oxygen to vital organs is maintained to ensure survival.

Aquatic species may hibernate underwater beneath a layer of ice. Most of the water below the ice in lakes and streams will not drop below 4 °C. However, because the surface layer of ice prevents access to air, normal pulmonary respiration must be curtailed for these air-breathing animals. Some reptiles (e.g., *Chrysemys picta*, *Sternotherus odoratus*, and *Thamnophis sirtalis*) maintain aerobic metabolism from cutaneous respiration (Zug et al. 2001). However, because of the thickly cornified skin of reptiles, buccopharyngeal or even cloacal respiration may be required to maintain adequate oxygen levels for aerobic metabolism (Seymour 1982). Some aquatic turtles may burrow into the mud at the bottom of a lake or stream, preventing access to oxygenated water. The anoxic or hypoxic environment caused by burrowing in the mud leads to prolonged periods of anaerobic metabolism. However, even these turtles may shuttle back and forth from the mud to open water, where they can switch to aerobic metabolism and flush their system of the accumulated lactic acid (Zug et al. 2001).

For terrestrial hibernators, physiological demands may not be as great, although even they must find shelter from freezing conditions. Typically, this means burrowing below the frost line. However, complete inactivity may not be possible. As the frost line descends, box turtles (*Terrapene carolina*) have been observed to burrow deeper (up to 0.5 m) to avoid freezing (Legler 1960). Hibernating snakes (*Elaphe* spp., *Crotalus* spp.) move to remain in the warmest part of their den or crevice (Zug et al. 2001). Intestinal response to long-term aphagia has been studied for infrequently feeding

snakes (Secor and Diamond 2000) and estivating amphibians (Secor and Diamond 1996). In each case, the intestine downregulates by changing function, morphology, or both to some extent to decrease performance. Amphibians and reptiles that inhabit temperate regions of the world hibernate, which like estivation may be characterized by extended periods of aphagia (Gregory 1982; Pinder et al. 1992). Since many of these temperate species feed frequently during the summer, they would be expected to narrowly regulate digestive performance during that time. However, the influence of these functional and structural responses on exposure is poorly understood and, more critically, greatly undervalued.

5.9.2.2 Freeze Tolerance

Freezing is lethal to most reptiles because the formation of ice crystals causes the lysis of cells. However, many temperate reptiles can withstand brief periods of supercooling (1 to 2 °C) in which ice crystallization does not occur (Lowe et al. 1971; Claussen et al. 1990; Claussen and Zani 1991; Packard and Packard 1995). Freezing of extracellular fluids results in dehydration. Because ice crystals form from pure water first, ions and other dissolved substances are excluded, raising the osmotic potential of the remaining fluids. This causes an osmotic imbalance, leading to dehydration of the surrounding cells and tissues. Freezing of extracellular fluids also interrupts blood and lymph flow and blocks transport of oxygen, CO_2, nutrients, and waste products.

Some reptiles have evolved physiological mechanisms to help them cope with freezing conditions. In some species, glucose is mobilized to act as a cryoprotectant, inhibiting freeze damage to the cells (Storey 1990). Water also may be redistributed from the tissues into the coelomic and subdermal spaces (Costanzo et al. 1993). By minimizing the amount of water in the tissues, damage from freezing can be reduced (Lee et al. 1990, 1992). However, a few species of reptiles (e.g., *T. carolina*, *C. picta*, and *Alligator mississippiensis*) are tolerant of some extracellular freezing (Hagan et al. 1983; Costanzo 1988; Storey et al. 1988; Storey 1990; Costanzo and Lee 1990; Costanzo et al. 1993; Packard et al. 1993).

5.9.2.3 Estivation

Unlike hibernation, estivation is associated with other environmental factors besides low-temperature avoidance and is usually correlated with water conservation (Espinoza and Tracy 1997; Storey 2002) or high-temperature avoidance (Lambert 1993; Bayoff 1995; Storey 2002). Estivation occurs predominantly in turtles and squamates in hot, arid environments where they retreat into shelters deep enough to avoid excessive heat and extreme temperature fluctuations (Voigt and Johnson 1976). During estivation, reptiles exhibit a reduced metabolic response to temperature (Abe 1995), but metabolic processes are not curtailed as significantly as during hibernation. Cellular mechanisms responsible for the metabolic torpor are similar to those during hibernation (Mauro and Isaacks 1989).

Temperature clearly has important implications for reptilian life cycles. Thermal pollution, therefore, may have a significant impact on reptiles by affecting metabolic rates and energy balances. This is particularly important for animals on restricted energy budgets, such as during hibernation or estivation. In addition, thermal pollution may have devastating impacts on the development of embryos in species that exhibit TSD.

5.10 PHYSIOLOGICAL ECOLOGY AND MULTIPLE STRESSORS: DEVELOPING A COMMON CURRENCY TO EVALUATE CHEMICAL EXPOSURES TO AMPHIBIANS AND REPTILES IN FIELD SETTINGS

Sparling et al. (2000a) clearly characterized existing data and applied research needs for the ecotoxicologists encountering amphibians and reptiles in the field. Although ecotoxicology had developed a process for evaluating chemical exposures in a few species of fish and wildlife, Sparling

et al. (2000a, 2000b) emphasized that the available literature through 1998 was, at best, sparse compared to publications focused on fishes and terrestrial vertebrates (particularly birds and mammals). Although awareness of herpetofauna has increased in the intervening period since publication of that first edition, we find a common refrain in this second edition (see Chapter 1, this volume); the literature focused on the herpetofauna again lags behind that for birds and mammals. The past 8 to 10 years have yielded a dramatic increase in research on amphibians and reptiles and the effects of environmental chemicals on these animals, yet much work remains to be conducted, if the herpetofauna are going to be sufficiently represented in the environmental risk assessment process. Few studies have characterized the role of nutritional or energetic interactions that influence exposure and mediate biological effects in amphibians and reptiles, be those direct, collateral, or indirect effects. Biological factors related to nutritional and bioenergetic interactions that influence exposure are generally not measured in screening level evaluations of chemical risks to fish and wildlife. However, if these factors are estimated or, better yet, measured, then exposures in the field might be better characterized, especially those involving long-term, low-concentration exposures. Furthermore, if data gaps were addressed using integrated field and laboratory studies (see, e.g., Linder et al. 1991; Sadinski and Dunson 1992), our risk evaluation process focused on the herpetofauna might well yield characterizations of risks that exceed expectations anticipated from tools currently applied to birds and mammals.

5.10.1 Research Needs: The Next 10 Years and Beyond

Our understanding of the biology of amphibians and reptiles has continued to increase in the past dozen years, perhaps outpacing the development of our capabilities to analyze exposure and effects of environmental chemicals when herpetofauna and stressors cross paths in the field. For example, research focused on the reproductive physiology and endocrinology of amphibians and reptiles has continued to develop over the past 2 decades, which has proven beneficial to recent ecotoxicological studies focused on endocrine disruptors and herpetofauna exposed in laboratory and field. Yet, additional work is required to better characterize endpoints and mechanisms of actions of endocrine disruptors relative to monitoring activities intended to benefit adaptive management programs across a wide range of field applications, such as discharges of treated wastewaters.

Beyond traditional, survival-based studies that often dominate screening level evaluations of risks, alternative, yet complementary endpoints must be refined or developed anew, particularly given the increasing awareness that life history attributes of the herpetofauna may require scrutiny and caution when comparing exposure and effects for environmental chemicals across a range of animal classes. For example, developmental effects in the herpetofauna, including traditional endpoints related to growth, may afford the most critical and sensitive endpoints linked to exposure to chemical stressors in their preferred habitats. As such, biomarkers of exposure and effects linked to developmental endpoints should be developed, or at least more fully characterized, for amphibians and reptiles, and these biomarkers must then be linked with population level effects. Long-term studies that evaluate effects over multiple life stages are required. Multigenerational studies must be completed, which is a shared research need across many animal classes. Similarly, outside of the ecotoxicological application, population level studies are available for only a handful of species, with much of that work a derivative of biodiversity concerns that have become increasingly confirmed for amphibians and reptiles, since the original statements that warned of their declining populations.

Although long overlooked and consistently undervalued, amphibians and reptiles have continued to gain appreciation among technical and lay communities as critical components within many aquatic, wetland, and terrestrial ecosystems. That heightened awareness among resource managers and members of the research community, however, must be matched by increased efforts to address data gaps in our existing knowledge of chemical toxicity to the wide range of species in these vertebrate groups. More importantly, the interrelationships of these animals with other ecosystem attributes and other physical and biological stessors must be characterized to enable amphibians and

reptiles to better serve as indicators of habitat quality and ecosystems at risk. Indeed, for aquatic habitats such as wetlands, indigenous herptofauna are more important to evaluating system sustainability than presently appreciated. These animals must be more thoroughly considered in future research, particularly as that relates to enhancing our understanding of their ecotoxicology, and the role that long-term, low-level chemical exposures play in their future.

At present, adopting the perspective of a physiological ecologist in conducting ecotoxicological research remains secondary to the commonly encountered application of ecotoxicology to the ecological risk assessment process. Despite the increased focus on herpetofauna in that process, data sources remain relatively scarce for the wide range of species potentially at risk to chemical exposures. And, given the ecological risk assessment tradition developed over the past 20 to 25 years, herpetofauna will likely benefit more from being considered as critical receptors in the risk assessment process, despite having their risks to chemical exposure bound by great uncertainties. In part, these uncertainties may be better addressed if the physiological ecologist weighs in on the research needed to improve the risk assessment process for herpetofauna. It is not simply a matter of collecting more threshold concentrations across a wider range of species, but we must delve into the ecological fabric that constitutes exposure. Although a quantitative energetics basis is long from being available to risk assessors, recognizing and developing tools that ensure a truly ecological basis for evaluating risks, especially within the context of multiple stressor exposures, should be fostered and developed to move exposure models beyond the simple "you are what you eat" tools commonly applied in today's oftentimes regulatory-driven risk assessment process. Much has been accomplished since publication of Sparling et al. (2000a), but our knowledge of the ecotoxicology of the herpetofauna still lags behind that of birds and mammals. Playing "catch up," however, should enable our developing tools and compiling research findings to better serve these long undervalued vertebrates.

DEDICATION

We dedicate this chapter to Wes Birge, whose early work with amphibians encouraged those of us who followed. The herpetofauna have lost an advocate, and we have lost a colleague and friend.

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6 Effects of Current-Use Pesticides on Amphibians

Christine M. Lehman and Bethany K. Williams

CONTENTS

6.1 HISTORY OF STUDIES INVOLVING AMPHIBIANS AND PESTICIDES

For many years, amphibians were understudied in the ecotoxicological literature. In 1989, the Canadian Wildlife Service published a comprehensive review of studies examining the effects of contaminants on amphibians (Power et al. 1989). Just 10 years later, the same organization published an updated review that included twice the number of studies (Pauli et al. 2000), indicating rapid growth in the field of amphibian ecotoxicology. However, Sparling et al. (2000) point out that the number of amphibian ecotoxicological studies remains modest relative to research utilizing other taxa. Relyea and Hoverman (2006) also report that amphibian data appear to be lagging behind other taxa, despite an increasing number of ecotoxicological studies involving freshwater ecosystems in general.

6.2 ROLE OF PESTICIDES IN AMPHIBIAN POPULATION DECLINES

Populations of amphibians have been declining worldwide for a number of years (Stuart et al. 2004), and pesticides have long been suspected as being at least partially responsible (Cowman and Mazanti 2000). The effects of pesticides on nontarget organisms such as amphibians can outlast the presence of the actual chemical in the environment. "New-generation," or current use, pesticides have largely replaced the organophosphates and chlorinated hydrocarbons that were heavily applied in the past. Although these newer products are formulated to break down quickly and be

effective at lower application rates, they can persist in the environment at concentrations adequate to impact amphibians either directly or indirectly. Thus, current use pesticides remain a threat to nontarget organisms such as amphibians. Despite the improvements in formulations, pesticides remain among the most frequently detected contaminants in surface water and groundwater worldwide (Gilliom 2007). Thus, the issue of pesticide contamination is still a clearly relevant topic for amphibian ecotoxicologists.

While increasing numbers of studies are focusing on pesticide effects on amphibians, few definitive links exist between pesticide contamination and actual population declines. Linking pesticide usage to amphibian declines can be problematic for a number of reasons. Boone et al. (2009) point out the following: 1) chemicals are less acutely toxic than in previous generations, and so their effects on nontarget wildlife will be more subtle; 2) species can differ with respect to their sensitivity to chemicals; 3) pesticide concentrations in the environment can fluctuate temporally and spatially; and 4) other stressors in the environment may cause declines or interact with the pesticides in unpredictable ways. Furthermore, there is a noticeable lack of long-term transgenerational studies that would shed light on the effects that larval exposure can have on adult traits (but see Rohr and Palmer 2005; Boone 2005) and subsequent population level effects.

Although pesticide contamination may seem an obvious cause of declines within agricultural landscapes, several confounding factors make attributing amphibian declines directly to pesticides difficult (Bonin et al. 2007). Increased pesticide inputs in agricultural areas occur simultaneously with other amphibian stressors, such as reductions in terrestrial habitat and altered hydrology. For example, Beja and Alcazar (2003) observed that a transition from temporary to permanent bodies of water in agricultural lands was a more important indicator of amphibian population persistence than chemical contamination. Furthermore, amphibians may not predictably demonstrate negative pesticide effects in the field. Both Piha et al. (2006) and Gilliland et al. (2001) conducted surveys in Finland and the United States, respectively, and observed that amphibian malformations were similar among agricultural vs. nonagricultural areas. Additionally, Murphy et al. (2006a, 2006b, 2006c) reported no significant relationship between field concentrations of atrazine and various anuran endpoints, including testicular oocytes and plasma steroid concentrations.

Specific instances of elevated pesticide concentrations within agricultural areas have, however, been linked to injury of amphibian populations. McDaniel et al. (2008) assessed amphibians from agricultural and nonagricultural areas and discovered that the number of testicular oocytes present in adult male *Rana pipiens* was correlated with mixtures of pesticides and nutrients, with the number of pesticides present being an important predictor. Hayes et al. (2002b, 2002c) also examined testicular abnormalities in *Rana pipiens* and correlated the number of testicular oocytes with high atrazine sales, as well as field-measured atrazine concentrations of greater than 0.2 ppb. Additionally, Knutson et al. (2004) reported that ponds near row crops were more turbid and had more nutrients and agricultural chemicals — all of which could reduce amphibian population sizes.

While attempting to document negative effects of pesticides in agricultural areas may be intuitive, amphibian declines due to pesticide contamination may also occur in areas with little or no intensive agriculture. Millions of tons of pesticides are used each year in urban and suburban settings (Kiely et al. 2004), and the contribution of urban areas to the insecticide load of streams may be comparable to that of agricultural areas (Hoffman et al. 2000). In addition, nonagricultural amphibian habitats may be impacted by agricultural pesticides introduced by runoff, overspray, or aerial drift and deposition.

Airborne pesticides can be transported great distances (Derek at al. 1990; LeNoir et al. 1999; Thurman and Cromwell 2000; Ryan and Hites 2002) and may be linked to amphibian declines, as suggested by several recent studies. Sparling et al. (2001) recorded lower cholinesterase levels in *Hyla regilla* collected from regions of California's Sierra Nevada Mountains containing higher

pesticide residues. Similarly, Fellers et al. (2004) observed very low *Rana muscosa* survival in the same region of California. Perhaps the most compelling correlations between large-scale patterns of amphibian decline and pesticide usage have been published by Davidson et al. (2001, 2002), Davidson (2004), and Davidson and Knapp (2007). These researchers used data from the Sierra Nevada Mountains to correlate recorded population declines for several amphibian species with upwind agricultural land use, even when a number of covariates, including the presence of predatory fish, were taken into consideration.

6.3 GOALS FOR THIS CHAPTER

In this chapter, we will discuss current use pesticides that are especially relevant today, from both an ecological and a practical perspective. We will focus primarily on widely used pesticides and biological endpoints directly linked to individual fitness.

Despite growing recognition of the complexity of pesticide effects and our increasing sophistication in uncovering those effects, the formula for pesticide exposure remains quite simple. In order to be a legitimate concern for nontarget organisms, a pesticide must be present in an organism's environment at levels adequate to induce a physiological response. Because patterns of use may be good indicators of environmental prevalence, we searched for amphibian studies on the most widely applied current use pesticides in the United States (arbitrarily defined as those that were applied in excess of 1,000,000 pounds active ingredient on a single crop in 2005, as reported by the USDA National Agricultural Statistics Service [2006]). Although US data were the most readily accessible in this case, the popularity of many of the pesticides holds worldwide. Given the relatively low persistence of many new-generation pesticides, it is especially important that ecotoxicological studies keep pace with changing pesticide use patterns. In the past 20 years, for example, popular herbicides such as alachlor and atrazine have been banned in the European Union, and cyanazine has been discontinued in the United States. Meanwhile, the broad-spectrum herbicide glyphosate has risen from relative obscurity to become the most commonly applied herbicide in the world (Kiely et al. 2004).

Many new genetic and biochemical techniques have been applied in amphibian ecotoxicology over the past decade (e.g., DNA microarrays), strengthening our ability to detect pesticide exposure and evaluate exposure effects. Although these techniques are vital to an integrated ecotoxicology program, we chose to limit the studies discussed here to those with response variables at the level of the individual and above. We also have not attempted to duplicate coverage of endocrine-disrupting effects of pesticides, or a detailed discussion on how pesticides can interact with other factors (see Chapter 14, this volume). In general, we limited our search of the amphibian ecotoxicological literature to studies published since the last edition of this book in 2000.

Using USEPA pesticide sales and usage data (Kiely et al. 2004), we chose to examine in greater detail the top 10 pesticides for which amphibian toxicological data exist. For 5 of these pesticides (the herbicides 2,4-D and acetochlor as well as 3 common fumigants), the recent literature regarding amphibians was sparse and will not be discussed in detail. In order of usage (with their rank by million pounds of active ingredient used per year in parentheses), the remaining 5 pesticides are glyphosate (1), atrazine (2), malathion (6), metolachlor-S (9), and metolachlor (10). Additionally, we elected to discuss carbaryl, one of the most widely used home and garden pesticides and the subject of considerable amphibian research (Kiely et al., 2004). While these 6 compounds may represent the most important pesticides currently being examined within the amphibian toxicological literature, many other pesticides have the potential to impact amphibian populations. Therefore, we also compiled a comprehensive summary of pesticide-related amphibian research from 2000 through 2008 (Table 6.1).

TABLE 6.1
Review of Amphibian Ecotoxicological Studies between 2000 and 2009

Pesticide	Endpoint	Test Type	Additional Factors	Species	Life Stage	Reference
2,4-D	Survival Metamorphic traits	Mesocosms	Pesticides Community structure	*Hyla versicolor* *Rana pipiens*	Tadpoles to metamorphs	Relyea 2009
2,4-D	Survival Metamorphic traits	Mesocosms	Community structure	*Ambystoma macuclatum* *Bufo americanus* *Hyla versicolor* *Pseudacris crucifer* *Rana pipiens* *R. sylvatica*	Tadpoles to metamorphs	Relyea 2005b
Acetochlor	Survival Metamorphic traits	Mesocosms	Pesticides Community structure	*Hyla versicolor* *Rana pipiens*	Tadpoles to metamorphs	Relyea 2009
Acetochlor	Thyroid hormones Escape behavior	Laboratory Acute exposure	na	*Rana catesbieana*	Tadpoles	Helbing et al. 2006
Acetochlor	Gene expression Metamorphosis	Laboratory	na or T3	*X. laevis*	Tadpoles through metamorphosis	Crump et al. 2002
Acrolein	Mortality	Laboratoryn Acute exposure	na	*Bufo arenarum*	Tadpoles	Venturino et al. 2007
Alachlor	Metamorphic traits Gonad/thymus histology	Laboratory Chronic exposure	na	*Rana pipiens*	Larvae to metamorphs	Hayes et al. 2006a
Amitrole	Predator avoidance behavior	Laboratory Acute exposure	Predator	*Rana temporaria*	Tadpoles	Mandrillon and Saglio 2007a
Amitrole	Activity level	Laboratory	Predator	*Bufo bufo*	Tadpoles	Mandrillon and Saglio 2007b
Atrazine	Mortality Parasite infection rates	Laboratory Chronic exposure	na	*Rana clamitans*	Tadpoles	Rohr et al. 2008

Atrazine	Reproduction Larval growth Adult fitness	Laboratory Chronic exposure	na	*Xenopus laevis*	Tadpoles to adults to tadpoles	DuPreez et al. 2008
Atrazine	Metamorphic traits Survival Gonadal morphology	Laboratory Chronic exposure	Predator	*Hyla versicolor*	Tadpoles to adults	LaFiandra et al. 2008
Atrazine	Organogenesis	Laboratory Acute exposure	na	*Xenopus laevis*	Tadpoles	Lenkowski et al. 2008
Atrazine	Gonadal morphology Metamorphic traits	Laboratory Chronic exposure	na	*Xenopus laevis*	Tadpoles to adults	Oka et al. 2008
Atrazine	Survival Metamorphic traits	Mesocosms	Pesticides Community structure	*Hyla versicolor* *Rana pipiens*	Tadpoles to metamorphs	Relyea 2009
Atrazine	Immune function	Laboratory Acute exposure	na	*Rana pipiens*	Tadpoles	Brodkin et al. 2007
Atrazine	Susceptibility to parasitic infestation	Laboratory Chronic exposure	na	*Rana sylvatica*	Tadpoles	Koprivnikar et al. 2007
Atrazine	Survival Metamorphic traits	Mesocosm	Nitrates Carbaryl	*Hyla versicolor*	Tadpoles to metamorphs	Boone and Bridges-Britton 2006
Atrazine	Sodium absorption	Laboratory	na	*Rana esculenta*	Adult	Cassano et al. 2006
Atrazine	Metamorphic traits Mortality Infection rates	Laboratory Chronic exposure	Iridovirus infection	*Ambystoma macrodactylum*	Larvae	Forson and Storfer 2006a
Atrazine	Time and size at metamorphosis ATV infection Peripheral blood leukocytes	Laboratory	Nitrate *Ambystoma tigrinum* virus	*Ambystoma tigrinum*	Larvae	Forson and Storfer 2006b
Atrazine	Metamorphic traits Gonad/thymus histology	Laboratory Chronic exposure	na	*Rana pipiens*	Larvae to metamorphs	Hayes et al. 2006b

(*continued*)

TABLE 6.1 (CONTINUED)
Review of Amphibian Ecotoxicological Studies between 2000 and 2009

Pesticide	Endpoint	Test Type	Additional Factors	Species	Life Stage	Reference
Atrazine	Combined trematode infection	Field study	Landscape and local characteristics of sites	*H. versicolor*	Tadpoles	Koprivnikar et al. 2006
Atrazine	Gonadal morphology	Field correlations	na	*Rana clamitans* *R. catesbeiana* *R. pipiens*	Adult frogs	Murphy et al. 2006
Atrazine	Larval development Sexual differentiation	Laboratory Chronic exposure	Nitrates	*Rana pipiens*	Tadpoles	Orton et al. 2006
Atrazine	Survival pre- and postexposure	Laboratory Chronic exposure	Food levels Hydroperiod	*Ambysoma barbouri*	Larvae to juveniles	Rohr et al. 2006
Atrazine	Cholinesterase activity	Laboratory Acute exposure	na	*Rana clamitans* *Xenopus laevis*	Tadpoles	Wacksman et al. 2006
Atrazine	LC50	Laboratory Acute exposure	na	*Rana catebeiana*	Tadpoles	Wan et al. 2006
Atrazine	Metamorphic traits Laryngeal development Gonadal development Aromatase activity Sex steroids	Laboratory Chronic exposure	na	*Xenopus laevis*	Tadpoles to metamorphs	Coady et al. 2005
Atrazine	Flow cytometry DNA Nuclei per cell Developmental stage	Laboratory Acute exposure	na	*Xenopus laevis*	Various stages of tadpoles	Freeman and Rayburn 2005
Atrazine	DNA characteristics Metamorphosis	Laboratory Chronic exposure	na	*Bufo americanus*	Tadpoles to metamorphs	Freeman et al. 2005
Atrazine	Gonadal morphology	Mesocosm Chronic exposure	na	*Xenopus laevis*	Tadpoles to metamorphs	Jooste et al. 2005

Atrazine	Mortality Metamorphic traits Behavior	Mesocosm Chronic exposure	Competitors Predators	*Rana sylvatica*	Tadpoles to metamorphs	Rohr and Crumrine 2005
Atrazine	Behavior Postmetamorphic water retention	Laboratory Chronic exposure Postmetamorph	na	*Ambystoma barbouri*	Larvae and metamorph	Rohr and Palmer 2005
Atrazine	Metamorphic traits Gonadal morphology	Laboratory Chronic exposure	na	*Rana clamitans*	Tadpoles to metamorph	Coady et al. 2004
Atrazine	Survival Time and size at metamorphosis Activity and shelter use	Laboratory	Food limitation drying	*Ambystoma barbouri*	Embryos to metamorphs	Rohr et al. 2004
Atrazine	Mortality	Laboratory Acute exposure	na	*Bufo americanus* *Psuedacris crucifer* *Rana clamitans* *R. sylvatica*	Tadpoles	Storrs and Kiesecker 2004
Atrazine	Development Mass Survival	Outdoor mesocosm	Carbaryl density hydroperiod	*R. sphenocephala* *B. americanus* *Ambystoma maculatum* *A. texanum*	Tadpoles to metamorphs	Boone and James 2003
Atrazine	Gonadal development Metamorphic traits Laryngeal morphology	Laboratory Chronic exposure	na	*Xenopus laevis*	Tadpoles to metamorphs	Carr et al. 2003
Atrazine	Mortality Growth Activity	Laboratory Chronic exposure	Water volume Hunger	*Ambystoma barbouri*	Larvae	Rohr et al. 2003
Atrazine	Mass and SVL at metamorphosis Days to metamorphosis Survival Hematocrit	Laboratory	Nitrate	*Xenopus laevis*	Tadpoles	Sullivan and Spence 2003
Atrazine	Gonadal development Laryngeal size	Laboratory Chronic exposure	na	*Xenopus laevis*	Tadpoles to metamorphs	Hayes et al. 2002a

(*continiued*)

TABLE 6.1 (CONTINUED)
Review of Amphibian Ecotoxicological Studies between 2000 and 2009

Pesticide	Endpoint	Test Type	Additional Factors	Species	Life Stage	Reference
Atrazine	Gonadal development Hermaphroditism	Laboratory Chronic exposure Field survey	na	*Rana pipiens*	Tadpoles Adults	Hayes et al. 2002b
Atrazine	Gonadal development	Laboratory Chronic exposure	na	*Rana pipiens*	Tadpoles to metamorphs	Hayes et al. 2002c
Atrazine	Mortality Metamorphic traits Immune function Parasitic infestation	Field Laboratory	na	*Rana sylvatica*	Tadpoles to metamorphs	Kiesecker 2002
Atrazine	Testis development	Laboratory Chronic exposure	na	*Xenopus laevis*	Tadpoles to metamophs	Tavera-Mendoza et al. 2002a
Atrazine	Ovarian development	Laboratory Chronic exposure	na	*Xenopus laevis*	Tadpoles to metamophs	Tavera-Mendoza et al. 2002b
Atrazine	Hemoglobin Malformities Mass Mortality Swimming Performance Ventilation	Laboratory Acute	na	*Bufo americanus* *Rana pipiens* *R. sylvatica*	Embryos Larvae Adults	Allran and Karasov 2001
Atrazine	Mortality Metamorphic traits Hematocrit	Laboratory Chronic exposure	Nitrates	*Rana pipiens*	Tadpoles to metamorphs	Allran and Karasov 2000
Atrazine	Hatching success Larval mortality Development time Number of metamorphs Malformation	Laboratory then mesocosms	UV MeHg chlorpyrifos	*Hyla chrysoscelis*	Embryos through metamorphosis	Britson and Threlkeld 2000

Atrazine	Mortality Metamorphis traits	Microcosms	na	*Hyla versicolor*	Tadpoles to metamorphs	Diana et al. 2000
Azadirachtin	Survival Fertilization Swimming performance	Laboratory Acute exposure	na	*Bufo marinus*	Tadpoles	
Azinphosiviethyl	LC50 Carboxylesterase activity	Laboratory Acute exposure	na	*Bufo viridis*	Tadpoles	Yesilada et al. 2006
Basudin	Survival Behavior Glycogen levels	Laboratory Acute exposure	na	*Ptychadena bibroni*	Tadpoles	Ezemonye and Ilechie 2007
Carbaryl	Survival Metamorphic traits	Mesocosms	na	*Bufo americanus* *Rana clamitans*	Tadpoles to metamorphs	Boone 2008
Carbaryl	Survival Metamorphic traits	Mesocosms	Pesticides Community structure	*Hyla versicolor* *Rana pipiens*	Tadpoles to metamorphs	Relyea 2009
Carbaryl	Mortality Parasite infection rates	Laboratory Chronic exposure	na	*Rana clamitans*	Tadpoles	Rohr et al. 2008a
Carbaryl	Survival Metamorphic traits	Mesocosms	Predation Competition fertilizers	*Ambystoma maculatum* *Bufo americanus* *Rana sphenocephala*	Tadpoles to metamorphs	Boone et al. 2007
Carbaryl	Survival Growth Skin peptide quantity	Laboratory	Chytrid	*Rana boylii*	Metamorphs	Davidson et al. 2007
Carbaryl	Survival Metamorphic traits	Mesocosms	Fertilizers Pathogen	*Rana catesbieana*	Tadpoles to metamorphs	Pugis and Boone 2007
Carbaryl	Oviposition site selection	Mesocosm	na	*Hyla chrysoscelis*	Adults	Vonesh and Buck 2007
Carbaryl	Survival Metamorphic traits	Mesocosm	Nitrates Atrazine	*Hyla versicolor*	Tadpoles to metamorphs	Boone and Bridges-Britton 2006
Carbaryl	Survival Metamorphic traits	Mesocosm	pH Predation	*Rana catesbieana* *Rana clamitans*	Tadpoles to metamorphs	Relyea 2006a

(*continued*)

TABLE 6.1 (CONTINUED)
Review of Amphibian Ecotoxicological Studies between 2000 and 2009

Pesticide	Endpoint	Test Type	Additional Factors	Species	Life Stage	Reference
Carbaryl	Metamorph survival Metamorph growth Overwintering success	Field Enclosures	Competition	*Rana blairi* *R. sphenocephala* *Bufo woodhousii*	Juvenile	Boone 2005
Carbaryl	Metamorphic traits Mortality	Mesocosm Chronic exposure	Nitrate	*Rana clamitans*	Tadpole to metamorph	Boone et al. 2005
Carbaryl	Mortality	Laboratory Acute exposure	na	*Bufo boreas*	Tadpole	Dwyer et al. 2005
Carbaryl	Metamorphic traits Lipid reserves Mortality Metamorphic success	Laboratory Chronic exposure	Density	*Ambystoma maculatum*	Larvae to metamorph	Metts et al. 2005
Carbaryl	Survival Metamorphic traits	Mesocosms	Community structure	*Ambystoma macuclatum* *Bufo americanus* *Hyla versicolor* *Pseudacris crucifer* *Rana pipiens* *R. sylvatica*	Tadpoles to metamorphs	Relyea 2005b
Carbaryl	Mortality Metamorphic traits	Field study Experimental	Competition	*Bufo woodhousii* *Rana sphenocephala*	Tadpoles to metamorphs	Boone et al. 2004
Carbaryl	Survival Metamorphic traits	Mesocosm	Competition Predation	*Rana sphenocephala*	Tadpoles to metamorphs	Mills and Semlitsch 2004
Carbaryl	Mortality Metamorphic traits	Mesocosms	Predation	*Rana catesbeiana* *Notophthalmus viridescens*	Tadpoles to metamorphs	Boone and Semlitsch 2003
Carbaryl	Mortality Metamorphic traits	Mesocosms	Densit Multiple exposures	*Rana clamitans*	Tadpoles	Boone and Bridges 2003a
Carbaryl	Mortality Metamorphic traits	Mesocosms	UV-B	*Rana sphenocephala*	Tadpoles to metamorphs	Bridges and Boone 2003

Carbaryl	Mortality	Laboratory Acute exposure	Predators	*Bufo americanus* *Hyla versicolor* *Rana catesbieana* *R. clamitans* *R. pipiens* *R. sylvatica*	Tadpoles	Relyea 2003
Carbaryl	Mortality Growth Activity	Laboratory Chronic exposure	Water volume Hunger	*Ambystoma barbouri*	Larvae	Rohr et al. 2003
Carbaryl	Survival Metamorphic response	Mesocosms	Competition Pond drying	*Bufo woodhouseii* *Notophthalmus viridescens* *Rana blairi* *R. clamitans* *R. sphenocephala*	Tadpoles to metamorphs	Boone and Semlitsch 2002
Carbaryl	Survival Metamorphic traits	Laboratory Field	na	*Hyla versicolor*	Tadpoles to metamorphs	Saura-Mas et al. 2002
Carbaryl	Mortality Metamorphic traits	Mesocosms	Competition Predation	*Bufo woodhouseii* *Hyla versicolor* *Rana clamitans*	Tadpoles to metamorphs	Boone and Semlitsch 2001
Carbaryl	Survival Metamorphic response	Mesocosms	Competition Multiple exposures	*Rana clamitans*	Tadpoles to metamorphs	Boone et al. 2001
Carbaryl	Genetic variation in tolerance	Laboratory	na	*Rana sphenocephala*	Tadpoles	Bridges and Semlitsch 2001
Carbaryl	Mortality	Laboratory Acute exposure	Predation	*Hyla versicolor*	Tadpoles	Relyea and Mills 2001
Carbaryl	Survival Metamorphic traits Malformities	Laboratory Chronic exposure	na	*Rana sphenocephala*	Tadpoles to metamorphs	Bridges 2000
Carbaryl	Genetic variation in tolerance	Laboratory	na	Various *Rana* spp.	Tadpoles	Bridges and Semlitsch 2000

(*continued*)

TABLE 6.1 (CONTINUED)
Review of Amphibian Ecotoxicological Studies between 2000 and 2009

Pesticide	Endpoint	Test Type	Additional Factors	Species	Life Stage	Reference
Carbaryl	Genetic basis for tolerance	Laboratory	na	*Hyla versicolor*	Tadpoles	Semlitsch et al. 2000
Chlorpyrifos	Cholinesterase activity Swimming speed	Laboratory	na	*Acris crepitans* *Hyla chrysoscelis* *Gastrophryne olivacea* *Rana sphenocephala*	Tadpoles	Widder and Bidwell 2008
Chlorpyrifos	Survival Metamorphic traits	Mesocosms	Pesticides Community structure	*Hyla versicolor* *Rana pipiens*	Tadpoles to metamorphs	Relyea 2009
Chlorpyrifos and oxon derivatives	96-hour median lethal concentration	Laboratory	na	*Rana boylii*	Tadpoles	Sparling and Fellers 2007
Chlorpyrifos	Growth Time to metamorphosis	Laboratory	na	*Smilisca phaeota*	Tadpoles to metamorphosis	Gallo-Delgado et al. 2006
Chlorpyrifos	Cholinesterase activity	Laboratory Acute exposure	Atrazine	*Rana clamitans* *Xenopus laevis*	Tadpoles	Wacksman et al. 2006
Chlorpyrifos	Cholinesterase activity Behavior Mass	Laboratory Acute exposure	na	*Rana sphenocephala*	Tadpoles	Widder and Bidwell 2006
Chlorpyrifos	Growth Swimming performance	Laboratory Acute exposure	na	*Xenopus laevis*	Tadpoles	Richards and Kendall 2003a
Chlorpyrifos	Mortality Deformity Biochemical endpoints	Laboratory	na	*Xenopus laevis*	Premetamorphs and metamorphs	Richards and Kendall 2003b
Chlorpyrifos	Mortality	Laboratory	na	*Rana pipiens*	Embryos	Gaizick et al. 2001
Cyclophosphamide	Immune response	Laboratory Chronic exposure	na	*Rana pipiens*	Tadpoles	Albert et al. 2007
Cyfluthrin	Metamorphic traits Gonad/thymus histology	Laboratory Chronic exposure	na	*Rana pipiens*	Larvae to metamorphs	Hayes et al. 2006a

Cypermethrin	Hatching success Mortality Deformities Growth/development	Laboratory Chronic exposure	na	*Rana arvalis*	Eggs to metamorphs	Greulich and Pflugmacher 2003
DDT	Immune response	Laboratory Chronic exposure	na	*Rana pipiens*	Tadpoles	Albert et al. 2007
DDT	Immune function	Laboratory Acute exposure	na	*Rana pipiens*	Adults	Gilbertson et al. 2003
Diazinon and oxon derivatives	96-hour LC50	Laboratory	na	*Rana boylii*	Tadpoles	Sparling and Fellers 2007
Diazinon	Survival Metamorphic traits	Mesocosms	Pesticides Community structure	*Hyla versicolor* *Rana pipiens*	Tadpoles to metamorphs	Relyea 2009
Dieldrin	Immune response	Laboratory Chronic exposure	na	*Rana pipiens*	Tadpoles	Albert et al. 2007
Dieldrin	Immune function	Laboratory Acute exposure	na	*Rana pipiens*	Adults	Gilbertson et al. 2003
Dimethoate	Mortality	Laboratory Acute exposure	na	*Hyla arborea*	Tadpoles	Sayim and Kaya 2006
Endosulfan	Survival Metamorphic traits	Mesocosms	Pesticides Community structure	*Hyla versicolor* *Rana pipiens*	Tadpoles to metamorphs	Relyea 2009
Endosulfan	Mortality Metamorphic traits Behavior	Mesocosm Chronic exposure	Competitors Predators	*Rana sylvatica*	Tadpoles to metamorphs	Rohr and Crumrine 2005
Endosulfan	Mortality Growth Activity	Laboratory Chronic exposure	Water volume Hunger	*Ambystoma barbouri*	Larvae	Rohr et al. 2003
Endosulfan	Survival Predator avoidance	Laboratory Acute exposure	Temperature	*Litoria citropa*	Tadpoles	Broomhall 2002

(*continued*)

TABLE 6.1 (CONTINUED)
Review of Amphibian Ecotoxicological Studies between 2000 and 2009

Pesticide	Endpoint	Test Type	Additional Factors	Species	Life Stage	Reference
Endosulfan	Survival Metamorphic traits Behavior	Laboratory Acute exposure Recovery period	Competitors	*Litoria freycineti*	Tadpoles	Broomhall and Shine 2003b
Endosulfan	Pheromonal system	Laboratory Acute	na	*Notophthalmus viridescens*	Adults	Park et al. 2001
Esfenvalerate	Mortality Metamorphic traits Immune function Parasitic infestation	Field Laboratory	na	*Rana sylvatica*	Tadpoles to metamorphs	Kiesecker 2002
Fenpropimorph	Activity Predator avoidance Metamorphic traits	Laboratory Chronic exposure	na	*Rana temporaria*	Tadpole to metamorph	Teplitsky et al. 2005
Fenvalerate	Cholinesterase activity	Laboratory Acute exposure	na	*Haplobatrachus tigerinus*	Tadpoles	Tilak et al. 2003
Glyphosate	Survival Metamorphic traits	Mesocosms	Pesticides Community structure	*Hyla versicolor* *Rana pipiens*	Tadpoles to metamorphs	Relyea 2009
Glyphosate	Mortality Parasite infection rates	Laboratory Chronic exposure	na	*Rana clamitans*	Tadpoles	Rohr et al. forthcoming
Glyphosate (Vision®)	Mortality	Laboratory Acute exposure	pH Food	*Rana pipiens*	Tadpoles	Chen et al. 2004
Glyphosate Glyphosate IPA Roundup® Touchdown® Roundup Bioactive	Mortality	Laboratory	na	*Crinia insignifera* *Heleioporus eyrei* *Limnodynastes dorsalis* *Litoria moorei*	Tadpoles, new metamorphs Adults	Mann and Bidwell 1999
Glyphosate (Vision)	Mortality	Caged larvae in wetlands	na	*Rana pipiens* *R. clamitans*	Tadpoles	Thompson et al. 2004
Glyphosate formulations	Mortality Malformations		na	*Scinax nasicus*	Tadpoles	Lajmanovich et al. 2003

Glyphosate plus surfactant (Rodeo®)	Mortality	Laboratory Acute exposure	na	*Rana pipiens*	Tadpoles	Trumbo 2005
Glyphosate (Roundup)	Mortality	Laboratory Acute exposure	na	*Rana sylvatica*	Tadpoles	Comstock et al. 2007
Glyphosate (Roundup)	Oviposition site selection	Mesocosm	na	*Hyla versicolor/ chrysoscelis*	Adults	Takahashi 2007
Glyphosate	Survival Metamorphic traits	Laboratory Chronic exposure	na	*Rana cascadae*	Tadpoles to metamorphs	Cauble and Wagner 2005
Glyphosate (Roundup)	Mortality	Laboratory Acute exposure	Predators	*Rana sylvatica* *R. pipiens* *R. clamitans* *R. catesbeiana* *Bufo americanus* *Hyla versicolor*	Tadpoles	Relyea 2005a
Glyphosate (Roundup)	Survival Metamorphic traits	Mesocosms	Community structure	*Ambystoma macuclatum* *Bufo americanus* *Hyla versicolor* *Pseudacris crucifer* *Rana pipiens* *R. sylvatica*	Tadpoles to metamorphs	Relyea 2005b
Glyphosate	Survival	Mesocosm	Sediment	*Bufo americanus* *Hyla versicolor* *Rana pipiens*	Tadpoles	Relyea 2005c
Glyphosate	Survival	Laboratory Acute exposure	na	*Bufo woodhouseii* *Hyla versicolor* *Rana sylvatica*	Metamorphs	Relyea 2005c
Glyphosate	Mortality Metamorphic traits Tail damage Gonadal abnormalities	Laboratory Acute (4 species) Chronic (1 species)	Several glyphosate formulations	*Rana clamitans* *R. pipiens* *R. sylvatica* *B. americanus*	Tadpoles and tadpoles to metamorphs	Howe et al. 2004

(*continued*)

TABLE 6.1 (CONTINUED)
Review of Amphibian Ecotoxicological Studies between 2000 and 2009

Pesticide	Endpoint	Test Type	Additional Factors	Species	Life Stage	Reference
Glyphosate (Vision)	LC50	Laboratory Acute exposure	pH	*Bufo americanus* *Rana clamitans* *R. pipiens* *Xenopus laevis*	Tadpoles	Edginton et al. 2004
Glyphosate (Vision)	Mortality Avoidance response Growth	Field enclosures	na	*Rana clamitans* *R. pipiens*	Tadpoles to metamorphs	Wojtaszek et al. 2004
Glyphosate (Kleeraway®)	Mortality Postexposure growth and development	Laboratory Acute exposure	na	*Pseudacris triseriata* *Rana blairi*	Tadpoles	Smith et al. 2001
Glyphosate	Mortality	Laboratory Acute exposure	na	*Litorea moorei*	Tadpoles	Giesy et al. 2000
Glyphosate (Roundup and Rodeo)	Mortality Abnormalities	Laboratory Acute exposure	na	*Xenopus laevis*	Embryos	Perkins et al. 2000
Malathion and oxon derivatives	96-hour median lethal concentration	Laboratory	na	*Rana boylii*	Tadpoles	Sparling and Fellers 2007
Malathion	Survival Metamorphic traits	Mesocosm	na	*Bufo americanus* *Rana clamitans*	Tadpoles to metamorphs	Boone 2008
Malathion	Survival Metamorphic traits	Mesocosms	Pesticides Community structure	*Hyla versicolor* *Rana pipiens*	Tadpoles to metamorphs	Relyea 2009
Malathion	Mortality Metamorphic traits	Mesocosms	Density Application frequency	*Rana sylvatica* *R. pipiens*	Tadpoles to metamorphs	Relyea and Diecks 2008
Malathion	Mortality Metamorphic traits	Mesocosms	Predators	*Bufo americanus* *Rana sylvatica* *R. pipiens*	Tadpoles to metamorphs	Relyea and Hoverman 2008
Malathion	Mortality Parasite infection rates	Laboratory Chronic exposure	na	*Rana clamitans*	Tadpoles	Rohr et al. 2008a
Malathion	Mortality Growth	Laboratory Acute exposure	na	*Rana ridibunda*	Tadpoles	Sayim 2008

Malathion	Survival Growth Food consumption	Laboratory Chronic exposure	na	*Limnonectus limnochairs*	Tadpoles	Gurushankara et al. 2007
Malathion	Survival Metamorphic traits	Mesocosms	Community structure	*Ambystoma macuclatum* *Bufo americanus* *Hyla versicolor* *Pseudacris crucifer* *Rana pipiens* *R. sylvatica*	Tadpoles to metamorphs	Relyea 2005b
Malathion	Mortality	Laboratory Acute exposure	Predation	*Bufo americanus* *Hyla versicolor* *Rana catesbieana* *R. clamitans* *R. pipiens* *R. sylvatica*	Tadpoles	Relyea 2004b
Malathion	Behavior Cholinesterase levels	Field	na	*Bufo woodhousii*	Adults	Dickerson et al. 2003
Malathion	Immune function	Laboratory Acute exposure	na	*Rana pipiens*	Adults	Gilbertson et al. 2003
Malathion	Mortality Metamorphic traits Immune function Parasitic infestation	Field Laboratory	na	*Rana sylvatica*	Tadpoles to metamorphs	Kiesecker 2002
Malathion	Equilibrium posture Mortality Growth Development	Laboratory Chronic exposure	na	*Rana catesbeiana*	Tadpoles	Fordham et al. 2001
Metalaxyl	Metamorphic traits Gonad/thymus histology	Laboratory Chronic exposure	na	*Rana pipiens*	Larvae to metamorphs	Hayes et al. 2006a
Methoxychlor	Mortality Malformity Growth Reproductive measures	Laboratory Acute exposure Chronic exposure	na	*Xenopus laevis*	Eggs Adults	Fort et al. 2004a

(*continued*)

TABLE 6.1 (CONTINUED)
Review of Amphibian Ecotoxicological Studies between 2000 and 2009

Pesticide	Endpoint	Test Type	Additional Factors	Species	Life Stage	Reference
Methoxychlor	Mortality Malformity Sex ratio Gonadal measures	Laboratory Chronic exposure	na	*Xenopus tropicalis*	Egg to metamorph	Fort et al. 2004b
Methoxychlor	Hatching Startle response	Laboratory Acute exposure	na		Larvae	Eroschenko et al. 2002
Methoxychlor	Behavior	Laboratory Acute exposure	Predators	*Ambystoma macrodactylum*	Larvae	Ingermann et al. 2002
Methoxychlor	Behavior	Laboratory Chronic exposure	na	*Ambystoma macrodactylum*	Egg to tadpole	Verrell 2000
Methyl parathion	LC50 Metamorphic traits Malformities	Laboratory Acute exposure Chronic exposure	na	*Rana tigrina*	Larvae to metamorphs	Kennedy and Sampath 2001
Metolachlor	Survival Metamorphic traits	Mesocosms	Pesticides Community structure	*Hyla versicolor* *Rana pipiens*	Tadpoles to metamorphs	Relyea 2009
Nicosulfron	Metamorphic traits Gonad/thymus histology	Laboratory Chronic exposure	na	*Rana pipiens*	Larvae to metamorphs	Hayes et al. 2006a
Octylphenol	Mortality Growth Activity	Laboratory Chronic exposure	Water volume Hunger	*Ambystoma barbouri*	Larvae	Rohr et al. 2003
Organochlorines	Tissue residues	Field survey	na	*Rana lessonae* *R. esculenta*	Tadpoles Adults	Fagotti et al. 2005
Organochlorines	Tissue residues	Field survey	Inorganics	*Rana temporaria*	Tadpoles	Hofer et al. 2005
Organochlorines	Tissue residues Hatching success	Field survey	na	*Ambystoma gracile* *Rana aurora*	Eggs	de Solla et al. 2002
Organochlorines	Tissue residue	Field survey	na	*Rana clamitans*	Eggs Tadpoles Adults	Gillilland et al. 2001

Organophosphates	Mortality Avoidance response Glycogen levels	Laboratory Acute exposure	na	*Ptychadena bibroni*	Tadpoles	Ezemonye and Ilechie 2007
Permethrin	Survival Metamorphic traits	Mesocosm	na	*Bufo americanus* *Rana clamitans*	Tadpoles to metamorphs	Boone 2008
Propiconizole	Metamorphic traits Gonad/thymus histology	Laboratory Chronic exposure	na	*Rana pipiens*	Larvae to metamorphs	Hayes et al. 2006a
S-Metalochlor	Metamorphic traits Gonad/thymus histology	Laboratory Chronic exposure	na	*Rana pipiens*	Larvae to metamorphs	Hayes et al. 2006a
Tebupirimphos	Metamorphic traits Gonad/thymus histology	Laboratory Chronic exposure	na	*Rana pipiens*	Larvae to metamorphs	Hayes et al. 2006a
Toxaphene	Tissue residue	Field survey	na	*Hyla regilla*	Tadpoles	Angermann et al. 2002
Triclopyr	Mortality Malformations	Laboratory Acute exposure	na	*Bufo americanus* *Rana clamitans* *R. pipiens* *Xenopus laevis*	Embryo Larvae	Edginton et al. 2003
Triphenyltin	Behavior Survival Metamorphic traits	Laboratory Chronic exposure	na	*Ambystoma barbouri*	Larvae to metamorphs	Rehage et al. 2002
λ-Cyhalothrin	Metamorphic traits Gonad/thymus histology	Laboratory Chronic exposure	na	*Rana pipiens*	Larvae to metamorphs	Hayes et al. 2006a
Multiple pesticides						
2,4-D Acetochlor Atrazine Carbaryl Chlorpyrifos Diazinon Endosulfan Glyphosate Malathion Metolachlor	Survival Metamorphic traits	Mesocosms	Community structure	*Hyla versicolor* *Rana pipiens*	Tadpoles to metamorphs	Relyea 2009

(*continued*)

TABLE 6.1 (CONTINUED)
Review of Amphibian Ecotoxicological Studies between 2000 and 2009

Pesticide	Endpoint	Test Type	Additional Factors	Species	Life Stage	Reference
Atrazine S-Metalochlor 7 other pesticides	Metamorphic traits Gonad/thymus histology	Laboratory Chronic exposure	na	*Rana pipiens*	Larvae to metamorphs	Hayes et al. 2006a
Atrazine Carbaryl	Survival Metamorphic traits	Mesocosm	Nitrates	*Hyla versicolor*	Tadpoles to metamorphs	Boone and Bridges-Britton 2006
Atrazine Chlorpyrifos Metolachlor	Survival Metamorphic traits	Laboratory Macrocosms	na	*Bufo americanus* *Hyla versicolor* *Rana catesbieana* *R. clamitans* *R. sphenocephala* *Psuedacris crepitans*	Tadpoles to metamorphs	Mazanti et al. 2003
Carbaryl Malathion Permethrin	Survival Metamorphic traits	Mesocosm	na	*Bufo americanus* *Rana clamitans*	Tadpoles to metamorphs	Boone 2008
Butachlor Dichlorvos	LC50	Laboratory Acute exposure	na	*Bufo melanostictus* *Fejervarya multistrada* *Polypedates megacephalus* *Micohyla ornate*	Tadpoles	Geng et al. 2005
Glyphosate Malathion	Survival Metamorphic traits	Mesocosm	Predation	*Bufo americanus* *Hyla versicolor* *Rana pipiens*	Tadpoles to metamorphs	Relyea et al. 2005
Aldicarb Atrazine Dieldrin Endosulfan Lindane Metribuzine	Immune function	Laboratory Acute exposure	na	*Rana pipiens* *Xenopus laevis*	Tadpoles	Christin et al. 2004
Imidacloprid RH-5849	LC50 DNA damage	Laboratory Acute exposure	na	*Rana N. hallowell*	Tadpoles	Feng et al. 2004

Multiple pesticides	Cholinesterase activity	Field survey	na	*Hyla regilla*	Tadpoles	Sparling et al. 2001
Aldicarb Atrazine Dieldrin Endosulfan Lindane Metribuzin	Immune function	Laboratory Chronic exposure	na	*Rana pipiens*	Juveniles	Christin et al. 2003
Carbaryl Diazinon Glyphosate Malathion	Survival Growth	Laboratory Chronic exposure	Pesticide combinations	*Bufo americanus* *Hyla versicolor* *Rana catesbieana* *R. clamitans* *R. pipiens*	Tadpoles to metamorphs	Relyea 2004a
Multiple chemicals	Tissue residues	Field survey	na	*Rana muscosa*	Adults	Fellers et al. 2004
Azoxystrobin Cyanazine Esfenvalerate MCPA Permethrin Pirimicarb	Mortality Growth	Laboratory Acute exposure Chronic exposure	na	*Rana temporaria*	Eggs to metamorphs	Johansson et al. 2006
Triclopyr Butoxyethyl ester	LC50 Avoidance response Growth	Field Acute In situ enclosures	na	*Rana clamitans* *R. pipiens*	Larvae	Wojtaszek et al. 2005
Atrazine Metolachlor	Parasite load	Laboratory and mesocosms	Trematode exposure	*R. sylvatica* *R. clamitans*	Tadpoles	Griggs and Belden 2008
Atrazine Metribuzin Aldicarb Endosulfan Lindane Dieldrin	Parasite penetration Parasite establishment and reproduction Immune function	Laboratory	Lungworm exposure	*R. pipiens*	Juveniles	Gendron et al. 2003
Multiple pesticides	Body weight Liver retinoid stores	Field survey	na	*R. catesbeiana*	Adults	Boily et al. 2005

6.4 ATRAZINE

Atrazine is one of the most widely used herbicides in the United States and worldwide. Approximately 76 million pounds of active ingredient are used on US crops annually. Atrazine is thought to have a half-life of 3 to 90 days (Solomon et al. 1996), but can persist in the environment for long periods of time at concentrations found to cause effects to amphibians (see discussion below). Research on the effects of atrazine on amphibians has grown steadily since Hayes et al. (2002a) published their findings on the effects of this pesticide on frogs at levels previously thought to be safe for wildlife.

Studies involving the effects of atrazine on amphibians can be broken down into several categories: estrogenic effects, direct toxicity, and indirect toxicity. Many of these studies include more than a single factor. By far, most recent research has focused on estrogenic effects, which are covered in detail in Chapter 8 of this book.

6.4.1 Estrogenic Effects

Tavera-Mendoza et al. (2002a, 2002b) were among the first to discover the estrogenic effects of atrazine on amphibian gonads. Soon thereafter, Hayes et al. (2002a) reported *Xenopus laevis* tadpoles exposed in the laboratory to atrazine concentrations as low as 0.01 μg/L developed reproductive anomalies, including testicular oocytes and supernumerary ovaries. Subsequent research has corroborated these findings in *Xenopus*, as well as in the US native *Rana pipiens* (Hayes et al. 2002c). Field studies were also conducted in an attempt to correlate atrazine usage in agricultural settings with incidence of reproductive abnormalities (Hayes et al. 2002b). Other investigators have failed to observe similar effects in both the field and laboratory studies (Carr et al. 2003; Coady et al. 2004; Jooste et al. 2005; Murphy et al. 2006b; Oka et al. 2008).

Hayes et al. (2006b) suggest that the mechanism by which atrazine induces gonadal malformations is by decreasing levels of androgens and increasing production of estrogen. Storrs and Semlitsch (2008) suggest that sensitivity to the estrogenic effects of atrazine on amphibians may be taxon specific. They report that species exhibiting accelerated somatic development (e.g., *Bufo americanus, Hyla versicolor*) also have delayed ovarian development. These species demonstrate fewer effects from exposure to estrogenic compounds because sexual differentiation occurs during or after metamorphosis. They suggest that species such as *Rana sphenocephala* may be more susceptible to estrogenic effects because sexual differentiation occurs during the fully aquatic larval stage.

6.4.2 Direct Effects

Direct effects are those defined as being toxic to the organism. Examples of direct toxicity include impairment of the immune system, changes in behavior, alterations in growth or length of the larval period, and outright mortality.

Because atrazine acts on photosynthetic systems, the herbicide's direct toxicity on amphibians is generally sublethal. In fact, atrazine has not caused direct mortality in a variety of species even at concentrations up to an order of magnitude above expected environmental concentrations (*Ambystoma barbouri*, Rohr et al. 2003; *Bufo americanus*, Allran and Karasov 2001; *Hyla versicolor*, Diana et al. 2000; *Rana pipiens*, Allran and Karasov 2000, 2001). However, Storrs and Kiesecker (2004) did observe mortality at low doses (3 ppb), and Boone and Bridges-Britton (2006) recorded an increase in mortality when atrazine and a fertilizer were combined.

Atrazine can delay development of *Xenopus laevis* in the laboratory (Freeman and Rayburn 2005), but can increase the size at metamorphosis of *Hyla versicolor* (Relyea 2009) in mesocosms. It can also increase sodium absorption in adult *Rana esculenta*, which could lead to a disequilibrium that would increase metabolism (Cassano et al. 2006). Atrazine can also impair immune system function of amphibians. Brodkin et al. (2007) report that adult leopard frogs (*Rana pipiens*) exposed to environmentally realistic concentrations of atrazine exhibited increased thioglycolate-stimulated recruitment

of white blood cells and decreased activity of these cells, but not outright mortality. Forson and Storfer (2006a) found that *Ambystoma tigrinum* virus (ATV) infection rates of *A. tigrinum* larvae exposed to atrazine and sodium nitrate were higher than those of control larvae or those exposed to either stressor alone. The same researchers (2006b) found similar results for *A. macrodactylum* when exposed to iridovirus and atrazine.

In addition to increased viral infection rates, several studies have linked atrazine exposure to increased susceptibility to parasitic infection. While Griggs and Belden (2008) found that mixtures of atrazine and metolachlor did not increase parasite load in ranid tadpoles, Kiesecker (2002) found atrazine to be among the pesticides that increased trematode cyst infestation in *Rana sylvatica* tadpoles. These parasitic cysts have been implicated in many cases of amphibian limb deformities (Johnson et al. 2002). Koprivnikar et al. (2007) also found that *Rana sylvatica* tadpoles exposed to 30 μg/L atrazine had a higher number of trematode parasites (*Echinostoma trivolvis*) than did tadpoles exposed to 0 or 3 μg/L concentrations. However, they noted that infection rates did not differ from controls when parasites and tadpoles were exposed simultaneously to atrazine, suggesting that high atrazine concentrations may reduce infectiousness of parasites. When Rohr et al. (2008a) exposed green frog (*Rana clamitans*) tadpoles and parasitic cercariae to several pesticides, they found that atrazine was the only chemical that reduced cercarial survival. They suggested that when tadpoles and cercariae were exposed to atrazine simultaneously, the net effect atrazine in the environment would be increased infection rates among tadpoles. Finally, in a field study, Rohr et al. (2008b) discovered that the best predictor of trematode infection rates in *Rana pipiens* tadpoles was concentration of atrazine in the environment.

Atrazine may also impact larval amphibian behavior. Koprivnikar et al. (2007) found that tadpole activity was not decreased by atrazine. In fact, Rohr et al. (2003) observed increases in spontaneous tadpole activity, potentially as a result of direct effects of atrazine on the nervous system. Rohr and Crumrine (2005) also found increased tadpole activity with atrazine exposure and attributed the pattern to decreased periphyton concentrations and the subsequent increase in tadpole foraging effort. The high rate of foraging noted by Rohr and Crumrine (2005) could potentially increase vulnerability to predation, if tadpoles do not decrease movement when predators are present. Allran and Karasov (2001), however, observed that *Rana pipiens* tadpole feeding behavior actually declined in the presence of atrazine, despite increases in buccal and thoracic ventilation.

Although atrazine seldom exceeds concentrations of 30.0 μg/L in the field (Solomon et al. 1996), levels adjacent to agricultural lands have been measured at as high as 500 μg/L (Kadoum and Mock 1978) due to runoff and overspray. Many of atrazine's effects have been observed at levels above expected environmental concentrations (EECs; e.g., Diana et al. 2000 [200 to 2000 μg/L]; Allran and Karasov 2001 [2000 μg/L]). So, while concentrations at which direct effects have been observed are possible in the field, they are not likely.

The effects of atrazine exposure can extend well beyond the larval period in which most exposures occur. Rohr and Palmer (2005) found that the direct effects of atrazine can be delayed up to 8 months postexposure. In their study, *Ambystoma barbouri* salamanders exposed to atrazine demonstrated accelerated water loss and increased risk for desiccation 4 and 8 months postexposure. Rohr et al. (2006) subsequently found lower survival rates in *Ambystoma barbouri* 14 months after exposure to ≥4 ppb atrazine — a concentration just 1 ppb higher than the USEPA drinking water standard (USEPA 2006).

6.4.3 Indirect Effects

Indirect effects are defined as those that affect an ecosystem component that, in turn, affects the species of interest. For example, because atrazine is an herbicide, it targets the photosynthetic systems of plants that serve as food for tadpoles. In this manner, a decrease in algal resources attributable to atrazine application may have an indirect effect on developing amphibian larvae (DeNoyelles et al. 1982).

Boone and James (2003) found that atrazine had negative effects on the size at metamorphosis of larval *Bufo americanus* and *Rana sphenocephala*, and attributed this effect to a reduction in chlorophyll levels (a measure of periphyton concentration). They also found atrazine to lengthen the larval period for the salamander *Ambystoma texanum* but suggested this may be a more direct effect of atrazine toxicity. Rohr and Crumrine (2005) also showed atrazine to have an indirect effect on developing *Rana sylvatica* tadpoles. Tadpoles reared in mesocosms with atrazine had longer larval periods and smaller body sizes upon metamorphosis, which corresponded to a decrease in periphyton.

6.5 CARBARYL

Carbaryl is an insecticide that has recently received considerable attention in the amphibian toxicological literature (Boone and Bridges 2003b). Although it is not widely used in commercial agriculture, carbaryl is one of the most widely used insecticides in the United States in the home and garden market (Kiely et al. 2004). It is relatively nontoxic to wildlife, and despite significant within- and among-species variation with respect to carbaryl sensitivity (Bridges and Semlitsch 2000, 2001), amphibians demonstrate LC50s that are 2 to 3 times higher than environmentally expected concentrations (Boone and Bridges 1999; Bridges et al. 2002; Dwyer et al. 2005).

Aside from being relatively nontoxic to amphibians, carbaryl has a relatively short half-life of 1 to 4 days (Boone and Semlitsch 2002; Boone and James 2003). Therefore, concentrations of carbaryl expected in the natural environment are generally not lethal to amphibians. However, carbaryl can interact with other factors to become directly lethal. UV radiation can increase its toxicity in the laboratory (Zaga et al. 1998), although a similar increase did not occur under field conditions (Bridges and Boone 2003). In the laboratory, low, environmentally realistic concentrations of carbaryl were lethal when 6 species of tadpoles were simultaneously exposed to predators (Relyea and Mills 2001; Relyea 2003). However, when the same exposures occurred in outdoor mesocosms, this pattern was no longer evident (Relyea 2006a). The conflicting results of laboratory versus mesocosm studies emphasize the importance of examining the effects of pesticides in natural settings.

The effects of carbaryl in a more natural environment (i.e., mesocosms) appear to be complex and have been studied extensively. Boone and Semlitsch (2001, 2002, 2003) and Mills and Semlitsch (2004) found that tadpole survival to metamorphosis was actually higher in ponds that had been dosed with carbaryl. Although increased survival is a counterintuitive response to pesticide exposure, this result was shown to be an indirect effect. By killing zooplankton, carbaryl reduced numbers of predators of zooplankton — many of which also feed on tadpoles. The opposite pattern was observed in newts and salamander larvae, which depend on zooplankton for a food source (Boone and James 2003; Boone and Semlitsch 2003; Boone et al. 2007). Carbaryl can also indirectly increase tadpole size at metamorphosis by removing zooplankton that would ordinarily compete with tadpoles for algal resources (e.g., Boone et al. 2007).

Interactions of carbaryl with other naturally occurring stressors have also been well studied. Biotic factors such as competition (e.g., Boone and Semlitsch 2001; Mills and Semlitsch 2004; Boone et al. 2007) and predation (Relyea 2004a), and abiotic factors such as UV radiation (Bridges and Boone 2003), hydroperiod (Boone and Semlitsch 2002), disease (Davidson et al. 2007; Pugis and Boone 2007; Rohr et al. 2008b), and other contaminants (e.g., Boone and James 2003; Boone et al. 2005; Boone and Bridges-Britton 2006) can interact with carbaryl, altering the consequences of exposure for amphibians developing in a natural environment (reviewed in Boone and Bridges 2003b).

6.6 GLYPHOSATE

Glyphosate is the most widely used pesticide in the United States (Kiely et al. 2004), with sales of a global end user market value of nearly $1.5 billion in 1997. Until 2000, this chemical received little attention in the amphibian toxicological literature (but see Mann and Bidwell 1999), but has recently received considerable attention because of its dominance of the herbicide market.

Commercial formulations generally contain substances (e.g., surfactants, carriers, corrosion inhibitors) to increase the efficacy of the active ingredients. Tests determining the toxicity of pesticides often include only the active ingredients, as these other ingredients are considered inert. However, many of these "inert" additives can possess considerable toxicity apart from the active ingredient. A growing body of work has addressed the toxicity of glyphosate end use products and associated inert ingredients.

Alone, the compound glyphosate has a moderate toxicity to developing amphibian larvae (Giesy et al. 2000). However, glyphosate is never applied as the active ingredient alone, and must therefore be tested in its end use form. Glyphosate is the active ingredient of several widely used commercial formulations, including Roundup® (i.e., Vision® in Canada) and Rodeo®. Roundup products were formulated strictly for terrestrial use and include surfactants to help the herbicide adhere to vegetation, while Rodeo was formulated for aquatic environments and contains no surfactants. In a FETAX assay, Perkins et al. (2000) found that Roundup was more toxic to developing *Xenopus laevis* tadpoles than was Rodeo. This difference was attributed to the toxicity of the polyethoxylated tallowamine (POEA) surfactant in Roundup, which had an LC_{50} as low as the active ingredient itself. Rohr et al. (2008a) found no effect on survival of *Rana clamitans* tadpoles when using glyphosate rather than a commercially available glyphosate formulation. Howe et al. (2004) also demonstrated that end use glyphosate products appear to have greater toxicity than the active ingredient alone. In another study examining the toxicity of a glyphosate formulation, Smith (2001) found the formulation Kleeraway® to be just as toxic to *Pseudacris triseriata* and *Rana blairi* as Roundup.

In addition to being acutely toxic, Roundup can increase the length of the larval period and slow the growth rate of a variety of anuran species (Howe et al. 2004; Relyea 2004b; Wojtaszek et al. 2004; Cauble and Wagner 2005) and increase susceptibility to trematode infestation in *Rana clamitans* (Rohr et al. 2008b). Takahashi (2007) found that female gray treefrogs (*Hyla* spp.) selected oviposition sites that were free from Roundup, suggesting that adults may have the ability to avoid exposure to their developing offspring.

Using Roundup, Relyea (2005a) found that several species of North American tadpoles experienced mortality at concentrations that were similar to expected environmental concentrations, particularly *Rana sylvatica*. The effects of this formulation were even more deadly when predators were added to the system (Relyea 2005a) and at higher environmental pH values (Chen et al. 2004; Edginton et al. 2004), and were independent of the presence of a soil substrate (Relyea 2005c).

There is some debate as to whether Roundup — a product formulated for application on land — can be found in aquatic habitats at concentrations that are toxic (Thompson et al. 2006; Relyea 2006b, 2006c). Unfortunately, glyphosate is not generally a part of large-scale water quality monitoring programs (Battaglin et al. 2005) and data on environmental concentrations are lacking. A few studies have documented fairly widespread presence of glyphosate and its degradates in surface water (e.g., Battaglin et al. 2005; Kolpin et al. 2006). Therefore, it is likely that many amphibian populations are exposed at least periodically to glyphosate. This may be especially true for amphibians breeding in habitats vulnerable to herbicide overspray or runoff. Thompson et al. (2004) investigated whether vegetative buffers protect amphibian habitat from overspray during applications of Vision, an end use glyphosate formulation identical to Roundup. They suggested that when Vision was sprayed according to the product label as well as Canadian environmental guidelines, harmful effects on native amphibians should be negligible.

6.7 MALATHION

Malathion is the most widely applied insecticide in the United States (Kiely et al. 2004). It is an acetylcholinesterase inhibitor and has a half-life of up to 26 days. Among other uses, malathion is applied to control mosquito populations, and is key in combating mosquito-borne diseases like malaria and West Nile virus.

Malathion concentrations that are lethal to amphibians tend to be much higher than EECs (Fordham et al. 2001; Relyea, 2004a, 2004b; Gurushankara et al. 2007; Sayim 2008). Thus, most of the effects of ecologically relevant concentrations of malathion on amphibians appear to be indirect. In fact, malathion can appear to have a positive effect on amphibian survival and biomass in mesocosms, as a result of increased mortality among predaceous insects (Relyea 2005b; Relyea et al. 2005; Relyea and Hoverman 2008). However, through a series of trophic effects (e.g., mortality of zooplankton leads to increased phytoplankton levels and subsequent decreases in periphyton available for tadpole grazing), malathion can negatively impact the growth and development of *Rana pipiens* (Relyea and Diecks 2008; Relyea and Hoverman 2008).

Gilbertson et al. (2003) found that malathion reduced immune function in *Rana pipiens*, but only if the frogs were exposed to pathogens prior to insecticide exposure. While recovery of the immune system was observed, it was not until 20 weeks postexposure. Dickerson et al. (2003) observed a trend toward higher cholinesterase in *Bufo woodhousii* caged in areas where malathion was detected.

Like that of many other pesticides, malathion's toxicity can be altered by interactions with other factors. For example, Relyea (2004b) found that malathion is twice as toxic to *Hyla versicolor* tadpoles when they are simultaneously exposed to predators. Boone (2008) found that malathion increased the mass at metamorphosis of *Bufo americanus* tadpoles, unless tadpoles were exposed simultaneously to another acetylcholinesterase inhibitor, carbaryl. When *Rana clamitans* tadpoles were exposed to the same combination of chemicals, mass at metamorphosis was greater than that of control animals, or when animals were exposed to either chemical alone.

6.8 METOLACHLOR

Metolachlor is one of the most frequently detected herbicides in surface water in the Midwest (e.g., Battaglin and Goolsby 1999; Battaglin et al. 2000, 2003, 2005; Clark and Goolsby 2000), occurring in up to 100% of stream samples (Battaglin et al. 2000; Lerch and Blanchard 2003) and up to 50% of groundwater samples (Battaglin et al. 2000). Despite the widespread presence of this herbicide in aquatic habitats, very few studies have examined potential effects of exposure on amphibians. Wan et al. (2006) reported a 96-hour LC50 of 14 mg/L for *Rana catesbeiana* tadpoles — a value similar to those found for rainbow trout (*Onchorhynchus mykush*) and Chinook salmon (*Oncorhynchus tshawytscha*). The apparent toxicity of the end use formulation Primextra II Magnum was considerably lower (96-hour LC_{50} = 56 mg/L). In another study with *R. catesbeiana* tadpoles, the metolachlor formulation Dual-960E induced DNA damage at concentrations as low as 0.272 mg/L (Clements et al. 1997). Metolachlor can also decrease growth and cause edema in *Xenopus laevis* embryos, and appears to become teratogenic after degradation to 2-ethyl-6-methylaniline (Osano et al. 2002).

As part of a large study involving 9 pesticides, Hayes et al. (2006a) determined that S-metolachlor (a compound consisting primarily of the more herbicidally active S-isomer pair of metolachlor) elicited no negative effects on *Rana pipiens* larval survival, growth, or development at a low, environmentally relevant concentration (0.1 ppb). However, S-metolachlor did increase the frequency of damage to the thymus. Interestingly, the compound also appeared to act as an "effector," significantly enhancing the toxicity of atrazine when tadpoles were exposed to the 2 herbicides simultaneously (as either a simple mixture or the commercial atrazine-metolachlor formulation Bicep II Magnum).

Mazanti et al. (2003) exposed larval anurans (*Hyla versicolor*) to a similar atrazine-metolachlor formulation (Bicep II) as well as the insecticide chlorpyrifos in a laboratory setting, with treatment concentrations based on runoff data. Exposure to the higher of 2 herbicide treatments (2.54 mg/L metolachlor, 2.0 mg/L atrazine) caused slower growth and modest delays in metamorphosis, while tadpoles exposed to the lower treatment level (0.25 mg/L metolachlor, 0.2 mg/L atrazine) performed similarly to control animals. All tadpoles in the high herbicide/high insecticide treatment (2.50 mg/L metolachlor, 2.0 mg/L atrazine, 1.0 mg/L chlorpyrifos) died. In a second experiment, Mazanti et al. (2003) simulated spray-overs of experimental wetlands with the same 3 active ingredients

and then sampled naturally occurring tadpoles for 4 months postspray. Although the effects of the herbicide mixture cannot be isolated from those of the insecticide, there were pulses of mortality in both low (0.25 mg/L metolachlor, 0.2 mg/L atrazine, 0.1 mg/L chlorpyrifos) and high (2.54 mg/L metolachlor, 2.0 mg/L atrazine, 0.1 mg/L chlorpyrifos) treatments relative to controls.

The above pesticides represent the chemicals that have received the greatest amount of attention in the amphibian literature. We now turn our attention to the various types of studies that have been undertaken in the amphibian ecotoxicologial literature. The types of studies using amphibians vary greatly and range from laboratory experiments to mesocosm experiments to field studies. Each type of study aims to further our understanding of the impacts of pesticides on amphibians at mechanistic and/or ecological levels.

6.9 TYPES OF STUDIES USED TO EXAMINE PESTICIDE EFFECTS ON AMPHIBIANS

Beginning with the simplest, Frog Embryo Teratogenesis Assay–*Xenopus* (FETAX; ASTM 1998) was developed as a standardized test to examine the effects of toxicants (e.g., pesticides, metals, effluents) on early life stage amphibian larvae. While this assay has proven a useful tool in evaluating acute effects of contaminants on survival and development, there have been several criticisms raised because the test subject (i.e., *Xenopus*) is in a family that is very different from most other amphibian species, which may limit the test's relevance (Mann 2005). Other criticisms include the sensitivity of the species and the length of the assay (96 hours).

AMPHITOX is a laboratory test developed by Herkovits and Perez-Coll (1999) that is similar to FETAX, with more flexibility in choice of life stage and species used during exposure (Herkovits and Perez-Coll 2003). Both FETAX and AMPHITOX are standardized tests that allow the comparison of relative toxicity of various compounds. The lack of concrete ecological relevance notwithstanding, both of these assays are useful in determining the direct effects pesticides can have on individual traits and developmental processes.

Laboratory studies without formal protocols are more flexible than FETAX or AMPHITOX assays and offer a high degree of control with limited external noise, but lack the complexity of natural exposures. While laboratory studies allow us to examine the mechanisms of pesticide toxicity, there are a number of examples of laboratory results conflicting with those from more complex mesocosm studies. For example, Zaga et al. (1998) found that the toxicity of carbaryl was increased when exposed to UV light, but Boone and Bridges (2003a) found no such effect in mesocosms. Similarly, while Relyea and Mills (2001) found that the presence of predators made carbaryl more toxic in the laboratory, Boone and Semlitsch (2001, 2003) observed no such phenomenon in mesocosms. This difference emphasizes the care that must be taken when attempting to predict pesticide effects in the field using laboratory-collected data.

In the study of pesticide effects, mesocosms offer a greater degree of ecological realism than laboratory studies while allowing relatively easy manipulation of multiple factors experimentally. Mesocosms are becoming an important tool in amphibian pesticide research. Boone and James (2005) note that there has been a steady increase in their use since Rowe and Dunson (1994) first drew attention to the usefulness of mesocosms in ecotoxicological testing. However, Boone and James (2005) also point out that these studies have examined only a small number of responses of a few species to a limited number of contaminants and encourage their broader use.

Aquatic enclosures placed in situ can serve as a preliminary examination of whether chemical contaminants in the environment can affect amphibians under seminatural conditions (Bishop and Martinovic 2000) and have been used to demonstrate pesticide effects in nature (Kiesecker 2002; de Solla et al. 2002; Thompson et al. 2004; Wojtaszek et al. 2004; Boone et al. 2008 [golf course ponds]). However, while these studies create realistic exposures, they can suffer from high variability among experimental units.

More recently, larger-scale field studies have become a more common way to examine pesticide effects on amphibian populations. Correlations can be made between patterns of pesticide use and observed amphibian responses (Davidson et al. 2001, 2002; Sparling et al. 2001; Hayes et al. 2002b, 2002c; Davidson 2004; Davidson and Knapp 2007). Additionally, developing amphibians can be exposed to samples taken directly from the environment (Bridges and Little 2003), or entire ponds can be manipulated in a controlled manner (Boone et al. 2004).

Increasingly, the connection of studies with the natural environment is an important component of experimental design. Studies with little or no environmental relevance have limited value, regardless of how elegant the design or clear-cut the results may be. The most successful ecotoxicology programs will integrate a wide variety of methods — laboratory exposures to establish causation and mechanisms of effect, seminatural experiments to examine effects in a more realistic context, and field and landscape level studies to uncover population and community responses (Semlitsch and Bridges 2005).

6.10 CONCLUSIONS

The field of amphibian ecotoxicology has undergone tremendous growth in the years since the first comprehensive review of the literature was published (Power et al. 1989). We now know more about the lethal and sublethal effects of contaminants on multiple life stages of amphibians than at any other time in history. As amphibian ecotoxicology has matured as a discipline, researchers have begun to design studies that integrate laboratory, mesocosm, and field techniques while incorporating explicit connections with the natural world. These advances will be necessary for understanding the complex effects of pesticides on amphibians. Given the reality of amphibian population declines and the widespread nature of pesticide residues, the number of pesticide studies on amphibians should continue to grow for many years.

The challenges to amphibian ecotoxicology remain formidable, however. New pesticide active ingredients are constantly being developed. Even for compounds that have been in use for decades, studies frequently expose new effects and interactions, especially as we move beyond traditional endpoints of mortality, behavior, and metamorphic characteristics. The expiration of patents on popular active ingredient molecules such as glyphosate has led to a proliferation of new end use formulations, emphasizing the importance of studying the contributions of "inert" ingredients to pesticide toxicity. In addition, although studies are slowly expanding to include life stages beyond the larval period, we know almost nothing about potential transgenerational effects of pesticide exposure.

Although the body of knowledge on pesticides and amphibians has exploded relative to the early days of ecotoxicology, a disconcerting number of commonly used pesticides have never been studied at all in regard to amphibians (Hayes et al. 2006a). Amphibian pesticide research must expand both the number of pesticides and the variety of species used in testing. Well-designed studies with a high degree of ecological relevance will improve our understanding of contaminant effects on nontarget organisms, while also contributing to the conservation of amphibian populations.

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7 Ecotoxicology of Pesticides in Reptiles

Bruce D. Pauli, Stacey Money, and Donald W. Sparling

CONTENTS

Editor's Note: Since the previous edition of this book was printed in 2000, there has been relatively little information published regarding the effects of nonorganochlorine (non-OC) pesticides on reptiles. The authors and editors felt that due to the paucity of recently published data on the effects of pesticides on reptiles, the completion of an entirely new chapter, which would contain sufficiently novel information to represent a new peer-reviewed publication, would not be possible. Therefore, the following chapter is a near verbatim reprint of the text of a chapter with the same title included in the first edition of the present book (Pauli and Money 2000), with 1 difference: more recent studies are inserted in the appropriate sections of the present chapter. In total there were only 23 new, open-literature publications added for this updated version of the original chapter. The chapter now reviews the literature describing the effects of non-OC pesticides on reptiles up to and including studies published through early 2010. Following the format of the original chapter, information is included on pyrethroid, organophosphate, and carbamate pesticides, and piscicides, herbicides, and fungicides, with descriptions of sublethal, lethal, and potential population level effects. It should be noted that the more recent literature has shifted in focus to examining the effects of pesticides on reptiles in the laboratory and in the field rather than simply reporting residues, as was the case prior to 2000; some of these studies are discussed elsewhere in this volume. The original chapter (Pauli and Money 2000) included extensive appendices listing pesticide residue measurements in reptiles. More than 90% of that data, however, dealt with chlorinated pesticides such as DDT, mirex, toxaphene, etc. We have not included these older studies in the present chapter.

7.1 INTRODUCTION

Pesticides applied to control fishes, rodents, insects, or vegetation can inadvertently harm reptiles. However, pesticides also may be used in attempts to control "nuisance" reptiles, most commonly snakes and lizards. Pesticides also are employed in reptile veterinary medicine, usually for the control of parasites. Following pesticide field applications, regardless of application procedures, exposure of reptiles generally occurs as a result of the animals' consuming pesticide-contaminated prey or through dermal or respiratory exposure.

To the year 2000 the number of reports concerning the toxicity of pesticides to reptiles was surprisingly low. In fact, there has been little research conducted on this group since an early review by Hall (1980) emphasized that more work was urgently required. Hall (1980) compiled evidence indicating that pesticides, particularly organochlorines (OCs), can kill reptiles when used in standard agricultural practices. Hall (1980) warned that the accumulated information on the effects of pesticides and other environmental contaminants on reptiles was not only severely limited, but also that the data were of questionable relevance because they had been collected using various test methods, analytical procedures, and means of reporting results. Hall (1980) concluded that "despite nearly 40 years of study, we have only scant knowledge of which chemicals may be particularly hazardous to reptiles." He further cautioned that few generalizations can be made about reptiles from the extensive literature on the effects of contaminants on birds (but see below).

In a review published a decade later, Hall and Henry (1992) noted that almost no experimental evaluations of the sensitivity of reptiles to environmental contaminants had been made. One of the objectives of the review was to assess whether reptiles would be adequately protected by the nontarget toxicity tests required at that time for the commercial registration of pesticides and other chemicals. Remarkably, there was only a single study, on 1 species of lizard, that had been conducted in a manner that allowed comparison of the sensitivity of reptiles to other vertebrate groups: Hall and Clark (1982). This single study revealed that reptiles show sensitivity similar to that of mammals and birds in terms of their response to cholinesterase-inhibiting insecticides. Hall and Henry (1992) concluded that far too little was known to safely conclude that guidelines based on tests conducted with other vertebrate taxa offer adequate protection for reptiles, and they recommended a research strategy to fill the existing knowledge gaps.

A review by Lambert (1997a) was more localized in its scope: the author reviewed the published literature and other reports on the effects of pesticides on reptiles in sub-Saharan Africa. One reason given for the review was to identify gaps in the literature concerning reptiles, given that pesticide use in Africa was likely to increase substantially. The author placed special emphasis on collecting information on reptile residue burdens and on the effects of pesticides following field applications. The result was a compilation of a fragmented and somewhat anecdotal literature on the effects of pesticides on tropical reptiles. The review included reports of reptiles killed or otherwise adversely affected — either directly or indirectly through a reduction of their prey base — as a result of applications of OC insecticides such as dichlorodiphenyltrichloroethane (DDT), dieldrin, endosulfan, and toxaphene. While most of the references related to reptile mortality from OCs used to control tsetse flies (*Glossina* spp.) (e.g., Wilson 1972), data included from unpublished sources also revealed that organophosphorus (OP) insecticides, such as cyanophos and chlorpyrifos, and the carbamate insecticide bendiocarb, might harm reptiles following their use for insect control. The author concluded that more information was required to determine both the direct threat of pesticides to reptiles and the potential secondary poisoning of reptile predators, such as raptorial birds, from their consumption of contaminated prey. The latter concern was earlier expressed by Koeman et al. (1978).

Hopkins (2000) also expressed concern about the general lack of ecotoxicological information on reptiles. He noted that life history traits, carnivorous and insectivorous food habits, relatively long lifespan, limited movements in some species, and prolonged time to maturation enhance the need for contaminant studies on reptiles as well as their potential value in monitoring contaminant exposure.

Campbell and Campbell (2002) provided a brief summary of ecotoxicological studies on reptiles with an emphasis on snakes and lizards. They noted that of the 15 families of lizards, 11 had

no contaminant data available. Snakes were not represented any better — of the 10 families in Serpentes, only 4 had contaminant studies published. The number of pesticide-related studies was a fraction of the total number of contaminants and reptiles papers published. The authors specifically cite the need for more papers on cholinesterase-inhibiting pesticides and pyrethroids.

Sparling et al. (2000) and Chapter 1 of this book both point out the continued deficiency of contaminant-related, and especially pesticide-related, papers that focus on reptiles. This deficiency is made even more noteworthy due to an apparent global decline in reptile populations that is akin to that seen in amphibians (Gibbons et al. 1998). This is also in spite of the fact that there are model species of lizards that can be raised in the laboratory and are sensitive to contaminants (Talent et al. 2002), allowing ecotoxicology investigations to be completed.

In summary, despite the data and information presented in the original chapter and in the reviews mentioned above, there is still a scarce amount of information available concerning the effects of contaminants, particularly modern, in-use pesticides, on reptiles. Further studies are required to determine how vulnerable these disappearing species are to contamination. The available information on specific non-OC pesticides is presented here.

7.2 PYRETHROID INSECTICIDES

Synthetic pyrethroids are neurotoxins that act on axons in the peripheral and central nervous system by interacting with sodium channels. While pyrethroids are generally used to control insects, they may also be employed in reptile veterinary medicine, and they have been studied for their ability to directly kill reptiles. As a result, there is information on the acute toxicity of pyrethroids following direct applications to the animals during reptile control programs or veterinary treatment; in addition, there is some scattered information on effects following applications for insect control. The few available studies suggest that pyrethroids can be acutely toxic when directly applied to reptiles, but field studies have not confirmed significant effects on the local reptile fauna when pyrethroids are broadcast sprayed to control insects.

Abe et al. (1994) documented that the pyrethroid insecticide prallethrin (Etoc®) could kill vipers when the snakes were sprayed with an oil-based formulation. The authors concluded that pyrethroid insecticides, which show little toxicity to birds and mammals, appear to be exceedingly toxic to snakes. Further, the pyrethroid seemed to affect the nervous system of treated snakes in a manner similar to that seen with target insects.

A similar conclusion was made following a study of the potential use of synthetic pyrethroids for ectoparasite control in snake and lizard veterinary medicine. Because some ticks and mites of reptiles are resistant to OP insecticides, Mutschmann (1991) decided to study the efficacy of various pyrethroids against these parasites and the ability of certain snake and lizard species to tolerate external applications of the pyrethroids. The pyrethroids examined included deltamethrin, cypermethrin, flumethrin, and permethrin. Among the snakes tested were boa constrictor (*Constrictor constrictor*), red-sided garter snakes (*Thamnophis sirtalis parietalis*), western ribbon snakes (*T. proximus*), rainbow boas (*Epicrates cenchria*), corn snakes (*Elaphe guttata*), and white-lipped tree vipers (*Trimeresurus albolabris*). Among the lizards tested were brown basilisks (*Basiliscus vittatus*) and leopard geckos (*Eublepharis macularius*). Deltamethrin, cypermethrin, and flumethrin produced toxicity in the animals at low doses (<0.074 mg/kg externally applied), with symptoms (such as hyperactivity and ataxia) similar to those seen in endotherms with pyrethroid intoxication. Permethrin proved to be slightly less toxic: 0.05 and 0.1 mg a.i./kg of a 1.0% pour-on solution was tolerated by the animals and provided acceptable ectoparasite control of OP-resistant ticks.

Williams (1989) provided a case report following an attempt at snake ectoparasite control with d-phenothrin. When d-phenothrin was used for mite control on 6 neonatal and juvenile animals of 6 different snake species (14 days to 3 months old), the snakes became ill 6 to 8 hours after application of the insecticide to their cages. The snakes were removed while their cages were "fogged" with

the pyrethroid, and returned to their cages 15 to 20 minutes after the treatment. The snakes later developed symptoms of pyrethroid poisoning. Again, the symptoms appeared similar to those seen in mammals, with twitching, convulsions, and hyperactivity; 1 animal died 27 hours after being returned to its cage. The author concluded that while d-phenothrin might be acceptable for mite control in reptiles of large mass (because the compound had been frequently used without incident in Australia), its use with juvenile reptiles was contraindicated. As in the previous study, the snakes were exposed to the insecticide through dermal uptake — in this case from contaminated surfaces in their cages — but these animals also obtained residues through ingestion of the skink prey that they consumed in their cages. The author noted that the most severely intoxicated snakes were those that had consumed the most skinks; 3 of the snakes regurgitated skinks the morning following the treatment, with the 1 snake that died regurgitating several partially digested lizards. Because the application rate was not provided and residues in the cages and on the skinks were not measured, an extrapolation of the dermal and oral exposures an animal might encounter following a similar pyrethroid application for insect control in the animals' natural environment could not be made.

Talent (2005) conducted a study to determine if ambient temperature had an effect on the toxicity of natural pyrethrins. He obtained a commercial mite and lice bird spray containing 300 mg/L pyrethrins and 3000 mg/L piperonyl butoxide, a pesticide synergist. Adult green anoles (*Anolis carolinensis*) were dipped into the solution up to their heads, while controls were dipped into reagent-grade water. Animals were then placed in incubators and held for 48 hours at constant temperatures ranging from 15 to 38 °C. Mortality closely followed an inverse dose-response relationship: 30% of the anoles died at 38 °C, whereas 100% died at 20 and 15 °C. The temperature that resulted in 50% mortality was calculated as 33.4 °C. Dose-dependent mortality also varied with ambient temperature. In a second test it was found that the LC50 for the pyrethrin solution at 20 °C was 77.6 mg/L, but exposure to the solution at 300 mg/L resulted in only 45% mortality at 35 °C, and no LC50 could be calculated at that temperature.

A study conducted in South Africa with the pyrethroid deltamethrin (in its Decis® formulation) attempted to extrapolate oral exposure to pyrethroids following their use in insect control operations. Stewart and Seesink (1996) set out to determine the risk to reptiles consuming brown locusts (*Locustana pardalina*) contaminated with deltamethrin as a result of applications to large groups of the insects during locust control programs. Further, because the reptiles could be exposed to deltamethrin from direct spray, through exposure to contaminated vegetation, or through ingestion of contaminated grasshoppers (collected as surrogates for the target locusts), sprayed vegetation and locusts were also analyzed for deltamethrin residues. To complete the exposure assessment, 2 species of small sand lizard (*Pedioplanis lineoocellata* and *P. namaquensis*, mean body weight of 2.5 g) were brought into the laboratory to determine their maximum daily consumption of brown locusts. Following a 3-day fast, the captive lizards (total of 21 replicates) consumed both live and dead locusts, the smaller lizards consuming a higher mass of locusts per body weight. Based on the residues measured in the grasshoppers, and the maximum consumption of locusts by lizards in the laboratory, the authors calculated that the maximum daily dose of deltamethrin a lizard could obtain by eating contaminated locusts would be approximately 34 mg/kg body weight. Whereas data do not exist to determine the effects an oral exposure of this magnitude might have on these lizards, because an external deltamethrin dose of less than 0.074 mg/kg resulted in toxicity in Mutschmann's (1991) experiments, there are some grounds for concern. In support, Owadally and Lambert (1988) reported that deltamethrin (K-Othrin® formulation) is used as a bait with sugar to remove geckos from houses on Mauritius.

Impacts at the population level were not noted, however, in 2 studies that monitored effects on reptiles following operational applications of deltamethrin in Zimbabwe (Grant and Crick 1987; Lambert 1994). Since 1988, deltamethrin has replaced DDT for tsetse fly control in Zimbabwe (Lambert 1994), warranting investigations into its toxicity to nontarget wildlife. Grant and Crick (1987) followed a series of 5 aerial applications of deltamethrin applied at approximately 2-week intervals to assess effects. Applications were made from a fixed-wing aircraft at a rate of 250 mg a.i./ha. An assessment of the impact of the sprays on reptiles was based on transect censuses of rainbow skinks (*Mabuya*

quinquetaeniata margaritifer), a common insectivorous lizard inhabiting the treated area and a nearby untreated control area. However, skink numbers were extremely variable on a daily basis, probably because of environmental and climatic changes, and this variability may have prevented determination of any effects of the pesticide on skink numbers. Nevertheless, the authors concluded that no effect of deltamethrin could be detected on the population of skinks inhabiting the treated site.

A similar conclusion was reached by Lambert (1994) following his study of the effects of single, ground-based treatments of deltamethrin applied selectively to tsetse fly resting sites on tree trunks in 8 areas of northwestern Zimbabwe. The same methodological problems apply to this study, as impacts were again assessed only by transect censuses of lizards in treated and untreated areas (the data were based on the number of trees occupied by lizards before and after the treatments). The single deltamethrin application did not appear to decrease abundance of the dwarf gecko, or the mabuya (*Mabuya striata wahlbergii*) in the short term, although changes in the animals' basking behavior may have confounded the analyses. The author also concluded that any long-term effects stemming from an insecticide-induced reduction of the lizards' invertebrate prey base appeared to be unlikely.

Also working with deltamethrin, Alexander et al. (2002) directly sprayed captive lizards (*Meroles suborbitalis* and *P. namaquensis*) with deltamethrin at a rate equivalent to either 17.5 or 25 g a.i./ha. The authors did not say if the pesticide was in formulation or not. They also indirectly exposed the same species at the same rates but focused the spray onto soil within the animals' holding tanks (lizards were haphazardly directly exposed during the application). Under both exposure regimens sublethal effects, including loss of motor coordination, spasms, and hyperactivity under direct light, appeared. These effects were seen in all 4 treatments, but they were more obvious at 25 g/ha than at 17.5 g/ha in the direct exposure. Effects were slower to occur and were less severe in the indirect application than in the direct application. Although effects dissipated within 24 hours, mortality at 2 months postexposure in all treatments was greater than in controls.

In the same study, Alexander et al. (2002) demarcated three 1 ha plots of similar plant composition in the same general area and sprayed 2 of these plots with 17.5 g a.i./ha delamethrin. They sampled each plot 1 week before application and at 1, 4, and 18 weeks following application. In the treated plots, population numbers of both species were less at 1 and 4 weeks postapplication compared to preapplication, while there was no difference between pre- and postapplication numbers in the control plot. Numbers at 4 weeks were lower than those at 1 week in the treatment plots, indicating that the effects of deltamethrin last longer than a few days. After 18 weeks, population numbers had returned to those prior to application in the treatment plots. *P. namaquensis* showed greater reductions in numbers in the field trial than did *M. suborbitalis*. The authors suggested that this difference may be related to the food habits of the 2 species in that *P. namaquensis* was seen ingesting dead, possibly contaminated prey, whereas *M. suborbitalis* is an ambush predator and more likely to consume only living prey.

7.3 ORGANOPHOSPHORUS AND CARBAMATE INSECTICIDES

7.3.1 Organophosphates

Studies of the effects of OP insecticides on reptiles include the following:

- field monitoring projects;
- controlled-dose laboratory studies that have established LD50s, examined sublethal effects, and investigated compounds that might be useful in controlling reptiles;
- anecdotal or observational reports following field applications or spills; and
- analysis of the effects of these pesticides on cholinesterase.

Özelmas and Akay (1995) studied the effects of chronic malathion exposure on the small (3 g) insectivorous dwarf lizard *Lacerta parva*. The lizards received a daily gavage dose for 16 weeks of

1, 2, or 3 mg/kg of technical malathion in sunflower oil, were gavaged with oil alone, or were held as untreated controls. No mortality could be attributed to the malathion exposure, but the results were confounded by dose-independent mortality of more than half the animals during the 16-week experiment. Further, while dose-dependent damage to the liver and kidneys was observed, and degenerative changes in the small intestine were noted in the treatment groups, degenerative damage in the kidneys of the sunflower oil control group was also noted.

Meenakshi and Karpagaganapathi (1996a, 1996b) studied the toxicity of phosphamidon to the variable agama, *Calotes versicolor*. The treatment animals were administered a single oral dose of phophamidon in distilled water, while the control animals received distilled water only. A 24-hour LD50 of 2.3 mg/kg was determined, and a no observed effect level (NOEL) of approximately 0.33 mg/kg. The authors also reported the remarkable behavioral changes and symptoms of OP poisoning brought about by administration of the OP to the agamas (although dose levels for these changes were not provided): irregular movements, quivering, tremors, convulsions, wriggling, gasping, blinking, shedding of body scales, and color changes (Meenakshi and Karpagaganapathi 1996a) and anemia in individuals following a single sublethal dose (0.77 mg/kg body weight) of technical phosphamidon (Meenakshi and Karpagaganapathi 1996b).

Japanese researchers studied compounds that might be used to kill the *habu*, or yellow-spotted pit viper (*Trimeresurus flavoviridis*; Kihara and Yamashita 1978). They used the small lizard *Eumeces oshimensis* as a surrogate for the viper and monitored the time to death after a dermal exposure to pesticides (administered as dusts or through pesticide-impregnated paper in the animals' holding cages). Among the 42 pesticides they tested were several OPs, but an LD50 value for the lizards could not be calculated. In another experiment, Lambert (1997b) showed that lizards could be killed by contact with soil heavily contaminated with OP and OC residues, but the separate contribution of the OPs to the toxicity could not be determined.

Since the first edition version of this chapter, there have been only a few additional papers published on the toxic effects of OP pesticides on reptiles. Holem et al. (2006), for example, looked at sprint speed in the western fence lizard (*Sceloporus occidentalis*). Locomotory ability is important to lizards for procuring prey, avoiding predators, and mate selection. Lab-reared juveniles were treated with 0.2 to 200 mg/kg malathion in corn oil via gavage. At 200 mg/kg, 20% (2 out of 10) of the treated lizards died but no mortalities occurred at lower dosages. Sprint velocity was measured at 24 hours before dosing and at 4, 24, 120, and 312 hours postdose. Somewhat surprisingly, lizards treated with the highest concentration of malathion demonstrated an increase in sprint velocity. Sprint velocities of lizards receiving single 0.2, 2.0, and 20 mg/kg doses of malathion were not different from vehicle control animals, while lizards exposed to 200 mg/kg showed on average a 23% increase in sprint velocity following exposure. This increase was apparent at 4 hours postdose and continued through 312 hours postdose. The heightened velocity occurred even though 70% of the lizards at this dose displayed signs of OP toxicity, including body/limb tremors and twitching. The authors were not able to fully explain the increased velocity.

Repeated exposures to malathion at 2.0, 20, or 100 mg/kg did not affect growth, food consumption, or body condition index of western fence lizards (Holem et al. 2008). The animals received a total of 3 gavage doses of malathion given as a single dose repeated every 27 days. At 20 mg/kg, the researchers observed 8% mortality. At 100 mg/kg, 23% of the lizards died and 85% of the animals displayed signs of organophosphorus toxicity. Twitching and tremors usually ceased after 24 hours postexposure. This study examined both terrestrial locomotory ability, by determining running velocity within a racecourse, and arboreal locomotory ability, by measuring sprint velocity along a 2.5-cm dowel rod. Terrestrial locomotory ability was not affected by malathion at 100 mg/kg, but arboreal locomotory ability was significantly impaired at this concentration.

Fenitrothion is used to control locust outbreaks in Australia, but the effects of this pesticide on native fauna there are not well known. To study this, Bain et al. (2004) dosed central bearded dragons (*Pogona vitticeps*) with corn oil (controls) or 4 or 20 mg technical-grade fenitrothion per kilogram body mass. Dragons given 20 mg/kg demonstrated significantly depressed total cholinesterase (ChE) levels

2 hours postdose; ChE depression lasted for at least 120 hours postdose. In contrast, animals dosed with 4 mg/kg fenitrothion showed ChE depression for only 8 hours. Fenitrothion did not appear to affect standard metabolic rate but may have weakly influenced thermoregulatory preferences in that mean body temperatures in both of the dosed groups were marginally higher postdose compared to predose.

7.3.2 Carbamates

Stinson et al. (1994) conducted a survey of the effects on wildlife of carbofuran applications to corn fields, mainly by searching for dead and debilitated wildlife on 44 treated fields on 11 farms. Carbofuran was applied as granules (Furadan 15G® formulation) and applications were carefully made according to specific risk reduction measures instituted for field applications of the Furadan 15G formulation. One dead eastern garter snake (*Thamnophis sirtalis*) was found in a treated field, but the cause of death was equivocal because no residues of carbofuran were found in the carcass and other pesticides had been used in the area.

The effects of carbaryl on swimming speed were tested on diamondback water snakes (*Nerodia rhombifer*) and black swamp snakes (*Seminatrix pygaea*) (Hopkins et al. 2005). Of the 2, the black swamp snake has a more permeable skin and is substantially more aquatic. The authors speculated that the greater skin permeability might make the black swamp snake more susceptible to pesticide effects than the diamondback water snake. In 1 experiment, snakes of both species were placed in water containing 0, 2.5, or 5 mg carbaryl/L for 48 hours and then tested for swimming speed along a 2-m track. At 5 mg/L swimming speed was reduced in both species compared to controls, and the decrease was significantly greater in the black swamp snake than in the diamondback. In a second, related, experiment Hopkins et al. (2005) found that the snakes were able to recover their swimming speeds around 96 hours after being removed from the treated waters. Again, the black swamp snake showed greater effects due to carbaryl than did the diamondback water snake. A subsequent experiment (Hopkins and Winne 2006) determined that carbaryl can also reduce the swimming speed of *Natrix taxispilota* and *N. fasciata*.

DuRant et al. (2007a, 2007b) studied the effects of carbaryl on western fence lizard locomotor ability and energy acquisition. Lizards were dosed via gavage with 2.5, 25, and 250 μg a.i./g body weight using a liquid Sevin® formulation (DuRant et al. 2007a). They were then tested for terrestrial and arboreal locomotory ability as in Holem et al. (2006, 2008). No mortalities occurred, but 58% of the lizards at 250 μg/g displayed tremors and twitching. At 2.5 and 25 μg/g terrestrial velocity actually increased by 11 to 23% compared to pretreatment velocities. At the highest dose, terrestrial velocity decreased by 33 to 37%. In the arboreal test, there were significant dose-dependent differences in the number of lizards refusing to cross the dowel rod and in the number that fell when trying to cross. Sprint velocity slowed only with the 250 μg/g dose. Locomotory ability returned to normal within 96 hours of exposure. The authors stated that impaired locomotory ability in terrestrial or arboreal environments could reduce survival of wild lizards. In a follow-up study, carbaryl, at the same doses as above, did not affect overall total energy expenditure in western fence lizards (DuRant et al. 2007b). However, lizards dosed with 250 μg/g allocated their energy differently than those given lower doses. The high-dosed animals used 16% to 30% more energy in maintaining standard metabolic rates and 45% to 58% less energy in additional expenditures above their metabolic rate. High-dosed animals also reduced food consumption by 30% to 34%, with a net energy loss, compared to controls or animals given lower doses. The authors concluded that energy acquisition and energy balance are complex systems and that further research is needed to determine the consequences of the alterations observed following the high-dose exposure.

7.3.3 Cholinesterase Inhibition Studies

A controlled dosing study was conducted using green anoles and 4 OP insecticides (Hall and Clark 1982). The objectives were to establish LD50 values for these compounds and determine

dose–response curves for brain acetylcholinesterase (AChE) inhibition. A related objective was to assess whether lizards could be used to monitor OP applications. In other words, could the measured level of cholinesterase inhibition in a field-collected reptile be used to indicate exposure to OPs or OP-induced mortality? The 4 OPs (parathion, methyl-parathion, azinphosmethyl, and malathion) were administered in single doses to the anoles by stomach intubation, LD50s were calculated, and brain AChE activity was measured. The results indicated that anoles are similar to birds and mammals in terms of their high sensitivity to OPs, and are unlike other poikilothermic vertebrates in this respect. Other literature supports this finding. A review of avian LD50 data by Baril et al. (1994) allows comparisons of the average sensitivity to OP pesticides of various reptile and bird species. Although the amount of data is limited, when the available LD50 values for reptiles and birds are ranked, there is a remarkable concurrence between both the ranking and the absolute values for those OP compounds for which there are LD50 data for both groups.

Hall and Clark (1982) were interested in whether reptiles could be used to monitor wildlife exposure to, and effects of, OPs. A decade later, a series of studies (Fossi et al. 1995; Sanchez et al. 1997) were conducted to determine whether Gallot's lizard (*Gallotia galloti*) could be used as an indicator of exposure and effects of OP applications in the Canary Islands. Because approximately 10 kg/ha of OPs are used in agricultural areas on the islands each year, the researchers wanted to determine whether the lizard might provide a more appropriate indicator than did birds, which typically are used to monitor environmental effects of OPs. In particular, they hoped to establish a nonlethal method of monitoring OP effects based on the activity of serum butyrylcholinesterase (BChE) measured in blood samples collected in the field from live animals. Because brain AChE measurements had become an established method of monitoring OP contamination and effects (Ludke et al. 1975; Zinkl et al. 1979, 1980; Grue et al. 1991), the authors also wanted to test whether this method could be used with reptiles.

Fossi et al. (1995) conducted a detailed examination of the effects of the OP trichlorfon in lizards using acute and chronic laboratory exposures. The main objectives were to measure inhibition of cholinesterase following administration of the OP and to test for a possible correlation between the inhibition of serum BChE and brain AChE. The existence of the latter relationship would allow serum BChE to be used as a nonlethal field method of measuring exposure and impacts of agricultural OP applications. In an acute experiment, brain AChE, serum BChE, microsomal carboxylesterase (CbE), and 7-ethoxyresorufin-O-deethylase (EROD) activity were measured 24 hours after a single gavage dose of 0, 5, 50, or 100 mg trichlorfon/kg lizard body weight. Six lizards were used per treatment group. There was significant inhibition of AChE and BChE at 50 mg/kg (on average, 25% inhibition of AChE and 70% inhibition of BChE) and 100 mg/kg (53 and 90%, respectively). There was also a high degree of correlation between the activities of the 2 cholinesterases at each dose level. A comparison of the inhibition of all 4 enzymes 24 hours after the 100 mg/kg dose indicated that serum BChE showed the highest inhibition, followed by EROD, CbE, and brain AChE. The chronic study was designed to examine the recovery rate of serum BChE after a single oral dose of 50 or 100 mg/kg. Blood samples were taken from the posterior orbital sinus under anesthesia every 24 hours for 22 days (50 mg/kg group) or 35 days (100 mg/kg group). The high dose of trichlorfon (100 mg/kg) caused an almost complete inhibition of the activity of serum BChE (99% inhibition at 24 hours after administration), and it took 21 days for recovery of serum BChE to 50% of normal activity levels.

Because of the sensitivity of BChE to the OP and because no mortality was observed in either investigation, the authors argued that their objectives were met: the high degree of inhibition without mortality and the extremely slow recovery of serum BChE following exposure confirm the utility of this species as a bioindicator of OP contamination (the slow recovery of BChE activity extends the period during which the animals can be sampled following an application). However, in a field study conducted in association with these experiments, no inhibition of serum BChE was measured in lizards collected in an area that had been treated 24 or 48 hours previously with 10 kg/ha of a trichlorfon formulation (Dipterex sp80, 80% trichlorfon). The authors also do not discuss how a relatively invasive procedure such as blood sampling from the orbital sinus may have influenced the results, or whether they observed any detrimental effects on the animals manipulated in this way.

Following Fossi et al.'s (1995) work, further studies by the same group were carried out on the effects of parathion on *Gallotia galloti* (Sanchez et al. 1997). Single doses of 0.5, 2.5, 5.0, or 7.5 mg a.i./kg parathion were administered by oral intubation, and brain AChE, serum BChE, and serum and microsomal CbE were measured 6 or 24 hours after dosing. In a second experiment, 0.5 or 7.5 mg a.i./kg parathion was administered 3 times over a 44-day period, and recovery of serum BChE activity was monitored using sequential blood sampling. As with trichlorfon, administration of parathion resulted in significant inhibition of both serum BChE and brain AChE activity following a single dose. The 7.5 mg/kg dose caused brain AChE activity to be reduced to approximately 15 and 30% of the normal level at 6 and 24 hours after dosing, respectively, and serum BChE was reduced to approximately 2% of normal activity levels at both sampling times. Both cholinesterases were inhibited in a dose-related manner, but brain AChE activity recovered much more quickly. The serum CbE inhibition pattern was similar to that of serum BChE, but the absolute level of inhibition was lower. Microsomal CbE showed a more complex pattern, being unchanged or induced, and was inhibited only at the high-dose level. As in the previous study with this species, no animals died, although the authors mention that symptoms of cholinergic poisoning (muscle twitching and tremors) were observed in the high-dose group. Results of the multiple-dose experiment were similar to those reported by Fossi et al. (1995): serum BChE activity was severely reduced (to 4% of normal) and recovered slowly following administration of the high dose. Brain AChE activity was also slow to recover: 30 days following the third dose of 7.5 mg/kg, brain activity was only 52.5% of normal activity levels. Because no mortality occurred, it would appear that this lizard species can tolerate relatively high levels of cholinesterase inhibition.

The reasons for lack of cholinesterase inhibition in Fossi et al.'s (1995) field-collected animals are unknown, and the potential of Gallot's lizard as an indicator of OP contamination remains unclear. The diet of *G. galloti* is exclusively vegetarian, and the preferred diet of the animals in the study area was fruit (Fossi et al. 1995). The expected residue levels on fruit would be approximately 50 mg/kg (Hoerger and Kenaga 1972; USEPA 1986) following an 8 kg a.i./ha application of trichlorfon. Assuming exposure to this level of residues, and the observed cholinesterase inhibition from trichlorfon exposures in the laboratory, it is unclear why there was no cholinesterase inhibition in the field-collected lizards. One possibility is that the rate of food consumption by the lizards was not sufficient for them to acquire a dose that would cause significant inhibition. Data for the smaller, insectivorous sand lizards studied by Stewart and Seesink (1996) indicated that they consumed about 20% of their body weight per day when fed grasshoppers ad libitum after a 3-day fast. Extrapolation of this ingestion rate to the larger, frugivorous Gallot's lizard results in an estimated daily consumption of about 10 mg trichlorfon/kg if the animals consumed only fruit contaminated with the maximum calculated residue level. This "dose" is below that which resulted in significant inhibition in the laboratory. Alternative explanations might be that the 6 lizards retrieved at each of the 2 posttreatment sampling times had recently moved into the relatively small treated area (300 m^2), or that the collected animals had consumed a small amount of treated fruit and, experiencing an adverse effect, avoided the contaminated fruit thereafter.

Two other field studies failed to detect significant inhibition of reptile AChE following application of OPs. McLean et al. (1975) collected anoles (*Anolis c. coelestinus*) in forested areas of Haiti 24 to 36 hours after the forest had received the second of 2 aerial applications of malathion. The 2 application rates were reported as 0.4 and 0.3 kg/ha, respectively, but the amount of active ingredient applied was not stated. Lizards collected in the treated area had levels of brain AChE activity comparable to levels measured in anoles collected prior to the malathion applications (i.e., no inhibition was measured). Although the spray killed fishes, the lush tree canopy may have intercepted most of the pesticide sprayed over the forest and prevented direct exposure of the lizards. Similarly, when the OPs phosphamidon and dicrotophos were applied to control the forest tent caterpillar (*Malacosoma disstria*), no adverse effects were seen in snakes encountered in the sprayed areas (Oliver 1964). On the other hand, a report from the Ukraine (Karpenko and Myasoedov 1978) mentioned that aerial applications of malathion ("carbophos") to oak forests at 0.6 L/ha (amount of active ingredient unknown) caused mortality of birds, reptiles, and small mammals.

Brain AChE inhibition following parathion exposure has also been measured in turtles. Yawetz et al. (1983) studied various responses of polychlorinated biphenyl-treated or untreated male Caspian terrapins (*Mauremys caspica rivulata*) following administration of parathion and drew comparisons between birds and reptiles. Capsules containing parathion were fed to turtles that had been collected from a sewage canal. Yawetz et al. (1983) noted that this turtle may be the only vertebrate present in these highly polluted habitats (where it reproduces successfully). Therefore, studies on its ability to tolerate contaminants were of interest. According to the authors, in comparison to invertebrates and some vertebrates, a relatively high LD50 for parathion was measured in the turtles (Table 7.1). This prompted the authors to speculate on the biochemical parameters in the turtles that might protect them from the lethal action of parathion. One of the mechanisms mentioned might be the relatively low rate of AChE inhibition observed in the turtles as compared to birds; the rate of AChE inhibition measured in turtle brain homogenates by paraoxon, the active metabolite of parathion, was 0.001 of that measured in the African bulbul (*Pycnonotus capensis*) and 0.004 of that seen in the barn owl (*Tyto alba*).

TABLE 7.1
LD50 Determinations in Reptiles Administered OP Insecticides, Compared with Medial LD50 Values Calculated from Various Bird Species

Pesticide	LD50 (95% CI) (mg/kg)	Species (Number of Species)[a]	Reference
		Lizards	
Phosphamidon	2.3[b]	*Calotes versiclor*	Meenakshi and Karpagaganapathi 1996
Parathion	>7.5	*Gallotia galloti*	Sanchez et al. 1997
Parathion	8.9 (4.7–13.2)[c]	*Anolis caronlinensis*	Hall and Clark 1982
Methyl-parathion	82.7 (56.2–188)[c]	*A. carolinensis*	Hall and Clark 1982
Azinphos-methyl	98[d]	*A. carolinensis*	Hall and Clark 1982
Trichlorfon	>100	*G. galloti*	Fossi et al. 1995
Malathion	170	*Lacerta parva*	Özelmas and Akay 1995
Malathion	2324 (1671–3234)[c]	*A. carolinensis*	Hall and Clark 1982
		Birds	
Phosphamidon	3.71[e]	(14)	Baril et al. 1994
Parathion	5.62[e]	(18)	Baril et al. 1994
Methyl-parathion	7.89[e]	(8)	Baril et al. 1994
Trichlorfon	53.1[e]	(10)	Baril et al. 1994
Azinphos-methyl	79.5[e]	(6)	Baril et al. 1994
Malathion	502[e]	(6)	Baril et al. 1994
		Turtle	
Parathion	≈15[f]	*Mauremys caspica*	Yawetz et al. 1983

Source: From Pauli and Money (2000).

[a] Number of species used in the calculation of the median LD50 (see Baril et al. 1994).

[b] 24-hour value.

[c] Determined by moving average method; values (95% confidence limits).

[d] Estimated value; confidence limits could not be calculated.

[e] Median LD50 calculated from various species.

[f] Actual value was 10 mg/kg; based on the mass of turtle hard tissue composed of the carapace and plastron, the authors estimate a soft tissue LD50 of 15 mg/kg.

Additional work on cholinesterase characterization in *Gallotia galloti* has been published by Sanchez-Hernandez since the publication of the first edition of this book. Sanchez-Hernandez and Sanchez (2002) found that BChE accounted for 83% of total serum cholinesterase and was essentially absent in brain tissue. Serum BChE and AChE activities increased with pH through pH 11.0, with some leveling off after pH 9.0. Brain AChE activity reached a peak at pH 9.0 and declined at higher and lower pHs. Pralidoxime (2-PAM) is known to reactivate AChE after inhibition with an orgphanophosphorus pesticide. The authors determined that BChE may also be reactivated with 2-PAM. These findings support the interpretation that BChE can be used as an effective indicator of organophosphorus pesticide toxicity. Due to its sensitivity to exposure, high activity rates, and abundance in serum, BChE may be more effective than AChE. However, BChE cannot be used to evaluate cholinesterase depression in reptilian brains.

Free-ranging *G. galloti* were captured from reference and agricultural sites in a field study located in the Canary Islands (Sanchez-Hernandez 2003), and animals were again assayed to determine if BChE and AChE are useful for determining exposure to cholinesterase-inhibiting pesticides. Serum collected from the postorbital sinus of *G. galloti* was tested for reactivation with 2-PAM and for activity of the cholinesterases. Incubation of samples in warm water for an hour was used to determine whether spontaneous reactivation consistent with carbamate exposure could be determined. Mean BChE activity rates from agricultural sites were significantly lower than those from reference sites. In 1 site 4% (5/125) of the captured animals were diagnosed as being BChE inhibited because their activity levels were lower than 2 standard deviations below the control group. At another site 22% (16/73) of the animals collected from the agricultural site had depressed BChE activity. When the samples were subjected to 2-PAM reactivation, evidence for inhibition in the animals increased to 18% (9/50) and 30% (17/56) in the 2 areas, respectively. There was no evidence of BChE inhibition in the 2 reference areas. Maximum BChE inhibition observed in the study was 94%. A third study (Sanchez-Hernandez et al. 2004) demonstrated that *G. galloti* exposed to carbamates in the field can show spontaneous recovery of BChE. These studies confirmed that lizard BChE can be an effective diagnostic tool of OP or carbamate exposure under field conditions.

7.4 PISCICIDES

The effects of piscicides on reptiles involve the compound rotenone. Rotenone acts by blocking reoxidation of the reduced form of nicotinamide adenine dinucleotide (NADH) by NADH-dehydrogenase, causing death through oxygen deprivation (Fontenot et al. 1994). It is often used to remove fish from managed ponds. Haque (1971) made an early observation that rotenone may be harmful to reptiles; 1 dead aquatic snake (species not given) was found in a fish-rearing pond that had been treated 48 hours earlier with approximately 1.0 mg/L of a rotenone formulation. However, another snake was seen entering the pond (to an unknown fate), and therefore the results are somewhat inconclusive. Fontenot et al. (1994) concluded that no good studies had been conducted to document the effect of rotenone applications on reptiles. They suspected, however, that animals utilizing a high degree of dermal respiration and that are slow to leave their aquatic habitat following treatment, such as certain turtles, are probably susceptible to rotenone treatments. This supposition was soon confirmed by McCoid and Bettoli (1996), who found dead and dying common mud turtles (*Kinosternon subrubrum*) in shallow coves around a reservoir where 3 mg/L of a 5% rotenone formulation had been applied to assess fish community structure. At least 60 turtles died in small coves (totaling only 6.7 ha surface area); the authors speculate that probably more were killed but their carcasses were not recovered.

7.5 HERBICIDES

Prior to 2000 only 1 report was found of a study of the direct effects of herbicides on snakes. Three other reports were found that contained anecdotal information concerning possible effects on snakes, turtles, and tortoises. However, after 2000 the effects of herbicides on reptiles were the

most active area of pesticide research for this class. Several papers were published on the effects of terrestrial herbicides, and 2 were published on aquatic herbicides. A few studies concerning the effects of the controversial herbicide atrazine and reptiles have been published recently, but since Chapter 8 of this volume reviews the effects of atrazine on amphibians and reptiles, these papers are not included here.

Littrell (1983) examined the toxicity of the herbicide thiobencarb (Bolero 10G®) to garter snakes. Thiobencarb is used in rice culture, and its use in the United States may overlap the range of the giant garter snake (*Thamnophis couchi gigas*). To assess the risk of herbicide applications to this rare snake, the mountain garter snake (*T. e. elegans*) was used as a surrogate in studies of the risk of toxicity to snakes through exposure to contaminated prey in the laboratory and aerial applications in the field. In the laboratory, thiobencarb was administered in gelatin capsules implanted in the flesh of fishes fed to the snakes. Doses of 158 to 623 mg thiobencarb/kg snake body weight did not adversely affect the 5 exposed snakes. As the estimated field exposure of a typical 200-g garter snake would amount to about 1.5 mg/kg through ingestion of contaminated food, the author concluded that an adequate margin of safety exists for the snakes consuming contaminated prey following thiobencarb aerial applications. In a field study, snakes were placed in 2 traps at the edge of a field that had been treated 2 hours previously with 45 kg/ha Bolero 10G, and 2 more snakes were placed in 2 traps in a nearby unsprayed ditch. No adverse effects were noted during a 5-day exposure of the snakes to the contaminated field or during an additional 8-day observation period after the snakes had been moved back to the laboratory.

Brown (1994) captured 2 adult smooth green snakes (*Opheodrys vernalis*) at the base of a power line pole where herbicide granules had been heavily applied to prevent growth of vines on the pole. Containers of the granular herbicide "Weed Blast-4G" and metribuzin were found nearby, and the snakes were captured among the herbicide granules, but few other details were provided.

In an early study, Pierce (1958) examined the effects of 2 applications of 2,4,5-T ("Kuron") to 2 areas along the shore of a pond. Turtles were consistently seen throughout the study period, but the results are not very conclusive; the herbicide was applied to the pond surface and may not have mixed into the pond water very thoroughly, as the applications apparently had no effect on the submersed pond weeds.

However, adverse effects of the herbicides 2,4,5-T and 2,4-D on tortoises were noted by Willemsen and Hailey (1989) during a survey they conducted on the status and distribution of turtles and tortoises in Greece. While the use of paraquat and atrazine for the removal of ground vegetation had no obvious toxic effects on tortoises (the animals were observed to consume vegetation contaminated with these herbicides), almost no tortoises were subsequently seen in areas where the scrub vegetation of low terrace walls was sprayed with 2,4-D or 2,4,5-T. Although the wall vegetation provided important cover for the animals, the authors concluded that the herbicides seemed to affect the tortoises through direct toxicity rather than indirectly through a reduction in their food or cover. This conclusion was supported by frequent observations of tortoises with swollen eyes and fluid discharge from the nose. Moreover, in 1 sprayed area that was intensively studied for a 5-year period, the number of tortoises declined substantially (44% reduction in numbers), apparently through mortality. The area was mapped into sprayed and unsprayed sectors and the movement of tortoises recorded. The numbers of tortoises decreased rapidly in the sprayed areas while remaining constant in the unsprayed sectors. The authors therefore concluded that the decrease in numbers was related to direct mortality in the sprayed areas and not to migration to unsprayed areas, but they acknowledged that physical disturbance to the treated sites was also a problem. Greater detail on this population was presented by Willemsen and Hailey (2001).

Jones et al. (2000) compared the effects of herbicide plus prescribed burning vs. herbicide alone on amphibian and reptile populations in an oak/hickory-dominated community in Oklahoma. They used the herbicide tebuthiuron following label application rates of 2 kg a.i./ha. Distinct differences were observed in the communities based on treatment. The control sites were mature oak/hickory

forests, the tebuthiuron-sprayed areas were mixed shrublands dominated by red cedar (*Juniperus virginiana*), and the areas treated with prescribed burning and herbicide were open parklands with scattered red cedar. Reptile abundance was greatest in the control sites and least in the herbicide-only sites; however, species diversity was nearly equal in all 3 habitat types. The authors concluded that the tebuthiuron-only areas had the least habitat diversity and that tebuthiuron spraying affected the animals negatively.

Glyphosate is a widely used herbicide in both terrestrial and aquatic environments. It is used extensively in agriculture as a preemergent herbicide and in ponds and lakes for controlling noxious or invasive plant species. Several studies conducted with amphibians have demonstrated that the surfactants used with glyphosate may be more toxic than the herbicide itself (see Chapter 6, this volume). In the United States, the bog turtle (*Clemmys buhlenbergii*) is a federally threatened species in the northeast portion of the country and is listed as endangered by several states within its distribution. It requires a habitat mosaic of open bogs, sphagnum moss, and moist grasslands. Because 1 method of maintaining these open habitats is to spray woody vegetation with herbicides, the US Fish and Wildlife Service wanted to determine if Glypro®, a formulation of glyphosate using LI700 as a surfactant, was safe to use. To test this, the red-eared slider (*Trachemys scripta elegans*) was used as a surrogate species (Sparling et al. 2006). Eggs were obtained from a commercial turtle farm and dipped in Glypro concentrations ranging from 1.3 to 95% Glypro and a 3% solution of LI700. These solutions resulted in calculated exposures of 0 to 11 200 mg a.i./kg of egg. The eggs were incubated at 27 °C until hatching. Turtles exposed to the highest concentration of Glypro had a significantly lower hatching success (73% compared to 100% in controls) and significantly lower body mass at hatching than controls. Genotoxicity, as determined by flow cytometry, increased with concentration of Glypro. At 6 days posthatch, turtles in the highest exposure categories had greater difficulty in righting themselves than did controls; by 9 days posthatch, there was no significant difference observed in righting ability. The authors concluded, however, that under typical spray operations there would be a low likelihood of harm from Glypro because the soil covering a turtle nest would substantially reduce exposure.

7.6 FUNGICIDES

No information on the toxicity of fungicides to reptiles following field applications was found either before or after 2000. Prior to 2000, 1 veterinarian case report on the benzimidazole fungicide thiabendazole was located, while another study dealt with physiological effects following injection of the antifungal antibiotic cyclohexamide into caimans, turtles, and chameleons. Holt et al. (1979) treated trematode infestations in 2 rat snakes (*Elaphe obsoleta quadrivittata* and *E. o. obsoleta*) with weekly doses of thiabendazole (Equizole®). Doses were as high as 110 mg/kg for 3 to 4 weeks. Although 1 of the snakes died within 24 hours of the final treatment, the authors attributed its death to the earlier infection. The other snake tolerated the thiabendazole and made a complete recovery following the treatments. The results of this case study suggest that there may be little risk to snakes as a result of their consumption of food items or contact with surfaces contaminated with this fungicide.

The antifungal antibiotic cycloheximide was used in agriculture but is no longer registered for agricultural use in the United States or Canada (Tomlin 1997). Coulson and Hernandez (1971) injected 1 or 10 mg/kg cycloheximide into spectacled caimans (*Caiman latirostris*), red-eared sliders, and green anoles, in which it blocked protein synthesis. This resulted in increased levels of free amino acids in tissues and body fluids. In 5 caimans, a single injection of 1 mg/kg resulted in effects noticeable for weeks (blood samples were taken from the tip of the tail for 21 days). Three of the original 5 animals had died by day 21; 10 mg/kg was invariably fatal within 3 to 4 days.

The fungicide methyl thiophanate (MT) is used on a variety of crops and ornamental shrubs. Studies on rats have shown that MT may have negative effects on adrenal and thyroid glands. De

Falco et al. (2007) separated male and female lizards (*Podarcis sciula*) into 4 groups as controls and those exposed to 1.5 g MT applied in water to their food, cage, and substrate, with each treatment group having a 15- or 30-day exposure. At the end of the exposures, lizards were bled and euthanized. Blood was evaluated for corticosterone, ACTH, and catecholamine concentrations. Adrenal histomorphology was also examined. Corticosterone concentrations significantly increased with MT treatment compared to controls, and the animals exposed to MT for 30 days had higher corticosterone concentrations than those exposed for 15 days. ACTH showed the opposite results: ACTH levels dropped in both treatment groups compared to controls and were lower in the 30-day than in the 15-day exposure. Adrenaline concentration increased in a similar fashion as corticosterone, and noradrenalin followed the ACTH pattern. Histomorphological differences in terms of adrenaline and noradrenaline cells and steroidogenic cords were consistent with the changes in hormone concentrations. The authors concluded that MT negatively affects the normal function of adrenal glands by interfering with the balance of hormone levels, duplicating endogenous hormone function and altering adrenal histomorphology.

The effects of MT on thyroid function and histology were examined by scientists within the same laboratory (Sciarrillo et al. 2008). In an acute study the authors injected lizards intraperitoneally with 350, 500, 700, 900, and 1000 mg/kg body weight. After 15 days survivors were euthanized. Mortality was observed at 500 mg/kg and increased with dose, peaking at 70% at 1000 mg/kg. The LD50 was calculated at 900 mg/kg. Other dose-dependent effects included hind limb paralysis and dyspnea.

7.7 VERTEBRATE PEST CONTROL AGENTS

Pesticide residues in various reptile tissues have been reported in the literature since the mid-1960s. Most of the data on tissue residues, however, were collected prior to 2000, when the emphasis in most reptile and contaminant studies was on determining tissue residues. Since 2000, as detailed in Chapter 1 of this volume, the emphasis of many studies has been on effects. Pauli and Money (2000) provided an extensive summary of the tissue residue data found for reptiles for both chlorinated and nonchlorinated pesticides. The vast majority of that, however, was on chlorinated pesticides. The reader is referred to Appendix 1 of that paper for more information. Campbell and Campbell (2001) reviewed residue concentrations in snakes and found only 1 paper containing information on organic contaminants that were not chlorinated.

A single study was found that mentioned pyrethroid residues in a reptile following an operational application. Bennett et al. (1983) collected 1 western ribbon snake (*Thamnophis p. proximus*) near an Arkansas cotton field that had received an aerial application of 0.112 kg a.i./ha fenvalerate 5 days before. The fenvalerate residue in the snake was 0.12 mg/kg wet weight (skin and gastrointestinal tract removed). This level was higher than those seen in various mice, bird, and amphibian species collected at the same site. Residues in fishes were also relatively high, which may suggest that the snake was consuming contaminated fish.

In a series of reports that detailed pesticide use and residues in a cotton-growing region and nearby areas of Texas, OP and OC residues were measured in lizards. Culley and Applegate (1966) reported parathion and methyl-parathion residues in tail muscle samples from 3 species of whiptail lizards (*Cnemidophorus tesselatus*, *C. tigris*, and *C. inornatus*) collected in cotton fields or from the adjacent desert. The lizards' diet was mainly termites, and residues ranged from nondetectable to approximately 5.0 ppm. Eggs, however, contained up to 5 times the concentration found in the muscle tissue of gravid females. Culley and Applegate (1967) reported similar, if slightly lower, residues of the same compounds in tail, brain, liver, coelem fat, and stomach contents of the same lizard species and the same pattern of OP accumulation in the eggs. Applegate (1970) recorded 0 to 0.7 ppm methyl-parathion and 0 to 0.1 ppm parathion in 36 whole lizards of 5 different species

trapped at Big Bend National Park, Texas. A note of caution concerning these residues was voiced by Hall (1980), however, who pointed out that most other investigators have been unable to confirm residues of these OPs in reptiles.

7.8 TISSUE RESIDUE DATA

A relatively large body of literature exists on the effects of vertebrate pest control agents (mainly rodenticides) on reptile populations. Some of this information concerns the application of gas fumigants, used for the control of nuisance rodents that occupy burrows, and their potential impact on burrow-dwelling reptiles. Other studies have examined pesticides for their potential to control reptiles, particularly snakes. Many of the reports, however, are anecdotal observations of the status of the local reptile fauna following the removal of rodents using pesticide-laced baits. Most of these studies have been conducted on oceanic islands that have introduced rodent or lagomorph species, and most have typically examined the response of the reptile population following removal of the mammals rather than the direct toxicity of the pesticides to the reptiles.

Various non-OC pesticides have been examined for their potential as snake control chemicals. Abe et al. (1994) documented that the pyrethroid insecticide prallethrin (Etoc®) sprayed onto the vipers *Agkistrodon blomhoffii brevicaudus* and *Trimeresurus flavoviridis* in an oil-based (kerosene) spray killed these snakes, typically within 4 hours of being sprayed. At 8 hours after being sprayed, there was 100% mortality of 5 snakes of each species treated, but there was no mortality of kerosene-sprayed controls. The 2 snake species were sprayed for 1 and 4 seconds, respectively, with a 0.3% prallethrin solution discharged at approximately 50 to 80 g/second. Symptoms included tremors, hyperactivity, and repeated attempts to bite the surrounding air.

On the other hand, in order to protect reptiles during vertebrate control operations, the US Fish and Wildlife Service (USFWS 1993) assessed the risk to reptiles from the application of 16 commonly used vertebrate control pesticides. A determination of risk, in terms of "no effect" or "may affect," was based on the species' habitat and ecology and resulting potential exposure. Three compounds, aluminum or magnesium phosphide and potassium nitrate, were determined to be potentially hazardous to several reptile species (mainly burrow-dwelling lizards, snakes, and tortoises), as it was concluded that registered use of these chemicals could constitute a threat to the continued existence of these reptile species if they were used to fumigate animal burrows or agricultural storage enclosures. The assessment of hazard was made essentially because the vulnerable animals inhabit the burrows of target species or burrows that might otherwise be fumigated during vertebrate pest control operations.

Only a few laboratory studies have examined the actual toxicity to reptiles of the pesticides used in rodent control activities. Braverman (1979) administered the rodent control chemical fluoroacetamide (Compound 1081) to 2 Palestine vipers (*Vipera palaestinae*), a Syrian black snake ("fire racer") (*Coluber jugularis*), and 2 Montpellier's snakes (*Malpolan monspessulanus*). The snakes were given 1 of 4 different regimens: a single dose of 0.1 or 0.4 mg/kg (the Palestine vipers), 4 doses totaling 1.6 mg/kg (Montpellier's snakes), or 4 doses totaling 3.2 mg/kg (the Syrian black snake). Because there was no mortality during the experiments, the author concluded that the use of Compound 1081 for rodent control in open fields was unlikely to harm snakes. This assessment was based on the fact that doses higher than those that might occur in poisoned small mammals in the field did not kill the snakes, but it did not take into account any sublethal effects.

Gopher snakes (*Pituophis catenifer*) were fed dead or moribund mice that had consumed grain-based baits containing 9 different rodenticides (Brock 1965). Over a 2-year period, only 17 snakes were used; snakes that did not react to the administration of 1 compound were used for further tests. The author acknowledged the flaw in the study in that using the same snake in subsequent tests might present the possibility of cumulative or synergistic effects resulting from exposure to

different toxicants. Compounds tested included sodium fluoroacetate (Compound 1080), strychnine, endrin, arsenic trioxide, zinc phosphide, thallium sulfate, and the anticoagulants Prolin, Warfarin, and Diphacin (diphacinone). There was no observable effect in snakes that consumed mice that had ingested lethal quantities of thallium sulfate or the anticoagulants. Snakes consuming the other compounds often regurgitated the mice and exhibited no further response. The rate of regurgitation was approximately 35% of the mice consumed. The 1 exception to this pattern occurred with strychnine; this compound caused tremors and irritability and the subsequent death of 5 snakes.

Detailed studies have been conducted in Australia with Compound 1080 and the shingleback skink *Tiliqua rugosa* (McIlroy et al. 1985; Twigg et al. 1988; Twigg and Mead 1990). In 1 region of Australia, sodium fluoroacetate occurs as a secondary compound in vegetation, and the skinks, through evolutionary exposure, have developed a remarkable resistance to its toxic effects. This resistance probably arose as a means to avoid depressed fertility rather than to prevent acute intoxication (Twigg et al. 1988). Skinks collected from this region show high fluoroacetate LD50 values (500 to 800 mg of 1080/kg) compared with animals of the same species from outside the region (LD50 of approximately 200 mg/kg) or animals of a target species such as the Norway rat (*Rattus norvegicus*) (LD50 of 0.22 mg/kg) (Tomlin 1997). McIlroy et al. (1985) showed that other Australian reptile species had lower LD50 values than did shingleback skinks, while another skink was comparable. In their studies bearded dragons (*Pogona barbata*), Gould's monitors (*Varanus gouldii*), and lace monitors (*V. varius*) all had approximate LD50s from Compound 1080 exposure of between 40 and 120 mg/kg, while the LD50 for the blotched blue-tongued lizard (*Tiliqua nigrolutea*) was 336 mg/kg. Despite the lower LD50 values for some species, McIlroy et al. (1985) calculated that most of the tested reptiles would have to eat large quantities of Compound 1080–impregnated bait to obtain a toxic dose, and this risk could be further reduced by decreasing the concentration of 1080 used in meat baits. Finally, Freeman et al. (1996) noted very little consumption of a cereal-based bait impregnated with Compound 1080 when the bait was offered to individuals of another skink species (*Oligosoma* [*Leiolopisma*] *maaccanni*) as their only food source during a 5-day laboratory study.

The anticoagulant rodenticides tested in these earlier studies (e.g., warfarin and diphacinone; Brock 1965) are typical of "first-generation" anticoagulants. These compounds generally have been replaced by "second-generation" anticoagulants such as brodificoum and flocoumafen, which show increased toxicity to rodents as a result of their accumulation and persistence in the liver (Eason and Spurr 1995). These compounds also control a wider range of rodent species, including those species resistant to other anticoagulants (Tomlin 1997). Brodifacoum and flocoumafen, both coumarin anticoagulants, are used to eradicate introduced mammals on islands where the introduced species are adversely affecting endemic wildlife and plant species. In New Zealand, for instance, where there is probably the most diverse fauna of geckos and skinks of any temperate archipelago, rodenticides are commonly being used to remove human commensals, mammalian predators, and introduced herbivores to reduce pressure on the resident fauna and flora, which includes many endangered reptile species (Merton 1987; Newman 1994).

Merton (1987) conducted a detailed investigation of the impacts of brodifacoum following its use to eradicate introduced rabbits from Round Island, Mauritius. The rabbits were overgrazing the island's vegetation and affecting its exceedingly rare fauna, which includes 6 endangered reptile species, 4 of which are endemic to Round Island. While no published data on the acute toxicity of brodifacoum to reptiles existed, Merton (1987) attempted a complete eradication of rabbits from Round Island using 2 applications of Talon 20 P pelletized baits (20 ppm brodifacoum). The 2 applications occurred at 4 and 5.7 kg/ha of the bait pellets, respectively. The choice of brodifacoum was supported by the following preliminary studies:

- Feeding and bait acceptance trials with both free-ranging and captive Telfair's skinks (*Leiolopisma telfairii*, one of the island's endemic reptiles) revealed little interest in brodifacoum baits on the part of the skinks.

- The pollard/bran pelleted bait was attractive to rabbits but was observed to be of little interest to both the skinks and their insect prey (in theory minimizing the chance of secondary poisoning of the skinks through ingestion of contaminated prey).
- Talon 50 P (a 50 ppm brodifacoum bait) had been used in rodent control for a decade by the New Zealand Wildlife Service with no reports of reptile mortality.
- Reptiles have a distinctly different blood coagulation chemistry than do mammals, and thus should not be affected by exposure to even relatively high levels of the anticoagulant (Merton 1987).

Despite all these precautions, however, skinks were observed eating rain-softened baits on Round Island, and more than 100 dead Telfair's skinks were eventually recovered. Yet, when 10 skinks were necropsied, only one showed signs of internal hemorrhage. The author speculated that the mortality was not the result of anticoagulation; rather, the animals appeared to have had problems thermoregulating. In spite of this apparent decimation of skinks, there were no long-term impacts on the population, and a follow-up study 3 years after the rabbit eradication (North et al. 1994) recorded increased numbers of 6 reptile species, including Telfair's skinks.

An increase in reptile populations following the removal of rodents is typically the case when these second-generation anticoagulants are used (e.g., Towns 1991, 1994; Newman 1994; Eason and Spurr 1995). Thus, it can generally be concluded that the improved habitat quality on islands following the removal of rodents more than compensates for any initial negative impacts on the reptile fauna. Nevertheless, it would obviously be prudent, given Merton's (1987) results with Telfair's skinks, to raise a captive colony of any species whose entire population resides on an island slated for intensive anticoagulant treatment.

The only post-2000 paper we were able to find on reptile control was the use of methyl bromide on brown tree snakes (*Boiga irregularis*; Savarie et al. 2005). The authors confined 18 snakes in cloth bags and placed them at random in a tarpaulin-covered cargo container commonly used by commercial airlines. They fumigated the container with 12 or 24 g/m^3 for 1 or 2 hours. All treatments except the 12 g/m^3 over 1 hour resulted in 100% mortality. The results were promising because brown tree snakes are a serious threat to the avifauna of the Pacific Islands. Except for potentially very destructive species such as the brown tree snake and highly venomous species living within human populations, the use of fumigants and poisonous baits to control reptiles is being replaced with humane removal and, if necessary, euthanasia.

7.9 ASSESSMENT AND CONCLUSION

Almost 3 decades ago, Hall (1980) summarized the available literature on the effects of pesticides on reptiles and concluded that there was very little information on the effects of environmental contaminants on reptiles. Hall (1980) also outlined research needs, including information on sublethal effects, behavioral and reproductive impacts, contaminant kinetics, the degree of cholinesterase inhibition that is diagnostic of lethal exposure to OP or carbamate insecticides, and the effects of newer compounds such as synthetic pyrethroids. The later review of Hall and Henry (1992) noted, however, that little new information had been generated, and concluded that there essentially have been no experimental assessments of reptile responeses to contaminants. Lambert (1997a) similarly reported that residue data for reptiles are lacking, effects levels are unknown, and the available information is insufficient for a complete synthesis. Additional appeals for further research were espoused by Pauli and Money (2000), Hopkins (2000) and Campbell and Campbell (2002). Despite these frequent requests, the area of pesticide ecotoxicology, indeed ecotoxicology in general, among reptiles is sorely underrepresented.

Studies on the effects of pyrethroids have been published for more than 25 years and yet are few in number. Many of those that have been published have focused on 1, deltamethrin, of the 14 or so pyrethroids. More organophosphorus pesticides have been examined than pyrethroids, but most

of the detailed studies have occurred with malathion, which is not the most toxic OP. One area that has received a fair amount of attention is the characterization of cholinesterases and their response to OP poisoning. Only 2 carbamate insecticides — carbofuran and carbaryl — have been used in tests on reptiles. While these may be the most widely used carbamates, there are others for which we have no information. We report 1 study that examined the effects of thiobencarb herbicide on lizards, while some other herbicides and some fungicides have been studied. Most studies of the individual chemicals cited above have been laboratory studies.

Thus, the potential effects on reptiles of field applications of modern pesticides remain essentially unknown. Following experimental exposures in the laboratory, through consumption of pesticide-laden baits, or as a result of direct applications to the animals in veterinary medicine or during reptile control activities, OP and synthetic pyrethroid insecticides have been demonstrated to be toxic to reptiles; the sensitivity of the tested lizard and turtle species to certain OP pesticides is comparable to that of birds. But it has been difficult to demonstrate impacts following field applications of non-OC pesticides. Therefore, field studies are warranted that might help determine the potential severity of the impacts following standard applications of the newer, nonpersistent pesticides. Studies that are critical to our understanding of reptile ecotoxicology should include genotoxic effects, endocrine disruption, long-term effects, toxicity to embryos, age-related toxicity, and maternal transfer studies. Granted, many of the nonchlorinated pesticides have relatively short half-lives that may reduce the importance of chemical fate and transfer studies, but we know little about the major pathways of exposure for reptiles. For instance, what are the relative roles of dermal absorption, inhalation, or dietary exposures? The involvement of pesticides in reptile "health" issues, such as the etiology of green turtle (*Chelonia mydas*) fibropapillomas (Hutchinson and Simmonds 1992; Aguirre et al. 1994; Herbst and Klein 1995) or the causes of disease or mortality in tortoises (e.g., Jacobson 1994), is uncertain.

There is evidence that certain groups of the more toxic vertebrate control compounds should not be used where reptiles might be present, particularly if they are applied as burrow fumigants. Rotenone applications may be harmful to turtles. Applications of bait formulations of the "second-generation" anticoagulants, such as brodificoum, when used to control populations of introduced rodents that are destroying reptile habitat, do more good than harm; these compounds may be toxic to reptiles that sample the baits, but the improvements in habitat quality following the removal of the rodents appear to more than offset any short-term adverse effects on the reptiles. Judicious selection of pesticide, bait type, and even bait color (Tershy and Breese 1994) may limit even the temporary, negative impacts on reptiles inhabiting an area undergoing rodent eradication. Finally, it is remarkable that no data appear to exist concerning the effects on reptiles of field applications of many groups of pesticides, including fungicides, modern herbicides (e.g., sulfonylureas), modern insecticides (e.g., microbial insecticides such as those based on *Bacillus thuringiensis*, viruses, or fungal agents), piscicides besides rotenone, or pesticides used as antifouling agents on boats (e.g., tributyltin) or in wood preservation (e.g., creosote).

The lack of information on the risk to reptiles from field applications of modern pesticides besides rodenticides is worrisome. This lack of knowledge corresponds to our lack of understanding of reptiles in general. As Chapter 1 of this volume indicates, the status of a large number of reptile species is very poorly known.

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8 Atrazine in the Environment and Its Implications for Amphibians and Reptiles

Christine A. Bishop, Tana V. McDaniel, and Shane R. de Solla

CONTENTS

Atrazine is one of the most widely used pesticides on a global basis. It is the most common pesticide detected in surface and ground water in the continental United States (Gillion et al. 2007). In areas with high corn production, atrazine concentrations in streams range as high as 3- to 10-fold greater than the 3 µg/L US Environmental Protection Agency (USEPA) drinking water standard (Thurman et al. 1991; USEPA 2006). While it is not highly persistent, atrazine is used often enough and persists long enough that amphibians, and to a lesser extent reptiles, can be exposed as eggs, juveniles, and adults to intermittent yet chronic concentrations of it throughout their lifetime. The effects of atrazine on amphibians in particular have received wide attention in recent years (Kiesecker 2002; Hayes et al. 2002, 2003, 2006a, 2006b; Hayes 2004; Hecker et al. 2004, 2005a, 2005b, 2006; Rohr et al. 2008a, 2008b), to the extent that the use of atrazine has been reviewed within the United States based solely on its potential to affect gonadal development in amphibians (Steeger et al. 2007). The ecosystems and food webs inhabited by herpetofauna may also be altered by atrazine (Rohr et al. 2008a, 2008b). Here, we explore the impacts of atrazine on the life history, health, and survival of amphibians and reptiles and on the aquatic communities they inhabit.

TABLE 8.1
Examples of Surface Water Concentrations (μg/L) of Atrazine

Country	Examples of Maximum Surface Water Concentrations (μg/L)	Reference
Serbia	4.13	Gasic et al. 2002
Belgium	3.7	Bintein and Devillers 1996
France	0.42	Bintein and Devillers 1996
France: Seine River	0.61–0.65	Guerit et al. 2008
England	7.5	Bintein and Devillers 1996
Scotland	4.2	Bintein and Devillers 1996
Netherlands	9.4	Bintein and Devillers 1996
Croatia	1.1	Gojmerac et al. 2006
South Africa: High velde area	9.3	DuPreez et al. 2005a
China: Liao-He and Yangtse Rivers	1.6	Gfrerer et al. 2002a
China: Yange River	6.7	Gfrerer et al. 2002b
China: Tiaozi and Zhaosutai Rivers	30–290	Li et al. 2007
Canada — Ontario	95	Takacs et al. 2002
		Struger et al. 2001
US: Michigan	250	Murphy et al. 2006a
US: Florida	18	Schuler and Rand 2008
Romania:		Kaloyanova 1998
Danube River	1.24	
Olt River	4.8	
Denmark	7.8	Helweg 1994
Switzerland	0.5	Johnen 1990
Rhine River:		Strosser et al. 1999
Netherlands	340	
Germany	250	

The physiochemical characteristics of atrazine make it both an effective herbicide and moderately persistent in the environment. The effectiveness of attrazine and its relative affordability have led to its widespread and intensive use worldwide. Initially registered by Ciba-Geigy in 1958, the triazine herbicide atrazine was registered for use in the 1960s in the United States, and swiftly replaced 2,4-D as the dominant herbicide for use on field corn since it offered selective weed control and reduced damage to crops. By the 1970s atrazine represented over 60% of herbicide use in corn row agriculture (Takacs et al. 2002). Other chemicals in the triazine herbicide group include cyanazine and simazine. In the 1980s, the triazines were the second most commonly sold group of pesticides in the United States, with 20 000 tonnes of atrazine being applied to corn crops in 1985 (Giddings et al. 2005). Annual use of atrazine in North America declined in the 1990s and 2000s, but its use remains widespread and intensive globally (Table 8.1). In Europe, atrazine was used on corn, orchards, vineyards, forestry, rose cultivation, and grassland management. Its use in France was 5000 tonnes in 1986, applied to approximately 3 million ha of corn (Bintein and Devillers 1996). Concerns regarding exposure to humans and wildlife from widespread detection in American surface waters prompted a review of triazine herbicide use by the USEPA in 1994 (Solomon et al. 1996). As a result, cyanazine was phased out of use in the United States in 2002 (USEPA 1996).

In the early 1990s, world consumption of atrazine was estimated at 70 000 tons/year, 90% of this applied to corn (Bintein and Devillers 1996). Atrazine has been registered for use in over 80

countries. In Europe, atrazine use has dropped since 1989 due to regulations restricting its use. Still, in the late 1990s, the world market for atrazine amounted to over US$400 million at the user level (Hicks 1998), and worldwide use was estimated at 149 000 to 160 000 tonnes (Short and Colborn 1999). In 1997, atrazine was applied to 53% of herbicide-treated corn in the United Kingdom and was used on 109 000 ha of grassland in 1997 (European Commission 2004). However, atrazine's use was banned throughout the European Union in 2003 (Garthwaite et al. 1997). In Asia, atrazine use is on the rise. In China, in 2002, use was estimated at 2273 tonnes per year and was expected to continue increasing (Ren et al. 2002).

The widespread use of atrazine can be attributed to its persistence, affordability, minimal crop damage, and selective control of a wide variety of broadleaf and grassy weeds. Its chemical formulation is $C_8H_{14}ClN_5$ (2-chloro-4-ethylamino-6-iso-propylanmin-s-triazine) (CAS 1912-24-9). It is sold primarily as a wettable powder or as a water-dispersible granular formulation, but is also available as a dry, flowable powder or a suspension. Where resistance has not developed, it effectively controls some broadleafed and grassy agricultural weeds such as clover, ragweed, pigweed, smart weed, wild buckwheat, lamb's quarters mustards, and purslane. Its primary use is on corn, which accounts for over 80% of its use. However, it is also used on sugar cane, sorghum, pineapples, nursery conifers, in the forestry industry, and to control algae in ponds (Stevens and Sumner 1991).

When used, atrazine is usually applied in the spring before crop emergence, dissolved in water with or without the use of oils or surfactants, and may be applied in conjunction with fertilizers. It is also occasionally used at preplanting or postemergence. Rates for application on corn typically vary between 1 and 1.6 kg ai/ha, with maximum application rates in the United States being 2.8 kg ai/ha, although pre-1990 maximum rates were 4.48 kg ai/ha (Giddings et al. 2005). While sugar cane accounts for a small proportion of atrazine use (3% of use in the United States), the application rates for this crop are up to 11.2 kg ai/ha. As of 2008, recommended application rates of the 11 atrazine products registered for use in Canada varied between 0.5 and 1.5 kg ai/ha.

The primary degradation pathway of atrazine in the environment is through microbial metabolism (Giddings et al. 2005; Takacs et al. 2002). Atrazine is chemically broken down by hydrolysis to produce hydroxyatrazine, which is not phytotoxic and therefore no longer an active herbicide. Atrazine is also metabolized by N-dealkylation via microbial action to deethylatrazine, deisopropylatrazine, and diaminochloro-s-triazine, all of which have reduced phytotoxicity (Huber 1993; Takacs et al. 2002). Atrazine has very low volatility with a vapor pressure of 0.04 mPa at 20 °C (Stevens and Sumner 1991), so loss from surface waters and soils through volatilization is minimal.

8.1 ATRAZINE IN THE ENVIRONMENT

8.1.1 Soil and Water

In aquatic environments contaminated with atrazine, residues are primarily present in the water column and do not sorb strongly to sediments (Giddings et al. 2005). While it is relatively stable in the water column, its chemical degradation by hydrolysis may be hastened by chemical components commonly found in surface waters, such as humic and fulvic acids, which significantly reduce its half-life in the water column. It is also degraded by photolysis in surface waters. The half-life of atrazine in the water column in freshwater aquatic environments can range from 41 to 237 days, with an average of 159 days (Giddings et al. 2005). However, in a study to assess wetlands designed to mitigate atrazine concentrations, the half-life of atrazine in a constructed freshwater wetland ranged from 16 to 48 days (Moore et al. 2000). The half-life of atrazine in the water collumn is thought to be influenced by a number of factors, including temperature, light, sediment type, bacterial community, and atrazine concentrations, leading to some variation in persistence (Giddings et al. 2005).

In estuarine systems, the half-life is reduced by increases in salinity and ranges from 3 to greater than 90 days (Solomon et al. 1996).

Atrazine is persistent in soils, with degradation time (DT50) varying widely between 20 and 385 days, depending on soil composition, pH, presence of organic acids such as fulvic acid, and soil microbial activity (Huber 1993). The DT50 tends to be lower in aerobic soils, with an average of 44 days, than in anaerobic soils and sediments such as those at the bottom of ponds and wetlands, where measured DT50 averaged 228 days (Burnett et al. 2000).

Atrazine primarily enters surface waters through runoff from agricultural fields (Giddings et al. 2005). Because of atrazine's high solubility in water, it can leach from soils during heavy rains that are common in the fall and during spring runoff or during the rainy season in tropical climates. In temperate climates, rates of leaching due to runoff vary from 18% to less than 3% of the amount applied (Huber 1993). Because atrazine's long half-life can be in excess of several months, in both soils and aquatic environments, it can persist in water between growing seasons. Other atrazine sources to aquatic habitats include wet and dry atmospheric deposition and ground water recharge. In some areas, particularly those isolated from agricultural runoff, atmospheric deposition may be the primary source of atrazine input to surface waters. Atrazine has been detected in alpine lakes in Switzerland (0.6 μg/L) and at the Experimental Lakes District in northern Ontario, Canada, both of which are isolated from agriculture. The herbicide was detected in rainwater in Norway 4 years after it was banned from use (Takacs et al. 2002). Rainwater has contained concentrations of atrazine as high as 0.45 μg/L in agricultural areas (Hall et al. 1993). Ground water recharge is another potential source of atrazine in surface waters (Hall et al. 1993).

In the Thames River watershed of southwestern Ontario, Canada, concentrations as high as 95 μg/L have been measured in tributaries draining agricultural areas (Takacs et al. 2002). Farm ponds and drains that receive direct atrazine inputs from agricultural runoff may contain higher than average atrazine concentrations than other surface waters. In corn-growing areas of the midwestern United States, concentrations up to 250 μg/L have been detected in field drains and agricultural wetlands (Murphy et al. 2006a, 2006b). Up to 57 μg/L of atrazine was measured in farm ponds in southwestern Ontario (Frank et al. 1990). While the above concentrations represent maximum atrazine concentrations, recent median concentrations of atrazine are typically much lower (Eisler 1989; Giddings et al. 2005). A risk assessment with a comprehensive summary of studies in Canada and the United States reported that the median of 90th percentile concentrations for surface waters ranged from 2.46 μg/L for regions of high atrazine use and high rainfall to 0.03 μg/L for areas of low atrazine use and low rainfall.

In larger water courses such as rivers and lakes, atrazine concentrations are much lower. However, they still may exceed water quality guideline concentrations established by various jurisdictions. A survey (1991 to 1993) of atrazine in surface waters in the United States indicated over 50% of 186 stream sites exceeded the USEPA maximum concentration of atrazine of 3 μg/L (Gillion et al. 2007). During a 4-year survey of tributaries of the Thames River in southwestern Ontario, Canada, atrazine concentrations exceeded the freshwater Canadian Water Quality Guideline for the protection of aquatic life of 1.8 μg/L in 5.4% of weekly samples and 49.7% of storm event samples (Takacs et al. 2002; Table 8.1).

A comprehensive survey of pesticide concentrations in streams, ground water, and biota in the United States between 1992 and 2001, carried out by the US Geological Survey, indicated that atrazine was nearly ubiquitous in surface waters and ground water. Surface water sampling was conducted in 51 watersheds, at 186 stream sites, for a total of 4380 water samples, while ground water was sampled from 5047 wells across the continental United States. It was the most frequently detected pesticide in streams from agricultural areas (over 75% of samples) (from 83 agricultural sites; Gillion et al. 2007). Eighteen percent of agricultural streams had atrazine concentrations that exceeded the benchmark for acute effects on aquatic plants of 18 μg/L (USEPA 2003; Gillion et al. 2007). Six percent of agricultural streams tested exceeded the benchmark for aquatic community effects of a 60-day average atrazine concentration of 17.5 μg/L. It was detected in urban streams in over 60% of samples from

30 urban sites. Atrazine and its metabolite deethylatrazine were also the most commonly detected pesticides in ground water, occurring in over 30% of samples (Gillion et al. 2007).

The majority of information in the literature on atrazine concentrations in the environment is reported from North America; however, there are an increasing number of studies from other parts of the globe (Table 8.1). In China, measured concentrations in some of the major rivers, such as the Liao-He and Yangtse Rivers, rarely exceeded 1.6 μg/L; an exception was the Yange River, which receives inputs from pesticide manufacturing plants. In China, as elsewhere, concentrations tend to be at their highest in the late spring, during the peak time of atrazine application, and lowest in the winter, outside of the growing season (Gfrerer et al. 2002a, 2002b). Some rivers in China have become contaminated with atrazine due to accidental industrial releases. In 1997, the Tiaozi and Zhaosutai Rivers in Liaoning Province had atrazine concentrations ranging from 30 to 290 μg/L due to an accidental discharge into the rivers from an industrial leak. Atrazine concentrations in the water were so elevated that it caused widespread failure of rice crops that were irrigated with water from these rivers (Li et al. 2007).

Soil concentrations of atrazine in Serbia ranged from 0.02 to 0.1 mg/kg soil within the top 15 cm of the soil (Gasic et al. 2002). In surface waters in Siberia, atrazine was at detectable concentrations in 83% of samples from agricultural areas. Atrazine was present in 60% of surface water samples from agricultural areas at 1 to 4.13 μg/L and in ground water at up to 0.3 μg/L (Gasic et al. 2002).

Amphibians often live in waters where they can be exposed to concentrations of atrazine that range from below detection limit up to several hundred micrograms per liter in wetlands that are close to atrazine sources. Atrazine can persist in water between growing seasons and therefore can be present when amphibians breed in early spring, or in tropical areas at the outset of the wet season. As the agricultural growing season progresses, atrazine concentrations will fluctuate through the period of development for amphibians. While atrazine residues in surface waters are well documented within watersheds, lakes, and higher-order streams (Solomon et al. 1996), they are less well documented at sites where highest atrazine concentrations likely occur, particularly shallow, small irrigation ponds or drains within or adjacent to farms. The lack of repeated sampling throughout amphibian breeding and development periods in wetlands means that the concentration and exposure periods for amphibians and reptiles in their typical habitats remain largely unreported. This information is essential to fully assess the risk of atrazine to amphibians and reptiles in the wild.

In 2003, water samples from south central Michigan were collected on a monthly basis from May to September during a study of atrazine concentrations in surface waters and biochemical response in livers of ranid frogs (Murphy et al. 2006c). Atrazine concentrations at nonagricultural sites ranged from nondetectable to 0.23 μg/L and did not exceed 1.2 μg/L at agricultural sites (Murphy et al. 2006a). In 2002, the same agricultural areas were sampled and atrazine concentrations did not exceed 2 μg/L at most sites, but a concentration of 250 μg/L was detected in 1 sample (Murphy et al. 2006b).

Du Preez et al. (2005a) measured atrazine concentrations in wetlands inhabited by amphibians from the western high veld corn-growing region of South Africa during the corn growing season. Maximum atrazine concentrations ranged from 1.2 to 9.3 μg/L. A second study of atrazine concentrations in amphibian-occupied wetlands in the same corn-growing region of South Africa, outside of the growing season, found atrazine concentrations between 0.12 and 1.23 μg/L (Du Preez et al. 2005b).

In Canada, Berube et al. (2005) measured atrazine concentrations in watersheds of the Yamaska River, a large river system draining agricultural areas in Southern Quebec and utilized by bullfrogs (*Rana catesbeiana*). Atrazine concentrations in the water ranged from below the detection limit to 220 ng/L, although they did not sample during runoff events. From 1991 to 1993, in an intensive vegetable production area of Ontario, up to 0.101 μg/L diazinon, 6.47 μg/L atrazine, and 0.210 μg/L azinphos-methyl were detected in surface waters of the Holland River during the amphibian breeding season (Bishop et al. 1999). In southern Ontario ponds that were utilized for breeding by amphibians within apple orchards, atrazine concentrations of 0.07 to 15.0 μg/L were found

in combination with azinphos-methyl concentrations of 0.06 to 1.0 µg/L, diazinon at 0.03 to 0.78 µg/L, and endosulfan at 0.51 to 0.53 µg/L (Harris et al. 1998). These studies document the mixture of pesticides typically found in wetlands inhabited by amphibians and reptiles within agricultural watersheds. In southwestern Ontario, farm pond and drain atrazine concentrations ranged from nondetectable to 3.13 µg/L and were detected in 37 out of 40 sites, many of which were utililized for amphibian breedings (McDaniel et al 2008). In each of these studies, other pesticides, including organophosphate insecticides, other herbicides and, in some cases, endosulfan, were also detected, emphasizing the reality that amphibians are rarely exposed solely to atrazine in the environment (Harris et al. 1998; Bishop et al. 1999; Berube et al. 2005).

8.1.2 Biota

Since triazines have a relatively short half-life in biological organisms, they are unlikely to bioaccumulate within food webs. Atrazine is relatively soluble in water, with a solubility of 28 mg/L at 20 °C and 70 mg/L at 25 °C. It has a moderate K_{ow} (2.3 to 2.8), making it an unlikely candidate for bioaccumulation in the food chain. Bioconcentration factors for most organisms tested are low. In fish, these values range from <0.27 to 12 (Giddings et al. 2005); algae were higher at 76, as were mayfly nymphs at 480 (Lynch et al. 1982). While no bioconcentration was found in bullfrog, *Rana catesbeiana*, tadpoles in an exposure study by Klassen and Kadoum (1979), a kinetics study with radiolabeled atrazine in *Xenopus laevis* tadpoles resulted in a bioconcentration factor of 1.5 to 1.6 ml water/g larvae (Edginton and Rouleau 2005). Allran and Karasov (2000) calculated that a bioconcentration factor for *Rana pipiens* tadpoles exposed for several weeks to atrazine was approximately 6.

In animals, atrazine is primarily metabolized by n-dealkylation of the side chains, in addition to conjugation with glutathione, to form cysteine conjugates, sulfides, and sulfoxides (Wu et al. 1998). Elimination of the parent compound in *Xenopus* is fairly rapid, at 48 minutes, whereas atrazine metabolites had a longer elimination half-life (72 hours) (Edginton and Rouleau 2005). Autoradiographic studies of atrazine in adult *Xenopus* revealed that most of the parent compound and metabolites were found concentrated in the liver, gall bladder, and intestines, similar to distribution in fish (Edginton and Rouleau 2005). A behavioral choice test between non-atrazine-treated soil and soil dosed with ecologically relevant concentrations of atrazine (1430 or 80 µg/kg based on those measured in agricultural fields in Missouri) was performed to examine uptake, distribution, and elimination of water dosed with ^{14}C-labeled atrazine. *Bufo americanus* did not avoid atrazine-laden soils. Atrazine crossed the pelvic patch rapidly and reached an apparent equilibrium within 5 hours. The majority of the atrazine was found in the intestines, whereas the greatest concentrations were detected in the gall bladder (Storrs Méndez et al. 2009).

8.2 TOXICITY TO AMPHIBIANS AND REPTILES

Since atrazine is often sprayed in the late spring to early summer season in temperate climates, and during the initiation of the rainy season in tropical areas, the highest exposures for amphibians can occur during egg and larval developmental periods as well as, chronically, throughout the year to adults. For reptiles, exposure of eggs, juveniles, and adults to water-soluble compounds such as atrazine is likely to be much lower due to their protective eggshells and scales. Nonetheless, many reptiles are aquatic and may be exposed through water consumption and cloacal and egg respiration.

8.2.1 Amphibians: Toxicity to Eggs, Larvae, and Metamorphs

8.2.1.1 Acute Toxicity

The LC50s of atrazine to amphibian larvae are generally higher than concentrations found in the environment. The 96-hour LC50 of atrazine to early-stage embryos and posthatch larvae of *Rana*

pipiens was reported as 7680 µg/L, and for *Rana catesbeiana* as 410 µg/L (Birge et al. 1980, 1983; Table 8.2), although the LC50s included both survivorship and occurrence of deformities. Acute exposures (96 hours posthatch) of *R. pipiens*, *R. sylvatica*, and *Bufo americanus* embryos to atrazine up to 20 mg/L did not affect hatchability, and 96-hour posthatch mortality of larvae was unaffected. Atrazine also had no effect on swimming speed of *Rana pipiens* tadpoles (Allran and Karasov 2001; Table 8.2). LC100 concentrations for *Rana pipiens* have been reported at 16 000 µg ai/L (Hovey 1975), but LC50s for late-stage *Rana catesbeiana* larvae are greater than 16 000 µg/L (Wan et al. 2006). Some studies indicate that the stage of amphibian development can have a significant impact on the acute toxicity of atrazine. Howe et al. (1998) exposed *Bufo americanus* and *Rana pipiens* tadpoles at early (Gosner 29; Gosner 1960) and late (Gosner 40) stages of larval development. The LC50 for atrazine was 47 600 µg/L for early-stage *Rana pipiens* larvae and only 14 500 µg ai/L for the older larvae. A similar trend was seen with *Bufo americanus*; the LC50 for Gosner stage 20 was 26 500 µg ai/L, while the LC50 for Gosner 29 larvae was 10 700 µg ai/L (Table 8.2).

Predicted no observed effect concentrations (PNOECs) for chronic exposures with an endpoint of mortality after 30 days were 5100 and 650 µg/L for early- and late-stage *Rana pipiens* tadpoles, respectively, and 1900 and 690 µg/L for early- and late-stage *Bufo americanus*, respectively (Howe et al. 1998). These values overlap with some of the highest atrazine concentrations found in surface waters. Threshold concentrations this high have not been documented, to date, to persist for 30 days or more in the environment. Predicted threshold values for population level impacts of atrazine (Birge et al. 1980, 1983; Howe et al. 1998) for 4-day posthatch *Rana pipiens* of 32.6 µg/L are within the range of environmental concentrations.

Amphibian dose-response curves following atrazine exposure may not be linear in some cases. Chronic (30 days) exposure of larval *Rana pipiens*, *Bufo americanus*, *Pseudacris crucifer*, *Rana sylvatica*, and *Rana clamitans* to low concentrations of atrazine (3, 30, and 100 µg/L) resulted in significantly higher mortality in all species in the lowest-dose group (3 µg/L) compared to the higher-dose groups, with the exception of late-stage *Bufo americanus* and *Rana sylvatica* (Storrs and Kiesecker 2004). There were differences in sensitivity between early- and late-stage larvae as well as variation in sensitivity among species (Storrs and Kiesecker 2004).

8.2.1.2 Mixtures

Atrazine can be more toxic in combination with other herbicides and insecticides due to additive or syngergistic interactions. Mixtures containing atrazine can be more toxic than the individual compounds. This is an important factor when interpreting the results of any field-based study on the effects of atrazine. It is rarely the sole pesticide present and/or detected in the environment, particularly in agricultural areas. Howe et al. (1998) reported synergistic effects when atrazine exposure was combined with equal concentrations of alachlor: LC50s for *Rana pipiens* exposed to an equal mixture of both chemicals ranged from 2100 to 6500 µg ai/L for early- and late-stage tadpoles, respectively, while values for *Bufo americanus* ranged from 1500 to 1800 µg ai/L for late- and early-stage larvae, respectively. Those LC50s for atrazine plus alachlor are less than half those for atrazine alone.

The effects on *Rana pipiens* and *Xenopus laevis* of 9 pesticides, including atrazine, were examined alone (0.1 µg/L) or in combination (Hayes et al. 2006b). Individually, some pesticides individually inhibited larval growth and development. Atrazine was one of these, showing a significant negative effect on size (snout-vent length and body weight) of the animals at metamorphosis. However, the pesticide mixtures had greater effects on body weight but similar effects on snout-vent length when compared with atrazine alone (Hayes et al. 2006b). In addition, atrazine and the 9-pesticide mixture, and a commercial mixture containing atrazine, increased the percentage of thymic plaques in *Rana pipiens* compared to controls. Mortality rates in animals treated with the 9-pesticide mixture at 10 µg/L all died after the first day of exposure, whereas exposure to atrazine and/or 7 other pesticides alone individually induced little mortality (mean = 4%) (Hayes et al. 2006b).

TABLE 8.2
Summary of Dose–Response Studies on Amphibians and Atrazine

Study	Dose Atrazine (µg/L)	Species	Stage	Exposure Time	Endpoint
			Immune Effects		
Brodkin et al. 2007	0, 0.01, 0.1, 1, 10, 21	*Rana pipiens*	Adult	8 days	White blood cell count[a] Phagocytic activity[a]
Forson and Storfer 2006	0, 1.84, 18.4, 184 Plus iridovirus	*Ambystoma macrodactylum*	6-week-old larvae	30 days	Body size[a] Suvivorship Time to metamorphosis[a] Infection rate of iridovirus[a]
Koprivnikar et al. 2007	0, 3, 30 Plus trematode	*R. sylvatica*	12-week-old larvae	31 days	Infection rate[a]
Rohr et al. 2008b	0, 201 Plus trematode	*Rana clamitans*	Stage 25–39 larvae	2 × 7 days (pre-post infection)	Trematode survival[a] Tadpole survival[a] Trematode infection rate[a]
Rohr et al. 2008a	0, 102 Plus trematode	*R. pipiens* *R. palustris* *R. clamitans*	Larvae	28 days	Survivorship[a] Liver melano-macrophages[a] Liver eosinophils[a] Trematode infection rates[a]
			Acute Effects		
Allran and Karasov 2001	0, 20, 200 2000, 20000	*R. pipiens* *R. sylvatica* *Bufo americanus*	Embryos, larvae	96 hours	Hatchability Swimming speed
Birge et al. 1983; Birge et al. 1980	28 to 48 000 µg/L	*R. pipiens* *R. catesbeiana*	Embryos	96 hours, 9 days	Survivorship[a] Deformity rates
Brodeur et al. 2009	1000–30 000	*Rhinella arenarum*	4 embryos, 25 larvae Pro-metamorphosis (39)	4, 14, 21 days	Acute toxicity[a] Time to metamorphosis[a] Deformities
Hovey 1975	0, 16 000	*R. pipiens*	Larvae		Survivorship[a]

Howe 1998	100, 1000, 10 000, 100 000, 1 000 000	*R. pipiens* *B. americanus*	Early and late larval stages	24, 96 hours	Survivorship[a]
Lenkowski et al. 2008	0, 10 000, 25 000, 35 000	*Xenopus laevis*	Larvae	48 hours	Development[a]
Storrs et al. 2009	1430 and 80 µg/kg soil	*Bufo americanus*	Juveniles	60 hours	Atrazine uptake[a] Behavioral avoidance
Wan et al. 2006	Not stated	*R. catesbeiana*	Larvae	24, 48, 72, 96 hours	Survivorship
			Chronic Effects		
Allran and Karasov 2000	0, 20, 200	*R. pipiens*	Posthatch to metamorphosis		Bioconcentration[a] Time to metamorphosis Survivorship Body size Hematocrit
Hayes et al. 2006b	0.1	*R. pipiens*	2 days posthatch to Gosner 46		Thymus histology[a] Time to metamorphosis Body size[a]
Kloas et al. 2009	0.01, 0.1, 1, 25, 100	*Xenopus laevis*	Embryo to metamorph		Growth Survivorship Time to metamorphosis Sex ratio Testes histology
Langerveld et al. 2009	0, 400	*Xenopus laevis*	Stage 43 larvae	Stages 43–62	Survivorship[a] Growth[a] Gene expression[a]
Rohr et al. 2003	0, 4, 40, 400	*A. barbouri*	Embryos to larvae	37 days	Hatching success Larval survival Body size Deformities Refuge use Activity level[a]

(*continued*)

TABLE 8.2 (CONTINUED)
Summary of Dose–Response Studies on Amphibians and Atrazine

Study	Dose Atrazine (µg/L)	Species	Stage	Exposure Time	Endpoint
Rohr et al. 2006	0, 4, 40, 400 Plus low food and water stress	*A. barbouri*	Embryos to larvae	37 days plus 14 m postexposure	Density-mediated effects on survivorship[a]
Storrs and Kiesecker 2004	0, 3, 30, 100	*R. pipiens* *Pseudacris crucifer* *R. sylvatica* *R. clamitans*	Early-stage larvae Late-stage larvae	30 days	Survivorship[a]
Sullivan and Spence 2003	0, 40, 320	*Xenopus*	Feeding stage to metamorphosis		Survivorship[a] Time to metamorphosis[a] Body size[a] Hematocrit[a]
			Endocrine Effects		
Carr et al. 2003	0, 1, 10, 25	*Xenopus*	Stage 25 to metamorphosis		*Gonad histology Gonad gross morphology Sex ratio Larynx diameter
Coady et al. 2005	0.1, 1.0, 10, 25	*Xenopus*	Stage 25 to postmetamorphosis		Gonad gross morphology Gonad histology Sex ratio Larynx diameter Aromatase activity *Circulating sex steriods
Du Preez et al. 2008	0, 1, 10, 25	*Xenopus*	Egg to 2 years postmetamorphosis of F1 Endpoints for F2 egg to metamorphosis	>2 years	Clutch size F1 Testes histology (F1 and F2) Survivorship F2 Sex ratio F2
Freeman et al. 2005	0, 250, 500, 800, 1000, 5000, 10000	*B. americanus Xenopus*	Stage 25 tadpoles	21 days	Nuclei stage and weight *Stage of development

Goulet and Hontela 2003	0, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} M	*Xenopus R. catesebiana*	Fat and adrenal cell culture	1 hour	*Corticosterone secretion *Adrenal cell viability
Hayes et al. 2003	0, 0.1, 25	*R. pipiens*	Stage 25 to metamorphosis		*Gonad gross morphology and histology
Hayes et al. 2006a	0, 0.1, 0.4, 0.8, 1, 25	*Xenopus*	Stage 25 to metamorphosis	50 days	*Gonad morphology and histology
Hayes et al. 2002	0, 0.1, 1, 10, 25, 200	*Xenopus*	Tadpole to metamorphosis		*Gonadal histology and morphology *Larynx diameter
Hayes et al. 2002	25	*Xenopus*	Adult males	46 days	*Circulating sex steroid levels
Hecker et al. 2005a	0, 10, 100	*Xenopus*	Adult males	50 days	Testicular histology Testicular index Aromatase activity *Sex steroid levels
Hecker et al. 2005b	0, 1, 25, 250	*Xenopus*	Adult males	36 days	Cyp 19 expression Testicular index Aromatase activit Circulating sex steriods
Larson et al. 1988	0, 75, 250	*A. tigrinum*	Eggs to metamorphosis		*Metamorphosis *Growth *Plasma corticosterone and thyroxine
Oka et al. 2008	0, 0.1, 1, 10, 100	*Xenopus* (wild type and ZZ all males)	Stage 49 tadpoles to metamorphosis		Aromatase activity *Sex ratio Gonad histology Vitellogenin induction (in vitro)
Orton et al. 2006	0, 10 In conjunction with nitrate	*R. pipiens*	Stages 2–24	10–12 weeks	Body size Survivorship Time to forelimb emergence *Sex ratio *Gonad histology

(*continued*)

TABLE 8.2 (CONTINUED)
Summary of Dose–Response Studies on Amphibians and Atrazine

Study	Dose Atrazine (µg/L)	Species	Stage	Exposure Time	Endpoint
Tavera-Mendoza et al. 2002a	0, 21	*Xenopus*	Stage 56	48 hours	*Testicular histology *Testicular index
Tavera-Mendoza et al. 2002b	0, 25	*Xenopus*	Stage 56	48 hours	*Ovarian histology
			Mecocosm Studies		
Detenbeck et al. 1996	15–25	*R. pipiens*	Larvae to metamorphosis	41 days	Metamorphosis Growth Survival
Boone and James 2003	0, 200	*R. sphenocephala* *B. americanus* *A. maculaturm* *A. texanum*	Larvae to metamorphosis		Survival *Time to metamorphosis *Body size Response to competition
Britson and Threlkeld 1998	0, 192	*Hyla chrysoscelis*	Eggs to metamorphosis	192 days	Deformities *Time to metamorphosis Survivorship *Body size
Diana et al. 2000	0, 20, 200, 2000	*H. versicolor*	Eggs to metamorphosis		*Body size Survivorship Time to metamorphosis
Jooste et al. 2005	0, 1, 10, 25	*Xenopus laevis*	Free swimming to 10 months after metamorphosis	1 year	Gonad histology Gonad morphology
Rohr and Crumrine 2005	0, 25	*R. sylvatica*	Larvae		*Developmental stage *Response to predation Response to competition *Activity level

[a] Significant difference between endpoint in the controls versus one or more atrazine treatments.

A comparative examination of the acute toxicity of atrazine, alachlor, and a 50:50 mixture of the 2 chemicals to early and late larval stages of *Rana pipiens* and *Bufo americanus* and to rainbow trout (*Onchorynchus mykiss*) and channel catfish (*Ictalurus punctatus*) revealed that effects of the mixture were greater than additive for most exposures, with 96-hour LC50s of 1.5 mg/L for late-stage *Bufo americanus* larvae. Rainbow trout and channel catfish were less sensitive than amphibian larvae (Howe et al. 1998).

From 2004 to 2006, in the fruit-growing area of the Okanagan Valley, British Columbia, Canada, where pesticides, water chemistry, and hatching success of the great basin spadefoot *(Spea intermontana*), pacific tree frog (*Pseudacris regilla*), western toad (*Bufo boreas*), and Columbia spotted frog (*Rana luteiventris*) were measured, it was atrazine concentrations in the water that correlated most strongly with reduced hatching success in spadefoots, but there was no correlation with tree frogs (Bishop et al. 2008). Predator proof cages containing early-stage eggs were placed in ponds in nonagricultural reference sites and in ponds in conventionally sprayed and organic orchards. Twenty pesticides were detected in the sprayed ponds. Of these, 4 were herbicides including atrazine. Pesticides in pond water occurred at parts per trillion concentrations as high as 1410 ng/L for diazinon, 25.3 ng/L atrazine, and 57 ng/L endosulfan-sulfate in sprayed sites. Chloride, sulfate, conductivity, nitrate, and phosphorus showed significant differences among sprayed, organic, and reference sites.

Great basin spadefoot mean hatching success ranged from 0 to 92% among sprayed orchards, whereas the range was 48 to 98.6% among organic orchards and 51 to 95.5% among reference sites. Mean hatching success for Pacific tree frog ranged from 22.1 to 76.1% among sprayed orchards, whereas the range was 83.4 to 97.1% among reference sites. Hatching success of western toad eggs in 2004 was as low as 0.6% in sprayed orchards and as high as 96% in organic orchards. For Columbia spotted frog in 2006, mean hatching success ranged from 0 to 67.6% among sprayed and 83.8 to 95.2% among reference sites.

Variables that correlated negatively with amphibian hatching success included 12 pesticides and 7 water chemistry parameters. However, for spadefoots, stepwise regression found that, in 2005, atrazine accounted for 79% of the variation in hatching success and, in 2006, atrazine, total nitrate, and chlorpyrifos accounted for 80%. For Pacific tree frogs there were no significant correlations with pesticide concentrations. Rather, hatching success correlated with water chemistry parameters (Bishop et al. 2008).

These findings and others (see also Section 8.2.1.4) emphasize the need to measure all pesticides occurring in the environment as well as water chemistry parameters during field studies that attempt to quantify impacts of any single pesticide on amphibian populations.

In a study examining interactions of atrazine and chlorpyrifos in 4 aquatic vertebrates, organisms were exposed to binary mixtures of these chemicals in bioassays (Wacksman et al. 2006). Atrazine alone did not affect organisms at concentrations up to 5000 µg/L; however, the presence of atrazine at 1000 µg/L did result in a significant increase in the acute toxicity of chlorpyrifos in *Xenopus laevis* tadpoles. Mixed results were found with *Pimephales promelas*, fathead minnow, with some bioassays showing greater than additive toxicity, while others showed an additive response. No effect of atrazine on chlorpyrifos toxicity was observed for bluegill (*Lepomis macrochirus*) or *Rana clamitans* tadpoles.

8.2.1.3 Malformations and Edema

There are some limited data suggesting high-dose atrazine exposure may result in deformities and/or edema in amphibians. Howe et al. (1998) reported the appearance of abdominal edema in early-stage *Rana pipiens* and *Bufo americanus* tadpoles (Gosner 29) exposed to atrazine concentrations ranging from 2800 to 23 000 µg/L for 96 hours, although the rates of edema were not included in the report. Birge et al. (1980, 1983) reported a 3% deformity rate in *Rana catesbeiana* tadpoles exposed to 410 µg/L atrazine. Exposure to atrazine at 20 000 µg/L resulted in significant deformity rates in *Rana pipiens*, *Rana sylvatica*, and *Bufo americanus*, with a no adverse effects level (NOAEL) for

deformity estimated at 2590 µg/L and the lowest adverse effects level (LOAEL) at 4320 µg/L (Birge et al. 1980, 1983). Deformities mainly consisted of abnormal tail shapes in tadpoles, including wavy tail and lateral flexure of the tail.

Exposures of streamside salamanders (*Ambystoma barbouri*) to 4, 40, and 400 µg/L of atrazine for 37 days, including the larval period, did not result in any increase in deformities (Rohr et al. 2003). There was a negative correlation between the number of eye deformities and atrazine concentration in a mixed exposure study (atrazine, chlorpyrifos, and monosodium methanearsonate) (Britson and Trelkeld 1998). There was also a dose-dependent increase of deformities in *Rana pipiens*, *Rana sylvatica*, and *Bufo americanus* larvae with increasing atrazine concentration (0.02 to 20 mg/L) (Allran and Karasov 2001). In a static renewal experimental treatment, the effects of 10, 25, and 35 mg/L atrazine from early organ morphogenesis through the onset of tadpole feeding were measured in larval *Xenopus laevis* (Lenkowski et al. 2008). There were significant dose-dependent increases in the percentage of atrazine-exposed tadpoles with malformations of multiple tissues, including the main body axis, circulatory system, kidney, and digestive system. Incidence of apoptotic cells also increased in midbrains and kidneys of atrazine-exposed tadpoles (Lenkowski et al. 2008).

8.2.1.4 Parasites and Disease

Primary hosts for trematodes are typically mollusks, while larval anurans are susceptible to trematode infections as secondary intermediate hosts. Parasitism may potentially lead to reduced survivorship and hind limb deformities. Kiesecker (2002) studied *Rana sylvatica* tadpoles by caging them within ponds exposed to agricultural runoff, including atrazine. An increased rate of trematode infections and hind limb deformities was found compared to tadpoles raised in ponds that did not receive agricultural inputs. *Rana sylvatica* tadpoles exposed to *atrazine* in ponds adjacent to agricultural fields developed a significant number of limb deformities. *Rana sylvatica* from agricultural runoff ponds exposed to cercariae were 37% smaller than their counterparts in the same ponds not exposed to cercariae And, in the agricultural runoff ponds, a significantly higher percentage (28.6%) of the *Rana Sylvatica* exposed to trematode infection developed limb deformities, compared with 0% among tadpoles shielded from trematode infection (Kiesecker 2002).

In the same study, a dose-response exposure was also reported in which *Rana sylvatica* tadpoles were exposed, individually, to atrazine, malathion, and esfenvalerate, at 3 or 30 µg/L for 4 weeks, and then cercariae of 2 species of trematodes, *Ribeiroia* and *Telorchis*, were exposed at both concentrations of atrazine (Kiesecker 2002). *Rana sylvatica* tadpoles exhibited statistically reduced immune response, as measured by the number of circulating leukocytes, and a significant increase in trematode infection (Kiesecker 2002). At all but the lowest exposure to malathion, all 3 pesticides had a similar effect on eosinophil numbers and proportion of cercariae that encysted. Pesticide exposure also had a treatment comparable effect on the amphibian responses to the 2 different parasites (Kiesecker 2002). Both field and laboratory results suggest that there is some common physiological effect on the immune response among pesticides despite the differences in mechanisms of action among chemicals.

Rana pipiens metamorphs were exposed to a mixture of atrazine, metribuxin, aldicarb, endosulfan, lindane, and dieldrin, representative of compounds and concentrations (ranging from 0.02 ng/L up to 21 µg/L) occurring in rivers of Quebec, Canada (Gendron et al. 2003), including 21 µg/L of atrazine. The frogs were exposed to an infection challenge with lungworms (*Rhabdias ranae*), a common frog parasite. Exposed frogs were infected with lungworms more quickly than nonexposed frogs. Although there was no significant difference in overall parasite burden, there were significantly more gravid female parasites found in the lungs of frogs from the highest-dosage group, suggesting that the exposure of the host to pesticides had altered the life history of the parasite, accelerating its life cycle.

In a related experiment with *Rana pipiens* and *Xenopus laevis*, Christin et al. (2004) used the same pesticide exposure regimen as Gendron et al. (2003). There were no effects on cellularity of

immune tissues and responses in *Rana pipiens* even at the highest pesticide concentrations, but the mixture significantly decreased the number of splenocytes in *Xenopus*. A significant reduction in the number of phagocytes with a dose-response trend was also found for *Xenopus*. In contrast, suppression of T-lymphocyte proliferation occurred at all concentrations in *Rana pipiens* and then recovered 3 weeks after exposure ended, while no modulations were observed in *Xenopus* (Christin et al. 2003, 2004). *Rana pipiens* were then challenged with *Rhabdias ranae*. No pesticide effects on phagocytosis and splenocyte numbers were detectable at the end of the exposure period, but these 2 parameters were diminished 21 days after the infection challenge in frogs previously exposed to the higher concentratons of pesticide mixture (Christin et al. 2003).

Results at 21 μg/L atrazine, in combination with other pesticides, are further supported by studies of exposure solely to atrazine (21 μg/L for 8 days), which affected the innate immune response of adult *Rana pipiens* in ways similar to acid exposure (pH 5.5) (Brodkin et al. 2007). Atrazine suppressed the thioglycollate-stimulated recruitment of white blood cells to the peritoneal cavity to "background" levels and also decreased the phagocytic activity of these cells (Brodkin et al. 2007).

Sometimes the interaction between parasites and pesticide exposure is in an unexpected direction, depending on the sensitivity of both the parasite and the host. In the aformentioned studies, only the amphibian host was exposed to atrazine, not the parasite. Koprivnikar et al. (2006) exposed the cercariae (infectious stage) of 4 species of digenetic trematodes to atrazine to determine if this impacted their survivorship and ability to infect anuran larvae. Exposure to 200 μg/L atrazine led to reduced survivorship and motility in some of the trematode species. There was also a significant reduction in infection rates of *Rana clamitans* tadpoles by exposed cercariae. This may counteract effects seen in the previous studies. When the hosts alone (*Rana sylvatica*) were exposed to atrazine at 3 and 30 μg/L, the rate of trematode infection was increased compared to *Rana sylvatica* raised without atrazine (Koprinvikar et al. 2007). When both hosts (*Rana sylvatica*) and trematode cercariae were exposed to 3 and 30 μg/L atrazine, infection rates in *Rana sylvatica* were no different from those raised without atrazine (Koprinvikar et al. 2007). Thus, it appears that atrazine may compromise both the amphibian host's ability to mount an immune response and the ability of the parasite to infect the host.

Similarly, in a short-term exposure of parasites and tadpoles to a mixture of metolachlor and atrazine, effects on the parasites and its snail host were more pronounced than in tadpoles and their susceptibility to infection. Changes in survivorship occurred in free-living trematode cercariae in low (10 and 15 μg/mL) and high (85 and 100 μg/mL) concentrations of mixtures of metolachlor and atrazine. A significant decline in cercarial survivorship in the high-concentration treatments at 14 hours was found. In a second experiment, the parasites, the second intermediate host tadpoles (*Rana sylvatica* and *Rana clamitans*), or both parasites and tadpoles were exposed to those mixtures for up to 10 hours. The mixtures had no significant effects on parasite load, but newly shed cercariae were more likely than 10-hour-old cercariae to infect tadpoles. They also used outdoor mesocosms to expose parasites, infected snail hosts, and *Rana sylvatica* tadpoles to those pesticide mixtures and found no significant effects on tadpole parasite loads in mesocosms (Griggs and Belden 2008).

Forson and Storfer (2006) exposed 6-week-old long-toed salamander larvae (*Ambystoma macrodactylum*) to *Ambystoma tigrinum* iridovirus (ATV) in conjunction with sublethal concentrations of atrazine ranging from 1.84 to 184 μg/L. They found that infection rates of ATV and mortality from ATV were significantly lower at all 3 atrazine concentrations when larvae were exposed to ATV in conjunction with atrazine, than when larvae were exposed to ATV alone, suggesting atrazine may compromise viral efficacy.

8.2.1.5 Growth and Metamorphosis

There are mixed results regarding the impact of low-dose atrazine exposure on larval growth and time to metamorphosis. These depend on the species and the concentrations of atrazine, and may

also depend on the somatic development rate of amphibians (Ogielska and Kotusz 2004; see also Section 8.2.2.2). In short-term experiments, few significant impacts have been reported in anurans using atrazine concentrations commonly found in the environment, but toxic effects at low concentrations in a long-term study of salamanders indicate that the effects in wild amphibian populations are very complex and possibly underestimated in previous shorter-term studies (Rohr et al. 2006; Forson and Storfer 2006).

Atrazine (20 to 200 µg/L), nitrate (0.5 to 20 mg/l NO_3-N/L), and a mixture of these compounds had no significant effects on development rate, percent metamorphosis, time to metamorphosis, percent survival, mass at metamorphosis, or hematocrit, although nitrate slowed the growth of *Rana pipiens* larvae exposed at Gosner stage 25 through to metamorphosis (Allran and Karasov 2000). Similarly, exposure of juvenile *Rana pipiens* for 21 days to a pesticide mixture that included 21 µg/L atrazine did not result in any changes in growth or condition compared to controls (Gendron et al. 2003). There was little impact on survivorship to metamorphosis, or hind limb length as a measure of size, in caged *Rana pipiens* tadpoles exposed to atrazine at 15 to 75 µg/L in treated stream mesocosms, although time to metamorphosis was accelerated in atrazine-treated animals (Detenbeck et al. 1996).

Larval *Xenopus laevis* were exposed to 1, 10, or 25 µg/L atrazine at 48 hours posthatch until metamorphosis was complete. Estradiol, dihydrotestosterone, and ethanol vehicle were also tested for effects on the larvae. None of the atrazine treatments affected posthatch mortality, larval growth, or metamorphosis (Carr et al. 2003). Similarly, larvae of *Xenopus laevis* were exposed to 0.01 to 200 µg/L atrazine throughout larval development. Atrazine at these concentrations had no effect on mortality, time to metamorphosis, length, or weight at metamorphosis (Hayes et al. 2002). Time to metamorphosis was not affected in exposures of *Xenopus laevis* to 0.01 to 100 µg/L (Hosmer et al. 2007; Steeger et al. 2007).

Six-week-old *Ambystoma macrodactylum* larvae were exposed for 30 days to 1.84, 18.4, and 184 µg/L of atrazine (Forson and Storfer 2006). Exposure to the highest concentration of atrazine resulted in significantly decreased time to metamorphosis and a corresponding reduction in snout-vent length and body weight at metamorphosis. There was no significant impact on survivorship. When Larson et al. (1998) exposed tiger salamander (*Ambystoma tigrinum*) larvae to 250 µg/L atrazine, a decrease in body mass at metamorphosis was found without a change in time to metamorphosis, whereas larvae exposed to 75 µg/L metamorphosed significantly later than controls but with no reduction in body mass. Streamside salamander (*Ambystoma barbouri*) larvae exposed from egg stage to 37 days to 4, 40, and 400 µg/L atrazine did not show a reduction in hatching success, survivorship, or growth (Rohr et al. 2003). However, salamanders exposed to ≥4 µg/L atrazine had significantly lower survival than did control animals 14 months postexposure (Rohr et al. 2006).

8.2.1.6 Toxicity to Adults

No LC50 data exist for adult amphibian exposures either orally or through skin exposures or injections. However, based on data we can extrapolate from small mammals, adverse effect doses are expected to be higher for adult animals. The oral LD50 for atrazine is 3090 mg/kg in rats, 1750 mg/kg in mice, 750 mg/kg in rabbits, and 1000 mg/kg in hamsters (Ecotoxnet http://extoxnet.orst.edu/pips/atrazine.htm).

Cutaneous exposure of adult *Rana pipiens* for 14 days to up to 20 mg/L atrazine resulted in a significant increase in ventilation rate, which may be indicative of respiratory distress. This effect was significant at the 12 mg/L atrazine concentration for thoracic ventilation and at 4.32 mg/L for buccal ventilation, although thoracic and buccal ventilation rates dropped at 7.2 and 12 mg/L atrazine, respectively (Allran and Karasov 2001). The estimated NOAEL for ventilation was 2.59 and 7.2 mg/L for buccal and thoracic ventilation, respectively. Frogs exposed to the highest atrazine concentrations (20 mg/L) showed no feeding response throughout the duration of the exposures but

did not decrease in mass, possibly due to compensatory fluid gain from edema. The NOAEL for a reduction in feeding response was 12 mg/L (Allran and Karasov 2001).

8.2.2 Endocrine Disruption: Receptor Binding and Modes of Action

Atrazine has demonstrated both estrogenic and anti-estrogenic activities in a number of *in vivo* and in vitro studies (Sanderson et al. 2001; Seung et al. 2003). Generally, there is little evidence that atrazine's estrogenic or anti-estrogenic activities are mediated directly through the modulation of estrogen receptors. Through the use of *in vivo* exposure of immature female Sprague-Dawley rat uterii, and in vitro exposures of estrogen-responsive MCF-7 human breast cancer cell lines and the estrogen-dependent recombinant yeast strain PL3, Connor et al. (1996) demonstrated that the estrogenic and anti-estrogenic activities of atrazine were not mediated by the estrogen receptor. Neither atrazine nor simazine induced typical estrogenic responses in Sprague-Dawley rats *in vivo* (increased rat uterine wet weight, cytosolic PR binding, or uterine peroxidase activity), while both herbicides inhibited estrogen-induced PR binding and uterine peroxidase activity (Connor et al. 1996). Uterine estrogen receptor-binding capacity in ovariectomized rats fed triazines at 300 mg/kg for 2 days was reduced by 30% (Tennant et al. 1994). However, atrazine was not able to competitively bind to receptors in the presence of estradiol, nor was there any displacement of ligand binding (Tennant et al. 1994; Roberge et al. 2004).

The prevailing hypothesis is that at least some of the effects of atrazine are mediated through alterations in the expression or activity of cytochrome P450 19 (aromatase). Aromatase activity is modulated by phosphodiesterase (PDE), which converts cyclic adenosine monophosphate (cAMP) to 5'AMP; cAMP in turn can increase mRNA expression of aromatase (Sanderson et al. 2001). Atrazine can inhibit PDE, thus resulting in elevated concentrations of cAMP, which in turn increases the expression of aromatase (Sanderson et al. 2001; Roberge et al. 2004; Fan et al. 2007). Aromatase, in turn, converts testosterone to estrogen (and androstenedione to estrone). Aromatase has been induced by exposure to atrazine in vitro, in chelonian testis cell lines (Keller and McClellan-Green 2004), and human carcinoma cell lines (Sanderson et al. 2001).

Nevertheless, there is still some potential for atrazine to demonstrate estrogenic or antiestrogenic activity independent of aromatase. First, estrogen receptors of nonmammalian taxa (i.e., reptiles or amphibians) may be more sensitive to atrazine. Vonier et al. (1996), for example, found that alligator (*Alligator mississippiensis*) estrogen receptors could bind with atrazine, whereas mammalian receptors typically do not bind with atrazine (Roberge et al. 2004). Both atrazine and cyanazine displaced [^{3}H]17ß-estradiol from the α-estrogen receptor in alligators, and at 20.7 and 19 μM these 2 compounds inhibited 50% of the estradiol from binding to the receptor (Vonier et al. 1996). Thus, interactions between atrazine and the estrogen receptor are still possible, at least in nonmammalian taxa. Furthermore, Kniewald et al. (1995) found that atrazine inhibited 5α-dihydrotestosterone-specific receptor complex formation in rats exposed *in vivo* and in vitro. Similarly, the metabolite, deethylatrazine, also inhibits 5α-reductase (Babic-Gojmerac et al. 1989) in a similar fashion as atrazine. Reduction of 5α-reductase in atrazine-exposed male rats decreases the conversion of testosterone to 5α-dihydrotestosterone (Kniewald et al. 1979), which is a potent androgen. Consequently, both atrazine and its metabolites can act as antiandrogens. Although atrazine did not affect estrogen binding to estrogen receptors, atrazine did reduce 5α-dihydrotestosterone binding to androgen-binding protein by about 40% (Danzo et al. 1997). Atrazine inhibition of 5α-dihydrotestosterone-specific receptor complex formation appears to be reversible, with the number of available binding sites for 5α-dihydrotestosterone returning to normal after 7 to 14 days postexposure in atrazine-treated rat prostates (Šimić et al. 1991). Dihydrotestosterone is not convertible to estrogen; thus, the inhibition of 5α-reductase by atrazine may increase the pool of testosterone available for conversion to estrogen. Exposure to exogenous testosterone, though not dihydrotestosterone, can cause feminization in turtles (Gutzke and Bull 1986), through the conversion to estrogen via aromatase.

Furthermore, atrazine can inhibit 3 β-hydroxysteroid dehydrogenase in exposed rats (Kniewald et al. 1979), which catalyzes the conversions of pregnenolone, 17-hydroxypregnenolone, and dehydroepiandrosterone to progesterone, 7-hydroxyprogesterone, and androstenedione, respectively. The mechanism(s) of endocrine disruption by atrazine is not fully understood, but the current focus on atrazine's interaction with aromatase should not be overemphasized to the exclusion of other viable hypotheses to its actions.

Activity of 7-ethoxyresorufin (EROD) is directly associated with the induction of hepatic activity of the cytotchrome P450 1A1 enzyme. Similarly, *O*-demethylase (MROD) activity is another hepatic biomarker that is not as highly inducible as EROD but is more sensitive than some other monooxygenase enzymes in amphibians (Murphy et al. 2006c). Liver somatic index (wet weight of the liver divided by the total wet body weight of the fish multiplied by 100) can increase as the liver increases in size to allow greater detoxification of pollutants over long periods of exposure. In wild ranid frogs collected from agricultural and nonagricultural sites in Michigan, EROD and MROD activities were measurable in both adult and juvenile frogs and were similar among sites (Murphy et al. 2006c). Juvenile frogs had greater EROD and MROD activities than adult frogs. *Rana catesbeiana* and *Rana pipiens* had greater activities than *Rana clamitans*. Atrazine concentrations in water from the ponds were significantly and negatively correlated with MROD activity in adult male green frogs. Liver somatic index, EROD, and MROD activities of adult female and juvenile *Rana clamitans* were not significantly correlated with atrazine concentrations in water. However, liver somatic index values in adult male frogs differed significantly between agricultural and nonagricultural sites (Murphy et al. 2006c).

8.2.2.1 Adrenal Function

The individual effects of cadmium (10^{-8} to 10^{-1} M), endosulfan, and atrazine (both at 10^{-8} to 10^{-4} M) on corticosterone secretion and viability of adrenal cells of *Xenopus laevis* and *Rana catesbeiana* were assessed using in vitro bioassay (Goulet and Hontela 2003). The ratio of the lethal concentration needed to kill 50% of the cells and the 50% effective concentration (LC50:EC50) was calculated with LC50 as the concentration that killed 50% of the steroidogenic cells and EC50 as the concentration that impaired corticosterone secretion by 50%. The higher the ratio, the greater the potential for endocrine disruption. Atrazine had no effect on cell viability and on corticosterone secretion in *Xenopus laevis*, but its endocrine-disrupting potential was high in *Rana catesbeiana* (Goulet and Hontela 2003). For comparison, LC50:EC50 ratios for cadmium and endosulfan in *Xenopus* were 26.07 and 1.23, respectively, and for atrazine, cadmium, and endosulfan in *R. catesbeiana* they were 909, 41, and 3, respectively, indicating that there was variation in species sensitivity, with atrazine being the most toxic chemical (Goulet and Hontela 2003).

8.2.2.2 Sexual Development

The potential for atrazine to alter the sexual development, in particular gonadal development and associated sex steroid concentrations, has been studied in several species of amphibians, but primarily in *Xenopus laevis*. Studies have measured effects of atrazine concentrations in dose-response studies and field scenarios at environmentally relevant concentrations in the range of 0.1 μg/L to several hundred micrograms per liter. Commonly measured endpoints include gonadal anomalies such as testicular oocytes, intersex, hermaphroditism, decreased testosterone concentrations in plasma of male amphibians, alterations in gonadal somatic index, sex ratios, reduction in laryngeal muscle size, and aromatase activity.

To examine the effects of atrazine on sexual development in amphibians, larvae of *Xenopus laevis* were exposed to a range of atrazine concentrations from 0.01 to 200 μg/L, or to untreated/control conditions, throughout larval development. The cross-sectional diameter of larynges was significantly reduced in size in males produced from the exposed larvae at or above 1 μg/L atrazine (Hayes et al. 2002). All doses tested, except 0.01 μg/L atrazine, produced up to 20% occurrence of multiple gonads, whereas these abnormalities were not observed in the control animals. In sexually

mature males, plasma testosterone concentratons were significantly lower and similar to testosterone concentratons in females when *Xenopus laevis* tadpoles were exposed every 3 days for 46 days to 25 µg/L atrazine (Hayes et al. 2002). The gonadal malformations induced by 0.1 to 25 µg/L atrazine in *Xenopus laevis* were then compared to those induced by an androgen receptor antagonist or 17 β estradiol. The combined frequency of multiple testes (single-sex polygonadalism), hermaphroditism, and nonpigmented ovaries was 10% or more in all concentrations of atrazine, whereas the frequency was less than ~2% in controls. Similar malformations were induced by estradiol except nonpigmented ovaries, which occurred only in the atrazine groups. The occurrence of gonadal malformations was significantly higher at all concentrations of atrazine compared to controls, but there was no clear linear dose-response at concentrations increasing from 0.1 to 25 µg/L (Hayes et al. 2006a).

In 2003, Carr et al. reported no effects on sex ratio in larval *Xenopus laevis* exposed to 0, 1, 10, or 25 µg/L atrazine at 48 hours posthatch until metamorphosis. At 25 µg/L atrazine, the incidence of intersex animals increased to 4.7%, which was significantly different than controls, and the incidence of intersex increased with atrazine concentrations. Atrazine at the tested concentrations did not reduce the size of the laryngeal dilator muscle, which is sexually dimorphic in *Xenopus laevis.* In contrast, *Xenopus laevis* tadpoles were also exposed under static conditions to atrazine (21 µg/L) for 48 hours during sexual differentiation (Tavera- Mendoza et al. 2002). The histology of the gonads indicated 57% reduction in testicular volume among atrazine-exposed tadpoles, primary spermatagonial cell nests were reduced by 70%, and nursing cells that provide nutritive support for the developing germ cells declined by 74%. Testicular resorption was observed among 70% and failure of full development of the testis occurred in 10% of atrazine-exposed tadpoles.

In a longer-term study, *Xenopus laevis* was exposed to 0.1 to 25 µg/L atrazine from 72 hours posthatch until 2 to 3 months postmetamorphosis in a 3-day static renewal system (Coady et al. 2005). Mortality, growth, gonadal development, laryngeal muscle size, and aromatase activity in juveniles were not affected. Male frogs exposed to 1 µg/L atrazine had lower estradiol concentratons, although there was not a consistent dose-response.

Similarly, male *Xenopus laevis* exposed to 10 or 100 µg/L atrazine for 49 days showed neither gonadal abnormalities nor significant differences in plasma estradiol or testosterone concentrations. The gonadal somatic index was significantly higher in males exposed to 10 µg/L atrazine, but there was no consistent dose-response (Hecker et al. 2005a). Also, male *Xenopus laevis* were exposed to 1, 25, and 250 µg/L atrazine for 36 days and testicular aromatase activity and cytochrome P450 19 (aromatase) gene expression, testosterone, and 17ß-estradiol and gonad size were measured (Hecker et al. 2005b). Aromatase gene expression was measured because at relatively high concentrations atrazine can upregulate P450 19 gene expression in the human adrenocarcinoma cell line (Sanderson et al. 2001). There were no effects found at any concentration except testosterone concentrations in plasma, which were significantly lower in males exposed to 250 µg/L atrazine (Hecker et al. 2005b).

Effects of atrazine on sex differentiation were studied using "wild-type" *Xenopus laevis* tadpoles and all-ZZ male cohorts of *X. laevis*, produced by mating wild-type ZZ male to sex-reversed ZZ male (female phenotype) (Oka et al. 2008). Stage 49 (Nieuwkoop and Faber 1994) tadpoles were exposed to 0.1 to 100 µg/L atrazine or 0.27 µg/L 17ß-estaradiol induction until all larvae completed metamorphosis. Atrazine had no effect on metamorphosis of developing wild-type or all-male *X. laevis* larvae. A statistical increase in female ratios was observed in 10 and 100 µg/L atrazine exposures relative to a control group. However, no hermaphroditism or sex reversal was found, and P450 aromatase mRNA in the gonad and hepatic vitellogenin were not induced in the atrazine treatments. The authors suggest that effects of atrazine on sexual differentiation were not caused by estrogenic action, and hasatrazine had no induction ability of P450 aromatase in the gonad (Oka et al. 2008).

To assess the transgenerational effects of atrazine, reproductive success and development of F2 offspring from F1 adult *Xenopus laevis* exposed to atrazine throughout larval development and as sexually mature adults were tested using larvae exposed to 1 of 4 nominal concentrations of

atrazine (0, 1, 10, and 25 µg/L) beginning 96 hours after fertilization and continuing through 2 years postmetamorphosis (Du Preez et al. 2008). Clutch size and survival of offspring were used as measurement endpoints of reproductive success of the F1 frogs. Larval survivorship and time to metamorphosis were used to measure developmental success of the F2 offspring from atrazine-exposed frogs. Testes in F1 and F2 frogs were examined for incidence of anomalies, such as testicular ovarian follicles, and sex ratios in F2 offspring were investigated to determine if exposure to atrazine caused transgenerational effects (effects on F2 individuals due to exposure of F1 individuals). There were no effects from any of the concentrations of atrazine on clutch size of F1 frogs. There were also no effects on hatching success or time to metamorphosis. Sex ratios did not differ between F2 offspring among treatments. There was no evidence to suggest a transgenerational effect of atrazine on spawning success or reproductive development of *Xenopus laevis*.

However, in atrazine-treated male *Rana pipiens* (0.1 and 25 µg/L) rates of 36% gonadal dysgenesis (underdeveloped testes with poor structure and closed lobules and low to absent germ cells) were found at 0.1 µg/L, and the rate was 12% at 25 µg/L. At 0.1 µg/L, 29% of the animals exhibited varying degrees of sex reversal, while only 8% showed this response at 25 µg/L. Sex-reversed males contained oocytes in testes while control males showed no oocytes in testes; 2 control males showed degenerating extragonadal oocytes and 1 had gonadal dysgenesis (Hayes et al. 2003).

Rana pipiens were exposed to 10 mg/L nitrate or 10 µg/L atrazine or combined exposure of both of these treatments and compared to controls (Orton et al. 2006). Testicular oocytes were found in nitrate-only, atrazine-only, and the control groups, but not in the combined treatment. In the atrazine-alone, nitrate-alone, and combined treatments, there were a decreased percentage of spermatocytes, an increased percentage of spermatids in testes, and an increased mature ovarian follicle size in females (Orton et al. 2006).

In a dose-response study conducted using a protocol developed with and approved by the USEPA (Hosmer et al. 2007; Steeger et al. 2007), exposures of *Xenopus laevis* to 0.01 to 100 µg/L atrazine were conducted under flow-through conditions and no testicular oocytes or intersex cases occurred in atrazine exposed groups (Hosmer et al. 2007; Steeger et al. 2007). The use of flow-through conditions contrasts with methods used in most previous studies of atrazine exposure and effects on gonadal development in amphibians, in which static renewal conditions were used, making comparisons among results of studies difficult. Flow-through conditions are generally not representative of conditions in small farm ponds and ditches, where amphibians are most likely to experience the exposure without substantial water flow.

A number of field surveys of frogs collected in agricultural areas (Reeder et al. 1998; Hayes et al. 2003; Hecker et al. 2004; Jooste et al. 2005), especially those areas where corn production and atrazine use were dominant, have found physical and physiological abnormalities that are consistent with those reported in laboratory dosing studies with atrazine, although many of these surveys have failed to find a dose-response relationship specific to environmental atrazine concentrations. While field studies are limited in the sense that they cannot be used to establish a cause-effect relationship, they are useful in exploring whether the effects seen in the laboratory are apparent in or relevant to wild populations.

Several investigations have examined the occurrence of atrazine effects in the native, wild populations of *Xenopus* in South Africa. *Xenopus laevis* were collected from wild populations in corn-growing regions with relatively typical surface water exposures of atrazine (greatest mean concentrations among sites of 3.5 to 4.1 µg/L) and non-corn-growing regions (Hecker et al. 2004). The trends in the combined data among sites showed negative correlations between plasma testosterone and atrazine exposure and its degradation products deisopropylatrazine, deethylatrazine, and tertbuthylazine in females, and between testosterone and diaminochloroatrazine in males. Estradiol in females exhibited a significant negative correlation with atrazine and deethylatrazine. No correlations were found between gonadal aromatase activity or gonadal somatic index and any chemicals measured. In the same study areas, there were no significant differences in laryngeal mass in *X. laevis* males from corn-growing and non-corn-growing areas (Hecker et al. 2004). Mean percent

fractional volume of seminiferous tubule distribution of testicular cell types, such as percentage of spermatogonia, spermatocytes, and spermatozoa, was not different among sites, nor was the incidence of testicular oocytes (Smith et al. 2005).

Xenopus laevis larvae were exposed to atrazine concentrations of 1, 10, and 25 µg/L in microcosms until just after metamorphosis (Jooste et al. 2005). They exhibited no significant increase in incidence of testicular oocytes relative to controls, although the rate in the control group was 57%. The mean number of testicular oocytes per individual was 9.5 in the control group and 11.1 in the 25 µg/L treatment group. Ten months after metamorphosis, another subset of juveniles was examined and the maximum number of testicular oocytes found was 5 per individual (Jooste et al. 2005).

In the early 1990s, species native to North America were starting to be examined in the wild in the context of pesticide exposure and effects on sexual development. Cricket frogs (*Acris crepitans*) from 2 general areas (study A and study B), including several sites each, in Illinois were collected to assess the effects of environmental contamination on the prevalence of intersex gonads and sex ratios. Of 341 frogs collected (1993 to 1995), 2.7% were intersex individuals (Reeder et al. 1998). There was no significant correlation between chemical compounds detected (65 herbicides, insecticides, and fungicides; 13 organochlorine pesticides; total polychlorinated biphenyl (PCBs) and polychlorinated dibenzo-furans (PCDFs), and dioxins; and lead and mercury in sediments and water) and cricket frog intersexuality. However, in study A, in 1 year (1994), there was a weak association approaching significance ($p = 0.07$) between the detection of atrazine and occurrence of intersex individuals. Pesticides detected in sediment included atrazine, cyanazine, and metolachlor (where detected concentrations ranged from 3 to 17 µg/L), and chlorpyrifos was detected in water at 3.1 mg/L. In study B, the sex ratios of juvenile frogs differed significantly between PCB/PCDF/PCDD-contaminated sites and reference sites ($n = 16$ frogs collected per site).

In a 2001 field study of *Rana pipiens* collected from ponds in 8 agricultural sites across a gradient of atrazine concentrations up to 12 µg/L (atrazine application rates in these areas ranged from 0.4 kg/km^2 to >28.7 kg/km^2), all sites associated with atrazine rates of more than 0.4 kg/km^2 and/or higher than 0.2 µg/L in water contained male *Rana pipiens* that displayed sex reversal similar to that found in dose-response studies on the same species (Hayes et al. 2003). However, there was no linear dose-response association of increasing gonadal malformation and concentrations of atrazine and its breakdown products at the time of sampling (i.e., sites with the highest atrazine concentration did not exhibit the highest rates of abnormalities in gonads among the 8 study areas). There were other pesticides used in these sites and analysis was conducted on water for these chemicals. However, atrazine and its metabolites were detected at all sites, but among the other pesticides, only metalochlor was detected, and only at 1 site (Hayes et al. 2003). During the field collections juvenile *Rana pipiens* were abundant at all of the collection sites, suggesting that the gonadal anomalies may be reversible, some percentage of the population is unaffected, these morphological anomalies do not impair reproduction, or resistance to atrazine may occur in the population (Hayes et al. 2003). Genetic variation within populations of amphibians can explain a significant amount of the variation in tolerance to insecticide (e.g., carbaryl) (Semlitsch et al. 2008), thereby suggesting it is a heritable genetic trait that could contribute to resistant populations of amphibians arising in agricultural areas.

In 2002 and 2003, frogs were collected from field sites in Michigan where atrazine occurred (nondetectable to 2 µg/L at all but 1 site, which had a concentration of 250 µg/L measured in 1 year of the study). In these field exposures, aromatase activity was measurable in less than 11% of testes in adult male *Rana clamitans*, and less than 4% of testes in juvenile males. Atrazine concentrations in field sites did not correlate with aromatase activity or with plasma steroid concentrations in *Rana clamitans (*Murphy et al. 2006b).

In the family Bufonidae, male toads possess rudimentary ovaries, called Bidder's organs, which are attached to the testes (Pancak-Roessler and Norris 1991). In field collections of *Bufo marinus* from sugarcane- and non-sugarcane-growing areas and urban sites in Florida, toads were examined for the presence of developed Bidder's organs in 3 separate studies, which found similar trends. In

the first study (Sepulveda and Gross 2003), there was an increased incidence of intersex (ovarian tissue in Bidder's organ) in toads identified as having testes. Approximately 29% of males from one agricultural site (Belle Glade) and 39% of males from another (Canal Point) were intersexed, while no intersex frogs were identified among the toads from a nonagricultural site. Vitellogenin, a female-specific protein, was present in intersex toads at concentratons similar to those in females and was about double that in male toads (Sepulveda and Gross 2003). Atrazine concentrations in the agricultural site range from <0.01 to 24.24 µg/L during a 6-month sampling period, but other pesticides were also used in these sites. In this same study, southern toad (*Bufo terrestris*) was also examined and had increased incidence of intersex (Bidder's organ containing ovarian tissue) in both agricultural (14% at Belle Glade and 22% at Fisheater Creek) and nonagricultural sites (33%).

In a second study, in the same general area of south Florida, similar results were found in that there was an increased incidence of the development of the Bidder's organ in males collected in sugarcane agricultural areas. At these sites, water samples contained up to 12.6 µg/L atrazine at the time of the toad collections, although there were a variety of other pesticides in use as well as historical contamination by organochlorine compounds in the agricultural area that were not measured (Gross et al. 2007). In a third study that included other areas of south Florida, and the Belle Glade and Canal Point sites, rates of males with abnormalities in Bidder's organ and a maximum number of gonadal abnormalities in *Bufo marinus* found per site increased with increasing intensity of agriculture (McCoy et al. 2008).

A 3-year field survey (2003–2005) of wild *Rana pipiens* and *Rana clamitans* from agricultural sites in southwestern Ontario and nonagricultural reference sites (McDaniel et al. 2008) found gonad abnormalities in male *Rana pipiens* at the microscopic level, and testicular ovarian follicles occurred in significantly more males from agricultural regions (42% in southwestern Ontario) than in those from reference sites (7%). Although the frequency of gonad abnormalities was not correlated directly with environmental atrazine concentrations, they did correlate with a mixture of pesticides and nutrients, particularly atrazine and nitrate. The number of pesticides detected at each site was also important. No other estrogenic signals, such as changes in sex steroid concentrations or vitellogenin induction, in males were found (McDaniel et al. 2008).

Ogielska and Kotusz (2004) identify some important aspects of amphibian ovarian development not previously taken into account during studies of amphibian exposure to atrazine. They described the variation in rate of ovarian development that occurs prior to and after metamorphosis among amphibian species. Many amphibians exhibit heterochronic somatic and ovarian development, meaning that at metamorphosis the ovaries are still developing with some species (e.g., *Bufo americanus*) following a retarded rate in which the ovary will take 3.5 weeks of further development post-metamorphosis, while for others the ovary develops in only a few weeks after metamorphosis (e.g., *Hyla versicolor*), and some species show an accelerated rate in which the ovary is developed at or just days after metamorphosis (e.g., *Rana sphenocephala)* (Ogielska and Kotusz 2004). This could be an important factor influencing the variation among species in sensitivity of sexual development to atrazine and other pesticides.

To test this hypothesis, 3 amphibian species with varying somatic and ovarian development rates were exposed to estradiol (10^{-7} M) or 3 concentrations of atrazine (1, 3, and 30 µg/mL [*Bufo americanus*, *Hyla versicolor*] or 2.7, 7.5, or 124 µg/mL [*Rana sphenocephala*]) or ethanol solvent control. Somatic and ovarian developmental stages and time to metamorphosis were measured. Each species exhibited heterochronic somatic and ovarian development.

In *Bufo americanus* and *Hyla versicolor*, somatic development measured as time to Gosner stages (Gosner 1960) and time to metamorphosis were affected by atrazine, whereas ovarian development was not. In *Rana sphenocephala*, somatic development in terms of Gosner stage or time to metamorphosis was not affected, but ovarian development showed an accelerated development rate. The authors propose that amphibians with shorter larval periods and therefore quicker somatic rates are more susceptible to effects on this endpoint (i.e., prolonged time to metamorphosis). Species with relatively rapid ovarian development are more susceptible to gonadal treatment effects of estrogenic

compounds (Storrs and Semlitsch 2008). Interestingly, estradiol at 10^{-7} M caused a slowing effect on somatic and ovarian development in this study. The authors suggest that this concentration (10^{-7} M) may have a toxic effect on amphibians (Storrs and Semlitsch 2008).

Overall, studies attempting to understand the occurrence of effects of atrazine on gonads of amphibians have measured effects of atrazine concentrations at environmentally relevant concentrations in the range of 0.1 to several hundred micrograms per liter in dose-response studies. While effects of atrazine and concentrations inducing effects vary among studies, and most are conducted in static renewal tests, most have reported effects in at least one endocrine endpoint in amphibians at atrazine concentrations in the range of 20 to 25 µg/L (Tavera-Mendoza et al. 2002; Coady et al. 2005; Orton et al. 2006). There are exceptions, for example, Hecker et al. (2005a) found effects at only 250 µg/L. In a flow-through test, no effects were detected up to 100 µg/L by Hosmer et al. (2007). The lack of consistency in specific concentrations and effects among all studies has raised questions about methods and made it difficult for regulatory bodies to make comprehensive conclusions (Steeger et al. 2007) but does not discount the endocrine-disrupting potential of atrazine.

Where effects have been reported in both laboratory and field studies, they can occur in low atrazine exposures at rates that exceed those in the higher concentration exposures. At this point, the lack of a linear dose-response is considered by regulatory bodies to be problematic when determining the effects of atrazine on sexual development (Steeger et al. 2007). Perhaps there is a need to clarify whether there is a nonlinear response to atrazine that can be quantified. Possible hypotheses include nonlinear responses that may be caused by antagonistic effects at the receptor levels of higher atrazine concentrations, by atrazine breakdown products during immersion studies, or a methodological factor given that more than one study has encountered this type of result when examining sexual development and other endpoints (Storrs and Kiesecker 2004).

Although the endocrine effects of atrazine have been studied extensively in the past decade, there are still many questions to be answered. Only 7 of 19 studies on amphibian gonadal development, recently reviewed by USEPA (Steeger et al. 2007) for the period 2003 to 2007, examined species native to North America. The species used in most studies was *Xenopus laevis*. Only 8 studies were conducted on wild populations of amphibians (Steeger et al. 2007). Interpretation of the field study results is difficult, however; evidence produced by studies supported by the manufacturer of atrazine (Syngenta) and reviewed by USEPA (Steeger et al. 2007) suggest no effects are induced by atrazine exposure at environmentally relevant concentrations. Other studies (e.g., Hayes 2004; Storrs and Semlitsch 2008; Oka et al. 2008) have shown that tadpoles exposed to 10 µg/L atrazine or less demonstrate significant changes in somatic or ovarian development, immune functions, and sexual differentiation.

There is an obvious need to better integrate field and laboratory study designs and to fully identify all pesticides present in field localities where effects are being measured. The dose-response studies, even those conducted under specific protocols defined by USEPA (Hosmer et al. 2007), contained deficiencies in study conditions (Steeger et al. 2007). The lack of species diversity among studies to date also leaves the question of effects on endocrine systems in the majority of amphibians in the world unconfirmed. Of the 7 studies reviewed by USEPA since 2003, 3 reported endocrine effects of some type in native species (Steeger et al. 2007). There have been no thorough population or metapopulation level effect studies of the impacts of atrazine on amphibians despite its widespread use.

Atrazine exposures among study areas in field research to date may represent the low or median concentrations of atrazine that occur in the environment but may not represent the highest concentrations that could occur in small, shallow ponds near farms where amphibians often live (Eisler 1989; Solomon et al. 1996; Giddings et al. 2005). Water sampling for atrazine residues, as described within the published field studies on sexual development, is typically often conducted once and only during the sampling period for amphibians, while atrazine exposure, depending on rainfall events and spray timing, may have been highly variable throughout the egg and larval development period and through the lifetime of the adults producing the amphibians collected for toxicological studies.

Furthermore, it is rare for agricultural pond water to contain only atrazine, but most field studies report only concentrations of atrazine and/or its metabolites in the study areas. Water chemistry among sites is rarely taken into account. Therefore, results of field studies have been difficult to interpret. Correlational analyses are typically conducted, and none of the field studies have demonstrated a clear linear dose-response between effects and atrazine and its breakdown products at the time of sampling. Some studies found alterations in gonadal and other hormone-sensitive tissues, and steroid concentrations, while others have not, which may be dependent on species, site, and the effects of atrazine or combined effects with atrazine and other stressors.

8.2.3 Toxicity to Aquatic Communities Including Amphibians

In a comprehensive effects assessment of atrazine in surface waters in North America, phytoplankton were the most sensitive organism to atrazine, followed by macrophytes, benthic invertebrates, zooplankton, and fish (Solomon et al. 1996). All of these organisms are important as food and/or shelter to amphibians and reptiles. The authors concluded that effects of atrazine are likely to be transient, and quick recovery of the ecological system is expected even in small streams vulnerable to agricultural runoff (Solomon et al. 1996). They acknowledge that a subset of surface waters, principally small reservoirs, in areas with intensive use of atrazine may be at greater risk of exposure to atrazine. They recommended site-specific risk assessments be conducted. However, such risk assessments have rarely been conducted on small ponds. Recent studies in the lab, microcosms and mesocosms, and field studies examining the effects of atrazine on aquatic communities confirm that atrazine effects are extensive, complex, and can be detrimental to amphibian health and survival in aquatic communities.

In a mesocosm study, the effects of 2 pulses (separated by 2 weeks) of atrazine (25 μg/L) and endosulfan (10 μg/L); (individually and in combination) were evaluated on the presence and absence of *Rana sylvatica* tadpoles, adult snails (*Planorbella trivolvis*), and caged dragonfly larvae (*Anax junius*) in a freshwater community (Rohr and Crumrine 2005). Tadpoles, snails, and chironomid larvae (*Polypedilum* sp.) competed for periphyton. Neither pesticide affected dragonfly survival, but endosulfan directly reduced zooplankton (*Daphnia* sp.) and atrazine indirectly reduced chironomid abundance. Atrazine also directly decreased periphyton, and endosulfan severely reduced chironomid larvae, resulting in changes in competition for both snails and tadpoles. Compared to endosulfan, atrazine tended to decrease snail mass and reproduction and reduce tadpole mass, development, inactivity, refuge use, and dragonfly avoidance. The indirect benefit of endosulfan on snail mass was greater in the presence of caged dragonfly larvae, and endosulfan's indirect benefit on tadpole mass was greater in the absence of snails. The effect of pesticides on tadpole activity was influenced by caged dragonflies and snails. Environmentally relevant concentrations of these pesticides shaped species responses and community composition, but the initial composition of the community influenced the pesticide effects (Rohr and Crumrine 2005).

In microcosms with *Rana sphenocephalus*, *Bufo americanus*, *Ambystoma maculatum*, and *Ambystoma texanum*, density, hydroperiod, and carbaryl and atrazine concentrations (0 and 200 μg/L atrazine) were manipulated to test for effects on development, mass, and survival of amphibian larvae. Exposure to atrazine had negative effects on body size, development, and time to metamorphosis in the anurans, which were associated with reduced chlorophyll concentratons. A significant interaction between atrazine and carbaryl resulted in smaller and less developed *Ambystoma maculatum* larvae compared to control ponds. Atrazine appeared to moderate the negative effects of carbaryl on *Ambystoma maculatum* (Boone and James 2003).

Artificial pond microcosms with pond water containing phytoplankton, periphyton, macrophytes, and larval *Hyla versicolor* were treated with atrazine at concentrations of 20, 200, and 2000 μg/L. Dissolved oxygen concentrations were reduced to approximately 20 to 40% of preexposure concentratons in 200 and 2000 μg/L groups within 1 day of atrazine exposure. Dissolved oxygen concentratons returned to control concentrations within 10 days postexposure but then declined to

60% to 80% of control concentrations 21 days postexposure and remained depressed. Similarly, in 200 and 2000 µg/L treatments, pH and oxygen decreased within 1 day of atrazine treatment and both increased to control concentrations within 16 days. Frogs in 200 and 2000 µg/L groups were 5% shorter and had 10% lower body mass at metamorphosis than in the control or lower-dose groups, whereas no differences in length or body mass at metamorphosis were detected in the lowest-dose group. No effects on survival rate were found (Diana et al. 2000).

Atrazine was applied to 0.45 ha experimental ponds in 2 replicate concentrations of 20, 100, and 500 µg/L and the impacts on invertebrate communities were observed. In this case, atrazine did not affect water temperature or oxygen concentrations. Macrophyte production decreased in a dose-response fashion, although macroalga (*Chara* sp.) was not affected below 100 µg/L. Abundance of emerging individuals of the chironomid *Labrundinia pilosella* was significantly reduced at 20 µg/L atrazine, and other less abundant species showed similar declines. Benthic species richness, species equitability, and total emergence all declined significantly at 20 µg/L atrazine, while predatory insects were unaffected. Emergence periods for several herbivorous insect species were earlier in atrazine-treated than in control ponds (Dewey 1986).

In a field study of 18 wetlands in Minnesota, atrazine had the strongest fit of over 240 plausible predictors of abundance of larval trematodes in *Rana pipiens* (Rohr et al. 2008b). The combination of atrazine, and its metabolite desethylatrazine, and phosphate accounted for 74% of the variation in the abundance of larval trematodes in *Rana pipiens* collected from these sites (atrazine and desethylatrazine together accounted for 51%). Analysis of field data supported the hypothesis that agrochemicals increase exposure and susceptibility to larval trematodes by augmenting snail intermediate hosts and suppressing immunity. This study also measured a large number of pesticide and water chemistry parameters, unlike most studies before it. Thirty pesticides plus their metabolites and chloride, nitrate, phosphate, and sulfate were measured in water samples. Twenty-six trace metals were measured in sediments and pesticides, and PCBs were also measured in amphibian tissues (Rohr et al. 2008b). A comparative experiment to the Minnesota field study was then conducted in which 4 species of larval amphibians (*Ambystoma maculatum*, *Hyla versicolor*, *Rana palustris*, and *Rana clamitans*), 2 snail, 1 beetle, 2 water bug, and 1 dragonfly species were exposed to a single dose of atrazine (mean concentration 117 µg/L) in 800 L mesocosms for 4 weeks (Rohr et al. 2008b). Atrazine tanks contained immunosuppressed tadpoles, significantly more attached algae, and snails and tadpoles with elevated trematode loads (Rohr et al. 2008b), consistent with findings from the field study by these authors and other mesocosm and laboratory-based research.

The multiple effects of atrazine occurring within the simple ecosystems of mesocosms emphasize the implications atrazine could have for wild populations of amphibians and reptiles and, to some extent, the resiliency of community response to stress caused by pesticides. However, effects of controlled stressors in the laboratory under ideal conditions for amphibians are generally expected to underestimate the effects that can occur in the wild where the environmental conditions are much harsher. For example, in a laboratory setting, higher deformity rates were found in *Rana pipiens* tadpoles exposed to ultraviolet (UV) radiation plus extracts of pond water from sites where gross morphological deformities in amphibians were common vs. a non-UV treatment with the same pond water extracts (Bridges et al. 2008). Previous studies where amphibians were exposed to pond extracts from the same area and where deformities in metamorphs had been reported found no effects of the pond water where the animals were not UV exposed (Fort et al. 1999). Similarly, even variation in water temperature during egg rearing, which must be the most basic of factors affecting amphibian physiology, can affect the toxicity of an insecticide such as endosulfan on tadpole growth in the Australian frog (*Limnodynastes peronii*; Broomhall 2004).

8.2.4 Effects of Atrazine Exposure on Reptiles

Crocodilians, many turtles, and some lizards exhibit temperature-dependent sex determination (TDSD), where the temperature during egg incubation determines the sex of the embryo. Most

studies on sex determination in reptiles have focused on those species with TDSD. Reptilian gonads do not initially produce significant amounts of aromatase and sex steroids until after the sexual differentiation of the gonads (White and Thomas 1992; Smith et al. 1995). In most reptiles with TDSD, estrogen production in embryos is highest at female-producing temperatures (Elf et al. 2002). Exposure to exogenous estrogen during egg incubation produces female embryos at all temperatures (Rhen and Lang 1994). Aromatase converts androgens to estrogens, and thus regulates both primary sexual characteristics and sex determination in reptiles with TDSD. Thus, aromatase has a critical role in the control of sexual development. For example, inhibiting aromatase (by the inhibitor fadrozole) induces male development at female-producing temperatures (Rhen and Lang 1994). Furthermore, exogenous exposure to testosterone, which is convertible to estrogen via aromatization, produces females at male-producing temperatures (Rhen and Lang 1994); aromatase inhibitors also prevent the feminization effect of aromatizable androgens (Crews and Bergeron 1994). At the beginning of the critical sex determination period, putative female alligator and turtle embryos had greater aromatase activity in the brain than putative males, suggesting that aromatase activity induces alterations in the neuroendocrine axis that controls gonadal sex steroid hormone production (Willingham et al. 2000; Milnes et al. 2002). The weight of evidence indicates that incubation temperature regulates the expression of aromatase enzymes, which in turn affect estrogen production and the sexual differentiation of the gonads. Thus, sexual development of reptiles may be affected by compounds that affect aromatase activity (Keller and McClellan-Green 2004; Willingham 2005; de Solla et al. 2006).

Results of research on the effects of atrazine on gonadal development or sex determination in reptiles have been mixed. Keller and McClellan-Green (2004) demonstrated that atrazine was capable of inducing aromatase activity in an immortal cell line of green turtles (*Chelonia mydas*). The cells were exposed to 3 known inducers of aromatase (dexamethasone, 8BR-cyclic AMP, and human chorionic gonadotropin), yet they failed to induce aromatase, except for dexamethasone at the highest dosage (1 μM). However, all concentrations of atrazine used (0.1, 1, and 10 μM) successfully induced aromatase compared to the controls (Keller and McClellan-Green 2004).

The ability of atrazine to affect aromatase in embryonic alligators, as opposed to cell cultures, is ambiguous. Crain et al. (1997) reported that male neonatal alligators exposed to atrazine had aromatase activity that was not significantly different than that of either untreated females or untreated males, although these latter 2 treatments were different from each other. Atrazine was topically applied to the eggshell, using a 95% ethanol vehicle, at 0.14, 1.4, and 14 ppm, at both male- (33 °C) and female- (30 °C) producing temperatures, respectively. A subsequent study (Crain et al. 1999) found no difference in aromatase activity, or any other endpoint, between neonates from atrazine-treated and control eggs.

Regardless of the effect of atrazine on aromatase activity, atrazine exposure affects sex determination in some cases. Although topical application of atrazine (at 5 ng/10 g egg) alone at male-producing temperatures did not affect the sex ratio of red-eared sliders (*Trachemys scripta*), near the transitional male-female temperature (29.2 °C) it did cause a female-biased sex ratio compared to controls, although the sex ratios, surprisingly, did not differ between temperature regimens alone (29.2 °C vs. 26 °C) (Willingham 2005). Snapping turtle (*Chelydra serpentina*) eggs exposed to atrazine-treated soil at typical and 10 times typical application rates (1.48 and 14.8 kg ai/ha) and incubated at male-producing temperatures (25 °C) exhibited normal hatching success, incidence of deformities, and gonadal development (de Solla et al. 2006), with only ~3% intersexes or females found in the atrazine treatments. All hatchlings at male-producing temperatures appeared to be normal males, based upon histology of their gonads. Although there is always a possibility of species-specific differences in sensitivity, differences between the studies may be explained by methods of dosing. Hatchlings from atrazine-exposed alligator eggs (Crain et al. 1999) or caiman (*Caiman latirostris*) eggs (Beldomenico et al. 2007) did not show sex ratios that differed from those of the controls. It is unknown how much exposure turtle eggs are likely to have from soil or other substrate that contains

atrazine. Furthermore, only Willingham (2005) incubated eggs near the transitional temperature; it is possible that sex determination is more likely to be affected by xenobiotics near temperatures that produce mixed sex ratios.

Atrazine may also affect growth of embryos in exposed eggs. Both Willingham (2005) and Beldomenico et al. (2007) found that atrazine exposure affected hatchling mass after eggs were exposed to atrazine. Laboratory exposures of caiman eggs to atrazine (0.2 ppm) resulted in increased egg weight loss and reduced hatchling mass (Beldomenico et al. 2007). Egg weight loss was generally due to evaporation of water through the eggshell (Manolis et al. 1987), but Beldomenico et al. (2007) argued that there might be some metabolic costs for the embryo associated with atrazine exposure. Conversely, Willingham (2005) found that atrazine-exposed eggs had significantly heavier hatchlings than the controls, although only at lower temperatures (26 °C).

8.3 CONCLUSIONS

At present, atrazine is banned in the European Union and has been voluntarily withdrawn by the manufacturer from use in British Columbia, Canada. Its recent use in the United States has been reviewed solely on the basis of the potential for atrazine to affect amphibian gonadal development. In June 2003, a Special Advisory Panel (SAP) reviewed a White Paper prepared by USEPA (Steeger and Teitge 2003) that critically evaluated the available data from 17 laboratory and field studies with respect to the ability of atrazine to induce amphibian gonadal abnormalities. The 2003 White Paper/USEPA (Steeger and Teitge 2003) concluded that while there was sufficient information to formulate a hypothesis that atrazine exposure can affect amphibian gonadal development, there was insufficient information to refute or confirm the hypothesis. The SAP agreed with the USEPA that additional studies were warranted and that a tiered testing approach was needed (USEPA 2003). In 2004, EPA issued a data call-in (DCI) notice to Syngenta Crop Protection, Inc. and other atrazine registrants, requiring that registrants conduct a study consistent with the first tier of testing described in the 2003 White Paper. After the agency and the registrant agreed to a protocol using a flow-through exposure for testing potential effects of atrazine on gonadal development of amphibians in 2005, the registrant submitted to us EPA in June 2007 a final report (Hosmer et al. 2007) focusing on effects of atrazine alone on amphibian gonadal development.

In 2007, the USEPA concluded that a review of the open literature since the 2003 Special Advisory Panel did not provide any additional information that could be used to refute or confirm the hypothesis that exposure to atrazine alone causes adverse developmental effects in amphibian gonads. They also concluded that the atrazine exposure concentration profile is reasonably characterized and sufficient for documenting the potential effects of atrazine over a broad range of exposure concentrations. At the same time, USEPA conducted an evaluation of laboratory-based studies submitted in response to a data call-in and concluded that those studies did not provide sufficient evidence to support the hypothesis that atrazine causes adverse gonadal development in amphibians due to methodological or other flaws. The USEPA also concluded that the designs available in the open literature are not appropriate for evaluating the hypothesis that atrazine affects amphibian gonadal development. Combined with the results of the DCI study, the USEPA concluded that atrazine does not cause adverse effects on gonadal development in *Xenopus laevis* when tested under experimental conditions recommended by the USEPA. The USEPA further concluded that atrazine at concentrations of up to 100 µg/L does not cause adverse effects on *Xenopus laevis* gonadal development exposed in a flow-through exposure apparatus. This concurs with a risk assessment of atrazine to amphibians in which data were reviewed in the context of estrogen-mediated, androgen-mediated, and thyroid-mediated mechanisms, adverse effects on gonadal development in amphibians, and effects at the population level in exposed amphibians (Solomon et al. 2005). Although the White Paper contained a thorough review of all the published papers and the data call-in results and concluded that there was no perfect study (Steeger et al. 2007), it was concluded that no further studies are warranted to further test the hypothesis that atrazine alone causes adverse developmental

effects in amphibian gonads. In summary, based on a thorough examination of these studies and their results, USEPA concluded that atrazine does not adversely affect amphibian gonadal development, and there is no compelling reason to pursue additional testing of atrazine for amphibian gonadal effects.

However, in a review of the same data, the Federal Insecticide, Fungicide and Rodenticide Act Science Advisory Panel (FIFRA SAP 2008) concluded differently than the USEPA (Steeger et al. 2007). It concluded that the while the DCI study was well designed and executed, the transformation products of atrazine were not measured and the flow-through design would have limited their accumulation in the exposure system. The panel recognized the need to evaluate field monitoring data to establish whether any transformation products of atrazine accumulate in the environment at concentratons that warrant direct evalulation of toxicity. The FIFRA SAP (2008) deduced that the results of the DCI study do not sufficiently address the hypothesis that atrazine at environmentally relevant exposure concentrations adversely affects amphibian gonadal development. While the FIFRA SAP (2008) agreed the concentrations of atrazine in the DCI experiment were sufficient for documenting gonadal effects in the concentration range of 1.0 to 100 µg/L atrazine, it was less confident that the exposures at the lower concentratons (0.01 and 0.10 µg/L) were well maintained in the experiment. The FIFRA SAP (2008) noted that the 2003 SAP recommended that studies with *Xenopus laevis* be followed up with comparable studies using a North American species. Such comparative studies have not yet been performed, and the FIFRA SAP (2008) expressed uncertainty in extrapolating the results from *Xenopus laevis* to North American frog species. In conclusion, the FIFRA SAP (2008) stated that the studies on *Xenopus laevis* were not sufficient to refute the hypothesis that atrazine, at environmentally relevant exposure concentrations, adversely affects amphibian gonadal development. It was only sufficient to refute the hypothesis that at environmentally relevant concentrations, atrazine adversely affects gondal development in *Xenopus laevis.* In this, the panel disagreed with the USEPA.

Atrazine regulation in Europe and Canada contrasts with events in the United States (Graymore et al. 2001). In 1980, the European Union's Drinking Water Directive specified 5 µg/L as the maximum allowable concentraton of any pesticide in drinking water (European Council 1980), followed by a maximum allowable concentraton of 0.1 µg/L of any one pesticide in 1998 (European Council 1998). The potential to contaminate ground water was the impetus for restrictions of atrazine in Europe. Both Italy and Germany banned atrazine in 1991 (Ackerman 2007), followed by a ban on atrazine throughout all EU member states in 2004 (European Commission 2004). The ban went into effect in 2005, although there were some extensions for limited uses, which expired in 2007 (European Commission 2004). In Canada, atrazine was voluntarily removed by Syngenta from the market in British Columbia in 2007 (Health Canada 2003, 2004, 2007a, 2007b).

Similar legislation has occurred in the United States, albeit on a much smaller scale (Graymore et al. 2001). For example, Wisconsin has prohibited atrazine use, by the Atrazine Rule, Chapter Ag 30, in areas in which atrazine has contaminated ground water (Hanson et al. 1997). Furthermore, new labeling regulations required the application rate of some pesticides to depend on type of soil, so as to minimize ground water contamination (Hanson et al. 1997). In areas with high corn production, atrazine concentrations in streams have been 3- to 10-fold higher than the 3 µg/L maximum contaminant concentraton allowed by the USEPA (Thurman et al. 1991).

There is an extensive literature on the impact of atrazine on amphibians for both individual endpoints and, more recently, mesocosm and field studies that identify the extensive and cascading effects atrazine has on aquatic communities that amphibians and reptiles inhabitat as prey and predators. We must now go beyond the debate on effects of atrazine on sexual development in amphibians and focus on atrazine's broader impact in the environment where amphibians and reptiles continue to be exposed. Amphibian and reptile populations are under a multitude of stresses, and habitat quantity and quality are the keys to their survival. The presence of atrazine degrades the quality of the remaining habitats, many of which persist mainly in agricultural landscapes.

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9 Ecotoxicology of Organic Contaminants to Amphibians

Donald W. Sparling

CONTENTS

By definition, an organic molecule is composed of oxygen, hydrogen, and carbon, but in practice other elements such as nitrogen and sulfur may also be present and the molecule is still considered to be "organic." There are literally tens of thousands of chemicals produced by humans and found in the environment that fall within the classification of being organic. As such, organic contaminants could include pyrethroid, organophosphate, and carbamate pesticides as well as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), organochlorine pesticides (OCs), dioxins, furans, and phenolics. Recently registered pesticides that represent "new" chemistries (Chapter 15, this volume) increase the list of candidate organic contaminants even more. However, the regulatory community, especially the US Environmental Protection Agency (USEPA), tends to separate these chemicals by the laws that govern their use and registration within the United States. Thus, pesticides that are covered by the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA; 7 USC s/s 136 et seq. 1996) are distinguished from other chemicals that are covered under the Toxic Substances Control Act (TSCA; 15 USC s/s 2601 et seq. 1976). We generally follow this convention in this book, with Chapter 6 reviewing nonhalogenated pesticides and this chapter reviewing other organic contaminants, but I include chlorinated pesticides here because many are no longer being released in North America.

Chlorinated pesticides and PCBs are totally synthetic chemicals. They were first developed in the early part of the 1900s, but many ceased being used during the mid-1970s in the United States, Canada, and most developed nations due primarily to human health concerns. However, a few OCs such as endosulfan are still in use, and others are being manufactured and used in third world nations. DDT, for instance, remains the chemical of choice for mosquito control in malaria-ridden areas. Both PCBs and chlorinated pesticides are persistent, so their residues are still found in environmental matrices around the globe. Dioxins and furans are products of combustion, including

forest and grassland fires, power generation, and other sources. As a result, they are released continually by natural and human processes. Many PAHs also occur naturally, but others are used in manufacturing processes, especially those associated with petroleum. Their persistence varies with the specific chemical.

Concern about organic contaminants and amphibians rises from several factors. First, some organic contaminants are extremely toxic to aquatic organisms, having median lethal concentrations (LC50s) in the low parts per billion (μg/L) range. Second, they may exert a variety of sublethal effects, including genotoxicity, carcinogenesis, reduced growth and developmental rates, and endocrine disruption. Third, these contaminants are noted for their environmental persistence, bioaccumulation, and biomagnification. Whereas larval amphibians typically do not occupy high trophic levels, the biphasic life history of amphibians makes them important links in food chains and connections between aquatic and terrestrial environments. Fourth, many organic contaminants have sufficient volatility to be transported by atmospheric currents and may have effects hundreds of kilometers from their sources.

Sparling (2000) reviewed the residue concentrations and effects of organic contaminants in amphibians. The present chapter will summarize some of the findings of that review and supplement it with new information obtained since 1999.

9.1 CELLULAR METABOLISM OF ORGANIC CONTAMINANTS

The sensitivity of an animal to an organic contaminant is a function of the toxicity of the parent compound, the ability of an organism to metabolize the compound, and the toxicity of the metabolites. Most vertebrates, including amphibians, have a variety of enzymatic mechanisms that metabolize xenobiotics and intracellular toxins. Examples of these mechanisms include mixed-function oxidases (MFOs), including cytochromes P450, and glutathione (GSH).

Cytochromes P450 are hemoproteins found in cells throughout the body but have especially high activities in liver. Exposure to xenobiotics can increase the concentration or induce these cytochromes. Two classes of inducible cytochromes, CYP1A and CYP2B, are currently recognized. Associated with the cytochromes P450 are a host of enzymes referred to as monooxygenases, or MFOs. These enzymes increase the polarity of lipophilic xenobiotics, thus enhancing their detoxification and elimination. However, they can also increase the toxicity of certain xenobiotics in that the oxidized metabolites may be more toxic than the parent compounds. This is true for PCBs, some organochlorine and several organophosphorus pesticides, and some PAHs (Melancon 2003). Thus, species that are inefficient in metabolizing these compounds may be less affected than those species that metabolize them efficiently. An MFO that has been well studied as an indicator of contaminant exposure is ethoxyresorufin-*O*-dealkylase (EROD) (Melancon 2003).

Based on the limited data available at that time (e.g., Schwen and Mannering 1982; Noshiro and Omura 1984), Sparling (2000) suggested that the activities of cytochromes P450 and MFO systems in amphibians were well below those of mammals and somewhat above those of fish. However, Nandi et al. (1997) surveyed P450 activity in fishes, rodents, and amphibians and found that the highest specific content of cytochromes P450 was in the liver microsomes of cane toads (*Bufo marinus*) and rats, and that rats showed significantly higher content of cytochrome B5 than cane toads, bullfrogs (*R. catesbeiana*), or 2 species of fish. EROD induction was highest in the toad and rather low in the frog. The authors compared their data to other studies and concluded that generalizations among classes of vertebrates in cytochromes P450 may be premature.

Glutathione helps protect cells from oxidative stress that can be related to PAHs, chlorinated hydrocarbons, and heavy metals. The glutathione complex, consisting of reduced glutathione (GSH), oxidized glutathione (GSSG), and glutathione reductase, scavenges free radicals, restores damaged molecules by hydrogen donation, reduces peroxides, and maintains protein thiols in the reduced state (Cavas and Tarhan 2003). Various other cellular factors (peroxidase, catalase, superoxide dismutase, ascorbate, and alpha-tocopherol) assist in this function. The GSH/GSSG ratio can be used

as a bioindicator of toxicant exposure. Glutathione functionality develops as tadpoles mature into metamorphs (Cavas and Tarhan 2003). For instance, total, reduced, and oxidized glutathione activities increased with developmental stage from the fifth through the eighth weeks posthatch in *Rana r. ridibunda* and *Bufo viridis.*

9.2 POLYCHLORINATED BIPHENYLS, DIOXINS, AND FURANS

Polychlorinated biphenyls are a group of 209 congeners composed of 2 phenolic rings with 1 to 10 chlorine atoms attached to the carbons (the figures for these structures are in Chapter 10, this volume). When they were sold, several congeners were sold together by the manufacturer as units called Aroclors. Each Aroclor was given a number that reflected its degree of chlorination, with higher numbers denoting greater chlorine content by mass. The geometry of the phenol rings may be such that their planes are at right angles to each other (nonplanar), or they may lie in the same plane (coplanar); coplanar PCBs are more biologically active than nonplaner forms, and the degree of chlorination is positively related to the toxicity of the congener. Production of PCBs began in the 1920s, and the final ruling on the ban of the manufacture, processing, distribution in commerce, and use of PCBs, except in totally enclosed systems or by special exemption by the USEPA, occurred in 1977. Acute exposure to PCBs can result in a wasting syndrome, immunosuppression, and hepatomegaly. Chronic toxicity is evidenced by endocrine dysfunction, carcinomas, and mortality in humans and other animals.

Dioxins (polychlorinated dibenzo-p-dioxins [TCDD]) and furans (polychlorinated dibenzofurans [PCDF]) are chemically related to PCBs as aromatic heterocyclic compounds. They can have 1 to 8 chlorine atoms, resulting in numerous congeners of either compound. As with PCBs, the toxicity of dioxins and furans tends to increase with degree of chlorination, with 2,3,7,8-TCDD and 2,3,7,8-TCDF being the most toxic forms of each group. For a review of PCBs, dioxins, and furans see Rice et al. (2003).

9.2.1 Amphibian Residues

Most studies that measured PCB residues in amphibians have focused on adults. This is logical because PCBs can bioaccumulate and biomagnify, and residues in older, insectivorous adults would likely be higher than in detritivorous larvae from the same habitats. Early studies (Hall et al. 1985; Hernandez et al. 1987; Russell et al. 1995; Phaneuf et al. 1995; Bonin et al. 1995; Vojinovic-Miloradov et al. 1996; Gendron et al. 1997) reported PCB concentrations ranging from below detection limits to 58 196 µg/kg wet body mass in the ovary of a mudpuppy (*Necturus maculosus*) collected from a heavily contaminated site. Most of the studies suggested that total PCB concentrations in all but the most highly contaminated sites should range between 100 and 500 µg/kg wet body mass. Table 9.1 presents information on PCBs in field-collected specimens.

Fewer studies have reported dioxin or furan residues in amphibians, but concentrations of these pollutants are orders of magnitude less than for PCBs. Green frogs (*Rana clamitans*) and northern leopard frogs (*R. pipiens*) collected from a reference site had 0.057 µg/kg wet body mass total dioxins, whereas those sampled from a site affected by a chemical burn had up to 0.404 µg/kg body mass in whole bodies; furan concentrations in these sites were below detection limits to 0.249 µg/kg body mass, respectively (Phaneuf et al. 1995). The highest field concentration of dioxins reported was in common toads (*Bufo terrestris*), with 1.36 µg/kg (Young and Cockerham 1987). However, Jung and Walker (1997) reported a concentration of 4.10 µg/kg in a laboratory population of American toads (*B. americanus*) exposed to water with 0.03 µg/L TCDD.

As indicated in Chapter 1 of this book, most of the recent studies on contaminants and amphibians have focused on effects rather than on residues. However, there have been a few papers that documented concentrations in contaminated sites.

The Fox River in east central Wisconsin has received considerable study due to contamination from pulp and paper mills and other industries. The river is polluted with heavy metals, PCBs, and

TABLE 9.1
Concentrations of Polychlorinated Biphenyls (PCBs) (µg/kg Wet Weight unless Specified) and Related Compounds in Amphibians Collected from Field Studies (Life Stage Is Adult unless Otherwise Specified)

Species	Compound	Tissue	Concentration	Comments	Reference
Ambystoma gracile	Total PCBs	Eggs	575–1231	Collected from 4 sites in British Columbia, Canada	de Solla et al. 2002
Bufo sp.	TCDD	Whole	200	Collected from field	Fanelli et al. 1980
Bufo terrestris	TCDD	Whole	1360	As above	Young and Cockerham 1987
Rana clamitans and *R. pipiens*	Total PCBs	Whole	7.5	Collected from reference site	Phaneuf et al. 1995
	Total PCBs	Whole	93.86	Collected from contaminated site	As above
	Total dioxins	Whole	0.108 (<DL–0.404)	Collected from contaminated site	As above
	Total dioxins	Whole	0.057	Collected from reference site	As above
	Total furans	Whole	0.095 (<DL–0.249)	Collected from contaminated site	As above
	Total furans	Whole	<DL	Collected from reference site	As above
Rana clamitans	Various PCB congeners	Whole	<DL–3.28	Field site; PCB 138 and 180 highest	Russell et al. 1997
R. clamitans	Total of 13 PCB congers	Whole	2.8–15.92	As above	As above
Rana perezi	Total PCBs	Muscle	50–108	National park in Spain over 3 years	Rico et al. 1987
Rana ridibunda	PCB 28	Liver	1.12–16.92	Collected from field in Serbia	Vojinovic-Miloradov et al. 1996
	PCB 52	Liver	0.65–9.52	As above	As above
Necturus lewisi	PCB 1254	Carcass less GI tract	400	Collected from field site in North Carolina	Hall et al. 1985
Necturus maculosus	Total PCBs	Female gonads	408	Collected from Ottawa River, Canada	Bonin et al. 1995
	Total PCBs	Whole	113–1082	Collected from St. Lawrence River	As above
Necturus maculosus	Total PCBs	Liver	751–2192	St. Lawrence and Ottawa Rivers	As above
	Total PCBs	Female gonads	437–58196	From 4 sites in St. Lawrence River	Gendron et al. 1997
	Aroclor 1260	As above	292–847	St. Lawrence River	As above

	Mono-ortho coplanar PCB	As above	50.9–615	As above	As above
	Non-ortho coplanar PCB	As above	1.06–256	As above	As above
	Total PCBs	As above	408–4423	From 5 sites in Ottawa River	As above
	Aroclor 1260	As above	141–3553	As above	As above
	Mono-ortho coplanar PCB	As above	57.7–407	As above	As above
	Non-ortho coplanar PCB	As above	0.68–5.59	As above	As above
Mixed species	Aroclor 1260	Carcass	318–1260	Paducah Gaseous Diffusion Plant	DeGarady and Halbrook 2003
	Congener 153	Carcass	<DL–62	As above	As above
R. sphenocephala	Aroclor 1260	Lipid	41–1859	As above	DeGarady and Halbrook 2006
R. catesbeiana	As above	As above	568–886	As above	As above
B. fowleri	As above	As above	55.6–894	As above	As above
H. cinerea	As above	As above	89.6–901	As above	As above
R. pipiens	Total PCB	Whole body	14.9–568	Contaminated sites in Green Bay and Fox River, Wisconsin	Karasov et al. 2005
R. pipiens	Total PCB	Whole body	3.3–54.7	Reference sites in Green Bay and Fox River, Wisconsin	As above
R. temporaria	PCB 52	Liver	0.052–2.00	Along an altitudinal gradient in Scandinavia	Ter Schure et al. 2002
	PCB 183	As above	0.036–0.564	As above	As above
	PCB 206	As above	0.002–0.016	As above	As above
	Total of 5 congers	As above	0.47–5.044	As above	As above
R. aurora	Total PCBS	As above	421–1083	Collected from 2 sites in British Columbia	de Solla et al. 2002
Pseudacris regilla	Total PCBs	Tadpoles	1.57–243	21 sites in the Sierra Nevada Mountains, California	Angermann et al. 2002

(continued)

TABLE 9.1 (CONTINUED)
Concentrations of Polychlorinated Biphenyls (PCBs) (µg/kg Wet Weight unless Specified) and Related Compounds in Amphibians Collected from Field Studies (Life Stage Is Adult unless Otherwise Specified)

Species	Compound	Tissue	Concentration	Comments	Reference
R. clamitans	Total PCBs	Adults	13.2–232[a]	5 sites near Kalamazoo River, Michigan	Glennemeier and Begnoche 2002
	As above	Tadpoles	200–826[a]	2 sites near Kalamazoo River, Michigan	As above
R. clamitans	Total PCBS	Adults	19.62	Mean from 7 sites in SW Michigan	Gillilland et al. 2001
	As above	Juveniles	19.12	Mean from 2 sites in SW Michigan	As above
	As above	Tadpoles	7.63	Mean from 7 sites in SW Michigan	As above
	As above	Eggs	79.11	Mean from 5 sites in SW Michigan	As above
R. perezi	Total PCBs	Adults	9.3a	Rice fields in Spain	Pastor et al. 2004
	As above	Tadpoles	11.2a	As above	As above
	As above	Adult fat	105	As above	As above
	As above	Tadpole fat	101	As above	As above

[a] Reported on dry weight basis.

dioxins. Karasov et al. (2005) collected sediments from reference sites along the shores of Green Bay, into which the Fox River flows, and along the river up to its mouth. Reference sediments generally had PCB concentrations below detection limits, but river sites ranged from 0.07 to 22 mg/kg dry mass. Concentrations of heavy metals were positively correlated with PCB concentrations. Although amphibians were found at all sample sites, species richness declined inversely with an index of contamination based on PCB and heavy metal concentrations. To determine the effects of these pollutants on green frog and northern leopard frog, egg masses were placed in Nitex bags at each of the sample locations and allowed to develop through metamorphosis. PCB concentrations in whole body tadpoles and adults correlated with those in the sediments of corresponding locations. Coplanar PCBs constituted approximately 99.8% of the PCB loads in whole bodies of frogs and tadpoles. Concentrations of total PCBs in the contaminated sites ranged from 105 to 114 μg/kg wet mass in embryos, 30 to 312 μg/kg in tadpoles, and 14 to 568 μg/kg in frogs (metamorphs and juveniles). Corresponding concentrations from reference sites were 6.3 μg/kg for embryos, 26 to 55 μg/kg for tadpoles, and 3.3 to 22 μg/kg for frogs. Hatching success of caged embryos was negatively correlated with degree of contamination, but survival and growth of tadpoles were not related to sediment pollution.

According to a global fractionation theory (Wania and Mackay 1993, 1996) persistent organic pollutants such as PCBs should be partitioned along a north-south axis with more volatile congeners being distributed farther north (in the northern hemisphere) than less volatile congeners. This partitioning is due to a plume of contamination spreading from industrial areas in the mid latitudes to higher latitudes and selective deposition of denser, less volatile congers in the south. Ter Schure et al. (2002) reported that PCBs in amphibians adhered to this theory in Sweden. They examined the distribution of 5 congeners (52, 153, 183, 201, and 206) in common frog (*Rana temporaria*) livers along a 1500 km transect between 53 and 68° N latitude. All 5 congeners decreased as latitude increased, with their sums going from approximately 5000 ng/kg wet weight to 470 ng/kg. However, the more volatile congeners, 53, 153, and 183, had higher concentrations in the north than did congeners 201 and 206.

Several species of amphibians have demonstrated severe population declines in the Sierra Nevada Mountains of California (Fellers and Drost 1993; Drost and Fellers 1996) and contaminants have been associated with these declines (Davidson et al. 2001; Sparling et al. 2001). Pesticides from the Central Valley of California and industrial contaminants from urbanized areas west of the Sierras are blown into otherwise pristine montane areas where they can impact the health of amphibians. Pacific treefrog (*Pseudacris regilla*) tadpoles were collected from 21 sites throughout the Sierras, from 610 to 3267 m elevation, and sampled for PCB and toxaphene concentrations (Angermann et al. 2002). Total PCB concentrations in whole bodies of tadpoles ranged from 1.6 to 243.8 μg/kg wet weight. Treefrogs collected from east-facing slopes protected from prevailing westerly winds had lower concentrations of PCBs and toxaphene than those on more exposed west-facing slopes. There were no significant relationships between total PCB concentrations and latitude or elevation when adjusted for east-west-facing slopes. The authors speculated that the contaminants could cause immunosuppression, making amphibians more susceptible to disease.

The Kalamazoo River (Michigan) was contaminated with PCBs over several decades by paper mills, resulting in a 128-mile stretch above its mouth that has been declared a Superfund site. Similarly, Saginaw Bay in Lake Huron is contaminated by industrial wastes including PCBs. In a survey of 15 wetlands (Glennemeier and Begnoche 2002) sediment concentrations of PCBs ranged from below detection limits in reference wetlands to 39 mg/kg dry weight in contaminated sites. Green frog adults or tadpoles were found at only 5 of 9 sites associated with the Kalamazoo River. PCB concentrations in adults were less than 2% of those found in sediments and ranged from 13.2 to 232 μg/kg dry body mass. Tadpole PCB concentrations were 200 and 826 μg/kg and represented 7.5 and 17.8% of those found in sediments, respectively.

In Paducah, Kentucky, a uranium enriching plant of the US Department of Energy has been in operation since the 1960s and has released many contaminants, including Aroclor 1260, into the

environment. Water flows from the plant through outfalls into Big and Little Bayou Creeks and has discharged heavy metals, PCBs, dioxins, PAHs, and radionuclides. Ten species of adult anuran amphibians were collected from 7 outfalls and 3 reference sites (DeGarady and Halbrook 2003). Mean concentrations of Aroclor 1260 in collective frog carcasses without kidney or liver ranged from 381 to 1260 µg/kg wet weight. The most frequently occurring congeners were 153 and 180. In a subsequent paper (DeGarady and Halbrook 2006), no interspecific differences in lipid-corrected Aroclor 1260 concentrations were found among adult green frogs, northern leopard frogs, Fowler's toad (*Bufo fowleri*), or green treefrogs (*Hyla cinerea*). However, juveniles collectively had higher lipid-corrected Aroclor 1260 concentrations (mean = 919 µg/kg) than did adults (mean = 354 µg/kg). The authors concluded that anurans, particularly larvae, can be useful biomonitors of PCB contamination.

9.2.2 Effects of PCBs, Dioxins, and Furans

Prior to 2000 comparatively little was known about the effects of PCBs, dioxins, or furans on amphibians, but much of what was available and studies on fish suggested that lethal effects might be expected at environmentally realistic concentrations of the contaminants. One finding was that sensitivity to PCBs varied with species and age. For example, the 96-hour static renewal LC50s for Aroclor 1254 in northern leopard frogs, American toads, and Fowler's toads at hatching were determined as 3.5, 10.3, and 38.2 µg/L, respectively. Four days posthatch the respective LC50s were 1.0, 2.0, and 3.7 µg/L. Similar differences were noted for Aroclors 1242 and 1016 (Birge et al. 1978). In addition, toxicity decreased with lower chlorination (e.g., Aroclor 1254 > 1242 > 1016), which is consistent with what has been observed in other species (Eisler 1986; Eisler and Beslisle 1996). Amphibians appeared to be 100- to 1000-fold less sensitive to dioxins than were fish in concentration-matched exposures (Jung and Walker 1997). A 24-hour exposure to 30 µg/L dioxin did not result in reduced hatching or survival in American toad or green frog embryos. However, 3 µg/L dioxin increased mortality in northern leopard frog hatchlings.

Some of the residue data cited above came from field studies that also investigated the effects of PCBs and other contaminants on free-ranging amphibians. Along the Kalamazoo River, Glennemeier and Begnoche (2002) failed to find any correlation between species richness of anurans and sediment PCB concentrations. Despite a positive correlation between tissue and sediment concentrations of PCBs in Paducah, Kentucky, no adverse effects in amphibians could be attributed to the contaminants (DeGarady and Halbrook 2003). The Fox River study (Karasov et al. 2005) provided a stronger test on the effects of PCBs on amphibians because of the in situ experiment. However, since heavy metal concentrations correlated with those of PCBs, effects due to PCBs alone could not be discerned. Hatching success of northern leopard frogs and green frogs was negatively correlated with the index of sediment contamination. Among surviving tadpoles, neither growth nor developmental rates were correlated to the contaminant index.

Several studies have examined the effects of PCBs on amphibians under laboratory conditions. African clawed frogs (*Xenopus laevis*) and common frogs were fed diets with no PCBs (control), 0.2 mg/kg PCB 126, or a mixture of PCB congeners (Clophen A50) at 2 and 200 mg/kg; exposures lasted from shortly after hatching until at least 75% of the animals had metamorphosed (76 and 51 days, respectively; Gutleb et al. 2000). Mortality of common frogs in the chronic test was control = 0%, group exposed to PCB 126 alone = 20%, 2 mg/kg Clophen = 33%, and 200 mg/kg Clophen = 47%. The higher concentration of Clophen also delayed metamorphosis compared to other treatments. Body burdens of PCBs in common frogs and *X. laevis* increased with increasing PCB concentrations. Common frog metamorphs had mean lipid-corrected total PCB concentrations of 5.4, 12.0, and 560 mg/kg for the 0.2 mg/kg PCB 126, 2 mg/kg Clophen, and 200 mg/kg Clophen treatments, respectively. The resulting bioconcentration factors were 27, 6, and 2.8, respectively. In *X. laevis,* but not common frogs, higher frequencies of malformations were found in PCB-exposed larvae. These malformations included bent tails, missing eyes, edema, and depigmentation of the skin and were most common in the 0.2 mg/kg PCB 126 treatment group. The concentrations of PCBs used

in this study appear to be higher than those found in plankton (0.2 to 1.0 µg/kg) on which tadpoles feed but fall within the values of highly contaminated sediments (200 mg/kg; Eisler 2000a).

The study along the Kalamazoo River (Glennemeier and Begnoche 2002) also investigated the effects of PCB-laden sediments on northern and southern (*R. sphenocephala*) leopard frogs in the laboratory. For northern leopard frogs, they spiked food with either 100 or 1000 µg/kg PCB 77, a low-chlorinated PCB congener. For southern leopard frogs, they used diets ranging from 0.01 to 100 µg/kg PCB 77. Analysis of whole body northern leopard frog tadpoles exposed to 100 µg/kg PCB 77 for a month revealed a bioconcentration factor of 2.1. For northern leopard frogs, control mortality was 60% (which is higher than the 20% normally considered as acceptable for controls), but for 100 and 1000 µg/kg PCB 77 it increased to approximately 80%. In southern leopard frogs, control mortality was 10% but increased to approximately 70% in tadpoles exposed to 1.0 µg/kg PCB 77 and 100% in those exposed to 10 and 100 µg/kg PCB 77. Growth was inhibited in both species. Glennemeier and Denver (2001) reported that chronic exposure of northern leopard frog tadpoles to PCB 77 (0 to 1000 µg/kg mixed into food) resulted in decreased activity, reduced competitive performance, and reduced corticosterone concentrations. Competitive performance was determined by placing northern leopard frog and wood frog (*R. sylvatica*) tadpoles in the same tanks and feeding them either control or PCB 77-contaminated food. When given control food, paired northern leopard frogs grew almost twice as rapidly as unpaired individuals. With PCB 77, however, no difference was observed between paired and unpaired tadpoles. Interestingly, wood frog tadpoles displayed even more dramatic effects; while growth was slower in unpaired animals fed control food than in paired animals, unpaired tadpoles given PCB 77-dosed food grew twice as rapidly as paired animals, thus showing a very significant interaction.

In another study, wood frog tadpoles were exposed to sediments from a PCB-contaminated wetland next to the St. Lawrence River in New York. The investigators placed sediment containing 325 µg/g of low-chlorinated congeners into half of a tank. The other half of the tank was separated by a porous wall so that water but not sediment flowed between the 2 halves. Controls were exposed to noncontaminated sediment. Nearly 70% of the tadpoles that were exposed to contaminated sediments died, whereas those exposed to contaminated water only had 15% mortality and less than 5% of the controls died. These results indicate that sediment exposure, while toxic, was not required for lethality. General activity levels decreased significantly in PCB-exposed tadpoles compared to controls. Tadpoles exposed to PCB water only had 25 to 27% of the PCB body burdens as those in contact with sediments. Body burdens in sediment-contact tadpoles were approximately 39% of the sediment concentrations.

Aroclors 1242 and 1254 at 10 µg/L increased the frequency of abnormal testes in *X. laevis* from no abnormalities in controls to a 50% frequency of occurrence. There was also a greater frequency of forelimb malformations that could have impaired males from successfully copulating (Qin et al. 2005).

Another test of reproductive effects by PCBs was provided by Mikkelsen and Jenssen (2006). Instead of using larval amphibians, the authors injected Aroclor 1254 subcutaneously into adult common frogs. Concentrations ranged from 0.01 to 100 mg PCB/kg body mass. After 14 days the frogs were euthanized and sampled for tissue, blood, and mensural characteristics. Lipid-based concentrations in livers were positively correlated with dose. No significant differences in serum testosterone concentrations due to PCB dose were reported, but all treatments had reduced testosterone levels, with those at 1 mg PCB/kg body mass having a mean testosterone concentration less than half of that of controls.

Some studies have investigated the toxicokinetics of PCBs and dioxins in amphibians, including uptake, transformation, and depuration. In 1 study (Huang and Karasov 2000), crickets were injected with ^{14}C radiolabeled PCB 126 to derive a dose of 0.35 or 5 mg PCB 126/kg body mass of adult northern leopard frogs. Frogs were bled, euthanized, and necropsied at periodic intervals up to 226 days postdose. By 48 hours, frogs had assimilated between 85 and 90% of the available PCB 126. PCB 126 concentrations remained comparatively stable in fat bodies through the entire period, although the amount of fat diminished. After an initial spike in carcass, muscle, skin, and

liver, concentrations decreased within the first 20 days. In females up to 23% of the ^{14}C-derived radioactivity was deposited into eggs. By 226 days over 60% of the initial PCB 126 dose was still present in the frogs. Depuration of PCB 126 occurred through feces and shed skin. No mortality, lesions, or dramatic weight loss due to PCB 126 was observed.

As cited above, various studies have exposed amphibians to PCBs via the diet, by either making a slurry of food and PCBs for tadpoles or spiking insects with PCBs and feeding them to adults. Johnson et al. (1999) spiked soil with 67 μg/g dry weight Aroclor 1260. The soil was also spiked with 1000 μg trinitrotoluene (TNT)/kg dry weight. Earthworms (*Lumbricus terrestris*) that had been placed in treated soil for 10 days were fed to tiger salamanders (*Ambystoma tigrinum*) and spotted salamanders (*A. maculatum*) in a 2 × 2 design of untreated and treated soils and food. For tiger salamanders, median dry weight body residues of PCBs were controls = 9 μg/kg, animals in untreated soil and with treated food = 1960 μg/kg, those in treated soil with untreated worms = 550 μg/kg, and those in treated soil and given treated food = 1965 μg/kg. Residue concentrations for both the dermal and the oral routes were statistically different from controls, indicating that both routes were important. However, the oral route led to significantly higher body concentrations than the dermal route.

Coplanar PCBs accumulate in frog tissues more readily than do dioxins and furans. Moreover, the more toxic 2,3,7,8-dioxins and -furans accumulate more readily than other dioxins and furans. Also, Japanese brown frog (*Rana japonica*) adult males tend to have higher concentrations of all of these compounds than do females collected from the same sites (Kadokami et al. 2002). An important cause for the difference between male and female body burdens of PCBs, dioxins, and furans is that females transfer a large proportion of their burdens to their eggs (Kadokami et al. 2004). A substantial amount of chemicals accumulated in the bodies of female frogs can be transferred to their eggs with each spawn.

Although PCBs are hydrophobic, their K_{ow} values range over 4 orders of magnitude. Thus, while PCBs occur in higher concentrations in fat than in other tissues, the rate of uptake and depuration could be expected to be both tissue and congener specific. We could expect, for example, that PCBs with lower K_{ow} values, which also tend to have lower molecular weights and toxicity, would be more water soluble and hence depurated more quickly than congeners that had higher K_{ow}s. The more toxic congeners would therefore be retained until some event, such as metamorphosis, depleted fat reserves and released the PCBs into the organism's system. That the fugacity or activity of PCB congeners changes with developmental stage has been shown by Leney et al. (2006a), who found that congeners with log K_{ow} values greater than 5.85 increased in tissues during metamorphosis. Metamorphic green frogs were determined to have greater MFO activity than either tadpoles or adults (Leney et al. 2006b). Whereas this increased metabolic function may help eliminate PCBs at this critical stage of development, it may also increase the toxicity of certain congeners and make metamorphs the most sensitive life stage in anurans.

9.3 POLYCYCLIC AROMATIC HYDROCARBONS

Polycyclic aromatic hydrocarbons (PAH) are hydrocarbons with 2 to 7 fused benzene rings. They generally have low solubility in water, low melting and high boiling points, and low vapor pressure. PAHs are naturally occurring and can be formed by green plants, fungi, and bacteria. They are also products of organic combustion. Thus, natural sources include forest and grass fires, oil seeps, and volcanoes. Forest and prairie fires release substantial amounts of PAHs, approximately 19 513 metric tons per year (Eisler 2000b). Fossil fuels are rich in PAHs, and their combustion contributes to the worldwide balances of the chemicals. Other anthropogenic sources include industrial and commercial uses of petroleum products, wastewater, and runoff from pavements. About 43 000 metric tons of PAHs are discharged into the atmosphere each year, and another 230 000 tons enter aquatic environments. Of that, anthropogenic sources, including burning related to agriculture, contribute

some 23 850 metric tons (Eisler 2000b). PAH toxicity is expressed as interference with cellular membrane functions and enzyme systems. Metabolic processes often convert parental PAHs into more toxic epoxides and dihydrodiols, which may bind to DNA and cellular proteins. Toxic signs include developmental malformations, tumors, and cancer. Intermediate 4- to 6-ring structures have greater potency than 1- to 3-ring or 7-ring PAHs (Albers 2003). Despite considerable interest on the effects of PAHs on fishes, aquatic mammals, and birds, little research has been conducted with amphibians.

9.3.1 Amphibian Residues

There are few, if any, sources of information available on PAH residues in amphibians under natural conditions, and few from laboratory studies. In a survey of organic contaminants and metals in common frog tadpoles in montane regions of the Hohe Tauern National Park of Austria, fluoranthene concentrations ranged between 19 and 39.5 mg/kg dry weight. No other PAHs were reported (Hofer et al. 2005).

In a laboratory study using newts (*Pleurodeles waltl*), bioconcentrations of benzo[a]pyrene (BaP) were as high as 200 times the exposure concentrations of 0.075 to 0.3 µg/L but declined within a day to 150 times (Grinfeld et al. 1986). Similar rapid depuration was seen in newts that had been injected with 100 µg/g BaP (Marty et al. 1989).

Garrigues et al. (2004) studied the toxicokinetics of several PAHs in *P. waltl.* Anthracene, phenanthrene, pyrene, and BaP were ^{14}C-radiolabeled and mixed into sediments at concentrations ranging from 2.26 to 3.57 ng/kg, well below those found in many contaminated sites. The rates of assimilation and depuration by the newts were generally inversely related to the K_{ow} of each PAH. It appeared that whole body concentrations at 24 hours were approximately 2200, 1450, and 155 µg/kg wet weight for phenanthrene, pyrene, and BaP, respectively. After 10 days the concentrations had declined to about 600, 250, and 75 µg/kg, respectively.

9.3.2 Effects of PAHs

Based on several studies that have been conducted on the effects of oil and the PAHs it contains on amphibians, embryos may be relatively insensitive to these chemicals. Used crankcase oil at 100 mg/L did not impair hatching success in green treefrogs, but it did reduce rates of growth and metamorphosis in larvae at concentrations of 55 mg/L or below (Fernandez and l'Haridon 1994). Bullfrog tadpoles became bloated, lethargic, and floated on the surface of water that had been sprayed with Bunker C, No. 6 fuel oil; older tadpoles were more sensitive than younger ones (McGrath and Alexander 1979). In 10-week-old tiger salamander larvae, the 24-hour LC50 for used motor oil was 31.63 ml/L (Lefcort et al. 1997). Water-soluble fractions of oil appear to be much less toxic than emulsions or floating layers. For wood frogs, the 96-hour LC50 value for floating used crankcase oil was 1.5 ml/L, but for an emulsion of No. 2 fuel oil, it was 0.026 ml/L, and for a water-soluble fraction it was 413 ml/L (Hedtke and Puglisi 1982).

Many PAHs such as BaP are highly genotoxic and carcinogenic. One protocol for evaluating the genotoxicity of a PAH or other organic contaminant counts the number of micronucleated (MN) cells per 1000 erythrocytes (Grinfeld et al. 1986). BaP can induce MN cells at concentrations as low as 0.01 mg/kg. Exposure to ultraviolet radiation can increase genotoxicity 100-fold. Under subdued natural daylight with no UV-A component, the concentration of benzo[a]anthracene necessary to induce MN in *Pleurodeles waltl* was 187.5 µg/L, but, with simultaneous exposure to UV-A, only 3.12 µg/L was necessary to have the same effect (Fernandez and l'Haridon 1992). Compared to irradiation of the water only, toxicity of benzo[a]anthracene increased when both tadpoles and water were irradiated. Similarly, when newts were exposed to 500 µg/L BaP under fluorescent lighting no mortality occurred. When they were exposed to both BaP and full daylight, all the animals died within 10 days. Total mortality occurred at 12.5 µg/L BaP presented with

UV-A irradiation (Fernandez and l'Haridon 1994). The authors also stated that the jelly coats around embryos provided some protection against BaP. A more recent study substantiated the protective function of the jelly coat (Marquis et al. 2006). Significantly greater mortality occurred to embryos that had their jelly coats removed and exposed to 3 different PAHs than those that had intact coats.

Huang et al. (2003) developed a quantitative structure-activity relationship (QSAR) model for benzene. They quantitatively determined the 12-hour LC50 for 51 benzene derivatives to predict their toxicity in Japanese frog tadpoles to other derivatives based on molecular structure. The LC50 values ranged from 1.6 to 3.0 mol/L. Different relationships with hydrophobicity could be developed for halogen substituted, alkylated, and nitroaromatic forms of benzene. Factors that reflected hydrophobic and electrophillic properties of the molecules provided a robust QSAR.

A potentially important source of PAHs in urban and suburban environments is runoff from paved surfaces such as roads, parking lots, and driveways. These surfaces are coated regularly with either coal tar or asphalt sealants (Mahler et al. 2005), which have high concentrations of PAHs and, along with oil, ethylene glycol, gasoline, and tire particles, can be washed into waterways. This may pose a particular problem for the endangered Barton Springs salamander (*Eurycea sosorum*), whose habitat is surrounded by parking lots within the city limits of Austin, Texas (Mahler et al. 2005). Compared to asphalt sealants, coal tar sealants have high concentrations of PAHs (9500 to 83 000 mg/kg sealant vs. 110 to 2000 mg/kg) with runoff concentrations as high as 9000 mg/kg vs. 770 mg/kg for asphalt sealants (Mahler et al. 2005). Nominal concentrations of 3, 30, and 300 mg/L flaked coal tar sealants were placed in chambers containing *Xenopus laevis* embryos. By 10 days of exposure all the larvae in the 300 mg/L treatment had died, and by 14 days there was a significant reduction in developmental and growth rates among treatments.

9.3.3 Phenol-Derived Chemicals

Phenol-derived chemicals are a group of monocyclic organic molecules that are chemically related to PAHs, come from many of the same sources, are highly toxic, and can be potent endocrine disruptors. These are chemicals that have a single phenol ring that may or may not be connected to an aliphatic chain. Important chemicals in this group include phenol, nonylphenol, and octylphenol.

Phenol is a single 6-carbon organic ring with a single hydroxyl group attached. It is a common industrial chemical used in making phenolic resins for strand board production, panels, insulation, paints, lubricants, creams, adhesives, brake components, and electrical components. Various industrial sources include pulp and paper mills, metal products, petroleum refining, and municipal wastes (Breton et al. 2003). The toxicity of phenol to aquatic organisms varies with pH, temperature, and water hardness. Phenol is more toxic in acidic conditions than at pH 6.5 to 8 and in hard water than in soft; its effects generally increase with water temperature, possibly due to increased metabolism. The 5 -to 9-day LC50s for amphibian larvae ranged from 0.04 to 11.2 mg/L (Birge et al. 1980). Early hatchling stage appeared to be more sensitive than later larval stages or embryos in northern leopard frogs. Rainbow trout and northern leopard frogs were found to be at least 2 orders of magnitude more sensitive to phenol than many aquatic invertebrates (Breton et al. 2003).

When phenol is alkylated it combines with a hydrocarbon chain, and the name of the resulting structure defines the number of carbons in the alkyl group; for example, nonylphenol has 9 carbons in the alkyl group and octylphenol has 8. These molecules also have wide uses in industry, particularly as detergents for various purposes. They are relatively stable and widespread and often occur at concentrations exceeding several parts parts per billion (USEPA 2005). They can be ethoxylated by bonding with ethylene oxide and increase in water solubility. These molecules may be used as surfactants in pesticide formulations. Nonylphenol, tert-octylphenol, and nonylphenol ethoxylate (NPE) have potent estrogenic effects. The endocrine-disrupting effects of nonylphenol are of sufficient importance to prompt the European Union to enact legislation banning its production and use. Instead, the safer (from an endocrine-disrupting effect) alcohol ethoxylate (AE) has been substituted in surfactants and detergents.

The acute toxicities of NPE and AE were tested on early larval stages of 4 species of Australian anurans, *Xenopus laevis,* and the cane toad in a 48-hour exposure (Mann and Bidwell 2001). Rather than determining lethal concentrations, however, the authors looked at the concentrations necessary to produce mild or full narcosis, defined by decreased activity and reduced reaction to external stimuli. Effective concentrations (EC50s) ranged from 1.1 to 2.9 mg/L for mild narcosis and 2.3 to 12.1 mg/L for full narcosis with 2 formulations of NPE. For AE mild narcosis occurred at 5.3 to 11.0 mg/L and full narcosis at 6 to 25.4 mg/L. Low dissolved oxygen at 30 °C increased the narcotic effect of both ethoxylates. Given enough time (≥72 hours), some of the individuals recovered from the narcosis.

In vertebrates testosterone is a precursor molecule found in both sexes. In healthy females and in animals exposed to estrogenic compounds, such as 17β-estradiol, testosterone is converted to estrogen via mediation of the enzyme aromatase. Some endocrine-disrupting chemicals block the activity of aromatase and are antiestrogenic; exposure to these chemicals may result in masculinized females. Other chemicals stimulate the activity or mimic the functionality of aromatase and produce feminized males. Evidence of endocrine disruption includes production of vitellogenin by genetic males, abnormalities in gonads and secondary sexual characters, and sex reversals. Unfortunately, sex determination in many species of amphibians is poorly known; some are predetermined by genetic constitution, others display various forms of environmentally mediated sex determination. This variation in normal sex determination complicates investigations of endocrine disruption. MacKenzie et al. (2003) compared the potency of NPE to that of known estrogens and antiestrogens. At a concentration of 10 µg/L NPE the sex ratio was 1:1, but 30% of the treated northern leopard frog metamorphs were classified as intersexes — that is, their gonads displayed characteristics of both testes and ovaries. This compared to 5% of control animals being classified as intersexes. At 100 µg/L NPE the F:M ratio was 10:7 (not statistically significant) and 26% of the animals were classified as intersexes. In comparison, 1 µg/L estradiol resulted in 28% of the animals as intersexes. However, at 10, 50, and 100 µg/L 90 to 100% of estradiol-exposed animals were classified as females. Similarly, ethinylestradiol, a synthetic estrogen, caused 25 and 30% intersex rates at 1 and 10 µg/L, respectively, and skewed the sex ratio in favor of females. Wood frogs appeared more resistant to the effects of either ethinylestradiol or NPE than did northern leopard frogs. Control animals showed a 3% rate of intersexes; 10 and 100 µg/L NPE resulted in 13 to 14% intersexes with no skew in sex ratios; and ethinylestradiol did not result in skewed sex ratios and less than 7% intersexes.

In other studies, nonylphenol has been suggested to increase vitellogenin concentrations in male edible frogs (*Rana esculenta*; Kloas et al. 1999; Mosconi et al. 2002), to have direct effects on the pituitary, and to reduce follicle stimulating hormone (FSH) and luteinizing hormone (LH). At 30 days of exposure in black-spotted pond frogs (*Rana nigromaculatus*) 2, 20, and 200 µg/L nonylphenol appeared to stimulate the production of testosterone, but by 45 days of exposure this effect disappeared (Yang et al. 2005).

In addition to having an estrogenic effect, nonylphenol also affects metamorphosis, a process largely controlled by the thyroid. Mid-development (Gosner stages 35 to 37; Gosner 1960) bullfrog larvae were exposed to nonylphenol concentrations of 234, 468, and 936 µg/L with and without the thyroid hormone triiodothyronine (T3) for 7 days. Normal development under control conditions was characterized by reduction in tail length and width, alternations in cranial structure leading to development of jaws and other features, and hind limb formation. Animals exposed to T3 only showed these developmental features, but in an asychronized and variable fashion. Tadpoles presented with the highest concentration of nonylphenol had longer tails, accelerated leg development, and reduction of cranial changes compared to controls at comparable ages. These changes did not occur when T3 was presented along with nonylphenol. The overall effect seemed to be that nonylphenol inhibited endogenous T3 activity and retarded development, but that nonylphenol was not sufficient to retard leg development at sublethal concentrations. The concentrations of nonylphenol used in this study, however, were higher than values typically seen in the environment. Yang et al.

(2005) demonstrated that nonylphenol concentrations as low as 2 µg/L could inhibit total thyroxine levels in black-spotted pond frog tadpoles after 30 days of exposure.

In addition to having endocrine-disrupting effects, nonylphenol can be highly toxic to some species. The 96-hour LC50 for southern leopard frogs was 0.34 µg/L (Bridges et al. 2002) and 0.12 µg/L in boreal toads (*Bufo boreas*; USEPA 1999). For southern leopard frogs, comparable LC50s were 8.4 mg/L for carbaryl, 0.23 mg/L for copper, and 18.2 µg/L for permethrin (Bridges et al. 2002).

Octylphenol is also a potent endocrine disruptor. For example, a 24-hour exposure to 10^{-9} M octylphenol, an environmentally relevant concentration, accelerated gonadal development in male and female bullfrog tadpoles (Mayer et al. 2003). In addition to endocrine-disrupting effects, 4-tert-octylphenol can be lethally toxic. In northern leopard frogs and wood frogs, the LC50s for Gosner stage 26 tadpoles were 1.36 µM/L for octylphenol, 3.01 µM/L for ethinylestradiol, and 5.57 µM/L for estradiol. Wood frog tadpoles were almost twice as sensitive as northern leopard frogs (Hogan et al. 2006). At 500 µg/L, octylphenol delayed hatching in streamside salamander embryos. A 37-day exposure to 500 µg/L octylphenol reduced growth rates, increased the prevalence of limb deformities, and reduced larval activity (Rohr et al. 2004).

Bisphenol A, a compound of 2 phenolic rings connected to a molecule of acetone, has been known as a potent estrogenic compound for over 50 years. It is used in epoxy resins and polycarbonates and as a stabilizing antioxidant in food can coatings, plastic products, and dental sealants. *Xenopus laevis* tadpoles were exposed to 17β-estradiol or bisphenol A at 10^{-7} and 10^{-8} M concentrations until they metamorphosed. Both concentrations of 17β-estradiol resulted in statistically higher frequencies of phenotypic females than males, compared to controls, as did the 10^{-7} M concentration of bisphenol A. In another trial, tadpoles were exposed to 10^{-6}, 10^{-7}, and 10^{-8} M bisphenol A, and only the 10^{-7} concentration resulted in a significantly greater frequency of females. Such biphasic response curves can be explained in that while a critical concentration threshold has to be reached, higher concentrations may induce functions that metabolize or excrete the chemical; hormonal effects may be observed between these concentrations.

9.4 ORGANOCHLORINE PESTICIDES

Organochlorine pesticides (OCs) are a group of organic chemicals with 1 or more chlorine atoms and sometimes other atoms attached to a hydrocarbon base. Blus (2003) distinguished 5 classes of OCs, including DDT and its derivatives; cyclodienes such as endosulfan, chlordane, and dieldrin; hexachlorocyclohexane (HCH), like lindane; toxaphene and related chemicals; and mirex/chlorodecone. Whereas these groups differ among and within themselves in toxicity and sublethal effects, they share common characteristics of lipophilicity, persistence, and bioaccumulation. Lipophilicity means that the chemicals are much more soluble in lipids than in water. Thus, they often accumulate in fatty tissues during times of food abundance and are released when body stores are used, such as during metamorphosis. Many of the molecules, including DDT and its derivatives DDE and DDD, are very stable and may persist in the environment for decades. Others, including endosulfan, heptachlor, and aldrin, have shorter half-lives of weeks or months. In some instances, breakdown products are more toxic than parent compounds; such is the case with DDE, dieldrin, and 12-ketoendrin. Organochlorine pesticides often bioaccumulate, making tissue concentrations higher than those found in the environment. They also tend to bioconcentrate in that residues are passed from one trophic level to another. It is not unusual to find concentrations at a trophic level to be 30- or 100-fold greater than in the level below it. As a result, lethal concentrations may be expressed at the top levels of food webs. These molecules are readily absorbed dermally, which is probably the primary mode of exposure to amphibians. Many OCs are neurotoxins in that they interfere with ion transport across the neurolemma or cell membrane of the neuron. Other toxic effects include cancer, activation of enzymes, and endocrine disruption. DDT was the first organochlorine contaminant found to have estrogenic properties.

9.4.1 Residues in Amphibians

Because organochlorine pesticides are toxic, very persistent, and subject to biomagnification, considerable research on residues in the environment and in aquatic organisms, including amphibians, was available for the first edition of this book. Table 9.2 summarizes information prior to and since 1999 on residue concentrations from field-captured amphibians.

There are several salient characteristics about organochlorine pesticides available from the older literature. Body concentrations are usually higher than found in surrounding waters. Biological concentration factors (BCFs) in the laboratory, where exposure concentrations can be carefully documented, range from less than 100 for toxaphene and endrin (Hall and Swineford 1980) to well over 600 for DDT (Cooke 1972; Licht 1976b). BCFs are a function of age and body size; older, larger larvae or adults have higher concentrations of most OCs than do younger or smaller animals. OCs may especially concentrate in livers (Licht 1976a) and fat (Kirk 1988). Uptake of OCs, especially DDT, can be much faster than depuration. Licht (1976b) found that liver concentrations of DDT in wood frogs exposed to 3 μg/kg could be 1000 times greater than in controls within 7 hours. The jelly coat surrounding eggs, however, can be an effective barrier against DDT (Licht 1985). Finally, free-ranging amphibians frequently contain multiple OCs (Russell et al. 1995; Bonin et al. 1995; Gendron et al. 1997).

As mentioned before, populations of several species of amphibians in California are experiencing severe declines and have been extirpated from many areas that were part of their historical distribution (Fellers and Drost 1993; Drost and Fellers 1996; Davidson et al. 2001). We found that 86% of the Pacific treefrogs sampled from sites just west of Lake Tahoe had detectable levels of endosulfan or its degradate, endosulfan sulfate (Sparling et al. 2001). Subsequently, it was found that Pacific treefrog tadpoles had whole body toxaphene concentrations ranging from 1.57 to 243.75 μg/kg (Angermann et al. 2002). The site with the highest toxaphene concentration was near a large metropolitan area, whereas the other sites were in more natural habitats. The next highest concentration was 35.28 μg/kg from Sequoia/Kings Canyon National Parks. Like PCBs, the concentration of toxaphene decreased at higher elevations, and it was lower on the eastern-facing slopes of the Sierra Nevada Mountains that were not directly exposed to the westerly winds coming from agricultural areas in California than on western-facing slopes. Measurable concentrations of DDE, γ-chlordane, trans-nonachlor, HCH, and endosulfan were obtained in mountain yellow-legged frogs (*Rana muscosa*) collected from 2 ponds within Sequoia/Kings Canyon National Parks (Fellers et al. 2004), and higher concentrations of these and other pesticides were found at a site that historically had a substantial population of this species. This site is directly exposed to prevailing westerly winds and has not been able to maintain a population of mountain yellow-legged frogs despite efforts to reintroduce the species.

Further north in British Columbia, organochlorine pesticides, including DDT, DDE, DDD, cis-nonachlor, and trans-nonachlor, were found in eggs of the red-legged frog (*Rana aurora*) and northwestern salamanders (*Ambystoma gracile*), but at concentrations below what is believed to be harmful (de Solla et al. 2002). The authors did not speculate if the pesticides were absorbed from the environment or were transferred from their mothers. Maternal transfer has been shown to be a significant factor in the concentration of persistent organic pollutants in amphibians (Kadokami et al. 2004).

9.4.2 Effects of Organochlorine Pesticides

Along with information on residues, several previous studies have examined the effects of OCs on amphibians. Median lethal 96-hour tolerances (LT50s) were in the 0.1 to 0.3 mg/L range for endrin, toxaphene, dieldrin, aldrin, and DDT for both Fowler's toads and western chorus frogs (*Pseudacris triserata*; Sanders 1970). Age may be a factor in sensitivity. Embryos appear to be less sensitive than larvae, probably because the jelly coat around embryos provides protection. Older tadpoles may be

TABLE 9.2
Concentrations (mg/kg Wet Weight) and Biological Concentration Factors of Organochlorine Pesticides in Amphibians Collected in the Field

Species	Age[a]	Compound	Tissue	Concentration[b]	Comments	Reference
Pseudacris crucifer	A	α-HCH	Whole	0.37	Field samples collected from area sprayed with DDT 26 years earlier; concentrations are for lipid	Russell et al. 1995
	A	β-HCH	Whole	1.37	As above	As above
	A	γ-HCH	Whole	<DL	As above	
	A	Oxychlordane	Whole	1.74		As above
	A	trans-Chlordane	Whole	0.11		As above
	A	cis-Chlordane	Whole	0.08		As above
	A	trans-Nonachlor	Whole	0.73		As above
Pseudacris crucifer	A	Heptachlor	Whole	1.98	As above	As above
	A	Dieldrin	Whole	199.8	As above	As above
	A	p,p-DDT	Whole	160.6	As above	As above
	A	p,p-DDE	Whole	1001	As above	As above
	A	p,p-DDD	Whole	26.5	As above	As above
Rana clamitans	A	p,p-DDE	Whole	0.58–45.0	Range from 7 field sites in southern Ontario	Russell et al. 1997
		HCB		0.08–0.49		As above
		trans-Nonachlor		0.02–0.72		As above
Rana perezi	A	Total DDE	Muscle	<DL–190	National park in Spain over 3 years	Rico et al. 1987
	A	Total DDT	Muscle	50–550	As above	As above
	A	γ-HCH	Muscle	<DL–10	As above	As above
Rana pretiosa	A	p,p-DDD	Whole	166–403	Collected from forest sprayed with 0.6–0.71 kg DDT/ha in fuel oil, 6 live animals, 3 weeks postapplication	Kirk 1988
	A	p,p-DDE	Whole	91–173	As above	As above
	A	p,p-DDT	Whole	563–1750	As above	As above
	A	p,p-DDD	Lipid	16 600–30 500	As above	As above
	A	p,p-DDE	Lipid	9600–10 000	As above	As above
Rana pretiosa	A	p,p-DDT	Lipid	56 300–132 000	As above	As above
	A	p,p-DDD	Whole	1920–6670	20 dead animals	As above

TABLE 9.2 (CONTINUED)
Concentrations (mg/kg Wet Weight) and Biological Concentration Factors of Organochlorine Pesticides in Amphibians Collected in the Field

Species	Age[a]	Compound	Tissue	Concentration[b]	Comments	Reference
	A	p,p-DDE	Whole	96–366	As above	As above
	A	p,p-DDT	Whole	122–5670	As above	As above
	A	p,p-DDD	Lipid	15 500–487 000	As above	As above
	A	p,p-DDE	Lipid	7740–26 700	As above	As above
	A	p,p-DDT	Lipid	13 200–413 000	As above	Kirk 1988
Rana ridibunda	A	Total HCH	Liver	61–2636	As above	Vojinovic-Miloradov et al. 1996
	A	p,p-DDE	Liver	1.13–13.1	As above	As above
	A	p,p-DDT	Liver	<DL–10.3	As above	As above
Necturus maculosus	A	HCB	Female gonads	2.2–14.7	Field collected from St. Lawrence River	Gendron et al. 1997
	A	Octachlor-styrene	As above	1.6–28.1	As above	As above
	A	Nonachlor	As above	5.1–48.4	As above	As above
	A	Chlordane	As above	19.3–54.9	As above	As above
	A	Oxychlordane	As above	1.7–9.4	As above	As above
	A	Dieldren	As above	5.6–20.4	As above	As above
	A	p,p-DT	As above	<DL–1.5	As above	As above
	A	p,p-DE	As above	81.2–1659	As above	As above
	A	p,p-DDD	As above	14.5–22.4	As above	As above
	A	HCB	As above	1.9–6.0	Field collected from Ottawa River	As above
	A	Octachlor-styrene	As above	<DL–1.7	As above	As above
	A	Nonachlor	As above	16.4–70.2	As above	As above
	A	Chlordane	As above	2.1–24.9	As above	As above
	A	Oxychlordane	As above	2.0–6.1	As above	As above
	A	Dieldrin	As above	3.4–16.6	As above	As above
	A	p,p-DDT	As above	0.1–13.8	As above	As above
	A	p,p-DDE	As above	41.5–488	As above	As above
	A	p,p-DDD	As above	4.9–98.4	As above	As above
	A	HCB	Whole	0.2–4.3	St. Lawrence and Ottawa Rivers	Bonin et al. 1995
	A	DDD	As above	1.2–85	As above	As above
	A	DDE	As above	0.3–90.0	As above	As above
	A	DDT	As above	<DL–8.3	As above	As above
	A	Chlordane	As above	<DL–87	As above	As above
	A	Total HCH	As above	<DL–10.1	As above	As above
	A	trans-Nonachlor	As above	1.5–61	As above	As above

(*continued*)

TABLE 9.2 (CONTINUED)
Concentrations (mg/kg Wet Weight) and Biological Concentration Factors of Organochlorine Pesticides in Amphibians Collected in the Field

Species	Age[a]	Compound	Tissue	Concentration[b]	Comments	Reference
	A	Dieldrin	As above	<DL–94	As above	As above
	A	DDE	Gonads	86.7–195.6	As above	As above
	A	DDE	Liver	136–330	As above	As above
Necturus lewisi	A	DDE	Whole body less GI tract	60	Collected in field from North Carolina	Hall et al. 1985
	A	DDD	As above	40	As above	As above
	A	Dieldrin	As above	20	As above	As above
	A	cis-Chlordane	As above	20	As above	As above
	A	trans-Nonachlor	As above	40	As above	As above
R. perezi	L	DDT + DDD + DDE	Whole	51.1[b]	Collected from rice fields in Spain	Pastor et al. 2004
	L	α- and γ-HCH	As above	2.6[b]	As above	As above
	L	HCB	As above	2.9[b]	As above	As above
	L	Octlychloro-styrene	As above	1.6[b]	As above	As above
	A	DDT + DDD + DDE	As above	35.4[b]	As above	As above
	A	α- and γ-HCH	As above	0.5[b]	As above	As above
	A	HCB	As above	2.7[b]	As above	As above
	A	Octylchlorostyrene	As above	2.4[b]	As above	As above
	L	DDT + DDD + DDE	Lipid	462	As above	As above
	L	α- and γ-HCH	Lipid	23.3	As above	As above
	L	HCB	Lipid	26.4	As above	As above
	L	Octylchlorostyrene	Lipid	14.1	As above	As above
	A	DDT + DDD + DDE	Lipid	401	As above	As above
	A	α- and γ-HCH	Lipid	5.8	As above	As above
	A	HCB	Lipid	30.7	As above	As above
	A	Octylchlorostyrene	Lipid	27.4	As above	As above
R. temporaria	L	p,p-DDE	Carcass	<DL–5.5[b]	Collected from high elevations in the Hohe Tauren National Park, Austria	Hofer et al. 2005
	L	p,p-DDE	Intestine	<DL–1.4	As above	As above
	L	p,p-DDD	Carcass	<DL–25.8	As above	As above
	L	p,p-DDD	Intestine	<DL–9	As above	As above
	L	p,p-DDT	Carcass	<DL–1.7	As above	As above
	L	p,p-DDT	Intestine	<DL	As above	As above
	L	Lindane	Carcass	2.6–5.3	As above	
	L	Lindane	Intestine	2.5–7.0		

TABLE 9.2 (CONTINUED)
Concentrations (mg/kg Wet Weight) and Biological Concentration Factors of Organochlorine Pesticides in Amphibians Collected in the Field

Species	Age[a]	Compound	Tissue	Concentration[b]	Comments	Reference
R. clamitans	A	Total HCH	Whole	0.12	Collected from 7 sites in SW Michigan	Gillilland et al. 2001
	A	Total chlordane	Whole	0.05	As above	As above
	A	Total DDTs	Whole	1.24	As above	As above
	J	Total HCH	Whole	0.04	As above	As above
	J	Total chlordane	Whole	<DL	As above	As above
	J	Total DDTs	Whole	0.10	As above	As above
	L	γ-HCH	Whole	0.33	As above	As above
	L	Total chlordane	Whole	0.01–0.14	As above	As above
	L	Total DDTs	Whole	0.37	As above	As above
	E	Total HCH	Whole	0.34	As above	As above
	E	Total chlordane	Whole	0.29	As above	As above
	E	Total DDTs	Whole	7.91	As above	As above
Hyla regilla	L	Toxaphene	Whole	1.47–15.62	Collected from 21 sites in the Sierra Nevada Mountains of California	Angermann et al. 2002
R. aurora	E	Pentachlorobenze	Whole	52.6–83.3	Collected from 2 sites in British Columbia	de Solla et al. 2002
	E	HCB	Whole	52.6–83.3	As above	
	E	p,p-DDE	Whole	157.9–333.3		As above
A. gracile	E	HCB	Whole	<DL–76.9	Collected from 3 sites in British Columbia	As above
	E	trans-Nonachlor	Whole	<DL–76.9	As above	As above
	E	pp-DDE	Whole	151.5–384.6	As above	As above
					As above	As above
	E	pp-DDD	Whole	30.3–102.6	As above	As above
	E	cis-Nonachlor	Whole	<DL–71.4	As above	As above
R. muscosa	A	α-HCH	Whole	<DL–4.9	Collected from Sequoia National Park, California	Fellers et al. 2004
	J	α-HCH	Whole	<DL	As above	As above
	A	γ-HCH	Whole	<DL–0.70	As above	As above
	J	γ-HCH	Whole	<DL	As above	As above
	A	γ-Chlordane	Whole	<DL–1.2	As above	As above
	J	γ-Chlordane	Whole	<DL–2.8	As above	As above

(*continued*)

TABLE 9.2 (CONTINUED)
Concentrations (mg/kg wet weight) and Biological Concentration Factors of Organochlorine Pesticides in Amphibians Collected in the Field

Species	Age[a]	Compound	Tissue	Concentration[b]	Comments	Reference
	A	trans-Nonachlor	Whole	0.44–2.5	As above	As above
	J	trans-Nonachlor	Whole	<DL–8.1	As above	As above
	A	Endosulfan I	Whole	<DL–1.2	As above	As above
	J	Endosulfan I	Whole	<DL–1.4	As above	As above
	A	p,p-DDE	Whole	17–100	As above	As above
	J	p,p-DDE	Whole	13–51	As above	As above

[a] Age: A = adult, L = larva, J = juvenile, H = hatchling, E = egg, EL = early larva (pre-limb-bud), LL = late larvae (limb bud +).
[b] Water concentration in ppm.

less sensitive than recently hatched larvae (Cooke 1970, 1972). Prolonged exposure to OCs may result in a resistance in that northern cricket frog tadpoles (*Acris crepitans*) collected from a reference site were statistically more sensitive to dieldrin and endrin than were tadpoles collected next to a cotton field where the pesticides were sprayed on a routine basis (Ferguson and Gilbert 1967). It is not known, however, if the differences in sensitivity were physiological acclimations or genetically based. Sublethal effects of DDT and other OCs include weight loss, hyperactivity, decreased alertness, lordosis, scoliosis, deformed rostrums, skin discoloration, abnormal blood cell counts, and hormonal disruption (Kaplan and Overpeck 1964; Cooke 1970; Schwen and Mannering 1982; Gendron et al. 1997).

More recent research has contributed to our understanding of the lethal and especially sublethal effects of OCs. In the past few years emphasis has been on endosulfan, which is one of the few OCs that are still widely used; many have been banned in North America and the European Union. Endosulfan is extremely toxic to foothill yellow-legged frogs (*Rana boylii*), 1 of the species in decline in the California Sierra Nevada Mountains (Sparling and Fellers 2007). The LC50 for chronic (54-day) exposure was 0.33 µg/L, and the estimated lowest observed effects level (LOEL) was 0.003 µg/L; all tadpoles died at concentrations >0.8 µg/L. Until they died, foothill yellow-legged frog tadpoles experienced reduced growth and development at 12.5 µg/L. In comparison, Pacific treefrogs, which have not experienced declines as severe as those seen in foothill yellow-legged frogs, have a chronic LC50 of 3.6 µg/L. Within 2 days of first exposure, tadpoles of both species exposed to 50 or 200 µg/L endosulfan developed acute flexions immediately behind their heads, which caused them to swim in tight circles. Ambient concentrations of endosulfan have been reported within 60% of the LC50 for foothill yellow-legged frogs (McConnell et al. 1998), well above minimally toxic concentrations. In a broad-scale survey of over 20 organic contaminants, only endosulfan significantly discriminated between sites that still had healthy populations of amphibians and those that had experienced sharp declines (Sparling et al. unpublished). Endosulfan is usually present in the environment as 1 of 2 isomers (endosulfan I or II) and as a common degradate, endosulfan sulfate. Endosulfan I appears to be more toxic than II, but together they are more toxic at a given concentration than either compound alone (Wan et al. 2005). Endosulfan sulfate is also toxic to aquatic organisms. Unfortunately, many of the studies that have reported endosulfan concentrations in the field do not include all 3 forms; inclusion of the entire endosulfan concentration would provide a more complete presentation of hazards to amphibians and other biota.

The lethal and sublethal effects of endosulfan have been studied in other species and situations. Larval streamside salamanders experienced increased mortality, reduced growth rates, respiratory distress, limb abnormalities, and altered behavior when exposed to 10 µg/L endosulfan for 37 days (Rohr et al. 2004). The pesticide may also disrupt pheromone production and reproduction. In a very

creative sequence of experiments, a 5 µg/L dose of endosulfan to red-spotted newts (*Notophthalmus viridescens*), while not sufficient to produce effects on survival, growth, or food consumption, reduced the attractiveness of females to males and reduced female mating success. At 10 µg/L, endosulfan glands used in producing and secreting reproductive pheromones had smaller alveoli and lumens than those in controls and elicited weaker electrophysiological responses from male olfactory epithelium (Park et al. 2001). Temperature of incubation and endosulfan interacted to affect predator risk in *Limnodynastes peronii*, an Australian anuran (Broomhall 2004). Twenty-eight-day-old tadpoles that had been incubated as embryos at 20 °C were less susceptible to being caught by an odonate predator than were tadpoles that had been incubated at 14 °C. However, exposure to 0.03 or 1.3 µg/L endosulfan as embryos later made tadpoles more vulnerable, with the greatest difference from controls occurring among tadpoles incubated at 20 °C. The study is particularly interesting in that it suggests that temperature and endosulfan exposure in embryos may have long-lasting effects on vulnerability to predators. One other example of the sublethal effects of endosulfan is that it appears to be genotoxic, based on an increased frequency of micronucleated erythrocytes in *Hyla pulchella* exposed to 5 and 10 µg/L endosulfan for 96 hours (Lajmanovich et al. 2007).

Organochlorine pesticides can affect the immune system of amphibians, making them more susceptible to disease. Northern leopard frogs are subject to parasitism by the nematode *Rhabdias ranae*, which penetrate frog skin and migrate to the lungs. From there they are coughed up and swallowed into the gastrointestinal tract to mature and produce eggs that pass out with feces. Christin et al. (2003) exposed frogs to 3 different concentrations (plus controls) of a chemical cocktail composed of lindane, dieldrin, endosulfan, aldicarb, metribuzin, and atrazine. The first 4 chemicals are OCs, while metribuzin and atrazine are herbicides. Concentrations reflected 0.1×, 1.0×, and 10× the concentrations of each chemical measured from a sample taken from the St. Lawrence River. Half of the frogs were euthanized after 21 days of exposure to test *in vivo* immune responses, and the other half were exposed to *Rhabdias ranae*. The pesticide mixture reduced T-cell proliferation, a key measure of immune response. DDT and dieldrin also increased immunosuppression in northern leopard frogs (Gilbertson et al. 2003; Albert et al. 2007).

DDT and its derivatives have been associated with endocrine disruption. Although estrogenic properties have been confirmed in birds (Verreault et al. 2006), reptiles (Guillette et al. 2000), fish (Baatrup and Junge 2001), and *Xenopus laevis* (Palmer et al. 1997), they have not been well documented in other anurans. However, there seems to be multiple endocrine systems that can be affected by these contaminants. DDE, for example, affected the thyroid/hypothalamic axis in common frogs (Mortensen et al. 2006). Gene expression associated with TSH production was depressed with 3-day DDE exposures at 0.001 and 0.01 µg/L compared to controls. The depressed gene activity was associated with greater growth in body weight, total length, and tail length. DDE (0.01 to 10 mg/kg body mass) also affected liver retinol in a dose-dependent fashion but did not alter estrogen or testosterone concentrations in adult male common frogs (Leiva-Presa and Jenssen 2006). Bone density and growth are affected by a variety of hormones, including growth hormone (GTH). Injections of 1 mg/kg p,p'-DDE negatively affected bone density in common frogs (Lundberg et al. 2007), suggesting that it might have affected the expression of one or more of the hormones involved with calcium metabolism or deposition.

9.5 AMPHIBIANS AS BIOINDICATORS OF ORGANIC CONTAMINATION

When considering whether an organism can serve as a reliable bioindicator of contamination, the following factors should be considered (modified from Sparling 2000):

- The species shows some tolerance to the lethal or reproductive effects of contaminants so that it can co-occur with the contaminants at low to moderate concentrations.
- The species has a propensity to accumulate the contaminant in a positive relation with ambient concentrations.

- The species is sufficiently sedentary so that individuals are sampled from the same area in which they were exposed.
- The species has a sufficiently broad distribution to allow comparisons from different regions.
- The species has either a wide flexibility in habitat preferences, so that several different types of sites can be investigated, or a narrow preference that fosters selective sampling of specific habitats.
- The species is sufficiently common to allow harvesting or manipulation without undue concern for continued population survival — endangered or threatened species are usually not suitable.
- The animal has sufficiently large body size to extract and measure residues or measure physiological changes — technology is making this easier all the time in that many tests can now be conducted with several microliters or subgram amounts of sample.

Some amphibians meet all of these characteristics and make suitable candidates as biomonitors of many organic contaminants; other species may meet only some of the criteria and are less suitable as study animals. Through the careful selection of species based on life histories, habitat preferences, and distributions, investigators can address very specific questions. An important weakness in this regard is that, while we have toxicity data that show that amphibian sensitivity to organic contaminants is generally within the range found for fishes, we are still limited to a relatively few number of species and primarily to larval rather than adult stages. This review, for example, covered 35 species, including 7 caudates, 15 ranids, 5 bufonids, 6 hylids, and a handful of other taxa of the approximately 5400 species in the class Amphibia. Many of these species were tested on 1 or 2 chemicals, and very few have been used across several chemical families. Certain taxa such as the entire order of Gymnophiona (= Apoda) or caecelians, and the suborder Sirenoidea (sirens), are completely absent. Thus, the information base is focused on a relatively few taxonomic groups.

Anurans form a major component of community biomass and occupy multiple trophic levels. Most tadpoles are often considered to be herbivores or detritivores (Duellman and Trueb 1986), whereas adult frogs and toads are carnivores. Most caudates are carnivores throughout their life cycle. Although anuran tadpoles may occupy a lower trophic level than do adults or salamanders, they often live intimately with sediments, and thus may be exposed to contaminants through nondietary routes. Biological concentration factors for many organic contaminants in tadpoles are often greater than 10 and may exceed 600. Thus, residue samples in tadpoles could more reliably indicate presence and biological availability of a contaminant than do ambient concentrations.

Amphibians have considerable interspecific variation in habitats. Some species, such as plethodontid salamanders, are entirely terrestrial. Others, including American toads, Fowler's toads, ambystomids, and some hylids, migrate into wetlands to breed but then return to upland habitats; these integrate upland and wetland habitats at a landscape level. Many ranids breed within aquatic habitats and remain close to the aquatic/terrestrial interface except perhaps to disperse at certain times of the year. Therefore, for most amphibian species, body burdens or physiological responses of larvae would more represent conditions within the aquatic environment, while adults would have to be selected based on their movement patterns to match specific hypotheses.

There are several amphibian species with broad distributions. For example, bullfrogs are ubiquitous in eastern North American waters and have been introduced in many parts of the West, where they are subsequently becoming pest species (Kupferberg 1997; Kats and Ferrer 2003; Knapp 2007). Green frogs, American toads, Fowler's toads, and tiger salamanders are also common in most of the United States and southern Canada. Fortunately, we also have some information about the ecotoxicology of these species. Species of the leopard frog complex are widely distributed, but close relationships do not guarantee identical contaminant tolerances, and correct identification of species is essential. Pickerel frogs (*Rana palustris*) are widespread, but their habitat preferences are restricted and little is known about their response to contaminants. Other species are more restricted in distribution but may be important monitors of the environment on a regional basis.

Habitat selection by amphibians ranges from highly specific to general, depending on species. Breeding habitats may be more important to studies of contaminant ecology because adult amphibians concentrate and larvae are dependent on these areas for days to several months or longer. Bullfrogs and green frogs inhabit streams, semipermanent and permanent ponds, and lakes. Other ranids are more common in shallower wetlands. Some salamanders are closely related to streams, but mudpuppies often inhabit large river systems and lakes. Treefrogs and toads frequently breed in shallow, temporary waters that may be found in fields, woodlots, or forests. By carefully combining habitat preferences with proportion of annual cycle spent in or near wetlands or uplands, biomonitoring activities can potentially be very selective in choosing a species of amphibian for a particular set of objectives.

Because of the concern for declining amphibian populations, it would be irresponsible to recommend the harvesting of any species of amphibian indiscriminately. However, bullfrogs and cane toads have been introduced in parts of the West where they could probably be sampled with less concern for reducing populations than could native species. Because the mortality rate of embryos and tadpoles can be very high (Shoop 1974; Breven 1990), collecting early life stages of amphibians would have less effect on populations than would collecting adults. Monitoring through nonlethal methods would be preferable to harvesting, but few methods other than sampling blood have been developed that avoid direct harvest of individuals. Many tadpoles are approximately the same size as fathead minnows, Japanese medaka (*Oryzias latipes*), and mummichogs (*Fundulus heteroclitus*), fish species that often are used in ecotoxicological studies. Note, however, that tadpoles represent a life stage that is undergoing fairly rapid growth, development, and change, so age or stage of tadpole must be considered. Specific biological tests, including micronucleated erythrocytes in *Pleurodeles waltl* (Jaylet 1971) and the Frog Embryo Teratogenesis Assay–*Xenopus* (ASTM 2004), have been used with considerable success in monitoring organic contaminants.

9.6 RESEARCH NEEDS

There is a great lack of information on the ecotoxicology of amphibians to organic contaminants. It may even be easier to list what we know about amphibians and organic compounds than to list what we do not know (which, of course, is what I have just tried to do). Whereas the amount of information on amphibians and contaminants has more than doubled since 1999, there is still a lot we do not know. Many of the needs espoused in the first edition (Sparling 2000) are still pertinent in this second edition. Therefore, this list of information needs is not intended to be comprehensive, but it may serve as a step in delineating the most relevant and current research needs.

- How effective are amphibians in accumulating PCBs from the environment? What are the ecological sources of variation (e.g., dissolved and nondissolved organic matter, sediment factors) that affect uptake of PCBs? What effect does life stage have on the accumulation and biological concentration factor for the most toxic PCB congeners, dioxins and furans?
- How efficient are tadpoles in accumulating organic molecules such as oil, PAHs, and the host of anthropogenic organic chemicals that are found in aquatic systems for which there are scant data? To date we were unable to find any studies that measured PAHs in field-collected animals.
- What are the sublethal and lethal effects of dioxins and furans to amphibians? In the first edition I included PCBs in this list, but we now have some data on that, although the lethal and sublethal effects of dioxins and furans to larval or adult stages remain largely unknown. Are the toxic equivalency factors for this class similar to those for fishes?
- What is the ecological significance of the observed differences between amphibians and other classes of vertebrates in the responsiveness of P450 MFOs? Are amphibians more or less susceptible to organic contaminants than other vertebrates because of this? Some information on this need has been provided since 1999, but there is still a question of whether we can generalize among vertebrate classes.

- Are micronucleated erythrocytes a common amphibian response to organic toxicants? Can they be used as a monitoring tool with other species? The studies that have been conducted on *Pleurodeles waltl* need to be expanded to other species and chemicals. At least one other species produces micronucleated erythrocytes in response to contaminant exposure (Lajmanovich et al. 2007).
- There is a need for multiple-generation studies using amphibians. What happens to the reproductive capacity of adults that have been exposed to organic contaminants as larvae? What effects do organic contaminants in adults have on hatchability and survival of offspring? Multiple-generation experiments are difficult in most amphibians because of husbandry issues, but initial work might be carried out in *Xenopus laevis* or *X. tropicalis.*
- What are the effects of organic contaminants on population dynamics and distribution of amphibians? This is really the crux of our research needs.
- What effects do combinations of organic contaminants have on amphibians that are not seen with single compounds? Are organic contaminants synergistic, mutually competitive, or independent of each other? Again, some work has been conducted toward answering this question, but there is still considerable need.

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10 Organic Contaminants in Reptiles

Shane R. de Solla

CONTENTS

The purpose of this chapter is to give an overview of the ecotoxicology of organic contaminants to reptiles. Two questions come to mind: What is a reptile, and what are organic contaminants?

Until fairly recently, reptiles formed their own class, Reptilia, which included turtles, snakes, lizards, tuataras, and crocodilians. Reptilia, however, formed a paraphyletic group as it excluded their descendents: mammals and birds. There have been many schemes to replace the traditional evolutionary tree based upon more recent genetic, paleological, morphological, and philosophical reasons. One recent classification scheme replaced class Reptilia by class Sauropsida, with 2 subclasses: Anapsida and Diapsida (Figure 10.1). Note the position of the birds, Aves. Although treating birds as within the same group would dramatically increase the toxicological literature of reptiles (sauropsids?), I am taking the defunct but more recognizable view of Reptilia. In other words, "at best, the cladists suggest, we could say that the traditional Reptila are 'non-avian, non-mammalian amniotes'" (Tudge 2000, p 85). However "reptiles" are defined, evolutionary relationships may be important for predicting or understanding physiological responses of reptiles to toxicological stresses. The behavior of the aryl hydrocarbon (Ah) receptors (Hahn 1998), cytochrome P450 activity (Ertl et al. 1998), affinity of estrogen receptors to selected substrates (Vonier et al. 1996), and other processes are not independent of the constraints of evolutionary history. For example, while

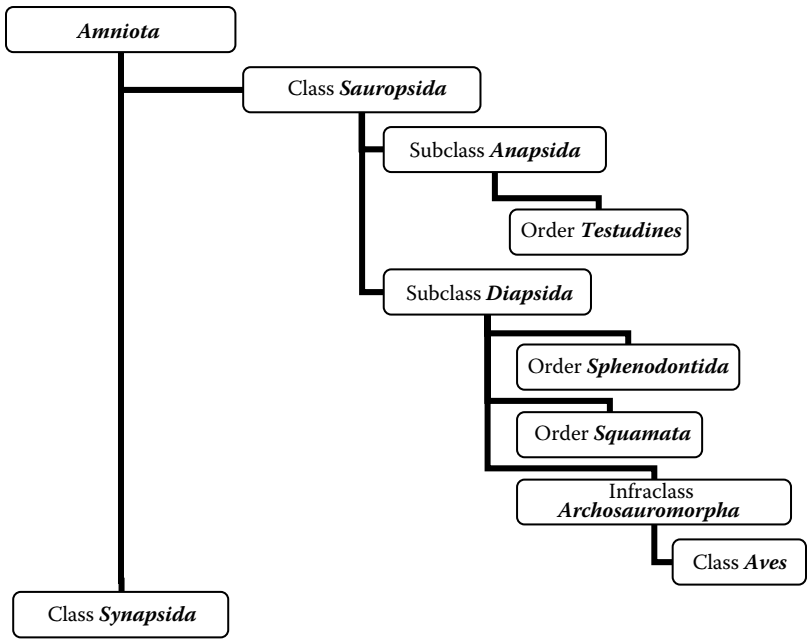


FIGURE 10.1 Cladistic classification of "reptiles" based upon monophyletic groupings (simplified from Benton 2005). Traditionally, the classification of reptiles is paraphyletic, as they excluded their descendents, birds and mammals. Class Synapsida is the outgroup to the extant animals traditionally called reptiles.

mammals have but 1 copy of the Ah receptor, different evolutionary nonmammalian lineages have, through gene duplication and lineage-specific gene loss, developed multiple forms of the Ah receptors (Hahn et al. 2006). Further, sex steroid receptors evolved from ancestral nuclear receptors from the Cambrian, which were initially relatively nonspecific in their ligand recognition (Baker 2001b). Over evolutionary time, the allele sequences of steroid receptors diverged among taxa, as did their specificity for ligands (Baker 2001a). Hydroxysteroid dehydrogenases, which regulate the conversions among forms of steroids, likely have different affinities among taxa for some xenobiotics, which suggests that phylogeny needs to be considered in evaluating endocrine disruption (Baker 2001a). Crocodilians, despite being "reptiles", are more closely related to birds (Figure 10.1) than they are to other sauropsids, and thus it is possible that they may respond more like birds than turtles, for example, to some toxicological stressors. Nevertheless, it will be some time yet before one could successfully publish a paper on crocodilians in the journal *Waterbirds* or a similar outlet.

Organic contaminants are not a well-defined class of compounds. Depending on who you ask, organic contaminants can include caffeine, fecal sterols, and polyethylene glycol. I will restrict this chapter to some of the more "traditional" organic contaminants examined in wildlife toxicology, such as polychlorinated biphenyls (PCBs), organochlorine (OC) pesticides, and polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs), as well as perfluorooctanesulfonate (PFOS), polybrominated diphenyl ethers (PBDEs), polycyclic aromatic hydrocarbons (PAHs), and petroleum products. Unlike the previous edition of this chapter (i.e., Portelli and Bishop 2000), I organized the contents by compound. Furthermore, I emphasized both exposure and fate of these compounds in relation to reptiles.

There are approximately 7370 species or reptiles (McDiarmid and Mitchell 2000), living in a wide range of habitats ranging from deserts to open oceans, and excluding extinct forms, found on every continent except Antarctica. They are also found in habitats that have substantial anthropogenic activity, including urban environments. Organic contaminants are now found in virtually every environment, both geographically and compartmentally; they are found in soil, sediment, water, air, and in the very food that reptiles consume.

Unlike many avian or some fish species, reptiles frequently have fairly small home ranges, and furthermore, they rarely travel more than a few kilometers from their core area in their home ranges; marine turtles are an extraordinary exception. Due to their limited movement, the contaminant burdens of the more sedentary reptiles largely reflect the contamination of their local environment. Although concentrations of organics generally tend to be higher in nonreptilian vertebrates, in some cases reptiles can bioaccumulate concentrations higher than other vertebrates from the same area. For example, high concentrations of DDE residues were found in avian, mammalian, and reptilian species in the Rio Grande and Pecos River drainages (Texas); a whiptail lizard (*Cnemidophorus* sp.) had the highest concentration (104 μg/g, whole body) of any vertebrate measured (White and Krynitsky 1986). PCBs in fat of snapping turtles (*Chelydra serpentina*) reached 3560 μg/g (lipid weight) total PCBs (Olafsson et al. 1983), in the upper Hudson River, New York.

Not only are there frequently observable differences in the concentrations of organic compounds in reptile tissues collected from different sites (e.g., Bishop et al. 1998), but reptiles are often sensitive enough indicators to differentiate regional use of different commercial mixtures (i.e., Aroclors) of PCBs (de Solla et al. 2007). Body burdens of organic contaminants in reptiles are also capable of reflecting localized spills, such as organochlorine pesticides following a spill in Lake Apopka, Florida (Guillette et al. 1999), and crude oil following the Ixtoc I oil spill in Mexico (Hall et al. 1983). As most reptiles are oviparous, they provide convenient tissues with a large lipid component suitable for measuring persistent organic pollutants. Some reptiles have been classified as "excellent sentinels" (Golden and Rattner 2003), largely due to their nonmigratory habits and trophic position.

In the earlier edition of this book, Portelli and Bishop (2000) reviewed the body burdens and effects of environmental contamination in reptiles. Until the 1990s, there were very few reports of contaminants measured in reptiles and even fewer studies on biological effects. Although this is still true, there are now a few locations where geographic and temporal trends in residue concentrations and/or effects are being repetitively sampled. Nevertheless, knowledge on the exposure, fate, metabolism, and toxicity of organic contaminants to sauropsids is woefully inadequate. As an illustration of the lack of data on the toxicology of reptiles, in a recent review on thyroid disruptors, Tan and Zoeller (2007, p 5) stated, "The articles in this issue review the thyroid systems of mammals … fish, amphibians, and birds, and the [methods] used to detect disruption of the thyroid system…. It must be noted that while reptiles represent an enormously important group, they were excluded because there was not enough information in the literature on thyroid toxicology in reptiles at the time that this series of reviews was drafted." This is likely a common theme, although, as this book attests, our knowledge of this field is improving.

10.1 EXPOSURE

Reptiles are potentially exposed to pesticides, agricultural fertilizers, and municipal or industrial effluents, industrial chemicals, petroleum products, and other chemicals, but there are little data to adequately estimate the relative importance of these factors to reptile populations. Reptiles are exposed to organic contaminants through their skin, consumption of contaminated food, eggshells, inhalation, and maternal transfer. Which mode of exposure is most important depends on the substrate that contains the organic compounds, the chemical properties of the organics, the physiological condition of the animal, the trophic feeding level of the exposed animal, and other factors. In all likelihood, there are usually multiple routes of exposure.

Much of the exposure of organic contaminants depends upon their chemical properties, such as solubility, stability in environmental and biological environments, and volatility. Most of the compounds considered in this chapter are moderately or highly lipophilic. After exposure, lipids in tissues are the prime factor controlling the transport, distribution, and storage of lipophilic compounds. Nonlipophilic contaminants tend not to cross cellular membranes except through facilitated or active transport sites, but lipophilic compounds will be associated with lipid membranes and lipid stores (Landrum and Fisher 1999). Hence, the partitioning of lipophilic contaminants will be

proportional to lipid content in tissues, and so concentrations tend to be highest in adipose fat or fat bodies followed by eggs, testes, liver, and kidneys with the lowest concentrations in muscle (Bryan et al. 1987; Bishop et al. 1995; Dabrowska et al. 2006; Perugini et al. 2006; Storelli et al. 2007).

Generally, lipophilic substances with octanol-water partition coefficients (log $K_{OW} \geq 5$) tend to biomagnify in fish (Fisk et al. 1998), and those with log $K_{OW} < 5$ do not (Kelly et al. 2007). However, air-breathing vertebrates can biomagnify compounds that have log $K_{OW} < 5$, but high octanol-air partition coefficients (K_{OA}). If metabolism is relatively slow, then air-breathing vertebrates can biomagnify compounds with $K_{OW} > 2$ and $K_{OA} > 6$, as these compounds would have slow respiratory elimination (Kelly et al. 2007). For example, dicofol, ß-endosulfan, ß-HCH, and trifluralin, none of which biomagnify in fish (K_{OW} of 3.5 to 4.4), can biomagnify in terrestrial vertebrates (Kelly et al. 2007). Given that reptiles breathe air, these and similar compounds have a high risk of biomagnifying in them.

The equilibrium lipid partitioning theory predicts that the concentrations of lipophilic compounds should be identical among tissues when expressed on a lipid basis (Russell et al. 1999). This implies that the lipophilic compounds in the blood plasma should reflect those of the body burden. However, Russell et al. (1999) found that the ratio of contaminants between eggs and maternal muscle of snapping turtles did not agree with the equilibrium partitioning model, and concentrations were approximately 2.4 times higher than expected in maternal muscle than in eggs based upon the ratio of lipids between tissues. Nevertheless, Bryan et al. (1987) suggested that the relative concentration of PCBs in tissues remained approximately the same regardless of the absolute concentrations. The lipid-adjusted ratios of maternal adipose burdens of OC pesticides to yolk burdens of American alligators from low, medium, and highly contaminated sites were close to 1 (0.94:1) (Rauschenberger et al. 2004). They suggested that these alligators were in steady state, and that OC pesticides in the eggs originated from lipid stores. For a good review of the routes of exposure for reptiles, see Smith et al. (2007) and references within.

10.1.1 Egg

Reptiles, with many exceptions among oviparous snakes and lizards, lay eggs in soil, sand, composting organic material, or similar substrates. Developing embryos can be exposed to organic contaminants within the substrate, from those dissolved in the aqueous phase in the substrate, or those in the gaseous phase through air exchange. Eggs of many reptilian species tend to be porous, and allow the passage of water across both the eggshell and membrane (Packard 1991; Marco et al. 2004a). The loose texture and poorly organized crystallites in chelonian eggshells allow substantial air and water exchange during development (Sahoo et al. 1997). Few studies have focused upon exposure of eggs to organic contaminants through soil, despite the potential of exposure of juveniles or eggs to agricultural or contaminated soils. Reptiles often nest within agricultural fields (Rauschenberger et al. 2004; de Solla et al. 2006), in substrates that have been exposed to oil (Van Meter et al. 2006), or in coal-ash-contaminated soils (Nagle et al. 2001), among others.

The transfer of metals in contaminated soils has been examined in lizards (arsenic and Iberian rock lizards [*Lacerta monticola*]; Marco et al. 2004b) and turtles (trace metals and slider eggs; Nagle et al. 2001). Bullsnake (*Pituophis melanoleucus*) eggs were incubated in a synthetic mix of Spanish moss and vermiculite that was dosed with aldrin, *p*,*p*'-DDT, dieldrin, endrin, heptachlor, and lindane (Cañas and Anderson 2002). There was absorption of 6 of the pesticides (lindane 19.0%, endrin 4.5%, aldrin 1.4%, heptachlor 1.0%, dieldrin 0.5%, and DDT 0.0%; Cañas and Anderson 2002). There was no relation between concentrations in the nesting substrate and concentrations in the egg, so concentration was not a factor in determining which compounds were absorbed. However, there was a negative relationship between absorption and lipid solubility (log K_{OW}). Eggs of free-ranging Morelet's crocodile (*Crocodylus moreletti*) collected from soil and plant material that were contaminated with hexachlorocyclohexane, *p*,*p*'-DDT, and methoxychlor had measurable concentrations of methoxychlor and DDE, although it was not clear what the contribution of maternal transfer was

relative to absorption from the nesting material (Wu et al. 2000). Nevertheless, most of the exposure of lipophilic compounds to embryos is likely from maternal transfer rather than exchange between the nesting substrate and eggshell. Highly lipophilic compounds, despite topical applications using vehicles to facilitate the transfer across the eggshell, do not readily pass through the eggshell, which typically results in a dosage much lower than the nominal values. Topical application of organochlorine pesticides to turtle and alligator eggs (Muller et al. 2007; Portelli et al. 1999) resulted in a relatively low, and highly variable, dosage to the embryo. Similarly, Podreka et al. (1998) found that only 8% of DDE that was topically applied to green turtle (*Chelonia mydas*) eggs was absorbed by the embryo. Following topical application to red-eared slider eggs, the eggshell retained 90 and 96% of the dose for PCB-126 and dioxin, respectively (Gale et al. 2002).

Contaminated nesting substrate has been shown to impact reptilian eggs. Van Meter et al. (2006) found that snapping turtle eggs exposed to crude oil in sand (and through topical application) had hatchlings with higher rates of deformities and reduced hatching success compared to controls. Red-eared sliders topically exposed to glyphosate (Glypro) and surfactant (LI700) (in vermiculite) had reduced hatching success, increased genetic damage, and reduced somatic indices, but only at dosages that exceeded likely environmental exposures (Sparling et al. 2006). Alligator snapping turtle eggs incubated in soils from agricultural sites growing cotton showed no differences in development to hatching, but did show reduced posthatch growth compared to hatchlings from control soil (Rauschenberger et al. 2004). The agricultural soils differed from the control soils in phosphorus, nitrates, and some metals, while the agricultural soils also received applications of current use herbicides and insecticides. Snapping turtles exposed to atrazine-treated soil at typical and 10× typical application rates exhibited normal hatching success, deformities, and gonadal development (de Solla et al. 2006), with only ~3% intersexes found in the atrazine-treatments. Slider turtle eggs (*Trachemys scripta*) incubated in ash-contaminated soil had reduced hatching success compared to those incubated in reference soil, but there was no difference in the concentrations of trace metals in the hatchlings from either group (Nagle et al. 2001). They suggested that the fine particles and cement-like characteristics of coal-ash-contaminated soil may interfere with O_2 exchange across the eggshell and membrane.

10.1.2 Dermal

Reptiles may also be exposed to organic contaminants through their skin. Although generally reptilian skin is not considered very permeable and the epidermis is generally covered with thick keratin scales, the dermal layer is fairly thin (Hildebrand 1988). There is little evidence that dermal exposure to lipophilic compounds represents an important route of exposure, except at very high dosages. High exposures may have occurred, however, in habitats where there have been direct applications of pesticides, and where exposures to reptiles have been either incidental or intentional. Pesticides have been used specifically to kill or repel snakes. DDT, aldrin, dieldrin, toxaphene, and heptachlorane (i.e., heptachlor) all have been suggested as means to repel snakes (Brock and Howard 1962; Savarie and Bruggers 1999). When discussing the use of organochlorines as snake repellents, Fitzwater (1974, p 181) noted, "Here again social acceptability is not necessarily a standard for reliability. One of the better snake repellents … contained eight different ingredients, the inclusion of chlorinated hydrocarbon chemicals in this list permanently incapacitated (Washingtonese for 'killing') individuals not influenced by the other additives." Much of the nature of these repellants was likely through the lethal nature of the organochlorines (Savarie and Bruggers 1999). DDT (50%) applied as a powder in buildings may repel or kill intruding snakes directly, or indirectly by eating contaminated mice that are dusted with DDT (Brock and Howard 1962). Snakes sprayed or dusted with DDT showed numerous physiological responses such as convulsions, erratic behavior, and death (Herald 1949; Munro 1949), while field applications of DDT, dieldrin, and endosulfan have caused deaths of snakes, lizards, and turtles (DeWitt et al. 1972; Koeman et al. 1978).

Transfer of OCs through the dermis to the internal tissues is not a unidirectional process only. Body burdens of contaminants may be eliminated through their discarded skin and scutes. Jones et al. (2005) found that corn snakes (*Elaphe guttata*) fed a diet that was enriched with chlordane, PCBs, and lindane over a 6-month period, at 2, 8, and 4 mg/kg, respectively, once a month, eliminated a portion of these compounds in shed skins. Mean concentrations in the shed skins were 0.18 µg/g chlordane, 5.72 µg/g PCBs, and 0.034 µg/g lindane. Shed skin of snakes may serve as an elimination route for OC contaminants, and may be used as a noninvasive, nondestructive indicator tissue for assessing OC contamination (Jones et al. 2005). OC pesticides (including endrin, methoxychlor, *p*,*p*'-DDE, and *p*,*p*'-DDT) may also be eliminated in caudal scutes of crocodilians, as all of these compounds were measured in the scutes of Morelet's crocodiles (*Crocodylus moreletii*) and American crocodiles (*C. acutus*) from Belize and Costa Rica (Rainwater et al. 2007). Mean concentrations ranged from 8 to 533.8 ng/g (dieldrin and methoxychlor, respectively) in the fat of the scutes. Mean metal and OC concentrations differed compared to those previously reported in crocodilian scutes from other localities in North, Central, and South America (Rainwater et al. 2007). Mercury concentrations were lower than those found in American alligators from the Florida Everglades and in southwestern South Carolina, but *p*,*p*'-DDT and methoxychlor in American crocodiles were substantially higher (Rainwater et al. 2007) than those recorded in Morelet's crocodiles from 2 other Belize study sites (DeBusk 2001). It is unknown how much of the OCs would be lost through scute loss or wear.

10.1.3 Diet

Diet is usually an important source of exposure to contaminants, and can be the dominant route of exposure if the compounds in question are persistent and highly lipophilic. Generally, compounds are considered potentially bioaccumulative if they are extremely lipophilic, such that their relative solubility in lipids is about ~100 000× that in water (i.e., octanol-water partition coefficient, or log $K_{OW} > 5$), although at high K_{OW} (~>9) bioaccumulation may be hindered sterically. However, the K_{OW} only predicts the tendency for a chemical to be partitioned between water and lipids, but the potential for bioaccumulation also depends on the ability of the animal to assimilate, metabolize, and excrete the chemicals. Chemicals that are considered highly bioaccumulative are lipophilic but are also slow to metabolize and/or excrete, such as highly chlorinated PCBs, many organochlorine pesticides, methylmercury, and others. Highly lipophilic compounds are sequestered in lipids in the prey items, and after ingestion are absorbed through the digestive tract. Subsequently, the contaminants are transported via the blood plasma to other tissues, and deposited into lipid stores. Binding with proteins can exacerbate the retention time of some compounds. For example, hydroxylated PCBs can bind to transthyretin (Rickenbacher et al. 1986), and thus not only displace thyroid hormones but also reduce the rate at which the PCBs are cleared.

Typically, animals at higher trophic positions in aquatic ecosystems have the greatest potential for bioaccumulation of POPs (Borgå et al. 2004). Reptiles run the gamut from obligate herbivores to obligate carnivores. Trophic position in reptiles appears to be related to body size, at least for those species that are omnivorous or carnivorous. For both Florida softshell turtles (*Apalone ferox*; Aresco and James 2005) and loggerhead turtles (*Caretta caretta*; Godley et al. 1998; Hatase et al. 2002), trophic position increased with body size, although trophic position was unrelated to body size for the more herbivorous turtles such as the Florida cooter (*Pseudemys floridana*) and slider (Aresco and James 2005), and green turtle (*Chelonia mydas*; Godley et al. 1998). Bergeron et al. (2007) found that freshwater turtles that had the highest tropic position had the greatest accumulation of mercury and methylmercury (Snapping turtles ≥ stinkpots [*Sternotherus odoratus*] > painted turtles [*Chrysemys picta*] > red-bellied turtles [*Pseudemys rubriventris*]). Changes in body size may be a factor in the ontogenetic shifts in diet observed in many lacertid lizards (Verwaijen et al. 2002). Herbivorous animals sometimes do not show increasing body burdens with increased body size.

McKenzie et al. (1999), for example, found that pesticide residues in plasma (lipid basis) of green turtles decreased with increasing body size, and this may have been due to a shift in diet toward plants in older/larger individuals.

Concentrations of PCBs and organochlorine pesticides tend to increase with body size in snapping turtles, such as in liver (Hebert et al. 1993) and blood plasma (de Solla et al. 1998), as measured on a wet weight basis. Similarly, OC pesticides and PCB body burdens increased with body size in adult viperine snakes (*Natrix maura*; Santos et al. 1999) and male cottonmouths (*Agkistrodon piscivorus*; Rainwater et al. 2005). The higher body burdens found in larger individuals of omnivorous or carnivorous species indicate that either the rate of accumulation exceeds the elimination rate for most tissues, the metabolic rate or ability to metabolize and eliminate contaminants slows in larger (and presumably older) individuals, or larger individuals are feeding on higher trophic levels than smaller individuals are and, thus, have greater exposure. Differences in trophic position may affect dietary exposure to persistent lipophilic contaminants, as higher-trophic-level animals generally feed on animals with greater contaminant body burdens (Hebert and Weseloh 2006).

Stable isotope ratios of nitrogen and carbon have been used to determine trophic position in many taxa, although the use of stable isotopes has been somewhat limited in reptiles. Stable isotope ratios (^{15}N:^{14}N, ^{13}C:^{12}C) in the proteins of consumers reflect those of the proteins in their diet in a predictable manner. The ratio of ^{15}N to ^{14}N generally exhibits a stepwise increase at each trophic level, and consequently, the $\delta^{15}N$ values in the tissues of consumers tend to be between 2.5 and 5% greater than those of their diets, although typically 3.4% is used in studies for assigning trophic position (Post 2002). Typically, the trophic position of primary producers is defined as 1, primary consumers as 2, and tertiary consumers as 3 or higher. Not unexpectedly, reptiles that are obligate carnivores, or have a predominantly animal diet, have $\delta^{15}N$ values typical of high trophic positions (3 or 4; Table 10.1). Snakes were uniformly found to be at higher trophic positions, whereas turtles and particularly lizards ranged from mid to higher trophic positions (Table 10.1). Generally, it is the reptiles at the higher trophic positions from which one would expect the greatest bioaccumulation of persistent lipophilic chemicals, all other factors (metabolic physiology, exposure period, maternal transfer, preferred prey species, etc.) being equal.

10.1.4 Maternal Transfer

Reptilian eggs have a high lipid content, often averaging about 4 to 14% of the wet weight mass (Speake et al. 2003; Ashpole et al. 2004; de Solla and Fernie 2004; Roosenburg and Dennis 2005), and lipophilic compounds are transferred maternally in the eggs by the female. A recent study demonstrated quite convincingly the importance of maternal transfer of lipophilic organic pollutants. Rauschenberger et al. (2007) exposed female alligators to organochlorine pesticides (chlordane, *p,p*'-DDE, toxaphene, and dieldrin), resulting in egg burdens similar to those found in free-ranging alligators. Lipophilic contaminants in the eggs are probably derived from daily dietary intake just prior to egg production rather than utilization of stored fats (Bishop et al. 1994). Chlorinated hydrocarbons in the lipid contents of the eggs are steadily absorbed into the embryos during embryonic development. They reach peak concentrations at or just before hatching and then begin to decline (Bishop et al. 1995; Alava et al. 2006). These peak concentrations are comparable to concentrations in the lipid contents of freshly laid eggs, indicating either that organochlorines are not metabolized by the embryo or that the rate of metabolism is low relative to the egg burden (Kleinow et al. 1999).

Nevertheless, sources to eggs may reflect both female diet and reallocation of female lipid stores. Because some OCs have clearance half-lives of over 6 months in birds (Norstrom et al. 1986; Clark et al. 1987), the contaminant burdens in eggs reflect not only local contamination, but also maternal burdens, which are transferred to the eggs. The maternal transfer of PCBs in birds ranges from

TABLE 10.1
Octanol–Water Coefficients for a Variety of Organic Contaminants

Compound	Log K_{OW}	Likelihood for Bioaccumulation
Polychlorinated biphenyls[a]	4.46–8.18	High
Polybrominated diphenyl ethers[b]	5.74–8.27	High
Polychlorinated naphthalenes[c]	4.06–7.70	Medium to High
p,p'-DDE[c]	6.5	High
Mirex[d]	7.01	High
Polycyclic aromatic hydrocarbons[e]	3.37–7.64	Medium
Polychlorinated dibenzo-*p*-dioxins[f]	4.2–8.6	High
Perfluorooctanoic acids[g]	Not measurable	Medium to high
Alkylphenols[h]	4.17–4.48	Low
Methylmercury[i]	~0.5–1.6	High
Polychlorobenzene[j]	2.84–5.73	Medium
Organophosphate pesticides[k]	5.07–6.15	Low
Phthalate esters[l]	1.48–10	Low to medium

Note: Compounds are considered (potentially) bioaccumulative and persistent if their bioaccumulation factor > 5000, or log KOW > 5, at least in fish.

[a] Hawker and Connell (1988).
[b] Braekevelt et al. (2003).
[c] Hackenberg et al. (2003).
[d] Veith et al. (1979).
[e] Data compiled or estimated by Neff and Burns (1996); see references within.
[f] Govers and Krop (1998).
[g] The interaction between octanol and water makes determination of log K_{OW} for perfluorooctanoic acid and sulfonate, and similar surfactants, impossible.
[h] Nonylphenol and octylphenol, and metabolites; Ahel and Giger (1993).
[i] Major et al. 1991; K_{OW} depends heavily on pH and concentration of chloride in water.
[j] Sangster et al. 1989 (chlorobenzene); de Bruijn et al. 1989 (hexachlorobenzene).
[k] Series of 6 organophosphates; de Bruijn et al. 1989.
[l] Estimated using QSAR; Parkerton and Konkel 2000.

2% to 22% of the maternal body burdens (see references within Bargar et al. 2001). Avian species that invest few lipids (e.g., 5 to 18% of maternal lipid reserves in eggs; Table 1 of Drouillard and Norstrom 2001) tend to have egg–maternal tissue PCB concentration ratios less than 1, and generally around 0.3 to 0.7. Female birds that do not deplete lipid reserves during egg formation rely primarily on diet as sources for the formation of yolk lipids (Brisbin 1969).

Organochlorine burdens are sometimes higher in male than female reptiles, due to maternal transfer of OCs from gravid females to their eggs (Mineau 1982; Bishop et al. 1994). Contaminants burdens tend to be higher in males than in females in turtles (Albers et al. 1986). Although OC pesticides and PCB body burdens increased with body size in both sexes of adult viperine snakes, the rate of increase was higher for males (Santos et al. 1999), although no sex differences were found in a similar study of body burdens of bullfrogs (*Rana catesbeiana*) or northern watersnakes (Fontenot et al. 2000). Concentrations of DDE in fat were correlated positively with body size in male cottonmouths, but not in females; the authors suggested that females had a slower rate of accumulation (Rainwater et al. 2005). Body mass and age do not correlate with OC concentrations in eggs, indicating that these compounds are accumulating and are transferred into eggs on an annual basis (Bishop et al. 1994).

10.2 METABOLISM

Cytochrome P450 enzymes (CYP) are a key component of the mixed-function oxidase (MFO) system, and are important for metabolizing endogenous and exogenous substrates, including organic contaminants. Although the MFO system has many endogenous functions, it is one of the key components for detoxifying and eliminating toxins, including those ingested or otherwise absorbed. Phase I metabolism by P450 enzymes entails the hydroxylation of the substrate, which increases the water solubility and rate of elimination of the metabolized substrate. Although CYP enzymes are found in every class of biota, there is considerable variation among taxa in both amino acid sequence (Bandiera 2001) and activity of P450 (Yawetz et al. 1997; Ertl and Winston 1998). Yawetz et al. (1998) demonstrated that painted turtles (*Chrysemys picta*) have at least 2 microsomal proteins associated with CYP1A forms, and thus may have 2 types of CYP1A genes in the liver. Furthermore, they may have multiple forms of CYP2B and CYP3A, as well as undescribed forms of CYP enzymes (Yawetz et al. 1998). Both snakes and alligators exhibited CYP1A-like activity (Jewell et al. 1989; Gunderson et al. 2004; Hecker et al. 2006; Mitchelmore et al. 2006), but alligators also had CYP2B forms (Ertl et al. 1998). The MFO response of alligators that were exposed to 3-methylcholanthrene was comparable to the CYP response of mammals similarly exposed (Jewell et al. 1989), although not necessarily in the level of induction.

The activities of different isoforms (1A, 2B, others) of P450 enzymes are often induced by organochlorine compounds, such as PCBs, dioxin, or pesticides. The pattern of chlorine substitution dictates which CYP isozyme is induced, and which organochlorines are metabolized by CYP P450 enzymes (Bandiera 2001). In general, dioxin-like compounds (e.g., dioxin, non-*ortho* PCBs, and some PAHs) bind with the aryl hydrocarbon (Ah) receptor, which increases the expression of CYP1A mRNA. *Ortho* PCBs and many pesticides induce CYP2B and/or CYP3A, for example (Bandiera 2001). The toxic responses of some organochlorine compounds are mediated through the induction of P450 enzymes, as they in turn metabolize endogenous compounds such as sex steroids and fatty acids. EROD (7-ethoxyresorufin-O'deethylase) is an enzyme that is catalyzed by CYP1A, and thus EROD activity is often used to indicate exposure to dioxin-like compounds. The activities of the aryl hydrocarbon receptor in response to PCB exposure are correlated with their corresponding toxicities and dioxin-like equivalents (TEQs) (Kafafi et al. 1993a).

EROD induction in snakes and turtles associated with exposure to dioxin-like compounds was lower than the induction frequently found in mammals and birds (Bishop et al. 1998; Hecker et al. 2006). The rates of induction of enzymes associated with CYP1A (i.e., EROD) by xenobiotic compounds in reptiles can be substantially slower than those reported for mammals and birds (Ertl et al. 1998; Hecker et al. 2006). Despite low EROD response, painted turtles are capable of oxidizing PCB 77 at rates similar to those of some bird species (Schlezinger et al. 2000), although not as rapidly as mammals. EROD and aryl hydrocarbon hydroxylase (AHH) are both catalyzed by CYP1A, and their inductions after exposure to xenobiotics are normally very similar. EROD and AHH activities are typically highly correlated with each other in fish (Monod et al. 1988; Gu et al. 2007), and in vitro studies have shown 1:1 correspondence in EROD and AHH induction of rat hepatoma H-4-II E cells exposed to dioxins (Kafafi et al. 1993b). However, although EROD induction in turtles tends to be considerably lower compared to mammals, AHH activity in turtles is comparable to that of other vertebrates (Yawetz et al. 1998). The different inductions of EROD and AHH in turtles by CYP1A following PCB exposure suggest that turtle CYP1A, and possibly CYP1A of other reptilian species, may differ structurally from that of other vertebrates (Schlezinger et al. 2000). Hence, it may be difficult to assess the ability of reptiles to metabolize xenobiotics by using EROD or similar enzymes. MROD (CYP1A2) showed a greater ability to discriminate between alligator hepatic microsomes than EROD (CYP1A1; Ertl et al. 1998). American alligators collected from 3 sites with varying contaminations in South Florida showed CYP1A1 induction, but no induction of

CYP1A2, or glutathione-S-transferase activity (Gunderson et al. 2004). EROD was highest in the site with the intermediate degree of contamination, possibly suggesting some inhibition of CYP1A expression or activity at the most contaminated site. Furthermore, Gunderson et al. (2004) found that at the least contaminated site, the EROD, MROD, and GST activities in alligators decreased with increasing body size. They also found that that there was no relationship between body size and hepatic enzyme activity found in alligators from sites with greater contaminant exposure, and suggested that the contaminants may have altered these allometric relationships.

10.3 ORGANIC CONTAMINANTS

10.3.1 PCBs, Dioxins, and Furans

Polychlorinated biphenyls (PCBs) are a class of chemicals used as lubricants, electrical insulators, heat transfer fluids, and surfactants. They were most commonly used in transformers, capacitors, and hydraulics, but were also found in a very wide range of products such as sealants, flame retardants, and plasticizers. They were useful because they were stable at high temperatures and were excellent electrical insulators. PCBs were first produced in 1929 by Swann Chemical Company, until Monsanto took over production in 1935. North American production peaked in the early 1970s, but by 1972 North American use of PCBs was limited to "closed systems" (i.e., products in which the PCBs were entirely contained). Production in North America ended in 1977, but production in Europe (e.g., France and Spain) continued until at least 1985. There are no significant natural sources of PCBs. There are 209 potential congeners of PCBs (Figure 10.2), although only 30 to 40 are frequently found at relevant concentrations in wildlife tissues. PCBs were produced and sold as commercial mixtures; in North America >95% of PCBs were produced as Aroclors (Monsanto; i.e., Aroclor 1242, 1254, 1016, 1260, 1248, etc.), but similar mixtures were produced in Europe (Bayer, Clophen, Progil Fabrique–France, Pyralene, etc.) and Japan (Kanegafuchi Chemical Industry, Kanechlor).

PCBs are highly persistent and lipophilic (Table 10.2), and the more chlorinated congeners (≥4 chlorines) are generally considered bioaccumulative. Like most POPs, exposures to animals tend to be highest in aquatic ecosystems, particularly in areas near large urban centers. Polychlorinated dibenzo-*p*-dioxins (PCDDs) and furans (PCDFs) were not manufactured as products, but instead were unintentional by-products of the production of PCBs and other chlorinated organic products. There are 75 congeners of dioxins and 135 congeners of furans (Figure 10.3), although only a few congeners comprise the majority of body burdens of free-ranging animals.

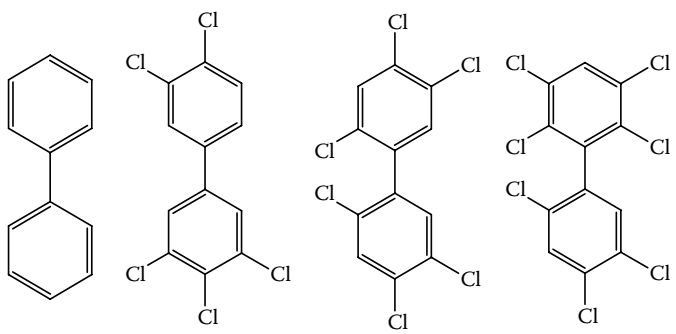


FIGURE 10.2 Biphenyl and polychlorinated biphenyls. Both non-*ortho* (PCB 126) and *ortho* (PCBs 153 and 187) chlorinated biphenyls are described.

TABLE 10.2
Trophic Position of Selected Turtles, Snakes, Lizards, and Alligators

Taxa	Adult	Juvenile	δN[e]	δN baseline[e]
Turtles				
Yellow-bellied slider (*Trachemys scripta*)[a]	3.3	3.5		
Florida cooter (*Pseudemys floridana*)[a]	2.3	2.3		
Stinkpot (*Sternotherus odoratus*)[a]	3.6			
Eastern mud turtle (*Kinosternon subrubrum*)[a]	4			
Florida softshell (*Apalone ferox*)[a]	3.8	3.2		
Snapping turtle (*Chelydra serpentina*)[a]	3.5			
Snakes				
Florida green watersnake (*Nerodia floridana*)[a]	3.4			
Banded watersnake (*Nerodia fasciata*)[a]	3.8			
Cottonmouth (*Agkistrodon piscivorus*)[a]	3.2			
Mud snake (*Farancia abacura*)[a]	4.6			
Chequered keelback (*Xenochrophis piscator*)[b]	3.4		9.9	1.7
Chequered keelback (*Xenochrophis piscator*)[b]		3.0	8.7	1.7
Rice paddy snake (*Enhydris plumbea*)[b]		3.0	8.5	1.7
Brahminy blind snake (*Ramphotyphlops braminus*)[b]	2.9		8.1	1.7
Red-tailed pipe snake (*Cylindrophis ruffus*)[b]		3.0	8.6	1.7
Small-spotted coral snake (*Calliophis maculiceps*)[b]	3.6		10.5	1.7
Indo-Chinese rat snake (*Ptyas korros*)[b]	3.5		10.2	1.7
Crocodilians				
American alligator (*Alligator mississippiensis*)[a]	3.3	2.7		
Lizards				
Side-blotched lizard (*Uta stansburiana*)[c] (coastal)	3.3			
Side-blotched lizard (*Uta stansburiana*)[c] (inland)	2.7			
Sail fin lizard (*Hydrosaurus pustulatus*)[d]	1.3		1.7	0.6
Panay monitor (*Varanus mabitang*)[d]	1.5		2.2	0.6
Reeves' smooth skink (*Scincella reevesii*)[b]	2.6		7.0	1.7
Speckled forest skink (*Mabuya macularia*)[b]	2.5		6.8	1.7

Note: Trophic position: Primary producers = 1, primary consumers = 2, secondary consumers = 3, tertiary consumers = 4 or greater. If δN is presented, no trophic position was listed, but was calculated *post hoc*.

[a] Aresco and James (2005).

[b] Kupfer et al. (2006).

[c] Barrett et al. (2005).

[d] Ulrich et al. (2002).

[e] The authors did not calculate trophic position; I used other data in the paper to estimate trophic position using the δN in the animals as well as in the matrix used as a baseline (e.g., Post 2002).

10.3.1.1 Body Burdens

PCBs are one of the most commonly measured organochlorine contaminants in biota, and are usually the most abundant (Figure 10.4). Large volumes of PCBs were discharged into the Hudson River from waste point source discharges of 2 General Electric capacitor manufacturing plants between 1950 and 1976 (Bopp et al. 1981). Concentrations of mean PCBs in the fat of adult male snapping turtles collected between 1976 and 1978 reached 2990 μg/g (lipid weight) in the Hudson River (Stone et al. 1980), and fat in 1 snapping turtle from Irondequoit Bay, New York, Lake Ontario, and

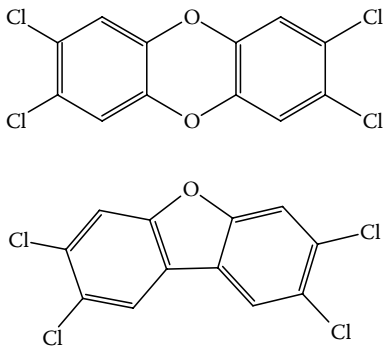


FIGURE 10.3 Polychlorinated dibenzo-*p*-dioxins and furans; the most toxic forms (2,3,7,8-chlorine-substituted) congeners are displayed.

a second from the upper Hudson River had 633 and 3560 µg/g total PCBs, respectively (Olafsson et al. 1983). From 1981 to 2004, snapping turtle eggs were collected from over 30 sites, including areas of concern (AOCs) in the Great Lakes–St. Lawrence River Basin in central Ontario and southern Quebec, Canada (Struger et al. 1993; Bishop et al. 1998; de Solla et al. 2001; Ashpole et al. 2004; de Solla and Fernie 2004; de Solla et al. 2007). Concentrations of PCBs were the highest among all compounds measured. Total PCB I eggs ranged from 0.057 to 737.7 µg/g among sites from the Great Lakes–St. Lawrence River Basin, whereas the mean total PCB concentrations in Algonquin Park eggs ranged from 0.187 µg/g in 1981 to 0.016 µg/g in 2001–2002 (Struger et al. 1993; de Solla and Fernie 2004).

Generally, the geographic variation in PCBs, PCDDs, PCDFs, and non-*ortho* PCBs, OC pesticides, and dioxin toxic equivalents (TEQs) in turtle eggs was similar among the different studies comparing the same sites. The contaminants in the eggs sampled from locations adjacent to or downstream from large industrial or municipal sources usually reflected their sources. Total PCBs in eggs from 2 clutches of snapping turtles from the Akwesasne Mohawk Nation (Turtle

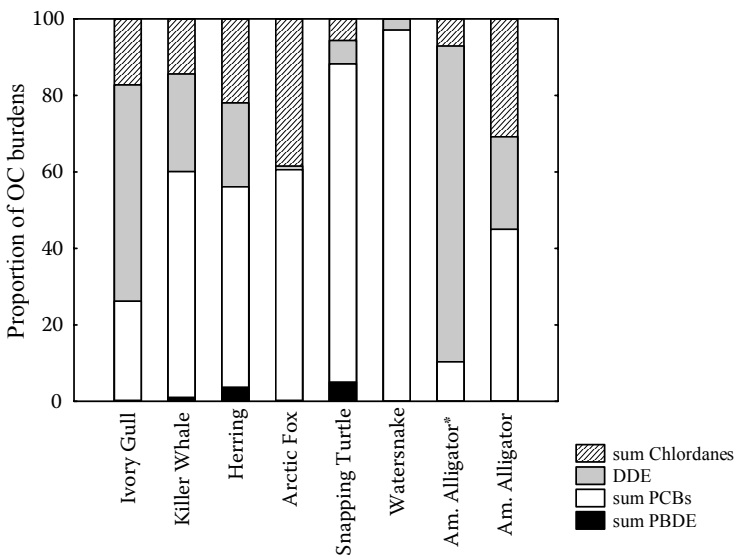


FIGURE 10.4 Examples of the relative proportion of body burdens of organochlorine pesticides (DDE, chlordane), PCBs, and PBDEs in a variety of animals, including watersnakes, turtles, and alligators. The alligator samples with an asterisk were from Lake Apopka following a pesticide spill. (From Guillette et al. 1999.)

Creek, New York) were 737.68 µg/g in 1998 (de Solla et al. 2001) and 60.96 µg/g in 1999 (Ashpole et al. 2004), and were among the highest recorded for free-ranging animals. Although there is no heavy industry within Akwesasne, the site is downstream of 3 Superfund sites: the General Motors foundry, Aluminum Company of America (ALCOA), and Reynolds Metals (since purchased by ALCOA). The GM central foundry site used hydraulic fluids that contained PCBs in their diecasting machines from 1959 to 1974, and both Reynolds Metals and ALCOA discharged hydraulic and heat transfer fluids that contained PCBs (Sokol et al. 1994). Turtle eggs from Hamilton Harbour had PCB concentrations up to 8.59 µg/g in 1990 (Struger et al. 1993). Hamilton Harbour received substantial industrial and municipal wastes, particularly from industries associated with steel production and municipal sewage. PCB concentrations were also relatively high on the south shore of Lake Erie, and concentrations ranged from 0.18 to 3.68 µg/g in eggs from Lake Erie AOCs (Dabrowska et al. 2006).

Concentrations of PCBs tend to be lower in sea turtles than in omnivorous freshwater turtles; some of this may be due to the lower trophic positions of some sea turtles, but much is likely due to the differences in contaminant exposure in marine environments. Loggerhead turtles from the eastern Mediterranean Sea had mean concentrations of total PCBs in their liver of 0.052 µg/g. Non-*ortho* PCBs (which have a toxicity similar to that of dioxins) typically are responsible for the majority of "dioxin-like" toxicity, based upon body burdens. PCB 126, the most toxic PCB congener, was responsible for 85% to 91% of the total (nominal) dioxin-like toxicity (TEQs) in green turtles (Miao et al. 2001). PCB 77, however, was the most important contributor to TEQs in loggerhead turtles, and accounted for >90% of the TEQs, although dioxins and furans were not included (Storelli et al. 2007).

American alligators can accumulate high concentrations of PCBs; mean concentrations in alligator eggs from Louisiana and South Carolina ranged from 0.0002 to 3.18 µg/g (lipid weight; Cobb et al. 2002), despite the relative remote location of some of these sites.

PCBs and pesticides were measured in chameleon eggs (*Chamaeleo chamaeleon*) from Southern Spain in 1997, in which eggs incubated in natural conditions had low hatching success. Although lead concentrations were sufficiently high to cause toxicity in other species (mean = 14.2 µg/g), mean concentrations of PCBs were only 0.017 µg/g (Díaz-Paniagua et al. 2002). In 2001, eggs were collected again from the same areas, and it appeared that PCB concentrations had increased. In 1997 PCB concentrations ranged from 0.003 to 0.033, whereas in 2001 they ranged from 0.025 to 0.040 µg/g (Gómara et al. 2007).

Although there are many exceptions, generally in freshwater systems PCBs tend to be the dominant organic contaminant in reptiles (Figure 10.4), followed by DDE. Where there was a dicofol/DDT spill in Lake Apopka, DDE concentrations were higher in American alligators than were PCBs (Figure 10.4).

10.3.1.2 Toxicity

Despite the large number of studies measuring PCBs in reptilian tissue, there are few studies examining the effects of these compounds. PCBs and dioxins/furans exhibit a wide range of physiological and developmental impacts on exposed animals. PCB exposure can induce neurological and behavioral dysfunctions in laboratory animals, and may involve alterations in cellular signaling processes and endocrine functions that influence neurofunction, the organization of the developing brain, and behavioral responses (Seegal 1996). Typical responses, as outlined by Safe (1993), are developmental and reproductive toxicity, endocrine disruption, hepatotoxicity, carcinogenesis, and the induction of diverse metabolizing enzymes (i.e., P450s and related enzymes). PCBs also impact the thyroid system by binding with thyroid hormone transporting proteins, and by induction of oxidizing enzymes, which in turn metabolize thyroid hormones (Rickenbacher et al. 1986; McKinney and Waller 1994; Hallgren et al. 2002). Much of the toxicity of PCBs is due to the "dioxin-like" properties of non-*ortho* (i.e., PCBs 77, 126, and 169) and mono-*ortho* (i.e., PCBs 105, 118, and 189) congeners. Mono-*ortho* and particularly non-*ortho* PCBs can form a "co-planar" position; that is,

the 2 biphenyls can form a flat configuration, which allows it to bind with the aryl hydrocarbon receptor (Ah; Safe 1990). Subsequently, the dioxin–Ah receptor complex forms DNA adducts and induces gene expression, such as enzymes responsible for metabolizing both endogenous and non-endogenous substrates (Safe and Krishnan 1995). Dioxin and related compounds can cause numerous developmental and physiological impacts, such as late-stage terata, thymic atrophy, chloracne, tumor promotion, hepatomegaly, cachexia, and death (reviewed in Birnbaum and Tuomisto 2000; Schecter et al. 2006). Cytochrome P450 (i.e., P450 1A), glutathione-S-transferase, NAD(P)H quinone oxidoreductase, and other enzymes are induced by dioxin, whereas dioxin inhibits estrogen-induced gene expression (Safe and Krishnan 1995). Many aspects of dioxin toxicity may be due to sustained interference of the normal functioning of the Ah receptor that is independent of xenobiotics (McMillan and Bradfield 2007). Liver neoplasms that are induced by PCB technical mixtures are primarily due to non-*ortho* and/or mono-*ortho* PCBs (Knerr and Schrenk 2006). Although the PCB congeners that do not have dioxin-like properties can also induce tumors, genotoxic assays generally show that individual congeners and technical mixtures are not active, indicating that they are likely nongenotoxic carcinogens (Knerr and Schrenk 2006).

Snapping turtle eggs collected from 1986 to 1991 from wetlands of Lakes Erie and Ontario, the St. Lawrence River, and a reference site in central Ontario in Algonquin Provincial Park were artificially incubated to assess developmental success (Bishop et al. 1991, 1998). While there were no correlations between organochlorine pesticide concentrations and abnormalities, there were significant positive correlations between PCBs and PCDD/Fs and rates of abnormalities (Bishop et al. 1991, 1998). In a later study (2001 to 2004), although there were differences in hatching success and deformities among sites, there were no associations with egg burdens of PCBs, PBDEs, or OC pesticides (de Solla et al. 2008). Generally, concentrations of PCBs and other compounds had declined from the earlier study (1986 to 1991; Bishop et al. 1998) to the later study (2001 to 2004; de Solla et al. 2008). Western pond turtle (*Clemmys marmorata*) eggs from Fern Ridge Reservoir, Oregon, were evaluated for PCB and OC pesticide contamination in relation to hatching success (Henny et al. 2003). The researchers found no significant difference in contaminant concentrations in eggs from nests in Oregon, where all turtle eggs failed to hatch, compared to those where some eggs hatched; PCB concentrations ranged from 0.0048 to 0.037 μg/g (Henny et al. 2003). Similarly, there was no evidence that the reproductive success of softshell turtles (*Apalone spiniferus spiniferus*) was compromised due to organochlorine contamination (de Solla et al. 2003), where mean PCB concentrations ranged from 0.77 to 1.49 μg/g. There was a positive correlation between PCB concentrations and egg viability, but the relationship was probably spurious (de Solla et al. 2003). The most important factor determining hatching success of eggs was predation, followed by egg viability and parasitism.

Bishop and Rouse (2000, 2006) examined the relationship between organochlorine contaminant burdens in plasma of Lake Erie water snakes (*Nerodia sipedon insularum*) with egg viability on Pelee Island, Canada. Concentrations of pesticides were low (≤0.1 ng/g) in all snakes, but mean PCB concentrations varied among sampling locations on Pelee Island. There were no significant correlations among body mass, snout-vent length, number of young per female, or per gram body mass of female snakes and contaminant concentrations in plasma. An interim estimate of a no effect concentration on embryonic survival in Lake Erie water snakes may be a maximum average concentration of 90.4 ng/g wet weight PCBs (Bishop and Rouse 2006).

PCBs, and their hydroxylated metabolites, can exhibit estrogenic or anti-estrogenic properties. Of 42 PCB congeners examined, only 104, 184, and 188 — all with 4 *ortho*-substituted chlorines — demonstrated an ability to bind with anole (*Anolis carolinensis*) estrogen receptors; 2 out of 7 hydroxylated PCBs also bound with the estrogen receptor (Matthew and Zacharewski 2000). Incidentally, 104, 184, and 188 are not commonly found in any Aroclor mixture (Frame 1997), and so are not likely to be a significant exposure risk. As sex determination in species with temperature-dependent sex determination is usually estrogen dependent, estrogenic or anti-estrogenic properties of contaminants can interfere with sexual development. Slider turtle eggs exposed to hydroxylated PCBs (4-HO-PCB 30, 4-HO-PCB 61) at 27.8 °C (male-producing temperature) through topical application

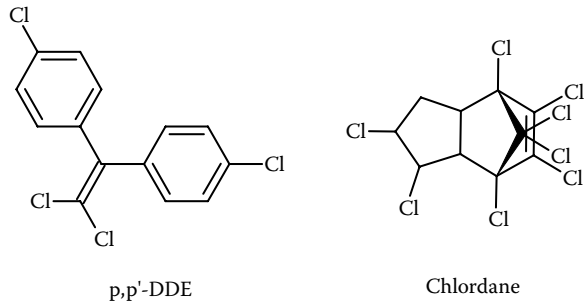


FIGURE 10.5 Two organochlorine pesticides, *p,p*'-DDE (metabolite of DDT) and chlordane, which are among the most common OC pesticides detected in biota.

produced significantly more females than expected (Bergeron et al. 1994). The metabolite 4-HO-PCB 30 produced 100% females at 100 μg per turtle, or just below 9 ppm. When slider turtle eggs were incubated at male-producing temperatures, in ovo exposure to Aroclor 1242 caused an increase in the number of female sliders (Willingham and Crews 1999). When administered with estradiol, Aroclor 1242 did not affect sex determination, but when applied with OC pesticides there was a significant increase in the number of females produced.

10.3.2 Organochlorine Pesticides

Organochlorine pesticides can generally be grouped into 2 classes (Coats 1990): DDT-like insecticides (e.g., DDT, dicofol, methoxychlor) and cyclodienes, including alicyclic insecticides (e.g., aldrin, dieldrin, heptachlor, endrin, chlordane, endosulfan). Figure 10.5 shows examples of the 2 main classes of organochlorine pesticides. Although most of these compounds are no longer being used, some are still active. DDT was perhaps the most economical insecticide ever produced (Ware 1989). Some orhanochlorine pesticides are still used (as of 2007) in North America, such as endosulfan, methoxychlor, lindane, and dicofol. Like PCBs, most organochlorine pesticides are usually highly persistent and lipophilic (Table 10.2), and are generally considered bioaccumulative.

10.3.2.1 Body Burdens

Generally, DDE is the organochlorine pesticide that is most frequently detected in biota (Figure 10.4), and tends to have the highest concentrations relative to other pesticides in a wide range of reptilian species throughout much of the world (e.g., cottonmouths, Rainwater et al. 2005; Morelet's crocodiles, Pepper et al. 2004; chameleon, Díaz-Paniagua et al. 2002; snapping turtles, de Solla et al. 2007). Very high concentrations of DDE were found in Australian freshwater crocodile (*Crocodylus johnstoni*) and estuarine crocodile (*Crocodylus porosus*) livers and adipose fat, in Ord River in Western Australia, where DDT and toxaphene were heavily applied to cotton from 1964 to 1974 (Yoshikane et al. 2006). Mean concentrations of DDE were as high as 252 μg/g (lipid weight) in liver and 53 μg/g (lipid weight) in adipose fat, whereas mean toxaphene concentrations were as high as 0.311 μg/g (lipid weight) in liver and 0.325 μg/g (lipid weight) in adipose fat (Yoshikane et al. 2006).

Occasionally, OC pesticides other than DDE can dominate; the most common pesticide in the livers of black turtle (*Chelonia mydas agassizii*), Pacific loggerhead (*Caretta caretta*), and olive ridley (*Lepidochelys olivacea*) turtles was chlordane (up to 65.1 ng/g), followed by endosulfan (up to 32 ng/g), lindane (up to 22.4 ng/g), hexachloro-benzene (HCB) (up to 18.6 ng/g), and then total DDT (up to 10.4 ng/g; Gardner et al. 2003). Mean concentrations of ΣDDTs, Σchlordanes, and dieldrin in unviable eggs of loggerhead turtle eggs (Florida) were 318 (7.88 to 1340 ng/g lipid), 161 (4.04 to 685 ng/g lipid), and 16.1 ng/g lipid (1.69 to 44.0 ng/g lipid), respectively (Alava et al. 2006). Mean ΣPCBs were the dominant organochlorine (904 ng/g).

Mean concentrations of OC pesticides (mostly *p,p*'-DDE and the cyclodiene pesticides dieldrin and chlordane) in American alligator egg yolk reached 31.8 μg/g in Emeralda Marsh Conservation Area, Florida, compared to alligator yolk from Lake Apopka (13.2 μg/g) and Lake Griffin (1.2 μg/g; Sepúlveda et al. 2006). Earlier reports of DDE in alligators or crocodiles have also found high concentrations; American alligators from Lake Apopka, Lake Griffin, and Lake Okeechobee had concentrations of DDE ranging from 0.1 to 7.6 μg/g (ww; Heinz et al. 1991). American crocodiles (*Crocodylus acutus*) had concentrations of DDE ranging from 0.37 to 2.9 μg/g in southern Florida (Hall et al. 1979).

Snapping turtle eggs from the St. Lawrence River tended to have relatively high concentrations of mirex (up to 0.438 μg/g; de Solla et al. 2001) relative to other areas within the Great Lakes. One of the major sources of mirex in the Great Lakes was the Oswego River, where there was a large discharge from the Armstrong Cork Company (Holdrinet et al. 1978), which used mirex as a flame retardant. The mirex plume extended into the St. Lawrence River and beyond (Comba et al. 1993), and subsequently turtles downstream of the plume had relatively high exposures to mirex compared to those throughout much of the Great Lakes region.

Following air raids in 1988, and the destruction of a pesticide store in Hargesia, Somaliland, the store was pillaged and 81 200 L of waste (largely composed of OC pesticides) was dumped into the local soil (Lambert 1997). The skinks *Mabuya s. striata* and *Chalcides ragazzii*, gecko *Hemidactylus parkeri*, and sandracer *Pseuderemias smithi* were monitored for whole body pesticide residues after the spill. Concentrations of dieldrin, total DDT, and β-HCH reached 80.4, 125.5, and 171.6 μg/g wet weight, respectively (Lambert 1997).

10.3.2.2 Toxicity

Organochlorine pesticides are primarily neurotoxins, although the mechanism of action varies among the different pesticides; DDT, for example, interferes with the Na and K pump mechanism in the neuronal membranes, causing disruption in calcium homeostasis (Colosio et al. 2003). Cyclodienes (e.g., chlordane, dieldrin) block GABA receptors and alter dopamine transporter and dopamine concentrations following exposure (Pomes et al. 1994; Kirby et al. 2002). Organochlorine pesticides affect the thyroid system (Mortensen et al. 2006), endocrine system (Sugiyama et al. 2005), induction or suppression of P450 enzymes (Barber et al. 2007), immune function (Gilbertson et al. 2003), and tumor promotion (Flodström et al. 1988). The acute toxic effects of organochlorine pesticides in animals are mediated by hyperexcitation of the nervous system, followed by respiratory failure, and possibly death (Coats 1990).

Some of the studies on toxicity of organochlorine pesticides to reptiles are centered on endocrine disruption, especially (anti)estrogenic and to a lesser degree (anti)androgenic interactions. Estrogen is responsible for much of the sexual differentiation of females, including metabolic, behavioral, and morphological changes throughout different life stages (Lange et al. 2002). Estrogenic assays have used a wide variety of taxa, and it generally should not be assumed that estrogen receptors, and their relative affinity to various ligands, do not differ among these taxa. Generally, there is strong structural homology of the estrogen receptor among taxa (Ankley et al. 1998), but differences in activity have been found between reptiles and mammals, for example. Vonier et al. (1996) found that alligator estrogen receptors could bind with atrazine, whereas mammalian receptors did not. Different ligand preferences and relative binding affinities for PCBs and hydroxylated PCBs have been found among taxa, including reptilian species (Matthews and Zacharewski 2000), using a semi-high-throughput competitive binding assay linked to the glutathione-S-transferase (GST) protein. These differences may be dependent on the assay used, as Sumida et al. (2003) found no differences among taxa, based on an estrogen-receptor-dependent transactivation using reporter gene assays, including caimans and whiptail lizards. Caution is required when attempting to generalize endocrinological function among species, including seemingly related taxa.

Most toxicological work has been on DDT's primary metabolite, *p,p*'-DDE (*p,p*'-dichlorodiphenyldichloroethylene). Much of the emphasis in wildlife toxicology of *p,p*'-DDE has been on birds, particularly focusing on eggshell thinning (e.g., Ratcliffe 1967). The main route of toxicity,

however, is through disruption of neurochemical function. Nevertheless, endocrine disruption has been frequently cited as an important aspect of the toxicity of DDE and other OC pesticides. Although at first it was hypothesized that DDE acted as an environmental estrogen, later work has demonstrated that it can be a much stronger anti-androgen. *p*,*p*'-DDE is an androgen antagonist in developing male rats, causing reduced anogenital distance at birth and the retention of thoracic nipples (Kelce et al. 1995). The researchers found, however, that DDE's ability to bind to the estrogen receptor was only about 1/1000th as effective at binding to the estrogen receptor as 17β-estrodial.

One of the best examples of the effects of organochlorine pesticides is the disruption of reproduction, development, and endocrine function of alligators at Lake Apopka, Florida. Given the very large literature on the subject, I will give an outline of the events, and suggest the reader refer to the review by Campbell (2003) for a more detailed account, as well as that by Guillette (2000). In 1980, there was a large spill by Tower Chemical of Kelthane (dicofol) and up to 15% DDT or DDT metabolites (Clark 1990) into Lake Apopka. Hatching success of alligators from Lake Apopka, after the spill, was sometimes as low as 4%, whereas hatching success ranged from 65 to 82% at the Lake Woodruff National Wildlife Refuge, Orange Lake, and the Everglades Water Conservation Areas in the same time period (Rauschenberger et al. 2007). From 1983 to 1986, egg viability of alligators from Lake Apopka declined, whereas in other lakes surveyed there was no trend (Woodward et al. 1993). The density of juvenile alligators from Lake Apopka fell during the same time period.

Although there has been considerably less research into the toxicity of dicofol compared to DDT, dicofol is related to DDT, and differs only in that there is a single hydroxyl on the carbon linking the 2 phenyl rings. It is likely that dicofol has mechanisms of toxicity similar to those of DDT. Dicofol has been shown to be a thyroid hormone (3,3',5-L-triiodothyronine) antagonist in *Xenopus* (Sugiyama et al. 2005), a weak estrogen agonist using the yeast-based steroid hormone receptor gene transcription assay (Hoekstra et al. 2006), a potent inducer of CYP2B in exposed Chinese hamsters (Flodström et al. 1990), and a weak aromatase inhibitor in human placental microsomes (Vinggaard et al. 2000), and has also showed some ability to bind with androgen receptors in MCF-7 breast cancer cells (Okubo et al. 2004).

In 2000 and 2001, dead alligator embryos and hatchlings were necropsied to examine relationships between contaminant exposure and pathology (Sepúlveda et al. 2006). Total organochlorine pesticide residues in yolk ranged from 0.1 to 52 μg/g, wet weight. The most common gross findings were generalized edema (34%) and organ hyperemia (29%), followed by severe emaciation (14%) and gross deformities (3%). Necropsies revealed histological lesions in 35% of the animals, most of which had pneumonia, pulmonary edema, and atelectasis (Sepúlveda et al. 2006). Clutches that had higher residues of pesticides had a higher prevalence of lesions. They concluded that growth retardation and respiratory abnormalities contributed to mortality, and that OC pesticides may increase the risk of exposed animals to various pathologic conditions. Rauschenberger et al. (2007) also found that organochlorine pesticides were likely impacting egg viability of alligators from contaminated lakes in Florida. They exposed female alligators to chlordane, *p*,*p*'-DDE, toxaphene, and dieldrin through diet such that the resulting concentrations in the eggs were similar to those of wild clutches; eggs of dosed females had reduced viability compared to control alligators. Concurrent field surveys showed that free-ranging alligators that had egg burdens similar to those in the laboratory dosing study also had reduced egg viability (Rauschenberger et al. 2007). These data suggest that pesticide contamination is likely causing low clutch viability in alligators that are exposed to high concentrations of organochlorine pesticides. Thiamine deficiency may be influencing alligator hatching success at the lakes with greater OC pesticide exposure (Sepúlveda et al. 2004), although whether it is through direct toxic action of the pesticides or indirect influence of differences in prey items is unknown.

Decreased egg viability was not the only impact that the pesticide spill had on the alligator populations. Juvenile alligators from Lake Apopka in the years following the spill had poorly developed testes and small phalli (Guillette et al. 1994) relative to alligators from reference lakes. Juvenile

alligators from Lake Apopka had significantly smaller penises and lower plasma concentrations of testosterone than alligators from reference lakes (Guillette et al. 1996); the anti-androgenic action of DDE was considered to be a possible cause of the differences in sexual development. They further asserted that the morphological differences observed in the alligators were not associated with the (then current) serum concentrations of the environmental contaminants that were measured, but instead they could have been due to embryonic exposures affecting development (Guillette et al. 1999). Laboratory dosing studies helped elucidate the effect of DDE exposure to sexual development of exposed alligators. In ovo exposure of alligators to DDE caused a female-biased sex ratio at intermediate temperatures, where both sexes were expected to be formed. Similarly, Matter et al. (1998) found that alligator eggs treated with DDE or 2,3,7,8,-tetrachlorodibenzo-*p*-dioxin and incubated at the male-producing temperature of 33 °C produced greater than expected female hatchlings. A mixture of nonestrogenic pesticides (chlordane) and weakly estrogenic pesticides (dieldrin and toxaphene) inhibited the binding of 17β-estradiol to alligator estrogen receptors.

Like DDE, chlordane has been shown to cause a number of endocrinological alterations in exposed reptiles. Red-eared sliders exposed as embryos to chlordane, trans-nonachlor, and DDE had reduced growth rates during fasting but increased growth rates while fed *ad libitum* (Willingham 2001). Furthermore, when slider turtle embryos were incubated at a male-producing temperature, the sex ratio of the hatchlings was female biased (Willingham 2004; Willingham and Crews 1999). Both chlordane and trans-nonachlor appeared to be more potent in altering sex determination than DDE, and were effective at 0.125 to 0.5 ng/egg compared to 7 to 28 ng/egg for DDE. There were synergistic interactions between chlordane and DDE with sex determination; mixtures of the 2 pesticides were more potent in altering the sex ratio than when applied singly (Willingham 2004). Trans-nonachlor, cis-nonachlor, Aroclor 1242, DDE, and chlordane all caused an increase in the number of female sliders produced at male-producing temperatures, although only chlordane caused sex reversal when applied along with estradiol (Willingham and Crews 1999). Sliders were treated with Aroclor 1242 (0.424 ng/10 g egg) or chlordane (0.451 ng/10 g egg) in the eggs at temperatures producing both sexes; males that were produced had reduced testosterone, while females had reduced progesterone, testosterone, and 5α-dihydrotestosterone compared to controls (Willingham et al. 2000). Although the interaction between these compounds and follicle-stimulating hormone (FSH) was examined, no interaction between these organochlorines and FSH in the sex steroid production was found (Willingham et al. 2000). DDE and chlordane exposure had also altered growth patterns in red-eared sliders, although not in a standard dose-response fashion (Willingham 2001).

Pesticide spills impacted crocodilians at localities other than Lake Apopka. Following a toxaphene spill from a cattle dip tank in 1978, Nile crocodiles may have declined in the Hluhluwe River, South Africa (Brooks and Gardner 1980), as some of the animals appeared to leave the area following the spill. The ovaries and testes of Australian freshwater crocodiles and estuarine crocodiles that had very high concentrations of DDE and toxaphene (range = 0.071 to 672 μg/g lipid weight for DDE) were also examined histologically for gonadal abnormalities and blood chemistry (Yoshikane et al. 2006). Although there were site differences in uric acid, cholesterol, high-density lipoprotein, and low-density lipoprotein concentrations in blood serum, they were not correlated with body burdens. Similarly, there were no obvious effects on gonad histology of the large burden of pesticides and their metabolites carried by exposed animals (Yoshikane et al. 2006). Laboratory exposures of eggs of *Caiman latirostris* to atrazine (0.2 ppm) and endosulfan (2 and 20 ppm) resulted in increased egg weight loss and reduced hatchling mass, though there was no effect on sex ratios (Beldomenico et al. 2007).

From 1987 to 1994, 19 eastern box turtles (*Terrapene carolina carolina*) from various locations on Long Island, New York, showed a number of pathological symptoms (Tangredi and Evans 1997). Elevated concentrations of chlordane and endosulfan metabolites were found in the livers of 2 and 1 animals, respectively. Tangredi and Evans (1997) argued that these 2 pesticides may have caused immunosuppression in exposed animals, relating to the infections that they observed.

Organochlorine pesticides can affect sexual development of gonadal tissue of exposed animals by interfering with normal sex steroid function independent of the sex steroid receptors. Although there have been earlier reports of xenobiotics affecting aromatase (P450 19) activity (e.g., ethanol; Gordon et al. 1978), as well as environmental contaminants (dioxin and PCB 126; Drenth et al. 1998), the concept that aromatase may be a target of environmental contaminants was popularized by recent studies on gonadal development of frogs exposed to current use pesticides. Induction of aromatase by atrazine has been hypothesized to cause feminization of leopard frogs (*Rana pipiens*; Hayes et al. 2003). Aromatase converts androgens to estradiol; thus, alterations in the induction of aromatase may alter the steroid environment without direct interaction with sex steroid receptors. *p,p*'-DDE, *o,p*'-DDT, and *o,p*'-DDE inhibited aromatase activity in exposed human term placental explants, which reduced estradiol secretion (Wojtowicz et al. 2007). A triazine pesticide, atrazine, and *p,p*'-DDE were exposed to a green sea turtle immortal testis cell line to examine their ability to alter aromatase (Keller and McClellan-Green 2004). Atrazine induced aromatase activity, while DDE inhibited aromatase, although only at a concentration (100 μM) that was cytotoxic (Keller and McClellan-Green 2004). Similarly, aromatase activity in alligator eggs was unaffected by exposure to DDE at 100 ppb, even though DDE significantly altered sex determination by favoring females (Milnes et al. 2005). Plasma testosterone, oviductal epithelial cell height, and phallus morphology were similarly unaffected from DDE exposure. Aromatase has a critical role in the control of sexual development in species with temperature-dependent sex determination (Bishop et al. 2009). The weight of evidence indicates that incubation temperature regulates the expression of aromatase enzymes, which in turn affects estrogen production and the sexual differentiation of the gonads (Crews 1996). Thus, sexual development of reptiles may be affected by compounds that affect aromatase activity (Keller and McClellan-Green 2004; Willingham 2005; de Solla et al. 2006).

Vitellogenin assays can be a useful biomarker for determining estrogenic actions of xenobiotics. Palmer and Palmer (1995) demonstrated that *o,p*'-DDT treatments induced vitellogenin in male slider turtles and *Xenopus* in a dose-related manner. Rainwater et al. (2007) examined vitellogenin in adult and juvenile Morelet's crocodiles that had body burdens of DDE ranging from below detection limits to 605 ng/g in plasma. They used an enzyme-linked immunosorbent assay (ELISA) developed specifically for Morelet's crocodiles. They found that although vitellogenin was observed in 9 out of 10 adult females, it was not detected in juvenile females ($n = 48$) or any males ($n = 202$).

Keller et al. (2004a) found that total chlordane concentrations in plasma of loggerhead turtles were negatively correlated with red blood cell counts, hemoglobin, and hematocrit, and suggested these alterations in blood parameters were consistent with anemia. Positive correlations were observed between most OC contaminants and white blood cell counts, and negative correlations were seen between both mirex and the sum of non- and mono-*ortho* PCBs with alkaline phosphatase (ALP) activity. There were also significant correlations between contaminant concentrations and plasma concentrations of blood urea nitrogen, albumin–globulin ratio, glucose, sodium, and magnesium. Keller et al. (2004a) suggested that OC contaminants might be affecting the health of loggerhead sea turtles at lower concentrations that accumulate in other wildlife. Relations between organochlorine contaminants and immune function were examined in free-ranging loggerhead sea turtles (Keller et al. 2006). Lysozyme activity was negatively correlated with whole blood concentrations of *p,p*'-DDE and total chlordanes, while lymphocyte proliferation responses were positively correlated with total PCBs. In vitro exposures of *p,p*'-DDE and Aroclor 1254 to peripheral blood leukocytes increased phytohemagglutinin- and phorbol 12,13-dibutyrate-induced lymphocyte proliferation at concentrations below those that affected cell viability. The similarities between the in vitro experiments and the mensurative study of free-ranging turtles suggest that loggerhead turtle immune function may be affected by OC exposure (Keller et al. 2006). Corn snakes (*Elaphe guttata*) fed with mice injected with α-chlordane, Aroclor 1254, and lindane at 2, 8, and 4 mg/kg, respectively, once a month for 6 months, showed no differences in peripheral blood leukocytes or in ratio between heterophil and lymphocytes (Jones et al. 2005). The 3 compounds 2,3,7,8-dioxin, *o,p*'-DDE, and *p,p*'-DDE altered T- and B-lymphocyte blastogenesis in American alligator hatchlings following in ovo

exposure, although not in a dose-response fashion (Peden et al. 1996). Holladay et al. (2001) found a relationship between lindane hexachloride, heptachlor epoxide, and oxychlordane concentrations in the liver with reduced serum and hepatic vitamin A, and with squamous metaplasia and aural abscesses in eastern box turtles.

10.3.3 Polycyclic Aromatic Hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) consist of 2 or more fused benzene rings. Although PAHs do not contain heteroatoms or carry substituents (i.e., consist solely of hydrogen and carbon), they usually contain up to 7 aromatic rings or occasionally more (Figure 10.6). However, aromatic compounds with heteroatoms such as nitrogen, oxygen, and sulfur, such as napthylamine and quinoline, often have activity similar to that of PAHs (McElroy et al. 1989). Generally, PAHs are hydrophobic (Hackenberg et al. 2003; see Table 10.2), although there is a large range in their water solubility. In aquatic ecosystems, PAHs have high affinity for organic particles and are deposited within sediments (Neff et al. 2005). PAHs in solution in ambient water or pore water of sediments are much more bioavailable and toxic than those adsorbed to particles (particularly combustion soot or "black carbon") (Gustafsson et al. 1997).

PAHs are formed by both anthropogenic processes and naturally occurring geological and biological processes. Naturally occurring (diagenic) PAHs can be formed from bacteria, fungi, and plants and other transformational processes (Neff 1979), and forest fires are a major source (Youngblood and Blumer 1975). Most environmental sources are from anthropogenic activity, such as fossil fuels (petrogenic-derived PAHs) and incomplete combustion of fossil fuels (pyrogenic or combustion-derived PAHs), although other sources for these types of PAHs do exists (i.e., forest fires). In aquatic environments near urban areas, the main sources of PAHs are from atmospheric deposition and runoff from impervious surfaces of PAHs produced through fossil fuel use (Gschwend and Hites 1981), whereas in marine environments municipal and industrial effluents are the main sources (NRC 1983). Although petrogenic PAHs appear to be bioavailable to a large extent, pyrogenic PAHs are often associated with soot particles in sediments and are not highly bioavailable (Neff et al. 2005).

PAHs have a wide range of biological effects, some of which are due to the large variation in their chemical structures, and thus function, relative to some other classes of organic contaminants.

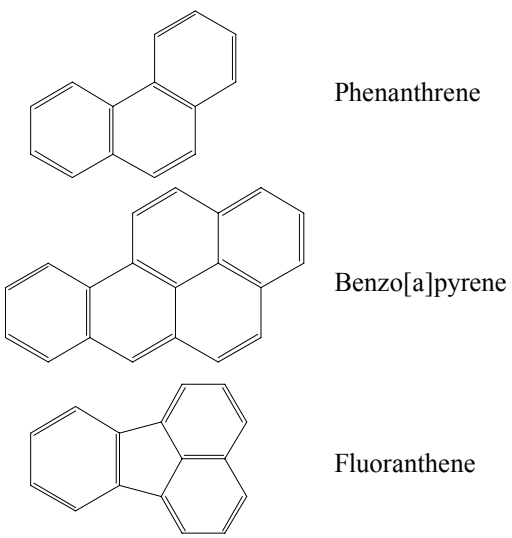


FIGURE 10.6 Three examples of polycyclic aromatic hydrocarbons (PAHs) often found in the environment.

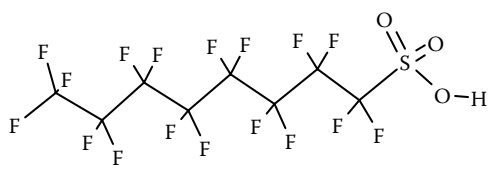
The toxicity of PAHs is highly dependent on their structure, with some isomers ranging from being nontoxic to extremely toxic. Their toxicity in part is mediated through the aryl hydrocarbon receptor (Ah receptor), which is the same receptor that dioxin-like PCBs, dioxin, and furans interact with. However, research based largely on mammalian *in vivo* and in vitro assays suggests that PAHs are also mutagenic and carcinogenic. Much of the toxicity of PAHs is due to their being metabolized *in vivo* to diol-epoxides.

PAHs have been implicated as genotoxic agents in free-ranging reptiles, although studies to date have been limited only to turtles. Matson et al. (2005) found that European pond turtles (*Emys orbicularis*), but not Caspian turtles (*Mauremys caspica*), had elevated chromosomal damage, estimated by using flow cytometry, from sites contaminated with 3-ring PAHs in sediment. Painted and snapping turtle embryos collected from PAH-contaminated sites in Pennsylvania had elevated deformity rates, including lethal abnormalities, compared to a reference site (Van Meter et al. 2006). There was a positive relationship between laboratory exposure to PAHs and severity of deformities in embryos collected from 2 of the clean reference sites (Van Meter et al. 2006), although not from embryos collected from a PAH-contaminated site. It is possible that the embryos previously exposed to PAH contamination were not affected by additional exposure to PAHs. Fish from highly contaminated areas sometimes exhibit phenotypic or physiological resistance to contaminant-induced toxicity, including PAHs (Wirgin and Waldman 2004). Whether slowly reproducing reptiles such as turtles are likely to evolve phenotypic defense mechanisms to anthropogenic-induced stress from contamination is unknown, although not likely.

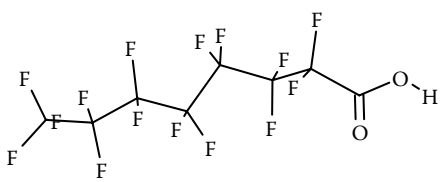
10.3.4 Perfluorooctanesulfonate (PFOS) and Perfluorooctanoic Acid (PFOA)

Although most halogenated organic compounds that have been measured in reptilian tissue have been organochlorines (i.e., PCBs, PCDDs, and pesticides), fluorinated organic compounds have also been measured recently. Perfluorooctane sulfonate (PFOS) has been manufactured for about 50 years, and is used in refrigerants, surfactants, and polymers, and as components of pharmaceuticals, fire retardants, paper coatings, and insecticides (Key et al. 1997). Unlike most other organic compounds considered in this chapter, PFOS and related compounds do not have any ringed components in their chemical structure (Figure 10.7).

Kannan et al. (2005) measured PFOS in the plasma of 5 snapping turtles from Lake St. Clair, which ranged from 105 to 169 ng/mL (mean: 137 ng/mL) in males and <1 to 8.8 ng/mL (mean: 6.13 ng/mL) in females. Kannan et al. (2005) attributed these sex differences to oviparous transfer of PFOS into eggs, although PFOS was only measured in the plasma of adults. Perfluorooctanoic acid



Perfluorooctane sulfonate



Perfluorooctanoic Acid

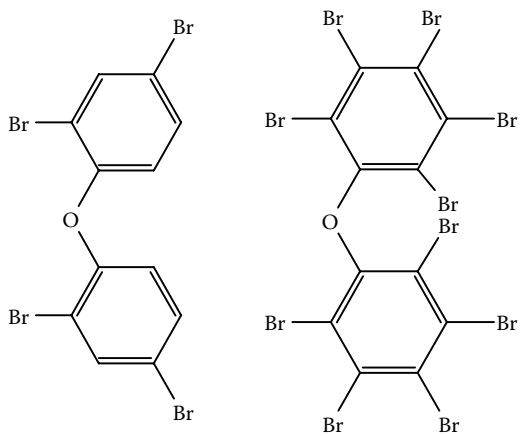
FIGURE 10.7 The surfactants perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA).

(PFOA) was below detection limits in turtle plasma. Although comparisons were difficult to make, given that different tissues and locations were measured, PFOS concentrations in turtle plasma were comparable to concentrations in liver, kidney, and muscle tissue of bald eagles (*Haliaeetus leucocephalus*), green frogs (*Rana clamitans*), and salmonids (Kannan et al. 2005). PFOS was also measured in the livers of yellow-blotched map turtle (*Graptemys flavimaculata*; Giesy and Kannan 2001) and ranged from 39 to 700 ng/ml. Keller et al. (2005) examined PFOS and PFOA in the plasma of loggerhead sea turtles (*Caretta caretta*) and Kemp's ridley sea turtles (*Lepidochelys kempii*) from South Carolina. Mean concentrations of PFOS and PFOA were 11.0 and 3.20 ng/mL for loggerhead turtles and 39.4 and 3.57 ng/mL for Kemp's ridley turtles. Perfluorocarboxylates (PFCAs) were also detectable, with PFCAs with 8 to 12 carbons more prevalent than those with 6 or 7 carbons, while 4 or 5 carbon PFCAs were undetectable. Mean PFOS concentrations were 2 to 12 times higher than typical mean PCB concentrations measured previously in sea turtle blood (Keller et al. 2005).

10.3.5 Polybrominated Diphenyl Ethers (PBDEs)

More recently, brominated organic compounds have also been measured in reptilian tissues. Polybrominated diphenyl ethers (PBDEs) are additive flame retardants that were produced commercially in North America in the form of penta-BDE until the end of 2004, but use of existing stock continues. Octa- and deca-PBDEs have been increasing at near exponential rates (at least up to the year 2000) in North American biota, and in particular in Great Lakes herring gull eggs and fish. PCBs and PBDEs have been shown to possess common mechanisms of toxicity, although not necessarily potency, and PBDEs can alter thyroid function, show aryl hydrocarbon receptor agonism, and cause neurotoxicity (Hallgren et al. 2002; Chen and Bunce 2003; Branchi et al. 2003). PBDEs are very similar to PCBs in their structure; they differ only by the substitution of chlorine by bromine (both halogens) and the addition of an oxygen between the 2 phenyl rings (Figures 10.2 and 10.8).

PBDEs have been measured in snapping turtle eggs (2001 to 2004) throughout the lower Great Lakes, and appear to be highest (mean up to 73.3 ng/g in Hamilton Harbour, Ontario) in urban settings (de Solla et al. 2007). The PBDE congeners found in snapping turtle eggs were consistent with exposure to penta technical mixtures, and not octa or deca technical mixtures. Concentrations of



PBDE 99 PBDE 209

FIGURE 10.8 Polybrominated diphenyl ethers (PBDEs); BDE 99 is typically found in penta formulations, whereas BDE 209 is found in the deca formulation.

PBDEs were approximately one-tenth those of PCBs (de Solla et al. 2007). PBDEs have also been recorded in plasma of Cumberland slider (*Trachemys scripta troosti*) and common musk turtle (*Sternotherus odoratus*) from the Tennessee River Gorge, Tennessee; means of 1.4 and 1.3 ng/g were found in musk turtle and slider plasma, respectively (Moss et al. 2009). In both species, concentrations of PBDEs appeared to be higher in males than in females. PBDEs were also measured in Chinese softshell turtles (*Chinemys reevesii*) from a small lake in Beijing, China, which receives effluent discharged from a large sewage treatment plant (Wang et al. 2007). Concentrations of total PBDEs (~1 ng/g, ww, unknown tissue) in Chinese softshell turtles were lower than those in any fish species measured, despite feeding at a higher trophic position (~3.5) than some of the fish species (2.5 to 3.9; Wang et al. 2007).

Polybrominated phenoxyanisoles are compounds related to polybrominated diphenyl ethers, although they may have either natural origins or anthropogenic origins as metabolites from biotransformation of anthropogenic polybrominated diphenyl ethers (Haglund et al. 1997). Concentrations of 0.2 to 0.24 μg/g (lipid weight) of 3,5-dibromo-2-(2',4'-dibromo) phenoxyanisole (BC-3, 6-MeO-BDE 47) were found in crocodile eggs (unspecified species) from Australia (Melcher et al. 2005). Concentrations of 2'-MeO-BDE 68 were lower than BC-3, 6-MeO-BDE 47 by about a factor of 3.5 (Melcher et al. 2005). It was not clear whether these compounds were of natural or anthropogenic origin in the crocodiles.

10.3.6 Petroleum Products

Petroleum, or the products derived from oil (i.e., diesel, gasoline, etc.), is a complex mixture of hydrocarbons, primarily paraffins, olefins, naphthenes, and aromatics (Figure 10.9). Heteroatoms, usually containing sulfur or nitrogen, are also found in petroleum. The PAHs in oil products may contribute to its toxicity, sometimes being the dominant toxic component. Nevertheless, oil contamination is usually associated with both accidental and purposeful releases in marine environments, and reptiles are sometimes exposed to oil following spills. Oil contamination may also occur through releases of hydraulic, lubricant, and other sources of oil, as well as products associated with petroleum use. Generally, the less lipophilic components of crude oil are more toxic, and weathering typically lowers the toxicity of the oil mixture (Di Toro et al. 2007).

Oil contamination certainly has been associated with reptile kills. Although sea turtles that were rehabilitated following an oil spill may have higher survival than similarly treated sea birds (Mignucci-Giannoni 1999), Hall et al. (1983) found oil both on superficial and in tissues of green turtles and Kemp's ridley turtles (*Lepidochelys kempi*) found dead after the Ixtoc I oil spill in Mexico. Compared to the effects of fishing and boating on turtle populations, oil mortality may be fairly minor. Two of 93 sea turtles found dead on the Canary Islands died from oil exposure, compared to 63 that died from boat collisions, net entanglements, and other activities from 1998 to 2001 (Orós et al. 2005). Nevertheless, oil can cause striking mortality in affected reptiles following exposure, and sometimes in unexpected ways. An oil tanker grounded on the island of San Cristobal (Galapagos) in 2001. Following a spill of 3 million liters of diesel from the grounded tanker, marine iguanas (*Amblyrhynchus cristatus*) on the adjacent Sante Fe Island suffered a massive 62% population decline (Wikelski et al. 2002). Nearby populations on unaffected islands did not experience population impacts. Originally, it was thought that only a few animals were likely to die from the direct effects of the oil spill, as much of the oil was quickly dispersed (Wikelski et al. 2002). However, after the oil had largely dispersed, 62% of the affected population died within the first year, likely from starvation after their gut bacteria were killed (Romero and Wikelski 2002). Starvation is the main cause of natural mortality in marine iguanas. Food deprivation typically induces a strong stress response characterized by increased corticosterone production and/or circulating concentrations in blood (Crespi and Denver 2005; Pravosudov and Kitaysky 2006).

Freshwater reptiles may also be exposed to and affected by petroleum. Nile crocodiles (*Crocodylus niloticus*) and African dwarf crocodiles (*Osteolemus tetraspis*) that were exposed in the laboratory

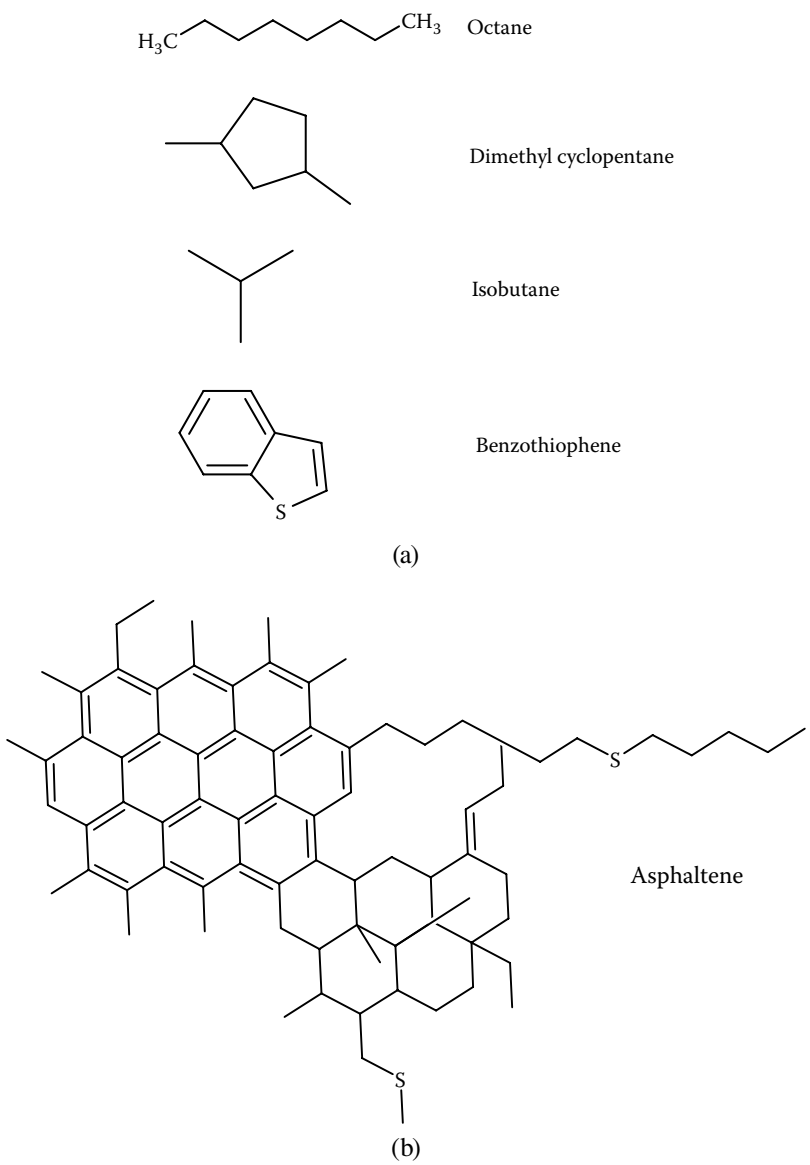


FIGURE 10.9 Hydrocarbons found in crude oil mixtures and in some petroleum products; asphaltene is highly variable. (Reprinted [Adapted in part from Gray 2008] with permission from Gray MR. 2008. Consistency of asphaltene chemical structures with pyrolsis and coking behavior energy fuels 17:1566–1569. Copyright 2008. American Chemical Society.)

to petroleum waste drilling fluid (concentrations 10 to 100%) showed avoidance behaviors to the petroleum. Only 2% of the crocodiles died in any treatment, suggesting that they were somewhat resistant to the drilling fluids, although delayed mortality was suspected (Ekpubeni and Ekundayo 2002). Saba and Spotila (2003) examined the effects of oil exposure and rehabilitation upon survivorship and movement of 4 species of freshwater turtles, although they did not find any effects. Following an oil spill in the Niger Delta, Nigeria, in 1988, turtle communities were monitored in affected and unaffected areas. There appeared to be a reduction of 50% in the species richness of turtles in the area of the spill and a marked reduction in the total numbers of animals found (Luiselli

and Akani 2003). Furthermore, at least one of the species made a large shift in habitat use following the spill (Luiselli and Akani 2003).

Acute (0.5 cm oil layer for 48 hours) and chronic (0.05 cm oil layer for 96 hours) exposure of crude oil to loggerhead turtles (*Caretta caretta*) adversely affected respiratory and salt gland function, blood chemistry composition, and integument surfaces (Vargo et al. 1986). Laboratory exposures of crude oil to juvenile loggerhead turtles indicated that following oiling, not surprisingly, turtles incidentally ingested the oil and oil was found in the throat, eyes, and feces (Lutcavage et al. 1995). The turtles also showed increased white blood cell counts, decreased red blood cell counts, acute inflammatory cell infiltrates, dysplasia of epidermal epithelium, and a loss of cellular architectural organization of the skin layers (Lutcavage et al. 1995). The turtles seemed to have at least partially recuperated after 21 days (Lutcavage et al. 1995), but as stated earlier, mortality can follow after protracted exposure to oil (Wikelski et al. 2002; Romero and Wikelski 2002).

Surfactants used to disperse oil in marine ecosystems following spills can also be toxic. Although not evaluated on reptiles, the surfactants Superdispersant-25® and Corexit 9527® can cause irreversible damage to respiratory organs of affected animals (Scarlett et al. 2005). Thus, there is a potential that remedial actions following oil spills may have their own suite of stressors that can affect reptiles.

10.4 POPULATION LEVEL IMPACTS

There are numerous examples of population level impacts of organic contaminants, particularly OC pesticides, on avian species (e.g., eggshell thinning in raptors). Yet it is not clear what impact organic contaminants have on populations of reptiles. Although there are examples where biodiversity of reptiles appeared to have declined following exposures to organic contaminants (e.g., Luiselli and Akani 2003), few data are available that show whether anthropogenic contamination has led to population declines.

One of the factors that will determine whether a species is susceptible to contaminant-mediated declines is its life history characteristics. Natural mortality of eggs and small juveniles is typically very high, sometimes exceeding 95% (Brooks et al. 1988). Conversely, adult survival in stable long-lived reptile populations is very high and often exceeds 90 to 95% annually (Cunnington and Brooks 1996). Therefore, changes in egg survivorship have little effect on the intrinsic population growth rate, whereas adult survivorship is the dominant factor affecting the population growth rate (Cunnington and Brooks 1996). With some exceptions, contaminants are acutely toxic to embryos or juveniles at lower exposures relative to adults. Thus, the development or mortality of embryos would be affected before later age classes. Second, contaminant-induced depression of hatching success would have little effect on populations, as opposed to depression of adult survivorship. Consequently, except at very high exposures, the expected outcomes of contaminant-related events in long-lived reptilians are reduced survivorship of embryos and sublethal alterations in the health of older age classes. Alternatively, short-lived reptiles are more reliant on recruitment for stability, and thus increased mortality of eggs or juvenile stages could have substantial impacts upon the population structure.

The life history characteristics of reptiles also have implications for sampling methodology. Due to the sensitivity of some reptilian populations to adult survivorship, the collection of eggs or blood plasma is a good alternative to lethal tissue sampling so as to minimize impacts upon populations (de Solla et al. 1998; Portelli and Bishop 2000; Bishop and Martinovic 2000; Keller et al. 2004b). Chorioallantoic membranes are another alternative tissue for measuring organochlorine contaminants (Cobb et al. 2003).

10.5 CONCLUSION

A major component that has not been addressed in this chapter is the relative importance that these organic contaminants have on wildlife. One of the most toxic compounds often measured in reptiles is dioxins/furans, whose LD50 (in rats) is considerably lower than those for PCBs, organochlorine

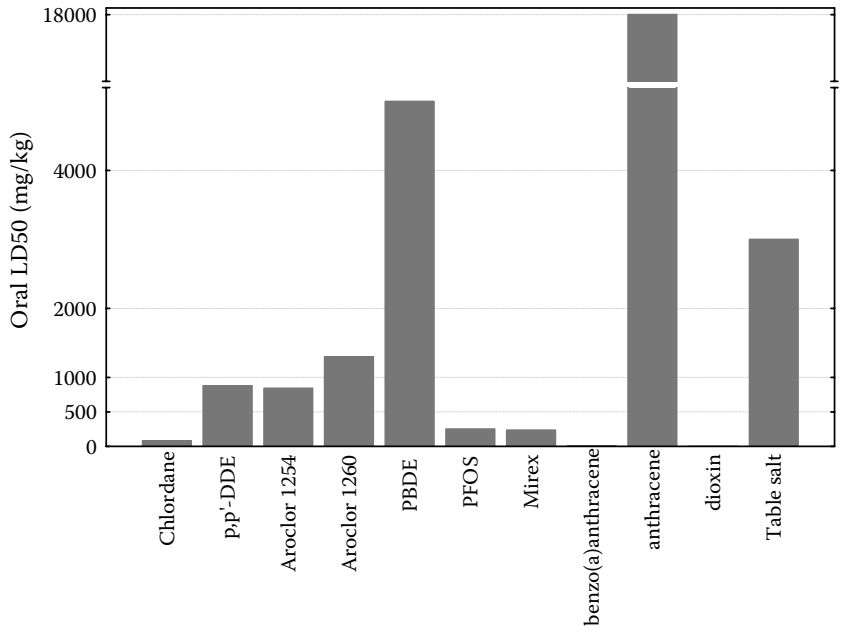


FIGURE 10.10 Oral LD50s (mg/kg) of selected organic contaminants to rats; the lower the value, the greater the toxicity. Table salt was included for comparative purposes. Note the break in scale between 4000 and 18000.

pesticides, and other organics (Figure 10.10). However, dioxins are found at much lower concentrations, and are orders of magnitude lower than PCBs or pesticides. When assessing the toxicity of these compounds in free-ranging wildlife, both the relative toxicity and exposure should be considered simultaneously, although few studies have done so. Synergistic, antagonistic, and additive interactions are generally not assessed (but see Chapter 14, this book). How these compounds interact with other stressors is another major gap in our knowledge. Laboratory studies demonstrating cause and effect would greatly advance our ability to interpret body burdens of free-ranging reptiles, as the risks of many of the compounds are not currently assessable. A particularly distressing gap in knowledge is the link between contaminant exposure and population level effects. Currently, we have no models or data linking alterations in survivorship or health due to toxicological stressors with population growth rates or distribution. There are many challenges yet to face in the field of ecotoxicology of organics and reptiles.

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11 Interdisciplinary and Hierarchical Approaches for Studying the Effects of Metals and Metalloids on Amphibians

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CONTENTS

Over the last 15 years ecologists have become increasingly focused on the effects of environmental pollutants on amphibians. Much of this interest has grown from concerns about the status of amphibian populations and the possibility that environmental contaminants could contribute to population declines at both local and regional scales (Sparling et al. 2001; Davidson et al. 2002; Collins and Storfer 2003; Stuart et al. 2004; Davidson 2004; Fellers et al. 2004; Lannoo 2005). In other cases, ecologists have realized that certain pollutants can be used to test fundamental ecological questions pertaining to amphibian interactions with other community constituents (Boone and James 2005; Relyea 2005; Relyea and Hoverman 2006). Taken together, the recent infusion of ecology into toxicology, and vice versa, has given rise to a wealth of published studies with exciting and sometimes unpredictable findings. Perhaps most notably, studies repeatedly demonstrate that amphibians respond quite differently to compounds in the field than in the laboratory. These situational differences occur for a variety of reasons, the most important of which are duration and mode of exposure, and because effects on amphibians are often mediated through impacts cascading through other community constituents. These important advances by ecologists have caused many to reevaluate current toxicological paradigms as we move forward to determine whether pollutants affect amphibians at the population level (Hopkins 2007).

Although amphibian ecologists have made remarkable achievements in recent years, their efforts have been almost entirely focused on pesticides and herbicides. Much less attention has been paid to ecological effects of metals and metalloids (hereafter referred to collectively as metals), despite their prevalence, toxicity, and persistence in the environment. Because protection of amphibian populations from harmful pollutants is a top priority for amphibian conservation, ecotoxicological studies of metals are of paramount importance. This brief essay highlights why metals in the environment are a potential threat to amphibian health, why previous laboratory approaches to evaluate the effects of metals are limited in their usefulness for ecological assessments, why purely ecological approaches can fail to identify causal relationships between metals and adverse effects in amphibians, and what we consider to be primary research priorities for the near future. We argue that the most significant progress will be achieved through hierarchical assessments spanning multiple levels of organization conducted by interdisciplinary teams of scientists.

11.1 WHY ARE METALS POTENTIALLY HAZARDOUS TO AMPHIBIANS?

Unlike modern synthetic pesticides, herbicides, and fungicides that are typically designed to kill specific taxa (with varying degrees of success in specificity), metals are often emitted into the environment as by-products from human activities. Metals are naturally occurring and many are essential for normal physiological function. However, exposure of organisms to nonessential metals or to essential metals in excessive concentrations can result in toxicity. In most cases in which metals occur in potentially toxic concentrations, anthropogenic activities are to blame. Human activities such as irrigation of metal-rich soils, fossil fuel extraction and combustion, mining, smelting, and urbanization/runoff have resulted in widespread contamination of water, sediments, soil, and air by metals. Whereas direct discharge or runoff to aquatic systems can produce localized areas of relatively high concentrations and risk (Rowe et al. 2001), atmospheric transport of metals such as Hg has resulted in widespread deposition to surface waters and terrestrial habitats (Driscoll et al. 2007). Thus, amphibian habitats can be contaminated with metals from a variety of sources at a range of spatial scales.

Anthropogenic activities release enormous quantities of metals into the environment, posing risks to amphibians and other wildlife. For example, according to the USEPA's toxic release inventory, release of persistent bioaccumulative toxic (PBT) metals far exceeds that of PBT organics (USEPA 2007). In 2005 (the most recent year for which data are available), the release of lead and lead compounds (213 million kg) accounted for 98% of all PBT chemicals. Mercury and mercury compounds also topped the list at 2 million kg. Similarly, release of carcinogenic metals into the environment far exceeds that of carcinogenic organics. Lead (213 million kg) and arsenic (85 million kg) accounted for a combined total of 71% of all carcinogens released in the United States in 2005. That same year, 24 million kg of carcinogenic chromium and chromium compounds were released in the United States.

Unlike many modern pesticides, which are specifically designed to degrade in the environment, metals resist degradation, and thus their release can result in chronic exposures to wildlife. Once released into the environment, many metals undergo complex chemical and physical interactions with particulate and dissolved materials and may be biologically altered (e.g., through conjugation), leading to changes in bioavailability and toxicity. For example, in cases where metals are sorbed to particulate matter they may become less bioavailable, reducing their toxic potential to amphibians and other animals. In other cases, such as in lotic habitats, bioavailability and risks to aquatic organisms may vary spatially from the source; transport of metals from the point source can result in localized dilution near the source but elevated concentrations in downstream sinks (e.g., pools, reservoirs, estuaries). Finally, chemical speciation of metals reflecting site-specific chemical and physical water properties can drastically alter bioavailability and toxicity. Perhaps the best-studied example is Hg, which poses the greatest risk to animals when it exists in the methylated rather than free ionic form. Given that many metals are released into the environment in enormous quantities, are highly toxic, and resist degradation, it is surprising that, relative to synthetic organic compounds, so little ecologically oriented research has been dedicated to quantifying their effects on amphibians.

11.2 WHAT ARE THE LIMITATIONS OF PRIOR STUDIES?

In amphibians, as in other animals, specific metals vary in toxicity, mode of action, and means by which effects are expressed. A comprehensive review of effects of metals on amphibians was recently provided by Linder and Grillitsch (2000), and we do not intend to reiterate the information presented in that document. Rather, we critically evaluate prior approaches in an effort to guide future work to achieve greater ecological relevance. Primarily, we identify what we believe are shortcomings of much work to date, while not leveling criticism at specific works. Most of the concerns that we raise stem from the need for interdisciplinary approaches to resolve complex conservation problems. Our discussion is intended to aid in bridging gaps between mechanistically and ecologically oriented assessments of effects of metals on amphibians. We emphasize that comprehensive assessments having both scientific merit and potential for practical application must draw upon the strengths of multiple disciplines. Our recommendations are targeted toward progress in research that will facilitate a more robust application of experimental results to natural systems contaminated with metals by considering both mechanism and response. Because the status of amphibian populations is a fundamental concern driving much research in amphibian ecotoxicology, it is vital that research be conducted with ecological realism and relevance to management and regulatory applications in mind.

Unlike recent work on pesticides, ecologists have seldom examined effects of metals on amphibians under conditions representative of natural habitats. Rather, until recently most metals have been studied with respect to lethal endpoints, typically in an effort to provide information on relative toxicity of different metals or for use in habitat-specific risk assessments or setting environmental quality standards. Given these goals, these studies have typically been reductionistic, acute laboratory assays that lack the inherent complexity associated with exposure to metals (or other stressors) in natural situations. As a result, much of what we currently understand about the effects of metals on amphibians largely lacks ecological context.

What do we know about effects of metals on amphibians under conditions representing those in natural habitats? The short answer seems to be "very little." Numerous features typical of many prior studies of metals on amphibians belie their application to natural systems. Table 11.1 lists what we perceive as primary limitations to interpreting historical studies of effects of metals on amphibians in the context of natural exposure regimes and ecological application. Doubtlessly it would be extremely difficult to address all of these issues in a given study, and depending upon the goals of the study, some approaches may be more relevant than others. We suggest that researchers consider these issues in the context of the desired application of their studies. These considerations will be critical for protocol development and interpreting results in an environmental context. Clearly some of what we consider to be drawbacks from an ecological perspective would be advantageous in mechanistic toxicological studies for which more reductionistic approaches are essential. This distinction is fundamental to our argument, since we contend that current understanding of effects of metals on amphibians are derived primarily from studies most often directed toward the latter ends. To gain a greater understanding of effects of metals on amphibians as they occur in natural systems, we must step beyond traditional laboratory methodology and accept the challenges of interdisciplinary approaches that simultaneously incorporate greater environmental realism and rigorous toxicological methodology. Such approaches will require collaborative efforts among scientists with different areas of expertise, but sharing the common goal of elucidating threats of metals to amphibian populations.

11.2.1 Biological Issues

The vast majority of studies on amphibians and metals have been concerned with relatively short periods of exposure, often encompassing periods of only days to weeks. Yet, with the exception of situations in which acute pulses of metals are released into or rapidly flushed through a system, or otherwise rapidly become biologically unavailable through chemical or physical interactions,

TABLE 11.1
Issues Limiting Application of Many Prior Studies of Effects of Metals on Amphibians to Ecological Questions

Issue	Drawbacks	Remedy
	1. Biological	
Acute exposure periods	Do not reflect chronic exposures reflective of natural habitats	Conduct exposure over entire duration of life stage of interest as dictated by conditions being modeled
Exposure to dissolved metals only	Potentially dominant routes of exposure (sediment, food) are overlooked	Quantify metals in environmental matrices and set exposures accordingly
Exposures typically address only embryonic/larval life stages	Do not capture effects on juvenile and adult life stages, which may strongly influence population dynamics	Incorporate studies of terrestrial life stages as applicable
Use of standardized test species	Responses are unlikely to apply broadly to natural systems	Choose study species based upon the communities inhabiting area of concern, closely related species, or species that has large geographical range
Artificial feeding regimes	Do not reflect natural resource limitations that may exacerbate effects on growth or survival Food-borne exposures could be higher than when resources are limited	Provide rations that allow for positive growth rates yet are not *ad lib*; pilot studies of dietary requirements would be required
Single species exposures	Do not account for indirect effects that may emerge through differential responses among competitors and predators	Apply hierarchical testing protocols to include both single and multispecies exposures
	2. Chemical and Physical	
Lack of monitoring, control, or reporting of water quality variables (pH, hardness, temperature), particularly in ecological studies (e.g., Rowe and Dunson 1994)	Speciation and complexation vary with chemicophysical properties of the media in which metals are present Water quality influences physiology and thus may mitigate or exacerbate effects of metals alone	Monitor and maintain chemical and physical exposure regimes reflective of those in natural systems Quantify variables that regulate speciation and complexation and employ chemical equilibrium models to estimate free ion concentrations
Exposure to single metal	Do not reflect most natural systems in which pollutant mixtures are present Synergistic or antagonistic interactions among metals cannot be identified	Provide exposures to realistic combinations of contaminants present in system of interest based upon field monitoring

amphibians are typically exposed to metals over long periods of time. With the exception of studies specifically designed to model such episodic exposure events, results of acute exposure studies are not useful for assessment of effects in most situations in nature. Furthermore, as there are vast differences among amphibian species in the duration of specific life stages, arbitrary exposure durations capture substantially different ontogenetic periods. For example, a 2-week exposure to a rapidly developing species will reflect a much different ontogenetic exposure period than would be experienced by a more slowly developing species treated similarly. Exposure over the entire life

stage(s) that naturally interacts with the metal(s) is much more applicable to conditions that amphibians experience in most environmental situations where metals are consistently present. For example, in cases where metals in the aquatic habitat are of primary concern for pond or stream breeding amphibians, exposures applied over the full embryonic and larval periods would be required if quantifying effects on recruitment to the terrestrial population is the goal of the study.

The route of exposure to metals employed in most studies of effects of metals on amphibians also may be inappropriate for assessing responses as they occur in some situations in nature. With very few exceptions, studies have employed aqueous exposures of dissolved metals to embryonic and larval amphibians. Yet in many contaminated habitats, metals are sequestered in sediments, soils, or food sources, providing an additional, and sometimes the predominant, route of exposure. While some metals are primarily available to amphibians in their dissolved forms (e.g., Al, Cu, etc.), for metals such as Hg and Se, the dissolved fraction may be an inconsequential portion of the total exposure (e.g., Pickhardt et al. 2006). Rather, the primary route of exposure may be dietary rather than aqueous. Measurement of metal concentrations in various matrices in some contaminated habitats has revealed high concentrations of metals in periphyton (Newman et al. 1985; Rowe et al. 2001; Unrine and Jagoe 2004; Unrine et al. 2005; James et al. 2005) and surface sediments (e.g., Hopkins et al. 1998) grazed by herbivorous or detritivorous larvae. In such systems, exposure studies using only dissolved metals likely provide unrealistic estimates of amphibian responses as they occur in the environment. It is critical that the relative contribution of metals from dissolved and dietary sources be evaluated prior to designing experiments that capture realistic exposure regimes.

The reader may note that the preceding discussion regarding exposure route and duration primarily addressed effects of metals on embryonic and larval life stages. This apparent bias reflects the unfortunate dearth of empirical information that exists regarding the effects of contaminants in general on terrestrial life stages of amphibians. The biphasic life cycle of most amphibians, putting them at risk of "double jeopardy" due to their occupation of distinct habitat types presenting multiple sources of stressors (e.g., Dunson et al. 1992; Rowe et al. 2003), has been invoked as a justification for their use as sensitive sentinels of environmental change. Yet, the research community has largely remained focused on studies of embryos and larvae, providing little empirical evidence to support this hypothesis and to evaluate relative influences of multiple stressors over a full life cycle. While there are certainly logistical justifications for focusing on easily collected and maintained embryos and larvae, logistics should not be the primary driver of environmental research. Studies on terrestrial life stages that have been conducted have sometimes revealed strong effects of metals and other chemical factors on behavior, survival, reproductive success, and distributions (Wyman and Hawksley-Lescault 1987; Horne and Dunson 1994a; James et al. 2004; Hopkins et al. 2006) that would otherwise have been overlooked in embryonic and/or larval assessments. Moreover, demographic models suggest that juvenile and adult vital rates are often primary drivers of amphibian population dynamics (e.g., Vonesh and De la Cruz 2004; Schmidt et al. 2005), and thus effects on embryos and larvae, unless occurring over numerous cohorts, may have relatively limited impacts on populations.

Regardless of taxa, a nearly universal feature of studies designed to assess the relative toxicity of contaminants is the use of standardized protocols using model species, vital for eliminating confounding of results arising from species-specific differences in sensitivity. Comparative toxicology of amphibians is no exception, and standard species have been adopted and widely applied (notably the African clawed frog, *Xenopus laevis*; Dumont and Schultz 1983; ASTM 2004). There is value in these studies when specifically employed to establish relative toxicological risks among different taxonomic lineages (e.g., fish vs. frogs), and different contaminants or chemical species of contaminants. Yet use of the amphibian model has extended beyond basic comparative toxicology, and has been used to assess ecological risks associated with contamination of natural habitats. Extreme caution must be used in such application of laboratory models to natural systems since an implicit assumption in such extrapolation is that the laboratory model possesses sensitivity to contaminants representative of local species of interest. Given the evolutionary and ecological diversity of amphibians, no single model species can possibly be representative of this entire class of

vertebrates. In fact, acute toxicity tests have clearly demonstrated that even different populations of the same species can differ widely in sensitivity to pollutants (Bridges and Semlitsch 2000). Thus, while model species may be useful for mechanistic studies and initial probing of the relative toxicity of a compound, it is difficult to justify their sole use when ecological assessment is the goal. In cases where surrogate species must be used for experimental manipulations, such as when assessing risk to a declining or rare amphibian species, great care should be taken to select closely related species with ecological attributes similar to those of the species of interest.

Proper provisioning of food resources in experimental exposures of amphibians to pollutants has rarely been carefully considered, even though regulation of individual and population growth and community structure through resource limitations is a paradigm of ecology (e.g., MacArthur and Wilson 1967). There is a large body of literature demonstrating that intra- and interspecific competition can be a primary determinant of growth and recruitment of amphibians under natural conditions (see critical review by Skelly and Kiesecker 2001). Yet the vast majority of laboratory studies of amphibian ecotoxicology are conducted under conditions of unlimited (*ad lib*) resource availability. In applying results of such studies to natural systems, several issues arise, such as 1) observed growth and survival rates, typical endpoints in ecotoxicological studies, are unlikely to reflect those in natural habitats; 2) in aqueous exposures, higher than natural growth rates may result in growth dilution of accumulated contaminants, resulting in body burdens different than would occur naturally; 3) in dietary exposure studies, contaminant exposures will exceed those experienced by individuals in the systems being modeled; and 4) physiological factors relating to nutritional state of the animal can greatly alter responses to contaminants (Hopkins et al. 2002, 2004).

Studies of effects of metals on amphibians have largely been conducted using single-species exposures. Certainly single-species studies have value in assessing potential direct effects of metals on that species. However, single-species studies fail to capture the biological complexity of natural systems that can mediate the effects stressors on a species of interest (e.g., Dunson and Travis 1991; Relyea et al. 2005). Testing multiple interacting species is challenging, especially when experimental conditions are meant to reflect those in nature. However, perhaps more so than researchers in any other discipline, amphibian ecologists have broadly employed multispecies testing in mesocosms to model stressor effects under conditions of ecological complexity (Wilbur 1989; Rowe and Dunson 1994; Boone and James 2005; Metts et al. 2005; Relyea and Hoverman 2006). Originally being applied in studies of nontoxicological variables, primarily competition and predation, multispecies mesocosm studies have been embraced by researchers studying organic contaminants (see review by Boone and James 2005), yet rarely have they been applied to study metals (but see Horne and Dunson 1995; Roe et al. 2006).

In suggesting that multispecies studies be applied more widely to future studies of metals, there are some caveats of such an approach that must be recognized (Hairston 1989). Depending upon the comprehensiveness and the desired rigor of the studies with respect to toxicological and ecological causes and effects, multispecies studies on their own may or may not be adequate to address the questions posed. When conducted in isolation, multispecies mesocosm studies typically preclude establishing pathways by which observed effects emerged. While results from these studies may be of greater applicability to nature than single-species laboratory studies, the mechanism by which the stressor elicited the response often remains speculative because of the complexity of these experimental systems. Thorough sampling and quantification of numerous biotic and abiotic variables can help to identify potential indirect pathways (e.g., reductions in food resources, increased competition, elimination of predators) by which the responses arose, but the relationships between community changes and responses of the amphibian of interest remain correlational. Thus, if understanding the effects of a metal at the species level as well as the community level is desired, multispecies testing alone is not satisfactory. Rather, multilevel, hierarchical studies that include laboratory tests to directly establish species-specific sensitivities and responses, in conjunction with more complex and environmentally realistic multispecies tests in the field or in mesocosms that capture effects *in toto* resulting from direct and indirect effects (Diamond 1986; Sadinski and Dunson

1992), can provide better assessment of cause (e.g., physiological response/species sensitivity) and effect (e.g., recruitment from a breeding site).

An additional caveat with respect to multispecies studies using mesocosms or field enclosures is that information derived from them is unlikely universal to other systems, and may essentially be unrepeatable (see Hairston 1989). Initial conditions, variations in community structure, interannual or geographic variations in climatic conditions (temperature, precipitation), and water quality can mediate ecological interactions, contaminant exposure regimes, and the nature and severity of response. Thus, in isolation, multispecies, community-level studies can only be rigorously evaluated with respect to the specific suite of biotic and abiotic conditions that prevailed throughout the study. As a result, their value to regulatory and management decisions is greatest when applied to local conditions or very specific scientific questions. For example, if single-species laboratory studies demonstrate that a particular metal decimates aquatic invertebrates but not amphibians, mesocosm studies can elucidate how the effect on invertebrates might cascade through a food web (e.g., starving predatory salamanders that eat invertebrates) when considered in a community context.

11.2.2 Chemical and Physical Issues

In addition to the biological issues discussed previously, there are several issues related to chemical and physical variables that need to be considered when designing and interpreting studies of metals and amphibians. Two such issues are particularly important to consider. First, physicochemical properties of water, sediment, and soil have profound influences on availability and toxicity of metals. Lack of control or monitoring of nontoxicological abiotic parameters thus confounds interpretations of the effects of the metals themselves and precludes rigorous comparisons of effects among different studies or field sites. Investigators should be sensitive to the problems in interpreting results with respect to actual contaminant exposures experienced by the individuals due to physicochemical properties inherent to outdoor mesocosms that can strongly influence contaminant partitioning, availability, and toxicity. Second, natural systems are rarely polluted by a single metal (or other contaminant). Therefore, potential additive, synergistic, or antagonistic effects of multiple contaminants in natural settings may have consequences for amphibians that are very different than those predicted from single-factor studies. Complex mixtures of metals are obviously common in industrial euents (e.g., coal combustion wastes; Rowe et al. 2002), yet even in relatively pristine habitats such as isolated vernal pools, mixtures of metals may pose risks to amphibians (e.g., Horne and Dunson 1994b).

Chemical and physical properties of water strongly regulate solubility, speciation, bioavailability, and toxicity of many metals. Factors including temperature, pH, and water hardness play key roles in determining solubility and thus potential toxicity of some metals (e.g., Al, Cd; Leuven et al. 1986; Freda et al. 1990; Freda 1991). As well, the propensity for dissolved organic compounds (Freda et al. 1990; Horne and Dunson 1995) to form complexes with metals can strongly influence the availability of metals for binding to sites of toxic action such as gill lamellae. Thus, it is important to distinguish between total and dissolved metal fractions when interpreting adverse effects to amphibians. Without monitoring or controlling such abiotic variables in laboratory and field studies, it is difficult or impossible to interpret total metal concentrations in a dose-response context. Establishing dose-response relationships based upon nominal rather than measured concentrations of toxicants is now nearly universally accepted as being problematic. However, in a physiological sense, measured concentrations in the absence of quantifying other parameters that affect bioavailability and toxicity are essentially nominal as well.

Comparing the toxicity of metals among multiple habitats is particularly challenging due to the extreme natural variation in physicochemical properties among sites (see Rowe and Dunson 1993; Skelly 2001; Brodman et al. 2003). While quantifying all possible factors potentially influencing metal availability and toxicity in natural systems is unlikely to be feasible, quantification of several primary drivers (pH, dissolved organic carbon [DOC], conductivity) can greatly aid in interpreting

dissolved concentrations of metals with respect to potential toxicity. Quantitative chemical equilibrium modeling tools such as MINEQL (Schecher and McAvoy 1992) are available for use in predicting speciation, and thus the availability and potential toxicity of numerous metals based upon physicochemical dynamics. In conjunction with water quality monitoring, applying such tools to estimate the bioavailable fraction of metals would greatly enhance assessments of risks to amphibians in natural systems.

As well as influencing availability and toxicity of contaminants, abiotic conditions regulate many physiological processes, thereby affecting susceptibility to and expression of contaminant effects. Perhaps most obvious is the influence of temperature regimes on traits of larval amphibians. With the exception of species having very short larval periods, most temperate species experience considerable changes in the thermal environment during development. Processes including growth, feeding and metabolism, and uptake and elimination of contaminants vary accordingly with temperature throughout development. Toxicity of organic contaminants to amphibians can be influenced by temperature (Berrill et al. 1993; Materna et al. 1995), and demonstrated effects of temperature on metal toxicity in other aquatic taxa (e.g., fish; Cairns et al. 1975) suggest that amphibians would be similarly affected. Therefore, chronic laboratory tests that neglect to regulate abiotic factors such as temperature such that they reflect seasonal fluctuations may produce results inconsistent with the system being modeled.

11.3 WHERE DO WE GO FROM HERE?

Ecotoxicological research on the effects of metals on amphibians lags far behind the recent advances made with pesticides and herbicides. We attribute this deficiency in metals research to the current bias by ecologists toward studies on synthetic compounds, and the lack of ecological context provided in the traditional amphibian bioassays commonly adopted by toxicologists and regulators. We believe that the most important pollution problems facing amphibians today cannot be resolved with either pure ecological or toxicological approaches. Instead, interdisciplinary teams adopting hierarchical approaches are needed to make significant progress.

We have highlighted what we perceive to be aspects of many studies of effects of metals on amphibians that most critically need to be considered and improved upon if future studies are to have meaningful application to natural systems and efforts in amphibian conservation. As teams of researchers move forward with interdisciplinary approaches, we hope that our critique will serve as a practical guideline for consideration. With this in mind, we close with a brief discussion of what we consider to be priorities in future research on the effects of metals on amphibians.

11.3.1 Community Level Assessments of Effects of Metals and Mixtures of Metals on Amphibians, Using Field Enclosures or Outdoor Mesocosms

Coupled with laboratory tests of individual species and monitoring of populations occupying contaminated habitats, community level experiments will aid in identifying potential indirect effects of metals on amphibians. Similar approaches are discussed at great length in the literature for pesticides (e.g., Boone and James 2005; Relyea and Hoverman 2006). However, to produce reliable information that is applicable to real-world situations, it is critical that ecologists team with chemists and toxicologists to ensure that interpretations are not compromised by unmeasured variables that obscure the effects of metals themselves, thus negating the potential usefulness of community level analysis.

11.3.2 Effects of Metals on Juvenile and Adult Life Stages

Despite the importance of these life stages to population dynamics, very little is known about how they respond to metals and other contaminants. Studies examining how sediment- and soil-borne metals may affect juveniles and adults through dermal contact and ingestion are critical to assessing the influence of terrestrial contaminants on amphibians relative to aquatic exposures. Endpoints

related to fitness traits, including growth, reproduction, and behavior, should receive priority. Additionally, studies examining physiological function, such as osmoregulation and immune system function, may be important for understanding the mechanistic basis for metal-induced changes in fitness-related parameters.

11.3.3 Maternal Transfer of Metals

Maternal transfer of pollutants has long been known to be one of the most important effects of certain compounds, especially certain organic compounds (e.g., DDT, PCBs, etc.) and inorganic pollutants (e.g., Se and Hg) that are readily transferred to the egg. Yet to date, only 1 study has quantified maternal transfer and adverse effects of contaminants in amphibians (Hopkins et al. 2006). As reproductive success is fundamental to population dynamics, and population status is a key endpoint in risk and damage assessments, a greater understanding of the relationships between adult body burdens and offspring performance and survival may have regulatory implications that will aid amphibian conservation efforts.

11.3.4 Trophic Transfer of Metals in Both Larval and Adult Amphibians

Trophic transfer has rarely been examined in amphibians (Unrine and Jagoe 2004; Unrine et al. 2004, 2005). Dietary exposure to Se and Hg has long been known to be the most important route of exposure for most wildlife, and both dietary and aqueous exposure to Pb represent important exposure pathways. The importance of dietary exposure to Cd has received less attention, but in certain systems fish and wildlife clearly accumulate Cd from their diet (e.g., Croteau et al. 2005). Much more extensive examination on the effect of food-borne metals on amphibian health and fitness is required. Controlled feeding studies combined with chemical/toxicokinetic analyses will provide information necessary to fill this knowledge gap.

11.3.5 Assessment of the Effects of Metals and Mixtures of Metals on Amphibian Populations

Of the research priorities we suggest, this may be the most important and the most difficult to adequately address. Nevertheless, conservation efforts will ultimately fail if we do not gain a better understanding of the influence of pollutants on population dynamics. Establishment of long-term monitoring programs in impacted and reference systems would undoubtedly be a tremendous step toward achieving this goal, yet they are increasingly not feasible due to economic limitations. Population models provide a practical and valuable alternative, yet they too are constrained by the availability of empirically derived estimates of vital rates of all life stages. However, through collaborative studies and sharing of data among researchers, and making well-reasoned estimates of parameters for which data do not exist for the species of interest, sufficiently robust models may be constructed to provide estimates of influences of metals on future population trends.

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12 The Ecotoxicology of Metals in Reptiles

Britta Grillitsch and Luis Schiesari

CONTENTS

12.1 INTRODUCTION

Environmental contamination with metals is a serious problem worldwide. All metals may be toxic above a certain threshold. No less than one-fourth of all metals are included in priority pollutant lists, and some metals such as mercury, lead, and cadmium rank highest among those of regulatory concern (Figure 12.1). Furthermore, metals are ubiquitous, at least in trace concentrations, and are remarkably diverse. More than half of all chemical elements are metals, and occur as dissolved ions and in a wide variety of inorganic compounds, metal complexes, and organometallic compounds, and form myriad alloys.

The study of metal toxicity in reptiles is particularly important because many reptilian species are experiencing declines. Among terrestrial vertebrates, the proportion of reptile species threatened with extinction roughly equals that of amphibians and considerably exceeds that of birds and mammals (reptiles: 8240 extant species with 30% being threatened with extinction; birds: 9956, 12%; mammals: 5416, 22%; amphibians: 6199, 31%; IUCN 2007). Directly or indirectly, chemical pollution is an important threat to reptiles (Gibbons et al. 2000; Hopkins 2000), yet reptiles remain underrepresented in ecotoxicological research (e.g., Hopkins 2000; Sparling et al. 2000a; Gardner 2006, Chapter 1, this volume).

Reptiles can provide an excellent source of information for understanding metal toxicity. For example, ecotoxicological investigations focused on American alligators (*Alligator mississippiensis*) in Florida developed from a classical study of contamination of wildlife with a high-priority metal (mercury) toward one linked to a critical effect category of high-priority concern, endocrine disruption in wildlife. Here, starting in 1989, long-term studies of chemical contamination in alligators were conducted amid intensive investigations on sources and impacts of mercury in the US Fish and Wildlife Service's southeast region (Facemire et al. 1995). The original work initiated in 1989 was motivated by a Florida panther (*Felis concolor coryi*) found dead in Everglades National Park. After it was determined that the panther had high mercury concentrations in liver tissue (110 ppm, wet weight), the region became one of the most intensively studied for wildlife metal contamination worldwide. In part, alligators were chosen as a focal species because of their importance as a regionally harvested game animal and as a source of meat for human consumption. Among findings related to the ecotoxicological kinetics and dynamics of mercury, this long-term monitoring also indicated that, although still high, mercury contamination in alligators in the Everglades declined between 1994 and 1999 (Yanochko et al. 1997; Rumbold et al. 2002), a pattern consistent with that observed in birds and fish. These observations may have been linked to increased rainfall during the observation period (Rumbold et al. 2002), which may have foreshadowed recent hypotheses linking climate change and contamination in any taxon.

However, Florida alligators also provided strong evidence for endocrine disruption in wildlife (Damstra et al. 2000). In 1980, Lake Apopka in Florida was subject to a major spill of organic and inorganic contaminants leading to dramatic, previously unseen adverse effects in male and female alligators such as altered plasma sex steroid concentrations, abnormal gonad and phallus morphology, and reduced clutch viability. As a consequence, the population of alligators in the lake plummeted by 90% within 4 years of the spill. For Lake Apopka, non-metallic organic compounds were considered the likely endocrine-active agents, but pollution-mediated endocrine effects appeared to be more widespread in alligators of the region and causal explanation is still not certain. Despite outstanding research efforts, the "Florida Alligator Case" exemplifies the serious difficulties in establishing cause-effect relationships between complex multi-stressor scenarios and complex multi-level responses in wildlife ecotoxicology (see Burger et al. 2000 and Campbell 2003 for a review of these studies, primarily conducted by Louis Guillette and collaborators).

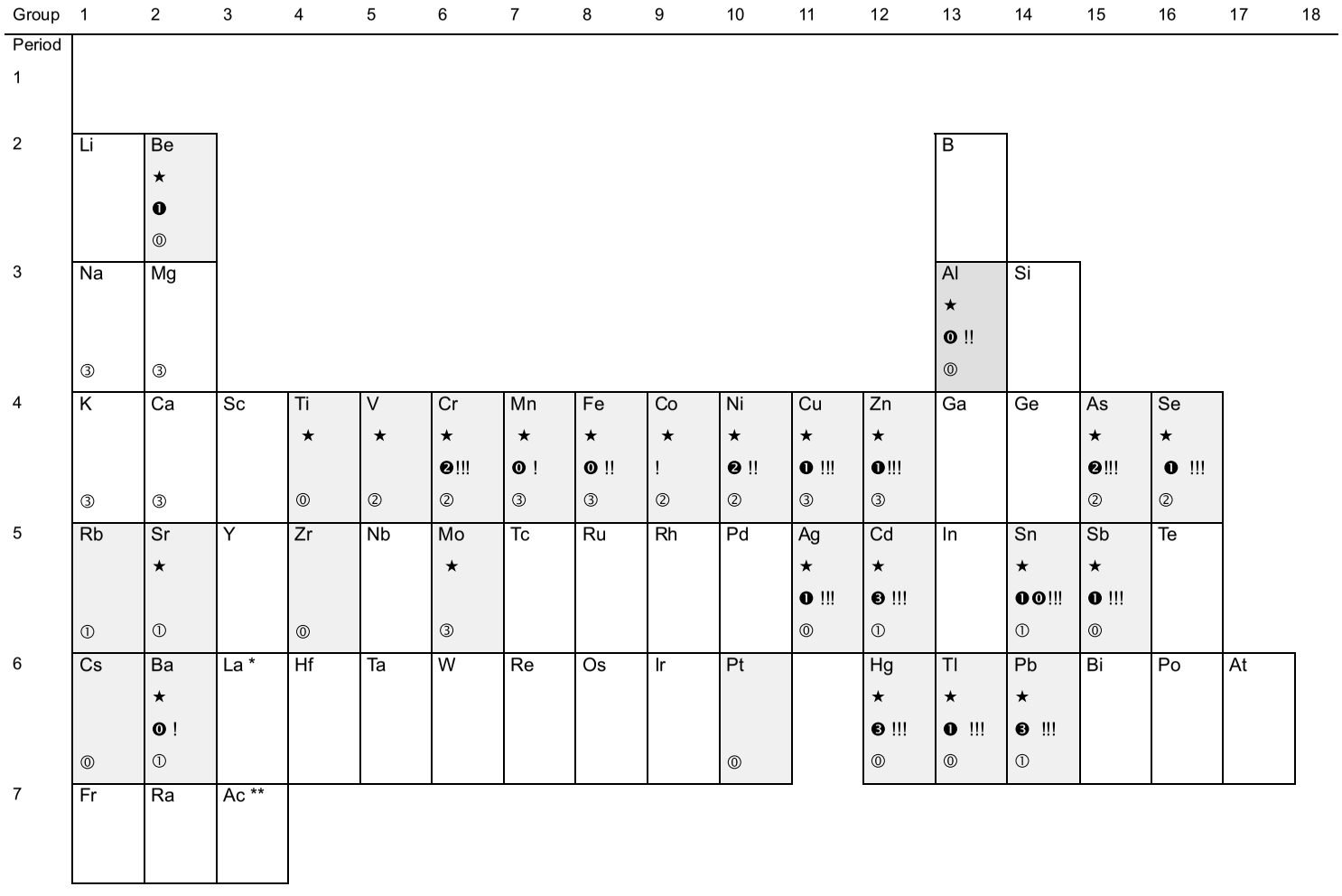

FIGURE 12.1 Periodic table of elements showing the metallic elements included in the present review, and their classification according to their biological and ecotoxicological relevance.

Symbols and abbreviations

Shaded background	Metallic elements in the present review.
★	"Metals and Metalloids of Primary Interest" (USEPA 2007)
❸❷❶⓿	Occurrence of metallic element in 3 current lists of chemicals of priority concern (CEPA [1999], current to March 2, 2006; EC 2001, 2007; USEPA 2006): [3] (present in all 3 lists); [2] (present in 2 out of 3 lists); [1] (present in 1 out of 3 lists); [0] listed as "nonpriority pollutants" in USEPA (2006).
!!! / !! / !	Ecotoxicological relevance of metallic elements: [!!!] high; [!!] medium; [!] "some" (Hellawell 1986; Freedman 1995; Hedgecott 1995).
③②①⓪	Essential metallic elements: [3] essential to most organisms; [2] essential to many organisms; [1] may be essential to some organisms; [0] not essential to all organisms (Merian 1991; Siegel 2002; USEPA 2007).
*	Lanthanide series.
**	Actinide series.

FIGURE 12.1 (Continued)

12.2 OVERALL STATE OF KNOWLEDGE

Numerous review publications on hazards of particular metals to wildlife include information on reptiles (Figure 12.2). Some of the most comprehensive are those compiled by Ohlendorf (1988), Wolfe et al. (1998), Eisler (2000, 2004, 2006), and Rattner et al. 2005. For radionuclides, reviews have been provided by Campbell and Campbell (2000, 2001) and Campbell (2003). Reviews focusing on specific metal sources related to the ecotoxicology of metals in reptiles include the ecotoxicological implications of aquatic disposal of coal combustion residues (Rowe et al. 2002), and geochemical, health, and ecotoxicological perspectives on gold and gold mining (Eisler 2004). Only relatively recently have reviews on the ecotoxicology of reptiles been published, wherein metals were considered among other contaminants (Pauli et al. 2000; Sparling et al. 2000b; Rattner et al. 2005; Gardner and Oberdoester 2006). The ecotoxicology of metals in reptiles was reviewed along with amphibians by Linder and Grillitsch (2000) in the first edition of this book, and subsequently, there has been an increasing number of reviews focused on environmental contaminants (including metals) in certain reptilian taxa such as crocodilians (Campbell 2003), squamata (Hopkins et al. 2001; Campbell and Campbell 2002), lizards (Campbell and Campbell 2000, 2005; Talent et al. 2002), snakes (Campbell and Campbell 2001), and marine turtles (Storelli and Marcotrigiano 2003). Meyers-Schöne and Walton (1994) wrote an earlier review on turtles. In addition, since the publication of the first edition of this book, the primary literature for the ecotoxicology of metals in reptiles has increased (Figure 12.2). During the last decade the number of publications has roughly doubled, and the number of datasets (sum of numbers of metals, reptile species, compartments, and locations analyzed per publication) has increased by a factor of 5, as indicated by counts in the Appendix tables and in Tables 12.5 and 12.6. Overall, the past 10 years indicate that the interest in the environmental chemistry and ecotoxicology of metals continues at and beyond its historic pace, resulting in a broad base of scientific knowledge, and a comparatively modest but expanding scientific interest in the ecotoxicology of reptiles has developed (Figure 12.2). Our chapter aims at systematically organizing and summarizing the intersection of these 2 fields of knowledge.

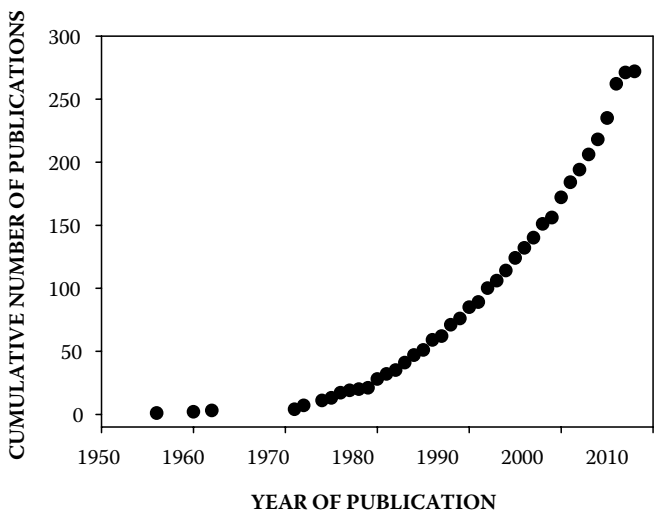

FIGURE 12.2 Chronology of the cumulative number of publications dealing with the ecotoxicology of metals in reptiles.

12.3 TOXICOKINETICS: THE FATE OF METALS IN REPTILES

Ecotoxicokinetics describes the fate of a chemical substance and its distribution among ecosystem compartments over time and space. Exposure characterizes the conditions of co-occurrence or contact of a substance with a biological receptor. A receptor may exist at any level of complexity, from molecule, cell, tissue, organ, and organism to population and community. Regardless of the level of complexity, environmental and biological availability, and eventually toxicological availability of a substance to a receptor at the molecular level are preconditions for a toxicological effects or effect cascades (Peakall and Burger 2003; Hopkins 2006). To predict ecotoxicological effects from exposure and vice versa, we need to understand the mechanisms determining the availability of a substance at different levels of ecosystem integration, in particular at the system barriers along major transfer routes (pathways).

In tables accompanying this section, the information extracted from the literature is considered at different levels of compression. Table 12.1 shows the distribution of information on metal concentrations in reptiles, as indicated by the numbers of publications per reptile species and metal. For the metals of highest-priority concern, Cd, Hg, and Pb, Table 12.2 summarizes the tissue levels observed in conventional (destructive) and alternative (minimally destructive) monitoring tissues, while Table 12.3 presents concentrations in transgenerational transfer. Finally, Table 12.4 presents a complete overview on the information currently available on the distribution patterns of metals among gonadal and early life stage compartments. The Appendix provides detailed results as mean, minimum, and maximum metal concentrations reported for each reptile species in each publication, complemented by key information on the studied organisms and locations.

12.3.1 Toxicokinetic Phases

12.3.1.1 Fate at the Organ System Level

Major routes of metal exchange in adult reptiles are ingestion and elimination through the digestive (particularly gastrointestinal) system barriers, and inhalation and exhalation through the respiratory (particularly pulmonary) system barriers. In addition, metals uptake and elimination may occur via the integumentary system, particularly for reptiles with comparatively high transcutaneous water and gas exchange rates (Lillywhite 2006). To our knowledge, few, if any, studies have differentiated between these co-occurring routes of metal fate in reptiles, and no studies have addressed the transfer of metals through the reptilian skin and lungs. In contrast, during the last decade, several studies have focused on the trophic transfer of metals in reptiles and will be addressed in Section 12.3.2.

As for other terrestrial wildlife (USEPA 2007), the 2 most important exposure pathways of metals to reptiles are the voluntary ingestion of food and the incidental ingestion of soil, sediment, or rocks (along with ingestion of prey, via face licking in geckonids, or as gastroliths in crocodylians) that are geogenically or anthropogenically rich in metals (Wings 2007). Of these, dietary ingestion of metals is documented for Se, As, Cd, Sr, and V from several studies by Hopkins and collaborators (2002, 2006); Cd in European wall lizards, *Podarcis carbonelli*, by Mann et al. (2006, 2007); Cd, Hg, Pb in corn snakes, *Elaphe guttata*, by Jones and Holladay (2006); Pb in crocodiles *Crocodylus porosus* by Hammerton (2003), and alligators by Camus et al. (1998) and Lance et al. (2006) (see Section 12.3.2). These studies confirm that ingested food is very important to the exposure of reptiles to metals, but also that the subsequent bioavailability of metals via trophic transfer (defined as the fraction of a substance present in the environment that is absorbed by an organism) might be overestimated.

Clearly, not all ingested metals are absorbed, and several factors mediate metal absorption and elimination. From a trophic-transfer perspective, one interesting mechanism of metal elimination in prey — the formation of metal-containing granules — is related to reduced metal absorption in predators. Bjerregaard and Anderson (2007) summarized the various types of metal granules hitherto described for snails, bivalves, oligochaetes, crustaceans, and insects, including the wood louse

TABLE 12.1
Distribution of Information of the Contamination of Reptiles with Metals According to Species, and Their Biogeographical and Ecosystem Distribution

Taxa	ZONE	ECO	Al	Ag	As	Ba	Be	Cd	Co	Cr	Cs	Cu	Fe	Hg	Mn	Mo	Ni	Pb	Pt	Rb	Sb	Se	Sn	Sr	Ti	Tl	U	V	Zn	Zr
Testudines																														
Actinemys marmorata 1)	NEA	F, T	1		1			1	1	1		1	1	1	1		1	1										1	1	
Aldabrachelys gigantea 2)	AFR	T																											1	
Apalone spinifera 3)	NEA	F						1																						
Caretta caretta	OCE	M, T	3		6	2		17	2	4		14	11	15	5	2	8	12			1	8	1	1		1		1	13	
Chelonia mydas	OCE	M, T	2	3	8	3		12	4	6	1	10	7	8	7	4	7	10		2	3	7		2		3		3	11	1
Chelonia sp. 4)	OCE	M, T	1							1		1	1	1	1			1											1	
Chelonoidis nigra 5)	NEO	T																											1	
Chelydra serpentina	NEA	F, T						3	1	1		1		10			1	2						1					2	
Chrysemys picta	NEA	F, T						1						1															1	
Cuora amboinensis	IND	F, T										1	1					1		1		1		1					1	
Cyclemys dentata	IND	F, T										1	1							1		1		1					1	
Dermochelys coriacea	COS	M, T		1	2			4		1		4	1	2			3	3				2							5	
Emys orbicularis	PAL	F, T										1	1	2								1								
Eretmochelys imbricata	OCE	M, T		2	2	1		4	1	1		3	1	2	2	2	2	3		2	1	3		1		1		2	4	1
Gopherus agassizii	NEA	T			1			1				1	1	1				1				1							2	
Kinosternon flavescens	NEA	F, T												1																
Lepidochelys kempii	OCE	M, T		1				1				2		1				1											3	
Lepidochelys olivacea	COS	M, T						3	1	1		3	3		3		2	3											5	
Macrochelodina rugosa 6)	AUS	F, T										1																		
Malaclemys terrapin	NEA	F, T			1			1		1				2	1			1				1								
Mauremys caspica	PAL	F, T																				1								
Pelodiscus sinensis 7)	IND	F, T										1	1							1		1		1					1	
Podocnemis unifilis	NEO	F, T												1																
Pseudemys rubriventris	NEA	F, T												1																
Sternotherus odoratus 8)	NEA	F, T												1															1	
Terrapene carolina	NEA	F, T																1											1	
Testudo hermanni	PAL	T										1	1																	

(*continued*)

TABLE 12.1 (CONTINUED)
Distribution of Information of the Contamination of Reptiles with Metals According to Species, and Their Biogeographical and Ecosystem Distribution

Taxa	ZONE	ECO	Al	Ag	As	Ba	Be	Cd	Co	Cr	Cs	Cu	Fe	Hg	Mn	Mo	Ni	Pb	Pt	Rb	Sb	Se	Sn	Sr	Ti	Tl	U	V	Zn	Zr
Testudo horsfieldii	PAL	T																											1	
Trachemys scripta	NEA	F, T			1			4	1	2		3		6	1			2				3		1					3	

Note: Distribution of information is indicated here by numbers of publications. Data derived from Appendix.

1) [*Clemmys marmorata*].
2) [*Geochelone gigantea*].
3) [*Trionyx spinifer*].
4) L: Papua New Guinea.
5) [*Geochelone elephantopus*].
6) [*Chelodina oblonga*].
7) [*Tryonyx sinensis*].
8) [*Kinosternon odoratus*].

Taxa	ZONE	ECO	Al	Ag	As	Ba	Be	Cd	Co	Cr	Cs	Cu	Fe	Hg	Mn	Mo	Ni	Pb	Pt	Rb	Sb	Se	Sn	Sr	Ti	Tl	U	V	Zn	Zr
Crocodylia																														
"Caiman" 1)																		1												
"Crocodilian" 2)																		1												
Alligator mississippiensis	NEA	F, T	2		5		1	6	1	4		4	4	17	3	1	2	8	1			7	2	1		1		1	6	
Alligator sinensis	IND	F, T			1			1		1		2	1	1	1			3											2	
Caiman crocodilus	NEO	F, T												1				1												
Caiman yacare	NEO	F, T																1												
Crocodylus acutus	NEO	F, T	1		2			3	1	1		3		3		1	1	3						1					2	
Crocodylus johnstoni	AUS	F, T				1		1	1			2	1		1		1	1						1			1		1	
Crocodylus moreletii	NEO	F, T			1			1				1		3				1											1	
Crocodylus niloticus	AFR, PAL	F, T	1		1			2	2	1		2	1	2	2	1	1	3				3		1					3	
Crocodylus porosus	AUS, IND	F, T	1			1			1	2		2	2	1	2		1	4				1		2	1		1		2	
Crocodylus rhombifer	NEO	F, T																1											1	
Paleosuchus palpebrosus	NEO	F, T																1												
Paleosuchus trigonatus	NEO	F, T																1												

Paleosuchus sp.	NEO	F, T												1															
Tomistoma schlegelii	IND	F, T															1												

Note: Distribution of information is indicated here by numbers of publications. Data derived from Appendix.
1) L: Brazil.
2) L: Brazil.

Taxa	ZONE	ECO	Al	Ag	As	Ba	Be	Cd	Co	Cr	Cs	Cu	Fe	Hg	Mn	Mo	Ni	Pb	Pt	Rb	Sb	Se	Sn	Sr	Ti	Tl	U	V	Zn	Zr
Squamata: Serpentes																														
"Brown snake" ("Linga") 1)												1																		
"Cobra" 2)																		1												
"Sea snake" 3)																	1											1		
"Sea snake"																													1	
"Snake"4)					1			1				1	1	1	1			1					1						1	
Acrochordus javanicus	IND, AUS	F, T	1							1		1	1	1	1			1						1					2	
Agkistrodon piscivorus	NEA	F, T	2		2	1		1	2	2	1	3	2	5	2		2	2		1		3	1	1			1	1	3	
Coluber constrictor	NEA, NEO	T	1		2			1	1	1		1	1	1	1		1	1				1	1						1	
Crotalus viridis	NEA	T																1												
Cylindrophis rufus	IND	T																											1	
Pantherophis guttatus 5)	NEA	T						1						1				1												
Eunectes murinus	NEO	F, T				1						1	1		1														1	
Lamprophis fuliginosus	AFR	T																				1								
Lapemis hardwickii	IND, AUS	M										1	1					1		1		1		1					1	
Leptotyphlops humilis	NEA	T																											1	
Natrix natrix	PAL	F, T												1															1	
Nerodia cyclopion	NEA	F, T	1		1			1	1	1		1	1	1	1		1	1				1	1						1	
Nerodia fasciata	NEA	F, T	1		5			3	1	3	1	4	1	2	2		1	2		1		5		3			1	3	1	
Nerodia rhombifer	NEA	F, T										1		1								1							1	
Nerodia sipedon	NEA	F, T			3			4		3				4	3			4				3							1	
Nerodia sp. 6)					1			1						1				1				1								
Nerodia taxispilota	NEA	F, T	1		1	1			1	1	1	1	1	1	1		1	1		1		1		1			1	1	1	
Pituophis catenifer 7)	NEA	T																•												

(*continued*)

TABLE 12.1 (CONTINUED)
Distribution of Information of the Contamination of Reptiles with Metals According to Species, and Their Biogeographical and Ecosystem Distribution

Taxa	ZONE	ECO	Al	Ag	As	Ba	Be	Cd	Co	Cr	Cs	Cu	Fe	Hg	Mn	Mo	Ni	Pb	Pt	Rb	Sb	Se	Sn	Sr	Ti	Tl	U	V	Zn	Zr
Pituophis melanoleucus	NEA	T						1		1				1	1			1				2							1	
Pseudonaja nuchalis 8)	AUS	T										1																		
Python molurus	IND	T										1	1					1		1		1		1					1	
Ramphotyphlops sp.																														1
Thamnophis radix	NEA	F, T																1												
Thamnophis sirtalis	NEA	F, T												2				1											1	
Vipera berus	PAL	T						1				1						1											1	
Waglerophis merremii 9)	NEA	T										1	1																	
Xenopeltis unicolor	IND	T																											1	
Boidae: 6 species 10)																													1	
Colubridae: 41 species 11)																													1	
Elapidae: 7 species 12)																													1	
Viperidae: 20 species 13)																													1	

Note: Distribution of information is indicated here by numbers of publications. Data derived from Appendix.

1) L: Western Australia.
2) L: India.
3) L: Saudi Arabia.
4) L: South Taiwan.
5) [*Elaphe guttata*].
6) L: Florida.
7) Not found in Pb table.
8) [*Demansia nuchalis*].
9) [*Xenodon merremii*].
10) *Boa constrictor*, *Candoia aspera*, *Charina bottae*, *Morelia spilota* [*M. argus*], *Python molurus*, *P. reticulatus*.
11) *Arizona elegans*, *Boiga dendrophila*, *Coluber constrictor*, *C. spinalis*, *Elaphe bimaculata*, *E. climacophora*, *E. flavirufa*, *E. flavolineata*, *E. guttata*, *E. longissima*, *E. mandarina*, *E. moellendorffi*, *E. obsoleta*, *E. radiata*, *E. rufodorsata*, *E. scalaris*, *E. schrencki*, *E. subocularis*, *E. taeniura*, *E. trivirgata*, *Enhydris plumbea*, *Farancia abacura*, *Gonyosoma oxycephalum*, *Heterodon simus*, *H. platyrhinos*, *Lampropeltis getulus*, *L. zonata*, *Masticophis flagellum*, *M. taeniatus*, *Nerodia clarkii*, *N. cyclopion*, *N. erythrogaster*, *N. fasciata*, *N. rhombifera*, *Opheodrys aestivus*, *Ptyas korros*, *Regina alleni*, *Spalerosophis cliffordi*, *Thamnophis elegans*, *T. sauritus*, *Trimorphodon tau*.

12) *Bungarus fasciatus*, *B. multicinctus*, *Micropechis ikaheka*, *Micrurus fulvius*, *Naja naja*, *N. nigricollis*, *Notechis scutatus*.

13) *Agkistrodon contortrix*, *A. piscivorus*, *Bitis arietans*, *B. gabonica*, *B. nasicornis*, *Bothrops asper*, *Cerastes cerastes*, *Crotalus atrox*, *C. basiliscus*, *C. cerastes*, *C. horridus*, *C. ruber*, *C. scutulatus*, *C. unicolor*, *C. viridis*, *Deinagkistrodon acutus* [*Agkistrodon acutus*], *Lachesis muta*, *Sistrurus miliarius*, *Trimeresurus stejnegeri*, *Vipera aspis*.

Taxa	ZONE	ECO	Al	Ag	As	Ba	Be	Cd	Co	Cr	Cs	Cu	Fe	Hg	Mn	Mo	Ni	Pb	Pt	Rb	Sb	Se	Sn	Sr	Ti	Tl	U	V	Zn	Zr
Squamata: Sauria																														
"Blue tongued lizard" 1)												1																		
"Lizard" 2)					1			1				1	1	1	1			1					1					1	
"Wall lizard" 3)																		1												
Helodermatidae: 2 species 4)																												1		
Iguanidae: 2 species 5)																												1		
Scincidae: 3 species 6)																												1		
Varanidae: 8 species 7)																												1		
Ameiva exsul	NEO	T												1																
Chamaeleo chamaeleon	PAL	T			1			2				2						2										2		
Egernia napoleonis	AUS	T										1																		
Heloderma horridum	NEA	T										1																		
Hemidactylus mabouia	OCE	T						2										2										2		
Iberolacerta monticola 8)	PAL	T			1			1										1										1		
Lacerta agilis	PAL	T						1										1												
Laudakia stellio 9)	PAL	T	1			1		1	1	1	1	1			1	1	1	1		1				1				1		
Lerista microtis 10)	AUS	T										1																		
Norops sagrei 11)	NEO	T			1			1		1				1	1			1				1								
Podarcis carbonelli	PAL	T						1																						
Podarcis muralis 12)	PAL	T						2										2												
Podarcis taurica 13)	PAL	T										1			1		1													
Pseudemoia trilineatum 14)	AUS	T										1																		
Sceloporus occidentalis	NEA	T																				1								
Sceloporus undulatus	NEA	T						1																						
Tarentola mauritanica	PAL	T	1		1	1		1	1		1	1	1		1	1	1	1		1	1	1		1		1		1	1	
Tiliqua rugosa 15)	AUS	T										1																1		

(*continued*)

TABLE 12.1 (CONTINUED)
Distribution of Information of the Contamination of Reptiles with Metals According to Species, and Their Biogeographical and Ecosystem Distribution

Taxa	ZONE	ECO	Al	Ag	As	Ba	Be	Cd	Co	Cr	Cs	Cu	Fe	Hg	Mn	Mo	Ni	Pb	Pt	Rb	Sb	Se	Sn	Sr	Ti	Tl	U	V	Zn	Zr
Varanus salvator	IND	F, T			1							1	1							1		1		1					1	
Varanus sp. 16)			1							1		1	1	1	1			1						1					1	
Zootoca vivipara 17)	PAL	T						4				2						4											2	

Note: Distribution of information is indicated here by numbers of publications. Data derived from Appendix.

1) L: Western Australia.
2) L: South Taiwan.
3) L: India.
4) Heloderma horridum, Heloderma suspectum.
5) Cyclura sp., Iguana iguana.
6) Corucia zebrata, Tiliqua nigrolutea, T. scincoides.
7) Varanus acanthurus, V. albigularis, V. exanthematicus, V. grayi, V. indicus, V. komodoensis, V. prasinus, V. salvadorii.
8) [Lacerta monticola].
9) [Agama s. stellio].
10) [Lygosoma microtis].
11) [Anolis sagrei].
12) [Lacerta muralis].
13) [Lacerta taurica].
14) [Lygosoma trilineatum].
15) [Trachysaurus rugosus].
16) L: Papua New Guinea.
17) [Lacerta vivipara].

Symbols and abbreviations

AFR	Afrotropic
AUS	Australasia
ZONE	Biogeographic zone
F	Freshwater or estuarine
IND	Indomalaya
L	Location(s) in the reference
M	Marine
N	Name in reference
NEA	Nearctic
NEO	Neotropic
OCE	Oceanic
PAL	Palearctic
ECO	Ecosystem type
T	Terrestrial
[ ]	Name(s) as given in the reference

Note: Distribution of information is indicated here by numbers of publications. Data derived from Appendix.

TABLE 12.2
Distribution of Information on Metals in Reproductive and Early Life Stage Compartments of Reptiles

Taxa	Compartment	Al	As	Ba	Be	Cd	Co	Cr	Cu	Fe	Hg	Mn
Testudines (sea turtles)												
Field												
Actinemys marmorata	Laid	c	c			c	c	C	C	C	C	C
Caretta caretta	Oviduct					Y,s,a	s,a,y		S,Y,A	Y,S,A	Y,S,A	Y,S,A
Caretta caretta	Hatched					H,AY,E					AY,H,E	
Caretta caretta	Hatched					Liver,S,Y			Liver,S,Y		Liver,sy	
Caretta caretta	Several					1)			1)	1)	1)	1)
Chelonia mydas	Several					1)	1)		1)	1)	1)	1)
Chelonia mydas	Hatched	S,h	s,h	S,H		S,h		S,h	S,H	H,S		H,S
Chelonia mydas	Hatched					E,YA,H					h,e,ay	
Chelonia mydas	Laid		s			S			S	S		
Chelydra serpentina	Laid										C	
Chelydra serpentina	Laid										C	
Chelydra serpentina	Laid										C	
Cuora amboinensis	Gonad								O			
Cyclemys dentata	Gonad								O,M	O,M		
Lepidochelys olivacea	Laid					S,ay	S,AY	S,AY	S,AY	S,AY		AY,S
Lepidochelys olivacea	Hatched					H,s	H,S	S,H	S,H	H,S		H,S
Malaclemys terrapin	Gonad		O			O		O				
Pelodiscus sinensis	Gonad							O		O		
Trachemys scripta	Laid	C,s				S,c		S,c	S,C			C,S
Field, laboratory												
Trachemys scripta	Laid					C,S°		S,C*			C,s*	S,C°
Trachemys scripta	Hatched		H			H		H	H			
Chrysemys picta	Several					2)						
Trachemys scripta	Gonad					F		F				
Crocodylia												

Farm									
Alligator sinensis	Laid	C,S*	S,C°	S,C*	S,C°	C,S*	C,S*	S,C*	
Alligator sinensis	Laid	C,B*	C,B*	B,C*	B,C*	B,C*	B,C*	B,C*	
Alligator sinensis	Gonad	F,M	F,M	F,M	F,M	F,M	F,M	F,M	

Note: When in a given study more than one tissue was analyzed for a given metal, tissues are listed in decreasing order of mean concentration levels. Data derived from Appendix.
Compartments analyzed:
1) Testis, oviduct, ovary, egg, shell, yolk, albumen (Figure 12.3).
2) Follicle wall, yolk, corpus luteum; yolk, albumen, and shell of oviductal egg; oviductal wall (Figure 12.3).

Mo	Ni	Pb	Rb	Se	Sn	Sr	Tl	V	Zn	Mass Basis	Reference
	C	c						C	C	DRY	Henny et al. 2003
	s,a,y	s,a,y							Y,S,A	WET	Sakai et al. 1995
		AY,H,e								DRY	Godley et al. 1999
		Liver,Y,S							Y,S	DRY	Kaska and Furness 2001
	1)	1)							1)	WET	Sakai et al. 2000b
	1)	1)							1)	WET	Sakai et al. 2000b
s,h	s,h						s,h	s,h	H,S	WET	Aguirre et al. 1994
		E,h,AY								DRY	Godley et al. 1999
	S	S								DRY	Celik et al. 2006
										—	Bishop et al. 1998
										WET	Bonin et al. 1995
										DRY	Ashpole et al. 2004
			O	O		O			O	DRY	Boman et al. 2001
			O,M	O,M		O,M			O,M	DRY	Boman et al. 2001

(*continued*)

TABLE 12.2 (CONTINUED)
Distribution of Information on Metals in Reproductive and Early Life Stage Compartments of Reptiles

Mo	Ni	Pb	Rb	Se	Sn	Sr	Tl	V	Zn	Mass Basis	Reference
	March	S,AY							S,AY	DRY	Sahoo et al. 1996
	H,S	H,S							H,S	DRY	Sahoo et al. 1996
				O						WET	Burger 2002
		O	O	O		O			O	DRY	Boman et al. 2001
	S,c	S,c			S,C			S,c	C,S	DRY	Tryfonas et al. 2006
		C,S*		C,S*						DRY	Burger and Gibbons 1998
				H						DRY	Nagle et al. 2001
										WET	Rie et al. 2001
									F	WET	Thomas et al. 1994
		S,C*							C,S*	DRY	Xu et al. 2006
		B,C*							B,C*	DRY	Xu et al. 2006
		F,M							F,M	DRY	Xu et al. 2006

Taxa	Compartment	Al	As	Ba	Be	Cd	Co	Cr	Cu	Fe	Hg	Mn
Crocodylia												
Field												
Alligator mississippiensis	Laid				o							
Alligator mississippiensis	Laid		C								C	
Alligator mississippiensis	Laid	O	o			o		O	O	O	o	O
Alligator mississippiensis	Laid											
Crocodylus acutus	Laid		C			C			C		C	

Crocodylus acutus	Laid	S,AY*		S,AY*	S,AY	S,AY*	S,AY*		AY,S*		
Crocodylus moreletii	Laid								C		
Alligator sinensis				S,B,A,Y			B,S,Y,A				
Field, farm											
Alligator mississippiensis	Gonad								F,M		
Alligator mississippiensis	Oviduct								D		
Field, laboratory											
Alligator mississippiensis	Hatched										
Squamata (lizards)											
Field											
Chamaeleo chamaeleon	Laid			c							
Chamaeleo chamaeleon	Laid		S,C	S,C			S,C				
Varanus salvator	Gonad		O				O	O			
Laboratory											
Iberolacerta monticola	Laid		S,E	S,E			S,E				
Sceloporus occidentalis	Gonad										
Sceloporus undulatus	Hatched			H,C°							
Squamata (snakes)											
Field											
Nerodia sipedon	Gonad		O,M	O,M		O,M			O,M	O,M	
Python molurus	Gonad						M				
Laboratory											
Lamprophis fuliginosus	Gonad										
Lamprophis fuliginosus	Laid										
Nerodia fasciata	Gonad		U	U			U				
Nerodia fasciata	Gonad		U	U			U				
Waglerophis merremii	Oviduct						C,O	C,O			

(continued)

TABLE 12.2 (CONTINUED)
Distribution of Information on Metals in Reproductive and Early Life Stage Compartments of Reptiles

Mo	Ni	Pb	Rb	Se	Sn	Sr	Tl	V	Zn	Mass basis	Reference
										WET	Heinz et al. 1991
										WET	Ogden et al. 1974
o	O	o		O			o	o	O	WET	Heinz et al. 1991
				O						DRY	Roe et al. 2004
		C							C	WET	Ogden et al. 1974
S,AY*	S,AY*	S,AY*				S,AY*				DRY	Stoneburner and Kushlan 1984
										WET	Rainwater et al. 2002
		S,B,A,Y							B,S,A,Y	DRY	Ding et al. 2001
										WET	Heaton-Jones et al. 1997
										WET	Heaton-Jones et al. 1997
				B,H						DRY	Roe et al. 2004
		C							C	WET	Diaz-Paniagua et al. 2002
		S,c							C,S	WET	Gomara et al. 2007
						O			O	DRY	Boman et al. 2001
		S,E							E,S	WET	Marco et al. 2004
				F,M*						DRY	Hopkins 2005b
										DRY	Brasfield et al. 2004
		O,M		O,M						WET	Burger et al. 2005
		M	M	M		M				DRY	Boman et al. 2001
				F						DRY	Hopkins et al. 2004
				O						DRY	Hopkins et al. 2004

	M	M	M	DRY	Hopkins et al. 2001
	F,M	F,M	F,M	DRY	Hopkins et al. 2002
				WET	De Jorge et al. 1971

Symbols and abbreviations

Mass	DRY	Concentrations based on dry mass
	WET	Concentrations based on wet mass
	—	Not given
Gonad	M	Male
	F	Female
	U	Sex unspecified
Oviduct	D	Oviduct tissue
Egg	Ovarial	
	Oviductal	
	Laid	
	Hatched	
Egg compartments		Capital letters indicate concentrations above detection limit; lowercase letters indicate concentrations below detection limit
	A	Albumen
	AY	Albumen-yolk
	C	Egg contents
	E	Embryo
	H	Hatchling (whole body)
	B	Eggshell membranes, chorioallantois membrane
	O	Egg, whole egg
	S	Shell
	Y	Yolk
Concentrations	[*]	Statistically significant intercompartment differences
	[°]	Statistically not significant intercompartment differences

TABLE 12.3
Concentrations of Cadmium, Lead, and Mercury in Reproductive and Early Life Stage Compartments of Free-Ranging Reptiles in Comparison with Environmental Water Quality Goals (USEPA 2006; EC 2007)

Cadmium (Cd)

Recommended Water Quality Criteria	Compartments	Concentrations (ppb, µg/L)	USEPA 2006
CCM (acute)	Freshwater	2.00	
CCC (chronic)		0.25	
CCM (acute)	Saltwater	40.0	
CCC (chronic)		8.80	
Suggested Environmental Quality Standards			EC 2007
Annual average (range depending on water hardness)	Inland surface waters	≤0.08–0.25	

Species	Compartments		Concentrations (ppm dry mass)			*N*	Locations	Reference
			Mean	Minimum	Maximum			
Lepidochelys olivacea	Hatchling	Whole	2.00	—	—	24	India	Sahoo et al. 1996
Crocodylus acutus	Egg	Shell	1.36	—	—	9	Florida	Stoneburner and Kushlan 1984
Lepidochelys olivacea	Egg	Shell	1.30	—	—	24	India	Sahoo et al. 1996
Caretta caretta	Embryo	Liver	1.16	—	—	22	Turkey	Kaska and Furness 2001
Dermochelys coriacea	Egg (posthatch)	Shell	0.90	—	—	—	Mexico; Pacific	Vazquez et al. 1997
Caretta caretta	Egg	Shell	0.65	—	—	22	Turkey	Kaska and Furness 2001
Chelonia mydas	Egg	Shell	0.58	—	—	12	Turkey	Celik et al. 2006
Caretta caretta	Egg	Yolk	0.36	—	—	22	Turkey	Kaska and Furness 2001

Caretta caretta	Hatchling	Whole	0.34	ND (<0.01)	1.45	16	Cyprus	Godley et al. 1999
Chelonia mydas	Embryo	Whole	0.33	ND (<0.01)	0.93	16	Cyprus	Godley et al. 1999
Chelonia mydas	Egg	Contents	0.27	ND (<0.01)	1.22	24	Cyprus	Godley et al. 1999
Caretta caretta	Egg	Contents	0.23	0.23	0.56	3	Cyprus	Godley et al. 1999
Chelonia mydas	Hatchling	Whole	0.23	ND (<0.01)	0.94	29	Cyprus	Godley et al. 1999
Caretta caretta	Embryo	Whole	0.21	ND (<0.01)	1.09	29	Cyprus	Godley et al. 1999
Trachemys scripta elegans	Egg	Shell	0.16	—	—	—	United States; Illinois	Tryfonas et al. 2006
Crocodylus acutus	Egg	Contents	0.13	—	—	9	United States; Florida	Stoneburner and Kushlan 1984
Crocodylus acutus	Egg	Contents	0.13	—	—	9	U.S.A.; Florida	Stoneburner and Kushlan 1984
Pituophis melanoleucus	Hatchling	Skin	0.12	0.06	0.20	46	U.S.A.; New Jersey	Burger 1992
Pituophis melanoleucus	Hatchling	Whole	0.12	0.06	0.18	16	U.S.A.; New Jersey	Burger 1992
Trachemys scripta	Egg	Contents	0.07	—	0.44	16	United States; South Carolina	Burger and Gibbons 1998
Crocodylus niloticus	Egg	Contents	0.05	ND(<0.030)	0.17	26	Zimbabwe	Phelps et al. 1986
Trachemys scripta	Hatchling	Whole	0.03	—	—	6	United States; South Carolina	Nagle et al. 2001
Trachemys scripta	Hatchling	Whole	0.03	—	—	6	United States; South Carolina	Nagle et al. 2001
Trachemys scripta	Hatchling	Whole	0.03	—	—	6	United States; South Carolina	Nagle et al. 2001
Trachemys scripta	Hatchling	Whole	0.03	—	—	6	United States; South Carolina	Nagle et al. 2001
Trachemys scripta	Egg	Shell	0.01	—	0.03	16	United States; South Carolina	Burger and Gibbons 1998
Lepidochelys olivacea	Egg	Contents	ND (<1.0)	—	—	24	India	Sahoo et al. 1996
Lepidochelys olivacea	Egg (posthatch)	Shell	ND (<1.0)	—	—	24	India	Sahoo et al. 1996
Actinemys marmorata	Egg	Contents	ND (<0.1)	ND (<0.10)	ND (<0.10)	14	United States; Oregon	Henny et al. 2003
Trachemys scripta elegans	Egg	Contents	ND (<—)	—	—	—	United States; Illinois	Tryfonas et al. 2006

(*continued*)

TABLE 12.3 (CONTINUED)
Concentrations of Cadmium, Lead, and Mercury in Reproductive and Early Life Stage Compartments of Free-Ranging Reptiles in Comparison with Environmental Water Quality Goals (USEPA 2006; EC 2007)

Cadmium (Cd)

Recommended Water Quality Criteria	Compartments		Concentrations (ppb, µg/L)					USEPA 2006
			Concentrations (ppm wet mass)					
			Mean	Minimum	Maximum			
Chelonia mydas	Testis		1.19	—	—	1	Japan	Sakai et al. 2000b
Caretta caretta	Testis		0.78	—	—	1	Japan	Sakai et al. 2000b
Chelonia mydas	Egg (posthatch)	Shell	0.20	—	—	3	United States; Hawaii	Aguirre et al. 1994
Chamaeleo chamaeleon	Egg	Shell	0.05	0.01	0.12	9	Spain	Gomara et al. 2007
Crocodylus acutus	Egg	Contents	0.05	0.05	0.05	5	United States; Florida	Ogden et al. 1974
Caretta caretta	Ovary		0.04	—	—	1	Japan	Sakai et al. 1995
Caretta caretta	Egg (oviductal)	Yolk	0.03	0.02	0.04	—	Japan	Sakai et al. 1995
Caretta caretta	Egg (oviductal)	Yolk	0.03	—	—	1	Japan	Sakai et al. 2000b
Chelonia mydas	Oviduct		0.03	—	—	1	Japan	Sakai et al. 2000b
Nerodia sipedon	Testis		0.02	—	—	3	United States, Tennessee	Burger et al. 2005
Caretta caretta	Egg (oviductal)	Whole	0.01	0.01	0.02	—	Japan	Sakai et al. 1995
Caretta caretta	Egg (oviductal)	Whole	0.01	—	—	1	Japan	Sakai et al. 2000b
Chamaeleo chamaeleon	Egg	Contents	0.01	0.005	0.02	9	Spain	Gomara et al. 2007
Crocodylus acutus	Egg	Contents	0.01	—	—	9	United States; Florida	Stoneburner and Kushlan 1984
Nerodia sipedon	Egg (gonadal)	Whole	0.006	—	—	8	United States; Tennessee	Burger et al. 2005
Malaclemys terrapin	Egg (gonadal)	Whole	0.0003	0.00001	0.001	8	United States; New Jersey	Burger 2002

Chelonia mydas	Hatchling	Whole	ND (<0.07)	ND (<0.07)	ND (<0.07)	3	United States; Hawaii	Aguirre et al. 1994
Chelonia mydas	Ovary		ND (<0.03)	—	—	1	Japan	Sakai et al. 2000b
Chelonia mydas	Egg	Whole	ND (<0.03)	—	—	1	Japan	Sakai et al. 2000b
Chelonia mydas	Egg	Shell	ND (<0.03)	—	—	1	Japan	Sakai et al. 2000b
Chelonia mydas	Egg	Yolk	ND (<0.03)	—	—	1	Japan	Sakai et al. 2000b
Chelonia mydas	Egg	Albumen	ND (<0.03)	—	—	1	Japan	Sakai et al. 2000b
Caretta caretta	Egg (oviductal)	Shell	ND (<0.01)	ND (<0.01)	ND (<0.01)	—	Japan	Sakai et al. 1995
Caretta caretta	Egg (oviductal)	Albumen	ND (<0.01)	ND (<0.01)	ND (<0.01)	—	Japan	Sakai et al. 1995
Alligator mississippiensis	Egg	Whole	ND (<0.01)	—	—	32	United States; Florida	Heinz et al. 1991
Caretta caretta	Egg (oviductal)	Shell	ND (<0.01)	—	—	1	Japan	Sakai et al. 2000b
Chamaeleo chamaeleon	Egg	Contents	ND (<0.008)	—	—	4	Spain	Diaz-Paniagua et al. 2002

Lead (Pb)

Recommended Water Quality Criteria	Compartments	Concentrations (ppb, µg/L)	USEPA 2006
CCM (acute)	Freshwater	65	
CCC (chronic)		2.50	
CCM (acute)	Saltwater	210	
CCC (chronic)		8.10	
Suggested Environmental Quality Standards			EC 2007
Annual average	Inland surface waters	7.2	

(*continued*)

TABLE 12.3 CONTINUED
Concentrations of Cadmium, Lead, and Mercury in Reproductive and Early Life Stage Compartments of Free-Ranging Reptiles in Comparison with Environmental Water Quality Goals (USEPA 2006; EC 2007)

Recommended Water Quality Criteria	Compartments		Concentrations (ppb, µg/L)					USEPA 2006
			Concentrations (ppm dry mass)					
Species	Compartments		Mean	Minimum	Maximum	*N*	Locations	References
Lepidochelys olivacea	Hatchling		20.00	—	—	27	India	Sahoo et al. 1996
Crocodylus acutus	Egg	Shell	16.42	—	—	9	United States; Florida	Stoneburner and Kushlan 1984
Lepidochelys olivacea	Egg	Shell	15.60	—	—	26	India	Sahoo et al. 1996
Dermochelys coriacea	Egg	Shell	11.60	—	—	—	Mexico; Pacific	Vazquez et al. 1997
Lepidochelys olivacea	Egg	Shell	11.00	—	—	24	India	Sahoo et al. 1996
Python molurus	Testis		11.00	—	—	1	Vietnam	Boman et al. 2001
Lepidochelys olivacea	Egg	Contents	3.60	—	—	25	India	Sahoo et al. 1996
Crocodylus acutus	Egg	Contents	3.35	—	—	9	United States; Florida	Stoneburner and Kushlan 1984
Caretta caretta	Embryo		2.48	—	—	22	Turkey	Kaska and Furness 2001
Pituophis melanoleucus	Hatchling	Skin	1.33	0.63	2.71	46	U.S.A.; New Jersey	Burger 1992
Caretta caretta	Egg	Yolk	1.31	—	—	22	Turkey	Kaska and Furness 2001
Trachemys scripta elegans	Egg	Shell	1.30	—	—	—	United States; Illinois	Tryfonas et al. 2006
Trachemys scripta	Egg	Contents	0.69	—	1.85	16	United States; South Carolina	Burger and Gibbons 1998
Chelonia mydas	Embryo	Whole	0.66	ND (<0.01)	3.41	16	Cyprus	Godley et al. 1999
Caretta caretta	Egg	Shell	0.63	—	—	22	Turkey	Kaska and Furness 2001

Pituophis melanoleucus	Hatchling		0.61	0.52	0.86	16	U.S.A.; New Jersey	Burger 1992
Trachemys scripta	Egg	Shell	0.22	—	1.37	16	United States; South Carolina	Burger and Gibbons 1998
Caretta caretta	Egg	Contents	0.19	ND (<0.01)	3.93	3	Cyprus	Godley et al. 1999
Caretta caretta	Hatchling	Whole	0.13	ND (<0.01)	10.56	16	Cyprus	Godley et al. 1999
Chelonia mydas	Egg	Shell	0.04	—	—	12	Turkey	Celik et al. 2006
Actinemys marmorata	Egg	Contents	ND (<1.50)	—	—	14	United States; Oregon	Henny et al. 2003
Caretta caretta	Embryo	Whole	ND (<0.01)	ND (<0.01)	6.48	—	Cyprus	Godley et al. 1999
Chelonia mydas	Hatchling	Whole	ND (<0.01)	ND (<0.01)	3.86	29	Cyprus	Godley et al. 1999
Chelonia mydas	Egg	Contents	ND (<0.01)	ND (<0.01)	1.61	24	Cyprus	Godley et al. 1999
Trachemys scripta elegans	Egg	Contents	ND (<—)	—	—	—	United States; Illinois	Tryfonas et al. 2006
			Concentrations (ppm wet mass)					
			Mean	**Minimum**	**Maximum**			
Chamaeleo chamaeleon	Egg	Contents	14.2	4.00	22.10	36	Spain	Diaz-Paniagua et al. 2002
Crocodylus acutus	Egg	Contents	0.64	—	—	9	United States; Florida	Stoneburner and Kushlan 1984
Chamaeleo chamaeleon	Egg	Shell	0.40	0.18	1.45	9	Spain	Gomara et al. 2007
Crocodylus acutus	Egg	Contents	0.34	0.20	0.50	5	Vietnam	Ogden et al. 1974
Nerodia sipedon	Testis		0.05	—	—	3	United States; Tennessee	Burger et al. 2005
Malaclemys terrapin	Egg (ovarial)	Whole	0.04	0.01	0.10	8	United States; New Jersey	Burger 2002
Nerodia sipedon	Egg (ovarial)	Whole	0.025	—	—	8	United States; New Jersey	Burger et al. 2005
Caretta caretta	Egg (oviductal)	Albumen	ND (<0.03)	—	—	7	Japan	Sakai et al. 2000b
Chelonia mydas	Egg (oviductal)	Albumen	ND (<0.03)	—	—	1	Japan	Sakai et al. 2000b
Chelonia mydas	Egg (oviductal)		ND (<0.03)	—	—	1	Japan	Sakai et al. 2000b
Caretta caretta	Ovary		ND (<0.03)	—	—	6	Japan	Sakai et al. 2000b
Chelonia mydas	Ovary		ND (<0.03)	—	—	1	Japan	Sakai et al. 2000b

(continued)

TABLE 12.3 CONTINUED
Concentrations of Cadmium, Lead, and Mercury in Reproductive and Early Life Stage Compartments of Free-Ranging Reptiles in Comparison with Environmental Water Quality Goals (USEPA 2006; EC 2007)

Recommended Water Quality Criteria	Compartments		Concentrations (ppb, µg/L)					USEPA 2006
			Mean	Minimum	Maximum			
Caretta caretta	Oviduct		ND (<0.03)	—	—	6	Japan	Sakai et al. 2000b
Chelonia mydas	Oviduct		ND (<0.03)	—	—	1	Japan	Sakai et al. 2000b
Caretta caretta	Egg (oviductal)	Shell	ND (<0.03)	—	—	7	Japan	Sakai et al. 2000b
Chelonia mydas	Egg (oviductal)	Shell	ND (<0.03)	—	—	1	Japan	Sakai et al. 2000b
Caretta caretta	Testis		ND (<0.03)	—	—	1	Japan	Sakai et al. 2000b
Chelonia mydas	Testis		ND (<0.03)	—	—	1	Japan	Sakai et al. 2000b
Caretta caretta	Egg (oviductal)	Whole	ND (<0.03)	—	—	7	Japan	Sakai et al. 2000b
Chelonia mydas	Egg (oviductal)	Whole	ND (<0.03)	—	—	1	Japan	Sakai et al. 2000b
Caretta caretta	Egg (oviductal)	Yolk	ND (<0.03)	—	—	7	Japan	Sakai et al. 2000b
Chelonia mydas	Egg (oviductal)	Yolk	ND (<0.03)	—	—	1	Japan	Sakai et al. 2000b
Caretta caretta	Egg (oviductal)	Albumen	ND (<—)	—	—	12	Japan	Sakai et al. 1995
Caretta caretta	Egg (oviductal)	Shell	ND (<—)	—	—	10	Japan	Sakai et al. 1995
Caretta caretta	Egg (oviductal)	Yolk	ND (<—)	—	—	11	Japan	Sakai et al. 1995
Alligator mississippiensis	Egg	Whole	—	ND (<0.20)	0.90	32	United States; Florida	Heinz et al. 1991
Chamaeleo chamaeleon	Egg	Contents	—	ND (<0.001)	0.02	9	Spain	Gomara et al. 2007
Mercury (total Hg)								
CCM (acute)	Freshwater			1.40				
CCC (chronic)				0.77				
CCM (acute)	Saltwater			1.80				
CCC (chronic)				0.94				
Suggested Environmental Quality Standards								EC 2007
Annual average	Inland surface waters			0.05				

Species	Compartments		Concentrations (ppm dry mass)			N	Locations	References
			Mean	Minimum	Maximum			
Chelydra s. serpentine	Egg	Contents	0.72	—	—	5	Canada	Ashpole et al. 2004
Crocodylus acutus	Egg	Contents	0.66	—	—	9	United States; Florida	Stoneburner and Kushlan 1984
Caretta caretta	Embryo	Liver	0.51	—	—	22	Turkey	Kaska and Furness 2001
Pituophis melanoleucus	Hatchling	Skin	0.28	0.05	0.63	46	U.S.A.; New Jersey	Burger 1992
Chelydra s. serpentine	Egg	Contents	0.25	—	—	4	Canada	Ashpole et al. 2004
Crocodylus acutus	Egg	Shell	0.21	—	—	9	United States; Florida	Stoneburner and Kushlan 1984
Caretta caretta	Egg	Contents	0.19	0.16	0.57	3	Cyprus	Godley et al. 1999
Pituophis melanoleucus	Hatchling	Whole	0.13	0.05	0.27	16	U.S.A.; New Jersey	Burger 1992
Chelydra s. serpentine	Egg	Contents	0.11	—	—	10	Canada	Ashpole et al. 2004
Chelydra s. serpentine	Egg	Contents	0.09	—	—	9	Canada	Ashpole et al. 2004
Chelydra s. serpentine	Egg	Contents	0.09	—	—	9	Canada	Ashpole et al. 2004
Chelydra s. serpentine	Egg	Contents	0.05	—	—	9	Canada	Ashpole et al. 2004
Trachemys scripta	Egg	Contents	0.04	—	0.24	16	United States; South Carolina	Burger and Gibbons 1998
Caretta caretta	Hatchling	"Hatched nest"	0.02	ND (<0.01)	0.75	16	Cyprus	Godley et al. 1999
Caretta caretta	Embryo	"Hatched nest"	0.01	ND (<0.01)	0.22	27	Cyprus	Godley et al. 1999
Chelonia mydas	Hatchling	"Hatched nest"	ND (<0.01)	ND (<0.01)	0.24	24	Cyprus	Godley et al. 1999
Chelonia mydas	Embryo	"Hatched nest"	ND (<0.01)	ND (<0.01)	0.12	18	Cyprus	Godley et al. 1999
Chelonia mydas	Egg	Contents	ND (<0.01)	ND (<0.01)	0.19	17	Cyprus	Godley et al. 1999

(*continued*)

TABLE 12.3 CONTINUED
Concentrations of Cadmium, Lead, and Mercury in Reproductive and Early Life Stage Compartments of Free-Ranging Reptiles in Comparison with Environmental Water Quality Goals (USEPA 2006; EC 2007)

Recommended Water Quality Criteria	Compartments		Concentrations (ppb, µg/l)					USEPA 2006
Trachemys scripta	Egg	Shell	ND (<0.002)	—	—	16	United States; South Carolina	Burger and Gibbons 1998
Caretta caretta	Egg	Shell	ND (<—)	—	—	22	Turkey	Kaska and Furness 2001
Caretta caretta	Egg	Yolk	ND (<—)	—	—	22	Turkey	Kaska and Furness 2001
			Concentrations (ppm wet mass)					
			Mean	Minimum	Maximum			
Alligator mississippiensis	Ovary		1.30	0.03	5.91	7	United States; Florida	Heaton-Jones et al. 1997
Alligator mississippiensis	Oviduct		1.20	0.06	5.42	7	United States; Florida	Heaton-Jones et al. 1997
Alligator mississippiensis	Oviduct		1.19	0.89	1.59	4	United States; Florida	Heaton-Jones et al. 1997
Alligator mississippiensis	Testis		1.17	0.31	2.35	8	United States; Florida	Heaton-Jones et al. 1997
Alligator mississippiensis	Ovary		0.70	0.39	1.34	4	United States; Florida	Heaton-Jones et al. 1997
Alligator mississippiensis	Egg	Contents	0.54	0.41	0.71	4	United States; Florida	Ogden et al. 1974
Nerodia sipedon	Testis		0.29	—	—	3	United States; Tennessee	Burger et al. 2005
Alligator mississippiensis	Oviduct		0.20	0.19	0.2	2	United States; Florida	Heaton-Jones et al. 1997
Alligator mississippiensis	Testis		0.19	0.01	0.48	5	United States; Florida	Heaton-Jones et al. 1997

Alligator mississippiensis	Testis		0.18	0.03	0.39	4	United States; Florida	Heaton-Jones et al. 1997
Crocodylus acutus	Egg	Contents	0.13	—	—	9	United States; Florida	Stoneburner and Kushlan 1984
Alligator mississippiensis	Ovary		0.12	0.11	0.13	2	United States; Florida	Heaton-Jones et al. 1997
Crocodylus moreletii	Egg	Contents	0.11	0.02	0.21	31	Belize	Rainwater et al. 2002
Crocodylus acutus	Egg	Contents	0.09	0.07	0.14	5	United States; Florida	Ogden et al. 1974
Crocodylus moreletii	Egg	Contents	0.07	ND (<0.02)	0.23	31	Belize	Rainwater et al. 2002
Nerodia sipedon	Egg (ovarial)	Whole	0.06	—	—	8	United States; Tennessee	Burger et al. 2005
Malaclemys terrapin	Egg (ovarial)	Whole	0.04	0.02	0.05	8	United States; New Jersey	Burger 2002
Caretta caretta	Oviduct		0.02	—	—	6	Japan	Sakai et al. 2000b
Caretta caretta	Ovary		0.02	—	—		Japan	Sakai et al. 2000b
Caretta caretta	Egg (oviductal)	Yolk	0.01	0.01	0.02	5	Japan	Sakai et al. 1995
Caretta caretta	Egg (oviductal)	Yolk	0.01	—	—		Japan	Sakai et al. 2000b
Podocnemis unifilis	Egg	While	0.01	0.01	0.02	—	Brazil	Aula et al. 1994 from Eisler 2004
Chelonia mydas	Testis		0.01	—	—	4	Japan	Sakai et al. 2000b
Caretta caretta	Egg (oviductal)	Whole	0.005	—			Japan	Sakai et al. 2000b
Caretta caretta	Egg (oviductal)	Whole	0.005	0.004	0.01	5	Japan	Sakai et al. 1995
Chelonia mydas	Ovary		0.005	—	—	6	Japan	Sakai et al. 2000b
Chelonia mydas	Oviduct		0.005	—	—	5	Japan	Sakai et al. 2000b
Caretta caretta	Egg (oviductal)	Shell	0.004	—	—		Japan	Sakai et al. 2000b
Caretta caretta	Egg	Shell	0.004	0.002	0.005	5	Japan	Sakai et al. 1995
Chelonia mydas	Egg (oviductal)	Yolk	0.003	—	—	9	Japan	Sakai et al. 2000b
Chelonia mydas	Egg (oviductal)	Whole	0.001	—	—	7	Japan	Sakai et al. 2000b
Chelonia mydas	Egg (oviductal)	Shell	0.001	—	—	8	Japan	Sakai et al. 2000b
Caretta caretta	Egg	Albumen	0.0005	0.0001	0.001	5	Japan	Sakai et al. 1995
Caretta caretta	Egg (oviductal)	Albumen	0.0005	—	—		Japan	Sakai et al. 2000b

(*continued*)

TABLE 12.3 CONTINUED
Concentrations of Cadmium, Lead, and Mercury in Reproductive and Early Life Stage Compartments of Free-Ranging Reptiles in Comparison with Environmental Water Quality Goals (USEPA 2006; EC 2007)

Recommended Water Quality Criteria	Compartments		Concentrations (ppb, µg/L)					USEPA 2006
Chelonia mydas	Egg (oviductal)	Albumen	0.00005	—	—	10	Japan	Sakai et al. 2000b
Alligator mississippiensis	Egg	Whole	ND (<0.03)	—	—	32	United States; Florida	Heinz et al. 1991
Trachemys scripta elegans	Egg (oviductal)	Whole	ND (<-)	—	—	4	U.S.A.; Texas	Flickinger and King 1972
Alligator mississippiensis	Egg	Whole	—	0.01	0.02	10	U.S.A.; South Carolina	Bowles et al. 1996 from Rainwater et al. 2002
Chelydra s. serpentine	Egg	Contents	—	ND (<0.02)	0.445	39	Canada	Bonin et al. 1995

Note: Data sets (lines) for reptiles are sorted by descending mean concentrations. Data derived from Appendix.

Abbreviations: CCC, criterion continuous concentration ("estimate of the highest concentration of a material in surface water to which an aquatic community can be exposed indefinitely without resulting in an unacceptable effect"; USEPA 2006); CCM, criterion maximum concentration ("estimate of the highest concentration of a material in surface water to which an aquatic community can be exposed briefly without resulting in an unacceptable effect"; USEPA 2006); ND, not detected.

TABLE 12.4
Concentrations of the Metals of Highest-Priority Concern (Cadmium, Lead, and Mercury) in Target Compartments (Liver, Kidney, Bone) and Potential Alternative Monitoring Compartments (Muscle, Blood, Integument, Tail Tips) of Free-Ranging Reptiles Based on Minimum, Maximum, and Mean Values Reported in the Literature per Case (i.e., per Metal, Species, Compartment, and Study)

Cadmium (Cd)	Compartments	Concentrations (ppm dry mass)								Concentrations (ppm wet mass)							
		Means				Maxima				Means				Maxima			
Taxa		MIN	MED	MAX	N	MIN	MED	MAX	N	MIN	MED	MAX	N	MIN	MED	MAX	N
Reptiles (all)	Liver	0.12	1.75 3.30	35.00	36	0.62 N1 = 0	19.01	148.00	19	ND (<0.01) N1 = 3	0.12 1.29	28.00	44	0.02	6.20	56.90	22
Testudines (sea turtles)	Liver	0.22	6.84	35.00	21	1.82	20.23; 27.10	148.00	16	0.96	6.40; 6.60	28.00	20	5.97	17.00	56.90	12
Testudines (others)		0.17	—	3.57	2	—	—	—	0	ND (<0.06) N1 = 1	0.07; 0.08	16.90	8	0.07		26.20	2
Crocodylia		0.28	—	0.53	2	—	—	—	0	ND (<0.02) N1 = 1	0.03	0.12	8	0.02	0.06; 0.07	0.11	6
Squamata (lizards)		0.40	0.60 0.71	27.18	8	0.62	0.68	4.01	3	—	—	—	0	—	—	—	0
Squamata (snakes)		0.12	0.17	0.50	3	—	—	—	0	ND (<0.01) N1 = 1	0.07	0.29	8	0.12	—	0.25	2
Reptiles (all)	Kidney	ND (<0.25) N1 = 1	16.96	153.00	23	0.37	140.00 158.00	653.00	14	ND (<0.01) N = 2	0.30 0.24	45.50	36	0.03	16.40	80.70	19
Testudines (sea turtles)	Kidney	2.49	30.50 57.20	153.00	16	23.73	158.00	653.00	13	5.01	26.00; 28.30	45.50	16	12.70	42.00	80.70	11
Testudines (others)		—	—	—	0	—	—	—	0	0.07	0.09; 0.24	9.87	6	—	—	—	0

(*continued*)

TABLE 12.4 (CONTINUED)
Concentrations of the Metals of Highest-Priority Concern (Cadmium, Lead, and Mercury) in Target Compartments (Liver, Kidney, Bone) and Potential Alternative Monitoring Compartments (Muscle, Blood, Integument, Tail Tips) of Free-Ranging Reptiles Based on Minimum, Maximum, and Mean Values Reported in the Literature per Case (i.e., per Metal, Species, Compartment, and Study)

Cadmium (Cd)	Compartments	Concentrations (ppm dry mass)								Concentrations (ppm wet mass)							
		Means				Maxima				Means				Maxima			
Taxa		MIN	MED	MAX	N	MIN	MED	MAX	N	MIN	MED	MAX	N	MIN	MED	MAX	N
Crocodylia		0.32	—	0.16	2	—	—	—	0	ND (<0.01) N1 = 1	0.03	0.16	7	0.03	0.09; 0.10	0.45	6
Squamata (lizards)		ND (<0.25) N1 = 1	0.25 0.50	7.50	4	—	0.37	—	1	—	—	—	0	—	—	—	0
Squamata (snakes)		—	0.03	—	1	—	—	—	0	ND (<0.04) N1 = 1	0.04	0.07	7	0.08		0.18	2
Squamata (lizards)	Whole body 1)	0.05	0.31	1.99	17	0.10	0.44	3.00	13	0.04	—	0.07	2	0.40	—	0.45	2
Squamata (snakes)	—	—	—	—	0	—	—	—	0	0.01	—	0.02	2	0.01	—	0.03	2

Note: Data derived from Appendix. Critical limits for the metals are for organs of cows in the view of food safety as compiled by de Vries et al. (2005).
Abbreviations: ND, not detected; N, numbers of cases; N1, number of cases below detection limit; —, no information available.
1) Gut contents excluded.

Cadmium (Cd)	Compartments	Concentrations (ppm dry mass)								Concentrations (ppm wet mass)							
		Means				Maxima				Means				Maxima			
Taxa		MIN	MED	MAX	N	MIN	MED	MAX	N	MIN	MED	MAX	N	MIN	MED	MAX	N
Reptiles (all)	Muscle	0.01	0.25 0.20	3.90	22	0.08	1.43	39.24	15	ND (<0.06) N1 = 1	0.05	2.5	24	0.02	0.13; 0.18	12.48	12
Testudines (sea turtles)	Muscle	0.01	0.35	3.90	19	0.15	1.43 1.45	39.24	14	0.01	0.07	2.5	13	0.12	0.26	12.48	7
Testudines (others)		—	—	—	0	—	—	—	0	ND (<0.06)	0.02	0.77	3	0.02		1.41	2
Crocodylia		0.11		0.20	2				0	0.03	0.03	0.06	3	—	0.03	—	1
Squamata (lizards)		—	0.07	—	1	—	0.08	—	1	—	—	—	0	—	—	—	0
Squamata (snakes)		—	—	—	0	—	—	—	0	0.01	0.02	0.02	5	0.05	—	0.10	2
Testudines (sea turtles)	Bone	—	—	—	0	—	—	—	0	0.03	0.09	1.36	5	—	22.79	—	1
Testudines (others)	Blood 1)	—	—	—	0	—	—	—	0	—	—	—	0	ND (<—) N1 = 1	—	0.45	2
Squamata (snakes)		—	—	—	0	—	—	—	0	0.003	0.01	0.01	5	0.01	—	0.09	2
Testudines (sea turtles)	Carapace	—	—	—	0	—	—	—	0	0.03	0.05; 0.07	0.13	4	—	—	—	0
Testudines (sea turtles)	Scale	—	—	—	0	—	—	—	0	0.02	0.04	0.09	5	—	—	—	0
Crocodylia	Osteoderm	—	0.10	—	1		0.13		1				0	—	—	—	0
	Scute	—	—	—	0	—	—	—	0	ND (<0.05) N1 = 1	0.34	1.90	5	—	—	—	0

(*continued*)

TABLE 12.4 (CONTINUED)
Concentrations of the Metals of Highest-Priority Concern (Cadmium, Lead, and Mercury) in Target Compartments (Liver, Kidney, Bone) and Potential Alternative Monitoring Compartments (Muscle, Blood, Integument, Tail Tips) of Free-Ranging Reptiles Based on Minimum, Maximum, and Mean Values Reported in the Literature per Case (i.e., per Metal, Species, Compartment, and Study)

Cadmium (Cd)	Compartments	Concentrations (ppm dry mass)								Concentrations (ppm wet mass)							
		Means				Maxima				Means				Maxima			
Taxa		MIN	MED	MAX	N	MIN	MED	MAX	N	MIN	MED	MAX	N	MIN	MED	MAX	N
	Skin	—	—	—	0	—	—	—	0	—	0.05	—	1	—	—	—	0
	Tail tip	—	—	—	0	—	—	—	0	—	0.10	—	1	—	—	—	0
	Tail regenerated	—	—	—	0	—	—	—	0	—	0.01	—	1	—	—	—	0
Squamata (snakes)	Skin	—	—	—	0	—	—	—	0	0.01	0.02	0.03	4	0.05	—	0.06	2

Cadmium (Cd)		Critical Limits (ppm dry mass)	
		Food Safety	Animal Health
Cow	Liver	0.5	1.4
	Kidney	1.0	5
	Meat	0.05	0.02

Note: Data derived from Appendix. Critical limits for the metals are for organs of cows in the view of food safety as compiled by de Vries et al. (2005).
Abbreviations: ND, not detected; N, numbers of cases; N1, number of cases below detection limit; —, no information available.
1) Data for blood fractions (not specified and whole blood) pooled.

Lead (Pb)	Compartments	Concentrations (ppm dry mass)								Concentrations (ppm wet mass)							
		Means				Maxima				Means				Maxima			
Taxa		MIN	MED	MAX	N	MIN	MED	MAX	N	MIN	MED	MAX	N	MIN	MED	MAX	N
Reptiles (all)	Liver	ND (<0.01) N1 = 7	0.83 0.85	19.85	34	0.06	1.84; 2.60	14.00	14	ND (<0.03) N1 = 7	0.12; 0.13	21.60	36	0.09	2.21	109.00	11
Testudines (sea turtles)	Liver	ND (<0.01) N1 = 6	0.13 0.15	3.55	18	0.06	1.84	14.00	11	ND (<0.03) N1 = 3	0.14	4.3	11	0.29	0.36; 0.99	33.09	4
Testudines (others)		—	—	—	0	—	—	—	0	ND (<—) N1 = 3	0.18	21.6	11	2.21	—	46.95	2
Crocodylia		ND (<—) N1 = 1	0.85	19.85	5	—	—	—	0	0.03	0.54	8.7	5	1.68	11.00	17.00	3
Squamata (lizards)		1.2	3.5	13.32	9	1.7	2.6	12.4	3	—	—	—	0	—	—	—	0
Squamata (snakes)		0.90	—	16.0	2	—	—	—	0	ND (<0.11) N1 = 1	0.05	0.14	9	0.09	—	109.00	2
Reptiles (all)	Kidney	ND (<0.01) N1 = 2	0.51	18.0	21	0.36	2.38; 2.63	69.89	12	ND (<0.03) N1 = 4	0.18	24.31	27	0.12	0.89	52.24	11
Testudines (sea turtles)	Kidney	ND (<0.01)	0.09 0.19	3.99	14	0.36	2.38	69.89	11	ND (<0.03) N1 = 3	0.14	2.44	9	0.21	0.28; 0.42	17.29	4
Testudines (others)		—	—	—	0	—	—	—	0	ND (<—) N1 = 1	0.16	24.31	7	4.83	—	52.24	2
Crocodylia		0.34	0.41	9.70	3	—	—	—	0	0.23	0.28; 0.60	1.60	4	0.89	2	2.20	3

(*continued*)

TABLE 12.4 (CONTINUED)
Concentrations of the Metals of Highest-Priority Concern (Cadmium, Lead, and Mercury) in Target Compartments (Liver, Kidney, Bone) and Potential Alternative Monitoring Compartments (Muscle, Blood, Integument, Tail Tips) of Free-Ranging Reptiles Based on Minimum, Maximum, and Mean Values Reported in the Literature per Case (i.e., per Metal, Species, Compartment, and Study)

Lead (Pb)	Compartments	Concentrations (ppm dry mass)								Concentrations (ppm wet mass)							
		Means				Maxima				Means				Maxima			
Taxa		MIN	MED	MAX	N	MIN	MED	MAX	N	MIN	MED	MAX	N	MIN	MED	MAX	N
Squamata (lizards)		ND (<1.25) N1 = 1	7.5	18.0	3	—	4.9	—	1	—	—	—	0	—	—	—	0
Squamata (snakes)		—	0.80	—	1	—	—	—	0	0.04	0.34	0.97	7	0.12	—	0.17	2
Squamata (lizards)	Whole body 1)	0.80	15.65 17.32	68.8	26	3.15	19.83	150.52	15	0.02	0.19; 0.28	0.52	6	0.1	—	0.87	2
Squamata (snakes)		9.6	—	69.7	2	—	—	—	0	0.10	—	0.48	2	1.33	—	1.56	2

Note: Data derived from Appendix. Critical limits for the metals are for organs of cows in the view of food safety as compiled by de Vries et al. (2005).
Abbreviations: ND, not detected; N, numbers of cases; N1, number of cases below detection limit; —, no information available.
[a] Gut contents excluded or not specified.

Lead (Pb)	Compartments	Concentrations (ppm dry mass)								Concentrations (ppm wet mass)							
		Means				Maxima				Means				Maxima			
Taxa		MIN	MED	MAX	N	MIN	MED	MAX	N	MIN	MED	MAX	N	MIN	MED	MAX	N
Reptiles (all)	Muscle	ND (<0.01) N1 = 4	0.38	32.00	31	ND (<0.09) N1 = 1	0.74; 1.23	19.78	12	ND (<0.005) N1 = 6	0.06	2.26	27	0.09	0.12 0.18	21.07	6
Testudines (sea turtles)	Muscle	ND (<0.01) N1 = 3	0.08	2.46	17	ND (<0.09) N1 = 1	1.23	19.78	11	ND (<0.03) N1 = 5	0.02	2.26	11	0.09	0.18	21.07	3

Testudines (others)		—	12.00	—	1	—	—	—	0	0.06	0.13 0.20	0.26	4	—	—	—	0
Crocodylia		ND (<—) N1 = 1	0.54	20.30	9	—	0.45	—	1	0.02	0.04	0.08	4	—	0.12	—	1
Squamata (lizards)		—	14.00	—	1	—	—	—	0	—	0.01	—	1	—	—	—	0
Squamata (snakes)		2.00	3.00	32.00	3	—	—	—	0	ND (<0.005) N1 = 1	0.06	0.66	7	0.12	—	0.42	2
Reptiles (all)	Bone	0.9	9.5	62.3	3	3.57	—	3.86	2	1.02	3.69	114.56	13	4.51	19.92 31.22	135.86	6
Testudines (sea turtles)	Bone	—	—	—	0					1.82	2.36	3.53	5	—	19.92	—	1
Testudines (others)		—	—	—	0					1.02	37.56	114.56	7	4.51	4.51	5.55 135.86	4
Crocodylia		—	—	—	0	3.57	—	3.86	2	—	7.98	—	1	—	31.22	—	1
Squamata (lizards)		0.9	9.5	62.3	3	—	—	—	0								
Squamata (snakes)		—	—	—	0	—	—	—	0	—	—	—	0	—	—	—	0
Testudines (sea turtles)	Blood 1)	—	—	—	0	—	—	—	0	—	0.01	1	1	—	0.03	—	1
Testudines (others)		—	—	—	0	—	—	—	0	0.11	0.81	6.00	5	0.09	0.09 0.22	11.59	4
Squamata (snakes)		0.04	0.04	0.10	3	—	—	—	0	0.04	0.05 0.06	0.11	6	0.14	—	0.25	2

Note: Data derived from Appendix. Critical limits for the metals are for organs of cows in the view of food safety as compiled by de Vries et al. (2005).
Abbreviations: ND, not detected; N, numbers of cases; N1, number of cases below detection limit; —, no information available.
1) Data for blood fractions (not specified and whole blood) pooled.

(*continued*)

TABLE 12.4 (CONTINUED)
Concentrations of the Metals of Highest-Priority Concern (Cadmium, Lead, and Mercury) in Target Compartments (Liver, Kidney, Bone) and Potential Alternative Monitoring Compartments (Muscle, Blood, Integument, Tail Tips) of Free-Ranging Reptiles Based on Minimum, Maximum, and Mean Values Reported in the Literature per case (i.e., per Metal, Species, Compartment, and Study)

Lead (Pb)	Compartments	Concentrations (ppm dry mass)								Concentrations (ppm wet mass)							
		Means				Maxima				Means				Maxima			
Taxa		MIN	MED	MAX	N	MIN	MED	MAX	N	MIN	MED	MAX	N	MIN	MED	MAX	N
Testudines (others)	Skin	—	—	—	0	—	—	—	0	0.07	—	0.35	2	0.16	—	0.44	2
Crocodylia		—	—	—	0	—	—	—	0	—	0.02	—	1	—	—	—	0
Squamata (snakes)		—	—	—	0	—	—	—	0	0.06	0.11 0.16	0.47	4	0.19	—	0.40	2
Testudines (sea turtles)	Carapax	—	—	—	0	—	—	—	0	1.56	2.30 2.42	3.10	4	—	—	—	0
Testudines (others)		—	—	—	0	—	—	—	0	0.98	12.33	33.01	3	25.28	—	63.18	2
Testudines (sea turtles)	Scale	—	—	—	0	—	—	—	0	0.07	0.08 0.09	0.14	4	—	—	—	0
Crocodylia	Osteoderm	2.22	3.4	25.2	5	3.05	9.4; 9.7	44.2	4	—	—	—	0	—	—	—	0
	Scute	—	—	—	0	—	—	—	0	ND (<0.05) N1 = 2	0.49	2.59	5	—	—	—	0
	Tail tip 1)	0.08	—	0.10	2	—	—	—	0	—	—	—	0	—	—	—	0

Note: Data derived from Appendix. Critical limits for the metals are for organs of cows in the view of food safety as compiled by de Vries et al. (2005).
Abbreviations: ND, not detected; N, numbers of cases; N1, number of cases below detection limit; —, no information available.
1) Data for tail pieces (tip, regenerate) pooled.

Lead (Pb)		Critical Limits (ppm dry mass)	
		Food Safety	Animal Health
Cow	Liver	0.5	2.0
	Kidney	0.5	3.0
	Meat	0.1	—

Mercury (total Hg)	Compartments	Concentrations (ppm dry mass)								Concentrations (ppm wet mass)							
		Means				Maxima				Means				Maxima			
Taxa		MIN	MED	MAX	N	MIN	MED	MAX	N	MIN	MED	MAX	N	MIN	MED	MAX	N
Reptiles (all)	Liver	0.13	0.78 0.94	41.03	24	0.67	1.20	8.76	11	0.01	0.49	39.99	41	0.03	1.26 1.62	99.48	22
Testudines (sea turtles)	Liver	0.13	0.70	2.41	13	0.67	1.20 1.37	8.76	10	0.02	0.29	8.15	15	0.03	0.47 0.64	8.15	10
Testudines (others)		0.19	—	1.59	2	—	—	—	0	0.46	1.14	3.49	7	3.30	—	8.77	2
Crocodylia		0.25	14.61 17.73	41.03	8	—	—	—	0	0.01	2.52	39.99	9	0.16	17.00	99.48	7
Squamata (lizards)		—	—	—	0	—	—	—	0	—	—	—	0	—	—	—	0
Squamata (snakes)		—	0.55	—	1	—	—	0.84	1	0.04	0.74 0.75	1.86	10	0.60	1.62	3.80	3
Reptiles (all)	Kidney	ND (<0.01) N1 = 1	0.90	36.42	15	ND (<0.01) N1 = 1	1.20 0.80	5.00	8	ND (<—) N1 = 1	0.21 0.24	25.85	42	0.04	0.33 0.44	65.33	16
Testudines (sea turtles)	Kidney	ND (<0.01) N1 = 1	0.47	1.40	9	ND (<0.01) N1 = 1	1.20 0.80	5.00	8	0.02	0.05 0.13	0.30	12	0.04	0.25	0.47	9
Testudines (others)		—	—	—	0	—	—	—	0	0.09	0.41	1.30	16	—	—	—	0

(*continued*)

TABLE 12.4 (CONTINUED)
Concentrations of the Metals of Highest-Priority Concern (Cadmium, Lead, and Mercury) in Target Compartments (Liver, Kidney, Bone) and Potential Alternative Monitoring Compartments (Muscle, Blood, Integument, Tail Tips) of Free-Ranging Reptiles Based on Minimum, Maximum, and Mean Values Reported in the Literature per Case (i.e., per Metal, Species, Compartment, and Study)

Mercury (total Hg)	Compartments	Concentrations (ppm dry mass)								Concentrations (ppm wet mass)							
		Means				Maxima				Means				Maxima			
Taxa		MIN	MED	MAX	N	MIN	MED	MAX	N	MIN	MED	MAX	N	MIN	MED	MAX	N
Crocodylia		0.78	4.82 12.59	36.42	6	—	—	—	0	ND (<—) N1 = 1	0.76 1.58	25.85	6	0.20	9.56	65.33	5
Squamata (lizards)		—	—	—	0	—	—	—	0	—	—	—	0	—	—	—	0
Squamata (snakes)		—	—	—	0	—	—	—	0	0.05	0.14 0.21	1.12	8	0.78	—	3.51	2
Testudines (others)	Whole body 1)	0.18	0.29	0.45	3	0.21	0.38	0.41	3	—	—	—	0	—	—	—	0
Squamata (lizards)	2)	ND (<0.08) N1 = 1	0.06	0.10	3	0.26	—	0.69	2	—	—	—	0	—	—	—	0
Squamata (snakes)	2)	0.08	—	0.12	2	—	—	—	0	—	—	—	0	—	—	—	0

Note: Data derived from Appendix. Critical limits for the metals are for organs of cows in the view of food safety as compiled by de Vries et al. (2005).

Abbreviations: ND, not detected; N, numbers of cases; N1, number of cases below detection limit; —, no information available.

1) Carapax excluded.

2) Gut contents excluded or not specified.

Mercury (total Hg)	Compartments	Concentrations (ppm dry mass)								Concentrations (ppm wet mass)							
		Means				Maxima				Means				Maxima			
Taxa		MIN	MED	MAX	N	MIN	MED	MAX	N	MIN	MED	MAX	N	MIN	MED	MAX	N
Reptiles (all)	Muscle	ND (<0.05) N1 = 1	0.42 0.43	5.57	24	0.09	0.29	1.92	10	ND (<0.0005) N1 = 1	0.16	2.96	60	0.12	0.61 0.66	6.05	20
Testudines (sea turtles)	Muscle	ND (<0.05) N1 = 1	0.09 0.11	0.69	12	0.09	0.29	1.92	9	ND (<0.0005) N1 = 1	0.02	0.210	16	0.12	0.43	0.66	5
Testudines (others)		—	—	—	0	—	—	—	0	0.03	0.10	0.17	13	0.36	—	0.50	2
Crocodylia		0.11	1.58 4.08	5.57	8	—	—	—	0	0.02	0.31	2.96	19	0.19	1.40	6.05	
Squamata (lizards)		—	—	—	0	—	—	—	0	0.02	—	0.18	2	—	—	—	0
Squamata (snakes)		0.24	0.60 0.70	0.90	4	—	0.53	—	1	0.17	0.67	1.31	9	1.02	—	1.63	2
Testudines (sea turtles)	Bone	—	—	—	0	—	—	—	0	0.002	0.002 0.01	0.014	4	—	—	—	0
Crocodylia		—	0.16	—	1	—	—	—	0	—	—	—	0	—	—	—	0
Testudines (sea turtles)	Blood 1)	—	0.02	—	1	0.07	—	0.14	2	—	—	—	0	—	—	—	0
Testudines (others)		0.001	0.02 0.03	0.05	6	0.5	—	1.4	2	—	—	—	0	—	—	—	0
Crocodylia		2.19	—	2.20	2	—	—	—	0	—	—	—	0	—	—	—	0
Squamata (snakes)		0.014	0.38	0.44	9	0.82	—	1.42	2	0.10	0.40	0.70	3	—	—	—	0
Testudines (sea turtles)	Carapax	0.002	0.003 0.04	0.16	4	—	—	—	0	—	—	—	0	—	—	—	0
Testudines (sea turtles)	Scale	0.003	0.005 0.04	0.28	4	—	—	—	0	—	—	—	0	—	—	—	0

(*continued*)

TABLE 12.4 (CONTINUED)
Concentrations of the Metals of Highest-Priority Concern (Cadmium, Lead, and Mercury) in Target Compartments (Liver, Kidney, Bone) and Potential Alternative Monitoring Compartments (Muscle, Blood, Integument, Tail Tips) of Free-Ranging Reptiles Based on Minimum, Maximum, and Mean Values Reported in the Literature per Case (i.e., per Metal, Species, Compartment, and Study)

Mercury (total Hg)	Compartments	Concentrations (ppm dry mass)								Concentrations (ppm wet mass)							
		Means				Maxima				Means				Maxima			
Taxa		MIN	MED	MAX	N	MIN	MED	MAX	N	MIN	MED	MAX	N	MIN	MED	MAX	N
Testudines	Scute	0.25	0.34	3.86	3	0.43	3.3	8.20	4	—	—	—	0	—	—	—	0
(others)							5.70										
Crocodylia	Scale	0.08	0.34	1.03	6	0.16	1.04	1.86	6	—	—	—	0	—	—	—	0
			0.35				1.1										
	Scute	0.07	—	0.10	2	—	—	—	0	0.52	5.83	4.58	6	—	—	—	0
	Skin	—	0.06	—	1	—	—	—	0	—	—	—	0	—	—	—	0
	Tail clip 3)	0.05	0.06	0.16	3	—	—	—	0	—	—	—	0	—	—	—	0
	Claw	—	—	—	0	—	—	—	0	1.67	—	2.69	2	—	—	—	0
Squamata	Skin	0.16	0.37	0.5	4	0.90	—	0.92	2	—	—	—	0	—	—	—	0
(snakes)			0.42														

Mercury (Total Hg)		Critical Limits (ppm dry mass)	
		Food Safety	Animal Health
Cow	Liver	0.05	2.0
	Kidney	0.05	14.0
	Meat	0.05	—

Note: Data derived from Appendix. Critical limits for the metals are for organs of cows in the view of food safety as compiled by de Vries et al. (2005).

Abbreviations: ND, not detected; N, numbers of cases; N1, number of cases below detection limit; —, no information available.

1) Data for blood fractions (not specified, whole blood, plasma, red blood cell) pooled.

2) Scute or scute scrape.

3) Data for all tail pieces (tip, clip, regenerate) pooled.

Porcellio scaber. This wood louse presents 3 basic types of granules in intestinal cells: type A mainly binds to Ca, Mg, Zn, and Pb by precipitation as phosphates; type B, containing Cu and Cd, binds to metallothionein; and type C binds excess Fe in the form of hemosiderin. There may be coevolution of a comparable mechanism in its predator, the woodlouse spider *Dysdera crocata*. The fraction of metals stored in granules in invertebrates might not to be available for assimilation by its invertebrate predators (Vijver et al. 2004) and, by analogy, might not be available to reptile predators. Recently, feeding experiments on subcellular compartmentalization of toxic metals in invertebrates included an experimental food-chain study with Western fence lizards, *Sceloporus occidentalis* (Inouye et al. 2007). Here, lizard-prey organisms (crickets, tenebroid beetle larvae, isopods) were exposed to Pb as lead nitrate in their food, yielding residual Pb in their tissues following a dose-dependent pattern. Subcellular fractions obtained from invertebrate whole body homogenates comprised a supernatant (cytosol, organelles, proteins), exoskeleton, cellular debris, and metal-rich granules (MRG). The exoskeleton contained a high proportion of the total Pb body burden, indicating that molting represents an important route of Pb elimination in these invertebrates. However, most of the remaining Pb body burden was associated with the MRG fraction. Based on the assumption that Pb in MRGs is toxicologically not available to either prey or predator organisms, only a fraction of the metal subject to trophic transfer to the reptile might be ecotoxicologically relevant (Inouye et al. 2007).

Metals that are ingested but not absorbed in the vertebrate gut are eliminated through the feces, which may sometimes contain very high metal concentrations. For example, As, Cd, Cr, Cu, Fe, Hg Mn, Pb, and Zn were found in the tissues, eggs, and feces of the Chinese alligator, *Alligator sinensis*, as well as in their fish prey, ambient water, and sediment. Except for Cu and Hg, however, all remaining metals were more concentrated in alligator feces than in any other compartment examined (Xu et al. 2006).

In all vertebrates, chemicals are absorbed from the environment through the alimentary and respiratory tracts, and then transferred primarily to the liver via the hepatic portal vein system for first metabilization, storage, and excretion (enterohepatic first-pass effect) before entering the systemic circulation. In reptiles, the dermal lymphatic system may also drain into the renal portal vein system, thus enabling presystemic renal metal clearance (Ottaviani and Tazzi 1977). Following their integumentary resorption, metals may also be directly transferred to the reptile kidneys where they may be subject to renal presystemic excretion, a mode of elimination that has not been investigated to date, yet may be critical to our understanding reptile toxicology. In addition to this renal excretion route, irreversible elimination of metals in terrestrial vertebrates occurs through presystemic or systemic enteral excretion routes (via bile fluid and gastrointestinal mucosa). For example, in mammals excretion of Cu takes place mainly via the bile, and similar patterns have been observed for the snake *Waglerophis merremii,* where high levels of Cu were found in biliary excretion (De Jorge et al. 1971). The biliary route is also important in the elimination of methyl-Hg (Nordberg et al. 2007), although this route of elimination has not been observed for American alligators. In this species, total Hg concentrations were high in the livers and kidneys but low in the bile (Heaton-Jones et al. 1997). Irreversible elimination of Hg and other metals may also occur through sequestration of these constituents into gonadal or integumentary tissues, which are subsequently subject to active removal (e.g., via egg laying; see Section 12.3.3) or passive loss (e.g., via scale shedding; see Section 12.3.4). The significance of these elimination routes in lowering whole body burden of reptiles is still under discussion (e.g., Heaton-Jones et al. 1997; Xu et al. 2006).

Once absorbed from the environment and having entered the blood stream, metals are rapidly distributed throughout the body, most often bound to plasma proteins and blood cells. Here, metals may be subject to reversible elimination (Section 12.3.1.2), transferred to different tissues where they may be sequestered (Section 12.3.4), and if not remobilized, stored in a form unavailable to the individual for his or her whole lifetime. Experiments conducted by Rie et al. (2001) showed a rapid decrease of blood Cd levels in *Chrysemys picta* after intravascular injection of ^{109}Cd at 6, 24, and 192 hours postinjection, suggesting systemic transport and redistribution of metals occurred much

as in other vertebrates. Redistribution of metals from storage depots into circulation may occur through an animal's life cycle, particularly during reproduction, and during the fasts common to hibernation or estivation, or consequent to pathological stress phases (Bergeron et al. 2007).

Conceptually, the biological half-life (defined as the time interval during which its concentration is reduced by half in a certain biological compartment) of a metal may be used to evaluate its bioaccumulation potential. However, only 1 well-documented study has considered the net elimination of any metal in reptiles, that of cadmium radioisotope (Cd-109) in the adult painted turtle, *C. picta*. In this study, following a single intravascular application, Cd excretion rate in the holding water (containing the pool excreted via feces, urine, and body surfaces) was relatively constant during the observation period (192 hours), approximately 4.5% of total injected dose per day (Rie et al. 2001).

12.3.1.2 Fate at the Subcellular Level

At the molecular level, the kinetic phases of metals may be described as a sequence of interactions between reactive ("free") metal ions and a diverse array of metal-binding ligands. The distribution of metals into tissues and cells is mediated by several relatively unselective carrier systems (e.g., albumin: Zn, Cd; metallothionein: Zn, Co, Cu, Ag, Cd, Hg). Metallothioneins (MTs) are low-molecular-mass, cystein-rich, high-affinity metal-binding intracellular peptides comprising a variety of subcategories and isoforms. MTs are involved in the regulation of essential metals (Cu and Zn in particular) and metal detoxification, which are interrelated metabolic processes primarily including metal binding and metal donation to other vital metalloproteins. MTs and MT-like proteins have been detected in nearly all phyla and are known from all vertebrate classes except Cyclostomata. Metal-binding proteins similar to mammalian MTs have been detected in several reptiles (see Linder and Grillitsch 2000). MTs are present in all tissues, but the capacity for MT induction is greatest in tissues that are involved in metal uptake, storage, and excretion such as gills, intestine, liver, and kidney. For reptiles, this pattern is supported by experimental study in which adult painted turtles were injected with cadmium chloride. After 192 hours, metal-binding protein induction was detected (as indicated by MT-like protein-binding activity in a modified cadmium-hemoglobin affinity assay) for all test concentrations in a variety of tissues. Induction was especially high in the liver, followed by the pancreas and kidney, but significant, albeit low, levels of Cd-binding protein were also detected in steroidogenic (gonadal and adrenal) tissues (Rie et al. 2001). For several metals, the distribution among liver sub-cellular fractions was described (Anan et al. 2001). For all reptile species studied, MT synthesis was induced by Cd administration, and in the case of the yellow pond turtle, *Clemmys mutica*, MT synthesis was also induced by Cu (Yamamura and Suzuki 1984).

Measurement of MT concentrations may be a suitable tool for routine monitoring of metal exposure and toxicity, a practice that has been supported by several studies. For example, Andreani et al. (2008) found positive correlations between Cu and Cd concentrations and Cu-MT and Cd-MT in liver and kidney of logger head (*Caretta caretta*) and green sea (*Chelonia mydas*) turtles. Unfortunately, MT concentrations varied with a number of endogenous factors (primarily ontogenetic and chronobiological), as well as physicochemical stress factors other than metals. Furthermore, induction of MTs by glucocorticoid hormones has been observed, which may indicate that this stress protein class is involved in the generalized adaptive syndrome in the course of the "general stress response." Thus, MT concentrations do not necessarily reflect the degree of exposure to metals, and as such, the usefulness of MTs as monitoring tools is still under discussion.

Other than MTs, various cell constituents or products are important in metal binding. Melanin and related pigments play important defensive roles in many organisms because they are capable of binding oxygen-derived radicals and cations, including Ca, Mg, Cu, Fe, and Zn (Klaassen 2007). Unusually high levels of Cu accompanied melanin-binding Hg (Heaton-Jones et al. 1994). In addition, melano-macrophages are considered to be important in detoxification and have been described in the liver of various reptile species (e.g., McClellan-Green et al. 2006). Other dermal and epidermal features of reptilian skin potentially moderate metal exposures. For example, keratins, the

major epidermal structural proteins, are sulfur-rich metal-binding proteins and concentrate thiol-reactive metal ions and metalloid compounds such as Hg, As, and Pb in the epidermis and its structures, such as scutes and nails in reptiles.

At the molecular level, the kinetics of metals may also involve biotransformation, that is, changes in the physicochemical form of a substance caused by a biological factor. Biotransformation may result in the detoxification of metals, but also in their toxification through changes in oxidation state and through methylation and demethylation, as known for As and Hg, or dealkylation, as known for organotin and organolead compounds wherein breaking 1 carbon-metal bond transforms the tetraethyl-metal into the highly toxic triethyl form. There is limited, and at best scattered, information available on the biotransformation of metals and the relationship between different organic forms of a metal in reptiles. For example, Kunito et al. (2008) reviewed accumulation patterns of As metabolites in marine mammals, birds, and sea turtles (*Caretta caretta* and *Chelonia mydas*). In these sea turtles, the speciation pattern of As among 6 compounds in liver tissue indicated that the major fraction occurred as arsenobetaine, followed by arsenocholine. In contrast to marine birds, cetaceans, and pinnipeds, concentrations of arsenobetaine and arsenocholine in turtles were particularly high. Such an observation suggested that feeding habits might influence formation of these arsenocompounds. Loggerhead turtles (*Caretta caretta*) preferentially consume jellyfish, which are rich in arsenobetaine, whereas green sea turtles (*Chelonia mydas*) preferentially feed on algae and sea grass, neither of which contains arsenobetaine, but do have rich stores of arsenosugars. The concentration of arsenosugars may subsequently be reduced via microbial transformation to arsenobetaine in the turtle's intestine; conversion of arsenocholine to arsenobetaine also ensures arsenobetaine stores are regulated as a function of environmental sources of the element (Kubota et al. 2003).

Accumulation and subcellular distribution patterns of As in sea turtles (*Chelonia mydas* and *Eretmochelys imbricata*) suggest that arsenobetaine is accumulated as an osmolyte along with glycine betaine (Fujihara et al. 2003). Also, in sea turtles, biotransformations of Hg are critical to the element's tissue-specific bioaccumulation. For example, the proportion of methyl-Hg to total Hg was observed at around 80% in muscle and 50% in liver tissue of *Caretta caretta* (Storelli et al. 1998), although the distribution pattern for Hg and methyl-Hg may differ for other tissues (Shen 2008). Biomagnification of Hg also varies as a function of biological processes in Hg exposures to reptiles in field settings. For example, biomagnification factors for methyl-Hg characteristic of trophic transfers of Hg from snails (*Littoria irrorata*) to female diamondback terrapins (*Malaclemys terrapin*) roughly exceeded those for total Hg by a factor of 10 (Blanvillain et al. 2007).

12.3.1.3 Toxicokinetic Phases Summary

Our understanding of the toxicokinetics of metals in reptiles is highly limited. In general, information on mechanisms that might help explain exposure of reptiles to metals and those linked to resistance to metals (as reviewed in Section 12.4) is sparingly available, and when available, only well characterized for few species. No comparative context for understanding the toxicokinetics of metals in reptiles is available, and future studies should focus on mechanisms mediating:

1) absorption of metals via integumentary and pulmonary routes (specific to many reptiles: intimate contact to soil and dust);
2) absorption of metals via gastrointestinal routes, in particular the importance of metal-containing granules in the trophic availability of metals (specific to many reptiles: invertebrate food);
3) presystemic elimination of metals through both liver and kidney (specific to many reptiles: subcutaneous lymphatic system and renal portal vein system);
4) elimination via metal-binding molecules or extracellular sequestration;
5) redistribution of metals during stress phases; and
6) kinetics of organometallic compounds.

12.3.2 Trophic transfer, Bioaccumulation, and Biomagnification

12.3.2.1 Trophic Transfer

Trophic transfer of metals in reptiles is suggested by several observational field studies detecting parallel patterns of contamination between reptiles and their prey, gut contents, or feces. These studies are important in that they provide the natural context of contamination, yet caution should be taken in interpreting results. Among many factors, differences in lifespan of predacious reptiles and their prey, or differences in seasonal or behavioral shifts in prey choice could lead to a lack of correspondence between contamination of reptiles and their food without necessarily negating the importance of dietary transfer of contaminants. Therefore, a much more direct demonstration for dietary transfer of contaminants is obtained in laboratory experiments where reptiles were fed contaminated prey. There is now relatively abundant evidence from both types of studies showing that trophic transfer is an important route of contamination of reptiles.

Regarding observational field studies, some important information comes from the Savannah River site in South Carolina, where coal combustion plants for the generation of electricity produce large quantities of solid waste in the form of coal ash. Coal ash is enriched with potentially toxic trace metals, including Se, As, Cd, Cr, Cu, Sr, and V (Nagle et al. 2001; Rowe et al. 2002). When disposed in landfills and settling basins, these metals invariably became associated with aquatic habitats. Metal contamination of turtles, water snakes, and alligators (Nagle et al. 2001; Roe et al. 2004), as well as other wildlife (see Nagle et al. 2001 for references) has been abundantly documented in these habitats, and could have resulted from ingestion of contaminated prey, maternal transfer, or absorption through skin or eggshell.

Nagle et al. (2001) studied adult female slider turtles (*Trachemys scripta*) from a coal-ash-polluted site that had higher concentrations of As, Cd, Cu, and Cr (not significant for Cu) in their livers than turtles from a reference site. Gut contents of these same turtles included Asiatic clams, crayfish, aquatic insects, and vegetation. Largely coincident with contamination patterns in turtles, metal residue levels in clams and crayfish (the only items collected in the field and analyzed) were higher in As, Cd, Cu, and Cr (not significant for Cr, and for Cu in clams). Likewise, metal contamination of the banded water snake *Nerodia fasciata* paralleled that of its prey (Hopkins et al. 1999). Arsenic, Cd, and Se were significantly higher in both water snakes and the anurans *Rana catesbeiana, Hyla cinerea*, *Bufo terrestris*, and the fish *Lepomis macrochirus* and *Gambusia affinis* collected from the contaminated site. In turn, contaminated site water snakes had nonsignificantly higher levels of Cr and Cu than reference site water snakes and no clear pattern was found among anurans and fish for these metals.

Other studies provide similar consistencies between reptile and prey contamination. Cook et al. (1989) reported that among 26 crocodilians from the New York Zoological Park, 3 (2 false gharials, *Tomistoma schlegelii*, and 1 Cuban crocodile, *Crocodylus rhombifer*) contained elevated blood levels of lead. In contrast to their 23 cohabitants, these 3 individuals had been fed wild-caught, urban pigeons, which displayed elevated bone lead levels. Difficulties in establishing a clear link between reptile predators and their prey, however, is more commonly encountered, as exemplified by a study comparing Hg contamination in diamondback terrapins (*Malaclemys terrapin*) with that of their most important prey, salt marsh periwinkles (*Littoraria irrorata*). Among sites in coastal areas of the southern United States, there was no significant correlation between total Hg or methyl-Hg in periwinkle and terrapin turtle scutes or blood. Yet, the site that displayed the greatest Hg contamination in periwinkles also displayed greatest Hg contamination in turtles, which suggested that trophic transfer of mercury may have occurred during exposures in the field (Blanvillain et al. 2007).

Additional support for the trophic transfer of metals is also apparent in studies comparing patterns of contamination between reptiles and their gut contents (Anan et al. 2001; Fletcher et al. 2006). For example, Fletcher et al. (2006) focused on Aznalcóllar in southern Spain, where a mine tailings dam collapsed in 1998 and released 2 million cubic meters of toxic mud containing 14 metals in the Guadiamar River floodplain. From 7 locations across an expected contamination gradient,

Moorish wall geckos (*Tarentola mauritanica*) were collected and tissue residues of 21 elements were compared to concentrations in gut contents (composed of arthropods and sediment). Spatial variation in the concentrations of several metals in geckos was in general agreement with those elemental concentrations measured in their gut contents, especially the high concentrations in both predators and prey on the mine-contaminated floodplain. Trophic transfer was hypothesized as the most likely route of exposure for geckos, although dermal exposure and ingestion of particulate matter amid prey or via eye and face licking were also considered important routes of exposure (Fletcher et al. 2006). In a second example, metal residues in liver, kidney, and muscle of green turtles and hawksbill turtles collected from the Yaeyama Islands in Japan (Anan et al. 2001) were evaluated. The authors found that green turtles, which had higher residue levels of V, Cr, Cu, Zr, Ag, Cd, Ba, and Pb, also presented gut contents with higher V and Cr than hawksbill turtles. In turn, hawksbill turtles, which had higher residue levels of Mn, Co, Se, Mo, and Hg, also presented gut contents with higher residue levels of Se (but also Zn) than green turtles. Other trophic transfer studies focused on reptiles include work with gopher snakes (*Pituophis melanoleucus*) where tissue residues of Se paralleled dietary concentrations of their prey (small mammals, birds, and bird eggs), which had been characterized in previous studies from the same site (Ohlendorf et al. 1988). Similarly, in *A. mississippiensis,* tissue residues of Se were higher in individuals fed fish (*Micropogon undulates*) containing 2.8 ppm Se dry weight than in those fed nutria (*Myocastor coypus*) containing 0.04 ppm Se dry weight (Lance et al. 1983).

Experimental studies have also demonstrated trophic transfer of metals to reptiles through feeding trials that manipulated contaminated food, dead prey, or live prey. Predator-prey pairs tested were lizards and crickets (*S. occidentalis* fed *Acheta domestica* and *P. carbonelli* fed *Nemobius sylvestris*), water snakes and fish (*N. fasciata* fed *Micropterus salmoides* and *Lepomis* spp), and terrestrial snakes (African house snake, *Lamprophis fuliginosus* and *E. guttata* fed mice). Again, some of the most comprehensive experimental studies were completed at the Savannah River Ecology Lab in South Carolina, and these results confirm the importance of trophic transfer in reptile exposure to environmental contamination. These studies include long-term experiments where snakes and lizards were fed prey from contaminated coal ash disposition sites, prey injected with metals, or prey fed with metal-contaminated chow (Hopkins et al. 2001, 2002, 2004, 2005a, 2005b; Jackson et al. 2003). Water snakes (*N. fasciata*) were fed fish from a coal-ash-contaminated site for 13.5 months, and concentrations of metals in liver, kidney, gonads, tail clips, blood, and shed skin were compared to those of snakes fed fish from a reference site; an overall effect of feeding treatment on accumulation of As, Cd, Se, Sr, and V, indicated in all cases that snakes fed the contaminated diet accumulated significantly higher concentrations of these metals compared to controls (Hopkins et al 2001).

Corroboration of these results was obtained in a 2-year experiment, where full-sibling juveniles of the same species were subject to 1 of 3 feeding regimens, that is, fed fish from a coal-ash-contaminated site, fish from a reference site, and fish from either site on alternating meals (Hopkins et al. 2002; Jackson et al. 2003). Contaminated fish contained elevated levels of As, Cd, Cu, Se, Sr, and V. As hypothesized for trophic transfer exposure, As, Cd, Se, Sr, and V accumulated 10- to 20-fold above control levels in the liver, kidney, and gonads of snakes fed contaminated fish, usually in a dose-dependent manner. Selenium, in particular, accumulated levels greatly exceeding established toxicity thresholds for other vertebrates. This particular result prompted 2 additional experimental lab studies directed at understanding the trophic and maternal transfer of selenium (Hopkins et al. 2004, 2005b). In the first paper, female *Lamprophis fuliginosus* were fed seleno-D,L-methionine injected mice at 1 of 3 levels (1, 10, 20 μg/g dry mass) for 10 months. Snakes exposed to excessive Se accumulated significant concentrations of Se in kidney, liver, and ovarian tissue in a dose-dependent manner, and transferred selenium to their eggs. Hopkins et al. (2005b) further demonstrated longer dietary transfer of selenium in a simulated food chain in which fence lizards, *Sceloporus occidentalis,* were fed crickets that had been fed Se-contaminated chow. As hypothesized, fence lizards accumulated Se, and this accumulation was influenced by tissue type (carcass, liver, gonad) and sex

(higher in females). Female gonad concentrations, in particular, reached very high concentrations (up to 14.1 μg/g Se dry mass), which can cause reproductive toxicity in fish and birds.

The studies by Hopkins and collaborators provide some of the strongest evidence for trophic transfer of Se and other metals (As, Cd, Sr, and V) in reptiles, but other studies demonstrate the importance of trophic transfer of metals in general, and additional elements such as Cd, Pb, and Hg in particular. For example, corn snakes fed mice contaminated with a mixture of Pb, Cd, and Hg accumulated significant amounts of all metals in their shed skins (Jones and Holladay 2006). Similarly, in a laboratory study, Mann et al. (2006, 2007) fed crickets contaminated with radiolabeled Cd (^{109}Cd as cadmium nitrate) to European wall lizards for 21 weeks. In 1 treatment, lizards were fed crickets that had been internally contaminated through a Cd-containing diet (lizards consumed a total of 8.85 ± 0.13 μg Cd); in another treatment, lizards were fed crickets that had been externally contaminated through Cd application between their wings (lizards consumed a total of 16.90 ± 0.25 μg Cd). Results of the study indicated that the majority of the Cd was retained within the gut of the exposed lizards and that transfer of Cd to internal organs (liver, kidney, carcass) was low. Cd accumulation patterns did not show significant differences between the 2 treatments.

Trophic transfer of Pb has also been considered experimentally. For example, motivated by the observation of elevated lead contamination in Australian estuarine crocodiles from sites where hunting with lead ammunition was common, Hammerton (2003) conducted an experiment where 3 juvenile crocodiles were fed single doses of 5 lead shots amid a meat bolus. Ingested lead particles were retained and slowly dissolved in the digestive system. From this lead shot source, Pb was absorbed and resulted in blood lead at 278 to 363 μg/dL at relatively steady-state concentrations 5 to 20 weeks after explosure. The blood Pb concentration-time curves followed a 1-compartment model with first-order loss kinetics that yielded an apparent biological half-life for Pb in blood of about 3.4 days. Similar studies also focused on alligator farms in Lousianna (Camus et al. 1998; Lance et al. 2006) where animals were fed lead-shot-contaminated meat of nutria. Captive and wild alligators were analyzed for lead in bone and ovarian egg yolk, and for lead, selenium, and cadmium in kidney and liver. Compared with wild alligators, captive alligators had extremely high tissue levels of Pb, but lower Se and similar Cd levels (Lance et al. 2006).

12.3.2.2 Bioaccumulation and Biomagnification

Evidence for biomagnification of metals exists for As, Cd, ^{137}Cs, Hg, Pb, Se, and Sn, among others, mostly in their lipid–soluble, organic forms where biological half-life is usually high. However, strongly compelling evidence is available for methyl-Hg and radiocesium,^{137}Cs only (Gray 2002; Wang 2002). Cs, Hg, Pb, Se, and Sn, but strongly compelling evidence is available for methyl-Hg and radiocesium,^{137}Cs, only (Gray 2002; Wang 2002). In part, a clear demonstration of biomagnification in other metals is hindered by the limited number of compartments and trophic levels analyzed. Assessing biomagnification is complicated by the diversity of trophic interactions in food webs, the frequent uncertainties regarding trophic links among species within food webs, and the presence of sometimes multiple food webs in the same environment. For example, metal concentrations in a given species of reptile may deviate substantially from that expected for its trophic position, in part because of particular spatial attributes reflected in the distribution of metals in the environment. Also, uptake of metals by organisms lower in the food web inevitably alters the transfer of constituents to reptiles. Since metals tend to concentrate in sediment, suspended particles, and bacteria, metal exposure and consequently bioaccumulation may achieve higher values in deposit- and/or suspension-feeding organisms within a food web.

Studies on trophic transfer suggest that metals have the potential to biomagnify in reptiles, and in food webs containing reptiles. Focusing on methyl-Hg, if this hypothesis is correct, 3 predictions can be made. First, in areas with mercury contamination reptiles should have higher mercury levels than their prey. Second, within a species, mercury levels should increase with stages, sizes, or sex, depending on differences in positions within the food chain. Third, carnivorous reptiles should have higher mercury levels in their tissues than omnivorous and herbivorous reptiles. Although several

of the available studies do not differentiate between inorganic and organic Hg, and are not based on whole body Hg concentrations in both predator and prey, some evidence in the literature indeed supports these 3 predictions for reptiles. First, higher Hg residue levels in reptiles relative to their prey have been characterized for *Malaclemys terrapin* and periwinkles in salt marshes, where an average biomagnification factor of 173.5 for methyl-Hg in females has been observed. Female terrapins had significantly higher mercury levels than males, possibly because they are larger and prey preferentially on larger periwinkles, which had higher mercury levels than smaller periwinkles (Blanvillain et al. 2007). Second, concentrations of Hg in the American alligators were higher than in fish; in addition, Hg concentrations increased with alligator body size, which was suggested to result from the larger proportion of higher trophic level prey in adult alligators than in juveniles (Yanochko et al. 1997). Third, carnivorous reptiles also contained higher Hg residue levels in their tissues than herbivorous reptiles from the same sites. For example, Hg concentrations in liver, kidney, and muscle of the carnivorous loggerhead turtles were consistently higher than in the generally herbivorous green turtles in the Mediterranean Sea (Godley et al. 1999).

Among freshwater species, the omnivorous common snapping turtle (*Chelydra serpentina*), which preys and scavenges on a wide range of other organisms, had higher mercury concentrations than the predominantly herbivorous *Trachemys scripta* (Meyers-Schöne 1989; Meyers-Schöne et al. 1993). Differences in trophic position and Hg contamination in these and other freshwater turtle species were later confirmed in ecotoxicological studies employing stable isotopes. Similarly, Bergeron et al. (2007) reported that total mercury and methyl-Hg concentrations were positively correlated with $\delta^{15}N$ across blood samples of 67 individuals of 4 species of turtles occurring in an Hg-contaminated river in Virginia. Isotopic and contaminant data were in overall agreement with the rank in carnivory reported in the literature (*Chelydra serpentina* ≥ *Sternotherus odoratus* > *Chrysemys picta* > *Pseudemys rubriventris*), although isotopic data suggested that all species were actually feeding at more than one trophic level.

Several studies, whether experimental or observational, have failed to support biomagnification of metals in reptiles. Selenium bioaccumulated but did not biomagnify in snakes (Hopkins et al., 2001, 2002, 2004, 2005a) and lizards (Hopkins et al. 2005b). In many cases, metal concentrations in lizards, especially herbivorous and detritivorous species, were markedly lower than those in their food (Fletcher et al. 2006; the same study found lizards contained higher levels of Rb and Sr, and similar levels of Se to their prey).

Factors describing bioconcentration, bioaccumulation, or biomagnification are frequently used for regulatory purposes (USEPA 2007) but are nearly unavailable for reptiles. Comparing female diamondback terrapin scutes with whole soft bodies of their snail prey, mean biomagnification factors were overall 11.3 for total Hg or 173.5 for methyl-Hg, reaching, respectively, 11.4 and 351.1 at the most contaminated site (Blanvillain et al. 2007). Hsu et al. (2006) analyzed soil and whole body residues of several elements in a variety of organisms (from fungi to mammals) in Kenting National Park (Taiwan) and found high bioconcentration factors (BCFs) for Cd, Hg, and Sn in lizards and snakes.

Collectively, these studies provide a clear demonstration of the role of trophic transfer in the contamination of reptiles in metals, and support biomagnification of methyl-Hg, but not of As, Cd, Cu, Pb, Se, or Zn.

12.3.3 Transgenerational Transfer

Through transgenerational transfer, exogenous agents may be passed from one generation to the next via reproductive tissues, eggs, and embryos, a process that continues until hatching. In this section we also consider transfers from the external breeding environment to offspring, which leads our discussion to different breeding strategies that range from ovipary to vivipary, and the role of maternal care (including breeding site selection) as a factor influencing exposure in early life stages of reptiles. Transgenerational transfer may result in contaminant exposure at the most critical

transition phases in the life history of organisms, namely gametogenesis and embryogenesis, and is closely related to reproductive toxicity, developmental toxicity, and endocrine disruption (see Section 12.4).

In viviparous and ovoviviparous animals, contaminant transfer to eggs and embryos typically takes place via the maternal gonadal system. In oviparous animals such as most reptiles, 2 not necessarily mutually exclusive proximate sources of contamination may be involved: the internal maternal environment (from ovulation and fertilization until deposition) and the external incubation environment (from egg deposition until hatching). This section considers the evidence for transgenerational transfer of metals in reptiles and the related implications for exposure and effect assessment. Metal concentrations observed in reproductive tissues of reptiles are presented in the Appendix. The distribution of the published information among metals, reptile species, and compartments of concern is further condensed in Table 12.2. Finally, all tissue concentrations reported for Cd, Hg, and Pb are summarized in Table 12.3, and compared with environmental quality standard levels for freshwater and saltwater, in the absence of such guidance for soils (USEPA 2006).

12.3.3.1 Transfer via Maternal Tissues

Several studies confirm the presence of metals of priority concern in male and female gonads, including ovarial oocytes. Field evidence is available for sea turtles (*Caretta caretta* and *Chelonia mydas*) caught by fishermen in Japan (Sakai et al. 2000b); turtles, lizards, and snakes from Vietnam (Boman et al. 2001); brackish water terrapins (*Malaclemys terrapin*) from New Jersey (Burger 2002); and water snakes (*Nerodia sipedon*) from Tennessee, including a Superfund site (Burger et al. 2005). Evidence from the field and farm is available for American alligators from Florida (Heaton-Jones et al. 1997) and Chinese alligators from a captive breeding center in China (Xu et al. 2006). Further evidence is derived from a series of controlled laboratory experiments with long-term dietary exposure for water snakes (*Nerodia fasciata*) fed field-collected coal-ash-contaminated prey (Hopkins et al. 2001) and for brown house snakes (*Lamprophis fuliginosus*; Hopkins et al. 2004) and fence lizards (Hopkins et al. 2005b) fed Se-dosed prey. In addition, several metals have been detected in oviduct tissue of alligators (Heaton-Jones et al. 1997), and in oviductal eggs of field-collected *Caretta. caretta*; Sakai et al. 1995) and of the snake *Waglerophis. merremii* (De Jorge et al. 1971).

Detailed experimental studies clearly demonstrate that cadmium, a priority concern metal, is transferred to gonadal tissues after intraperitoneal injection of cadmium chloride in female *Trachemys scripta* (Thomas et al. 1994) and after intravascular injection of ^{109}Cd radioisotope or cadmium chloride in female *Chrysemys picta* (Rie et al. 2001). The latter study confirmed maternal transfer of Cd to eggs via both the ovary and oviduct tissue (Figure 12.3). Cadmium concentrations were highest in the liver, the site of synthesis of egg yolk precursor proteins (vitellogenine) where Cd is bound, then transported via the blood stream to the female gonads, where it is incorporated into the yolk of developing oocytes. In contrast, unbound Cd seems to be transferred to egg albumen and shell via the epithelium of the oviduct.

Maternal transfer may also be safely concluded from the detection of metal contamination of freshly laid eggs collected in the field for olive ridley turtles, *Lepidochelys olivacea* (several metals including Cd and Pb; Sahoo et al. 1996), and American alligators (Se; Roe et al. 2004), and from eggs laid in the lab shortly after collection of females in the field for slider turtles (several metals, including Cd, Hg, and Pb; Burger and Gibbons 1998).

12.3.3.2 Transfer via Breeding Substrate

Metal contamination of reptile eggs collected in the field is well documented (Tables 12.3 and 12.4; see also Appendix), but could be due to both maternal transfer and transfer via the breeding substrate. As noted in Section 12.3.3.1, maternal transfer of some metals does occur, and both experimental

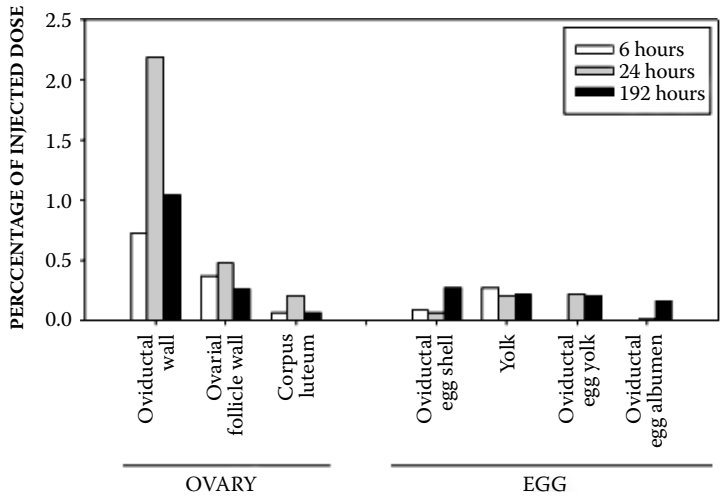

FIGURE 12.3 Distribution of ^{109}Cd among reproductive tissues in female painted turtles, *Chrysemys picta,* 6, 24, and 192 hours after intravascular injection (concentrations in oviductal eggs not analyzed at 6 hours postinjection). (Data from Rie et al. 2001.)

and observational studies demonstrate that metal transfer via the breeding substrate also occurs (but see Nagle et al. 2001 for a case where substrate contamination did not influence embryo metal loads). In experimental studies, eastern fence lizard (*Sceloporus undulatus)* hatchlings exposed as eggs to a concentration range of Cd in an artificial breeding substrate (1.48 to 14 800 μg Cd/g perlite) concentrated Cd in a dose-dependent manner; bioconcentration factors ranged from 0.04 to 0.92 (Brasfield et al. 2004). Similarly, in studies on As, Iberian rock lizard (*Lacerta monticola cyrenni)* eggs obtained from field-collected gravid females and incubated in an artificial breeding substrate watered with a range of As solutions (50 to 500 ng As/ml of water) concentrated As in a dose-dependent manner in eggshells and embryos; bioconcentration factors were negatively related to exposure concentration and ranged from 0.629 to 0.171 in eggshells and from 0.265 to 0.048 in embryos (Marco et al. 2004). Sahoo et al. (1996) compared metal concentrations among freshly laid eggs, fresh hatchlings and sand samples from nests of olive ridley turtles, found that concentrations of the nine metals in hatchlings exceeded those in freshly laid eggs and concluded that the turtle embryos accumulated metals from the nesting beach sand during incubation.

12.3.3.3 Distribution among Early Developmental Stage Compartments

Some information is available to characterize the distribution of metals in reptiles during their early developmental stages (Tables 12.3 and 12.4). The existing literature presents a relatively large but patchy dataset because the available studies focused on various reptile species; various exposure scenarios; ovarial, oviductal, or freshly laid eggs; and egg compartments as diverse as whole egg, eggshell, shell membrane, chorioallantoic membranes, albumen, yolk, albumen-yolk, egg contents, embryos, or hatchlings. Differences between wet or dry mass-based concentrations may further limit comparisons between studies. The compartments considered in a publication are listed in decreasing order of the mean concentration levels detected therein. Where published, statistically significant differences are indicated. See Linder and Grillitsch (2000) for references prior to 2000.

Concentrations of metals tended to be higher for Al and Cr in eggshells, while concentrations of Hg were higher in the egg contents. In most records, Cu levels were higher in the shell, whereas Fe and Zn were higher in the egg contents. For Cd, Pb, and other metals, distribution patterns were inconsistent (Tables 12.3 and 12.4).

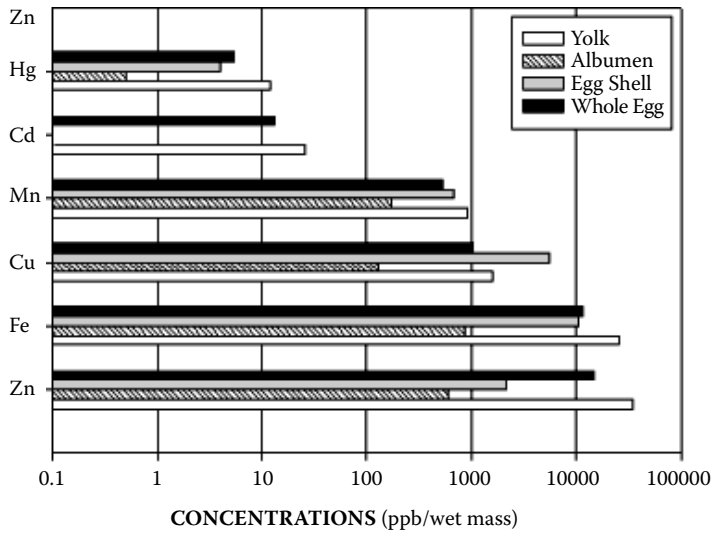

FIGURE 12.4 Distribution of mercury, cadmium, manganese, copper, iron, and zinc among oviductal egg compartments in loggerhead turtles, *Caretta caretta* (mean concentrations; Cd below detection limits in albumen and shell). Note log scale for metal concentrations. (Data from Sakai et al. 1995.)

For oviductal eggs from field-collected females, differences observed in the distribution of metals (all wet mass based) have not been subject to statistical analyses. Sakai et al. (1995), who analyzed Cd, Co, Cu, Fe, Hg, Mn, Ni, Pb, and Zn in yolk, shell, and albumen of oviductal eggs collected from 5 loggerhead turtles, was the primary source for much of the data for these metals, although Co, Ni, and Pb concentrations were below detection limits in all samples. Within an egg, yolk contained the highest concentrations for all detected metals except for Cu, which was more concentrated in the shell (Figure 12.4), and Cd, which was detected only in the yolk. No significant correlation was found between metal concentrations and egg mass. Largely in accordance with Sakai et al. (1995), concentration of Cu in oviductal egg contents of *Waglerophis merremii* exceeded whole egg concentrations by about a factor of 2, whereas Fe concentrations were almost equal for the 2 compartments, indicating that Fe is mainly concentrated in egg contents (De Jorge et al. 1971).

Rie et al. (2001) described maternal transfer and the time course of Cd distribution in reproductive tissues of turtle, *Chrysemys picta*, after intravascular injection of Cd-109 radioisotope or cadmium chloride. This study demonstrated that Cd distributation among oviductal eggshell, yolk, and albumen may vary with the time elapsed (6 to 192 hours) postinjection (Figure 12.3). Concentrations of As, Cd, Cu, Pb, and Zn in eggshell samples from common chameleon (*Chamaeleo chamaeleon*) exceeded those in egg contents except for Zn (Gomara et al. 2007). Xu et al. (2006) analyzed 9 metals in eggshells, shell membranes (used synonymously with chorioallantois membranes in this publication), and contents of eggs laid by Chinese alligators in a captive breeding center. All elements were detected in each of the 3 compartments. Chromium, Mn, and Pb were significantly higher in the shells, and As, Fe, Hg, and Zn were higher in egg contents (no differences for Cd and Cu). Comparisons between shell membranes and contents indicated that Cr, Fe, Hg Mn, Pb, and Zn were lower and only As and Cd were higher in the egg contents. Metal levels in the shell membranes showed significant correlations with those in the egg contents for Cu, Fe, and Zn, and those in the eggshells for As and Hg. No correlation was significant between eggshell and egg contents for any metal (Xu et al. 2006). As a final example of compartmental analysis in laid eggs, we consider Se. Roe et al. (2004) observed that Se concentrations were higher in chorioallantoic membranes than in eggs and hatchlings of the American alligator and that Se concentrations in chorioallantoic membranes seemed to parallel the degree of Se contamination of the breeding sites.

Kaska and Furness (2001) analyzed Cd, Cu, Fe, Hg, Pb, and Zn in samples (eggshells, remaining yolk, and liver) from hatchlings of loggerhead turtle collected from beaches of southwest Turkey; concentrations of Cd, Cu, Fe, Hg, and Pb were higher in embryo liver than in yolk (Hg below detection limit in all samples), whereas Zn concentrations in yolk exceeded those in liver.

12.3.3.4 Implications for Exposure Assessment

All metals analyzed in the reproductive tissues of field-collected reptile specimens were found more frequently above detection limits than below. Essential metals such as Cu, Fe, Mn, Se, and Zn were detected in all samples, and metals of highest-priority concern, like Cd, Hg, and Pb, were detected in the vast majority of samples. Among metals only sporadically analyzed, Ba, Rb, Sn, and Sr were above, and Be and Tl were below detection limits in all samples, whereas Mo and V were detected in only a few samples.

In general, concentrations of essential metals were generally higher than those for nonessential metals, when transgenerational transfers were considered. Furthermore, metal burdens in reproductive populations may be overestimated, if that estimation is based on high levels of essential metals in early life stage samples. Overestimation would likely result from simple numeric comparisons because the transfer of essential metals from maternal tissues to yolk and eggshell is a regulated process that affects different components of development: one, the development of yolk, and the other, formation of eggshell. These processes should be distinguished as being functionally linked to trophic and construction materials, respectively. Sex-specific differences in metal allocation of essential metals were anticipated and demonstrated in sexually mature *Sceloporus occidentalis*, where Se contents in ovaries exceeded those in testes (Hopkins et al. 2005b).

In contrast, observations of low metal levels detected in samples collected from early life stage may underestimate metal burdens in the reproductive population, as demonstrated by metal concentrations in gonadal tissues and early life stages (egg to hatchling) generally being lower than in maternal liver and kidney (Linder and Grillitsch 2000; Tables 12.2 and 12.3; see also Appendix). Relative to whole body metal loads, mean metal concentrations in whole ovaries were approximately 10% (Cu) or less (Fe > Zn > Mn > Hg > Cd), and those in oviductal eggs 3% (Cu) or less (Fe > Mn > Zn > Hg > Cd) (see Sakai et al. 2000b). Within eggs, metal concentrations were highest in the yolk, where roughly 60 to 100% of the whole egg burden occurred (Cu > Mn > Fe > Hg > Zn > Cd; see Sakai et al. 1995). In *Caretta caretta*, concentration levels of several metals in reproductive tissues paralleled those in the liver; concentrations in oviductal eggs were no more than 1 order of magnitude lower than those in ovary and oviduct; and concentrations of essential elements (Zn > Fe > Cu > Mn) in all tissues clearly exceeded those of the nonessential elements (Cd > Hg > Pb) (Figure 12.5; see Sakai et al. 2000b).

Eggshells, eggshell membranes, and chorioalantoic membranes may be alternative tissues for contaminant monitoring through nondestructive sampling (see also Section 12.3.4.1). Their use must satisfy the assumption that contaminant concentrations in these alternative tissues is a function of whole body concentrations (for example, in hatchlings). Unfortunately, the strength of the association of metal concentrations in eggshells and egg contents or hatchlings has rarely been examined in reptiles (Burger and Gibbons 1998). Comparisons between wet mass concentrations of metals detected in eggshell and egg contents of *Trachemys scripta elegans* ranked Zn > Al > Sn > Mn > Cu in egg contents but Zn > Sn > Cu > Cr > Pb > V > Mn > Ni > Cd in eggshells. For those elements detected in both compartments (Mn, Cu, Sn, Zn), only Zn presented a significant correlation between eggshell and egg content (Tryfonas et al. 2006).

Recent studies (Roe et al. 2004; Xu et al. 2006) suggest that chorioallantois membranes are suitable tissues for monitoring metal levels in reptile eggs, as has been previously demonstrated for organic contaminants (Pepper et al. 2004). In reptile eggs, the shell membrane is a multilayer, tertiary egg membrane secreted by the oviduct. In contrast, the chorioallantois membrane, which is apposed to the eggshell membrane, represents an extra-embryonic structure made up by fusion of

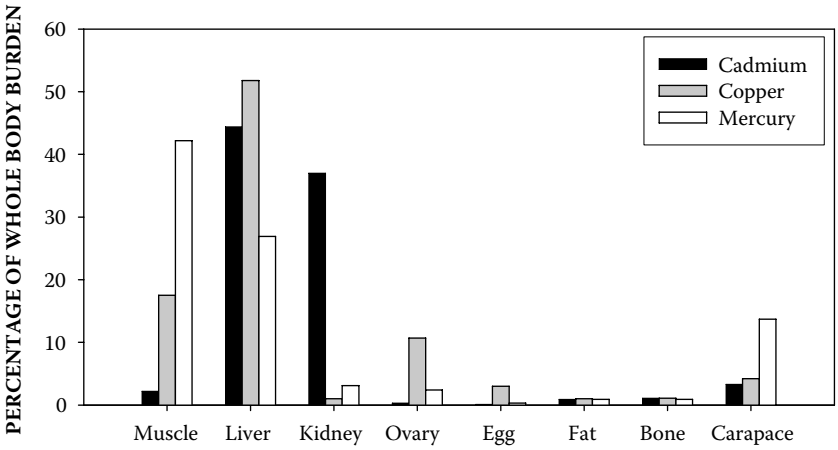

FIGURE 12.5 Distribution of cadmium, copper, and mercury among reproductive and nonreproductive tissues of female loggerhead turtle, *Caretta caretta.* (Data from Sakai et al. 2000b.)

the chorion and the allantois, and serves in homeostatical control of respiration in the developing embryo (Roe et al. 2004).

When using egg compartments for minimally invasive residue monitoring, potential sources of bias may be linked to cross-contamination associated with breeding substrate and other egg compartments. For example, earlier studies with reptile eggs did not include the presence of chorioallantois membranes when preparing analytical samples, which may explain, in part, between-study variation in observed residue levels. Similarly, improper handling of the contents of the allantoic sac, which serves as the embryonic urinary bladder and represents a waste storage site, may have introduced contamination of hatchling samples in earlier studies.

In summary, there are pros and cons for employing metal concentrations in egg compartments as indicators of exposure for monitoring metal contamination of reptiles in the field. Concentrations detected in egg compartments may be representative because these concentrations likely parallel those in maternal tissues. However, these estimates are potentially insensitive, given measurements may be lower than in maternal tissues (thus becoming a matter of analytical detection limit). While future research should address bias associated with interpretations of metal concentrations in egg compartments, reptile eggs are relevant to field monitoring, and metal contamination of critical life stages may be indicative of increased contaminant levels in reproducing populations of reptiles. For reliably employing egg compartments in the assessment of contaminant exposure, the structure and function of reptilian oviduct and eggshell have to be better understood. The reptilian oviduct structures and functions (such as albumen production, eggshell production, placentation, oviposition or parturition, and sperm storage) are diverse and little understood (Girling et al. 2002). Similarly, eggshell fine structure (Schleich and Kästle 1988), permeability, and chemical composition are varied (Sexton et al. 2005) and strongly influence gaseous exchange, water absorption, and uptake of pollutants. In the few studies that experimentally studied transgenerational transfer of metals in reptiles, structural and functional traits and the ecophysiology of species eggs have been incompletely considered.

12.3.3.5 Implications for Effect Assessment

Early life history stages may be particularly sensitive to pollutants, and several metals of priority concern have the potential to adversely affect fitness through disruption of reproductive function and the early development of offspring (e.g., Golub 2006; Apostoli et al. 2007). Linking tissue concentrations of a metal with particular direct or indirect toxicological effects, and understanding the underlying interactions of the metal at the molecular and cellular level represent challenges in

current metal toxicology. For reptiles (Hopkins 2006) and even humans (Hoyer 2006), evidence based on mechanistic animal model studies is largely missing, although the presence of a metal in target tissues may serve as an important first indicator of risk.

Overall, Cd, Hg, and Pb concentrations detected in reproductive and early life stage compartments of free-ranging reptiles (Table 12.3; see also Appendix) and birds (Rattner et al. 2005, 2008) indicated that metal residues occurred within the same orders of magnitudes. For example, comparisons of maximum concentrations indicate the following:

1) Cd levels in reptiles (2.0 ppm dry mass, hatchling, whole body; 1.19 ppm wet mass, adult male gonad) exceeded those in birds (0.013 ppm dry mass; 0.013 ppm wet mass egg contents, bald eagle, *Haliaeetus leucocephalus*);
2) Pb levels in reptiles (20 ppm dry mass, hatchling, *Lepidochelys olivacea*; 22.10 ppm wet mass, egg contents, *Chamaeleo chamaeleon*) attained higher values than in birds (6.7 ppm dry mass, 3.43 ppm wet mass, egg contents, Herring Gull, *Larus argentatus*); whereas
3) Hg reached higher concentrations in birds (5.27 ppm dry mass, egg contents, bald eagle, *Haliacetus leucocephalus*; 7.93 ppm wet mass, egg contents, sooty tern, *Sterna fuscata*) than in reptiles (0.75 ppm dry mass, hatchling, *Caretta caretta*; 5.91 ppm wet mass, ovary, *Alligator mississippiensis*).

The highest Cd, Hg, and Pb levels were all observed in animals closely associated with coastal marine or estuarine environments where both pollution and natural background levels with these metals may be relatively high.

In the absence of epidemiological studies, extrapolation from tissue concentrations to effect concentrations across vertebrate taxa would be a preliminary approach toward linking observed tissue levels and adverse effects. Both maximum and median concentrations of these priority pollutant metals reported for reptile "transgenerational" tissues fell within those levels known to cause reproductive and developmental effects in fish, birds, and mammals (Eisler 2000; Smit et al. 2000; EC 2005a, 2005b, 2005c; Lepper 2005; Golub 2006); therefore, related adverse effects to the reproductive fitness of some reptile wildlife may not be excluded.

Maximum levels observed in reptile transgenerational tissues nearly exceed the "Current National Recommended Water Quality Criteria" (EPA 2006) and suggested Environmental Quality Standards for priority substances by about a factor of 100 or 1000 (Table 12.3). Overall, such environmental quality target concentrations are a function of no observed effect concentration (NOEC) and bioconcentration factor (BCF) levels for aquatic fauna and flora. These target concentrations are intended to protect predators from secondary poisoning (Smit 2000; Lepper 2005) and may therefore demonstrate the relative degree of contamination of wildlife.

For tissue residues of metals in reptiles, observational or experimental studies linking exposure concentrations, concentrations in reproductive tissues, and reproductive or developmental effects are scarce. Some recent studies have linked experimental transgenerational metal transfer to reproductive and developmental effects (Section 12.4; Tables 12.5 and 12.6). For example, while no significant relationships were found between Se accumulation in ovary and reproductive output in brown house snakes (Hopkins et al. 2004), for slider turtles some relationships indicated there may be links between maternal transfer of Se and hatching rate. However, in the case of Se, surviving hatchlings displayed no adverse effects as indicated by days to hatch, carapace length, or total body wet mass (Nagle et al. 2001). Similarly, in *Sceloporus undulatus*, exposures to Cd via the breeding substrate did not explain observations related to hatching rates in eastern fence lizard (Brasfield et al. 2004). Freshly laid eggs from field-collected gravid Iberian rock lizards (*Lacerta monticola cyrenni*) previously exposed to As via breeding substate, eggshells, and embryos accumulated As in a dose-dependent manner. Arsenic treatments, however, did not affect embryo survival, incubation duration, or hatchling size, but there was a strong negative relationship between hatchling running speed and As exposure concentration (Marco et al. 2004). This study, however, did not allow for causal explanation of

the observed, ecotoxicologically important effects because additional metals (Cd, Cu, Pb, and Zn) other than As occurred in field exposures, and had concentrated in shells and embryos, suggesting concomitant uptake from breeding substrate or maternal transfer. The only robust link between the presence of a metal in reproductive tissues and a physiological response is evident in previously described experiments completed by Rie et al. (2001). Here, adult painted turtles (*Chrysemys picta*) were exposed to Cd-109 radioisotope (single dose via intravascular injection), and accumulation of the Cd isotope was observed. In addition, induction of metallothioneine-like proteins in steroidogenic tissue (particularly gonadal and adrenal-interrenal) and steroid hormone target tissue (particularly hepatic, where vitellogenin is produced under estrogenic control) was measured.

12.3.3.6 Transgenerational Transfer Summary

Overall, 43 publications reported concentrations of 21 metals in 18 different transgenerational compartments for 30 reptile species (Linder and Grillitsch 2000; Table 12.1). The compartments analyzed included the gonadal system (testis, ovary, or oviduct), eggs at different stages of development (and hence different locations, such as ovarian, oviductal, laid, hatched), whole egg or egg compartments (shell, membranes, yolk, albumen, embryo), and hatchlings. Since the toxicant burden of nonfeeding reptile hatchlings reflects the contamination of the eggs, they were also included in this section and were compared by study type and exposure scenario. Observational field studies are relatively numerous (20 species, 20 metals, 21 publications), whereas experimental studies completed under laboratory (8 species, 4 metals, 8 publications) or farm (2 species, 8 metals, 2 publications) conditions are scarce.

The largest datasets are available from observational field studies for sea turtles and crocodiles, especially *Caretta caretta* (6 publications) and *Alligator mississippiensis* (4 publications). In these studies, eggs lain in the field or in the lab from field-exposed *Trachemys scripta* females were subject to residue analysis or lab incubation (4 publications). In experimental laboratory studies, focus was put on metals in gonadal tissues from enteral administration, particularly Se (4 publications), and from parenteral administration of Cd in 2 turtle species, *Chrysemys picta* and *Trachemys scripta* (2 publications).

Among the 23 metals of priority concern or special interest (Table 12.1), 3 (Ag, Sb, and Ti) have little, if any, information readily available from the ecotoxicological literature. From the field studies, the dataset is most comprehensive for Cd (17 species in overall 30 publications), Pb (15 in 23), and Hg (13 in 22), followed by the essential metals Zn (15 in 19), Cu, Fe, Ni, Cr, Mn, As, and Se (13 in 13). In laboratory experiments, Cd (4 in 4) and Se (3 in 4) dominate existing literature. Overall, little information is available for Ba, Be, Mo, Rb, Sn, and Tl.

In summary, these studies confirmed, for several priority concern metals and reptile species, the following:

1) evidence for their presence in both male and female gonadal systems during gametogenesis, fertilization, and eggshell formation, as well as in all compartments of early developmental stages (embryos and hatchlings);
2) evidence for their transgenerational transfer via maternal tissues and external incubation substrates;
3) evidence for their accumulation in transgenerational compartments in a dose-dependent manner;
4) overall, mean concentration levels fall within the range coarsely reported for mammalian and bird tissues; reproductive and developmental toxicity thresholds are exceeded in some cases, and adverse effects to reptile fitness may not be excluded;
5) indication for association with effects of priority concern (behavioral and endocrine effects in particular);
6) lack of standardization and control in tissue selection and preparation; and
7) lack of consideration of the influence of reptilian ecophysiological traits that determine structure and function of barriers that exist between the embryo and its environment.

12.3.4 Monitoring Tissue Concentrations

12.3.4.1 Organotropism

In vertebrates, metals tend to have organ-specific affinities, and in turn, organs tend to serve as metal-specific locations for metal accumulation (organotropism). The resulting differences in the distribution patterns of metals among tissues and organs usually become more evident when exposures to high, yet nonlethal, concentrations are prolonged. Likewise, in reptiles such "correlation chaos" tends to occur at low exposure levels but lessens at high exposure levels (Burger et al. 2005). Also, in the assembled dataset organotropism was especially evident in specimens from contaminated sites (Tables 12.3 and Appendix).

12.3.4.1.1 General Distribution Patterns in Vertebrates

The typical distribution of metals among vertebrate tissues demonstrates that liver and kidneys have the highest concentrations of most metals. Muscle concentrations may be particularly high for Hg, especially methylmercury, and to a lesser extent Pb, after high-level, long-term exposure. Mineralized tissues such as bone are well known for their high storage capacity of some divalent metal ions, including Ba, Be, Pb, and Sr, which have physicochemical properties similar to Ca. In bone, after Ca the most abundant essential metals are Mg and Zn, followed by Cu and Mn at much lower levels. Bone is also the major storage site of aluminum in birds and mammals. Keratinized tissues such as epidermal structures (hair, nails, and scutes) may concentrate thiol-reactive metal ions and metalloid compounds such as Hg, As, and Pb. Uptake of metals into the brain is restricted by the blood-brain barrier and the blood–cerebral spinal fluid barrier, which can be easily passed only by lipid-soluble metal compounds such as tetraethyl-Pb, methyl-Hg, and elementary Hg. Typically, adipose tissues represent the storage depot for highly lipophilic toxicants such as organometallic compounds. Blood is the principal transport medium, also for metals, and links all tissue compartments of an organism. Except for initial phases of high-level exposure, blood metal concentrations will be well below those in storage tissues. Many metals show distinct partitioning among blood compartments being preferentially bound to blood cells (such as Cd, Pb, methyl-Hg, and partly inorganic Hg) or plasma proteins.

12.3.4.1.2 Liver and Kidneys

Highest tissue concentrations of most metals were consistently found in the liver and kidneys of reptiles (see Appendix). This was particularly evident for Cd and Hg (known to have very high metallothionen affinity), for example, for Cd, Cu, and Hg in several tissues of *Caretta caretta* (Figure 12.5) and for Cd in *Alligator mississippiensis* (Figures 12.6 and 12.7). Also, when comparing median values calculated for Cd, Hg, and Pb over the entire dataset (Table 12.2), Cd and Hg levels were consistently higher in the kidneys than in the liver (more pronounced when based on dry rather than wet mass), whereas the distribution of the Pb levels was inconsistent.

12.3.4.1.3 Bone and Muscle

Bone was the major storage site for Al, Pb, and Zn and to a lesser extent for As and Mn, but note that data on Al, As, and Mn are based on marine turtle samples only (see Appendix). In addition, high bone levels were reported for Sr. Across the entire dataset, bone Pb levels were higher than Cd and Hg (dry mass basis only; Table 12.2), although Cd and Hg were rarely analyzed in bone. When analyzed, Cd levels were inconsistent and Hg levels comparatively low. In muscle, Hg levels tended to exceed Cd and Pb levels by roughly an order of magnitude (only in the wet mass-based data); levels of Cd and Hg (but not Pb) in the muscle were consistently lower than in the liver and kidneys.

In marine turtles, highest As concentrations were found in muscle tissue (Storelli and Marcotrigiano 2003; Kunito et al. 2008). Examples demonstrating metal partitioning between bone and muscle include observations that total Hg levels in American alligators captured along a transect through the Florida Everglades in 1999 were lower in tail muscle than in liver, and highly correlated

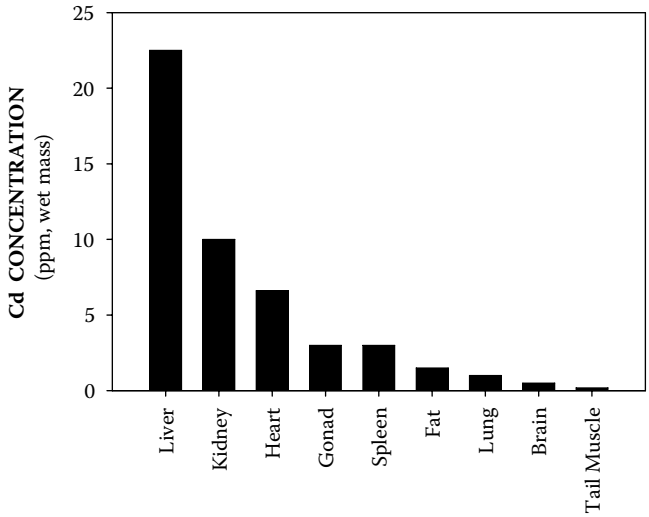

FIGURE 12.6 Distribution of cadmium among tissues of American alligators, *Alligator mississippiensis,* 10 days after cadmium administration (single intracardiac injection of $CdCl_2$, 1.0 mg/kg body mass). (Data from Bell and Lopez 1985.)

between these tissues (Rumbold et al. 2002). Also, the high proportion of Cd observed in muscle and liver but not in the kidneys (as shown in Figure 12.5) of a loggerhead turtle indicates longer-term high-level exposure to this metal (Sakai et al. 2000b). A comprehensive field study conducted by Torent et al. (2004) analyzed several metals in muscle, bone, liver, and kidneys in a collection of 78 *Caretta caretta* specimens stranded along the coasts of Gran Canary Islands. These individuals presented mean bone levels that were highest for Al and Zn. Tissue residues of As were also highest

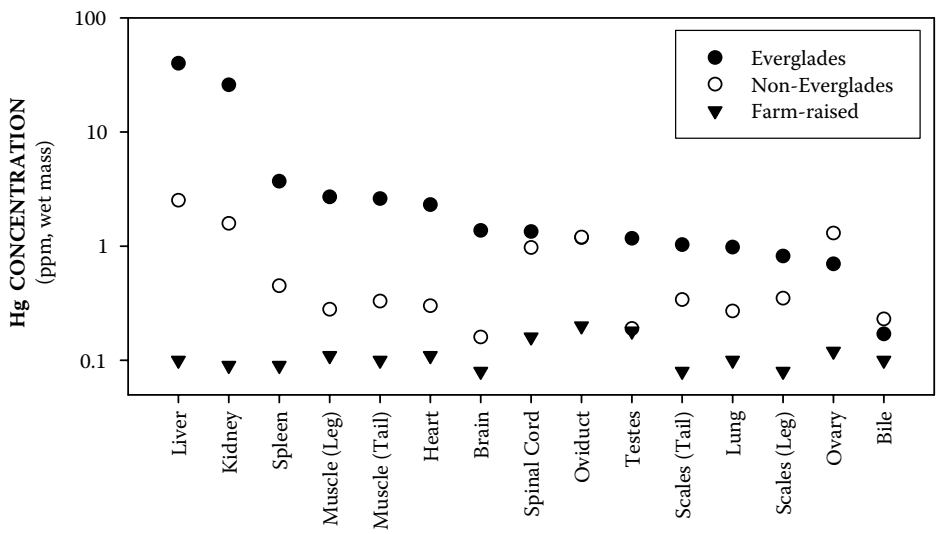

FIGURE 12.7 Mean mercury concentrations in tissues of wild and farm-raised American alligators, *Alligator mississippiensis.* Wild individuals came from sites inside and outside the severely polluted Everglades area (Florida). Tissues ordered by concentration in individuals from the Everglades. Note log scale for metal concentrations. (Data from Heaton-Jones et al. 1997.)

in the bone tissues, but comparatively low for Cd, Cu, and Fe in most individuals. Within the same sample, Cd, Cu, and Fe levels in bone and muscle tissue were consistently high.

Tail muscle is accessible for minimally invasive monitoring through biopsy. Golet and Haines (2001) compared the distribution of Hg concentrations in skeletal muscle tissue from front shoulder, back leg, and tail, as well as blood, liver, and marginal carapace of snapping turtles. Range of variation of Hg concentrations was the same in the muscle tissues and blood (50 to 500 ng/g wet mass) and in liver and shell (500 to 3300 ng/g wet mass). Total Hg concentrations in muscle were highly correlated among the 3 tissue locations, and significantly correlated with that in blood and carapace. Liver Hg concentrations showed no correlation with those of the other tissues. Golet and Haines (2001) concluded from the uniform distribution of Hg in muscle tissues from different body locations that analysis of small portions of muscle from any body location will produce representative results, and that there is no need to homogenize large amounts of tissue to estimate whole animal values.

12.3.4.1.4 Brain

Brain tissue was less frequently analyzed for metal residues than were for liver, kidneys, bone, and muscle, but metals of concern were detected in most brain samples when the tissue was analyzed (see Appendix). The poorly developed database on metals in nervous tissues of reptiles warrants additional research.

Among the 9 metals analyzed in up to 26 different tissues of sea turtles (*Caretta caretta* and *Chelonia mydas*), all were detected in the brain with the exception of Pb and Co, for which concentrations were low, often below detection limits (Sakai et al. 2000b). For mercury, a few studies illustrate partitioning among tissues; for example, brain/liver proportions (mean, ppm, wet mass) were 0.039/0.4 for *Caretta caretta* (Japan; Sakai et al. 2000b), 0.04/0.41 for *Caiman crocodilus* (Surinam; Vermeer et al. 1974), and 0.46/4.30 for *Alligator mississippiensis* (Okefenokee National Wildlife Refuge Georgia, Jagoe et al. 1998). The latter example for alligators was consistent with observations of an Hg contamination gradient in Florida that presented brain/liver partitions ranging from 0.08/0.10 in farm-raised animals, to 0.16/2.52 outside the Everglades, up to 1.37/39.99 at the most contaminated site in the Everglades (Heaton-Jones et al. 1997).

Lead brain/liver/bone proportion was <0.03/0.08/3.53 (mean, ppm, wet mass) in *Chelydra serpentina* collected from the Missouri Old Lead Belt area, and only weakly paralleled the expected contamination gradient that was observed in bone samples. Partitioning among these tissue compartments ranged from 0.166/0.177/1.015 outside the lead mining belt (Meyers-Schöne and Walton 1994) to 0.190/0.300/37.560 within the mining belt. Depending on spatial location, partitioning of lead among these tissue compartments varied; for example, upstream of tailing piles presented lower tissue residues, while turtles collected downstream of tailing piles in the mining belt presented partitioning of lead in tissues up to 0.292/0.490/114.563 (Overmann and Krajicek 1995; Figure 12.8). As illustrated in Figure 12.5, Cd was detected in the brain of *Caretta caretta*. Cadmium brain/liver/kidney proportions (mean, ppm, wet mass) were 0.26/8.18/41.9 in *Caretta caretta* caught in commercial fishing nets (Sakai et al. 2000b). Similar distribution pattern of Cd was observed, *Trachemys scripta* and *Waglerophis merremii* (see Appendix).

12.3.4.1.5 Reproductive Tissues

As metal residues in brain tissues may suggest potential for neurotoxicity, reproductive tissues are critical compartments for evaluating potential developmental toxicity and endocrine disruption for some metals of priority concern. Several metals, including Cd, Hg, and Pb, which are all known or suggested to interact with reproductive function (e.g., Golub 2006), were detected in maternal reproductive tissues (see Section 12.3.3 for details).

12.3.4.1.6 Blood

Blood is a tissue of choice in minimally invasive biomonitoring for several reasons: sampling blood is relatively easy in the field; it has a relatively low impact to the animal; and it is a practice that is

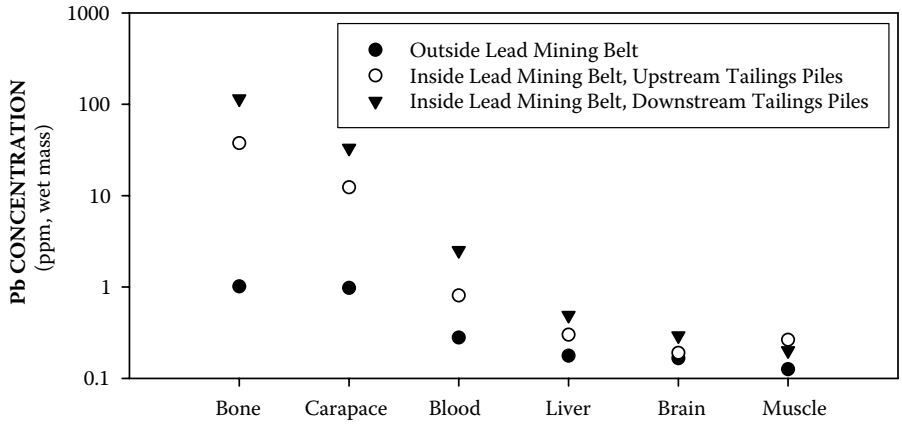

FIGURE 12.8 Mean lead concentrations in tissues of *Chelydra serpentina* in the Old Lead Belt region (Missouri). For several tissues, the graph presents a comparison among individuals from 1 site outside the mining area, 1 site inside the mining area but upstream from a tailings pile, and 1 site inside the mining area and downstream from a tailings pile. Note log scale for metal concentrations. (Data from Overmann and Krajicek 1995.)

well established in human and veterinary medicine. Hence, routine protocols for preparation and analysis of blood are available. Also, many biomarkers of exposure and effect can be simultaneously obtained from a single blood sample.

Preferential partitioning of metals among blood compartments has been observed, for example, in *Malaclemys terrapin,* where red blood cells contained 91.2% of the total Hg present in the whole blood with a consistently positive correlation between the Hg concentrations in red blood cells and plasma, indicating an equilibrium between the 2 Hg fractions (Blanvillain et al. 2007). However, in *Caretta caretta* the positive correlation between blood mercury concentration and hematocrit reflected the higher affinity of mercury species for erythrocytes than plasma (Day et al. 2007). Because of this potentially confounding effect of metal-specific partitioning among blood fractions, we considered only whole blood metal concentrations in the following overview.

Information on the concentration of metals in whole blood relative to other tissues is available from several observational studies. Several observational studies (see Appendix), in addition to the Cd, Hg, and Pb dataset (Table 12.2), showed that, except for initial phases of high-level exposures, whole blood metal concentrations tended to be consistently below those in other tissues. There is rapid transfer of Cd from the blood to the liver after parenteral application in turtles. In *Chrysemis picta* rapid partitioning was demonstrated by the blood/liver proportions at 3 postinjection time points: 87/1.42 (6 hours), 19.3/18.3 (24 hours), and 9/63.01 (192 hours) (wet mass; cpm/mg ^{109}Cd; single intravascular injection; Rie et al. 2001).

Blood proved to be the most accurate overall predictor of internal mercury burden in *Caretta caretta* (Day 2003; Day et al. 2007). Kenyon et al. (2001) provided a comprehensive overview of metal concentrations in whole blood samples from 106 Kemp's ridley sea turtles, *Lepidochelys kempii*. Overall, the observed mean levels (ppm, wet mass, range in parentheses) were: Ag, 0.00094 (0.000042 to 0.00274); Pb, 0.011 (0.00 to 0.0343); Hg, 0.0180 (0.00050 to 0.0673); Cu, 0.524 (0.215 to 1.300); and Zn, 7.500 (3.280 to 18.900). These values may represent baseline levels for the species in the Gulf of Mexico region (sampling took place from 1994 to 1995), although the samples presented high variation of within- and between-metal concentrations.

Metal concentrations in blood reflect recent uptake and redistribution of metals during the course of longer-term exposures, although turnover times for metals in blood may be shorter for metals that

have not reached accumulation thresholds (Burger et al. 2006). As a consequence, extrapolation from concentrations found in the blood to those in other tissues is limited in humans and perhaps more so in wildlife. In lower-level, multimetal exposure scenarios, we may expect particularly high variability and correlation chaos between blood and other tissues (Burger et al. 2006).

For reptiles, correlations between metal concentrations in blood and other tissues have been described for Hg in turtles (*Chelydra serpentina* by Golet and Haines 2001; *Caretta caretta* by Day et al. 2005), alligators by Yanochko et al. (1997), and snakes (*Nerodia sipedon* by Burger et al. 2005). For example, the range of variation for Hg in blood and muscle of snapping turtles was roughly the same (50 to 500 ng/g wet mass) as in carapace and liver (500 to 3300 ng/g wet mass; Golet and Haines 2001). Burger et al. (2006) compared blood with muscle levels of 17 metals and found a high degree of variation in metal levels within but not between the 3 snake species analyzed water moccasin (*Agkistrodon piscivorous*, *Nerodia fasciata*, *N. taxispilota*; see Appendix).

For Se, a significant correlation between concentrations in blood and other tissues (gonad, liver, kidney, eggs) was described in snakes (*Lamprophis fuliginosus* and *Nerodia fasciata*) by Hopkins et al. (2005a). In the correlation matrix of the concentrations of 7 metals in water snake blood relative to 4 other tissues (kidney, liver, muscle, and tail tip skin), the only significant, positive correlations were between blood Hg and liver Hg, blood Hg and muscle Hg, and blood Mn and kidney Mn. A significant negative association was found between blood Se and liver Se (Burger et al. 2007). But for metals other than Hg (as methyl-Hg), distribution patterns between blood and other tissues appeared less consistent, perhaps as a consequence of the high methyl-Hg half-life and the degree of enterohepatic circulation among other toxicokinetic peculiarities (Shen 2008).

In a few comprehensive field studies, blood levels of several metals and species were examined for associations with exposure gradients (see Appendix). At highly contaminated sites, blood levels seemed to parallel environmental contamination levels in turtles (see Appendix) for Cd (Hays and McBee 2007), Pb (Clark et al. 2000; Bergeron et al. 2007; Blanvillain et al. 2007), and Hg (Bergeron et al. 2007; Blanvillain et al. 2007). For other metals and study sites, association of blood metal concentrations with exposure gradients was inconsistent or not detected at all (Clark et al. 2000; Burger et al. 2005; Campbell et al. 2005; Burger et al. 2007), which reinforces the call for additional studies given the paucity of existing literature.

When comparing across species, life history stages, or between sexes, marked differences in exposure may occur, even within a single site. For example, food is a major source of metals (see Section 12.3.2.1), and blood concentrations of metals have been experimentally shown to parallel food concentrations. Examples are available for As, Se, and Sr in *Nerodia fasciata* (Hopkins et al. 2001); Se in *Lamprophis fuliginosus* and *Nerodia fasciata* (Hopkins et al. 2005a); and Pb in *Alligator mississippiensis* (Camus et al. 1998) and *Crocodylus porosus* (Hammerton et al. 2003). Because species, life history stages, and sexes frequently differ in microhabitat use and diet, understanding the feeding ecology of a particular species is a basic prerequisite for understanding metal kinetics in reptiles. For example, within a single site Bergeron et al. (2007) found differences in total Hg concentrations in the blood of free-ranging turtles that were consistent with their rank in carnivory (*Chelydra serpentina* ≥ *Sternotherus odoratus* > *Chrysemys picta* > *Pseudemys rubriventris*).

Although blood is a tissue of choice for biomonitoring, species-specific or habitat-linked variability poses major limitations to the use of blood for biomonitoring in wildlife medicine. Aitio et al. (2007) explained that an understanding of toxicokinetics of specific metals is fundamental to interpreting concentrations of those metals in blood. For metals with high affinity to blood cells, plasma or serum levels are considerably lower than whole blood or blood cell levels, and concentrations may fall close to or below detection limits, potentially indicating false negative results during an exposure assessment. The availability of cellular components in blood may influence the concentration of certain metals in the whole blood, which may affect analytical detection limits when measuing metal levels in blood for biomonitoring purposes. Consequently, whole blood

measurements may require correction for hemoglobin content or hematocrit to minimize variability when using metal blood concentrations as biomarkers of exposure (Aitio et al. 2007; Day et al. 2007). Blood values may also be affected by capture, confinement, and handling stress. For this reason, a standardized operating procedure should be developed.

12.3.4.1.7 Integumentary Structures

Structures of the integument employed for minimally destructive tissue monitoring of metals in reptiles included: 1) for squamates, molted skin (shed or sloughed) and separate scales and tail tips (all keratinized epidermal structures); 2) for crocodylia and testudines, scales differentiated into plates, scutes, or shields or scales underlain by bony plates (ossified dermal structures), called osteoderms or osteoscutes; and 3) in testudines, osteoderms fused with vertebrae and ribs dorsally, and osteoderms with sternum ventrally, called carapace (dorsal shell) and plastron (ventral shell).

In general, metal distribution patterns among these compartments should be congruent with those generally described for keratinized and mineralized tissues in vertebrates and reptiles. Consequently, for the priority metals (Table 12.2) we expected high concentrations of As, Hg, and Pb in the keratinized structures and of Al and Pb (Ba, Be, and Sr) in the ossified structures. As expected, in the assembled data for Cd, Hg, and Pb, Pb levels in predominantly ossified tissue samples (e.g., ostederms and carapace, Table 12.2) were consistently high and conformed to those in the bone. Likewise, in predominantly keratinized tissue samples (skin, scales, scutes, tail tips, claws), Hg levels were relatively high. Cadmium levels were well below those in other storage tissues, but data are few and interpretation was constrained.

Rainwater et al. (2007) examined concentrations of the metals As, Cd, Cu, Pb, Hg, and Zn in caudal scutes of crocodiles from 2 sites in Belize (Morelet's crocodiles, *Crocodylus moreletii*) and 1 site in Costa Rica (American crocodiles [Crocodylus Actus]). Mercury was the most frequently detected metal occurring in all scutes from both crocodile species. Mercury and Cu were detected at all sites, whereas Cd, Cu, Pb, and Zn were below detection limits at some sites. Arsenic was not detected in any samples collected during this study, but Hg concentrations in scutes corresponded to previous findings on river sediment and crocodile egg contamination (Rainwater et al. 2002, 2007).

In addition, 3 comprehensive publications exemplify the metal-specific distribution patterns in integumentary structures of reptiles, which are consistent with trends observed throughout the vertebrates. Sakai et al. (2000b) provide data for *Caretta caretta* that allow comparisons between metal concentrations co-occurring in scales and carapace, which confirmed the expected distribution for Hg in keratinized tissue and Pb in the ossified tissue. The authors demonstrated the importance of the carapace as a selective metal sink, with nearly all Pb and more than half of the Mn and Zn whole-body burden in the body were accounted for by residues in carapace (more than 15% of the sea turtles' total body mass).

Metal distribution patterns were also reported for skin in juvenile *Alligator mississippiensis* (Burger et al. 2000) and in adult *Nerodia sipedon* (Campbell et al. 2005); shed skin in water snakes, *Nerodia fasciata* (Hopkins et al. 2001); and in osteoderms of freshwater crocodiles, *Crocodylus johnstoni* (Jeffree et al. 2005; Appendix).

Although metal distribution patterns among tissues are generally consistent, correlation chaos limits extrapolation from metal concentrations in integumentary structures to those in internal organs. Ratio estimators such as bioconcentration factors (BCFs) have characterized associations between metal residues in integumentary or other tissues and metal concentrations in exposure matrices. Khan and Tansel (2000) evaluated Hg in scutes of American alligator relative to Hg concentrations in the exposure matrix and derived BCFs for Hg from literature data. Their results indicated that BCF differed considerably between age classes and underscored the importance of biotic modulating factors in the toxicokinetics of metals.

In *Caretta caretta*, Hg in keratinized tissues was a better predictor of liver Hg than estimators derived from other tissues (Day 2003). For 9 metals in the carapace of loggerhead and green turtles, concentrations of Hg, Mn, and Zn were correlated with whole body burdens (Sakai et al. 2000b).

In contrast, Burger et al. (2007) found no consistent patterns in the relationships among metals in either blood or liver for As, Cd, Cr, Pb, Mn, Hg, and Se concentrations in blood, kidney, liver, muscle, and skin of adult water snakes (*Nerodia fasciata*, *N. sipedon*). However, for skin significant correlations with internal tissues other than blood were apparent, and Hg displayed significant corrections more frequently than the other metals evaluated.

Analysis of field studies, summarized by Khan and Tansel (2000), demonstrates the difficulties encountered for interpreting tissue concentrations. Several comprehensive observational field studies examined metal levels in integumentary structures and their association with exposure gradients (see Appendix). In-depth field studies confirmed scutes useful in Hg exposure monitoring in turtles (*Caretta caretta*, *Chelonia mydas* by Sakai et al. 2000b; *Chelydra serpentina* by Golet et al. 2001; *Caretta caretta* by Day et al. 2005), and scute Hg values paralleled environmental exposure gradients in diamondback terrapins (Blanvillain et al. 2007) and American Alligators (Yanochko et al. 1997; Jagoe et al. 1998; see Appendix). Mercury concentrations in both scales and soft tissues of American alligators increased with the degree of ambient Hg contamination, but Hg concentrations in the scales were comparatively low (Heaton-Jones et al. 1997). A similar range of variation of Hg levels in carapace and liver of snapping turtles was also observed by Golet and Haines (2001). Levels of several metals paralleled environmental contamination (see Appendix for *Terrapene carolina triunguis, Chelydra serpentina, Pseudomys melanoleucus,* and *Terrapene carolina*).

Jeffree et al. (2001) observed differential partitioning of metals in estuarine crocodiles captured in 3 catchments of Kakadu National Park, within the Alligator Rivers region of northern Australia affected by different types of human activities (mining, hunting). Tissue levels for 18 elements were determined, and 5 elements in tail muscle (Al, Ba, Cr, Ni, and Pb) and 5 elements in osteoderms (Co, Fe, Mg, Mn, and U) were significantly different among catchments.

In a long-term feeding experiment, Hopkins et al. (2001) observed that banded water snakes accumulated As, Cd, Cu, Se, Sr, and V from their prey fish, which had been exposed to waters from a coal-ash-contaminted site. Banded water snakes presented concentrations of As, Se, and Sr detected in shed skins that were significantly different between feeding treatments. Similarly, Jones and Holladay (2006) observed that corn snakes fed mice contaminated with a mixture of 3 metals (Pb, Cd, and Hg injected into dead at a dose of 2 mg/kg of each metal/month/snake) for 34 weeks accumulated significant amounts of all metals in their shed skins.

12.3.4.1.8 Biopsied Tail and Skin

Tissues accessed through biopsy included muscle, tail, and skin, the last 2 involving several tissue types. Composite tail tissue was demonstrated useful for monitoring of several metals in alligators (Burger et al. 2000), snakes (Hopkins et al. 2001; As, Se, and Sr in *Nerodia fasciata* by Jackson et al. 2003), and lizards (Se in *Sceloporus occidentalis* by Hopkins 2005b; *Tarentola mauritanica*, Fletcher 2006); skin tissue was analyzed in snake (*Nerodia sipedon*; Burger et al. 2005), and muscle tissue in freshwater turtles for Hg (Golet and Haines 2001).

Concentrations of As, Cd, Cr, Hg, Mn, Pb, and Se in liver of American alligators were highly correlated in at least 1 of the 3 biopsied tissues (skin, biopsied tail muscle, or tail tip tissue), and only Sn showed no significant positive correlation. Although no single tissue provided links for high prediction of liver levels for all metals, skin presented the highest correlation for Hg, and tail muscle provided the best overall correlation for Cd and Pb (Burger et al. 2000). In *Nerodia sipedon,* Burger et al. (2005) state that skin seemed to be a better predictor of total body burdens of As, Cd, Cr, Pb, Mn, Hg, or Se than was blood. Skin was a better bioindicator of internal metal levels than blood for Hg levels in internal tissues and a "moderately useful" bioindicator for Pb, Se, Cr, and Mn. Skin was particularly useful as a bioindicator for Cd and As. Comparing their findings with those from other pertinent studies (Burger et al. 2000; Clark et al. 2000; Hopkins et al. 2001), Burger et al. (2005) concluded that skin biopsies were easily and non-lethally collected and correlated with several metals of ecological importance, especially Hg.

Hopkins et al. (2001) and Jackson et al. (2003) evaluated the utility of shed skins, tail clips, and blood for nondestructive or minimally destructive sampling for trace element exposure assessment in banded water snakes. For 13.5 months, snakes were fed either fish from a coal-ash-contaminated site or uncontaminated food from a reference site. Snakes fed contaminated prey accumulated As, Cd, Se, Sr, and V in liver, kidney, and/or gonads. For As, Se, and Sr concentrations in tail clips, blood, and/or shed skins, differences between the 2 feeding treatments were statistically significant and afforded high predictive value for the tail clip levels.

Fletcher et al. (2006) employed Moorish wall geckos for indication of mining pollution. When comparing metal residues from biopsied tissues of gecko tail with whole body concentrations, 16 elements presented significant correlations for tail–whole body partitioning. In contrast, Cd, Cu, Ni, and Zn lacked statistically significant linear relationships between tissue residues in tail and whole body. Accumulation of Cd, As, Pb, and Se from mine-related contaminant exposures, however, was confirmed.

12.3.4.1.9 Whole Body

Whole body concentrations of metals in reptiles can be calculated from multicompartment studies or measured from whole organism analyses, as demonstrated for small reptiles. For example, Fletcher et al. (2006) relied on Moorish wall gecko to characterize direct and indirect effects of the catastrophic collapse of a mine tailings dam that released several million cubic meters of toxic mud and acidic water into the Guadiamar River Valley, southern Spain, in 1998. Remediation efforts removed most of the sludge from the floodplain, but contamination persisted. Cleanup activities also produced clouds of aerosolized materials that affected the surrounding landscape. Fletcher et al. (2006) measured whole body concentrations of 21 elements in the wall gecko collected from 7 locations that spanned an expected contamination gradient, including a rural and an urban non-mine-affected location, 2 mine-affected towns, and 3 locations on the contaminated floodplain. Multivariate analyses of whole body concentrations identified pollutants that increased across the expected contamination gradient, which was particularly evident for As, Pb, and Cd.

12.3.4.2 Modulators of Metal Tissue Levels and Distribution

Modulators of toxicity account for variation in exposure and effects that is not explained by contaminant concentration, and duration or frequency of exposure. Understanding uncontrolled variation, or at least taking uncontrolled variation into account, is critical to understanding the ecotoxicology of metals in reptiles, and may be easily viewed as a trade-off between variability and sample size in the statistical power function.

Any kinetic characteristic of a substance is a function of the properties of the substance itself (such as the chemical speciation of a metal) and the biological receptor (such as age, sex, or lipid content of an organism). Both attributes may be further influenced by several abiotic and biotic modulating factors (e.g., host factors; see Peakall and Burger 2003). Such abiotic and biotic factors interact, such as pH determining ionization equilibria of metals or microbiota transforming metals through methylation (Allen 2002; Paquin et al. 2003; Meyer et al. 2005, 2007), as well as age or sex determining body size, which in turn frequently determines the diet of an organism.

Metal and organ specificities are apparent in their distributional patterns within an animal. There is consistent information regarding the influence of concentration and duration or frequency of exposure for metal distributional patterns. There is further indication that tissue levels may vary less for essential than for nonessential metals; for example, the among-individuals coefficient of variation (CV) for tissue residues was consistently highest for Cd, followed by Cu and Zn in liver, kidney, and muscle of marine turtles, *Caretta caretta, Lepidochelys kempii,* and *Dermochelys coriacea* (Caurant et al. 1999). Similar findings were found for *Caretta caretta*: CV was high for Cd and Hg but low for Cu, Se, and Zn (liver, kidney, and muscle; Maffucci et al. 2005), and high for Cd and Pb but low for Cu, Fe, and Zn (yolk from laid eggs; Kaska and Furness 2001).

Another interesting modulator of tissue-metal distribution patterns is metal-metal interactions that influence the degree of accumulation of each metal. Frequently manifested as patterns of covariation in metal concentrations among tissues, only in some cases there are mechanistic underpinnings underlying observations of metal-metal interactions promoting accumulation are understood. For example, competitive inhibition (e.g., mediated by ionic or molecular mimicry) can mediate antagonistic interactions, whereas induction of metal-binding protein production (e.g., metallothionein and metallothionein-like proteins) can mediate synergistic interactions.

There are several examples of covariation of metal concentrations among reptile organs and several examples of covariation of metal concentrations among individuals, that is, where individuals exhibiting a high degree of contamination for one metal also exhibited a significantly high (or low) degree of contamination for other metals. This was the case for the 55 pairwise correlations among 21 elements found in the Moorish wall gecko (Fletcher et al. 2006). Also, some studies have characterized positive correlations among Cd, and Cu and Zn in all tissues analyzed (muscle, liver, and kidney) in, for example, *Chelonia mydas* (Sakai et al. 2000a), and in the liver and kidneys of loggerhead turtle (Maffucci et al. 2005); these correlations were attributed to the covariation of metallothionein induction by Cd. However, patterns of metal covariation in reptiles are frequently poorly understood mechanistically.

Potentially beneficial interactions between several Se compounds and metals, especially Cd and Hg, have been extensively examined in mammals and birds, which suggest similar patterns may be operating in reptiles. However, interactions among Se and other metals remain uncertain throughout the vertebrates, and studies to date display inconsistent outcomes. For example, selenite co-administered with methyl-Hg was expected to have a neuroprotective effect in test subjects, but studies have found both an increase and a decrease in Hg levels in the brain, and interactions were linked to reduced biliary elimination of methyl-Hg (Nordberg et al. 2007). Among reptiles, Se concentrations were more often positively correlated with Hg, for example, in liver and kidney in the green turtle (Gordon et al. 1998), which presents a pattern roughly similar to that for marine mammals (Storelli et al. 2005), and in the liver of the snake *Nerodia sipedon* (Burger et al. 2007). In contrast, co-exposure to Se was only marginally significant, although positively correlated to Hg concentrations in blood samples of individuals of *Chelydra serpentina*, *Sternotherus odoratus*, *Chrysemys picta*, and *Pseudemys rubriventris* from an Hg-contaminated site (Bergeron et al. 2007). Covariation in patterns of distributions of metals among tissues may have other causes. The remarkably high Zn concentrations found in *Hemidactylus mabouia* (liver; Schmidt 1986), *Lacerta vivipara* (bone, liver; Avery et al. 1983), *Laudakia stellio* (liver, carcass; Loumbourdis 1997), and *Tarentola mauritanica* (whole body; Fletcher et al. 2006), all collected from apparently severely contaminated sites, were associated with remarkably increased tissue concentrations of other toxic metals, which could be symptomatic of a breakdown in processes regulating metal homeostasis as a result of severe but not necessarily zinc-induced intoxication (Eisler 2000).

Differences in morphology, physiology, behavior, and life history that arise as a function of species, populations, age, stage, and sex are important modulators of metal tissue levels and distribution. Age and size are significant sources of variation within species; for example, net exposure to environmental chemicals is likely to increase with age because individuals have longer exposure, or more opportunities of exposure, to contamination. Such a positive effect of age should be particularly strong for metals with high biological half-life time and in long-lived species, provided, of course, that individuals are sufficiently robust to survive long-term, low-level exposures. For example, one could anticipate highest tissue levels of Cd, Hg, Pb, and Zn in long-lived species such as sea turtles and crocodiles, which the accompanying data corroborate (Tables 12.2 and Appendix). Age had a positive effect on Hg tissue concentrations in American alligators (Khan and Tansel 2000; but see Rumbold et al. 2002). Khan and Tansel (2000) suggested that the higher rates of growth, feeding, and metabolic activity in juveniles contributed to this pattern. Because older individuals are larger, one could predict a similar, positive effect of size on metal levels; however, results are mixed. In *Alligator mississipiensis*, this was observed for Hg levels in some studies (fat, liver, muscle, and

skin; Heaton-Jones et al. 1997; Yanochko et al. 1997; along with Pb in the liver; Burger et al. 2000), but not others (e.g., liver, tail muscle; Rumbold et al. 2002). In analysis of As, Cd, Cu, Pb, Hg, and Zn in *Crocodylus moreletii* and *Crocodylus acutus*, the only metal that correlated with body size was Cu in male *Crocodylus moreletii* (Rainwater et al. 2007).

Among marine turtles, body size significantly influenced levels of tissue residues of metals (Sakai et al. 2000a; Anan et al. 2001; Kenyon et al. 2001), but the sign of the correlation coefficient in these studies varied among metals. Seemingly inconsistent observations regarding metal residues in tissues were also noted in *Caretta caretta*, wherein body size was positively linked with Mn and Ni levels in adipose tissue, yet negatively associated with Zn levels in liver and Cu in adipose tissue (Franzellitti et al. 2004). Maffucci et al. (2005) also noted no effect on Cd, Cu, Hg, Se, and Zn in liver, kidneys, and muscle. Divergent associations were also noted by Storelli and Marcotrigiano (2000), who observed that body mass had a positive effect on the liver-muscle ratio for inorganic As, but not for organic As. Day et al. (2003) also noted allometric influences for partitioning relationships between blood and keratin Hg levels, as did Storelli et al. (1998) for Cd liver and muscle levels. Size and age were significantly correlated with the concentrations of 13 metals in *Crocodylus porosus*; however, the relationship was negative. Osteoderm metal concentrations were positively correlated to osteoderm Ca concentrations (Jeffree et al. 2001, 2005). Sex differences are also apparent in the existing data for metal residues in reptiles. Maffucci et al. (2005) noted significant sex-related differences for Cd, Cu, Hg, Se, and Zn in liver, kidneys, and muscle of *Caretta caretta* (Maffucci et al. 2005), as did Kenyon et al. (2001) for Ag, Cu, Hg, Pb, and Zn in *Lepidochelys kempii*. In contrast, no differences were noted for total Hg tissue levels in *Alligator mississippiensis* (liver or tail muscle; Rumbold et al. 2002) and in the levels of 13 metals in *Crocodylus porosus* (Jeffree et al. 2001, 2005), and in As, Cd, Cu, Pb, Hg, and Zn concentrations in *Crocodylus moreletii* and *Crocodylus acutus* caudal scutes (Rainwater et al. 2007).

12.3.4.3 Monitoring Tissue Concentrations Summary

During the last decade, the number of publications on metal residues in reptiles nearly doubled (Figure 12.1). Available information in these publications increased markedly because research trends shifted toward simultaneous analysis of several metals in multiple tissues for several species (Tables 12.1 and Appendix). Based on the number of species studied per biogeographic zone, most information is currently available for Nearctic species, followed by species from the Neotropical, Indomalayan, Palearctic, Oceanic, Australasian, and Afrotropical zones. Most species were terrestrial, followed by freshwater or estuarine, and marine. Among reptile orders, most information is available for testudines, particularly Nearctic terrapins and marine turtles (Table 12.1). Among metals (Figure 12.1), information is most abundant for the pollutants of particular concern: Cd, Hg, Pb, Cu, and Zn (Figure 12.9).

The most frequently employed tissues in conventional residue monitoring were the major storage tissues — liver, kidney, and bone — as evidenced by reports for Cd, Hg, and Pb (Table 12.2). However, there is an increasing number of studies that have evaluated tissues using minimally destructive methods that could be employed in alternative residue monitoring. These tissues include blood, different types of integumentary structures, and tail biopsies. Overall, the database for such "alternative tissues" is still comparatively small and patchy (Table 12.2). In contrast, egg compartments, which can also be considered alternative tissues for monitoring, capture a much larger collection of existing data (Table 12.3). Comparing across the dataset assembled for reptiles (Table 12.2), mean residue levels of Cd, Hg, and Pb in the major storage tissues (liver, kidney, and bone) fell within the range observed for other vertebrates, whereas maximum residue values in reptiles were among the highest ever reported (Eisler 2000; Rattner et al. 2005, 2008). The maximum levels these metals reached in reptilian liver, kidneys, and muscle markedly exceed critical limits for food safety and animal health (Table 12.2), which likely indicate long-term exposures to low to less than acutely toxic metal levels in the environment. Studies designed to assess critical organ tissue concentrations in vertebrates are few in number, and for reptiles in particular, and do not

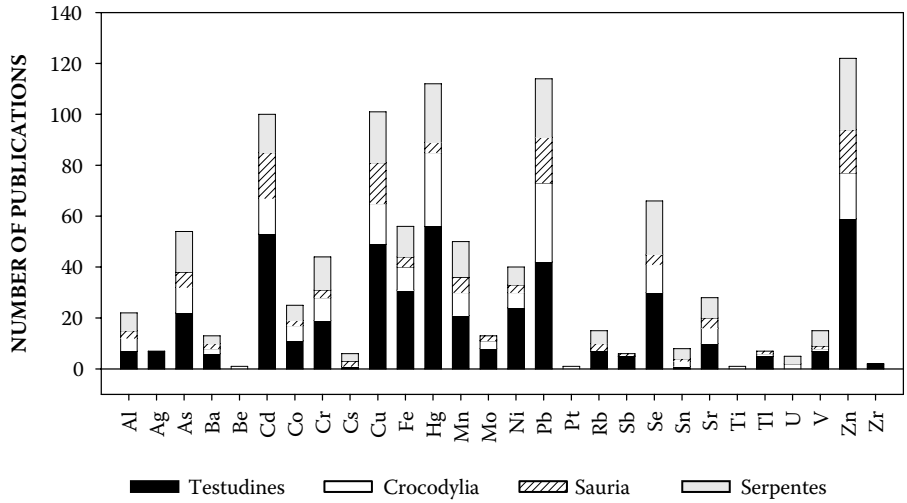

FIGURE 12.9 Distribution of information on metal concentrations in reptiles as indicated by the numbers of publications per reptile order and suborder, and metal. (Data summarized from the Appendix.)

allow conclusive interpretation. Furthermore, in contrast to wild birds and mammals, model-based approaches are infrequently employed in studies of the toxicokinetics of metals in reptiles. Such studies need specific designs and large sample sizes, which can most easily be obtained through minimally destructive sampling. Jeffree et al. (2005) demonstrated that the mean concentration of many metals in *Crocodylus porosus* varied considerably with age, size, and calcium concentration, and that these predictors need to be considered when comparing between populations regarded as controls and those exposed to particular metal contaminants. Interindividual variation of metal tissue concentrations has been extensively analyzed in many reptile species. Several statistically significant relationships between tissue levels and individual attributes have been observed, yet several combinations were consistently not significantly correlated. For example, Khan and Tansel (2000) focused on numerous studies on *Alligator mississippiensis* tissue and environmental metal levels and characterized findings that may foreshadow a better understanding of the influence of individual attributes on the tissue distribution of metals.

With a goal of understanding patterns of intra- and interindividual variation in metal tissue levels, research over the past decade has focused on descriptive, multimetal, multiorgan targeted studies that have yielded an inconsistent information matrix, a trend not unlike outcomes of ecotoxicological investigations completed on fishes in the mid to late 1960s through the 1980s. More recently, research trends have turned to mechanistic study designs (e.g., Hopkins et al. 2005b; Gardner et al. 2006; Bergeron et al. 2007; Blanvillain et al. 2007). Overall, monitoring metal levels in reptilian tissues may be characterized by the following conclusions:

1) Metal-specific distribution among organs follows the general vertebrate pattern.
2) Tissue levels parallel the degree of environmental contamination.
3) Trends were more evident for nonessential metals of priority concern having a relatively long biological half-life (Cd, Hg, Pb) in the metal-specific storage organs, along steep contamination gradients, in high-level, long-term exposure scenarios.
4) Observed high levels of metal residues in tissues indicate high net exposure of reptiles to metals.
5) Principal intraspecific modulating factors of tissue distribution are size (and related age and sex), diet, and chronobiological rhythm.

Both conventional and alternative methods have been employed in monitoring studies focused on metal-exposed reptiles. Comparing between conventional and alternative residue monitoring,

1) Metal concentrations obtained through conventional monitoring tended to be higher than those from alternative monitoring.
2) In multimetal exposure scenarios, conventional monitoring (particularly for liver or kidney) obtained more metal-positive results than single-tissue-based alternative monitoring.
3) Extrapolation of concentrations among organs is limited, particularly from alternative to conventional studies.
4) Risk of false positive tissue concentrations due to cross-contamination is particularly high for egg and integumentary tissues, which are typically used in alternative monitoring.

While research over the past decade has benefited existing data sources for evaluating metal effects on reptiles, the existing data remain limited. Conclusions developed from existing data should be viewed with caution, given differences readily apparent when independent outcomes of various studies are considered. These differences in study designs complicate data compilations and reduce the comparability of results. Future studies should strive for

1) harmonization at the subindividual level, such as in tissue selection and tissue preparation, particularly in alternative monitoring (note diversity in blood or eggshell fractions); and when reporting tissue concentrations (values should be reported on a wet or dry mass basis, and wet weight–dry weight conversion factors recorded as available);
2) harmonization at the individual level, particularly in major confounding variables (species, size or developmental stage, chronobiological rhythm);
3) information on environmental exposure and background levels;
4) information on tissue reference values and critical organ concentrations;
5) mechanistic toxicokinetic understanding; and
6) hypothesis-based, statistically sound study designs.

To overcome the observed limitations, particularly in alternative monitoring of tissue concentrations, future research should focus on

1) evaluating simultaneous multi-tissue-based alternative monitoring;
2) evaluating regional model indicator species suitable for both lab and field studies;
3) considering power analysis to assess sample sizes appropriate to the intra- and intersite variability, exposure gradient, and the number of effect variables analyzed; and
4) developing harmonized monitoring strategies linked to effect assessment as addressed in detail in the concluding section of this chapter.

Future research should focus on linking toxicology, physiology, and ecology in further condensing and statistically evaluating the existing data and integrating mechanistic and model-based approaches.

12.3.5 Toxicokinetics Concluding Remarks

During the last decade, three main areas of research on the ecotoxicology of metals in reptiles arose and proved that trophic (Section 12.3.2) and transgenerational transfer (Section 12.3.3) are key exposure pathways for reptilian metal contamination. A further focus was put on minimally invasive monitoring

of metal contamination in reptilian wildlife and now provides a solid basis for the development of harmonized monitoring tools (Section 12.3.4). In contrast, the mechanistic understanding of the toxicokinetics of metals in reptiles remains poorly developed and must be addressed in future research to characterize shortcomings for taxa and compounds, and to link toxicodynamics and toxicokinetics (i.e., the fate and effect of metals in reptiles). Mechanistic toxicology "is concerned with identifying and understanding the cellular, biochemical, and molecular mechanisms by which chemicals exert toxic effects on living organisms" (Klaassen 2007). As noted by Beckett et al. (2007) for mammals, if we are to understand the mechanisms mediating metal toxicokinetics, the following factors must be investigated in reptiles:

1) metal partitioning among the plasma protein-bound fraction and the "diffusable fractions" in plasma, interstitial, and intracellular fluid;
2) rate of biotransformation (metabolism of organometallic compounds in particular);
3) rate of organ vascular perfusion;
4) permeability of cell membranes to the metal as it occurs in plasma; and
5) availability and turnover rate of intracellular ligands for the metal.

Further complicating the evaluation of biological mechanisms are abiotic factors that influence exposure, including the various chemical forms in which metals can be present in organisms (Vijver et al. 2004; Bjerregaard and Andersen 2007):

1) free ionic or complexed ion form (e.g., chloride, phosphate, or carbonate complexes);
2) bound in the active center of low-molecular-weight peptides (enzymes) or functional proteins (e.g., hemoglobin, hemocyanin, zinc finger proteins, cytochromes, carbonic anhydrase, superoxide dismutase);
3) bound to low-molecular-weight organic acids (e.g., citrate);
4) bound to transport or sequestration proteins (metallothionein, ferritin, transferrin);
5) bound in vesicles of the lysosomal system, as intracellular granules;
6) precipitated in extracellular granules, mineral deposits, residual bodies, and exoskeletons; and
7) bound to cellular constituents (enzymes, ion channels, DNA).

If we are concerned with reptilian conservation, we should further extend the mechanistic concept from the organismic to the population and community levels. Until these mechanistic studies are initiated and outcomes of these studies become available to ecotoxicologists, questions related to reptile exposure and the sensitivity of reptiles to metals remain unanswered or clouded by uncertainty.

12.4 TOXICODYNAMICS: THE EFFECTS OF METALS IN REPTILES

The number of articles published on the ecotoxicological effects of metallic elements and their compounds in reptiles has increased significantly since the first edition of this book, as suggested by the summaries in Tables 12.5 and 12.6. In these tables experimental studies are defined as those where exposure to metals, or to environments contaminated with metals, was directly manipulated, whereas others are categorized as observational studies. These latter studies quantified subindividual, individual, or population level endpoints when comparing individuals or sites with measured or expected metal contamination, regardless of the strength of association between exposure and effect. If not indicated otherwise, detailed information on residue levels, specimens, and locations corresponding to the effects described below are provided in Table A.1.

TABLE 12.5
Effects of Metals in Reptiles According to Experimental Studies (Included Are All Studies That Directly Manipulated Exposure to Metals, or to Metal-Containing Substrates)

Species	Experimental Design	Effects Analyzed	Significant Effects Observed?
ARSENIC			
Lacerta monticola cyrenni	Stage exposed: Eggs Mode of application: Absorption via eggshell; egg incubated in substrate contaminated with arsenic pentoxide Exposure conditions: Long-term exposure and observation (~31 days); 5 treatments (0, 50, 100, 250, 500 ng As/g substrate) Reference: Marco et al. 2004 Observations: Cu, Cd, Zn, Pb were not manipulated but were detected in eggshells and embryo tissues, and therefore should have been maternally transferred. Cu, Cd, Zn contents in embryos were independent of As concentrations; there was a trend for higher Pb concentrations in embryos in the highest As concentrations.	Mortality Hatchling size Time to hatching Developmental abnormalities Hatchling running speed	0 0 0 0 –
CADMIUM			
Chrysemys picta	Stage exposed: Adult males and females Mode of application: Intravascular injection of single dose of cadmium chloride Exposure conditions: Short-term exposure and observation (8 days); 4 treatments (0, 0.05, 0.5, or 5.0 mg/kg) Reference: Rie et al. 2001	Metallothionein-like cadmium-binding protein induction	+
Trachemys scripta	Stage exposed: Eggs Mode of application: Absorption via eggshell; single, direct application of cadmium chloride to the eggshell Exposure conditions: Short-term exposure (single application at day 1 of egg incubation), long-term observation (up to 3 months after hatching); 3 treatments of a 5 µL CdCl2 solution in dimethylsulfoxide (0, 0.1, 1 µg Cd/g) Reference: Kitana 2005 Observations: All neonates were female	Hatchling body mass Hatchling size Number of germ cells Oocyte proliferation Oocyte apoptosis	0 0 – 0 +
Podarcis carbonelli	Stage exposed: Adults, both males and females Mode of application: Through diet (simulated food chain) Exposure conditions: Long-term exposure and observation (21 weeks); 3 treatments (lizards fed uncontaminated crickets, lizards fed crickets fed lettuce contaminated with $Cd(NO_3)_2$ solution, lizards fed crickets superficially contaminated with $Cd(NO_3)_2$ solution)	Mortality	0

TABLE 12.5 (CONTINUED)
Effects of Metals in Reptiles According to Experimental Studies (Included Are All Studies That Directly Manipulated Exposure to Metals, or to Metal-Containing Substrates)

Species	Experimental Design	Effects Analyzed	Significant Effects Observed?
		Physical condition	0
		Growth	0
		Liver somatic index	0
		Kidney somatic index	0
		Standard metabolic rate	0
		Brain AChE activity	0
		Plasma BChE activity	0
		Gut metallothionein concentration	0
			trend for (+)
		Liver metallothionein concentration	0
	Reference: Mann et al. 2006, 2007		
	Observations: Final Cd residue loads were 0.006 µg Cd in control lizards, 0.37 µg Cd in lizards fed crickets fed contaminated lettuce, and 1.03 µg Cd in lizards fed crickets superficially contaminated. Differences in final Cd residue loads between the 2 exposure treatments are partly a consequence of differences in ingested doses (0.5 and 0.9 µg/week, respectively). Doses considered realistic and relatively low.		
Sceloporus undulatus	Stage exposed: Eggs Mode of application: Absorption via eggshell; eggs incubated in substrate contaminated with cadmium chloride Exposure conditions: Long-term exposure and observation (60+ days); 5 treatments (0, 1.48, 14.8, 148, 1480, 14800 µg/g perlite) Reference: Brasfield et al. 2004	Mortality Hatchling size Blood [T3] (thyroid hormone) Blood [T4] (thyroid hormone) Blood [T3]:[T4]	
LEAD			
Trachemys scripta	Stage exposed: Hatchlings Mode of application: Intramuscular injection of single dose of lead acetate Exposure conditions: Short-term exposure, long-term observation (4 months); Exp I: 3 treatments (0, 0.05, 0.1 mg/g); Exp II: 4 treatments (0, 0.25, 1.0, 2.5 mg/g) Reference: Burger et al. 1998	Mortality Growth Behavior — time to begin righting Behavior — time to be under cover	
Trachemys scripta	Stage exposed: NA Mode of application: NA Exposure conditions: Long-term exposure (8 months) to 5 ppm lead (ii) acetate Reference: Lovelette and Wright 1996 Observations: This is an abstract. Trend for negative effect assumed from magnitude of reduction (73%; significance not reported).	Plasma 5-aminolevulinate dehydratase activity Hemoglobin	

(*continued*)

TABLE 12.5 (CONTINUED)
Effects of Metals in Reptiles According to Experimental Studies (Included Are All Studies That Directly Manipulated Exposure to Metals, or to Metal-Containing Substrates)

Species	Experimental Design	Effects Analyzed	Significant Effects Observed?
Crocodylus porosus	Stage exposed: Juveniles Mode of application: Through diet (ingestion of single dose of lead shots amid meat bolus; either voluntary or forced ingestion) Exposure conditions: Long-term exposure despite single application (see "Comments"), long-term observation (20 weeks); 2 treatments (control: pebbles; exposure: pebbles + lead shots) Reference: Hammerton et al. 2003 Observations: Lead shots remained for the duration of experiments in the stomachs; they were slowly dissolved and absorbed in blood. Small sample sizes ($N = 6$ total), no statistics.	Mortality Growth rate Physical condition Indication of hypochromic anemia Behavior: lethargy Behavior: anorexia Blood-packed cell volume	
Lacerta agilis	Stage exposed: Adults (9–12 g) Mode of application: Intraperitoneal injection of single dose of lead nitrate Exposure conditions: Short-term exposure and observation; time series in which each of 4 groups of lizards was sacrificed after 6, 12, 24, or 48 hours following injection of 20 µm pure lead/g and compared to a control group injected with saline solution Reference: Biczycki 1992a Observations: Effects observed, no statistical analysis	Histomorphology and histochemistry (SDH, LDH, NADHrt activities) of central nervous system	
Lacerta agilis	Stage exposed: Adults (9–12 g) Mode of application: Intraperitoneal injection of single dose of lead acetate Exposure conditions: Short-term exposure and observation; time series in which each of 4 groups of lizards was observed from 6, 12, 24, or 48 hours following injection of 20 µm pure lead/g and compared to a control group injected with saline solution Reference: Biczycki 1992b Observations: Effects observed, no statistical analysis	Histomorphology and histochemistry (SDH, LDH, NADHrt activities) of central nervous system	
Lacerta agilis	Stage exposed: Adults (9.5–11.5 g) Mode of application: Per os via tube solution Exposure conditions: Long-term exposure and observation (75 days); 3 treatments: control, lead nitrate, lead acetate administered daily at the dose of 4 µm pure lead/g (vs. saline in the control) Reference: Biczycki 1992c Observations: Effects observed, no statistical analysis	Histomorphology and histochemistry (SDH, LDH, NADHrt activities) of central nervous system	

TABLE 12.5 (CONTINUED)
Effects of Metals in Reptiles According to Experimental Studies (Included Are All Studies That Directly Manipulated Exposure to Metals, or to Metal-Containing Substrates)

Species	Experimental Design	Effects Analyzed	Significant Effects Observed?
Sceloporus occidentalis	Stage exposed: Juveniles Mode of application: Ingestion of single dose of lead acetate trihydrate via oral gavage Exposure conditions: Short-term exposure and observation (312 hours); 5 treatments (0, 1, 10, 100, 1000 mg/kg) Reference: Holem et al. 2006	Mortality Maximum sprint velocity Skin pigmentation	+ 0 + (informally reported)
Sceloporus occidentalis	Stage exposed: NA Mode of application: Ingestion of single dose of lead acetate via oral gavage Exposure conditions: Short-term exposure and observation (14 days); 9 treatments (0, 100, 200, 500, 1000, 2000, 3000, 4000, 8000 mg/kg) Reference: Salice et al. 2003	Mortality Food consumption rate Body mass	+ – 0
MERCURY			
Caretta caretta	Stage exposed: Adults and subadults Mode of application: In vitro exposure of peripheral blood leukocytes to methylmercury Exposure conditions: Short-term exposure and observation (136 hours); 7 treatments (0, 0.01, 0.03, 0.05, 0.1, 0.35, and 0.7 µg MeHg/g) plus presence of mitogens (PHA, PDB) or a control (RPMI-1640) Stage exposed: Adults and subadults Mode of application: In vitro exposure of peripheral blood leukocytes to methylmercury Exposure conditions: Short-term exposure and observation (136 hours); 7 treatments (0, 0.01, 0.03, 0.05, 0.1, 0.5, and 1.0 µg MeHg/g) Reference: Day et al. 2007 Observations: Hg concentrations bracketed environmentally realistic concentrations	PDB-induced B-lymphocyte proliferation PHA-induced T-lymphocyte proliferation Leukocyte viability	– – –
Trachemys scripta	Stage exposed: NA Mode of application: In vitro preparation of isolated urinary bladder exposed to Hg (mercuric chloride) Exposure conditions: Short-term exposure and observation (<4 hours); $HgCl_2$ manipulated within a range of 10^{-8} to 10^{-3} M Reference: Schwartz and Flamenbaum 1976	Membrane Na+ transport Membrane Cl– transport Membrane potential difference Short-circuit current Transepithelial resistance	– 0 – – 0

(*continued*)

TABLE 12.5 (CONTINUED)
Effects of Metals in Reptiles According to Experimental Studies (Included Are All Studies That Directly Manipulated Exposure to Metals, or to Metal-Containing Substrates)

Species	Experimental Design	Effects Analyzed	Significant Effects Observed?
Thamnophis sirtalis	Stage exposed: NA Mode of application: Through diet Exposure conditions: Up to 200 mg MeHg/g food Reference: Wolfe et al. 1998 Observations: No details given; results of a range-finding test	Consumption rate Sign of intoxication Appearance of progeny	0 0 0
Elaphe guttata	Stage exposed: NA Mode of application: Through diet (snakes fed dead mice injected with either carbon-filtered water or methylmercury chloride solution) Exposure conditions: Long-term exposure and observation (20 weeks); 5 treatments (0, 1.0, 2.5, 6.0, 12.0 mg methylmercury chloride per kg of snake) Reference: Bazar et al. 2002	Mortality Spatial learning ability	+ 0
SELENIUM			
Sceloporus occidentalis	Stage exposed: Juveniles Mode of application: Through diet (simulated food chain: lizards were fed crickets, which were fed chow contaminated with seleno-D, L-methionine) Exposure conditions: Long-term exposure and observation (98 days); 2 treatments (control vs. exposure) Reference: Hopkins et al. 2005a	Mortality Growth Prey consumption rate Body condition index (mass–SVL)	0 0 0 + (females) – (males)
Lamprophis fuliginosus	Stage exposed: Juvenile females Mode of application: Through diet (prey injected with seleno-D,L-methionine) Exposure conditions: Long-term exposure and observation (10 months); 4 treatments (0, 1, 10, 20 µg/g of prey) Reference: Hopkins et al. 2004 Observations: Overall, natural individual variability suggested to potentially mask true effects	Mortality Growth Body condition index (mass–SVL) Prey consumption rate % of females breeding Reproductive output per female Egg and hatchling mass	0 0 0 0 0 trend for (–) 0 trend for (–) trend for (+)

TABLE 12.5 (CONTINUED)
Effects of Metals in Reptiles According to Experimental Studies (Included Are All Studies That Directly Manipulated Exposure to Metals, or to Metal-Containing Substrates)

Species	Experimental Design	Effects Analyzed	Significant Effects Observed?
MERCURY, CADMIUM, LEAD			
Elaphe guttata	Stage exposed: Individuals > 1 year old		
	Mode of application: Through diet (prey injected with mixture of metals)	Sign of intoxication	0
	Exposure conditions: Long-term exposure and observation (34 weeks); 2 treatments (control: snakes fed uncontaminated prey; treament: snakes fed once a month, prey injected with enough mercuric chloride, cadmium chloride, and lead acetate to yield a dose of 2 mg of each metal per kg of snake; other meals with noncontaminated prey)		
	Reference: Jones and Holladay 2006		
COAL COMBUSTION WASTES			
Trachemys scripta	Stage exposed: Eggs	Hatching rate	0
	Mode of application: Maternal transfer or absorption via eggshell; eggs incubated in field-collected contaminated substrate	Hatching time	0
		Hatchling mass and length	0
		Hatchling metabolic rate	–
	Exposure conditions: Long-term exposure and observation (80 days); 2 × 2 factorial design [(eggs from females from contaminated vs. control site) crossed with (substrate from contaminated vs. control site)]		
	Reference: Nagle et al. 2001		
	Observations: Authors argue effects must be due to Se via maternal transfer for the following reasons. As, Cd, Cr, Cu, and Se found in hatchlings. No chemical differences were found between hatchlings from contaminated vs. uncontaminated incubation substrates; but hatchlings from contaminated vs. uncontaminated females differed in Se contents and not in any other metal analyzed.		
Nerodia fasciata	Stage exposed: Juveniles	Mortality	0
	Mode of application: Through diet (snakes were fed prey from contaminated site)	Growth	+
		Gonadosomatic index (gonad mass–body mass)	0
	Exposure conditions: Long-term exposure and observation (2 years); 3 treatments (fed only uncontaminated prey, fed uncontaminated and contaminated prey, fed only contaminated prey)	Standard metabolic rate	0
		Prey consumption rate	+
		Liver histology	–
	Reference: Hopkins et al. 2002, Ganser et al. 2003 for histology		
	Observations: Snakes accumulated As, Cd, Se, Sr, and V		

(*continued*)

TABLE 12.5 (CONTINUED)
Effects of Metals in Reptiles According to Experimental Studies (Included Are All Studies That Directly Manipulated Exposure to Metals, or to Metal-Containing Substrates)

Species	Experimental Design	Effects Analyzed	Significant Effects Observed?
LEAD AND ZINC MINE AND FOUNDRY, TREPCA; KOSOVO			
Testudo hermanni	Stage exposed: NA Mode of application: Exposure to contaminated field site Exposure conditions: Long-term exposure, short-term observation; turtles from uncontaminated site were transplanted for 8 months to the contaminated site (courtyar d of the mine and foundry) and to the control (courtyard of the university) Reference: Elezaj et al. 1983 Observations: Based on the information reported in this article, a contamination by lead is assumed, but not measured. Contrary to the findings of this experimental study, turtles sampled from the mine and foundry area exhibited decreased blood catalase and peroxidase activity relative to turtles from the reference site.	Blood catalase activity Blood peroxidase activity	+ 0
MASSACHUSETTS MILITARY RESERVATION WATER AND SEDIMENT			
Trachemys scripta	Stage exposed: Hatchlings Mode of application: Exposure via contaminated vs. reference site substrate and water in aquaria Exposure conditions: Long-term exposure and observation (1 year); 2 treatments (contaminated vs. reference substrate and water) Reference: Kitana 2005 Observations: Unreplicated experiment	Mortality Final body mass Hepatosomatic index (male, female) Hepatic CYP1A band density Hepatic CYP1A expression Male gonadosomatic index Spermatogonial proliferative activity Seminiferous tubule diameter Spermatogonial apoptosis Histological structure of testes (qualitative) Abnormal liquid filling in cystic spaces of testes found in 1 of the test site males and in none of the reference site males Female gonadosomatic index Oocyte proliferative activity Apoptosis in oocytes Histological structure of ovary (qualitative) Abnormal liquid filling in cystic spaces of ovary found in 1 of the test site females and in none of the reference site females	0 0 0 0 0 0 0 0 + 0 0 0 0 0 trend for (+) 0 0

Legend: +, significant positive effect; –, significant negative effect; 0, no significant effect. Significance or lack thereof is simply a report of the analysis found in the original publication; effects reported are between control and at least one of the treatments manipulating metal doses or concentrations.

TABLE 12.6
Effects Associated with Metal Exposure in Reptiles, According to Observational Studies

Species	Tissue Residues Analysis	Sampling Design	Effects Analyzed	Significant Effects Observed?	Comments
WETLANDS CONTAMINATED WITH COAL COMBUSTION WASTES, SOUTH CAROLINA					
Alligator mississipiensis	Metals analyzed: Se	Egg clutches collected in 3	Clutch size	0	Greater in site of intermediate contamination
	Metals detected: Se	sites (1 reference, 2 test)	Clutch mass	0	Greater in site of intermediate contamination
	Metal levels higher in test than in	and incubated in the lab;	Egg mass	0	Greater in site of intermediate contamination
	reference site animals: Se	7 clutches analyzed	Hatchling size	0	Greater in site of intermediate contamination
	Other contaminants detected: NA		Hatchling mass	0	Greater in site of intermediate contamination
			Egg viability	0	Trend for negative effect, i.e., greater in reference site
			Incubation period	0	Shorter in site of intermediate contamination
	Reference: Roe et al. 2004				
	Observations: No statistical analysis given due to small sample size (2–3 clutches per site); coal ash known to contain As, Cd, Cu, Cr, V				
Nerodia fasciata	Metals analyzed: As, Cd, Cr, Cu, Se	Animals collected in 2	Standard metabolic	+	
	Metals detected: As, Cd, Cr, Cu, Se	sites (1 reference, 1 test)	rates		
	Metal levels higher in test than in	and tested in the lab, 25			
	reference site animals: As, Se, Cd	individuals			
	Other contaminants detected: NA				
	Reference: Hopkins et al. 1999				

(*continued*)

TABLE 12.6 (CONTINUED)
Effects Associated to Metal Exposure in Reptiles, According to Observational Studies

Species	Tissue Residues Analysis	Sampling Design	Effects Analyzed	Significant Effects Observed?	Comments
WETLANDS IN THE MASSACHUSETTS MILITARY RESERVATION, MASSACHUSETTS					
Chrysemys picta	Metals analyzed: Cd	Juveniles and adults	FEMALES		
	Metals detected: Cd	sampled in 2 sites	Body mass	0	
	Metal levels higher in test than in reference site animals: Cd	(1 reference, 1 test);	Ovary mass	0	
		166 individuals	Oviduct mass	0	
	Other contaminants detected: NA		Gonadosomatic index	0	
			Relative oviduct mass	0	
			Plasma 17-beta estradiol	–	
			Plasma vitellogenin	–	
			Number of ovarian follicles	–	For 2 out of 5 follicular cohorts
			Clutch size	0	
			MALES	–	
			Body mass	0	Trend for negative effect
			Testis mass	–	
			Gonadosomatic index	0	Qualitative assessment
			Testicular histology	0	
			Sperm counts	0	Trend for negative effect
			Plasma testosterone	0	Not detected in males sampled
			Plasma 17-beta estradiol	0	Not detected in males sampled
			Plasma vitellogenin		

Reference: Rie et al. 2005; Rie 2000 for cadmium residues in turtle tissues

Observations: Wetlands surrounding the MMR have water and sediments contaminated with several metals (Al, Cu, Pb, Mn, and Cd) and organic compounds (the PAHs benzo(a)pyrene and phthalates, polychlorinated biphenyls, dieldrin, and pesticides such as methoxychlor). The test site is also adjacent to a groundwater plume contaminated with trichloroethene and ethylene dibromide. Increases in hepatic p4501A1 suggested that turtles were contaminated with organic compounds as well. Number of specimens is total; for each variable a subset of these turtles was analyzed.

Chrysemys picta	Metals analyzed: NA Metals detected: NA Metal levels higher in test than in reference site animals: NA Other contaminants detected: NA	Adults collected in 2 sites (1 reference, 1 test) and tested in the lab, 32 individuals	FEMALE Plasma steroid (17-beta estradiol) after injection of gonadotropin (ovine FSH) Plasma vitellogenin after injection of 17-beta estradiol MALE Plasma steroid (testosterone) after injection of gonadotropin (ovine FHS)	 – 0 0	 Testosterone of animals from test site took longer to return to baseline levels
	Reference: Kitana et al. 2006 Observations: Same site and species as above; therefore, similar pattern of contamination is expected				
WETLANDS IN TEXAS, INCLUDING FINFEATHER LAKE					
Trachemys scripta	Metals analyzed: As, Cu, Hg, Se, Zn Metals detected: Cu, Hg, Se, Zn in turtles from both sites; As in 1 turtle from reference site Metal levels higher in test than in reference site animals: None Other contaminants detected: DDE	Animals collected in 2 sites (1 reference, 1 test), 22 individuals 21 individuals	Damage to blood DNA Plasma cholinesterase activity	0 0	
	Reference: Clark et al. 2000 Observations: The test site was selected because of historic As contamination. However, no turtles from the test site contained As, whereas 1 turtle from the reference site contained As. Other wetlands and species were sampled, but not for presumed metal contamination. These are not reported here.				

(*continued*)

TABLE 12.6 (CONTINUED)
Effects Associated to Metal Exposure in Reptiles, According to Observational Studies

Species	Tissue Residues Analysis	Sampling Design	Effects Analyzed	Significant Effects Observed?	Comments
FINFEATHER LAKE, TEXAS					
Chelydra serpentina, *Trachemys scripta*	Metals analyzed (*T. scripta*): As, Cr, Cu, Hg, Ni, Se, Zn Metals detected: Cu, Hg, Se, Zn Metal levels higher in test than in reference site animals: NA Other contaminants detected: NA Reference: Cearley 1973; Clark et al. 1998, 2000	Not a comparison among sites; a report of die-off from 1973 and of residue analysis from 2000	Mortality Lesions	+ +	In 1973, Cearley reported on what appeared to be a die-off of turtles. He found 5 dead *T. scripta* and 1 dead *C. serpentina* around the margin of the lake; these turtles appeared to be blind prior to death. The tissues of the eyelid, eyeball, and nasal area were covered by a keratinized growth. Three live *T. scripta* also were blind. No turtles were again reported from Finfeather Lake until 1996, with reports of their absence in 1990, 1994, and 1995.
	Observations: Finfeather Lake received As-contaminated industrial wastes from 1940 to 1993 and contained high levels of As in water. In 1976 the lake was drained and contaminated sediment was removed. Clark et al. (1998) tested for As, Cr, Hg, Se, Zn and detected As, Cr, Zn in tadpoles of 3 species, which are potential prey to turtles.				
MISSOURI OLD LEAD BELT, MISSOURI					
Chelydra serpentina	Metals analyzed: Pb Metals detected: Pb Metal levels higher in test than in reference site animals: Pb Other contaminants detected: NA	Animals collected in 3 sites (reference site; moderately contaminated test site; heavily contaminated test site), 37 individuals	Body mass Carapace length Body condition index (mass–length) Liver mass Liver–body mass Turtle capture success Hematocrit Plasma delta–ALAD activity Plasma osmolality	0 0 0 0 0 0 0 – 0	Trend for negative effect

			Plasma chloride content	0	
			Plasma glucose	0	
			Hemoglobin	0	
	Reference: Overmann and Krajicek 1995				
	Observations: A Superfund site subject to large-scale lead mining and smelting between 1870 and 1970. Now 227 million tons of crushed rock in tailing piles cover 1200 ha. Pb in liver, carapace, and bone highly matched hypothesized gradient in contamination.				
TAR CREEK SUPERFUND SITE, OKLAHOMA					
Trachemys scripta	Metals analyzed: Pb, Cd	Animals collected in 3 sites (2 reference, 1 test), 211 individuals	Coefficient of variation of mean DNA content	0	
	Metals detected: Pb, Cd				
	Metal levels higher in test than in reference site animals: Cd for pooled samples		Aneuploidy	+	
	Other contaminants detected: NA				
	Reference: Hays and McBee 2007				
	Observations: Region heavily mined for Pb, Zn from 1890 to 1970. Seventy-five million tons of ore extract leftovers and 324 ha of tailing ponds are found in the test site, which is mainly contaminated with Pb, Cd, Zn. CV of mean DNA content is a metric of chromosome lesions.				
CALIFORNIA					
Gopherus agassizii	Metals analyzed: Cd, Cu, Fe, Hg, Pb, Se	Animals collected in 2 sites (4 healthy individuals from reference site, 9 sick individuals from test site); 13 individuals 390–4900 g	Incidence of upper respiratory tract disease (URTD)	+	
	Metals detected: Cd, Cu, Fe, Hg, Pb, Se in both diseased and healthy individuals		Thymus size	–	Thymus located in all 4 healthy individuals, but only in 2 out of 12 ill individuals; this was interpreted as a sign of atrophy. Cause is unknown, but both Hg contamination and starvation can lead to thymus atrophy.
	Metal levels higher in test than in reference site animals: Fe, Hg				
	Other contaminants detected: NA		URT histology	–	
			Bacterial abundance in URT	+	
			Hemoglobin	–	Anemia interpreted as a secondary response to disease

(*continued*)

TABLE 12.6 (CONTINUED)
Effects Associated to Metal Exposure in Reptiles, According to Observational Studies

Species	Tissue Residues Analysis	Sampling Design	Effects Analyzed	Significant Effects Observed?	Comments
			Red blood cell counts	0	
			White blood cell counts	0	
			WBC: % heterophils	0	
			WBC: % lymphocytes	0	
			WBC: % monocytes	0	
			WBC: % eosinophils	0	
			WBC: % basophils	0	
			WBC: % activated granular monocytes	0	
			WBC: azurophilic monocytes	0	
			Packed cell volume (%)	0	
			MCV	0	
			MCH	0	
			MCHC	0	
			Serum glucose	0	
			Serum sodium	+	Interpreted as a possible physiological response to conserve water and nutrients during period of environmental drought
			Serum potassium		
			Serum chloride	0	
			Serum calcium	0	
			Serum phosphorus	0	
			Serum CO_2	−	
				0	

			Serum BUN (urea)	+
				Interpreted as a possible physiological response to conserve water and nutrients during period of environmental drought
			Serum creatinine	+
			Serum uric acid	0
			Serum ALP (alkaline phosphatase)	0
			Serum SGOT activity	+
				Interpreted as a consequence of tissue damage
			Serum SGPT activity	0
			Serum cholesterol	+
				Interpreted as a possible response to fasting
			Serum triglyceride	0
			Serum bilirrubin	0
			Serum albumin	0
			Serum globulin	0
	Reference: Jacobson et al. 1991 Observations: By definition, the test site was associated with higher incidence of URTD because this study was a comparison between ill and healthy individuals and not between sites (i.e., 100% of the individuals coming from the test site were diseased; 100% of the individuals from the reference site were healthy). However, ill individuals also contained higher concentrations of Hg, Fe than did healthy individuals — therefore a + association between test site and incidence of disease. High Fe concentrations in the liver are interpreted as the result of erythrocyte breakdown and inability of reutilizing iron released from degraded hemoglobin. Hg concentrations are not considered high enough to be toxic, but potentially high enough to depress immunocompetence.			
Gopherus agassizii	Metals analyzed: As, Cd, Cr, Cu, Fe, Hg, Mo, Ni, Pb, Se, Zn Metals detected: As is the only one reported Metal levels higher in test than in reference site animals: As is the only one reported Other contaminants detected: NA Reference: Berry et al. 2001 Observations: Cites articles stating that population declines were correlated with appearance of 2 main diseases, URTD (a mycoplasmosis) and cutaneous diskeratosis.	Not a comparison among sites, but between 41 ill, dying, or dead individuals and an unspecified number of healthy individuals	Incidence of various diseases (not specified; ill tortoises exhibited metabolic disease, infectious disease, cutaneous dyskeratosis, and URTD)	+

(*continued*)

TABLE 12.6 (CONTINUED)
Effects Associated to Metal Exposure in Reptiles, According to Observational Studies

Species	Tissue Residues Analysis	Sampling Design	Effects Analyzed	Significant Effects Observed?	Comments
LAKE APOPKA, FLORIDA					
Alligator mississipiensis	Metals analyzed: As, Cd, Cr, Hg, Mn, Pb, Se, Sn Metals detected: As, Cd, Cr, Hg, Mn, Pb, Se, Sn Metal levels higher in test than in reference site animals: Considering all tissues, pattern is higher [Cd] but lower [Hg], [Pb] in test site Other contaminants detected: Higher p,p'-DDE, dieldrin, endrin, mirex, oxychlordane, ΣDDTs, and ΣPCBs in test site	Juveniles collected in 3 sites (2 reference, 1 test); 20–31 individuals	Population size Clutch viability MALES Abnormalities in testis histology Phallus size Testosterone levels Estradiol 17β (males) FEMALES Abnormalities in ovarian histology Abnormalities in ovarian steroidogenesis Estradiol 17β (females)	– – + – – 0 + + 0	

Reference: Guillette et al. 2000 for metal residues and Guillette et al. 1999 for organic residues from 50 juvenile individuals from same sites; Guillette et al. 1994, 1996, 1999 for effects (sampling design reported above is for effects)

Observations: Lake Apopka is contaminated with pesticides, dicofol, DDT and their metabolites, and sulfuric acid. Endocrine disruption at the embryonic stage is the predominant hypothesis for observed effects. Given the overall low concentrations of metals, and residue concentration pattern opposite to effects, metal intoxication was not considered a cause for the observed effects (Guillette et al. 1999).

Florida					
Alligator mississippiensis	Metals analyzed: Hg Metals detected: Hg in both reference and test site animals Metal levels higher in test than in reference site animals: Hg Other contaminants detected: NA	Individuals collected in 2 sites (1 reference, 1 test), plus a farm (the "negative control"); 30 individuals TL > 2.0 m	Behavior	0	"No clinical signs of neurotoxicosis, such as visual and auditory deficits and ataxia, were detected. All animals exhibited the ability to see, hear, and react to people and surroundings by bellowing, hissing, and accurately evading or attacking the air boats. The largest males and female with the highest brain concentrations … displayed good visual ability and coordination when attacking."
	Reference: Heaton-Jones et al. 1997		Histology	0	No histologic evidence of renal disease
ALLIGATOR FARM IN LOUISIANA					
Alligator mississipiensis	Metals analyzed: Pb, As Metals detected: Pb, As Metal levels higher in test than in reference site animals: Pb Other contaminants detected: NA	1-year-old individuals from 2 sites (test farm where alligators were fed nutria meat, reference farm where alligators were not fed nutria meat); 12 individuals	Histology	+	
	Reference: Camus et al. 1998				
	Comments: Alligators were fed ground nutria (*Myocastor coypus*, a rodent) meat, which was hunted with lead shots; nutria and alligators indeed contained lead bullets and fragments. Alligators from the facility with greater mortality rates (5% of 3000 animals) had higher lead contents in blood and kidney than did alligators from other facilities, 4 out of 7 with concentrations considered diagnostic of lead poisoning. Arsenic concentrations were low. For histological analysis, 3 contaminated individuals (2 moribund, 1 dead) were compared to 3 size-matched control animals that never ate nutria meat. Qualitative histological analyses revealed, in the test animals: wasting of tail musculature; little fat in tail; mild individual cell necrosis of proximal renal tubular epithelial cells; infrequent globular hyaline cytoplasmic inclusions containing basophilic nuclear fragments, interpreted as apoptotic bodies; epithelial cell nuclei often enlarged with marginated chromatin; acid-fast intranuclear lead inclusion bodies not present; and no evidence of basophilic stippling. One individual presented lungs partially filled by clear mucoid exudates infected with *Pseudomonas*; microscopic examination confirmed an acute mild heterophilic pneumonia.			

(*continued*)

TABLE 12.6 (CONTINUED)
Effects Associated to Metal Exposure in Reptiles, According to Observational Studies

Species	Tissue Residues Analysis	Sampling Design	Effects Analyzed	Significant Effects Observed?	Comments
Alligator mississipiensis	Metals analyzed: Pb, Cd, Se Metals detected: Pb, Cd, Se Metal levels higher in test than in reference site animals: Pb Other contaminants detected: NA	>160 cm individuals from 2 groups (farm where alligators were fed nutria meat; wild animals); 59 individuals	Liver TBA-reactive products Ovary TBA-reactive products Testicle TBA-reactive products	+ 0 0	
	Reference: Lance et al. 2006 Comments: Farm alligators were stated to have dramatically lower reproductive output than wild populations. Clutch size was high, but number of females nesting, egg fertility, and hatching rate were very low. In addition, eggs were commonly misshapen and poorly calcified. Wild alligators just outside the pens nested successfully. Thiobarbituric acid-reactive products were measured as an indicator of lipid peroxidation, which can cause reproductive problems in egg-laying vertebratess. Lead contamination can induce lipid peroxidation. Reproductive failure was attributed to ingestion of lead and of rancid frozen nutria meat.				
FERN RIDGE RESERVOIR, OREGON					
Clemmys marmorata	Metals analyzed: Al, As, Cd, Co, Cr, Cu, Fe, K, Mg, Hg, Mn, Na, Ni, Pb, V, Zn Metals detected: Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, Zn, Hg Metal levels higher in test than in reference site animals: NA Other contaminants detected: The OC pesticides p,p-DDE, oxychlordane, trans-nonachlor, and PCBs and PCB congeners were found in all clutches. Six other OCs found in part of the clutches.	Egg clutches collected in 2 sites in same area; 14 clutches analyzed; comparison not between sites, but of residue loads in clutches where hatching success = 0 vs. clutches where hatching success > 0	Hatching success	0	Organic and metal contaminant loads considered low and with small variability
	Reference: Henny et al. 2003 Observations: The study is not a comparison among populations or sites. Residue analyses were conducted in 1 egg per clutch.				

GREAT LAKES–ST. LAWRENCE RIVER BASIN, CANADA/UNITED STATES				
Chelydra serpentina serpentina	Metals analyzed: Hg Metals detected: Hg Metal levels higher in test than in reference site animals: NA Other contaminants detected: PCBs, PCDDs PCDFs, OCs Reference: Bishop et al. 1998	5–13 egg clutches collected in each of 8 sites and incubated in the lab	Hatchling success Hatchling morphological abnormalities	– +
	Observations: 5 eggs per clutch underwent chemical analysis. Hg concentrations considered low, and with low variability. Hg was not measured in the reference site. However, among clutches where Hg was measured, abnormalities declined with Hg concentration. This led the authors to state that Hg is unrelated to any toxic effect. In turn, incidence of egg abnormalities correlated with residue loads of several PCBs, PCDDs, and PCDFs.			
WETLANDS IN THE INDUSTRIAL ZONE OF SUMGAYIT, AZERBAIJAN, FSU				
Emys orbicularis	Metals analyzed: Al, As, B, Ba, Ca, Co, Cr, Cu, Fe, Hg, Mg, Mn, Mo, Ni, Pb, Se, Sr, Ti, V, Zn Metals detected: Turtles from both sites contained Al, As, Ba, Ca, Cr, Cu, Fe, Hg, Mg, Mn, Mo, Ni, Pb, Se, B, Sr, V, Zn; in addition, turtles from the reference site contained Co, Ti Metal levels higher in test than in reference site animals: Hg Other contaminants detected: Test site turtles contained significantly higher levels of PCB, heptachlor, DDD, HCB, chlordane, pentachlorobenzene, PAH, trans-nonachlor, alfa HCH and aldrin than reference site turtles. Nevertheless, reference site turtles contained a broad variety of organic contaminants. Reference: Swartz et al. 2003	Animals collected in 2 sites (1 reference, 1 test); 13 individuals	Micronucleus counts in erythrocytes	+

(*continued*)

TABLE 12.6 (CONTINUED)
Effects Associated to Metal Exposure in Reptiles, According to Observational Studies

Species	Tissue Residues Analysis	Sampling Design	Effects Analyzed	Significant Effects Observed?	Comments
	Observations: Major center for production of chlorinated industrial and agricultural products; known spillage of 1566 tons of mercury. Micronucleus counts were significantly correlated with Hg, heptachlor, DDD, HCB, trans-nonachlor residue levels. The authors also analyzed residue levels and micronucleus counts in the Caspian turtle, *Mauremys caspica*, but only 1 individual was collected from the reference site, therefore precluding meaningful comparisons. *M. caspica* individuals from the contaminated site contained roughly the same pattern of metal contamination as *E. orbicularis*, but lower contaminant loads.				
LEAD AND ZINC MINE AND FOUNDRY, TREPCA, KOSOVO					
Testudo hermanni	Metals analyzed: NA Metals detected: NA Metal levels higher in test than in reference site animals: NA Other contaminants detected: NA Reference: Elezaj et al. 1983	Animals collected in 2 sites (1 reference, 1 test), 37 individuals	Blood catalase activity Blood peroxidase activity	– –	
	Observations: Based on the information reported in this article, a contamination by lead is assumed, but not measured. Contrary to the findings of this sampling study, an experiment reported in the same article found that turtles collected in another reference site and transplanted for 8 months to the mine and foundry courtyard exhibited increased blood catalase activity (and no difference in blood peroxidase activity) relative to the control (turtles in the University courtyard).				
BRACKISH AND FRESHWATER WETLANDS IN NEW JERSEY/MARYLAND					
Chelydra serpentina	Metals analyzed: Cd, Cr, Cu, Hg, Ni, Pb, Zn Metals detected: Cd, Cr, Cu, Hg, Ni, Pb, Zn	Animals collected in 5 sites (1 reference, 4 test); 32 individuals	Population size	0	Trend for negative. Estimate of population density was not an objective of the study, but it is noteworthy that in the reference site and in the 2 least contaminated test sites, on average 1 individual was collected per 4–6 set-line days; in the 2 most contaminated test sites, no turtles were trapped despite an effort of 66 set-line days.
	Metal levels higher in test than in reference site animals: Cu, Hg, Zn; conversely, Cr, Ni in reference site > at least one of the test sites		Growth rates % lipid of visceral fat Plasma ALAD activity	– – –	Growth rates inferred from length-estimated age relationship. Most metal-contaminated site had lowest value. Most metal-contaminated site had lowest value (despite low Pb in tissues).

	Other contaminants detected: Chlordane, nonachlor, DDE, PCB found in turtles from all 3 sites; other organic compounds found in a subset of sites	Plasma albumin	+	Least metal-contaminated test site had highest value; suggested to be a consequence of age (individuals were oldest in this site).
		Plasma glucose	+	Least metal-contaminated test site had highest value; suggested to be a consequence of age (individuals were oldest in this site).
		Plasma total protein	+	Least metal-contaminated test site had highest value; suggested to be a consequence of age (individuals were oldest in this site).
		Blood hemoglobin	+	Reference site had lowest value; suggested to be due to lower salinity.
		Plasma osmolality	0	
		Blood-packed cell volume	0	
		Plasma triglycerides	0	
		Plasma uric acid	0	
	Reference: Albers et al. 1986			
	Observations: [Cd, Hg, Pb] considered low. Gradient of contamination for metals was, overall, New Jersey brackish water (test site) > New Jersey freshwater (test site) = Maryland freshwater (reference).			
GULF COAST, SOUTHEASTERN UNITED STATES				
Lepidochelys kempii	Metals analyzed: Ag, Cd, Cr, Cu, Hg, Pb, Zn Metals detected: Cr, Hg (presence of other metals cannot be inferred from sources) Metal levels higher in test than in reference site animals: NA Other contaminants detected: NA Reference: Peden-Adams et al. 2003; Keller et al. 2006	Juveniles sampled in 3 sites, 1 of which was contaminated with Hg; 36 individuals	Plasma T-lymphocyte proliferation	– [Hg] – [Cr]
	Comments: The study is not a comparison among populations or sites. Effects reported are significant correlations between plasma metal concentrations and the variable measured.			

(*continued*)

TABLE 12.6 (CONTINUED)
Effects Associated to Metal Exposure in Reptiles, According to Observational Studies

Species	Tissue Residues Analysis	Sampling Design	Effects Analyzed	Significant Effects Observed?	Comments
ATLANTIC COAST, SOUTHEASTERN UNITED STATES					
Caretta caretta	Metals analyzed: Hg Metals detected: Hg Metal levels higher in test than in reference site animals: NA Other contaminants detected: PCBs, DDE, chlordanes detected in blood of turtles from the same area	Subadults and adults sampled in ~50 sites along the coast; 9 individuals 12/58 individuals 28 individuals	Total plasma white blood cell counts	–	
			Plasma B-lymphocyte proliferation	–/0	Negative in preliminary tests with 12 individuals, null in larger sample with 58 individuals; suggested to arise to broader range of blood Hg concentrations in smaller preliminary test
			Plasma T-cell proliferation	0/0	
			Plasma lysozyme activity	+/0	
			Hematocrit	+	
			Plasma AST (enzyme) levels	–	
			Plasma CPK (enzyme) levels	+	
			Plasma lymphocyte counts	–	
			Plasma heterophil counts	0	Trend for negative
			Total protein, albumin, globulin, glucose, urean nitrogen, uric acid, calcium, phosphorus, sodium, potassium, chloride in plasma	0	

Reference: Day 2003; Day et al. 2007; data for organic contaminants in blood from Keller et al. 2006

Comments: The study is not a comparison among populations or sites. Effects reported are significant correlations between plasma metal concentrations and the variable measured; dead stranded turtles had significant higher blood and keratin mercury concentrations than did live turtles.

HAWAII					
Chelonia mydas	Metals analyzed: Al, As, Ba, Be, Cd, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Se, Tl, Va, Zn Metals detected: Al, As, Ba, Cd, Cr, Cu, Fe, Mn, Mo, Ni, Se, Tl, Va, Zn Metal levels higher in test than in reference site animals: NA Other contaminants detected: No. PCBs and organochlorine, organophosphate, and carbamate pesticides were screened, but none were detected. Reference: Aguirre et al. 1994 Comments: The study is not a comparison among populations or sites. Although several metals were detected, all were considered below levels reported to be normal in other animal species.	Individuals collected in 7 sites (10 juveniles afflicted with GTFP; 1 juvenile and 1 pelagic individual without GTFP used as "control"); 12 individuals	Incidence of green turtle fibropapillomatosis	0	
ZOO					
Crocodylus rhombifer	Metals analyzed: "Heavy metals" Metals detected: Zn Metal levels higher in test than in reference site animals: NA Other contaminants detected: NA Reference: Cook et al. 1989	Adult (10 kg); 1 individual	Behavior	0	The single individual displayed anorexia, depression, weight loss.

Source: Cook et al. (1989).

Note: Included here are all studies that compared traits of individuals sampled or collected in the field, for which metal contamination was demonstrated, and from which subindividual, individual, or population level endpoints were measured but in the absence of controlled manipulation of exposure other than selection of field sites (test vs. reference). It includes studies that found both inconclusive and conclusive evidence (supporting or refuting) for a causative role of metals in generating the observed effects.

Legend: +, significant positive effect; –, significant negative effect; 0, no significant effect. Significance or lack thereof is simply a report of the analysis found in the original publication; effects reported are for test site (or for at least one of the test sites) relative to the control site. "Metals detected" refers to all metals that were above detection limits. N sites and specimens refer to effects measured, not residue analysis. NA = not applicable, analyzed, or reported.

12.4.1 Subindividual Level Effects

Subindividual level indicators of change in biological systems are often referred to as biomarkers, and the development of biomarkers has become one of the most powerful tools for detecting exposure to and effects of contaminants in environmental toxicology. There are several definitions of biomarkers available in the literature (NRC 1987; Mitchelmore et al. 2006), but a biomarker is essentially an indicator that signals an event in a biological system (NRC 1987). The term "biomarker" could be defined at any level of biological organization, but the term's most frequent application refers to measurements at or below the individual level, especially in the biochemical or cellular modifications of body fluids, cells, or tissues (NRC 1987). This is the definition we will follow in this section, where tested biomarkers of exposure and effect to metals in reptiles will be presented and related to their functional significance.

12.4.1.1 Hematological Effects

Chemical and morphological blood parameters provide for a wide range of biomarkers, and interest in their use has greatly increased because sampling is potentially quick and minimally destructive. Here, we focus on hematological parameters not directly related to endocrine, immune, genetic, or neural function, which will be discussed in later sections.

Various metals alter enzyme activities, some in a toxicant-specific manner. For example, lead interferes with enzymes involved in heme synthesis, especially inhibition of metabolism of aminolevulinic acid. One of the most sensitive biological indicators of lead exposure in fish, birds, and mammals is decrease in erythrocyte δ-aminolevulinic acid dehydratase (ALAD) activity. Relatively few studies focused on ALAD activity in reptiles yet the enzyme appears to be an effective bioindicator of lead exposure. In addition to ALAD, other bioindicators in blood used for evaluating metal exposure in reptiles include hematocrit, lipid content, total serum proteins, total cholesterol, number of reticulocytes and the enzymes SGOT (serum glutamic oxaloacetic transaminase, a synomym for AST [aspartate aminotransferase]), CPK (creatine phosphokinase), catalases and peroxidases (Elezaj et al. 1983; Day et al. 2007).

12.4.1.2 Immunological Effects

Metals in both inorganic and organic forms can cause a variety of immunological alterations, such as immunosuppression (e.g., organotin, Cd, Pb, both inorganic and organic Hg, Ni), nonspecific stimulation (e.g., Hg, Pb), hypersensitivity (e.g., Be, Cr, Co, Au, Hg, Ni), and autoimmunity (e.g., Cd, Hg). In general, altered immune function may contribute to increased risk of disease (see Hultman 2007). Notably, mercury and mercury compounds have a broad variety of immunotoxic effects in mammals (Hultman 2007), which has also been suggested by studies with sea turtles and other reptiles.

For example, Day and collaborators (Day 2003; Day et al. 2007) observed that there was a significant negative correlation between blood Hg concentrations and lymphocyte cell counts among 28 wild loggerhead sea turtles captured along the coasts of South Carolina, Georgia, and Florida. They also conducted an assay with blood from a subsample of 12 individuals and demonstrated a significant negative correlation between plasma concentrations of Hg, and β-lymphocyte proliferation after stimulation by the mitogen, phorbol 12,13-dibutyrate (PDB). In turn, evidence questioning an immunotoxic effect for Hg included the observation that the negative relationship between blood Hg concentrations and lymphocyte cell counts was not statistically significant when sample size was increased to 58 individuals, and the observation that loggerheads from this study area were contaminated with a host of organic compounds including polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethylene (DDE), and chlordanes in addition to Hg (Keller et al. 2006). However, an important role for Hg in the immunotoxic effects observed was confirmed by a final in vitro assay where Day et al. (2007) treated blood samples with methyl-Hg in 6 environmentally relevant (i.e., bracketing concentrations found in the blood of wild turtles) plus a control and either

PDB or phytohemaggultinin (PHA) mitogens to stimulate B- and T-lymphocyte proliferation, respectively. The investigators observed significant dose-dependent decreases in both B- and T-lymphocyte proliferation activities, with a companion assay indicating that the decreases were not a result of reduced cell viability. These findings suggested that individual loggerheads that presented elevated blood Hg levels had both fewer lymphocytes and lower lymphocyte activity. Although lymphocyte counts and activities comprise only 1 aspect of the complex responses of the immune system, these studies clearly suggest that Hg may have subtle, yet important immunotoxic effects at environmentally relevant concentrations.

Other metals may also have immunotoxic effects in reptiles. Peden-Adams et al. (2003) found Hg, Cr, Ag, Cd, Cu, Pb, and Zn in blood samples of 36 juvenile Kemp's ridley turtles from the Gulf Coast. There was a negative correlation between T-lymphocyte proliferation and Hg (and also Cr blood concentrations; Peden-Adams et al. 2003; Keller et al. 2006). Further studies, however, are needed to test an immunotoxic effect of Hg, Cr, and other metals in reptiles. If metals can have an immunosuppressive effect to reptiles, metal contamination may increase reptile susceptibility to diseases. For example, several marine turtle species, including green turtles, olive ridleys, flatbacks (*Natator depressus*), and loggerheads, are afflicted by fibroepithelial tumors. Although benign, these tumors are linked with sublethal adverse effects and are potentially lethal. Historically, the associated disease state, green turtle fibropapillomatosis (GTFP), has episodically reached epidemic proportions in some areas of the world and posed significant threats for sea turtle populations worldwide (Herbst and Klein 1995). GTFP appears to be caused by a virus (possibly a herpes virus), and several studies with captive and wild turtles demonstrate that infected and morbid individuals present with depressed immune functions as indicated by lower lymphocyte counts and proliferation activity, lower albumin–globulin ratios, and higher heterophil counts (Keller et al. 2006). Because disease occurrence appeared more prevalent in degraded habitats, environmental contamination may be a contributing factor in this disease (Herbst 1994). Studies have quantified contaminant residues in turtles with GTFP (Keller et al. 2006), including characterization of metal residues in tissues of green turtles. Data are severely limited and more studies are needed to understand whether environmental contamination in general, and of metals in particular (such as Hg), plays a role in green turtle papillomatosis.

Another reptile disease with a plausible link to contamination, but without any identified immunological link, is the upper respiratory tract disease (URTD) reported to have killed free-ranging desert tortoises, *Gopherus agassizii*, in various populations (Jacobson et al. 1991). Both diseased and healthy tortoises had comparable Cu, Cd, and Pb liver residue levels, but diseased tortoises had much higher concentrations of Fe and Hg. Elevated levels of Fe were considered to be a consequence of red blood cell breakdown and of an inability to reuse iron released from hemoglobin, but mercury may have immunotoxic effects in chelonians (Jacobson et al. 1991). Desert tortoises diagnosed with URTD and other diseases had elevated levels of As (Berry et al. 2001; Seltzer and Berry 2005). Detailed chemical analyses of 66 elements in soil, stream sediment, and plants from 6 desert tortoise habitat sites (but not of tortoises themselves) in the Mojave and Colorado deserts revealed high As concentrations throughout the study region. Interestingly, among 27 plant species sampled, and stated to be consumed by these herbivorous tortoises, the highest As concentrations were found in 13 plant species considered preferred foods of tortoises. Arsenic was suggested as a major cause for the high incidence of the disease (Berry et al. 2001), although other metals associated with mining activities (Au, Cd, Hg, Sb, and W) were locally abundant and occurred in relatively higher concentrations in soils and/or plants from the region; Pb was also elevated and associated with road traffic in the area (Chaffee and Berry 2006).

12.4.1.3 Endocrine and Reproductive Effects

One of the most frequently discussed classes of effects from contaminants is endocrine disruption (ED). An endocrine disruptor is an exogenous substance or mixture of substances that alters functions of the endocrine system, and as a consequence, exposure to endocrine-disrupting

chemicals (EDCs) causes adverse health effects in an organism, its progeny, or its population (Damstra et al. 2002). Several metals including Hg, Cd, Pb, Al, As, Br, Se, Sn, are suspected EDCs (Henson and Chedrese 2004; IEH 2005; Virgolini et al. 2005; Golub 2006). Reptiles may be particularly vulnerable to EDCs. The cleidoic egg, which prevents water loss but also the elimination of excretions, facilitates exposure of the developing embryo to potentially high levels of contaminants acquired via maternal transfer. Furthermore, many reptiles (all crocodilians, most turtles, and many lizards) have temperature-dependent phenotypic sex determination, and several endocrine disruptors (such as steroid hormones, PCBs, and organochlorines; see Wibbels and Crews 1995) override the effects of temperature in sex determination, leading to a dramatic change in the development of the individual and providing an obvious endpoint of ED for researchers. Whereas reptiles are relatively understudied in terms of their development and endocrinology, taxa within the order — the alligators of Lake Apopka, Florida (Damstra et al. 2000) — present one of the strongest cases for ED in wildlife.

In 1980, Lake Apopka was subject to a major chemical spill of dicofol, DDT and its metabolites DDD and DDE, and sulfuric acid. In the years following the spill, male and female alligators displayed a variety of notable adverse effects. For example, plasma sex steroid concentrations were dramatically altered: males from Lake Apopka had half the testosterone levels and twice the estradiol levels than males from a reference lake; indeed, estradiol levels in males were comparable to those of females (Guillette and Gunderson 2001). Alligators of both sexes had abnormal gonad morphology and altered gonadal steroidogenesis, males had significantly reduced phallus size, and clutch viability was dramatically reduced. As a consequence, the population of alligators in Lake Apopka plummeted by 90% within 4 years of the spill (see Damstra et al. 2002 and Campbell 2003 for reviews of these studies).

Alligator eggs and adults from Lake Apopka were contaminated with 17 metals (Burger et al. 2000; see Appendix). However, metal concentrations were generally low and only Cd appeared elevated relative to other Florida Lakes. Furthermore, because several of the organic compounds found in tissues and eggs at high concentrations are known endocrine disruptors, metals were not considered as primary causes directly linked to ED. Their role as contributing factors was not completely characterized, and several of the organic compounds identified in tissues and eggs occurred at high concentrations and were endocrine disruptors presumptively linked to observations of ED among the alligators of Lake Apopka. Arsenic, Be, Cd, and their compounds, Cr VI, and Ni compounds are *known* carcinogenic to humans; Pb and inorganic Pb compounds are *probably* carcinogenic to humans; Co sulfate and other soluble Co salts, organic Pb compounds, metallic Ni and Ni alloys, and V pentoxide are *possibly* carcinogenic to humans (IARC 2007).

Other examples of ED in reptiles occur in the literature, including studies focused in part on the relationship between ED and metal exposures. Painted turtles from the Massachusetts Military Reservation (MMR) — a Superfund site contaminated with heavy metals (Al, Cu, Pb, Mn, and Cd) and various organic compounds (benzo(a)pyrene, polychlorinated biphenyls, pesticides, dieldrin, and phthalates; Rie et al. [2005]) — were subject to a series of studies focused on endocrine endpoints. Rie et al. (2005) showed that female turtles from a contaminated site had lower estradiol 17β (E2) plasma levels (at the preovulatory and ovarian recrudescence phases) and lower vitellogenin (in the ovarian recrudescence phase) than turtles from a reference site. To test more specifically the step where endocrine disruption occurred, Kitana et al. (2006) injected field-caught turtles with either gonadotropin (ovine FSH) or E2. Injection of gonadotropin elicited an increase in E2 secretion in female turtles from the reference site but not from the contaminated site; in turn, injection of E2 yielded no change in secretion of vitellogenin in female turtles from either group. Therefore, the authors concluded that the animal's hepatic response was normal, and endocrine disruption occurred in steroid biosynthetic pathways (Kitana et al. 2006). Males from the contaminated site had normal plasma testosterone levels (Rie et al. 2005); gonadotropin (o-FSH) injection elicited similar responses in males from both groups, except that testosterone levels of contaminated site individuals took longer to return to baseline conditions than the control's (Kitana et al. 2006).

Comparing populations of the contaminated and reference sites, no differences in body weight, ovary weight, gonadosomatic index, and oviduct weight in females were observed, but contaminated site females had lower numbers of follicles in some cohorts (Rie et al. 2005). In turn, males from the reference site were heavier and had greater total and relative testicular weight than males from the contaminated site. No differences were found in testicular histology, sperm count, or sperm viability. Both organic and inorganic contaminants found at the site and in turtles could be capable of causing the endocrine and reproductive effects observed at the military reservation (Rie et al. 2005; Kitana 2005). Among the inorganic contaminants, cadmium, which causes reproductive toxicity and is an ED element, reached both steroidogenic tissue (particularly gonadal and adrenal-interrenal) and steroid hormone target tissue (particularly in liver where vitellogenin is produced under estrogenic control) in adult turtles. Rie et al.'s (2005) findings are consistent with the hypothesis that maternally transferred cadmium could mediate the observed endocrine alterations in MMR painted turtles.

Our review encountered only 1 study focused solely on endocrine disruption by metals in squamates (Brasfield et al. 2004). Here, the authors experimentally tested the effects of Cd-contaminated soils on thryoid function in eastern fence lizard eggs. Laboratory exposures to Cd-contaminated soils, as a single spike of a cadmium chloride solution to perlite $CdCl_2$ at 1.48, 14.8, 148, 1480, or 14 800 μg Cd/g perlite, were evaluated through measurements of thyroid hormones (T3, T4) in whole body homogenates. No effect of cadmium dose on whole body T3 or whole body T4 was observed, and the only evidence for an effect of cadmium was a significantly lower T3:T4 ratio in embryos from a single-exposure concentration (148 μg Cd/g perlite treatment group).

12.4.1.4 Genetic Effects

Chemical contaminants and radiation may lead to changes in the genetic material through direct interactions with DNA or through indirect interactions with the cellular apparatus that regulates the fidelity of genomic replication. These changes in the genetic material can result in deleterious effects, including cell, gamete, and embryo death, abnormal development, neoplasia (i.e., cancer) and heritable mutations (Novillo et al. 2006). Several metals can have genotoxic effects in mammals. For example, As, Be, Cd, Ni, and their compounds, and Cr VI are *known* carcinogens to humans; Pb and inorganic Pb compounds are *probable* carcinogens to humans; Co sulfate and other soluble Co salts, organic Pb compounds, metallic Ni and Ni alloys, and V pentoxide are *possibly* carcinogens (IARC 2007). In addition, metallic radionuclides such as plutonium-239, radium-224, radium-226, radium-228, thorium-232, and their decay products are also *known* carcinogens to humans (IARC 2007). In this chapter we do not exhaustively review effects of metallic radionuclides.

Not surprisingly, very little is known about genotoxicity in reptiles, but we present findings that metals classified as genotoxic to humans are present in the natural environment and the tissues of reptiles, including eggs and early life stages (Tables 12.3 and 12.4; Novillo et al. 2006). Studies that quantified biomarkers of genotoxicity in reptiles in environments contaminated with metals include Clark et al. (2000) and Swartz et al. (2003), and with metal radionuclides, Meyers-Schöene et al. (1993), Bickham et al. (1988), Lamb et al. (1991), and others. Whereas some of these studies could establish associations between environmental exposure to metals and/or radionuclides and genotoxicity endpoints in observational field studies, cause-effect relationships have not been experimentally established. For example, Meyers-Schöene et al. (1993) found that pond slider turtles and snapping turtles from a lake used as a settling basin for low-level radioactive and nonradioactive wastes had more DNA single-strand breaks in hepatocytes than turtles from a reference site. Similarly, tissues of turtles from the contaminated site had significantly higher levels of ^{90}Sr, ^{137}Cs, ^{60}Co, and Hg.

Using flow cytometry, Clark et al. (2000) determined genetic damage in 4 species of reptiles — diamondback water snake *Nerodia rhombifer*, blotched water snake *N. erythrogaster*, cottonmouth *Agkistrodon piscivorus*, and red-eared slider turtle occurring in 5 water bodies in Texas. Two of these water bodies were contaminated (one with nonmetallic agrichemicals, the other with As) and

3 were used as reference sites. Although reference site comparisons reflected uncertainties commonly encountered in reconnaissance studies, no differences among sites were observed for genetic damage in erythrocytes from any species. Interpretation of these findings must be guarded, as the species-by-site matrix contained many empty cells (i.e., species rarely came from the same sites) and sample sizes were small. Subsequent studies at similar contaminated sites (Hays and McBee 2007) did not find intersite differences in erythrocyte DNA content in red blood cells in *Trachemys scripta* collected from a Pb-, Zn-, and Cd-contaminated Superfund site and 2 reference sites. Similarly, Swartz et al. (2003) detected 19 metallic and 50 organic contaminant residues (including PAHs, PCBs, DDT, and other OCs) in the tissues of European pond turtles, *Emys orbicularis*, and Caspian turtles, *Mauremys caspica*, from a wetland adjacent to an industrial wastewater treatment plant in Sumgayit, Republic of Azerbaijan (former Soviet Union). Pond turtles from Sumgayit displayed a trend toward higher erythrocyte micronucleus counts (a biomarker of genotoxic effects) than pond turtles from a reference site. Small sample size (5 and 8 individuals, respectively) and high variability may have provided insufficient statistical support for interpretation, as the trends observed were not significant. Nevertheless, genotoxic effects were suspected, given the highly significant positive correlation between micronucleus counts and residue levels of 5 contaminants (including Hg) among turtles from the contaminated site.

Overall, the genotoxicity of metals in reptiles remains uncertain, although comparative data and existing literature suggest that future work more clearly characterize adverse genotoxic effects associated with metal exposures. DNA damage could be the basal mechanism for higher-order effects such as development of histopatologies, neoplasias, and morphological malformations. In many cases there is no strong evidence that higher-order effects associated with environmental contamination have a genetic basis, although the reproductive effects of cadmium chloride in red-eared slider turtles and painted turtles urge caution in developing general statements. Painted turtles from the MMR displayed cadmium residues and a metallothionein-like protein in liver, kidneys, and gonads. Adverse reproductive effects were indicated by lower gonadosomatic index and sperm number in males, and lower oviduct weight, follicle number, and levels of plasma estradiol and vitellogenin in females from the military reservation than in turtles from a reference site (Rie 2000; Kitana 2005). Kitana (2005) also demonstrated that these reproductive effects could have been caused by Cd in studies with red-eared slider eggs. In these studies, eggs exposed to environmentally relevant concentrations of Cd as $CdCl_2$ yielded Cd-contaminated yolk, a nearly 60% reduction in embryogenital germ cells, and fewer oocytes in the gonads of neonate turtles due to higher apoptosis.

12.4.1.5 Neurological Effects

Metals play a critical role in the functioning of the central nervous system. Many metals are potent neurotoxicants, especially As, Al, Cd, Hg, Mn, and several organometallic compounds (e.g., Moser et al. 2007). Exposure to metal contamination, even at low levels, may have adverse effects ranging from discrete neurological dysfunction to a multitude of ethophysiological changes. This is well established for vertebrates in general but largely underinvestigated in reptiles.

Only a few investigations have dealt directly with the effects of exposure to metals on the nervous system of reptiles. Biczycki (1992a, 1992b, 1992c) studied sand lizards, *Lacerta agilis,* for the effects of inorganic (lead nitrate) and organic lead (lead acetate) compounds on the hypothalamic secretory centers after short-term and prolonged treatments. Histomorphological and histochemical analyses showed degeneration of cell nuclei after prolonged intoxication, and that both compounds induced changes in neuro- and ependymosecretory activities. Brain and plasma cholinesterase activities were unaffected in European wall lizards fed cadmium-contaminated crickets for 21 weeks (Mann et al. 2007).

12.4.1.6 Hepatic, Renal, and Adrenal Effects

In reptilian homeostasis and metabolism, the liver and the kidney-adrenal complex perform critical functions. The liver is responsible for storage and filtration of blood, synthesis of bile, and

the metabolism and storage of elements, carbohydrates, proteins, lipids, vitamins, and other molecules. The adrenal-kidney complex, in turn, is involved in excretion, acid-base balance, osmoregulation, and maintenance of blood pressure. The latter organ in particular is involved in the synthesis and metabolism of hormones, including sexual steroids and stress hormones such as epinephrine, norepinephrine, and glucocorticoids. Finally, and of special importance to this chapter, both organs are involved in the accumulation and detoxification of xenobiotics in general, and of metals in particular (McClellan-Green et al. 2006). Accumulation of several metals such as Cd (which is potentially adrenotoxic and nephrotoxic) and Pb and Hg (which are potentially nephrotoxic; Pottinger 2003; Virgolini et al. 2005) can cause severe damage in these organs, in part by modifying cell membrane structure and function. Beyond accumulation, few studies actually investigated the effects of metal intoxication on form or function in these organs. An exception is the study by Ganser et al. (2003), who observed that 29% of the southern watersnake juveniles experimentally fed trace metal-contaminated prey (especially As and Se, but also Cd, Cu, Sr, and V) for 1 or 2 years had liver cellular and histological pathologies (as opposed to 0% in the controls). The most common pathology was liver fibrosis, caused by proliferation and infiltration of collagen fibers into vascular and other interhepatocyte areas. Torrent et al. (2004) examined an unusually large collection of 78 *Caretta caretta* stranded along the coasts of Gran Canary Islands, Spain. Among these, 3 of the turtles with high As concentrations in the liver had severe hepatic degeneration, and 2 with high Pb concentrations in the kidney had renal lesions. Interestingly, no renal lesions were observed in 7 sea turtles containing Cd levels in kidneys exceeding up to 3 times concentration thresholds associated with renal damage in marine mammals.

12.4.2 Individual Level Effects

Clinical signs of metal intoxication in reptiles are neither specific to metals nor widespread in reptiles. As detailed in the following reptiles exposed to metals may have increased mortality rates, morphological malformations, and changes in growth, development, and behavior. However, to our knowledge, none of these responses, as reported, are specific to metals. Similar responses could be caused by other chemicals or nonchemical stressors. Nor are these responses universal, as effects reported for a given metal or species are not necessarily observed for other metals or species.

12.4.2.1 Severe Toxicosis Including Mortality

There are few reports in the literature that provide obvious signs to aid in a clinical diagnosis of severe toxicosis in reptiles caused by metals. Red-eared slider turtles and snapping turtles in arsenic-contaminated Finfeather Lake in Bryan, Texas were blinded by an anomalous keratinized growth on eyelid and eyeball that extended to the nasal area, nasal passages, and palate (Crearley 1973). Camus et al (1998) were called to investigate the causes of alligator mortality in a Louisiana farm. Alligators displayed anorexia, weight loss, poor growth, lethargy, and death. Autopsies and radiographs of affected alligators revealed that they contained Pb fragments in their stomachs. The authors found that alligators were fed a ground mixture of dry pellet chow and nutria that were hunted with lead shot. Another study (Hammerton et al. 2003) focused on a scenario similar to that observed by Camus et al. (1998) except that outcomes from experiments did not present any effects. In this study, the authors fed estuarine crocodiles a single meal of meat containing lead shot. The lead shot was retained in their stomachs for up to 20 weeks, slowly dissolving, and resulting in very high blood Pb levels that were sustained over several months. Nevertheless, the crocodiles remained in apparent good physical condition and displayed no clinical signs of lead toxicosis.

Experimental work clearly showed that mortality can be a sign of metal intoxication in reptiles. Lead was lethal to *Trachemys scripta* and *Sceloporus occidentalis*, and cadmium to *Sceloporus undulatus* (Salice et al. 2003; Brasfield et al. 2004; Holem et al. 2006). However, several studies failed to detect mortality of reptiles exposed to metals, sometimes even under doses considered high

or very high when compared to birds and mammals (Hopkins et al. 2004 and 2005a for Se; Hopkins et al. 2002 and Nagle et al. 2001 for coal combustion wastes; Hammerton et al. 2003 for Pb; Marco et al. 2004 for As).

12.4.2.2 Development, Growth, Maintenance, and Reproduction

Several publications of the past decade focused on developmental effects linked to exposure to xenobiotics, especially during the sensitive embryonic phase. However, fewer experimental studies analyzed effects to growth, maintenance, and reproduction, which usually require selection of more tractable species, longer-duration studies, and often more complex experimental designs. A series of studies investigating coal ash contamination in water bodies in the Savanna River site provide some of the best documentation on the effects of metals on reptile growth, development, maintenance, and reproduction. Coal combustion generates large quantities of solid waste in the form of coal ash, which is disposed in landfills and settling basins invariably associated with aquatic habitats where turtles, water snakes, and alligators are found. Coal ash is enriched in several potentially toxic trace metals, especially Se and As, but also Cd, Cr, Cu, Sr, and V (Rowe et al. 2002), that could come into contact with reptiles through ingestion of contaminated prey, maternal transfer, or absorption through skin or eggshell. Indeed, several studies documented that alligator eggs and field-caught turtles and snakes had been exposed and were contaminated with these constituents (Nagle et al. 2001; Rowe et al. 2002).

In 1 experiment, Hopkins et al. (2002) fed full-sibling juvenile water snakes, *Nerodia fasciata*, prey from a reference uncontaminated site (control), prey from a coal-ash-contaminated site (high exposure treatment), or prey from alternating reference and contaminated sites (low exposure treatment) for 2 years. Prey from the coal-ash-contaminated site contained As, Cd, Cu, Se, Sr, and V, and all elements but Cu accumulated in snake tissues. The authors observed treatment differences and sex differences in prey consumption rates that had consequences for growth. Males exposed to trace metals had higher consumption rates than unexposed males; females presented a weaker response with a tendency toward higher consumption rates at intermediate exposure levels. There was a slight increase in standard metabolic rates in one of the treatments, but these results were only a weak indication of a treatment effect because these snakes also had higher metabolic rates at test initiation. This finding was contrary to a previous study that indicated that water snakes collected from a coal-ash-contaminated site had 32% higher average standard metabolic rate per unit mass (Hopkins et al. 1999). No treatment-related differences were observed in overwintering weight loss. Surprisingly, as a consequence of increased consumption rates, snakes fed contaminated prey grew significantly more than snakes fed noncontaminated prey.

These studies also found that Se, known to be a teratogen and to be maternally transferred to offspring, accumulated to remarkably high levels in snake tissues. Concentrations of Se in water snakes greatly exceeded concentrations known to induce reproductive failure in birds and fish (Lemly, 1993, 1996). Hopkins et al. (2004) employed the African brown house snake to characterize consequences of chronic Se exposure. African brown house snakes reach reproductive size within 10 months, breed up to 8 times a year, and lay relatively large clutches; hence, the species was more amenable to experimentation of reproductive effects of contaminants than other snakes in the region. Female house snakes were fed Se-contaminated prey (0, 1, 10, and 20 μg of seleno-D,L-methionine per g of prey, dry mass) for 10 months. After the sixth month, Se-exposed females were placed with mature males to copulate. Surprisingly, no treatment effects were observed in survival (all but 1 individual survived), growth, or condition. Selenium, which can harm reproduction even in individuals that appear externally healthy (Lemly 1999), had no significant effects on house snake reproduction, although there were trends in decreased percentage of females breeding, total number of eggs produced, number of eggs per clutch, and clutch mass. Interindividual variability may have obscured adverse effects, given that large quantities of Se were maternally transferred to the eggs. However, hatching rate and hatchling snout-vent length (SVL) were similar across treatments, and

mass was 30% greater in heavily contaminated hatchlings (i.e., from the 20 µg treatment) than in background contaminated hatchlings (i.e., from the 1 µg treatment).

Other studies by the same research group demonstrated that the top predators of a simplified laboratory food chain (the fence lizard) accumulated Se at high doses when fed crickets exposed to contaminated chow. Despite the accumulation, all lizards survived, reached sexual maturity, and grew irrespective of treatment. The only observed difference was in body condition index (BCI = mass/SVL); contaminated males had lower BCI, but females had higher BCI when compared to controls (Hopkins et al. 2005a). Additional studies focused on the effects of coal ash contamination were conducted with turtles. Nagle et al. (2001) collected female *Trachemys scripta* from contaminated and reference sites and induced egg laying. The eggs were then distributed in replicated artificial nests divided in 2 halves, one containing soil from the contaminated site, and the other containing soil from the reference site. Following this design the authors attempted to separate effects of contamination via maternal transfer from effects of contamination via the incubation substrate, considering that both females and substrate from the contaminated site had higher levels of As, Cd, Cr, and Se. Turtle hatchlings were contaminated with all these elements, but substrate type did not influence their concentrations, and only selenium concentrations were significantly higher in hatchlings from contaminated site females than uncontaminated site females. As for effects, eggs incubated in ash substrate had lower survival than eggs incubated in reference site soil. However, this difference was hypothesized to arise from physical rather than chemical characteristics of the substrate. In turn, despite differences in Se concentration, hatching rate, time to hatch, and mass and length at hatching were not affected regardless of their being offspring of mothers collected from contaminated or uncontaminated locations. Hatchlings from ash-contaminated females, however, had significantly decreased metabolic rates than controls. In summary, although teratogenicity associated with Se exposure in birds and fish is well documented (Lemly 2002; Hamilton 2004), most of these studies demonstrated little or no effect on 4 species of reptiles exposed to coal combustion wastes or Se.

Few other studies have considered effects of metal contamination or of metal-contaminated environments on reptile reproduction, development, and growth. Of these endpoints, metal effects on reproduction are poorly characterized in reptiles, which is highly problematic considering its role in population ecology and evolution. For example, observational studies with female painted turtles from the Massachusetts Military Reservation, a site contaiminated with various metals and organic compounds, presented lower plasma estradiol and vitellogenin levels, and fewer numbers of follicles, than turtles from a reference site, yet they did not differ in ovarian weight, oviduct weight, gonadosomatic index, and clutch size, as determined by counting the number of shelled oviductal eggs in spring animals (Rie et al. 2005).

Even if reproduction successfully occurs, metal exposure may still influence individual performance by depressing egg or hatchling viability. For example, eggs of alligators collected in coal-ash-contaminated sites had higher selenium content (2.1 to 7.8 ppm) and lower viability (30% to 54%) than eggs collected at a reference site (1.4 to 2.3 ppm Se content and 67 to 74% viability; Roe et al. 2004). Similarly, eggs of *Clemmys marmorata* collected in a contaminated site in Oregon contained low-level residues of 9 organochlorine pesticides, various PCBs and PCB congeners, and 9 metals, but clutches that failed to produce a single hatchling did not differ in residue loads from those that produced at least one hatchling (Henny et al. 2003). Beyond these observational studies, experimental studies have evaluated effects of metal exposure on reptile eggs and hatchlings. For example, Burger et al. (1998) conducted 2 experiments where hatchling slider turtles were exposed to Pb via a single, intramuscular injection of lead acetate. The first experiment (0, 0.05, 0.1 mg/g lead acetate) failed to detect any growth effects in the 4 weeks or 6 months following injection. At doses of 0.25, 1.0, and 2.5 mg/g (plus control) dose-dependent effects on mortality were detected after 4 months and hatchling length and mass after 6 months. As detailed previously, Brasfield et al. (2004) studied the consequences of Cd-contaminated soil on eggs of Eastern fence lizards and found that cadmium chloride solutions in perlite (1.48, 14.8, 148, 1480, or 14800 µg Cd/g perlite) caused 100%

egg mortality at the 2 highest concentrations, less than 30% at the 2 intermediate concentrations, and 0% at the lowest concentration and in the control, and no size differences among treatments in hatchling size or mass. Similarly, Marco et al. (2004) incubated eggs of Iberian rock lizard in vermiculite containing different concentrations of As (50, 100, 250, and 500 ng/ml in substrate water) and found no effects on time to hatch, hatchling survival, hatchling size, or morphological abnormalities in hatchlings.

Desynchronized early development and growth may influence size at maturity, morphometry, organosomatic indices, and increase the incidence of teratologies and neoplasias. These effects are traditional endpoints in ecotoxicology and each is critical in evaluating effects of metal exposures in reptiles (see Linder and Grillitsch 2000).

12.4.2.3 Behavior and Locomotion

Neurological impairment and endocrine disruption could translate into a variety of behavioral changes that influence individual performance, such as thermoregulation, locomotion, feeding, foraging and predator avoidance, social interactions, mating, nest site selection, and parental care. Food consumption was evaluated in 2 recent laboratory studies with snakes, but only 1 of them detected significant effects. Hopkins et al. (2004) found no differences in feeding rates (i.e., prey refusal rates) of brown house snakes among treatments of 0, 1, 10, and 20 μg of seleno-D,L-methionine per gram of prey, dry mass. In Hopkins et al. (2002) prey consumption rates by water snakes were affected by exposure, and outcomes were sex and dose dependent. Males water snakes exposed to trace metals had higher consumption rates than males whose diet did not include trace metals, whereas females were less responsive with a tendency toward higher consumption rates at intermediate exposure levels. For each sex, the observed differences in feeding behavior resulted in significant differences in growth rates among treatments.

Locomotor performance has been evaluated in some studies. Holem et al. (2006) examined the consequences of acute exposure to Pb (a single oral dose of lead acetate trihydrate at 1, 10, 100, or 1000 mg/kg plus a distilled water vehicle control) to the running speed of juvenile *Sceloporus occidentalis*. No effects of Pb on running speed were observed. In contrast, in studies with As Marco et al. (2004) incubated Iberian rock lizard eggs in substrates containing 50, 100, 250, and 500 ng As/g and observed a significant negative relationship between hatchling running speed and arsenic exposure of eggs, indicating that exposure to metals can impair locomotor performance.

Field studies have also included observations of integrative responses to metal exposure. For example, Heaton-Jones et al. (1997) compared total Hg concentrations in the brain and spinal cord of *Alligator mississippiensis* from various sites in Florida, and alligators from the most contaminated sites studied had significantly higher Hg concentrations than those from less contaminated sites. Surprisingly, no clinical signs of neurotoxicosis were detected in these animals.

The few published studies indicate that exposure to metals has the potential to alter behavior in ways that reduce individual fitness. However, the results are not always straightforward or easily interpreted. In general, the available literature reports a very narrow subset of behavioral traits that could be used as indicators of exposure (see Burger 2006).

12.4.3 Population Level Effects of Metal Exposure

No published study clearly demonstrates population level effects of exposure to metals in reptiles. However, an early observational study provides a basis for anticipating future investigations linked to metal exposures. Crearley (1973), followed by Clark et al. (1998), reported on field investigations at Finfeather Lake, Bryan, Texas, where industrial production of calcium-arsenate- and arsenic-based compounds had occurred. Crearley reported a turtle die-off, with 5 red-eared slider turtles and 1 snapping turtle found dead and blinded by anomalous keratinized growth on eyelid, eyeball, and nasal area; 3 live-captured slider turtles were also blind. All signs were consistent with As contamination in domestic animals; hence, As contamination was presumptively identified as the cause

for these dramatic effects. In 1976 the industry was ordered to drain Finfeather Lake and remove the contaminated sediments; the lake was refilled in 1983. Following remediation, lake-averaged concentrations of As decreased. By 1996 turtles were again observed at Finfeather Lake (Clark et al. 1998). The ecological impacts appeared in other biota inhabiting Finfeather Lake, as there were records of pathomorphologies in fish and impoverishment of fish communities (1976–1991), and the apparent absence of tadpoles and snakes (1994–1996), which were common in other lakes of the region. Tadpoles collected in 1994 contained As, Cr, and Zn at concentrations that might be toxic to their predators (such as turtles), with As concentrations ranging from 4.32 to 9.52 ppm (wet mass) in *Acris crepitans* tadpoles (Clark et al. 1998). However, As concentrations detected in the blood of 2 *Trachemys scripta* from the same region (1994–1995) were relatively low (within 2 times the detection limit, 0.1 ppm), which confounded the characterization of cause-effect relationships.

Few studies in the existing ecotoxicological literature focus on linking detrimental effects of metals on individual traits, such as fecundity and mortality, directly with altered population dynamics. If we restrict our review to reptiles, the available literature linking metals with adverse population level effects is practically nil; published studies reporting demographic data for reptiles are few, and those focused on demographic endpoints linked to metal exposure are at best antecdotal. Demographic data are consistently absent or are not a main objective of the studies, and hence limited in their collection (e.g., a comparison of effort devoted to collect individuals in reference site and contaminated test sites such as that reported by Albers et al. 1986) or subjective (e.g., population considered healthy or stable based on abundance as reported by Rainwater et al. 2005). Considering the extent and magnitude of environmental contamination with metals and the demonstrated effects of metal contamination on subindividual and individual endpoints, data are sufficient to design integrated field and laboratory studies focused on metal contamination and detrimental population level effects to reptiles.

12.4.4 Toxicodynamics Concluding Remarks

Over the past 10 to 15 years there has been an increase in the number of studies analyzing the effects of metal exposure in reptiles. In addition to mortality, there is now evidence that metals can have serious sublethal effects through immunological, genetic, and neurological toxicity, as well as endocrine disruption. Metals can also lead to histological and morphological malformations. Finally, metal exposure can impact individual behavior, growth, and development. Several studies, however, failed to detect lethal effects (e.g., Hopkins et al. 2004 and 2005 for Se; Hopkins et al. 2002 and Nagle et al. 2001 for coal combustion wastes; Hammerton et al. 2003 for Pb; Wolfe et al. 1998 for Hg; Marco et al. 2004 for As), changes in morphology such as body proportions (Hopkins et al. 2004 for Se), sizes at specific stages (hatchling size: Hopkins et al. 2004 for Se; Nagle et al. 2001 for coal combustion wastes; Brasfield et al. 2004 and Kitana 2005 for Cd; Marco et al. 2004 for As), physical condition (Hammerton et al. 2003 for Pb), appearance of progeny (Wolfe et al. 1998 for Hg), and developmental abnormalities (Marco et al. 2004 for As). Finally, not all studies detected behavioral changes, including no responses in prey consumption rate (Hopkins et al. 2004, 2005b) or in running speed (Holem et al. 2006). Indeed, an overview of Tables 12.5 and 12.6 suggests that reptiles may be considerably robust against metal intoxication. Although a simple count of the total number of variables measured in experiments manipulating metals, metal-contaminated substrate, or metal-contaminated food is a subjective and oversimplified evaluation of the overall ecotoxicity of metals to reptiles, it is nevertheless remarkable that 67% ($N = 48$) of all variables measured detected no significant effects of metal exposure. Several of these studies employed experimental designs that included exposures with metal concentrations considered high and known to be severely toxic to birds and mammals (e.g., Wolfe et al. 1998; Hopkins et al. 1999; Hammerton et al. 2003) and long-term exposures that encompassed various life history phases (e.g., 10 months from juvenile to reproductive age in *Lamprophis fuliginosus*, Hopkins et al. 2004; 80 days for the whole egg incubation period for *Trachemys scripta*, Nagle et al. 2001). Of the remaining 33% of the

variables ($N = 24$), only 15 could be considered negative effects (e.g., increased mortality, decreased growth rates, increased incidence of malformations, decreased immune response), whereas 9 are more difficult to interpret (e.g., increased food consumption rates, changes in body proportions, changes in metabolic rates, changes in skin pigmentation). Given the paucity of studies conducted to date, and especially those quantifying multiple endpoints and interactions among stressors, it is too early to state that reptiles, considered sensitive indicators of organic contamination and endocrine disruption, are relatively insensitive to environmental contamination by metals. Clearly, exposure and effects of metals in reptiles remain critical avenues of future research.

12.5 CONCLUSIONS AND FUTURE DIRECTIONS

Reptiles constitute a diverse vertebrate class with a high proportion of species threatened with extinction worldwide. One important threat challenging reptiles is chemical pollution. Metals may be particularly influential as chemical stressors because of their ubiquity, abundance, persistence, and chemical diversity. Reptiles potentially experience very high metal exposures that result from both natural and anthropogenic sources, because these vertebrates often live in close contact with metal-rich substrates (such as soil and dust, sediment, brackish and saline water) and feed on metal-rich diets (which in part reflect their occupying high trophic positions) over long periods of time (because of their relatively long lifespans). Among metals found in high concentrations in critical reptile tissues, there are several metals of priority concern (Figure 12.1) known or suspected to cause a diversity of serious health effects (Table 12.7).

Several of these effects have been reported in the reptilian literature, but overall, reptiles appear to be particularly robust against metal intoxication. Testing the veracity of this statement is one of the most important avenues for future research, because long-term exposure scenarios and interactions among multiple stressors have seldom been manipulated, effects of priority concern have rarely been measured, and critical tissue levels have never been established. If indeed reptiles are robust against metal intoxication, it remains to be seen if mechanisms resulting in relatively high resistance for high-level exposures are also protective of adverse effects potentially linked to long-term, low-level exposures, such as endocrine, neurobehavioral, and transgenerational effects.

TABLE 12.7
Potential Adverse Health Effects Caused by Metals of Priority Concern Detected in Tissues of Reptiles

Health Effects	Al	As	Cd	Cr(VI)	Cu	Hg	Mn	Ni	Pb
Cancer	N	●	●	●	U	U		●	●
Reproductive and developmental disorders	N	●	●	●	N	●		N	●
Endocrine disruption	●	●	●	N	N	●	●	N	●
Immune function disorders		●		●	●	●		●	●
Renal dysfunction		●	●	●	●	●			●
Hepatic dysfunction		●		●	●				
Neurotoxic disorders	●	●				●	●		●

Source: IEH (2005); Nordberg et al. (2007); Kegley et al. 2008. Note that information is only based on the metallic forms of the elements.

Symbols and abbreviations: N, no available weight of the evidence summary assessment; U, unclassifiable.

Despite the considerable increase of ecotoxicologic studies on metals in reptiles during the last decade (Figure 12.2), reptiles are still underrepresented in metal ecotoxicology and risk assessment. Existing information is mainly descriptive, and experimental studies aimed at mechanistic understanding are few to rare in number. Similarly, hypothesis-based, statistically sound study designs, and standardized testing methods are wanting. The development, evaluation, and validation of model reptile species appropriate to both lab and field studies are critical to future research. Experimentation is necessary to improve our understanding of both the toxicokinetics and toxicodynamics of metals in reptiles. Realistic exposure scenarios can be achieved by manipulation of sublethal, long-term exposure of metals, organic metal compounds, and mixtures of metals, or metals with other chemical, physical, and biological stressors. Among the latter, there is a full range of modulators of toxicity for which there is virtually no experimental knowledge with respect to reptiles. If both aspects of reptile ecotoxicology were investigated through the same or similar experiments, then we would greatly strengthen our ability to make mechanistic linkages between toxicokinetics and toxicodynamics.

There is much to be learned in toxicodynamics. Expanding our knowledge of effects of priority concern, including endocrine, developmental and reproductive, neurobehavioral, and transgenerational effects, is critical to characterizing reptile-metal interrelationships. Given the concern of declining reptile populations (Gibbons et al. 2000), endpoints that can be directly translated to population dynamics, and therefore of immediate use for conservation biology, should be given priority. These include information that is basic for the construction of population projection matrices such as reproductive output, egg viability, time to maturation, and stage and probabilities of age-based mortality given exposure to pollutants.

Monitoring of reptile contamination in the field is an integral part of ecotoxicological research. Unfortunately, the literature indicates that we lack standardization in field sampling, tissue choice, tissue preparation, tissue analysis, and data reporting. Likewise, monitoring studies are frequently plagued by inappropriate sampling protocols and inadequate sample designs (e.g., sample sizes). Experimentation should be conducted to harmonize protocols and validate the use of alternative tissues in biomonitoring through nonlethal sampling, as these tools may be increasingly required in study designs focused on threatened or endangered species. Collectively, these issues should be addressed by the development of harmonized guidance that allows for comparability among results developed by a wide range of users.

Our objectives for this chapter focused on establishing common ground for reptilian ecotoxicology, conservation biology, conservation medicine, and ecological risk assessment with respect to environmental contamination with metals. Our approach was to comprehensively review available literature, organize and condense these quantitative and qualitative data, synthesize the available information (particularly that regarding general patterns and trends in the toxicokinetics and toxicodynamics of metals in reptiles), and critically evaluate the information to identify research needs. Progress in the area of the ecotoxicology of metals in reptiles has occurred since the first edition of this book, but clearly much remains to be done.

One of the greatest challenges we now face is to link exposure in the field, tissue residue levels, and adverse effects at the subindividual, individual, and population levels, accounting for the range of reptile species and field settings critical to their long-term sustainability. To accomplish this task, future field and laboratory studies should focus on linking observational with experimental and predictive ecotoxicology through integrating mechanistic physiological and ecological approaches.

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13 Solar UV Radiation and Amphibians
Factors Mitigating Injury

Edward E. Little and Robin D. Calfee

CONTENTS

As a result of stratospheric ozone depletion, concerns about increasing levels of solar ultraviolet (UV) radiation gave impetus for research to determine the impact of UV exposure to amphibians. Worldwide observations of declining amphibian populations were considered, in part, as outcomes potentially linked to changes in solar irradiance that have occurred over large landscapes during the past 25 to 30 years (Blaustein et al. 1994a; Carey 1994). Ozone concentrated in the stratosphere partially absorbs and ultimately governs the amount of solar ultraviolet B (UV-B) over the wavelength range of 290 to 320 nm radiation penetrating to the earth's surface. Ozone has little effect on the ultraviolet A (UV-A) wavelength range (320 to 400 nm) and visible (400 to 800 nm) radiation. The amount of UV radiation reaching the earth's surface is strongly influenced by diurnal, seasonal, and latitudinal variations in solar angle, which determines the thickness of ozone column traversed by UV radiation. Stratospheric contamination by chloroflurocarbons (Rowland 1982), brominated hydrocarbons (Wuebbles et al. 1999), and contamination associated with volcanic eruptions (Sigurdsson 1990) has resulted in ozone depletion evident as reduced ozone concentrations and concomitant increases in UV-B, especially in early spring over large areas of Antarctica and to a lesser extent over Arctic regions (Blumthaler 2003). Sulfer dioxide, nitrogen dioxide, and other greenhouse gases absorb UV light in the upper atmosphere. The intensity of UV penetrating the lower atmosphere is also influenced by cloud cover, aerosols, and altitude (Herman et al. 1996). In northern temperate latitudes, average surface exposure to biologically damaging UV-B radiation has increased by about 3 and 6% in south temperate latitudes since 1980 (McKenzie et al. 2006), with the greatest increases occurring in the spring when amphibians breed. Transient changes in

UV-B in excess of 20% have been observed, although such changes occur over brief durations (1 to 3 days) over limited areas (Siani et al. 2002; Iwao and Hirooka 2006).

Amphibians vary in their tolerance to UV exposure, which is influenced by the interplay between the ecological niche occupied by the organism and its UV sensitivity. Throughout their various developmental life stages, amphibian habitats and habits will likely complement the organism's tolerance of UV. Early laboratory studies demonstrated the sensitivity of early life stages of amphibians to UV and indicated species differences in sensitivity (Crump et al. 1999). Early studies established that UV exposure could result in reduced hatching success (Blaustein et al. 1994a, 1997; Ovaska et al. 1997; Langhelle et al. 1999). Significant mortality occurred among embryos of *Bufo americanus*, *Hyla versicolor*, *Rana catesbeiana*, *Rana clamitans*, and *Rana pipiens* exposed in the laboratory to elevated UV irradiance (Grant and Licht 1995). There were also differences in the sensitivity of different life stages of the same species, with larval amphibians generally found to be more sensitive to UV than embryos (Crump et al. 1999; Hofer and Mokri 2000). Tests with tiger salamanders (*Ambystoma tigrinum*) indicated that embryos were considerably more sensitive than larvae (Little 2005). Laboratory exposures conducted at environmentally realistic UV irradiance intensities from early embryos to metamorphosis demonstrated that some species, such as the boreal toad (*Bufo boreas*), tolerate UV at irradiance levels exceeding that of their habitats (Little et al. 2003a). In contrast, other species were highly sensitive to UV exposure and suffered injury when exposed at the upper range of UV irradiance measured in their habitats. *Bufo boreas* larvae were tolerant of laboratory exposures at 372 Joules/cm^2 as UV-B, *Bufo woodhousii* were more sensitive at 190 Joules/cm^2 (Little et al. 2003a), *Hyla versicolor* at 1.4 Joules/cm^2, and *Xenopus laevis* at 0.8 Joules/cm^2 (Zaga et al. 1998), and tiger salamanders were highly sensitive at 0.34 Joules/cm^2 (Little 2005).

The range of UV-induced injuries reflecting the severity of exposure has also been described in the literature. The severity of injuries ranged from sunburn and lesions, to sublethal effects on development and reproduction, to the ultimate effect, mortality.

Exposure to natural sunlight was lethal to *Rana pipiens* and *Rana septentrionalis* embryos and larvae in the absence of shading structure or natural waters (Peterson et al. 2002; Tietge et al. 2001). Solar radiation was implicated as the cause of impaired embryonic development of *Hyla cadaverina* during in situ solar exposures in the southern Sierra Nevada (Anzalone et al. 1998). *Bufo boreas* eggs were highly tolerant of exposure to natural solar radiation during in situ studies in northern Colorado (Corn 1998). Similar studies in the Cascade Mountains showed that hatching success of eggs from various indigenous amphibian populations, including *Bufo boreas,* was impaired under ambient solar conditions, and was significantly enhanced when ambient solar radiation was blocked with filters (Blaustein et al. 1998). Natural sunlight was also lethal to larval *Bufo boreas* (Kiesecker et al. 2001). These investigations suggest that different populations of *Bufo boreas* may differ in sensitivity. However, most studies were hampered by limited means with which to measure UV, making it difficult to estimate dose and intensity, and often the study designs could not differentiate population differences in UV sensitivity from unknown experimental factors that might have been at play.

Injuries from UV exposure in laboratory and field studies are often initially evident as sunburn or erythema (Figure 13.1), progressing to lesions and edema (Calfee et al. 2006). Fungal infections are commonly associated with these injuries and usually result in death of the organism. For example, aquatic tiger salamanders developed lesions and subsequently fungal infections over the dorsal skin areas within 7 days of exposure to simulated UV irradiances as low as 2 μW/cm^2 (Little 2005). Tiger salamanders are in decline or are disappearing, frequently in localized die-offs, in areas where they were formerly abundant (Jancovich et al. 1997). Similar injuries were observed in spotted salamander (*Ambystoma maculatum*) larvae exposed to UV in the lab (Calfee et al. 2006). Cellular damage was found in the epidermis of surviving alpine newt (*Triturus alpestris*) larvae exposed to both simulated and solar UV radiation (Nagl and Hofer 1997). Injury to skin and eye lens

FIGURE 13.1 Sunburn and lesions on a juvenile *Ambystoma tigrinum* exposed to low-level irradiance (0.91 μW/cm^2 as UV-B) in the laboratory. (Taken from Little and Fabacher 2003. Reproduced by permission of the European Society of Photobiology).

opacities developed in tadpoles of 2 frog species, *Hyla regilla* and *Rana aurora*, exposed to ambient and simulated UV radiation (Flamarique et al. 2000). In addition to mortality, UV exposure resulted in morphological deformities particularly under high irradiance conditions (Blaustein et al. 1997). Commonly, these included spinal curvature, a kinking or clubbing of tail fins, and a stunted body trunk development (Worrest and Kimeldorf 1976). Bilateral limb deformities were also induced by exposure of *Rana pipiens* to low-intensity UV from the embryo stage through metamorphosis (Ankley et al. 2000).

Reduced larval growth has been consistently reported to result from UV exposure often at irradiance levels found in the organism's natural habitats. The response to UV has energetic costs associated with cellular repair and may cause reduced mass, body length, and head length in tiger salamander larvae after exposure to UV (Table 13.1). Pahkala et al. (2003) found that in addition to increased developmental anomalies, *Rana temporaria* larvae exposed to enhanced UV radiation as embryos metamorphosed at a smaller size, indicating there may be carryover effects from the exposure at the earlier life stage. Reduced growth has also been shown in other species (Nagl and Hofer 1997; Zaga et al. 1998; Calfee et al. 2006), and is known to impact reproductive success and fitness (Semlitsch et al. 1988).

Behavioral changes also result from UV exposure. Behavioral activity levels were reduced in larval *Xenopus laevis* and *Hyla versicolor* during UV exposure. Both species need to swim in order to feed on suspended algae (Seale 1982; Lawler 1989). Decreased activity can affect the tadpole's ability to acquire enough food to develop to metamorphosis or to evade predation (Lawler 1989). In some species, exposure to UV eliminated responsiveness to predator odors, which could ultimately lead to increased mortality rates (Kats et al. 2001). Increased tadpole survival is related to rapid growth and larger body size, which are correlated with food acquisition (Morin 1987); thus, behavioral impairments such as decreased activity can influence population demographics (Little 2002).

The laboratory and field studies have effectively demonstrated the potential hazards of UV exposure to developing amphibians and have determined that a range of injuries can be induced by such exposures. A common limitation of such studies has been that the exposure design deprived the organisms of shade from passing clouds, vegetation, canopy or cover, or UV-absorbing dissolved organic matter. In the absence of these mitigating variables, exposure and associated effects of UV would likely be overestimated.

TABLE 13.1
Mean Wet Weight, Total Body Length (SD) for *Ambystoma tigrinum* Larvae from A) a 2553 M elevation pond (Mud Lake) and B) a 1583 M elevation pond (Limon Pond) Exposed to 2 Simulated Solar UV–B Intensities for 28 Days (Taken from Little and Farbacher 2003)

A) Mud Lake Larvae

Growth Parameter	0.002 μW/cm²	0.91 μW/cm²
Wet weight (g)	0.0482	0.0373[a]
	(0.0049)	(0.0044)
Total body length (cm)	1.6481	1.4550[a]
	(0.1879)	(0.0887)
Head length (cm)	0.4038	0.3150[a]
	(0.0647)	(0.0328)

Source: Little (2005).
[a] Denotes significance from the reference UV-B treatment.

B) Limon Pond Larvae

Growth Parameter	0.002 μW/cm²	0.91 μW/cm²
Total body length (cm)	1.4820	1.2444[a]
	(0.1346)	(0.1937)
Head length (cm)	0.3374	0.2233[a]
	(0.1014)	(0.0667)

Source: Little (2005).
[a] Denotes significance from the reference UV-B treatment.

13.1 MITIGATING FACTORS IN UV EXPOSURE

A context for understanding the vulnerability of amphibians to solar radiation must consider the layers of protection that mitigate injury from UV exposure, including 1) atmospheric filtering by ozone, cloud cover, aerosols, and dust; 2) habitat filtering from shading provided by vegetation and substrate, turbidity, and dissolved organic carbon; and 3) organismal defenses, including protective pigmentation, and physiological photorepair processes and adaptive behavioral responses, such as diurnal or seasonal activity patterns, and spatial selection. Injuries induced by exposure to UV radiation are dose-dependent. A number of factors can influence dose, including intensity of exposure, spectral composition of the irradiance, and duration of exposure. Each of these may be influenced by climate and habitat.

13.1.1 Climate Conditions

Stratospheric ozone depletion has been the focus of many investigations concerning UV impacts. Depletion of ozone concentration in the stratosphere reduces the filtering capacity of the ozone layer, resulting in increased UV irradiance reaching the earth's surface. Ozone depletion has been most severe over polar regions in early spring. In temperate regions, ozone is reduced as it mixes with the depleted polar air masses. On average, ozone depletion of about 3% has occurred in the past decade over the United States, but has been considerably greater in the southern hemisphere (Herman et al. 1999; Middleton et al. 2001). In addition, brief periods (1 to 3 days) of elevated UV can occur over localized regions as a result of irregularities in atmospheric ozone concentrations

TABLE 13.2a
Albedo of Soil Covers

Soil	Albedo %
Black earth, dry	14
Black earth, moist	8
Grey earth, dry	25–30
Grey earth, moist	10–12
Ploughed field, moist	14
White sand	24–40
River sand	43
Light clay earth (leveled)	30–31

Source: Muneer et al. (2004).

(Siani et al. 2002; Iwao and Hirooka 2006). Tropospheric accumulation of ozone diminishes UV, but can be highly toxic as an air pollutant. Gases such as SO_2, NO_2, and methane can also absorb UV-B in the upper atmosphere (Madronich 1993).

The amount of UV reaching the earth's surface also is strongly influenced by variables such as clouds, aerosols, and reflective or absorbing (albedo) features. Clouds strongly influence UV irradiance depending on the size, depth, shape, altitude, and composition, especially when the solar disk is covered by clouds (Blumthaler 2003). Under partially cloudy conditions, UV radiation can be heightened to a considerable degree over clear sky irradiance as a result of reflection from cloud surfaces (Lovengreen et al. 2005).

UV is variously reflected by water, land, and vegetation, and these variables can play a major role in reflecting and amplifying UV radiation as well as absorbing UV. Albedo or the ratio of reflected to incident radiation can be influenced by snow and ice, dust storms, or changes in vegetation (Table 13.2; Madronich 1993). Fresh snow cover increased UV at 320 nm by about 30% compared with snow-free conditions, and under cloudy conditions the albedo effect was magnified through multiple reflections between snow and clouds (Blumthaler 2003). Changes in albedo are anticipated to result from climate change.

Changing weather patterns can also influence UV dose. For example, the number of sunny days in Central America has increased significantly over the past decade (Middleton et al. 2001). Such

TABLE 13.2b
Albedo of Vegetative Covers

Class of Vegetation	Species at Maximum Ground Cover	Albedo %
Farm crops	Grass	24
	Wheat	26
	Tomato	23
	Pasture	25
Natural vegetation and forests	Heather	14
	Bracken	24
	Deciduous woodland	18
	Coniferous woodland	16

Source: Muneer et al. (2004).

TABLE 13.2c
Albedo of Natural Surfaces

Surface	Albedo %
Fresh snow cover	75–95
Old snow cover	40–70
Rock	12–15
Densely built-up areas	15–25
High dense grass	18–20
Sea ice	36–50
Water surfaces, sea	3–10
Lawn: High sun, clear sky	23
Lawn: High sun, partly cloudy	23
Lawn: Low sun, clear sky	25
Lawn: Overcast day	23
Dead Leaves	30

Source: Muneer et al. (2004).

changes in weather patterns significantly correlated with the loss of amphibian species. Aside from changes associated with solar angle, clear sky irradiance should not vary greatly from day to day, but the cumulative UV dose under clear skies is considerably larger than that occurring under cloudy conditions. Increased surface temperatures, possibly as a result of global warming trends, could lead to the stratification of the water column in lakes. In turn, increased thermal stratification could result in increased water clarity and UV irradiance of the upper water column, where a majority of juvenile forms of aquatic organisms occur (Zepp et al. 2007).

Aerosols and trace gases can also influence UV irradiance at the earth's surface by absorbing or scattering UV. Aerosols associated with air pollutants such as soot, NO_2, methane, and ozone effectively absorb UV, particularly near urban areas. Others, such as mineral dust, scatter UV and actually increase diffuse UV radiation even in locations shaded from sunlight (Blumthaler 2003).

13.1.2 Habitat Characteristics

Degradation and destruction of aquatic habitats often include the removal of trees, rocks, aquatic vegetation, and other structures that protect aquatic organisms from solar radiation. There can be greater solar impact in the water column in these habitats and greater potential of increased exposure of aquatic organisms to solar UV-B compared to undisturbed habitats. It is important that aquatic organisms have shade that protects them from much of the solar radiation spectrum.

As with other aquatic organisms, amphibians have adapted to certain levels of sunlight and exhibit different levels of tolerance to UV-B. Species inhabiting shallow, clear water habitats that are fully exposed to sunlight should be more tolerant to high levels of UV-B than species using more shaded habitats. The presence of shade-providing structures, including vegetation in riparian and littoral zones, is important. Floating and submerged aquatic vegetation also provide protection from solar UV-B. Irrigation or other water diversions reduce water volumes in aquatic habitats, and channelization results in erosion of stream banks. Consequent excess deposition in side channels could reduce water depth, resulting in heightened UV exposure. Aquatic organisms could also be exposed to increases in UV-B radiation with declining turbidity. In general, any activity that increases water clarity, such as the release of reservoir water or the presence of introduced zebra mussels (*Dreissena polymorpha*), could increase exposure of freshwater aquatic organisms to potentially harmful levels of solar UV-B radiation.

Water quality characteristics of the habitat can also affect the UV-B dose received by aquatic organisms. Dissolved organic carbon (DOC), especially the yellow-colored chromophoric dissolved

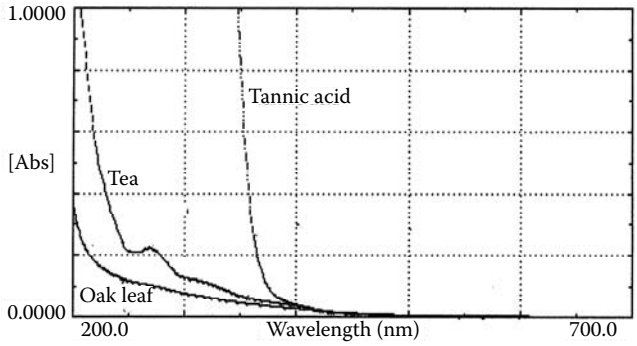

FIGURE 13.2 Absorbance scans for tannic acid, tea, and water from oak leaves soaked for 2 months.

organic material (CDOM), plays a major role in limiting UV-B in the water column and has been extensively investigated in recent years (Skully and Lean 1994; Schindler et al. 1996; Lean 1997). Dissolved organic material (DOM) includes a range of substances that are generated during the degradation of organic material that ultimately yields CO_2 and mineralized carbon. DOC originates from diverse sources, especially terrestrial vegetation; hence, the chemical composition of DOC may vary considerably among watersheds and reflect the unique vegetation and soil chemistry of the site (Diamond et al. 2002). As a consequence, it is likely that its UV filtering characteristics will vary as well (Figure 13.2). DOM can significantly limit UV in aquatic systems (Skully and Lean 1994; Lean 1997). Moreover, UV irradiance in the aquatic habitat is considerably more dynamic than at the earth's terrestrial surface, and can vary by orders of magnitude depending on DOC concentrations. For example, investigations in Minnesota have shown that slight reductions in DOC resulted in dramatic increases in water column UV-B irradiance (Figure 13.3). DOC is probably the single most significant indicator of habitat sensitivity to solar radiation. Because slight changes in DOC concentration in the water column influence exposure to UV by orders of magnitude, the impact that reduced DOC has on biological effects far outweighs those projected by the 5% to 10% increases in solar irradiance from ozone depletion. DOCs such as humic acids are broken down by environmental acidification (Schindler et al. 1996); thus, acid deposition is of concern for amphibian habitats, not only for the direct impacts of reduced pH to the organism, but also for potentially harmful increases in water column UV that may result. Humic acids are also degraded by UV radiation, which are particularly apparent in the bleaching of the yellow chromophoric dissolved organic matter, as UV cleaves chemical bonds and reconfigures isomers or photolytically converts CDOM polymers into

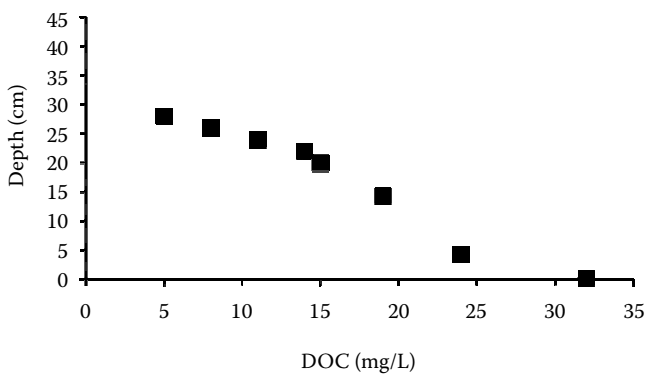

FIGURE 13.3 Decreases in dissolved organic carbon increases UV-B exposure in the water column in sites in Minnesota.

smaller units (Brinkmann et al. 2003; Osborn and Morris 2003). As a result, the protection provided by DOC may not persist under constant sunny conditions as the UV-absorbing properties of CDOM are reduced (Williamson et al. 1999). Because terrestrial sources of DOC are critical to aquatic systems, reductions in transport during drought can limit DOC levels and increase water column UV. Moreover, climate change could affect UV exposure to amphibians in their aquatic habitats, since changes in plant communities adjacent to aquatic habitats may lead to different chemical constituents comprising DOC, and influence the transport of organic material to these aquatic habitats (e.g., enhanced stream bank filtration of DOM entering from the surrounding watershed).

13.2 PHOTOPROTECTIVE DEFENSES OF THE ORGANISM

Protection of freshwater aquatic organisms from UV-induced injury is dependent on a variety of factors that can function as photoprotective mechanisms, including adaptive behavioral and physiological characteristics. When UV radiation breaches photoprotective mechanisms sufficiently, UV-induced injury will occur. Yet amphibians that exploit surface or shallow water habitats probably evolved adaptations to tolerate the high UV exposure in such habitats (Williamson 1996). These organisms likely exploit habitats that are at the limits of their UV tolerance, and such tolerance may change during development (Palen et al. 2002). For example, *Bufo boreas* embryos develop near the surface in open, shallow, clear water in high-altitude environments, and their larvae inhabit unshaded clear water habitats, often breaking the surface of the water as they swim at the surface (Little 2005). *Bufo woodhousii* inhabit lower-altitude habitats, which tend to have periodic turbidity and elevated DOC concentrations. Their egg masses are deposited in the water column where the embryos develop. Larvae of this species exist in clear water habitats, but tend to remain in the water column in contrast to the surface habitat of *Bufo boreas*. *Ambystoma tigrinum* exists over a range of altitudes in habitats from clear water to turbid conditions. UV is often minimal in these habitats because of the presence of DOC (Table 13.3). *A. tigrinum* eggs are deposited on the bottom of these habitats, often in vegetation, and it is here that the embryos and larvae develop.

13.2.1 Behavioral Mechanisms

Selection of habitats suitable for survival and reproductive success requires that organisms seek beneficial habitats and avoid less favorable environmental conditions, balancing resource exploitation against risks of predation. Many species live at their limits of UV tolerance, such that changes in environmental quality such as water clarity, or shifts in seasonal irradiance, could pose hazards to survival and reproductive success (Damkaer 1982). Several studies have shown that numerous species probably already exist at their limits of tolerance for UV-B (Hunter et al. 1982; Little and Fabacher 1994; Nagl and Hofer 1997). Evolutionarily, UV-B may have been a limiting factor in the distribution and abundance of organisms (Damkaer 1982). Nocturnal or crepuscular activity regimes would clearly limit UV exposure, as would selection of UV-limiting habitats. *Rana pipiens* intermittently surface, then shelter among floating vegetation (personal observation). The roiling movements of the larval mass *Bufo boreas* could provide a degree of shade as individuals are displaced from direct exposure to sunlight at the water surface. Similarly, during exposure of *Bufo boreas* toadlets to UV in the laboratory, individuals were often found on the vertical surface of the exposure chamber oriented in a head-up position that may have served to minimize body surface exposure to UV (Little and Calfee personal observations). In the field, adult *Bufo boreas* are often found in full sun, frequently in aggregations (clumps) that undoubtedly reduce UV exposure, as individuals change position within the aggregation.

Many aquatic organisms are behaviorally responsive to UV radiation, showing both attraction and avoidance usually in a manner that is consistent with their sensitivity to UV (Leech and Johnson

TABLE 13.3
UV Irradiance Levels (μW/cm²) and Corresponding Amphibian Habitats Listed by Species and Altitude

Site	Limon	Magnolia	Mud Lake	Porcupine	Mexican Cut	Collegiate Peaks	4-Mile Creek	Hartenstein	Riverside
Species[a]	AT	AT	AT	AT	AT	BB	BB	BB	BW
Elevation (meters)	1583	2560	2553	3507	3416	2987	3400	3475	1368
Longitude/latitude	107E 30'W 39E 34' N	105E 31'W 39E 56' N	105E 30'W 39E 58' N	105E 25'W 39E 19' N	107E03'W 39E 01' N	106E 19'W 38E 48' N	n/a	106E 21'W 38E 49' N	104E 14'W 40E 19' N
DOC (mg/L)	15.0	10.7	9.48	3.73	1.56	1.7	n/a	1.2	6.1
Surface UV-B	310	333	340	376	372	361	383	382	317
Surface UV-A	5119	4450	5649	5944	6122	5492	6425	6256	5309
Subsurface UV-B	90	11	98.6	127	155	149	132	166	66.4
Subsurface UV-A	3260	728	1992	2503	2839	2725	2513	2881	1664
10 cm UV-B	13	0.03	0.42	3.7	72	38	8	4	0.03
10 cm UV-A	159	124	1371	515	1785	1253	622	451	228

Source: Little et al. (2003a).

[a] AT = *Ambystoma tigrinum*, BB = *Bufo boreas*, BW = *Bufo woodhousii*.

2003). These organisms possess photoreceptor pigments that have absorption maxima in the mid to upper UV-A range, or are physiologically or behaviorally responsive to UV stimuli within this range. The extent of behavioral responsiveness toward the lower UV-B wavelengths is unknown but suggested on the basis of diurnal movements under UV-B-shielded conditions. For example, *Litoria aurea* and *Litoria peronii* tadpoles spent significantly more time under UV-B-blocking filters than under UV-transparent filters (Van de Mortel and Buttemer 1998). Strawberry (*Dendrobates pumilio*) and green (*D. auratus*) poison dart frogs of Costa Rica had similar preferences for test chamber positions that were covered by UV-absorbing Mylar filters, avoiding those covered by UV-transparent acetate (Han et al. 2007). However, the organisms could have responded to optical qualities of the treatment filters unrelated to UV-B. Pahkala et al. (2003) found no evidence for UV-B avoidance by larval *Rana arvalis*, *R. temporaria*, and *Bufo bufo*, but did determine that they preferred to remain under different filter treatments regardless of the presence or absence of UV, possibly because of preference for the slightly lower temperature beneath Mylar filters than under other filters. Temperature selection dominated spatial selection by larval *B. boreas*, *P. regilla,* and *R. cascadia* independent of high UV irradiance levels (Bancroft et al. 2008a). These organisms sought warmer water temperatures, which favor more rapid development in spite of high UV irradiance levels. In the absence of other environmental cues, there was no evidence of UV avoidance in these species. Other studies suggest that amphibians and fish indirectly avoid UV-B by seeking positions lower in the water column or by seeking shade to avoid intense visible or UV-A radiation (Hawryshyn 1992). Since solar visible and UV-A radiation would not increase with ozone depletion, higher levels of solar UV-B would go undetected by the organism and cause harmful effects (Williamson 1995).

Another important behavioral adaptation to UV is seeking UV-limited conditions for rearing their young. An example of this behavior is the wrapping of eggs in vegetation by the marbled newt, *Triturus marmoratus*. Possibly, this protects the developing embryos from predators, but it also effectively reduces their UV exposure. Unwrapped embryos quickly succumbed to UV exposure (Marco et al. 2001). The northwestern salamander, *Ambystoma gracile*, is very sensitive to UV, modifying egg-laying behavior by increasing oviposition depth with increasing water transparency to UV-B (Palen et al. 2002). The average oviposition depth for this species in Mt. Rainier National Park lakes was 1 m or greater, a depth at which most, if not all, UV-B would be attenuated by DOC in these lakes. The availability of structure and vegetation was probably also important for oviposition site selection (Figure 13.4).

13.2.2 Photoprotective Substances in Aquatic Organisms

Physiologically, UV causes injury by directly altering DNA and changing the structure of proteins and lipids in membranes. UV also generates highly reactive free radical oxygen species in water and tissue, such as hydroxyl radical (OH^-), singlet oxygen ($^1O_2$), superoxide radical (O_2^-), and hydrogen peroxide (H_2O_2), that react with proteins, lipids, and DNA, causing oxidative stress (Kieber et al. 2003). Countering such insults are photoprotective substances, including pigments, that absorb UV or sequester the highly reactive oxygen free radicals or other reactive species that are generated by UV and result in DNA damage and other cellular injuries (Krinsky 1979). UV-shielding pigments can be extrinsic environmental substances, or they can be synthesized by the organism.

There are several types of photoprotective substances found in aquatic organisms. General characteristics of pigments include resonating π-electron systems of conjugated bond structures that occur in alternating single and double bonds in linear and aromatic or cyclic molecules, and in aromatic and cyclic compounds containing electron resonance. The overlapping orbits of π-electrons have absorption maxima in the UV region that cause an energetic transition from the highest occupied bonding π-orbitals (HOMO) to the lowest unoccupied antibonding π-orbitals (LUMO). The energy difference between ground and excited state, termed the HOMO-LUMO gap, determines the wavelengths absorbed. When visible wavelengths are absorbed, as with body

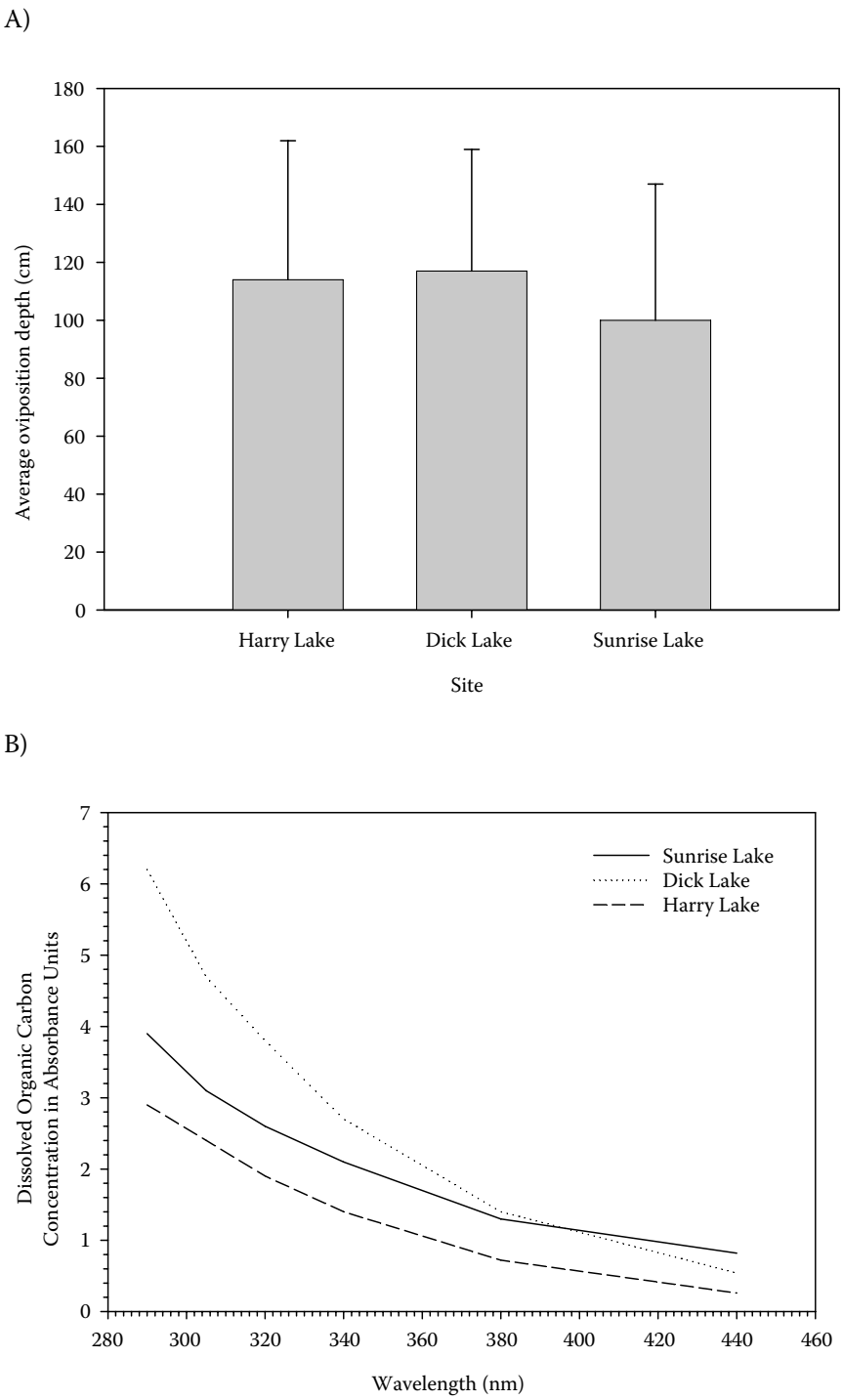

FIGURE 13.4 A) Average oviposition depth for *Ambystoma gracile* egg masses at 3 sites within Mt. Rainier National Park, Washington. Vertical lines represent standard deviation. B) Dissolved organic carbon concentration of water from 3 sites within Mt. Rainier National Park, Washington.

coloration pigments, the reflected wavelengths will be perceived as color. When UV energy is absorbed, the photon energy generated by the reaction is then directly transferred to a biomolecule, such as melanin, or it can be transferred to oxygen, creating singlet or triplet excited states (Cockell and Knowland 1999). Similar reactions occur when contaminants such as polycyclic aromatic hydrocarbons (PAHs) absorb UV radiation and result in enhanced toxicity (Diamond 2003). The absorbance characteristics and the irradiance spectra attenuated by the substance will vary with the structure of the conjugated molecule. Thus, over the course of evolution, changes in the molecular structure of photoprotective substances have developed for specific absorbance characteristics. An organism can screen a broad spectrum of UV-B and UV-A wavelengths by synthesizing a range of photon-absorbing molecules.

UV-absorbing molecules can be synthesized by the organism, or they can be absorbed or accumulated from the food chain. A colorless methanol-extractable substance in fish skin had a peak absorbance in the UV-B wavelength range (Fabacher and Little 1998). The concentration of this substance was indicative of UV tolerance in fish. The photoprotective substance is secreted by the cells of the epidermis and concentrated in the overlying mucus. However, it has not been determined whether this substance is accumulated through diet or induced by ambient UV-B radiation, or whether nature has selected for tolerant individuals with large amounts of this substance.

Mycosporine-like amino acids, another type of photoprotective substance, have been found in a diversity of organisms ranging from bacteria to fish (Cockell and Knowland 1999). The 19 kinds of mycosporine-like amino acids identified have maximum absorbance ranges from 309 to 360 nm. Certain organisms contain several of these substances that broadly screen UV (Karentz et al. 1991). The concentrations of these substances increase proportionately with the intensity of UV irradiance to which they are exposed (Karentz et al. 1991; Gleason 1993; Shick et al. 1995). Mycosporine-like amino acids are probably not synthesized by invertebrates, fish, or amphibians, but acquired through the diet, especially from grazing on algae (Shick et al. 1994). Gadusol, also believed to be photoprotective, is structurally related to mycosporine-like amino acids and is found in the eggs of cod and Mediterranean fish (Grant and Plack 1980) and in brine shrimp (Grant et al. 1985).

Photoprotective melanins are found in a diversity of vertebrate and invertebrate organisms and are polymers formed from 5,6-dihydroxyindole, a phenolic and indolic compound (Kollias et al. 1991). Melanins are complex molecules that broadly absorb UV and visible radiation (Figure 13.5), but show no specific absorption maximum. However, their absorption increases with decreasing wavelength (Crippa et al. 1978; Menon et al. 1983). In addition to photoprotection, melanin and melanin precursors can provide protective antioxidant activity by substantially reducing the formation of lipid peroxidation products (Schmitz et al. 1995). Melanin is produced in melanophores that then deposit melanin on subcellular organelles called melanosomes, which are often positioned above the nucleus (Gilchrest et al. 1996). Exposure to UV can cause an increase in melanin production and the number of melanosomes during long-term exposures to UV (Cockell and Knowland 1999). In frogs (Figure 13.6), melanin occurs in the epidermis, where photoprotection appears to be related to the amount of melanin and its distribution (Little et al. 2003a). UV-B-tolerant boreal toads have a distinct double layer of melanin. Related Woodhouse's toads (*Bufo woodhousii*) that live in lower-altitude habitats also have a double melanin layer, but the melanin appears to be diffuse and less concentrated. Nocturnal grey tree frogs (*Hyla versicolor*) have a single layer of melanin and are sensitive to UV-B. Tiger salamanders have a diffuse and limited distribution of melanocytes and are highly sensitive to UV-B (Little 2005). The concentration of melanin in Patagonian amphibians was correlated with the photic levels of their habitats and was inversely correlated with the absorbance properties of their egg jellies. Also, the degree of melanization of *Pleurodema bufonium* decreased during development from 14 mg/g tissue to 1.5 to 4 mg/g tissue in larvae (Perotti and Diéguez 2006).

The photic environment may be important for the development of adaptive mechanisms for UV tolerance in early life stage amphibians. For example, boreal toad tadpoles previously exposed to

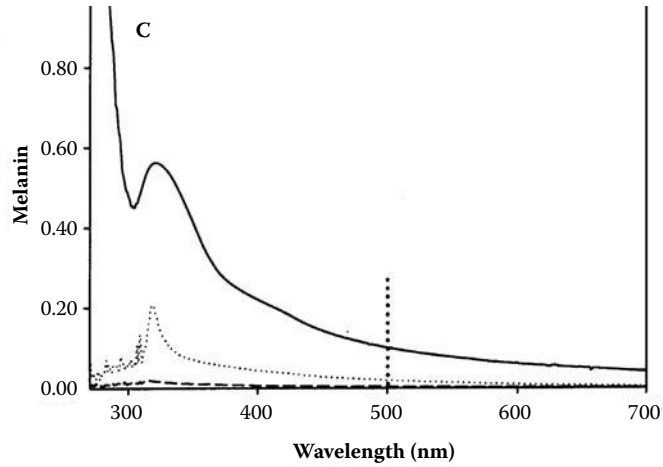

FIGURE 13.5 Absorption spectrum of melanin. (Reprinted from Perotti MG, Diéguez Mdel C. 2006. Effect of UV-B exposure on eggs and embryos of patagoniamn anurans and evidence of photoprotection. Chemosphere 65:2063–2070. With permission from Elsevier.)

natural sunlight prior to laboratory exposures were more tolerant of UV-B than those held in the laboratory prior to exposure, whereas tadpoles cultured under limited laboratory lighting conditions during early development developed deformities during UV exposures. This suggests that culture conditions can affect UV tolerance, a factor that would be important to consider when interpreting laboratory results (Little 2005; Figure 13.6). Larval newts (*Taricha granulosa*) and salmanders (*Ambystoma gracile*; Langhelle et al. 1999) and grey tree frog larvae (Zaga et al. 1998)

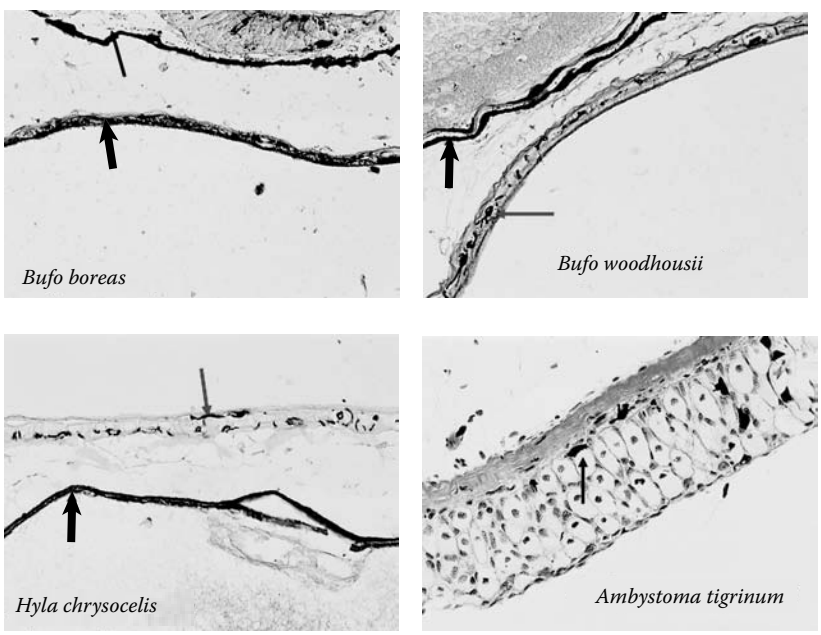

FIGURE 13.6 Skin melanization of *Bufo boreas*, *Bufo woodhousii*, *Hyla chrysocelis*, and *Ambystoma tigrinum* showing dense melanin layers beneath the skin with variable amounts in the epidermal cells. Thick arrows represent the dermis and the thin arrows represent the epidermis. (Reproduced by permission of the European Society of Photobiology.)

became significantly darker following exposure to UV. However, organisms with darkened body colors as a result of being held on dark backgrounds were not less sensitive to UV. Belden and Blaustein (2002) found that skin darkening did not prevent erythema; however, the photoprotective substances responsible for UV adaptation may be different from those involved with cryptic coloration changes.

Carotenoid pigments are conjugated chains of alternating single and double bonds that occur widely among crustacean zooplankton, although the composition and quantities vary with species (Siebeck et al. 1994). They are produced by algae, bacteria, and plants, and transferred to invertebrates and vertebrates through the food chain (Britton and Goodwin 1981). Carotenoids have limited UV-filtering capacity; however, in cladocerans the carotenoids are thought to play an important photoprotective role by sequestering oxygen free radicals (Krinsky 1979; Kieber et al. 2003). In amphibians carotenoids provide yellow and red variations in coloration, and could also bind free radicals.

Pterines are aromatic ring compounds, including pteridine, guanine, uric acid, and adenine, that play a significant role as pigments in amphibians (Sugiura 1968) and could also provide photoprotection from UV exposure. These compounds absorb UV radiation and combine with cholesterol to form previtamin D-3 (Norman 1998).

13.2.3 Egg Jelly

Jelly capsules surrounding amphibian egg masses may provide some protection from exposure to UV-B (Grant and Licht 1995; Ovaska et al. 1997; Licht 2003). The egg jellies from 12 species representing 2 orders and 5 families of amphibians were determined to absorb UV radiation within the range of 280 to 320 nm, and thereby protect the embryos from injury. Embryos of *Bufo americanus* and *Rana clamitans* removed from the jelly had significantly greater mortality than embryos exposed with the jelly intact (Licht 2003). In spectrophotometric analysis of *Ambystoma gracile* egg gel, Grant and Licht (1995) discovered a broad absorbance peak that would provide protection to the developing embryos (Figure 13.7). This same absorbance characteristic of egg jelly was independently determined in *Ambystoma maculatum* by Smith et al. (2002; Figure 13.8), and also appears in scans of egg jellies from *Bufo boreas*, *Bufo woodhousii*, and *Ambystoma tigrinum* (Figure 13.9). In contrast, we determined a small absorbance peak at 276 nm with minimal absorbance at longer wavelengths in the jelly of *Ambystoma gracile* egg masses collected in Mt. Rainier National Park, Washington (Figure 13.10). Within the UV-B range the absorbance was low and nonspecific, and possibly characteristic of a refraction of irradiance. Ovaska et al. (1997) reported similar UV absorbance from their studies of the egg gels of *Hyla regilla* and *Rana aurora* and concluded that the jelly offers minimal photoprotection. Hansen et al. (2002) determined a small peak of absorbance at 276 nm in gels of *Hyla regilla* and *Bufo canorus* from the inner and outer capsules, which also include a nonspecific tail of absorbance extending through the UV-B spectrum that would likely offer little protection within the ambient range of UV radiation. In addition, there was no difference in embryo survival or hatching success between organisms that had been exposed to UV in the presence and absence of the jelly. Similar results were observed in dejellied *Rana temporaria* embryos (Rasanen et al. 2003). Detritus and algae adhered to the egg gel could also effectively shade the developing embryo, therefore increasing absorbance and protection. The optical absorbing properties of habitat water and egg jelly from 3 species of Patagonian amphibians varied considerably, with eggs of *Pleurodema thaul* receiving less than 1% of incoming radiation compared to 10% in *Pleurodema bufonium* and *Bufo spinulosus* (Perotti and Diéguez 2006). It is clear from the discussion above that jellies from different species vary in their UV absorption properties. It also appears that there may be population differences in the absorbance properties of egg jelly. Site-specific or population-specific differences in chemical composition may be responsible for the apparent disparity in the results of these studies. We determined a considerable difference in the absorbance properties of the egg jelly of *Ambystom gracile* egg masses collected from 3 lakes in Mt Rainier National Park

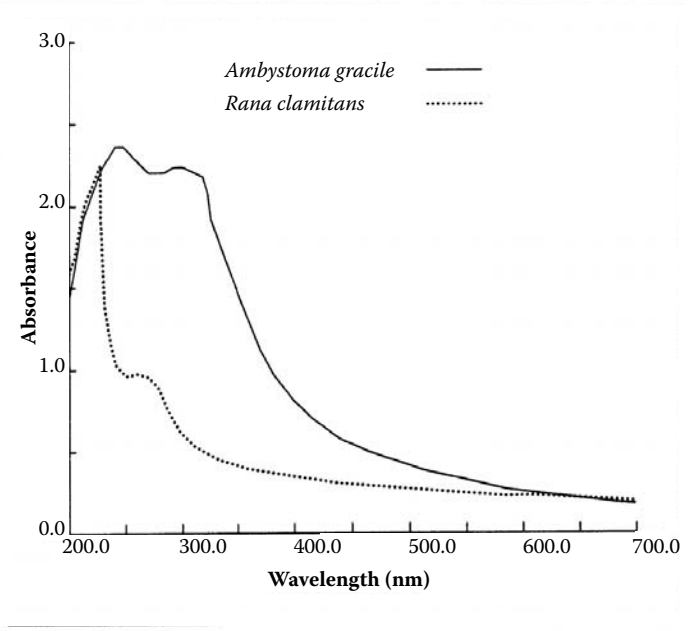

FIGURE 13.7 Egg jelly absorbance scans for *A. gracile* and *R. clamitans*. (Reprinted from Licht LE 2003, Shedding light on ultraviolet radiation and amphibian embryos. Bioscience 53: 551–561, with permission.)

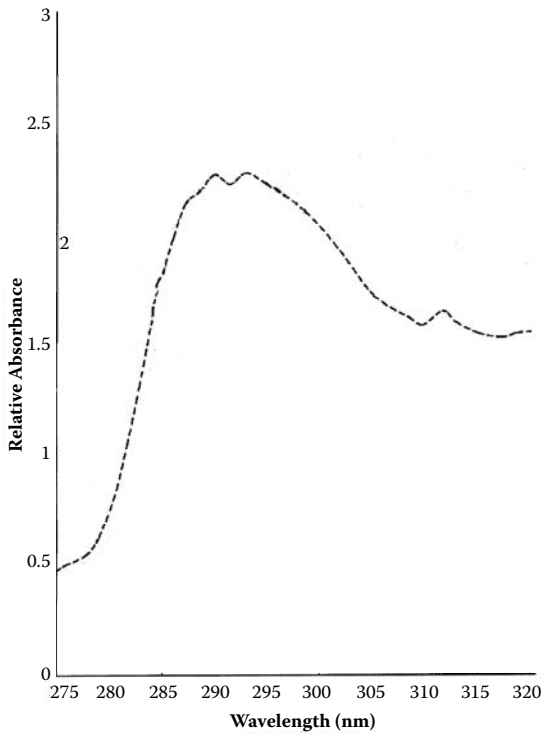

FIGURE 13.8 Absorbance scan for the yellow-spotted salamander. (Taken from Smith et al. 2002.)

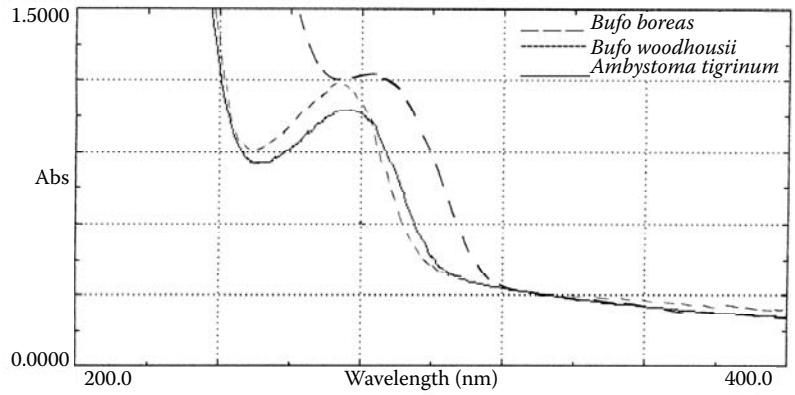

FIGURE 13.9 Egg jelly absorbance scans for *Ambystoma tigrinum, Bufo boreas,* and *Bufo woodhousii*.

(Figure 13.10). It is likely that the difference in absorbance may have been a function of the refractive index of the multilayered egg jelly rather than spectral absorbance by a pigment, since the absorbance maxima were at wavelengths below the UV-B range.

13.2.4 Photoreactivation and Photorepair

Once the protective atmospheric, habitat, and integumentary defenses of an organism have been breached by UV exposure, injury will occur. DNA is particularly vulnerable to UV because it induces the formation of cross-linkages, or dimers, in the pyrimidine base thymine. Such cross-linkages include cyclobutane-type dimers of thymine, cytosine, and uracil; pyrimidine adducts; photohydrates; and DNA-protein cross-links (Tevini 1993) that can interfere with DNA

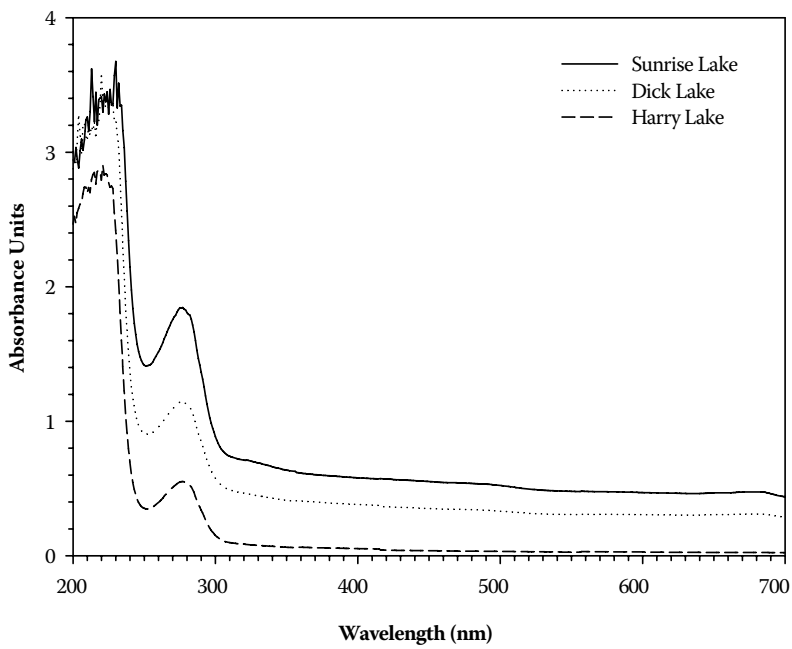

FIGURE 13.10 Absorbance spectra of *Ambystoma gracile* egg mass jelly as measured by a spectrophotometer.

replication and protein synthesis necessary for cell division in growth and replacement, and can lead to the development of tumors, as well as lesions. Most organisms are capable of repairing the DNA damage induced by UV-B through excision repair, photoreactivation, and postreplication repair (Tevini 1993). Among these, photoreactivation is promoted by the DNA photolyase, an enzyme that binds to the cyclobutane dimer, becomes activated by absorbing photons from UV-A and visible light, then cleaves the dimer from the ring before unbinding (Mitchell and Karentz 1993). Blaustein et al. (1994a) found that the amount of this enzyme in embryonic amphibians is directly correlated with the UV-B tolerance of that species. Although this correlation does not demonstrate a higher rate of dimer repair among tolerant organisms than among sensitive organisms, it does suggest a cellular basis for photorepair efficiency. However, results from studies with the wood frog (*Rana sylvatica*) indicated variation in response to photolyase, depending on environmental conditions, and led to the conclusion that estimating amphibian photorepair is a complicated process and previous conclusions regarding the relationship between photorepair and amphibian population decline must be reevaluated (Smith et al. 2000). Photorepair efficiency in fish varied by as much as 500% between 2 closely related species (Regan et al. 1982). Photorepair is likely ubiquitous among amphibians given the range of aquatic and terrestrial plant and animal species for which evidence for photorepair has been found.

Excision repair involves damage recognition, incision of the DNA chain near the site of the lesion as DNA is excised and resynthesized around the damaged site, and ligation following detachment of DNA polymerase (Mitchell and Karentz 1993). Species may be capable of both types of repair mechanisms and may vary as to which one predominates. For example, much of the DNA repair occurred during daylight hours through photorepair, and remaining repair occurred in darkness through excision repair (Siebeck et al. 1994). If conditions are appropriate for UV radiation to penetrate sensitive cellular molecules, and if cellular repair mechanisms are unable to keep up with the rate of cellular damage, UV-induced injury is inevitable.

13.3 MULTIPLE STRESSORS

Organisms are subjected to a complex host of biotic, physical, and chemical stressors that can interact additively to overwhelm the organism's homeostatic balance and induce injury (Little et al. 2003b). Although much of the initial interest in UV was focused on this stressor as a major cause of amphibian decline, it is generally implicated with other variables as a causal factor. A recent meta-analysis of potential interactive effects of UV with environmental acidification, contaminants, and disease indicated significant synergistic interactions resulting in greater than additive mortality than would have been induced by the individual stressor (Bancroft et al. 2008b). Each population is likely to be challenged by a unique interaction of stressors that may or may not include UV. Clearly, habitat alteration through anthropogenic actions and through climate change poses enormous challenges to amphibians. Two challenges may be outstanding relative to interactive effects with UV: disease and environmental contamination.

13.3.1 Disease

Disease is a significant cause of mortality in natural frog and salamander populations, and UV-B is thought to play a significant role in immunosuppression in vertebrates, which results in increased susceptibility to opportunistic infections. Studies with mammals have determined localized immune suppression in response to UV exposure through an urocanic acid receptor at the surface of the skin (DeFabo and Noonan 1983; DeFabo et al. 1990; Noonan and DeFabo 1992). Urocanic acid has also been measured in fish epidermis (Fabacher et al. 1994). For example, a time-dependent progression of UV-B-induced effects (sunburn, then fungal infection, followed by mortality) occurred in fish,

which suggested immunosuppression, while UV-tolerant fish showed no effects of the radiation (Little and Fabacher 1994).

Amphibians exposed to UV-B radiation may experience depressed immune function, making them more susceptible to pathogens (Zeeman and Brindley 1981; Knowles 1992). While little is known about the effect of UV-B on amphibian immune systems, montane amphibians are dying of facultative pathogens normally held in check by healthy immune systems. Carey et al. (1999) implicated bacterial infections in the extinction of many populations of *Bufo boreas* and *Rana pipiens* in the Colorado Rocky Mountains. In such cases, adults and metamorphs are usually affected by such infections. Fungal infections (*Saprolegnia* sp.) appeared to be associated with the mass mortality of eggs of several montane species of amphibians in the Cascades (Blaustein et al. 1994b). Disease was not evident among the toad species (Little et al. 2003a), and attempts to determine immunological response of salamanders, such as spleen condition, were unsuccessful given the small size of the organism (Little 2005). However, disease was a consistent result of the exposure of salamanders even under low irradiance conditions. The onset of fungal infections occurred within 7 days of exposure, and appeared to result in the death of the salamanders. The extreme sensitivity of the salamander larvae, however, may reflect their poor adaptation to handling in the laboratory environment. Epidermal lesions from sunburn allow invasion by pathogens, especially fungi (Pickering and Richards 1980). During laboratory studies, fungal hyphae were frequently observed at the margins of the sunburned fish skin within 1 or 2 days of the initial sunburn (Little and Fabacher 1994). These fungal infections progressed over the dorsal surface of the fish and the fish died soon after. Extensive fungal infection was also noted among tiger salamander larvae that had developed skin lesions during UV exposure (Little et al. 2003a).

In mammals, UV-B-induced immunosuppression may occur through isomerization of urocanic acid from the *trans* to the *cis* form (DeFabo and Noonan 1983; DeFabo et al. 1990; Little et al. 2003a). The *cis* form of urocanic acid may modulate cell-mediated immunity by binding to receptors (Norval et al. 1990; Palaszynski et al. 1992). Urocanic acid occurs in mammalian skin predominantly as the *trans*-isomer. Upon UV-B irradiation of the skin, urocanic acid isomerizes to the *cis*-isomer concurrent with suppression of the immune response. In mammals this is thought to be a protective mechanism by preventing uncontrolled autoimmune destruction of sun-damaged skin cells (Little et al. 2003a). Exposure to high levels of UV-B could cause hyperstimulation of this mechanism with subsequent increased susceptibility to pathogens. In a preliminary study, a substance that appeared to be *trans*-urocanic acid was found in the skin of UV-B-exposed and unexposed rainbow trout (Fabacher et al. 1994). A similar mechanism of immunosuppression may be induced by UV-B in amphibians. Although it remains to be established that urocanic acid functions similarly in amphibian epidermis as it does in mammals, the detection of this chemical in poikilotherms raises the possibility that localized immunosuppression may also be responsible for epidermal infections observed in amphibians and other aquatic organisms (Fabacher et al. 1994).

Peptides on the skin surface have been shown to play an immunological role by binding to pathogens in amphibians (Jacob and Zasloff 1994; Nicolas and Mor 1995; Carey et al. 1999). The amino acid composition of these peptides may be vulnerable to photodecomposition or phototransformation by UV, causing the peptide to be less effective as a bactericidal or fungicidal agent. In such cases, UV could directly lead to increased vulnerability to epidermal infection. Sustained exposure to solar radiation may play an important role in the initiation of disease outbreaks in aquatic amphibians. Suppression of the immune system may occur in UV-B-exposed organisms, making them more susceptible to disease. Conversely, low-level infection by pathogens may increase the vulnerability of the skin of fish and amphibians to ambient levels of solar UV-B radiation. In these studies, very low doses of UV-B were apparently sufficient to enhance the rate of breakdown in skin structure initiated by ectoparasites. Thus, conditions that affect the integrity of the epidermis prior to, and during, exposure to solar UV radiation can lead to increased susceptibility of the fish to UV-B radiation.

13.3.2 Contaminants

In aquatic habitats UV can interact additively or synergistically with certain contaminants, increasing their toxicity and severity of injury (Little et al. 2000). Chemicals of anthropogenic origin that have molecular characteristics similar to those of photoprotective substances may be altered by absorbed UV. This interaction may generate free radicals or singlet oxygen that can alter DNA, enzymes, or lipoproteins, leading to cellular injury and rapid death. For example, polycyclic aromatic hydrocarbons (PAHs) and other components of crude and refined petroleum increase in toxicity by as much as 1800 fold in the presence of UV (Oris and Geisy 1987). A water-accommodated fraction of crude oil was not lethal to larval southern leopard frogs, *Rana sphenocephala*, when simultaneously exposed to UV, but became significantly lethal when the exposure increased from 12 to 17 $\mu W/cm^2$ (Little et al. 2000). The toxicity of anthracene was 65 µg/L after 30 minutes of sunlight exposure and 25 µg/L after 5 hours of sunlight (Kagan et al. 1984). Walker et al. (1998) found that brief exposure to environmentally relevant aqueous concentrations (10 µg/L) of the cyclic aromatic hydrocarbon, fluoranthrene, caused significant injury to the skin of bullfrog (*Rana catesbeiana*) and changes in locomotory activity at 40 to 60 µg/L. In studies conducted under natural sunlight, exposure to 25 µg/L fluoranthene caused significant mortality among larval *Rana pipiens*, *Xenopus laevis*, and *Ambystoma maculatum* within 5 hours of exposure to sunlight (Hatch and Burton 1998). Survival, as measured as time to death, was markedly decreased under full sunlight conditions. After 48 hours of depuration in clean water, sufficient body residues of fluoranthrene remained in *Rana pipiens* larvae previously exposed to 2 to 10 µg/L fluoranthrene, which proved to be lethal when they were subsequently exposed to UV-A (Monson et al. 1999). *Rana pipiens* tadpoles exposed to a complex mixture of lipophilic compounds collected at sites of amphibian deformity in Minnesota in the presence of UV had higher rates of deformities than controls, indicating that the unknown substance was photoactivated by UV (Bridges et al. 2004).

UV may also change the chemical structure of the substance to a more toxic form. UV degrades ferrocyanide compounds to release free cyanide, which is toxic to fish and amphibians (Calfee and Little 2003; Little et al. 2007). Pesticides, plastics, and pharmaceuticals may also be transformed to more toxic substances (Zaga et al. 1998). Thus, photosensitization and sunburn-like lesions can occur at solar irradiance levels that would otherwise be harmless.

It is apparent that a variety of factors, acting singly or as multiple stressors, can contribute to UV-induced injury in freshwater organisms. Because many amphibian species are often restricted to shallow aquatic habitats and frequently exhibit preferences for full solar exposure, they may become captives of their own evolution in the face of increasing environmental UV irradiance (Blaustein and Bancroft 2007). Climate change may become a major driver directly increasing UV exposure through drier, sunnier climates, or through increasing water column UV as a result of accelerated decomposition and depletion of UV-absorbing DOC and altered carbon cycling. There may also be exchanges of trace gases such as methyl bromide that will influence ozone depletion, increasing availability of iron, copper, and other metals potentially toxic to food chain organisms, which in turn may alter carbon and nitrogen cycling (Zepp et al. 2007). It also appears that parasitic and infectious diseases of amphibians will increase as the climate warms (Blaustein and Dobson 2006; Pounds 2006).

13.4 SUMMARY

A range of injuries have been reported in amphibians as a result of exposure to solar UV, including deformities, erythema, skin necrosis, increased susceptibility to disease, impaired development, and reduced mass at metamorphosis, leading to reduced fitness and mortality. The solar wavelengths typically responsible for UV-induced injuries are in the UV-B wavelength range (290 to 320 nm) and, to a lesser extent, the UV-A wavelength range (320 to 400 nm). The aquatic life

stages of amphibians appear to be especially vulnerable to the harmful effects of UV radiation, given their shallow aquatic habitats and, in some species, their sun-basking habits. It is likely that there is a considerable range of sensitivities exhibited among species. UV tolerance exhibited by an organism can be linked to the concentration and distribution of photoprotective pigments contained in the integument, the efficiency of cellular photorepair mechanisms, and behavioral characteristics of the species. Habitat characteristics, in turn, also play a role in the extent to which organisms are exposed to UV. These characteristics may be large scale, such as the latitude and altitude of the organism's habitat, or small scale, such as the degree of shading provided by canopy or substrate cover. In addition, the chemical composition of the aquatic habitat (particularly the composition and concentration of dissolved organic carbon) mediates UV penetration into the water column. Moreover, UV in the aquatic habitat can vary by orders of magnitude depending on water clarity. A number of climatic conditions can contribute to UV-induced injuries, including extended periods of elevated UV associated with ozone depletion, changes in cloud cover or extent of sunny conditions, and global warming that may give rise to increased water clarity because of water column stratification. Drought can reduce water depth, and changes in watershed characteristics can alter the composition and input of dissolved organic material. The presence of certain chemical contaminants can additively or synergistically increase UV injury in organisms, even in habitats having low UV irradiance, and there is growing evidence that aquatic pathogens may also be facilitated by UV exposure. Moreover, UV exposure is regarded as an interactive stressor among a range of biotic and abiotic insults that additively increase hazard to amphibian populations.

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14 Multiple Stressors and Indirect Food Web Effects of Contaminants on Herptofauna

Rick A. Relyea

CONTENTS

Ecotoxicology is an active and exciting field of applied research, and studies of amphibians and reptiles have been making important contributions to this body of work. During the past decade, there has been a tremendous increase in research on the toxicology of these groups as evidenced by the number of publications on the topic (Figure 14.1). A large proportion of this research has been focused on amphibians with considerably less attention given to reptiles despite repeated calls for more studies of reptile toxicology (Hall 1980; Hopkins 2000). One of the driving factors responsible for the disproportionate interest in amphibian toxicology has been the global decline in amphibian populations and the associated hypothesis that contaminants may be playing either a primary or secondary role in these declines (Alford and Richards 1999; Collins and Storfer 2003; Stuart et al. 2004).

In toxicology studies, the traditional approach of assessing the risk of different chemicals has been to use short-term experiments that expose an organism to a range of concentrations of some contaminant. In these types of experiments, no individuals die at the lowest concentrations, all individuals die at the highest concentrations, and one can estimate the lethal concentration that would kill 50% of a population (termed the LC50). From this comes the paradigm that "the dose makes the poison," in that it is simply the concentration of any particular contaminant that determines its lethality. These LC50 experiments are highly effective at testing a large number of chemicals in a standardized way to allow direct comparisons to previous studies on other taxa and other contaminants. However, as noted by several previous authors, LC50 studies can have serious limitations when extrapolating the results to organisms in nature (DeNoyelles et al. 1994; Fleeger et al. 2003; Relyea and Hoverman 2006). In essence, the limitations of LC50 studies stem from the very same features that make them quick and efficient assessments. They are short-term experiments that focus on a single species in a single (typically nonstressful) environment. For amphibians at least, there is growing evidence that data from

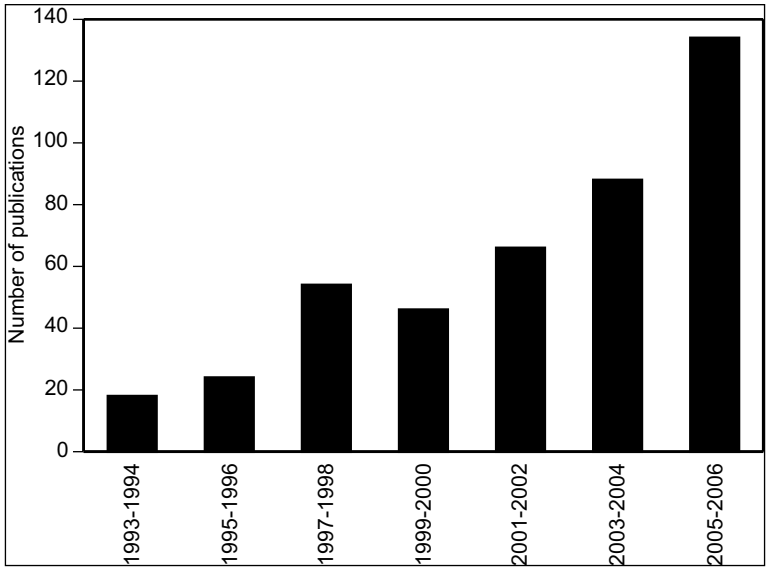

FIGURE 14.1 The growth of amphibian toxicology papers from 1993 to 2006. The number of publications was determined using the Web of Science database and a number of keywords: (amphibian or frog or toad or tadpole or salamander or snake or lizard or turtle) and (pesticide or toxicol* or contaminant).

LC50 studies may serve as an important first step in estimating lethality and risk (see Chapters 6, 9, and 11, this volume), but they may not be a good indicator of the impact that contaminants have on survival and performance (i.e., growth, behavior, physiology, and life history) in nature. That is, the dose does not always make the poison. Below, I highlight the insights gained by a number of researchers that have moved from traditional LC50 tests to incorporate natural stressors and other taxa found in amphibian and reptile communities, thereby moving from a traditional toxicological approach to a more ecotoxicological approach. While I strive to incorporate both amphibian and reptile examples, reptile studies that incorporate natural environmental variation and community interactions are quite sparse. Moreover, the body of work on amphibians is largely restricted to larval amphibians (mostly larval anurans) and to only the most commonly applied pesticides. Given that previous chapters discuss direct toxicity of several common contaminants (Chapters 6 to 11), here I focus on how contaminants interact with natural stressors and cause indirect effects in natural communities.

14.1 INTERACTIVE EFFECTS OF ENVIRONMENTAL STRESSORS

14.1.1 Abiotic Stressors

Amphibians and reptiles live under a wide range of abiotic stressors, including extremes in temperature, pH, desiccation, salinity, and UV-B radiation. As a result, one might expect these abiotic changes to alter the toxicity of contaminants either by changing the molecular configuration of the contaminant or by making an animal more susceptible to the contaminant. For example, Chen et al. (2004) found that an increase in pH from 5.5 to 7.5 had no impact on the survival of leopard frog tadpoles (*Rana pipiens*) in the absence of the herbicide Vision® (marketed in the United States as Roundup®). When the herbicide was present at 0.75 mg a.e./L, however, there was "complete mortality in pH 7.5 and minimal mortality in pH 5.5" (Chen et al. 2004, p. 828). Similarly, Edginton et al.'s (2004) study of 4 species of tadpoles found that the 96-hour LC50 values for Vision were lower at a pH of 7.5 than at a pH of 6.0 (*Rana clamitans*, LC50 = 2.1 vs. 0.9 mg a.e./L; *Rana*

pipiens, LC50 = 1.1 vs. 0.8 mg a.e./L; *Bufo americanus*, LC50 = 2.1 vs. 1.2 mg a.e./L; and *Xenopus laevis*, LC50 = 2.0 vs. 0.9 mg a.e./L). The mechanism underlying this interaction is not understood, but it is thought that the surfactant added to the herbicide (POEA; polyethoxylated tallow amine) is the causative agent of the mortality. As noted by Edginton et al. (2004, p. 821), the observation that pH affects the toxicity of Vision and Roundup is critical when assessing the risk of this herbicide to amphibians, "We concluded that, at EEC levels [expected environmental concentrations], there was an appreciable concern of adverse effects to larval amphibians in neutral to alkaline wetlands. The finding that the mean pH of Northern Ontario wetlands is 7.0 further compounds this concern."

Motivated by concern over acid precipitation and the increased solubility of metals at low pH, numerous studies have found that pH changes also can have interactive effects with metals (Clark and Hall 1985; Andrén et al. 1988; Beattie and Tyler-Jones 1992; Horne and Dunson 1994; Jung and Jagoe 1995). For example, Clark and Hall (1985) found that American toad embryos (*Bufo americanus*) suffered greater mortality from increased concentrations of aluminum (60 to 75 μg/L) when the pH was 4.3 but not when the pH was 4.8. This effect was not observed in wood frog (*Rana sylvatica*) embryos, suggesting that the synergism can be species-specific (for similar results, see Andrén et al. 1988). In experiments using tadpoles, Jung and Jagoe (1995) found that 250 and 400 μg/L of aluminum caused no significant mortality in green tree frogs (*Hyla cinerea*) when the pH was 5.5, but caused 60% to 85% mortality when the pH was 4.5. Moreover, total length and swimming speed of the tadpoles also exhibited interactive effects, with reductions in both traits occurring more in the lower pH environment than in the higher pH environment. In short, it has become clear that pH can interact with metals to affect a variety of lethal and sublethal response variables.

UV-B radiation has also received considerable attention from amphibian biologists as a potentially harmful component of the natural environment that has increased during the past century (Bancroft et al. 2007). Emerging from this concern over increasing exposure to UV-B radiation, a number of studies have been conducted that combine UV-B exposure with different contaminants. For example, in studies using relatively high concentrations of the insecticide carbaryl (sold commercially as Sevin®) and a range of UV-B concentrations (blocked, low [4 μW/cm^2], and high [65 μW/cm^2]), Zaga et al. (1998) observed greater embryonic mortality from carbaryl plus UV-B in African clawed frogs (*Xenopus laevis*; 3% mortality from high UV-B alone, 30% mortality with 15 mg/L of carbaryl alone, and 100% mortality with 15 mg/L of carbaryl plus high UV-B) and gray tree frogs (*Hyla versicolor*; 0% mortality from high UV-B alone, 77% mortality with 15 mg/L of carbaryl alone, and 100% mortality with 15 mg/L of carbaryl plus high UV-B). In contrast, Bridges and Boone (2003) found no interaction between carbaryl and UV-B in southern leopard frog (*R. sphenocephala*). Similarly, Ankley et al. (1998) found no interaction between UV-B and the insecticide methoprene in the survival of leopard frogs (*R. pipiens*). In studies of amphibian exposure to nitrate fertilizer, Hatch and Blaustein (2003) examined high- and low-elevation populations of Pacific tree frogs (*Pseudacris regilla*) and long-toed salamanders (*Ambystoma macrodactylum*) and found that only in the high-elevation tree frogs did UV-B interact to cause higher mortality. All of this suggests that interactive UV-B effects may be quite specific to the contaminant used and the species that is tested. Interestingly, although many contaminants can be photodegraded in sunlight, none of the above studies has found reduced mortality with UV-B and contaminants.

Temperature can also affect the lethality of pesticides. For example, Boone and Bridges (1999) found that the insecticide carbaryl became more toxic to green frog tadpoles (*R. clamitans*) at high temperatures. In contrast, Talent (2005) found that green anole lizards (*Anolis carolinensis*) suffered higher mortality from a natural pyrethrin pesticide under low temperatures (15 °C to 20 °C) than under high temperatures (38 °C). Given the ease in which one can conduct short-term experiments while manipulating temperature, it is rather surprising that we do not have many more studies on temperature-pesticide interactions.

In examining this collection of studies, it is clear that we currently have too few studies on abiotic stressors to draw any strong conclusions about their ability to interact with contaminants. However,

some conclusions are clear. First, we must be cautious to not assume that a particular abiotic condition is stressful simply because it differs from a standard testing protocol. For example, changes in pH (i.e., from 5.5 to 7.5) may not present any physiological stress to an animal, yet a contaminant may change in lethality because the contaminant changes conformation (in some case affecting solubility) or because the animal becomes more permeable to the contaminant (Chen et al. 2004; Edginton et al. 2004). Moreover, an environment that is stressful may not be the environment that causes a contaminant to be more lethal. For example, as noted above, Talent (2005) found that green anole lizards suffered higher mortality from a pyrethrin pesticide under lower temperatures; however, higher temperatures were actually the more stressful environments for the lizards. This underscores the importance of understanding the mechanisms by which contaminants interact with abiotic conditions. We currently appear to have no data identifying such mechanisms, yet determining the mechanisms would provide a powerful way to predict a priori the conditions and contaminants under which interactive effects should occur.

14.1.2 Biotic Stressors

Given that abiotic conditions can alter the toxicity of pesticides, one might also expect biotic stressors such as predation, competition, parasites, and pathogens to affect the toxicity of pesticides. One of the most extensively studied areas of biotic stress has been the effect of predatory stress. This phenomenon was first discovered by Relyea and Mills (2001), who found that 0.05 to 0.5 mg/L of the insecticide carbaryl (a carbamate insecticide that inhibits acetylcholine esterase) became 2 to 4 times more deadly to grey tree frog tadpoles (*Hyla versicolor*) in the presence of chemical cues from predatory spotted salamanders (*Ambystoma maculatum*) than in the absence of predatory cues. Because the predators were caged, the observed mortality was not due to predation but simply from the stress of smelling the waterborne cues of predators in the environment. Subsequent experiments by Relyea (2003) detected a synergy between carbaryl and predator cues (in this case from red-spotted newts, *Notophthalmus viridescens*) in 3 of the 6 species of tadpoles tested. To determine whether this synergy was restricted to carbaryl, Relyea (2004a) investigated potential interactions between predator cues and the insecticide malathion. Malathion, like carbaryl, is an inhibitor of acetylcholine esterase, but it is from a different class of chemicals (an organophosphate). Relyea (2004a) found that malathion also interacted with predator cues from red-spotted newts in 1 of the 6 species tested. Finally, to examine whether the synergistic phenomenon was restricted to insecticides or was a more general phenomenon, Relyea (2005b) conducted experiments with predator cues and the herbicide Roundup (i.e., glyphosate + POEA) and found that the smell of predators made the herbicide more lethal in 1 of the 6 species tested. Collectively, these studies suggest that a variety of contaminants (both insecticides and herbicides) can become considerably more toxic when there is simultaneous exposure to the smell of predators in the water. Given that most species of amphibians live with predators, the lethal effects of pesticides on amphibians may be substantially larger than we currently appreciate.

The above examples make it clear that variations in the biotic and abiotic conditions have a profound impact on the toxicity of contaminants to amphibians. However, to have any predictive power for these synergisms, it is essential to know the mechanisms underlying these synergies. In the case of both abiotic and biotic stressors, we seem to have little understanding of the mechanisms that make the specific contaminants more lethal. One hypothesis is that sublethal concentrations of contaminants and biotic stressors may each induce an increase in stress hormones. When the 2 stressors are combined, stress hormone levels may increase either additively or synergistically and lead to amphibian death. To my knowledge, no study has examined the combined effects of predator cues and contaminants on stress hormones. If the hypothesized mechanism is correct, then a variety of natural stressors that induce increases in stress hormones (e.g., competition, food limitation, parasites, and pathogens) could make sublethal concentrations of contaminants become lethal (Glennemeier and Denver 2002; but see Rohr et al. 2004). Given the ubiquity of interactions

between pesticides and predatory stress, we need to give increased attention to potential interactions between pesticides and other biotic stressors to determine how our LC50 estimates are affected.

14.2 INDIRECT EFFECTS IN FOOD WEBS

14.2.1 Two-Way Interspecific Interactions

To understand how contaminants affect amphibians and reptiles under more natural conditions, we need to not only include natural stressors but also include the other taxa with which amphibians and reptiles interact in nature. A growing number of studies have documented a variety of interesting ways in which contaminants can alter the outcome of 2-way species interactions. Typically conducted in laboratory tub experiments, this approach has frequently been applied to larval amphibian systems in which most contaminants have a depressive effect on movement and foraging behavior (Weis et al. 2001; Zala and Penn 2004). Insecticides have been particularly well studied in this regard, and the common observation is that sublethal concentrations of insecticides generally reduce the swimming and foraging activity of amphibian larvae (Bridges 1997, 1999; Relyea and Mills 2001; Rohr et al. 2003; Broomhall 2004; Punzo 2005). As a result, one would predict that larval amphibians exposed to these contaminants would consume their food resources at a slower rate and therefore grow slower. This outcome has been observed in a number of laboratory studies that control per capita food rations (Relyea and Mills 2001; Relyea 2004b). In more realistic conditions containing much more diverse communities, growth outcomes can be substantially different (see below).

Pesticide-induced changes in behavior can also impact the rate at which predators consume prey. If a contaminant is of sufficient concentration to kill the predators but not the prey (e.g., insecticides killing larval insect predators but not larval amphibians), then we should observe a positive indirect effect of increased prey survival (e.g., Relyea 2005a). However, amphibian toxicologists have been more interested in the effect of pesticide at concentrations that are sublethal to both the predator and the prey. Because the interactions between amphibian prey and their predators are a function of detectability, catchability, and consumption, there is the potential for pesticides to affect these stages of predation through both effects on the predator and effects on the prey. In many cases, several insecticides cause reduced tadpole movement, and this should translate into reduced predation risk (Bridges 1997, 1999; Relyea and Mills 2001; Rohr et al. 2003; Widder and Bidwell 2006). However, few studies have actually documented a reduction in predation risk that could be tied to this effect (Boone and Semlitsch 2001; Broomhall 2002, 2004; Mandrillon and Saglio 2007b). In some cases, a change in prey survival is not observed because invertebrate predators were used and these predators typically die at concentrations of contaminants that are only sublethal to the amphibians (Widder and Bidwell 2006). In other cases, exposure to a contaminant precludes larval amphibians from appropriately responding to a predator (Lefcort et al. 1998), or even prevents the detection of a predator at a future date (Mandrillon and Saglio 2007a). In what appears to be the only study examining contaminant effects on reptile predator-prey interactions, Bain et al. (2004) found no effect of the insecticide fenitrothion (up to 20 mg/kg) on the foraging ability of bearded dragons (*Pogona vitticeps*) on invertebrate prey. The collective evidence suggests that continued studies will find that contaminants commonly alter predator-prey interactions.

A less investigated 2-way interaction that can be impacted by contaminants is that of pathogens or parasites and their hosts. As in the case of predator-prey interactions, contaminants can make hosts either more or less likely to become infected by a parasite, depending upon how the contaminant affects the 2 interacting species. For example, 1.84 to 184 µg/L of the herbicide atrazine reduces infection by a ranavirus in salamander larvae (*Ambystoma macrodactylum*) from 25% to 13%, possibly by reducing the efficacy of the virus (Forson and Storfer 2006). Immunosuppression due to exposure to some contaminants (e.g., organophosphate insecticides) has been widely documented

across a diversity of vertebrates and invertebrates via inhibiting critical enzymes, damaging immune organs, or altering the signals that induce immune responses (reviewed in Galloway and Handy 2003). Consistent with this finding are several studies that have documented how certain pesticides (or mixtures of pesticides) can cause amphibians to develop compromised immune systems and become more vulnerable to infections from parasitic trematodes (Kiesecker 2002; Linzey et al. 2003), nematodes (Christin et al. 2003, 2004; Gendron et al. 2003), and bacteria (Hayes et al. 2006). In some cases, although immunosuppression is not confirmed, it is suspected based on observations of increased susceptibility to a pathogen (bacteria; Taylor et al. 1999).

There are 2 particularly well-studied pathogens of amphibians, the fungus *Batrachochytrium dendrobatidis* and the water mold *Saprolegnia* (Blaustein and Kiesecker 2002; Hatch and Blaustein 2003; Rachowicz et al. 2005; Gomez-Mestre et al. 2006; Lips et al. 2006), yet these 2 pathogens have received very little attention from the perspective of contaminants and immunosuppression (but see Davidson et al. 2007). While it is too early to know the importance of the immune system in combating these diseases, it is intriguing that in the western United States there are patterns of amphibian populations declining downwind of agricultural pesticide applications (Sparling et al. 2001; Davidson et al. 2001, 2002; Davidson 2004). In these areas, the concentrations of pesticides appear to be too low to directly kill the amphibians (Zabik and Seiber 1993; Aston and Seiber 1997; McConnell et al. 1998; LeNoir et al. 1999; Hageman et al. 2006), and many of these declining populations are dying of chytridiomycosis (the disease that results from *B.d.* infections). Given these patterns, it is important that we investigate the potential for contaminants to affect susceptibility to these major amphibian diseases. For example, there is growing evidence that the skin peptides of adult amphibians may play an important role in resistance to *B.d.* via antifungal properties (Rollins-Smith et al. 2006), yet the impact of pesticides on amphibian skin peptides has thus far received little attention (Davidson et al. 2007). Indeed, Rollins-Smith et al. (2006, p. 840) recently concluded, "Other factors, such as pesticide exposure, may also inhibit immune defenses allowing a controlled infection to become lethal. The interaction of pesticides with the immune system and the impact on disease development is an important area of future research." In general, as we continue to learn more about the pathogens and parasites of amphibians and reptiles, we will probably conclude that pesticides play an important role in mediating these interactions via changes in the behavior, physiology, and immune response of 1 or both of the interactors.

14.2.1.1 More Complex Interactions in Diverse Food Webs

Understanding how contaminants affect relatively simple 2-way species interactions allows us to subsequently examine how contaminants affect more complex communities. In this case, our insights primarily come from outdoor experiments using larval amphibians in mesocosms ranging in size from tens of liters to >1000 L. The point of such experiments is to simulate many of the natural conditions of real wetlands in which these animals find themselves in nature. The most simple mesocosm communities (Figure 14.2, left panel) include plant litter (which provides nutrients and a substrate for periphyton growth), phytoplankton and periphyton (which can compete for nutrients and light), zooplankton (which consumes phytoplankton), and tadpoles (which consume periphyton).

In such a simplified community, one can arrive at a number of different outcomes via a variety of different mechanisms. For example, at high concentrations (relative to a tadpole's LC50), contaminants can directly eliminate some or all of the tadpoles (Relyea 2005a; Relyea et al. 2005; Roe et al. 2006). At somewhat lower doses, the tadpoles will not be directly killed, but their growth and development can be impacted by behavioral changes induced by the contaminant. For example, Boone and colleagues (Boone et al. 2005; Boone and Bridges-Britton 2006) observed that high concentrations of the insecticide carbaryl (7 mg/L), which can shut down tadpole foraging in the short term (until the carbaryl breaks down), allow ephemeral increases in periphyton, and this can lead to a greater mass at metamorphosis (although this effect may vary with larval period). At lower concentrations (0.01 mg/L) or when the contaminant breaks down before tadpoles are added to the

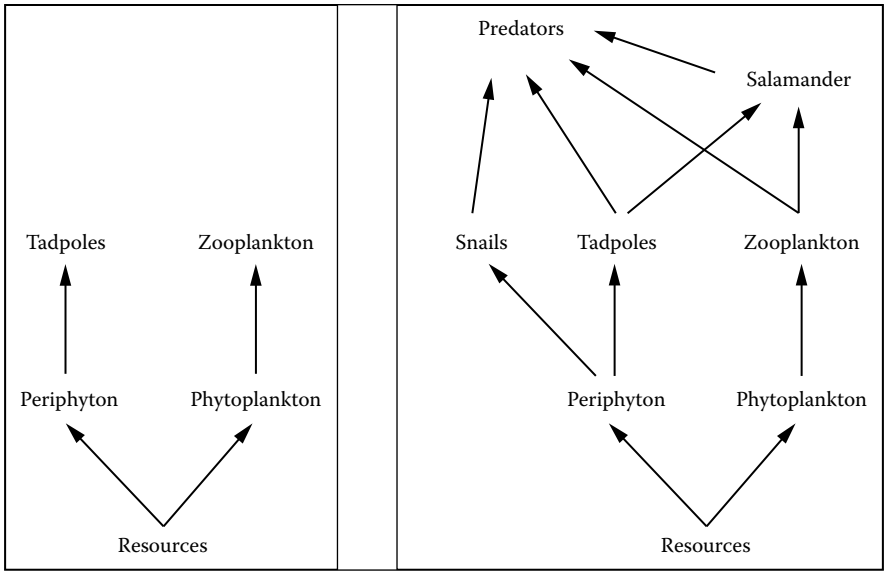

FIGURE 14.2 Simplified food webs used in mesocosm experiments examining larval amphibians embedded into aquatic communities. Many mesocosm experiments use relatively simple communities consisting of algae (periphyton and phytoplankton), zooplankton, and tadpoles (left panel), whereas other experiments include a greater diversity of taxa to represent the multitude of interspecific interactions that can occur in a natural amphibian habitat (right panel). Arrows indicate energy flow through the food web via consumption.

community, tadpole behavior is not expected to be affected by the contaminant, but other taxa may be affected in ways that cause trophic cascades that indirectly affect the tadpoles. For example, Mills and Semlitsch (2004) added carbaryl to mesocosms and allowed the insecticide to break down before adding the tadpoles. They witnessed a decline in cladoceran zoopankton (which have LC50s that are nearly 2 orders of magnitude lower than tadpoles), an increase in phytoplankton (due to relaxed herbivory), a decrease in periphyton (due to shading by the phytoplankton), and a decline in tadpole mass at metamorphosis. Similarly, Relyea and Diecks (2008) used low concentrations of another insecticide (0.01 mg/L of malathion) and found a similar trophic cascade in which malathion killed nearly all of the cladoceran zooplankton, and this allowed a bloom of phytoplankton. The phytoplankton bloom then reduced light transmission, thereby decreasing periphyton biomass and slowing the growth and development of leopard frog tadpoles enough to prevent a large proportion of them from metamorphosing before the tanks dried. As a result, a very low concentration that could not directly kill the leopard frog tadpoles indirectly caused nearly 40% of the leopard frogs to die.

As we consider more diverse communities that include salamanders, herbivorous snails, invertebrate predators (e.g., dragonfly larvae, aquatic beetles, and hemipterans), and vertebrate predators (e.g., fish), the variety of indirect pesticide effects becomes quite complex yet tractable. For example, insecticides are typically highly toxic to zooplankton, thus removing a major food source for larval salamanders and thereby reducing salamander growth and survival (Metts et al. 2005; Boone et al. 2007). Insecticides also can be highly lethal to invertebrate predators, which can reduce predation pressure on larval amphibians (Relyea 2005a; Relyea et al. 2005). Obviously, many indirect effects will reinforce or oppose each other, requiring that we have a solid understanding of the mechanisms responsible. Thus far, the vast majority of community-based approaches in amphibian systems have focused on insecticides. However, if one builds a foundation by determining the toxicities of any contaminant for all of the major players in an amphibian community (i.e., LC50

studies) and determining the behavioral and growth/developmental effects of sublethal concentrations, one should similarly be able to develop a priori predictions about how a given contaminant might impact amphibians both directly and indirectly in nature. Moreover, by developing these insights for groups of contaminants with similar modes of actions, we can likely proceed without testing all of the major registered chemicals that occur in the environment.

14.3 CONCLUSIONS

Larval amphibian communities have rapidly risen as outstanding systems for testing multiple stressor and indirect effects of contaminants. Increasingly, community ecologists are joining with more traditional toxicologists to consider the impact of evaluating contaminants under more natural ecological settings and documenting multiple stressor effects and indirect food web effects. We have made tremendous progress by examining a few globally common pesticides, yet a great many other contaminants (including nonpesticide contaminants) have received little or no testing. Unfortunately, our knowledge base currently is heavily biased toward larval anurans. We know little about the impacts of contaminants on adult anurans living in terrestrial communities, salamanders living in any life stage, or caecilians. Equally disconcerting is the fact that while we still require a great deal of research to understand contaminant effects on amphibians, we know almost nothing about the effects of contaminant effects on reptiles, especially in the realm of multiple stressors and indirect food web effects, despite repeated calls for more reptile research (Hall 1980; Hopkins 2000).

While we are certainly in the early stages of discovery, we are making rapid progress. The synergistic interactions between contaminants and stressors are being discovered at a rapid rate, we understand the mechanisms by which some of these synergies occur (e.g., pH and metals), and we have testable hypotheses for some of the other synergies (e.g., stress hormone responses to predators and pesticides). The indirect effects of contaminant food webs (via both lethal and sublethal mechanisms) are continuing to garner the attention of herpetologically oriented community ecologists, and the mechanisms underlying food web changes are thus far proving to be relatively straightforward, suggesting that we might expect a great deal of progress in this realm. While most of this community work has taken place in aquatic systems, the principles should be highly applicable to terrestrial systems of amphibians and reptiles as well. Regardless of the system, progress will undoubtedly be favored by traditional toxicologists and ecologists working together to arrive at a better understanding of how contaminants impact amphibians and reptiles.

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15 Emerging Contaminants and Their Potential Effects on Amphibians and Reptiles

Laura L. McConnell and Donald W. Sparling

CONTENTS

Serious threats to the health and sustainability of global amphibian and reptile populations have been well documented over the last few decades (Stuart et al. 2004). As many authors of this book have already indicated, habitat destruction and encroachment, increased ultraviolet B radiation, fungal diseases (e.g., chytridiomycosis), parasites, climate change, introduction of exotic species, and pollution have been cited as factors in these declines (Burrowes et al. 2004; Lips et al. 2006). Effects from pollutant exposure on wild populations are often difficult to discern due to their sublethal nature and interaction with other stressors that confound clear understanding to the causes behind population declines. The overall number of laboratory ecotoxicological studies carried out on amphibians and reptiles are small relative to other aquatic organisms, such as rainbow trout (*Oncorhynchus mykiss*), *Daphnia magna*, fathead minnow (*Pimephales promelas*; Chapter 1, this volume). A large number of these existing studies on amphibians have focused on metals or organohalogens such as DDT (4, 4'-(2, 2, 2-trichloroethane-1, 1-diyl) bis(chlorobenzene)) or polychlorinated biphenyls (PCBs), which have been banned for many years (Sparling et al. 2000). Other studies have included some second-generation pesticides like the organophosphate, carbamate, and pyrethroid insecticides (Cowman and Mazanti 2000; Chapters 6 and 7, this volume).

Over the last 5 to 6 years, many compound classes have been identified as "emerging contaminants." Emerging contaminants may generally be defined as natural or synthetic chemicals or microorganisms that fall outside the normal list of typical pollutant classes and are released to the environment with the potential for toxic effects on humans or biota. Environmental science

has expanded in scope beyond the banned organochlorine insecticides, polychlorinated biphenyls, and pesticides in widespread use to include additives in industrial and consumer products and pharmaceuticals used by humans and in concentrated animal feeding operations. A classic example of an emerging contaminant is perfluorinated surfactants (PFOS) such as Scotchguard®, and their metabolites, which, after many decades of use, have become global contaminants with potential for adverse effects (Renner 2004). This discovery contributed to significant expansion in the area of environmental science focused on the fate and transport of perfluorinated chemicals.

As the capacity and technological capability of analytical chemistry have improved, development of methods to measure organic chemicals of varying properties in environmental matrices has become more rapid. Capillary gas chromatography with high-resolution mass spectrometry or tandem mass spectrometry has allowed for unambiguous measurement of complex mixtures of organic pollutants and their degradation products in samples as varied as air and precipitation to fish and whale blubber (Aono et al. 1997; Xie et al. 2007b; Yao et al. 2007; Young et al. 2007). More recently, high-performance liquid chromatography–mass spectrometry (HPLC-MS) equipment has become standard in most environmental science laboratories. The development of the electrospray interface between the HPLC and MS components combined with triple-quadrupole MS has increased both sensitivity and selectivity of analytical methods for more polar and higher-molecular-weight compound classes. Recent reviews of scientific publications related to developments in environmental mass spectrometry have documented the development in this research area (Richardson 2001, 2004; Richardson and Ternes 2005).

In the United States, several ongoing and newly formed efforts are aimed at identifying pollutants that may be causing serious toxic effects in humans or wildlife populations. In 1976, the Toxic Substances Control Act (TOSCA) Interagency Testing Committee was formed to identify chemicals with the potential for toxicity where minimal toxicological or environmental fate data are available. Reports from this committee are made annually to the administrator of the US Environmental Protection Agency (http://www.epa.gov/oppt/itc/), and chemicals are added to the Priority Testing List and testing information is requested from the manufacturer. The 1996 Food Quality Protection Act required that contaminants such as pesticides be screened for effects on the endocrine systems of humans and wildlife. From this USEPA has developed an Endocrine Disruptor Screening Program where substances are prioritized for uniform testing and results are made public (http://www.epa.gov/endo/). Environment Canada manages the Toxic Substances Research Initiative, which includes support for research on endocrine-disrupting substances (EDS) (http://www.hc-sc.gc.ca/sr-sr/finance/tsri-irst/index_e.html).

The purpose of this chapter is to provide information on several "new" or "emerging" chemicals that may pose risks to amphibians and reptiles (Tables 15.1 and 15.2). We do not intend this list to be exhaustive; that would require more space than this book allows. Rather, we selected a few chemicals that, based on production volume, usage, known toxicity, and other factors, may pose greater risks than others. Certainly, many readers would be able to add other chemicals to our list that pose equal or even greater risks. Many of these emerging contaminants are released along with wastewater treatment effluents; therefore, an evaluation of several compound classes associated with residential or industrial wastewater streams is included. The use of fungicides in US agriculture has increased over the last few decades and a number of new chemistries have been introduced to the environment, while little is known of their risk to amphibian or reptile populations. Outcomes from this effort are expected to identify research needs with respect to toxicological and developmental studies of amphibian species. The area of emerging contaminants and their study is a rapidly developing field, and undoubtedly several new papers will have been published by the time this chapter gets to press. Nevertheless, this is our perspective at the time of writing (July 2009).

TABLE 15.1
List of Specific Chemical Contaminants, Abbreviations, and Basic Physical Chemical Properties

Common Name	Chemical Name	Abbreviation	CAS Registry Number	Chemical Formula	Molecular Weight (g/mol)	Melting Point (°C)	Aqueous Solubility (mg/L)	Vapor Pressure (Pa)	Log K_{ow}
Brominated flame retardants									
Decabromodiphenylether[a]	2,3,4,5,6-Pentabromo-1-(2,3,4,5,6-pentabromophenoxy)benzene	Deca-BDE or BDE 209	1163-19-5	$C_{12}Br_{10}O$	959.17	304–307	<0.001 at 25 °C	4.63×10^{-6}	6.3–12.6
Hexabromocyclododecane[b]	1,2,5,6,9,10-Hexabromocyclododecane	HBCD	3194-55-6	$C_{12}H_{18}Br_6$	641.7	180–185	3.4 at 25 °C	6.27×10^{-5} at 21 °C	5.625 at 25 °C
Pentabromodiphenylether[c]	1,2,4-Tribromo-5-(2,4-dibromophenoxy) benzene	BDE-47	32534-81-9	$C_{12}H_5Br_5O$	564.69	–7 to –3	0.0109	1.45×10^{-5} at 25 °C	6.46–6.97
Tetrabromobisphenol-A[d]	2,2',6,6'-Tetrabromo-4,4'-isopropylidenediphenol	TBBPA	79-94-7	$C_{15}H_{12}Br_4O_2$	543.88	181	1.26 at 25 °C at pH 7	1.19×10^{-7} at 20 °C	8.024 at 25 °C
Perfluorinated Compounds									
N-Methyl perfluorooctane sulfonamidoethanol		*N*-MeFOSE	24448-09-7	$C_{11}H_8F_{17}NO_3S$	557.23				
N-Ethylperfluorooctane sulfonamide	*N*-Ethyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-octanesulfonamide		4151-50-2	$C_8F_{18}SO_2NHCH_2CH_3$	527.19				
Perfluorooctanesulfonic acid[e]	1-Octanesulfonic acid	PFOS	1763-23-1	$C_8F_{17}SO_3^-$	500.13	>400	519–570 at 25 °C at pH 7	3.31×10^{-4} at 20 °C	
Perfluorooctanoic acid[f]	Pentadecafluorooctanoic acid	PFOA	335-67-1	$C_7F_{15}COO^-$	414.07	45–50		128–96 500 at 59–190 °C	
Perfluorooctane sulfonamide	*N*-Ethyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulfonamide	PFOSA	754-91-6	$C_8F_{17}SO_2NH_2$	499.14				
Anionic Surfactants									
Nonylphenol[g]	4-Nonylphenol	NP	104-40-5	$C_{15}H_{24}O$	220.35	43–45	6.2×10^6 at pH 7	4.55×10^{-3}	3.8–4.77
Octylphenol[h]	4-(1,1,3,3-Tetramethylbutyl)phenol	OP	140-66-9	$C_{14}H_{22}O$	206.33	79–82	17–19 at 22 °C	0.21 at 20 °C	4.12 at 20.5 °C
Antibacterial Compounds									
Triclosan[i]	5-Chloro-2-(2,4-dichlorophenoxy)phenol		3380-34-5	$C_{12}H_9Cl_3O_2$	289.54	180	1.97–4.6		4.8

(*continued*)

TABLE 15.1 (CONTINUED)
List of Specific Chemical Contaminants, Abbreviations, and Basic Physical Chemical Properties

Common Name	Chemical Name	Abbreviation	CAS Registry Number	Chemical Formula	Molecular Weight (g/mol)	Melting Point (°C)	Aqueous Solubility (mg/L)	Vapor Pressure (Pa)	Log K_{ow}
Methyl triclosan	5-Chloro-2-(2,4-dichlorophenoxy)anisole		4640-01-1	$C_{13}H_9Cl_3O_2$	303.57				
Triclocarban[j]	3-(4-Chlorophenyl)-1-(3,4-dichlorophenyl) urea		101-20-2	$C_{13}H_9Cl_3N_2O$	315.59	255.3	0.11 at 20 °C at pH 6.1–6.3	<1 hPa at 50 °C	4.2 at 22.6 °C
			UV Filters[k]						
Benzophenone-1	2,4-Dihydroxybenzophenone		131-56-6	$C_{13}H_{10}O_3$	214.22	144.5–147			
Benzophenone-2	2,2',4,4'-Tetrahydroxy-benzophenone		131-55-5	$C_{13}H_{10}O_5$	246.22	198–200			
Benzophenone-3	2-Hydroxy-4-methoxy-benzophenone	BP-3	131-57-7	$C_{14}H_{12}O_3$	228.24	62–65	210 at 25 °C		3.79
Ethylhexyl methoxycinnamate	(3-(4-Methoxyphenyl)-2-propenoic acid 2-ethylhexyl ester	EHMC	5466-77-3	$C_{18}H_{26}O_3$	290.4	–25	150 at 25 °C		5.8
4-Methylbenzylidene camphor	(3-(4'-Methyl-benzylidene)bornan-2-one)	4-MBC	36861-47-9	$C_{18}H_{22}O$	254.37	66–69	5.1 at 25 °C		4.95
Octocrylene	2-Cyano-3,3-diphenyl-2-propenoic acid 2-ethylhexylester	OC	6197-30-4	$C_{24}H_{27}NO_2$	361.48	14	0.2 at 25 °C		7.35
Fungicides									
Trifloxystrobin[l]	Methyl (*E*)-methoxyimino-{(*E*)-a-[1-(α,α,α-trifluoro-*m*-tolyl) ethylideneaminooxy]-*o*-tolyl}acetate		141517-21-7	$C_{20}H_{19}F_3N_2O_4$	408.4	72.9	0.61 at 25 °C	4.5×10^{-4} Pa at 25 °C	
Azoxystrobin[m]	Methyl (*E*)-2-{2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate		131860-33-8	$C_{22}H_{17}N_3O_5$	403.388	116	6.0	1.1×10^{-10} Pa at 25 °C	
Fluoxatstrobin[n]	(*E*)-{2-[6-(2-Chlorophenoxy)-5-fluoropyrimidin-4-yloxy]phenyl} (5,6-dihydro-1,4,2-dioxazin-3-yl) methanone *O*-methyloxime		361377-29-9	$C_{21}H_{16}ClFN_4O_5$	458.8	103–105	2.3 at pH 7	5.63×10^{-10} at 20 °C	2.86
Ketoconazole	1-[4-[4-[[(2*S*,4*R*)-2-(2,4-dichlorophenyl)-2-(imidazol-1-ylmethyl)-1,3-dioxolan-4-yl] methoxy]phenyl]piperazin-1-yl]ethanone		65277-42-1	$C_{26}H_{28}Cl_2N_4O_4$	531.43	148–152			
Tetraconazole[o]	(*RS*)-2-(2,4-Dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propyl 1,1,2,2-tetrafluoroethyl ether		112281-77-3	$C_{13}H_{11}Cl_2F_4N_3O$	372.16	–29.2	159	1.8×10^{-4}	3.56 at 23 °C

Prothioconazole[p]	(*RS*)-2-[2-(1-Chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-1,2,4-triazole-3-thione	178928-70-6	$C_{14}H_{15}Cl_2N_3OS$	344.26	139.1–144.5	5.0 at pH 4 at 20 °C	$<4 \times 10^{-7}$ at 20 °C	3.82 at pH 7
Cyazofamid[q]	4-Chloro-2-cyano-*N*,*N*-dimethyl-5-*p*-tolylimidazole-1-sulfonamide	120116-88-3	$C_{13}H_{13}ClN_4O_2S$	324.9	152.7	0.107 at pH 7 at 20 °C	$<1.33 \times 10^{-5}$ at 35 °C	
Dimethomorph[r]	(*EZ*)-4-[3-(4-Chlorophenyl)-3-(3,4-dimethoxyphenyl)acryloyl] morpholine	110488-70-5	$C_{21}H_{22}ClNO_4$	375.9	125–149	18 at pH 7 at 20 °C	1.0×10^{-6} to 9.7×10^{-7} at 25 °C	2.63–2.73 at 20 °C
Boscalida[s]	2-Chloro-*N*-(4'-chlorobiphenyl-2-yl) nicotinamide	188425-85-6	$C_{18}H_{12}Cl_2N_2O$	343.21	143.4–143.6	6 at 20 °C	7×10^{-7} Pa at 20 °C	2.96 at 21 °C
Famoxadone[t]	(*RS*)-3-Anilino-5-methyl-5-(4-phenoxyphenyl)-1,3-oxazolidine-2,4-dione	131807-57-3	$C_{22}H_{18}N_2O_4$	374.39	140.3–141.8	111 at pH 7 at 20 °C	6.4×10^{-7} Pa at 20 °C	4.65 at pH 7

[a] EPA (2008).
[b] Data from USEPA High Production Volume Information System (http://iaspub.epa.gov/oppthpv/quicksearch.display?pChem=100412).
[c] Vapor pressure values from Tittlemier et al. (2001) and other values from Peltola and Ylä-Mononen (2000).
[d] Data from USEPA High Production Volume Information System (http://iaspub.epa.gov/oppthpv/quicksearch.display?pChem=100413).
[e] OECD (2002).
[f] Melting point from Boit (1975) and vapor pressure from Kaiser et al. (2005).
[g] EPA (2005a).
[h] Data from USEPA High Production Volume Information System (http://iaspub.epa.gov/oppthpv/quicksearch.display?pChem=100234).
[i] Estimated values of melting point, vapor pressure, and Log K_{ow} from Halden and Paul (2005).
[j] Data from USEPA High Production Volume Information System (http://iaspub.epa.gov/oppthpv/quicksearch.display?pChem=101315).
[k] Solubility and Log K_{ow}s of UV filters from Díaz-Cruz et al. (2003).
[l] USEPA (1999).
[m] USEPA (1997).
[n] USEPA (2005b).
[o] USEPA (2005c).
[p] USEPA (2007).
[q] USEPA (2004).
[r] USEPA (1998).
[s] USEPA (2003a).
[t] USEPA (2003b).

TABLE 15.2
Results of Toxicological Studies of Amphibian Species with Selected Emerging Contaminants Arranged by Contaminant Class

Species	Compound(s)	Experiment Type	Exposure Type, Level, and Duration	Results	Reference
		Brominated Flame Retardants			
Pseudacris regilla	Tetrabromobisphenol-A	Thyroid hormone-mediated activity on tadpoles	Aqueous at 5.4 and 54.4 ug/L over 96 hours	May act as agonist of TH action and potentiate TH-mediated gene expression leading to accelerated anuran metamorphosis	Veldhoen et al. 2006a
Rana rugosa	Tetrabromobisphenol-A	Thyroid hormone agonist activity on tadpoles	Aqueous at 540, 54, and 5.4 µg/L over 9 days	Agonist activity to T_3 at lowest treatment	Kitamura et al. 2005
Xenopus laevis	BDE-47	Effect on time to complete metamorphosis	Single intraperitoneal injection of 1 or 100 µg/tadpole	Significant effect at 100 µg/tadpole	Balch et al. 2006
	BDE-99	Effect on time to complete metamorphosis	Single intraperitoneal injection of 1 or 100 µg/tadpole	No observable effect	Balch et al. 2006
	BDE-206	Thyroid hormone agonist activity on tadpole tail tips	Culture media at 0.88 to 880 µg/L for 6 days in presence of T_3 or 880 µg/L alone	Significant reduction in tail tip regression in all treatments with T_3; no effect with BDE-206 alone	Schriks et al. 2006
	DE-71 commercial formulation	Effect on time to complete metamorphosis	Single intraperitoneal injection of 0.6, 6, or 60 µg/tadpole	Significant effect at 60 µg/tadpole	Balch et al. 2006
	DE-71 commercial formulation	Effect on time to complete metamorphosis	Dietary at 1, 1000, or 5000 µg/g for 14 days	Significantly inhibited metamorphosis in all 3 treatments	Balch et al. 2006
	HBCD	Thyroid hormone agonist activity on tadpole tail tips	Culture media at 0.64 to 640 µg/L for 6 days in presence of T_3 or 640 µg/L alone	Significant reduction in tail tip regression in all treatments with T_3; no effect with HBCD alone	Schriks et al. 2006
Xenopus tropicalis	BDE-47	Effect on time to complete metamorphosis for tadpole	Dietary at 100, 1000, or 10 000 µg/g for 14 days	Significant mortality at 10 000 µg/g; reduced hind limb length and body length at 1000 µg/g	Carlsson et al. 2007
	BDE-99	Effect on time to complete metamorphosis	Dietary at 100, 1000, or 10 000 µg/g for 14 days	Significant mortality at 10 000 µg/g; reduced hind limb length and developmental stage at 1000 µg/g	Carlsson et al. 2007

TABLE 15.2 (CONTINUED)
Results of Toxicological Studies of Amphibian Species with Selected Emerging Contaminants Arranged by Contaminant Class

Species	Compound(s)	Experiment Type	Exposure Type, Level, and Duration	Results	Reference
			Perfluorinated Chemicals		
Rana pipiens	PFOS	Effects on survival and development from early embryogenesis through complete metamorphosis	0.03 to 10 mg/L through metamorphosis	Survival was significantly decreased at 10 mg/L (90%), but survival was not effected at the lower treatment levels' an increased time to initial metamorphosis was observed in the 3.0 mg/L treatment	Ankley et al. 2004
Xenopus laevis	PFOS	LC50	96-hour FETAX	LC50 = 15.6 mg/L, and NOEC and LOEC were calculated as 4.82 and 7.97 mg/L, respectively	Beach et al. 2006 and references within
			Anionic Surfactants		
	NP	Developmental effects on tadpoles	Aqueous flow through at 6.25 to 100 mg/L	Exposure resulted in a significantly increased proportion of females at nominal concentrations of 100 and 25 mg/L, but not at 50, 12.5, or 6.25 mg/L	Blandin et al. 1996
	NP	Developmental effects on embryos compared with natural estrogen			Bevan et al. 2003
	OP	Examine interaction between OP sublethal exposure and UV-B radiation on mRNA expression in the brain and effects on metamorphosis, specifically growth rate and hind limb emergence	Aqueous static with 48-hour renewal at 1 nM and 1 µM for 10 days to OP alone at 2 different dose levels; to subambient UV-B radiation alone; and to 2 combinations of OP and UV-B	Combined 1 µM OP and UV-B treatment were heavier than other treatments, and displayed significant acceleration of hind limb emergence	Crump et al. 2002

(*continued*)

TABLE 15.2 (CONTINUED)
Results of Toxicological Studies of Amphibian Species with Selected Emerging Contaminants Arranged by Contaminant Class

Species	Compound(s)	Experiment Type	Exposure Type, Level, and Duration	Results	Reference
			Antibacterial Products		
Rana catesbeiana	Triclosan	Changes in metamorphosis process	Aqueous at 0.3 to 30 ug/L over 4 days	Effects on thyroid hormone function at lowest exposure level	Veldhoen et al. 2006b
Rana pipiens	Triclosan	Activity level, startle response, survivorship, and growth on tadpoles	Aqueous at 0.23 to 230 µg/L over 24 days	Activity level reduced in all treatments; startle response and survivorship were lower at 230 µg/L treatment; no interaction with acetaminophen exposure	Fraker et al. 2004
			Fungicides		
Rana temporaria	Azoxystrobin	Acute toxicity to tadpoles	Aqueous at 0.5, 0.13, and 0.03 mg/L	Negative effect on survival at highest dose and negative effect on body length at all concentrations	Johansson et al. 2006
	Azoxystrobin	Chronic toxicity to tadpoles	Aqueous at 10 and 1 µg/L	No negative effects observed	Johansson et al. 2006

15.1 EMERGING COMPOUND CLASSES AND RELEVANCE TO HERPETOFAUNA

15.1.1 Brominated Flame Retardants

There are 3 major types of brominated flame retardants (BFRs): 1) Tetrabromobisphenol-A (TBBPA) is added during the production of epoxy and polycarbonate resins used in circuit boards and other products. It becomes part of the polymer backbone, making it less available for loss to the environment. However, this chemical is also used as an additive in acrylonitrile-butadiene-styrene plastics for products like television casings (BSEF 2007). 2) Polybrominated diphenyl ethers (PBDEs) are added to different polymers, but they are not chemically bound to the polymer backbone and thus are easily released to the environment. 3) Hexabromocyclododecane (HBCD) is added to polystyrene insulation foams used in building construction and is used in the back coating of textiles like upholstered furniture.

At present, the risk from TBBPA is deemed low due to its incorporation into the polymer backbone, and this product remains in heavy use. This chemical has primarily been detected in sewage sludge and in sediment samples collected near industrial sources (Law et al. 2003). A recent report by Xie et al. (2007a) described decreasing atmospheric concentrations of TBBPA with increasing latitude from the North Sea to the Arctic, suggesting that the potential for long-range transport is

limited. TBBPA is similar in structure to thyroxine (T_4) and can alter thyroid hormone-responsive genes in the laboratory amphibian model *Xenopus laevis* (Jagnytsch et al. 2006). Developmental effects have also been observed in *Rana rugosa* at 10^{-8} to 10^{-6} M (Kitamura et al. 2005), and in the Pacific tree frog, *Pseudacris regilla,* at 10^{-8} to 10^{-7} M (Veldhoen et al. 2006a).

PBDEs are a class of chemical compounds in which up to 10 bromine atoms are attached to a diphenyl ether molecule. There are 209 different possible compounds, called congeners, depending on the number and position of the bromine atoms. PBDEs have properties similar to those of polychlorinated biphenyls (PCBs), are persistent in the environment, and undergo long-range atmospheric transport, as demonstrated through their bioaccumulation in the arctic marine food web (Muir et al. 2006). PBDEs function in multiple ways to inhibit thyroid activity and act as endocrine disruptors. Two industrial PBDE mixtures, called penta-BDE and octa-BDE, were banned in the European Union in 2004 due to concern over their potential for toxicity and biomagnification. Analysis of archived guillemot (*Uria algae*) eggs from the Baltic Sea revealed increasing PBDE concentrations from early 1970 to approximately 1990, followed by a steep decline through 2001 (Sellström et al. 2003). A similar study of tawny owl (*Strux aluco*) eggs from Northern Europe observed an average decline of 6% per year in total PBDEs between 2001 and 2004, reflecting a decline in usage in the region (Bustnes et al. 2007). The primary manufacturers of these products in the United States have voluntarily phased out their production as of 2004.

PBDEs residues have been measured in turtles and other reptiles (e.g., de Solla et al. 2007; Wu et al. 2008; see Chapter 10, this volume). However, studies have not gone beyond residue determination into exploring the possible effects of these chemicals in reptiles.

The deca-BDE formulation, primarily used in plastics for electronics equipment, is still widely used in the United States and Europe. The higher brominated congeners appear to be less prone to bioaccumulation (Ciparis and Hale 2005). Amphibians and reptiles are important components of many food webs and may become exposed by eating contaminated prey or become a source of BDEs for organisms at higher trophic levels. The decabrominated congener BDE-209 has been detected in Peregrine falcon (*Falco peregrinus)* eggs in Sweden (Lindberg et al. 2004). Uptake by fish such as rainbow trout (*Onchorhynchus mykiss*) and common carp (*Cyprinus carpio*) appears to be minimal, but biotransformation of BDE-209 to lower brominated PBDE congeners was observed in the liver of these species (Stapleton et al. 2006). PBDEs were also detected in the livers of frog samples (*Rana temporaria*) collected along a transect of the Scandinavian Peninsula (ter-Schure et al. 2002). The tetrabrominated congener BDE-47 was the most frequently detected PBDE, but PBDE concentrations were 10 to 100 times lower than the most abundant PCB congeners. Recent research by Balch et al. (2006) with *Xenopus laevis* and by Carlsson et al. (2007) with *Xenopus tropicalis* has demonstrated the potential for developmental effects from exposure to BDE-47.

HBCD is a widely used BFR with a reported global production of 600 000 metric tonnes per year in 2000 (Alaee et al. 2003). The HBCD commercial mixture contains 3 diastereomers, α-, β-, and γ-HBCD, which may be preferentially assimilated. From work by Zegers et al. (2005) it appears that the β- and γ-HBCD are susceptible to enzyme-mediated biotransformation while α-HBCD is resistant. Each of the 3 diastereomers also have 2 enantiomers (Janák et al. 2005), making the environmental fate of these chemicals in biological systems all the more complex. Alpha-α-HBCD caused thyroid disruption in *Xenopus laevis* tadpoles in laboratory studies (Schriks et al. 2006), but further investigations are required under environmentally relevant concentrations.

The relative risk of BFRs to herpetofauna is difficult to assess. Clearly the potential for thyroid function-disrupting or developmental effects on amphibians in the environment exists for all 3 types of BFRs. However, exposure to toxicologically significant levels of BFRs may be limited to amphibian populations downstream or downwind from urban or industrial areas. Despite the reduction in

usage of the penta-BDE formulation, a huge reservoir of BFRs exists in consumer products still in use, and the potential for exposure by amphibian populations will continue into the future.

15.1.2 Perfluorinated Chemicals

Perfluorinated chemicals (PFCs) are industrial products utilized in a variety of consumer and agricultural products. They are used as refrigerants, agrochemicals, chemical catalysts/reagents, surfactants, and in fire-fighting foams. The strength of the carbon-fluoride bond, and the presence of multiple C-F bonds in PFCs contribute to their resistance to biotic and abiotic degradation (Key et al. 1997). The study of PFCs in the environment is an active area of research.

Perfluorooctane sulfonyl fluoride is the primary building block used in polymers to treat fabrics and other textiles to repel water and stains. Some common perfluorooctanesulfonyl fluoride-derived products are N-methyl perfluorooctane sulfonamidoethanol and N-ethyl perfluorooctane sulfonamidoethanol. Another PFC, N-ethyl perfluorooctane sulfonamide, is an insecticide used in ant bait products. These neutral, semivolatile fluorinated chemicals have the potential for long-range atmospheric transport and have been detected in arctic air (Shoeib et al. 2006). Perfluoroalkyl sulfonamido ethanols and fluorotelomer alcohols are precursors to perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), the most commonly detected perfluorinated environmental contaminants.

Evidence for global environmental contamination by PFOS and PFOA was initially published in 2001 (Giesy and Kannan 2001; Hansen et al. 2001; Kannan et al. 2001). Subsequent investigations and improvements in analytical methods have led to the identification of additional PFCs in the environment (Powley et al. 2008). Results from a survey of PFCs in the arctic food chain (zooplankton to whale) suggest that PFOS biomagnifies in the food chain; however, biotransformation of other PFCs to PFOS is a complicating factor in determining biomaginfication factors (Tomy et al. 2004). PFOS and PFOA are chemically persistent, highly water soluble, and can even be used as tracers for ocean circulation patterns (Yamashita et al. 2008).

Published results from standard laboratory toxicity testing experiments indicate that PFOS is toxic to many aquatic organisms, but effects are generally seen at concentrations well above measured environmental concentrations (Ankley et al. 2005). For example, PFOS affected the survival of the aquatic midge (*Chironomus tentans*) at an EC50 of 92 μg/L in a chronic life cycle test, but no toxic effects were seen for PFOA (MacDonald et al. 2004). In another study of PFOS toxicity, 2 green algae species, a floating macrophyte, and 2 invertebrates were tested, and an overall acute toxicity concentration of 100 mg/L was determined from the most sensitive species (Boudreau et al. 2003).

In an experiment that exposed northern leopard frog (*Rana pipiens*) larvae to 0.03 to 10 mg/L of PFOS (Ankley et al. 2004), survival was affected at the highest concentrations and metamorphosis was delayed at lower concentrations. During these same experiments, Ankley also found that tadpoles bioaccumulated PFOS via the water. Beach et al. (2006) reported a lowest observable effects level (LOEL) for PFOS of 7.97 mg/L and an LC50 of 15.6 mg/L using a 96-hour embryo teratogenesis assay with *Xenopus laevis.*

PFOS has been measured in tissues of turtles, so we know that they are being exposed. In a study on the Great Lakes, Kannan et al. (2005) determined that PFOS was the most common perfluorinated compound in the food chain. Zebra mussels accumulated PFOS concentrations that were approximately 1000 times greater than in the water, and concentrations in predators were 5 to 10 times greater than in their prey. The livers of 2 male snapping turtles had PFOS concentrations of 105 and 169 ng/g, while 2 female turtles had only 1 and 8.8 ng/g. For comparison, adult green frog livers contained 50 to 285 ng/g PFOS. Keller et al. (2005) found that among 12 PFCs, PFOS had the highest concentration in plasma of loggerhead (*Caretta caretta*) and Kemp's ridley (*Lepidochelys kempii*) sea turtles; mean concentrations were 3.2 to 11.0 ng/mL in loggerheads and

3.57 to 39.4 ng/mL in Kemp's. However, there are no studies that we know of that have examined the effects of perfluorinated chemicals in any reptile.

As an endocrine disruptor, PFOS did not appear to have a strong effect on the thyroid hormone system of rats when introduced orally (Chang et al. 2008). However, results from a recent study with mice by Johansson et al. (2008) suggested that PFOS and PFOA are potential developmental neurotoxins. In aquatic organisms, endocrine-disrupting effects of PFCs have been observed by measuring estrogenic activities in cultured tilapia hepatocytes (Liu et al. 2007). Estrogenic activity was also expressed in minnows exposed to PFOA concentrations of 3 to 30 mg/L over 14 to 28 days (Wei et al. 2007).

Although information on PFC exposure by amphibian populations is not available at present, the stability of PFCs in water and their potential for long atmospheric range transport suggest that amphibian populations in all regions of the world have a potential for exposure to PFCs. Aquatic reptiles such as turtles and crocodilians may also be at risk. An aquatic benchmark to protect aquatic organisms of 1.2 µg/L PFOS has been proposed by Beach et al. (2006). Recent measurements of PFOS in river samples collected upstream and downstream from a wastewater treatment plant effluent in Germany were 0.8 to 3.5 ng/L and 0.7 to 15 ng/L, respectively (Becker et al. 2008). In a study conducted in rivers in China, PFOS concentrations ranged from 0.90 to 99 ng/l and <0.01 to 14 ng/l in samples from the Pearl River and Yangtze River, respectively (So et al. 2007). Therefore, despite widespread contamination, measured concentrations of the more bioaccumulative perfluorinated chemical, PFOS, does not appear to pose a significant threat to amphibians or aquatic reptiles.

15.1.3 Anionic Surfactants

Anionic surfactants are widely used in consumer products to improve the effectiveness of detergents, pesticides, and other products. While many different types of surfactants are used, one of the most studied groups of surfactants is the 4-alkylphenol polyethoxylates. The technical product is a complex mixture of 4 to 20 carbon chain ethoxylates with differing levels of branching (Giger et al. 1981). The ethoxylate chains are easily biodegraded under anaerobic conditions, leaving behind the more persistent metabolites, mono- and diethoxylates, nonylphenol, and octylphenol (Ahel et al. 1994). Alarmingly high concentrations of these surfactant metabolites were detected in sewage treatment sludge in the early 1980s (Giger et al. 1984).

Octylphenol and nonylphenol have been observed in sediments and surface waters in many regions of the world (Rice et al. 2003; Li et al. 2004; Vitali et al. 2004; Nagy et al. 2005; Chen et al. 2006; Li et al. 2007b; Fiedler et al. 2007) and have recently been found in the air and surface waters of remote regions like the North Sea (Xie et al. 2007b). Their hydrophobic nature favors partitioning into sediments, generally creating a zone of high concentrations around sources, that is, wastewater treatment plant discharges.

Research carried out since the 1990s established that 4-nonylphenol in particular acted as an endocrine disruptor in aquatic organisms (Jobling and Sumpter 1993; White et al. 1994; Jobling et al. 1996; Baldwin et al. 1997; Gimeno et al. 1997; Ashfield et al. 1998; Bistodeau et al. 2006) and in amphibians (Blandin et al. 1996; Bevan et al. 2003). Endocrine disruption by nonylphenol in embryonic diamondback terrapins (*Malaclemys terrapin*) and snapping turtles was confirmed when application of the chemical caused sex reversal in embryos incubated at male-inducing temperatures (Place et al. 2001). Also, octylphenol may disrupt hypothalamic development in young snapping turtles (Trudeau et al. 2002) and amphibian larvae (Crump et al. 2002). Extensive research shows that 4-nonylphenol and related compounds are estrogenic in various fish species (Ackermann et al. 2002; Arsenault et al. 2004). This chemical is often used to evaluate new bioassay methods and biomarkers to detect estrogenic activity (Allner et al. 1999; Belt et al. 2003). Herpetofaunal exposure to octyl- and nonylphenols and mono- and diethoxylates will be greatest in urban and suburban streams and wetlands receiving effluents from wastewater treatment systems.

The National Recommended Ambient Water Quality Criteria for nonylphenol are not to exceed a 1-hour average concentration of 28 µg/L or a 4-day average concentration of 6.6 µg/L more than once over 3 years (King 2006). A recent review of previous measurements of alkylphenol ethoxylates and related metabolites in surface waters of the United States and subsequent exposure analysis concluded that 97% of samples fell below the National Recommended Ambient Water Quality Criteria for nonylphenol. Therefore, chemicals related to 4-alkylphenol polyethoxylate surfactants are a concern with respect to endocrine-disrupting effects on aquatic organisms and herpetofauna, but exposure levels of these chemicals in aquatic environments may fall well below those that cause endocrine-disrupting effects. Further work to determine the sensitivity of various species to this class of chemicals is needed.

15.1.4 Antibacterial Products

Triclosan and triclocarban are bactericides used in numerous consumer products like cosmetics, toothpaste, hand soaps, shampoos, and plastics. Triclocarban has been included in the high-production-volume chemical challenge by the EPA (http://iaspub.epa.gov/oppthpv/quicksearch.display?pChem=101315). Due to the nature of the products in which they are used, residues of these chemicals are generally rinsed into wastewaters very quickly after use. Therefore, wastewater effluents and solids from wastewater processing have been identified as the most important sources of these 2 chemicals. Both chemicals are relatively lipophillic, with estimated log K_{ow} values of 4.9 for trichlocarban and 4.8 for triclosan (Halden and Paull 2005), and both chemicals are persistent in soils under anaerobic conditions. However, triclosan has a shorter half-life in aerobic soils than triclocarban (18 vs. 108 days, respectively; Ying et al. 2007).

Triclosan and its methyl derivative, methyl triclosan, have been found in surface waters, wastewaters, sediments, biosolids, and fish (Adolfsson-Erici et al. 2002). Triclosan is susceptible to photodegradation in aqueous solutions under certain conditions (Lindström et al. 2002), but in general is relatively stable in surface waters. Examination of triclosan fate in modern wastewater treatment plants indicates it is very effectively removed from influent water (87 to 95%), with methyl triclosan concentrations higher in effluent waters than in the influent (Bester 2005). Both biological degradation and sedimentation were important removal processes during waste treatment, thereby concentrating triclosan in resulting biosolids material (Ying and Kookana 2007).

In an aquatic toxicity assessment of triclosan using chronic and acute toxicity measurements across 14 species of fish, invertebrates (no amphibians), and algae (Capdevielle et al. 2008), algae were more sensitive than the other species with a no observable effects concentration (NOEC) of 0.69 µg/L. The resulting risk assessment concluded that risks to aquatic species were low even near wastewater discharge points. Similar conclusions of minimal risks to aquatic organisms were made in a separate ecological risk assessment of triclosan (Reiss et al. 2002). Reiss et al. (2009) published a risk assessment of the chemical in terrestrial systems and concluded that risk to birds and mammals was low, but they did not include amphibians or reptiles in either study. Results from a study of triclosan toxicity on *Bufo americanus* tadpoles were inconclusive in that the intermediate exposure level (2.3 µg/L) had highest mortality rates, but the highest survivorship was observed at the highest exposure level (230 µg/L; Smith and Burgett 2005). A similar study with *Rana pipiens* tadpoles (Fraker and Smith 2004) with the same exposure levels as Smith and Burgett (2005) found lower survivorship and startle response at the highest exposure level.

Triclocarban, triclosan, and methyl triclosan residues bioaccumulated in algae and snails immersed in water near the outfall of a wastewater treatment plant (Coogan and La Point 2008). Recent reports on triclocarban indicate that while it is not an endocrine disrupter, it enhances the activity of estradiol (E2)-dependent or testosterone-dependent activation of estrogen- and androgen-responsive gene expression (Ahn et al. 2008), suggesting a new mode of action for endocrine-disrupting chemicals. Much less information has been published on the toxicity of triclocarbon relative to that of triclosan. This new information on the potential for triclocarban to enhance the activity

of other pollutants suggests that this chemical requires further examination with respect to herpetofauna toxicity.

15.1.5 UV Filters

Another group of chemicals associated with personal care products are ultraviolet light-filtering compounds used in sunscreens and cosmetics. Four of these compounds, benzophenone-3 (BP-3), 4-methylbenzylidene camphor (4-MBC), ethylhexyl methoxycinnamate (EHMC), and octocrylene (OC), have been found in surface waters, wastewaters, and fish tissue in Swiss lakes (Balmer et al. 2005; Buser et al. 2006), and 23 others were also listed in a recent publication by Diaz-Cruz et al. (2008). Examination of the fate of selected UV filters in wastewater plants suggests typical processing is only partially effective at removing residues from effluents (Li et al. 2007a). Some UV filters are lipophillic in nature with log K_{ow} values in the same range as other persistent organic pollutants (Table 15.1). UV filters such as BP-3, 4-MBC, and OC have exhibited estrogenic characteristics in rats (Schlumpf et al. 2001), and both in vitro and *in vivo* estrogenic effects were seen in rainbow trout and fathead minnows, with vitellogenin induction occurring at 435 µg/L with benzophenone-1 and 4900 µg/L with benzophenone-2 (Kunz and Fent 2006). Benzophenone-2 also was bioaccumulated in fathead minnow and exhibited negative effects on reproduction at a concentration of 1.2 mg/L, and complete cessation of spawning activity was observed at 9.7 mg/L (Weisbrod et al. 2007). Relatively little is known of the fate of these chemicals in the environment and potential exposure by amphibians and reptiles, and no published studies of toxicity or effects on reproduction on herpetofauna are available at present.

15.1.6 Fungicides

A number of new fungicide chemistries have been developed over the last few years, and fungicide use has increased significantly in the United States, with the invasion of Asian soybean rust disease beginning in 2004.

15.1.6.1 Strobilurins

Strobilurins include at least 9 products, including trifloxystrobin (Bayer), azoxystrobin (Syngenta), and fluoxatstrobin (Bayer) (www.alanwood.net/pesticides/class_fungicides.html), and are one of the classes of fungicides recommended for control of soybean rust disease. Their mode of action includes inhibition of the electron transport system. These chemicals are practically nontoxic to birds and mammals but are highly to very highly toxic to fish and other aquatic animals. Trifloxystrobin is a broad-spectrum fungicide, which, in different formulations, is registered for cucurbit vegetables, peanuts, pome fruits, grapes, turfgrass, and ornamentals. Application rates depend on formulation and crop. It has an LC50 of 0.014 mg/L for rainbow trout and 0.054 mg/L for bluegill (*Lepomis macrochirus*). However, it rapidly degrades in the environment with a half-life measured in hours or days to a more stable acid metabolite of unknown toxicity (USEPA 1999).

Similarly, azoxystrobin is registered for a variety of fungicide diseases on golf courses and turf farms. Application rates vary by crop. It is moderately to highly toxic in freshwater fish, with a 96-hour LC50 of 0.47 mg/L for rainbow trout and 1.1 mg/L for bluegill (USEPA 1997). The principal degradate of azoxystrobin has an LC50 in rainbow trout of >150 mg/L and is considered to be practically nontoxic. Aqueous exposures to relatively high concentrations of azoxystrobin (0.5 mg/L) were found to be acutely toxic to *Rana temporaria* tadpoles and had negative effects on body length, as well as at lower concentrations of 0.13 and 0.03 mg/L (Johansson et al. 2006). Chronic exposure to concentrations of 10 and 1 µg/L did not cause any measurable effects on growth, survival, or metamorphosis (Johansson et al. 2006). The only report on its stability is that the chemical is "chemically stable for at least 14 days" (USEPA 1997).

Fluoxastrobin is registered for controlling early and late blight, leaf spots and rust, and *Rhizoctonia solani* in peanuts, tuberous and corm vegetables, leaf petiole vegetable turf, fruiting vegetables, and seed potatoes (USEPA 2005b). Up to 2.5 kg/ha can be applied per season. Fluoxastrobin degrades slowly with a half-life estimated at 1 month to over 1 year. The 96-hour LC50 for rainbow trout is 0.435 mg/L, and the no observed adverse effects concentration (NOAEC) for a 28-day exposure was 0.055 mg/L, and predicted exposure concentrations were as high as 0.033 mg/L (USEPA 2005b).

As with many of the other compounds in this chapter, concern for these chemicals occurs for several reasons: 1) there are few or no data on measured concentrations in the field under actual operations; 2) whereas acute data exist for 1 or 2 species of fish, no data are available for amphibians or reptiles; 3) even if acute data were available for herpetofauna, toxicity tends to increase with exposure duration, so the studies should last for the entire larval period, and chemicals may be more toxic in one stage of the life cycle than others, so embryo and larval tests need to be conducted; and 4) mortality is only the most severe of effects — sublethal effects expressed at lower concentrations may be debilitating to individuals and populations.

15.1.6.2 Triazoles and Imidazole

These are fungicides that inhibit the CYP51-mediated enzyme 14α-demethylase, which is involved with sterol production and cell membrane formation (Hegelund et al. 2004). They are most effective in controlling fungi prior to spore formation and are often used as a preventative and as an early curative to fungal disease. The various types of triazoles and imidazoles are broadly used on many different crops and plants throughout the world (Fishel 2005).

Triazoles and imidazoles pose hazards to wildlife because their effects may not be limited to CYP51. Ketoconazole is a pharmaceutical that is considered a model for the functioning of triazoles and imidazoles (Ankley et al. 2007). Previous studies have shown that ketoconazole can decrease testosterone production in mammals by inhibiting other CYPs involved with steroid production (e.g., Feldman 1986). Ketoconazole also inhibits steroid production *in vivo* with fish gonadal preparations (Villeneuve et al. 2007).

Hegelund et al. (2004) investigated the effects of ketoconazole on CYP1A and CYP3A enzymes in rainbow trout and killifish (*Fundulus heteroclitus*). CYP3A enzymes are involved in liver and intestinal functions in vertebrates, especially with the metabolism of lipophilic substances that include many of the pharmaceuticals currently in use. CYP1A enzymes affect the metabolism of several organic pollutants, including polyaromatic hydrocarbons (PAHs; see Chapter 9, this volume). Thus, inhibition of either enzyme complex could have important secondary effects with an animal's ability to cope with contaminant exposure. The authors injected juvenile rainbow trout intraperitoneally with 12 to 100 mg ketaconazole/kg body mass and adult killifish with 25 mg/kg. Compared to controls, ketoconazole increased liver CYP1A protein levels and enzyme activity in rainbow trout at all dosages, but the responses to 12 and 25 mg/kg were greater than those to 50 and 100 mg/kg. Induction of CYP1A was also seen in intestines and kidney. Killifish did not show a response in CYP1A activity when injected with 25 mg/kg ketoconazole. CYP3A was inhibited at all dosages in rainbow trout and by 25 mg/kg in killifish, compared to their respective controls. In killifish induction of CYP3A was sex dependent, with greater protein induction occurring in females. Induction of CYP1A and CYP3A gene expression was at lower dosages of ketoconazole than those needed for induction in mammals or birds. The authors concluded that ketoconazole was more potent in inducing CYP1A than CYP3A enzymes in rainbow trout, but that the reverse relationship held for killifish. In either species, however, it was clear that the fungicide affected more than its targeted CYP51.

Tetraconazole, which was registered in 2005 as a fungicide on *Cercospora* leafspot and powdery mildew in sugar beets, is considered to be of concern to birds and mammals by the USEPA (2005c). Chronic risk levels of concern (LOCs) were exceeded for small birds and small mammals living in grasslands. Presumably, reptiles and terrestrial life stages of amphibians may be at similar risk, although no testing has been conducted with these vertebrate classes. Tetraconazole

is relatively persistent, with a half-life in soil, sediments, or water ranging from months to over a year. Its primary breakdown product, 1,2,4-triazole, also may be toxic at environmentally realistic concentrations. Terrestrial animals can be exposed to this fungicide through ingestion of vegetation or invertebrates; direct contact; inhalation of vapors, aerosols, or residues on dust; or ingestion of contaminated water. Secondary transfer can occur by ingesting vegetation that has taken up the chemical systemically. Aquatic organisms, including amphibian larvae, invertebrates, and fish, can be exposed through dermal absorption or uptake by gills. Tetraconazole is soluble up to 159 mg/L. In mallards (*Anas platyrhynchos*), tetraconazole significantly reduced egg laying, embryo survival, number of normal hatchlings, survival of chicks to 14 days, and chick body weight at 14 days. The NOAEC and LOAEC were 10 and 50 mg/kg diet, respectively. Reproductive effects in mallards and in rats may be due to endocrine disruption. Estimated doses to birds and mammals ingesting contaminated foods in natural settings ranged from 1 to 46 mg/kg. Greatest risk was to small (≤20 g) birds and mammals, which would also include many species of lizards and amphibians. Toxicity data on aquatic species are limited, but the 96-hour LC50 for bluegill was 3.85 mg/L and the 28-day LOAEC for growth in fathead minnows was 0.96 mg/L. These data, of course, are not very predictive for chronic effects in amphibian larvae. The greatest risk from tetraconazole to reptiles and amphibians is probably related to its reputed (but unproven) endocrine-disrupting effects, which would occur at exposure concentrations less than those necessary to kill animals outright.

Other recently registered conazoles are only slightly less problematic. Prothioconazole is used as a broadcast fungicide on barley, canola, chickpeas, oil seed crops, beans, lentils, and wheat. Its half-life in soils ranges from 553 to 1386 days. Under aerobic conditions it changes rapidly in water (half-life 15 to 20 days) into a comparably toxic degradate, and in anaerobic sediment its half-life is from 2 to 8 months (USEPA 2007). Limited data do not reveal any great, direct threat to aquatic animals, including amphibians. The substance is practically nontoxic to birds and mammals and, by extension, probably not a major threat to reptiles. However, prothioconazole and its degradate, prothioconazole-desthio, are highly toxic to freshwater aquatic plants and invertebrates. The greatest risk to amphibians and aquatic or semiaquatic reptiles, therefore, may be through habitat degradation.

Cyazofamid is classified as a cyanoimidazole fungicide for downy mildew on cucurbit vegetables and blight in tomatoes and potatoes. It has a short half-life of several days and is listed as practically nontoxic to birds and mammals (USEPA 2004). The water solubility of cyazofamid is not accurately known but is estimated as 0.107 mg/L. At 0.179 mg/L growth of larval fathead minnows was significantly reduced. This suggests that the fungicide may be problematic at ppb concentrations to larval amphibians. Significant reproductive effects, including reduction in nestling survival, thinned eggshells, reduced hatching success, and depressed body weights in adult females, were observed in northern bobwhite (*Colinus virginianus*) and Japanese quail (*Coturnix coturnix*). The EPA recommended that further endocrine testing with wildlife species should be done (USEPA 2004), but data from those studies, if performed, were not found.

Triazoles and imidazoles may present risk to both amphibians and reptiles, depending on the type of fungicide. There is potential risk due to endocrine disruption, habitat deterioration, and direct mortality at concentrations that are environmentally realistic. This risk is magnified by the environmental persistence shown by some of the chemicals included here. As with other contaminants in this chapter, there is a paucity of data and no field studies have been conducted. In fact, methods for detection of some triazoles and imidazoles in environmental matrices are not available. The use of these fungicides has expanded tremendously in the past few years, and their environmental consequences are yet to be determined.

15.1.6.3 Other Fungicides

Dimethomorph is a systemic morpholine fungicide registered for use on potatoes, tomatoes, grapes, and other vegetables and fruits (USEPA 1998). As the sole active ingredient in formulation, it has moderate toxicity to freshwater fish and should not pose extreme hazards to aquatic life stages of

amphibians. It is practically nontoxic to birds and mammals, in either acute or chronic presentations, and may not be of concern to terrestrial amphibians or reptiles when used appropriately. However, the Acrobat® formulation combines dimethomorph with the carbamate mancozeb. Neither pesticide alone seems to be highly toxic to fish. For example, the 48-hour LC50 for mancozeb is 2.2 mg/L in rainbow trout and 5.2 mg/L in catfish (*Ictalurus punctatus*; E. I. DuPont Nemours 1983). The combined toxicity, however, translates to a 96-hour LC50 of 0.03 mg/L dimethomorph and 0.26 mg/L mancozeb on freshwater fishes (USEPA 1998).

Boscalid is an extremely stable carboxamide fungicide registered for food crops, including "beans, berries, bulb vegetables, canola, carrots, fruiting vegetables, grapes, lettuce, peanuts, pistachios" and 10 other food groups (USEPA 2003a p 1). During the testing required for registration, half-lives under various conditions were not obtained. Instead, the pesticide fact sheet (USEPA 2003a p 13) states the following: "Boscalid is hydrolytically stable and is photolytically stable on soil and in water. The compound is not transformed to any significant extent in either aerobic or anerobic aquatic systems, but is relatively rapidly transferred (dissipation half-lives of <2 weeks) from the water phase to the sediment phase of sorbing to the sediment." "Based on the results obtained at 25 °C, the parent compound is not expected to hydrolyze in the environment, rendering hydrolysis an insignificant fate process for boscalid." "… photodegradation is not expected to be a significant route of dissipation for boscalid in the environment." "For assessment purposes, boscalid may be considered to be essentially stable to microbial degradation in anaerobic soils." The degradation of boscalid in aerobic soils was slow, with half-lives ranging from 96 to 578 days. The majority of the compound's apparent degradation is actually due to its transformation to bound residues rather than to actual degradation or complete mineralization of the compound. In other words, it appears that boscalid is inert to common degradation pathways, but that it binds to soil and sediment particles for indefinite duration. The binding may reduce the bioavailability of boscalid, but we cannot help but to question the possibility of long-term effects of such a stable compound and what it might mean for benthic organisms such as the larva of some species of amphibians. While boscalid is considered practically nontoxic to birds and mammals, the only aquatic toxicity data we could find was a 96-hour LC50 of 2.7 mg/L and a 97-day NOAEC of 0.12 mg/L in rainbow trout. "Boscalid is classified as 'suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential'" (USEPA 2003a). Can we say the same about benthic organisms?

Famoxadone is another fungicide that is very highly toxic to aquatic organisms and poses some threat to amphibians (USEPA 2003b). It is used in formulation with the fungicide cymoxanil on peppers, tomatoes, potatoes, curcubits, lettuce, and grapes. Famoxadone is not persistent and has a half-life of a few days. The fungicide can bioconcentrate with bioconcentration factors in fish ranging from 971 to 3608. The acute, 96-hour LC50 to bluegill is 13 µg/L, with an NOAEC of 9.3 µg/L. In chronic exposures the NOAEC = 1.4 µg/L and the LOAEC = 4.1 µg/L. "Agency analysis indicates that famoxadone presents the greatest risks to fish and aquatic invertebrates through spray drift and runoff in the dissolved phase as compared to the other taxonomic groups evaluated in this assessment" (USEPA 2003b). It is fair to include amphibians in that risk as well. The USEPA also states that the risk quotients for herbivorous and insectivorous birds and herbivorous mammals exceeded the levels of concern in wildlife food items and that there could be chronic risks. Similar warnings may be added for reptiles, although there are no toxicity data on this group for famoxadone.

15.2 CONCLUSIONS

It is axiomatic that to be at risk from a chemical contaminant, wildlife must be exposed to it at sufficiently high concentrations to cause acute, often lethal, effects, or at lower concentrations for a longer time to produce chronic sublethal effects. A major handicap in predicting the threat from a contaminant is that most toxicological studies in amphibians have been conducted in the laboratory.

These highly controlled studies provide information on possible lethal and sublethal effects but often are deficient in estimating risk under more complex field situations. For the most part, we lack even laboratory studies in reptiles. For many of the chemicals described in this chapter we have neither field nor laboratory data for either amphibians or reptiles. Data on other wildlife such as fish, birds, or mammals can suggest possible risk, but cannot confirm it. In addition, new methods are often required to detect some contaminants in the environment or in tissues. These methods are often lacking or are in development so that ecotoxicologists are not always aware that the contaminants are even present.

The chemicals highlighted in this chapter were chosen because they show at least some evidence of effects, are manufactured in high to very high quantities, and demonstrate either widespread dispersal or broadscale use. Fungicides, for example, are used extensively in many types of agriculture and are often aerially sprayed, thus increasing the potential for dispersal. They can contact wildlife directly or enter waterways through runoff and pose problems for aquatic herpetofauna. Biocides and pharmaceuticals do not appear to present much risk as direct exposures to herpetofauna, but they enter waterways through municipal water treatment plants and may locally cause problems. Brominated flame retardants appear to be the "new PCBs." They, like perfluorinated hydrocarbons, have become globally dispersed. While they do not appear to be acutely toxic at environmentally realistic concentrations, their possible sublethal effects on free-ranging populations of herpetofauna, including endocrine disruption, are not well known. Similar concerns can be raised about anionic surfactants whose endocrine-disrupting properties are better known. The bottom line is that we really do not know to what extent these chemicals pose risks to amphibian and reptile populations.

In this chapter we describe only a few of the thousands of emerging chemicals that potentially threaten wildlife. As we stated in the beginning, others may disagree with this list as being too limited or perhaps not including some candidates that they believe pose greater threats. The intent of this chapter was not to be exhaustive but to increase awareness.

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16 A Decade of Deformities
Advances in Our Understanding of Amphibian Malformations and Their Implications

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CONTENTS

Abnormal amphibians have been reported in the literature for centuries. Since at least the 1700s, malformed amphibians, especially frogs with multiple hind limbs, have been documented in research articles, illustrated in atlases, and preserved in museums (Vallisneri 1706; de Superville 1740; Van Valen 1974; Ouellet 2000). Generally, such reports have involved an isolated individual or, in rare cases, a few animals. But it was not until August 1995, when a group of Minnesota middle school students discovered a pond in which 50% of the frogs had missing limbs, extra limbs or feet, bony protrusions, and skin webbings, that the issue of malformed amphibians was catapulted to the forefront of the public's attention (Kaiser 1997, 1999; Schmidt 1997; Souder 2000). Subsequent field surveys discovered large numbers of abnormal frogs in other regions of North America (NARCAM 2008), particularly parts of the Midwest (Helgen et al. 1998; Hoppe 2000, 2005; Vandenlangenberg et al. 2003), the West (Johnson et al. 1999, 2002, 2003), Alaska (Reeves et al. 2008), and the Northeast (Converse et al. 2000; Kiesecker 2002; Eaton-Poole et al. 2003; Levey 2003). The causes of observed abnormalities and their potential implications for human health and wildlife conservation became a topic of intense scientific debate and controversy. Now, more than a decade later, we revisit the issue and evaluate scientific progress in our understanding of amphibian malformations and their environmental significance.

16.1 BACKGROUND AND HISTORICAL CONTEXT

A thorough review of the historical literature on amphibian malformations can be found in the first edition of this book (Ouellet 2000). In the current edition, we build upon this historical foundation by incorporating recent advances in the study of malformed amphibians and addressing the implications of contemporary observations. We review laboratory and field data and propose questions that involve complex, large-scale issues of land use and environmental change. Throughout the chapter, we highlight existing data gaps and recommend areas that need further investigation. For a review of the patterns and mechanisms involved in limb development and disruption, readers are referred to other sources (Bryant et al. 1987; Gilbert 1997; Stopper et al. 2002).

Any investigation of amphibian malformations must begin by identifying what is "normal" or expected in an amphibian population, for which some terminology must also be defined. An "abnormality" is a general term referring to "any gross deviation from the normal range in morphological variation," and includes both "malformations" (permanent structural defects resulting from abnormal development) and "deformities" (alterations, such as amputation, to an otherwise correctly formed organ or structure; Johnson et al. 2001a). Abnormalities occur in all organisms, but what prevalence is considered normal for amphibians? Normal frequencies of abnormalities have been estimated at 0 to 2% (Ouellet 2000) and 0 to 5% (Johnson et al. (2001b). Recent studies looking at large numbers of frogs across Canada and the United States support a baseline frequency of 5% or lower: Minnesota (2.5%; Hoppe 2000), Michigan (0.14%; Gillilland and Muzzall 2002), the midwestern and northeastern United States (1.4% to 2.6%; Converse et al. 2000; Schoff et al. 2003), western Canada (0.2%; Eaton et al. 2004), Vermont (1.6%; Taylor et al. 2005), and Illinois (0.4%; Gray 2000). Surprisingly, many disagreements over the relative importance of potential causes of amphibian malformations can be explained by simple differences in how the "problem" is operationally defined. Thus, our focus here is explicitly on reports of abnormalities in amphibian populations that significantly and consistently exceed 5% (as determined by the 95% confidence interval around the estimate, which will be influenced by the percentage of malformed animals observed and by the total sample size examined). The use of a 5% threshold in conjunction with statistical confidence intervals may underestimate the number of amphibian populations or species exhibiting an abnormal level of malformations, but we believe this offers a rigorous and defensible approach for consistently defining the problem. While estimates of abnormality frequency are subject to various forms of bias (e.g., malformed frogs may be easier to catch and therefore overestimated, or may be quickly eliminated by predators and therefore underestimated), the use of a clearly defined "null hypothesis" of what is expected in a population allows sampling efforts to determine whether observed patterns differ statistically from what might be considered normal.

Several recent studies have reported much higher levels of malformations in newly metamorphosed frogs, ranging from just above baseline to greater than 50% (Helgen et al. 1998; Johnson et al. 1999, 2002, 2003; Converse et al. 2000; Kiesecker 2002; Eaton-Poole et al. 2003; Lannoo et al. 2003; McCallum and Trauth 2003; Vandenlangenberg et al. 2003; Hoppe 2000, 2005; Bacon et al. 2006; Gurushankara et al. 2007; Reeves et al. 2008). Observed abnormalities vary by site and by species but generally affect the limbs, including missing and partially missing limbs, irregular skin pigmentation, abnormal or deficient bone formation, extra limbs and limb elements, and a variety of other conditions that challenge conventional terminology (Figure 16.1). It is these sites and reports that have been the focus of more intensive studies to understand the factors responsible. Frogs with abnormal internal organs are rarely reported at high frequency and will not be emphasized in this review. This lack of information on internal malformations in wild frog populations is at least partially the result of difficulties inherent in documenting internal abnormalities without sacrificing large numbers of animals, which is not usually done in field investigations.

Historically, severe malformations are relatively uncommon in amphibian populations. Museum studies and resurveys of historic field sites suggest that, in some regions, abnormalities are increasing

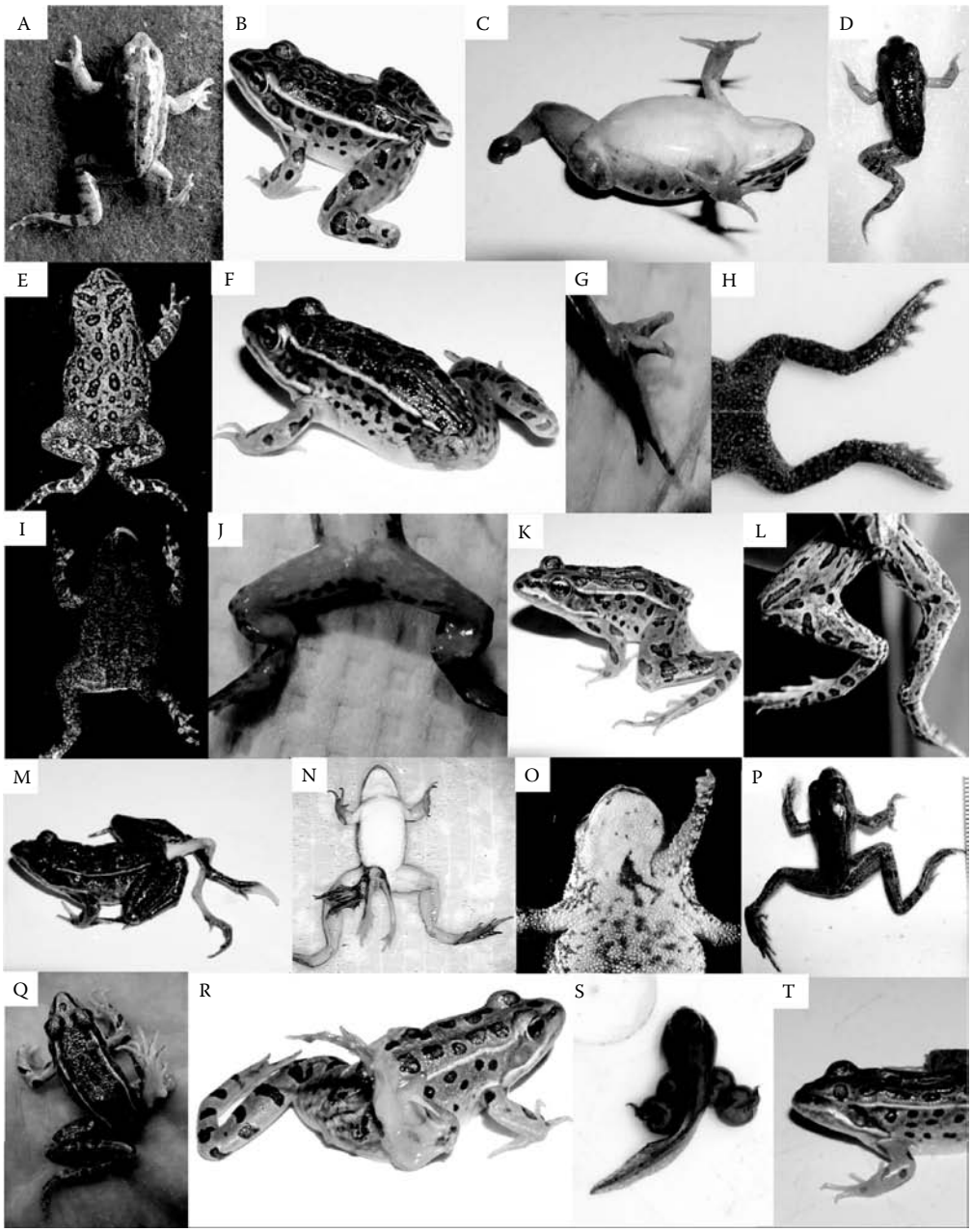

FIGURE 16.1 Abnormalities in wild-caught North American frogs. A) *R. sylvatica*, micromelia on right hind limb; B) *R. pipiens*, bilateral ectromelia; C) *R. pipiens*, apody on right hind limb and ectromelia at femur on left hind limb; D) *R. sylvatica*, ectromelia of right hind limb; E) *B. americanus*, ectromelia of left fore limb; F) *R. pipiens*, ectromelia of left hind limb; G) *R. pipiens*, syndactyly; H) *B. americanus*, brachydactyly of left hind limb; I) *B. americanus*, taumelia of right hind limb (note shortening of limb); J) *R. pipiens*, bilateral taumelia; K and L) *R. pipiens*, cutaneous fusion (skin webbing) between femur and calf on left hind limb; M) *R. pipiens* polymelia; N) *R. sphenocephala*, polymelia with extra ventral pelvis; O) *B. americanus*, polymelia of forelimb; P) *R. pipiens*, polydactyly with anteversion of left hind limb; Q) *R. pipiens*, taumelia and polydactyly of right hind limb; R) *R. pipiens*, hind limb malformation that challenges categorization; S) *R. draytonii*, bilateral edema of limbs; T) *R. pipiens*, anophthalamia of left eye. See Glossary at the end of the chapter for a description of terminology.

(Johnson et al. 2003). Working in Minnesota, Hoppe (2000, p 86) used both of these techniques and concluded that frog abnormalities were "more frequent, more varied, more severe, and more widely distributed in 1996 and 1997 than in 1958–93." Similarly, McCallum and Trauth (2003) reported a steady increase in amphibian abnormalities in Arkansas, from 3.3% (1957–1979) to 6.9% (1990s) to 8.5% in 2000. Johnson et al. (2003) noted that accounts of "mass malformations," in which >5% of the amphibian population exhibits limb malformations, are extraordinarily rare in the historical literature in the United States (1900–1990), particularly in contrast to the number reported since 1990 (e.g., NARCAM 2008). Collectively, these findings support the hypothesis that recently observed patterns of amphibian malformations deviate from the historical precedent. In the sections that follow, we review and evaluate available evidence for proposed causes of such malformations.

16.2 POSSIBLE CAUSES

Isolating the causes of abnormalities in amphibians is a necessary step in determining the implications of the phenomenon and the potential risks for both amphibians and humans. While no single cause can explain all deformities, several features have proven consistent. A review of the recent and historic cases yields the following observations: 1) abnormalities primarily involve the hind limbs, whether in the form of missing, extra, or malformed limbs (Hebard and Brunson 1963; Ouellet et al. 1997; Helgen et al. 1998; Hoppe 2005); 2) juvenile or premetamorphic frogs are the age class most commonly affected (Merrell 1969; Volpe 1977; Reynolds and Stephens 1984; Sessions and Ruth 1990; Johnson et al. 2001b, 2002); and 3) most abnormal individuals come from lentic or still-water habitats (Bishop 1947; Houck and Henderson 1953; Cunningham 1955; Hauver 1958; Lopez and Maxson 1990). Few cases of abnormal stream- or river-dwelling amphibians have been reported (see Cooper 1958; Ruth 1961; Banta 1966). These features direct our search toward causative agents acting on larval frogs with developing hind limbs, suggesting the causative agent is either in the water or affects amphibians while in an early aquatic stage.

Numerous factors have been proposed to explain contemporary observations of abnormal amphibians, including parasite infection, injuries from predation, chemical contaminants, UV-B radiation, ground-level ozone, radioactive mineral deposits, nutritional deficiencies, teratogenic viruses and fungi, acid precipitation, extreme temperatures, and even reformulated gasoline (Ouellet 2000; Blaustein and Johnson 2003). Genetic mutation is undoubtedly responsible for a portion of the abnormalities, and at least one gene is known to cause malformed limbs in amphibians (Droin and Fischberg 1980). However, the occurrence of abnormal amphibians at high frequency, in multiple species, and across a broad geographic scale has caused many to discount natural mutation as an important factor (Meteyer et al. 2000; Loeffler et al. 2001; Blaustein and Johnson 2003; Ankley et al. 2004), and genetic analyses of normal and malformed animals have revealed no signs of inbreeding (Williams et al. 2008). In this chapter, we focus on the causative agents that are likely to impact larger areas and have received extensive research attention: parasitic trematodes, injury and attempted predation, chemical contaminants, and UV-B radiation (Ouellet 2000; Cohen 2001; Loeffler et al. 2001; Blaustein and Johnson 2003; Ankley et al. 2004). We also discuss multiple stressor interactions among these factors and their potential significance for amphibian population viability.

16.2.1 Parasite Infection

Trematode parasite infection is probably the most thoroughly studied cause of amphibian limb malformations thus far. This hypothesis was first suggested by Sessions and Ruth (1990), who reported high frequencies of malformed Pacific chorus frogs (*Pseudacris regilla*) and long-toed salamanders (*Ambystoma macrodactylum croceum*) in a California pond. Upon clearing and staining specimens collected from the pond, Sessions and Ruth (1990) discovered high concentrations of trematode

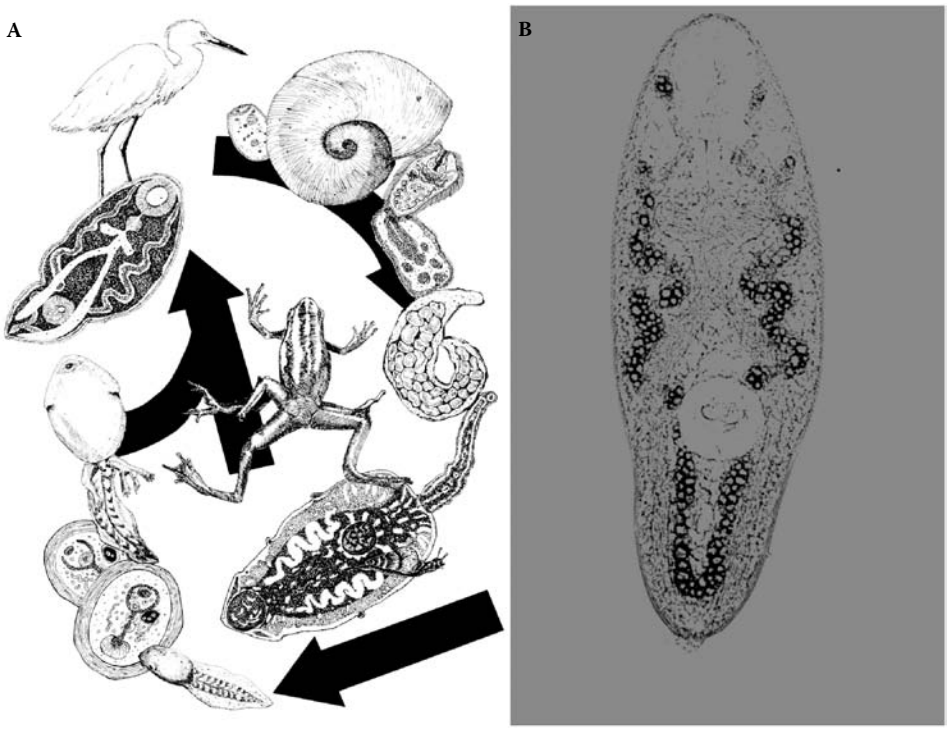

FIGURE 16.2 The digenetic trematode *Ribeiroia ondatrae* causes severe limb malformations in amphibians. A) The complex life-cycle of the parasitic trematode, *Riberoria ondatrae* (drawing courtesy of Brandon Ballengée); B) Metacercaria of *R. ondatrae* isolated from a malformed amphibian (image courtesy of D. Sutherland).

cysts around the limbs of affected individuals. Subsequent work later identified the parasite responsible as *Ribeiroia ondatrae* (Johnson et al. 1999, 2003), a digenetic trematode with a multihost life cycle (Figure 16.2). *Ribeiroia* moves sequentially among freshwater snails (rams horn snails in the genera *Planorbella* and *Helisoma*), larval amphibians, and finally, birds or, less frequently, mammals (Johnson et al. 2004). Infected snails release large numbers of mobile cercariae, which seek out and infect larval amphibians. Because cercariae specifically encyst around the developing limbs of amphibians, they can cause severe disruptions in limb growth leading to malformations. Interestingly, these malformations may aid transmission of the parasite by increasing the vulnerability of infected amphibians to bird predators, which become infected when they consume the amphibian host. The parasite completes its life cycle in the bird, wherein it develops into a sexually mature adult and releases its eggs in the feces of the bird (Figure 16.2).

Extensive experimental evidence now supports a causal link between *Ribeiroia* infection and limb malformations in amphibians. Exposure to realistic numbers of *Ribeiroia* cercariae causes increased mortality and severe malformations in species of frogs, toads, and salamanders (Johnson et al. 1999, 2001b, 2006; Kiesecker 2002; Stopper et al. 2002; Schotthoefer et al. 2003; Johnson and Hartson 2009). The frequency of malformations induced is often high, and can reach 100% among surviving animals. From this body of work have emerged several important patterns:

1) Malformations and mortality are dose dependent; higher levels of *Ribeiroia* exposure increase the risk and the severity of malformations produced, as well as the likelihood that animals die following exposure. Low levels of infection may or may not cause any obvious pathology.

2) The timing of parasite exposure influences the degree of pathology and the types of malformations. Malformations are most likely to occur when larval amphibians are exposed during early limb development (Bowerman and Johnson 2003; Schotthoefer et al. 2003). Exposure to *Ribeiroia* prior to limb growth also may increase the risk of mortality (e.g., Schotthoefer et al. 2003), whereas exposure after limbs are fully developed is unlikely to alter development. This suggests that amphibian species with differing larval development periods may exhibit different levels of infection or malformations, even within a single wetland (see Johnson et al. 2001a; Hoppe 2005).
3) Amphibian species vary in their responses to *Ribeiroia* infection. Exposure to identical levels of *Ribeiroia* infection may cause different types or frequencies of malformations in different species. For example, while Pacific chorus frogs exhibit predominantly extra limbs and digits in response to *Ribeiroia* (Johnson et al. 1999, 2002), American toads (*Bufo americanus*) suffer primarily from skin webbings and bony triangles, with very few extra limbs (Johnson and Hartson 2009). Some amphibian species (e.g., gray treefrogs [*Hyla versicolor*]) appear to be resistant to *Ribeiroia* infection, possibly owing to differences in innate immune response (Johnson and Hartson 2009).

Field evidence further substantiates the association between *Ribeiroia* and certain types of malformations. Working in the western United States, Johnson and colleagues (1999, 2002, 2003) have reported strong associations between the presence of *Ribeiroia* and the presence of severe limb malformations (significantly >5%). Importantly, higher concentrations of *Ribeiroia* infection are positively correlated with the frequency of malformations, emphasizing the functional relationship between parasitism and amphibian abnormalities in nature (see also Johnson and Chase 2004). Kiesecker (2002) reported similar patterns for a subset of wetlands in the Northeast, and showed that the experimental exclusion of *Ribeiroia* cercariae effectively eliminated malformations within field enclosures. *Ribeiroia* infection has also been linked to cases of severe malformations in the Midwest (e.g., Lannoo et al. 2003; Sutherland 2005; Johnson and Hartson 2009). It is important to emphasize, however, that not all methods used to detect parasites are equally effective. Clearing and staining of amphibians, which often involves removal of the skin and digestion of tissue, will often destroy parasites, leading to the false conclusion that *Ribeiroia* is absent (e.g., see Gardiner and Hoppe 1999 and Sutherland 2005 for contrasting findings for *Ribeiroia* infection in the "CWB" wetland in Minnesota). Examination of preserved frogs, frozen material, or histological sections can also obscure the identity of isolated parasites, as trematode metacercariae are often difficult to identify. Researchers should strive to examine freshly isolated, living parasites to detect key morphological features (see Sutherland 2005; Johnson and Hartson 2009).

Despite strong links between *Ribeiroia* infection and amphibian malformations in certain species and regions, *Ribeiroia* does not explain all accounts of malformations in amphibians. Some wetlands with a high frequency of abnormal frogs do not support *Ribeiroia* infection (Lannoo et al. 2003; Reeves et al. 2008). One of the advantages of trematode infection as a hypothesis to explain malformations is that it is very easy (and inexpensive) to test. If *Ribeiroia* is not found in amphibians following examination by a trained parasitologist, it is unlikely to be responsible for observed abnormalities. Using this method, researchers have eliminated *Ribeiroia* as a potential cause at several known malformation sites (see Lannoo et al. 2003; Linzey et al. 2003; Bacon et al. 2006; Reeves et al. 2008). If *Ribeiroia* is found in amphibians, it may or may not be responsible for frog abnormalities, depending on the amount of infection, the timing of infection, the amphibian species involved, and the types of abnormalities observed. For example, low levels of *Ribeiroia* or infection occurring outside of the limb development period are unlikely to cause malformations. In our experience, *Ribeiroia* is most commonly associated with specific types of malformations, such as skin webbings, bony triangles, shortened long bones, and missing or extra limb elements (Figure 16.1). However, extra limbs may or may not occur, depending on the amphibian species involved (see above). Thus, the recurring suggestion that *Ribeiroia* causes only (or even predominantly) extra

limbs is a gross oversimplification (e.g., Skelly et al. 2007). However, *Ribeiroia* is rarely associated with sites that produce exclusively missing limbs and/or digits in the absence of other malformation types, as reported at a number of wetlands (e.g., see Reeves et al. 2008).

Thus far, *Ribeiroia* is the only well-documented parasite to cause limb malformations in amphibians. Several other trematodes (e.g., *Alaria* and *Echinostoma*) failed to induce limb abnormalities in laboratory experiments (Fried et al. 1997; Johnson et al. 1999). However, growing evidence suggests that other parasites may also cause disruptions in limb development. For example, Rajakaruna et al. (2008) reported that an unidentified trematode (monostome type) caused missing and abnormal limbs in the common hourglass frog (*Hyla ebraccata*) in Sri Lanka. Whether such abnormalities also occurred at field sites with the parasite is unknown. Other parasites have also been linked to morphological abnormalities in amphibians. Murphy (1965) found that infections by an ectoparasitic mite were associated with limb and heart anomalies in pickerel frogs (*Rana palustris*). Similarly, Kupferberg et al. (2009) reported a significant association between ectoparasitic copepod infestations (*Lernaea* sp.) and limb abnormalities in foothill yellow-legged frogs (*Rana boylii*). Among larval and metamorphosing frogs, the prevalence of missing and abnormal limbs was 26% in copepod-infected individuals but only 1.1% in uninfected animals. Finally, Rostand and Darré (1969) suggested that a teratogenic virus excreted by fish was responsible for high rates of severe malformations observed over several decades in Europe (Ouellet 2000). When common frog (*Rana temporaria*) larvae were exposed to fish mucus during the first few days of development, digit malformations were produced (Surlève-Bazeille et al. 1969; Ouellet 2000). However, Surlève-Bazeille and Cambar (1969) were unable to produce the abnormality observed by Rostand and Darré (1969) when exposing *R. esculenta* to the fish mucus or bacterial cultures of it (Ouellet 2000). Whether the putative virus was ever isolated and identified is unclear, and to the best of our knowledge there has been little follow-up work by other researchers.

16.2.2 Predation

Predation is often cited as the single most important factor regulating amphibian survivorship and population size (Martof 1956; Calef 1973; Licht 1974; Cecil and Just 1979; Woodward 1983). Amphibian predators encompass a diverse collection of terrestrial and aquatic taxonomic groups, including mammals, birds, reptiles, fishes, aquatic insects, crayfish, and other amphibians (Martof 1956; Calef 1973; Licht 1974; Bradford 1989; Fauth 1990; Jennings et al. 1992; Kiesecker and Blaustein 1997). Tissue predation is almost certainly responsible for some fraction of observed abnormalities, but few studies have recorded baseline levels of injury in amphibian populations, making quantification of this component difficult (Martof 1956; Dubois 1979). To date, surprisingly little research has examined the potential role of predation in causing limb abnormalities, and predators are either ignored or discounted in most discussions of amphibian malformations (e.g., Lannoo 2008). The hypothesis implicating predation as a contributor to recent occurrences of amphibian abnormalities is often criticized on the grounds that: 1) a predator is far more likely to consume an entire frog rather than simply a leg; 2) injuries should produce scar tissue at the wound site, which would be an obvious indicator of earlier trauma in a metamorphic frog; and 3) a frog could not suffer an injury as severe as the loss of an entire limb without dying (Meteyer et al. 2000; Loeffler et al. 2001; Lannoo 2003, 2008). These assumptions may hold true for some predators and for injuries inflicted during certain periods, but there are many cases in which they are not supported. Although birds, mammals, turtles, and larger fish may consume an entire frog, smaller predators such as aquatic insects, small fish, and crayfish are much more likely to attack an exposed portion of an amphibian, such as a limb or a tail. Even more importantly, many of these predators will inflict injuries not on adult frogs, but on larval amphibians. Two important facts about predator attacks on larval amphibians are worth emphasizing. First, larval anurans have some capacity for tissue regeneration; thus, if the amputation or injury occurs during limb development, subsequent regeneration — while incomplete — is often sufficient to eliminate obvious

signs of trauma, creating a malformation (Fry 1966). Second, the loss of a tadpole limb — which is only partially ossified and incompletely vascularized — is likely to be far less severe than the loss of an adult frog limb. While many tadpoles likely die in response to such severe trauma (see Bohl 1997a, 1997b), those individuals that survive the injury to metamorphose can often exhibit missing or abnormal limbs.

Growing evidence now suggests that predators play an important role in causing certain types of abnormalities in amphibians. Recent experimental studies conducted with leeches, sticklebacks, and odonate naiads indicate that predation can cause a high frequency (e.g., >5%) of amphibian limb abnormalities. Two teams of researchers in Germany concluded that leeches (*Erpobdella octoculata*) were responsible for severe malformations in several populations of the common toad, *Bufo bufo* (Figure 16.3; Viertel and Veith 1992; Veith and Viertel 1993; Bohl 1997a, 1997b). In all cases, the abnormalities were dominated by partially and completely missing limbs, which exceeded 20% in some wetlands. While no genetic anomalies or contaminants could be linked to the abnormalities, experimental enclosures that permitted water but excluded predators eliminated the abnormalities in developing anurans (Bohl 1997a, 1997b). More importantly, investigators were able to reproduce the same suite of abnormalities in toads exposed to leech attack in the laboratory (Viertel and Veith 1992). While many larval toads died from the initial attack, those that escaped often developed abnormal limbs, similar to what was seen in field observations. Because anurans have partial regenerative ability

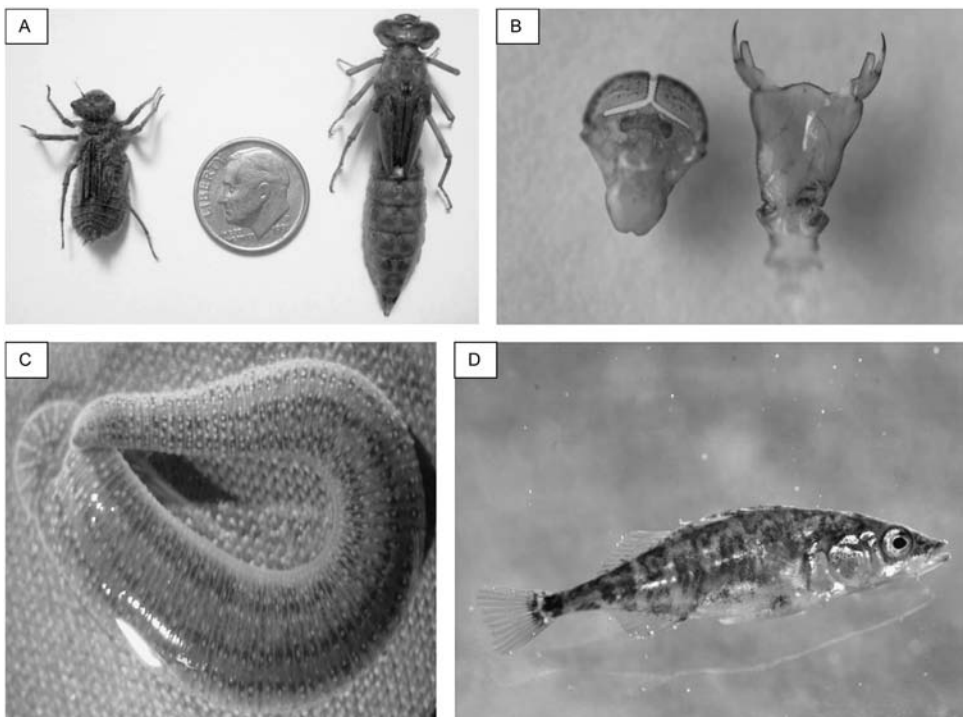

FIGURE 16.3 Aquatic predators capable of causing limb abnormalities. A) Two dragonfly larvae representing the families Libellulidae (left) and Aeshnidae (right), which both prey upon amphibian larvae (image courtesy of P. Jensen); B) Lower mandibles of libelluilid (left) and aeshnid (right) dragonfly larvae (image courtesy of J. Bowerman); C) Leeches (*Erpobdella* sp.) can attack and injure the developing limbs of tadpoles (Image copyright W. Moses, Smithsomian Institution, National Museum of Natural History, reprinted with permission); D) Three-spined sticklebacks (*Gasterosteus aculeatus*) have been linked to limb abnormalities in toads from Oregon (image courtesy of J. Bowerman).

prior to metamorphosis, some of the injuries became developmental malformations (also see Forsyth 1946; Fry 1966). Subsequent efforts to reduce the population of leeches within a wetland were successful in eliminating abnormalities in the toad population, offering compelling experimental evidence for the causal role of leeches (Bohl 1997b). Interestingly, however, because leeches were active primarily at night, investigators working during daylight did not initially realize their importance.

Similarly, Bowerman et al. (forthcoming) conducted a series of studies examining the importance of 3-spined sticklebacks (*Gasterosteus aculeatus*) and corduliid dragonfly larvae (*Somatochlora albicincta*) in causing amphibian abnormalities (Figure 16.3). Across a period of more than 10 years and >10 000 amphibians, they found that the annual abundance of non-native sticklebacks correlated positively with the frequency of limb abnormalities (missing limbs and digits) in western toads (*Bufo boreas*), which often exceeded 15%. As with the leech studies, experimental enclosures established within the lake precluded the occurrence of abnormalities in protected animals, while experimental laboratory trials revealed that sticklebacks will actively attack and injure the developing limbs of toad tadpoles, leading to abnormalities similar to those observed in the field (Bowerman et al. forthcoming). These same authors found that larval odonates (*Somatochlora albicincta*) can cause high levels (>15%) of missing and abnormal limbs in Cascades frogs (*Rana cascadae*). Through carefully designed experiments and sustained field observations, the authors established a link between the abundance of larval odonates and the types and frequencies of abnormalities observed among high-elevation wetlands in Oregon. On several occasions, odonates were observed attacking (and removing) the limbs of larval *R. cascadae*, and complementary experiments revealed that the addition of *S. albicincta* to enclosures induced the same types of abnormalities.

In the cases of leeches, sticklebacks, and larval insects, predators create an injury that either results directly in an abnormality (e.g., a missing limb) or creates a malformation during the regeneration process. This latter occurrence can be particularly important because the partially regenerated limb becomes a developmental malformation, even though it resulted from trauma. Literature on amphibian regeneration suggests that the timing and location of the injury are key determinants of the type of abnormality produced. Fry (1966), for example, found that if northern leopard frog (*R. pipiens*) limbs were amputated at early stages (i.e., prior to complete differentiation of the digits), these limbs regenerated into fully patterned albeit smaller limbs. When amputation occurred later in development, however, after joints and digits had already developed a pattern, the limb would heal into a stump lacking joints, toes, or other limb-like features (Fry 1966). More distal structures (feet and toes) retained regenerative ability longer than proximal structures (e.g., the femur). Reeves et al. (unpublished) found similar results for wood frogs (*Rana sylvatica*). Another study of 4 species of Japanese frogs produced comparable results: regenerative ability was present in all species, but varied among species and decreased with age at amputation (Kurabuchi and Inoue 1982). Taken together, these studies suggest that predator-mediated injuries early in tadpole development can cause abnormalities such as shrunken, missing, or truncated hind limbs, as documented in naturally occurring amphibian populations (see above and Reeves et al. 2008).

16.2.3 Biocides and Chemical Pollution

The hypothesis that anthropogenic chemicals in the environment are responsible for amphibian abnormalities is one of the most alarming. Amphibians are often cited as indicator species, showing heightened sensitivity to environmental conditions before they become deleterious for other species (Chandler and Marking 1975; Cooke 1981; Blaustein et al. 1994; Van der Schalie et al. 1999). Abnormalities in amphibians could represent a sublethal response to a toxic chemical in their habitat. If chemicals are the responsible agents, are they natural or anthropogenic, and what implications do abnormalities in amphibians have for human health and well-being?

Field and laboratory studies have explored whether chemical exposure plays a role in amphibian abnormalities. Field studies often focus on agricultural areas and compare abnormality prevalence in

areas with documented or presumed amphibian exposure to biocides with prevalence in "reference" areas. Laboratory experiments can be grouped into 2 categories: short-term tests with endpoints in the early-to mid tadpole stage (such as the 96-hour Frog Embryo Teratogenesis Assay–Xenopus [FETAX]) and tests with endpoints at the late tadpole stages (past hind limb emergence) or through metamorphosis. We assert that the most relevant laboratory studies for assessing possible causes of the types of abnormalities typically observed in the field are those that evaluate late-stage tadpoles or newly metamorphosed frogs. These studies should test a range of environmentally realistic and analytically documented contaminant concentrations.

In agricultural regions, amphibians are one of the nontarget groups most commonly affected by biocides, fertilizers, and their inert ingredients (Relyea 2005). This is largely because 1) the developmental timing of amphibian larvae often coincides with the season of heaviest biocide use, 2) the aquatic habitats used by amphibians may concentrate chemicals applied in the surrounding area, and 3) amphibian prey are one of the intended targets of a given biocide. Because of these spatial and temporal associations between biocides and breeding amphibians, agricultural chemicals and fertilizers have spurred numerous amphibian abnormality studies (see reviews in Ouellet 2000; Ankley et al. 2004).

Fieldwork has been an important component of investigations into agricultural and nonagricultural chemicals. Working in Canada, Ouellet et al. (1997) compared the frequency of anuran abnormalities on agricultural lands to those on nonagricultural lands. They recorded >10 times more abnormalities on agricultural sites. These results, while not statistically significant due to high variance, have been cited as suggestive and prompted additional studies. In western India, Gurushankara et al. (2007) found a higher prevalence of missing and shrunken limbs in rice paddies and coffee plantations than in forested areas used as reference sites. Taylor et al. (2005) found agricultural land use increased the risk of amphibian abnormalities in the northeastern United States. Piha et al. (2006), however, did not find such an association in agricultural areas in Finland.

Laboratory exposure studies have investigated the effects of agricultural chemicals on the prevalence of abnormalities. Here we summarize studies that noted external abnormalities. The many studies that examined whether exposure to atrazine results in gonadal abnormalities are summarized in Chapter 8. Grossly visible abnormalities have been induced by laboratory exposures to organochlorine compounds, including DDT (*Rana temporaria*, Cooke 1981), dieldrin (*Xenopus laevis, Rana catesbeiana*, Schuytema et al. 1991; *Rana perezi*, Alvarez et al. 1995), and lindane (*Xenopus laevis,* Marchal-Segault and Ramade 1981); organophosphates, such as folidol (*Rana perezi*, Alvarez et al. 1995), malathion (*Microhyla ornata,* Pawar et al. 1983), and guthion (*Xenopus laevis*, Schuytema et al. 1994); carbamates, such as ZZ-Aphox (*Rana perezi*, Honrubia et al. 1993, Alvarez et al. 1995), oxamyl (*Rana temporaria*, Cooke 1981), and carbaryl (*Rana perezi*, Bridges 2000); and certain herbicides, such as paraquat (*Rana pipiens,* Dial and Bauer 1984) and glyphosate (*Rana sylvatica*, Glaser 1998). Although the effects of these agricultural chemicals vary, it is clear they can cause skeletal abnormalities in developing amphibians. In laboratory experiments, carbamate and organophosphate pesticides caused scoliosis, shortening of the long bones, and twisted epiphyses in *Rana perezi* (Alvarez et al. 1995). The insecticide carbaryl caused 80% of exposed *Rana sphenocephala* tadpoles to develop abnormal curvature of the tail (Bridges 2000). The insecticides endosulfan and azinphosmethyl and the fungicide mancozeb caused skeletal and eye abnormalities in *Bufo americanus* and *Rana pipiens* (Harris et al. 2000). Recently, Brunelli et al. (2009) reported that endosulfan exposure of *Bufo bufo* from Gosner stage 25 (Gosner 1960) to metamorphosis resulted in an increased incidence of malformations, including bloated heads, kinked tails, and abnormal development of the mouth.

Nonagricultural pollutants have also been linked to amphibian abnormalities in the field. Amphibians exposed to coal combustion wastes exhibited a higher incidence of skeletal abnormalities in contaminated sites (18 to 37%) than in reference sites (0 to 4%; Hopkins et al. 1998, 2000). Flyaks and Borkin (2004) reported a high prevalence of hind limb abnormalities, including

polydactyly, reduction of limb segments, and asymmetric limbs in 3 anuran species (*Rana ridibunda, Bombina bombina*, and *Bufo viridis*) from the eastern Ukraine. Three areas were studied, 2 of which were highly industrialized. They measured tissue residues of metals and interpreted environmental data. The authors concluded that the frequency of abnormalities was correlated with the levels of environmental contamination.

A survey of cane toads, *Bufo marinus,* conducted from 2000 to 2003 in Bermuda, showed a high prevalence of limb abnormalities, particularly digit abnormalities and partially missing limbs (Bacon et al. 2006). To further investigate possible stressors, Fort et al. (2006a) conducted laboratory toxicity tests with *B. marinus* and *Xenopus laevis* using sediments and water collected from affected ponds. Chemical characterization of sediments indicated that metals, petroleum hydrocarbons, and ammonia occurred at potentially toxic concentrations. *B. marinus* embryos raised through metamorphosis in microcosms at the sediment-water interface exhibited abnormalities similar to those in free-ranging toads (Fort et al. 2006a, 2006b). Similarly, Sparling et al. (2006) conducted a dose-response study with *Rana sphenocephala* tadpoles using sediment from a shooting range spiked with concentrations of lead acetate. Surviving tadpoles demonstrated multiple skeletal malformations, including curvature of the spine and truncated and twisted femurs, long bones, and digits. All malformations were bilateral and essentially symmetrical, unlike most malformations observed in nature. Skeletal abnormalities occurred at concentrations of 540 mg/kg, far less than the highest contaminant concentration (5700 mg/kg) detected in wetlands near the range. Chen et al. (2006) also reported spinal deformities in northern leopard frog tadpoles exposed to lead in the water column. Scoliosis was detected in 92% of tadpoles exposed to 100 µg/L, and this condition was associated with abnormal swimming behavior. It persisted in some frogs after metamorphosis. A long-term laboratory exposure of green frogs (*Rana clamitans*) and northern leopard frogs (*Rana pipiens*) to PCB 126 through metamorphosis yielded few skeletal abnormalities (Rosenshield et al. 1999). Only the incidence of edema was significantly higher in both species exposed to the highest concentration (50 µg/L).

The fundamental challenge in investigating the role of contaminants in causing amphibian limb malformations in nature is determining which chemical(s) (or combinations thereof) to study. Testing for the full suite of these compounds and their various breakdown products in wetlands is prohibitively expensive and methodologically challenging. This has often caused investigators to focus their search on particular chemicals or classes of compounds. Beginning in the late 1990s, increased attention focused on the possible role of retinoids in causing amphibian malformations in nature (e.g., Gardiner and Hoppe 1999; Gardiner et al. 2003). Retinoids are vitamin A derivatives with well-documented tendencies to disrupt development (including limb growth) and pattern formation in vertebrates (Bryant et al. 1987; Bryant and Gardiner 1992; Maden 1993; Gilbert 1997; Gardiner and Hoppe 1999). The insect growth regulator methoprene, which was widely applied as an antimosquito agent and flea treatment for domestic pets (Harmon et al. 1995), initially received attention as a possible agent contributing to amphibian abnormalities because of the similarities between methoprene acid and retinoic acid (Henrick et al. 2002). Methoprene and its derivatives were initially found to cause developmental problems in *Xenopus* embryos even at low concentrations (Dumont et al. 1997; Degitz et al. 2000). Yet in later experiments, Degitz et al. (2003a) reported that the concentrations of methoprene required to cause developmental toxicity in amphibians were much more likely to cause mortality than developmental malformations (see also Ankley et al. 1998). Methoprene was also not correlated with the occurrence of amphibian malformations in nature (Sparling 2000; Henrick et al. 2002). Degitz et al. (2000, 2003b) arrived at a similar conclusion for the direct exposure of amphibians to exogenous retinoic acid. In experiments with both pulsed and continuous retinoic acid exposure, the authors reported that the conditions necessary to induce limb malformations in native amphibians were unlikely to occur in nature (see Ankley et al. 2004).

Building upon this foundation, Bridges et al. (2004) used a novel approach to investigate the possible role of contaminants in causing observed frog malformations. They deployed a series of semipermeable membrane devices (SPMDs), which are integrated samplers that effectively "soak up"

lipophilic organic compounds from the environment, in 2 Minnesota wetlands: one with a history of severe malformations in multiple amphibian species ("impacted" site) and one with few observed abnormalities ("reference" site). After a 30-day deployment period, the authors retrieved the SPMDs from the ponds and raised leopard frog (*Rana pipiens*) larvae in water containing extracts from the SPMDs. Intriguingly, extracts from the impacted site caused a high frequency of bony triangles and skin webbings in metamorphosing frogs, leading the authors to suggest that a chemical or combination of chemicals from the pond was teratogenic. Extracts from the reference site did not cause bony triangles. However, skin webbings were observed in >40% of animals exposed to extracts from the reference site — which did not support these abnormalities in nature — and in 20% of animals in the SPMD control treatment, suggesting that more work is needed to understand the effects of this approach on amphibian development. Linking these results to field patterns is further complicated by the finding that the impacted site in this study supports extremely high abundances of *Ribeiroia* infection (see Sutherland 2005). Nevertheless, integrated approaches such as this that attempt to identify potentially teratogenic compounds from field sites have enormous potential in helping to narrow and direct the search for environmentally important contaminants.

In summary, while it is clear chemicals can cause skeletal malformations in amphibians, and field studies suggest chemical contamination is sometimes correlated with the occurrence of abnormalities, it has only been rarely demonstrated that chemical contaminants directly cause abnormalities similar to those observed under field conditions (Fort et al. 2001, 2006a, 2006b; Bridges et al. 2004). This issue is complicated by the diversity of chemicals released into the environment, the difficulties (and expense) in detecting parent and metabolite compounds in the field, and the proclivity for chemicals to interact with one another to affect amphibians. Future tests need to incorporate more ecologically relevant approaches that critically evaluate the effects of chemical agents on limb growth in native amphibians (see Degitz et al. 2003a, 2003b; Ankley et al. 2004; Bridges et al. 2004). Boone and James (2005) reviewed the use of mesocosms in amphibian ecotoxicology and cited several studies that have used mesocosms to evaluate the prevalence of abnormalities (e.g., Bishop et al. 2000; Harris et al. 2001; Kiesecker 2002). A promising approach is the coordinated use of field surveys, laboratory exposures, and mesocosm studies supported by tissue residue analyses (e.g., Flyaks and Borkin 2004; Boone and James 2005; Bacon et al. 2006; Fort et al. 2006a, 2006b).

16.2.4 UV-B Radiation

Declines in the earth's ozone layer have been connected with seasonal increases in the level of UV-B penetration, which is suspected to have deleterious effects on wildlife (Kerr and McElroy 1993; McKenzie et al. 1999; see Chapter 13, this volume). Because many amphibians deposit their relatively unprotected eggs in shallow water, this group is perhaps particularly vulnerable to changes in UV-B. Blaustein et al. (1994) demonstrated that ambient levels of UV-B radiation in the Oregon Cascades significantly reduced the hatching success of 2 amphibian species known to be in decline. In addition to mortality, developmental abnormalities were observed in surviving embryos exposed to solar UV-B radiation. Abnormal growth and development of the tail, concave curvature of the spine, bloating or distention of the body cavity, and improper development of the cornea were recorded in the embryos of multiple species (Hays et al. 1996). Subsequent studies have also noted adverse effects from laboratory UV-B exposures. Elevated UV-B increased mortality in larval and embryonic amphibians and caused developmental abnormalities, including skin burns, spinal curvature, abnormal cornea development, eye cataracts, and delayed development (Worrest and Kimeldorf 1976; Grant and Licht 1995; Ovaska et al. 1997). To specifically investigate the connection between UV-B radiation and amphibian abnormalities, Blaustein et al. (1997) conducted a field-based experiment exposing spotted salamander (*Ambystoma macrodactylum*) embryos to ambient UV-B conditions. Embryos protected from UV-B had a 95% survival rate with less than 1% abnormality rate. In the unshielded condition, hatching success was less than 15% and the

frequency of abnormalities in surviving embryos was 86%. The predominant abnormalities were lateral flexure of the tail, blistering of the skin, and edemas (Blaustein et al. 1997).

While these studies underscore the potential danger of UV-B exposure for embryonic and larval amphibians, they do not directly address the importance of UV-B in causing limb abnormalities. Limited evidence suggests that, in some circumstances, UV-B exposure can cause limb malformations. Butler and Blum (1963), for example, reported that localized UV irradiation (at greatly elevated levels) could induce supernumerary limbs in salamander larvae (see also Ankley et al. 2004). More recent laboratory and outdoor studies have established that exposure to ambient UV-B can cause high frequencies of limb reductions or deletions in amphibians (Ankley et al. 2000, 2002). However, the resulting abnormalities were generally bilaterally symmetrical, unlike most field observations, causing the authors to question a direct role of UV-B in explaining recently observed malformations in amphibians (Ankley et al. 2002, 2004). Additionally, extensive field studies and risk analyses suggest that the levels of UV-B exposure to which amphibians are exposed in natural wetlands will often be insufficient to induce abnormalities (Peterson et al. 2002; Diamond et al. 2002). In nature, UV-B is rapidly attenuated in aquatic ecosystems, often within a few centimeters, owing to dissolved organic carbon in water (Palen et al. 2002; Diamond et al. 2002; Ankley et al. 2004). Thus, while UV-B may pose a threat to amphibian eggs (which are laid in shallow water by some species) or in exceptionally clear or shallow water, most evidence suggests that the UV-B hypothesis is unsupported as a major cause of limb abnormalities in nature (Blaustein and Johnson 2003; Ankley et al. 2004; Reeves et al. 2008). UV-B radiation is more frequently associated with problems of the cornea, skin, or neural and spinal development (Malacinski et al. 1974; Blaustein et al. 1994, 1997; Grant and Licht 1995; Ovaska et al. 1997; Ankley et al. 2004).

16.3 MULTIPLICITY OF CAUSES

Given the wide range in the composition of abnormalities, the large number of amphibian species recorded with abnormalities, and the broad geographic range affected, we suggest that multiple factors are almost certainly responsible for the amphibian abnormalities documented since the early 1990s. The involvement of multiple causative agents heightens the complexity of the investigation considerably. Sites with similar abnormalities may, in fact, be affected by unrelated causes. Alternatively, multiple, interacting causes may be operating within a single wetland.

Whereas all factors currently under investigation (chemicals, parasites, predators, or UV-B radiation) have the potential to induce abnormalities, it is difficult to assess if they actually cause abnormalities in situ. Studies need to be carefully designed to detect multiple, potentially interacting causes. This process has been hindered by the artificial division of causes into "natural" (parasite, injury) and "anthropogenic" (elevated UV-B radiation, xenobiotic chemical) sources. Such categories are misleading because they ignore the very real potential for interactions among these factors and because they (wrongly) suggest that natural factors are unlikely to be a problem. For example, malformations caused by nonnative predators or by emerging pathogens may pose a very real threat to amphibian populations, despite that both agents are natural. To illustrate this point, consider the case of emerging pathogens in human and wildlife populations. While most of these pathogens are natural, they can nevertheless pose a significant threat to affected hosts, often owing to changes in infection prevalence caused by underlying environmental changes (e.g., Daszak et al. 2000; Harvell et al. 2002). We argue that the putative causes of abnormalities should instead be classified as either *biotic* or *abiotic* (see Johnson and Lunde 2005), and suggest that interactions between these groups are more likely to be the rule than the exception.

Several studies have found evidence for multiple interacting factors in driving amphibian abnormalities, particularly in association with *Ribeiroia* infection. Kiesecker (2002), for example, reported that larval wood frogs exposed to common pesticides developed higher levels of *Ribeiroia* infection than did control animals. Based on the reduced levels of circulating eosinophils in the

blood of amphibians exposed to pesticides, Kiesecker (2002) suggested that contaminant exposure reduced the immune response of amphibians in response to parasitic invasion, leading ultimately to higher infection levels (see also Belden and Kiesecker 2005; Koprivnikar et al. 2007). More recently, Johnson et al. (2007) showed that eutrophication resulting from nutrient runoff can increase the levels of *Ribeiroia* infection. Excess nutrients stemming from agricultural fertilizers, livestock manure, or urbanization enhance algal growth in aquatic systems, ultimately promoting the growth and reproduction of the snail hosts for *Ribeiroia* (e.g., *Planorbella* spp.). In a large-scale mesocosm experiment, Johnson et al. (2007) found that nutrient enrichment led to increases in both the density of infected snails and the per snail production of infectious cercariae. This increase in parasite production translated directly to an increase in *Ribeiroia* infection in larval amphibians. Rohr et al. (2008) recently found evidence that both nutrient runoff and pesticides were contributing to elevated trematode infections in Minnesota amphibians, underscoring the complexity of the relationships between environmental change and host-parasite interactions.

The presence and composition of predators in freshwater ecosystems can also indirectly influence trematode infection. Larval amphibians often change their behavior in response to the chemical cues from fish or dragonfly predators, spending more time immobile or hiding to avoid detection. Such changes may increase their vulnerability to parasite infection. Thiemann and Wassersug (2000) found that amphibian larvae exposed to chemical cues from caged predators spent less time moving and developed significantly higher trematode infections. In a related example, Johnson et al. (2006) found that conspecific attack (i.e., cannibalism) by amphibians can increase the frequency of parasite-induced malformations. Larval salamanders frequently attack one another, often causing limb injury or loss (e.g., Crump 1983; Walls et al. 1993; Wildy et al. 2001). If injured animals were subsequently exposed to *Ribeiroia* during the regeneration process, the limbs were 3 to 5 times more likely to develop permanent malformations than uninjured limbs. Predators, however, also have the potential to reduce infection. By consuming trematode cercariae, dragonfly larvae and other predators could indirectly reduce infection and malformations in amphibians (Schotthoefer et al. 2007; Thieltges et al. 2008).

Finally, environmental contaminants have the potential to interact with factors such as UV-B radiation, pH levels, predator cues, and other contaminants (e.g., Relyea and Hoverman 2006). For example, Relyea (2005) found that commercial-grade Roundup®, containing the active ingredient glyphosate and a polyethoxylated tallowamine (POEA) surfactant, exposure was far more lethal to amphibians when combined with the presence of predators (as would occur in nature) than under artificial laboratory conditions. Such interactions underscore the complexity of investigating amphibian malformation phenomena in nature. To summarize: 1) amphibian abnormalities can be caused by a variety of abiotic and biotic agents, 2) there is frequently more than one causal agent present, 3) these agents vary in importance among sites and years, and 4) no single cause is likely to explain all high-frequency accounts of amphibian deformities in the wild (Carey et al. 2003). A brief overview of the US Fish and Wildlife Service's Abnormal Amphibian Project illustrates the complexities inherent to a causal analysis focused on malformations in amphibians.

16.4 CASE STUDY: US FISH AND WILDLIFE SERVICE, ABNORMAL AMPHIBIAN PROJECT

In 2000, the US Fish and Wildlife Service (USFWS) began a uniquely large-scale and collaborative program focused on understanding the geographic distribution and severity of amphibian abnormalities on US National Wildlife Refuges. The objectives were to 1) determine the prevalence of abnormalities in frogs on refuges; 2) evaluate how abnormality frequencies vary among sites, refuges, and years; and 3) investigate possible causes of the abnormalities through targeted follow-up studies.

During the first 6 years of the study (2000 to 2005), a total of 46 530 frogs and toads were collected and examined, with the vast majority released after inspection (USFWS 2009). Between 2000 and 2005, 112 refuges in 46 states were sampled at least once for abnormal frogs, and many refuges were sampled on multiple occasions. A total of 3093 individuals (6.6%) were classified as

abnormal for various reasons, including injury or signs of disease. Of these, 1239 (3.1%) of the total were classified as having skeletal abnormalities.

A subset of the frogs was inspected for parasites and analyzed with radiography. Guderyahn (2006) summarized and interpreted the radiographic analysis of 666 abnormal frogs collected during the 2003 and 2004 field seasons from refuges within 27 US states. The objectives were to use radiography to provide diagnostic information on the nature of the abnormalities and to compare types and frequencies across regions. Despite considerable differences in species and ecological factors (e.g., habitat type, climate, and land use), abnormalities were generally similar across regions of the country. The most commonly affected body part was the hind limb (80% of the 861 abnormalities). Hind limb brachydactyly (shortened digits, 27%) and ectromelia (missing limb segments, 23%) were the most frequently observed abnormality types, followed by ectrodactyly (missing digit[s], 7%), hemimelia (shortened limb, 5%), and bony expansions (5%). Other hind limb abnormality types comprised <3% of all observed abnormalities.

Research comparing the types of abnormalities in different regions as well as what stressors are present is currently under way in an attempt to identify presumptive cause-effect relationships at refuges with elevated incidences of abnormalities. In several cases, in-depth studies were conducted on individual refuges or on a group of refuges. These studies are summarized in the following subsections.

16.4.1 Great Bay National Wildlife Refuge

Great Bay National Wildlife Refuge (Newington, New Hampshire) was created on the former Pease Air Force Base, listed as an EPA Superfund (CERCLA) hazardous waste site due to high concentrations of trichloroethylene and nitrate in ground water; pesticides, polycyclic aromatic hydrocarbons (PAHs), and metals in sediment; and pesticides (including DDE) in fish tissue. The Great Bay in-depth study (Pinkney et al. 2006) was triggered by observations of abnormalities greater than 9%, 2 amphibian die-offs, and contaminant concerns. These concerns included the results of 140-day laboratory tests using *Xenopus laevis*, which exhibited increased mortality and delayed metamorphosis when exposed to refuge pond sediment (Turley et al. 2003). Laboratory and in situ toxicity tests with wood frogs and northern leopard frogs, chemical sampling of water and sediments, and abnormality surveys were conducted at 4 ponds. Decreased survival and increased time to metamorphosis occurred in wood frogs exposed either in situ or in the laboratory to passive sampler extracts, water, or sediments from several refuge ponds. The major observation in the in situ study was the high prevalence of a leg abnormality (rounded femurs), which resulted in impaired hopping ability. This was observed in 63% of the wood frogs from the refuge's Beaver Pond, compared with 0 to 1% in wood frogs from the 3 other refuge ponds. This leg abnormality was confirmed in x-rays as a probable malformation. There was, however, no evidence of a linkage between adverse effects in the in situ study with exposure to DDT or other pesticides. Pinkney et al. (2006) recommended abnormality and population monitoring, maintenance and monitoring of water levels in frog habitats, and further sediment sampling. While present on the refuge, *Ribeiroia* was extremely rare in field-collected frogs and therefore unlikely to significantly contribute to observed malformation patterns.

16.4.2 Alaskan National Wildlife Refuges

A study of 5 Alaskan National Wildlife Refuges, conducted in tandem with the national abnormal amphibian project, has indicated a pattern in the abnormality frequencies. Reeves et al. (2008) examined 9269 metamorphic wood frogs from 86 breeding sites from 2000 through 2006. The prevalence of skeletal and eye abnormalities at Alaskan refuges ranged from 1.5% to 7.9% and was as high as 20% at individual sites. The most common types of abnormalities were ectromelia, micromelia (shrunken limb or limb element), and unpigmented iris. Proximity to roads (as a proxy for human development) significantly increased the likelihood of skeletal abnormalities and skeletal malformations but not eye abnormalities. The authors suggested either chemical contaminants,

invertebrate predators, or an interaction between these factors was causing the abnormalities. *Ribeiroia* was not detected among any of the necropsied frogs from the region, and overall parasite richness and abundance were both very low. Their data did not support the UV-B or parasite hypotheses for wood frog abnormalities in Alaska.

16.4.3 Parasitological Investigations on NWRs

From a subset of refuges sampled across the country, metamorphosing amphibians (normal and malformed, if present) were necropsied to identify and quantify their parasites. While the focus was on *Ribeiroia*, data were collected on all encountered helminths (parasitic worms), including other trematodes (flatworms), cestodes (tapeworms), nematodes (roundworms), acanthocephalans (spiny-headed worms), and various protistan (protozoan) parasites (Sutherland 2005). To the best of our knowledge, these data comprise one of the largest amphibian parasite databases ever assembled. Thus far, we have examined more than 22 species of amphibians from 40 refuges and 28 states. More than 200 000 individual parasites have been identified and enumerated.

While this sampling effort is ongoing and much of the data have yet to be analyzed, several interesting patterns have already emerged. *Ribeiroia* appears to be relatively widespread but is highly variable in abundance, both among refuges and between years within a refuge. Fifteen of the 40 refuges sampled for parasites tested positive for *Ribeiroia*, including infections in 8 different amphibian species. Infection within amphibians ranged from 1 to 177 parasites in an individual frog. *Ribeiroia* also exhibited distinctive distributional patterns across the United States. Interestingly, we have recovered *Ribeiroia* only in frogs from the northern half of the United States, with no records below 37° latitude (Figure 16.4). This is somewhat surprising given the preponderance of *Ribeiroia* records in birds from Florida (see Johnson et al. 2004). In addition, very few trematodes (and no records of *Ribeiroia*) have been found in Alaska (despite the significant occurrence of abnormalities; see Reeves et al. 2008), possibly owing to the difficulties inherent in parasite overwintering or to the very short growing season in which transmission must occur. More data are needed to understand these intriguing patterns.

Ribeiroia-positive refuges occurred primarily along the West Coast, in the Northeast, and in the Midwest along the Mississippi River (Figure 16.4). This pattern likely underscores the importance of the major migratory bird flyways, including the Pacific flyway, the Mississippi flyway, and the Atlantic flyway, and is therefore consistent with birds acting as the major transport vector for *Ribeiroia*. Why we have not found more records along the southern half of the migratory pathways is unclear. In a study of helminths in wood ducks (*Aix sponsa*), Thul et al. (1985) found that *Ribeiroia* was a dominant member of the helminth fauna in migratory ducks in the Northeast, with a prevalence of 21%, whereas *Ribeiroia* was rare (<2% prevalence) among resident wood ducks in the Southeast, similar to our current investigations with frogs.

In summary, *Ribeiroia* infection is widespread but variable among refuges in the United States. At high infection levels, *Ribeiroia* is associated with the occurrence of particular malformation types that vary in frequency among species. These malformations are generally dominated by skin webbings, bony triangles, and truncated long bones. Extra limbs and limb elements occur in some species but not in others. Sites without *Ribeiroia* often exhibit abnormalities involving primarily missing digits, limb elements, or entire limbs. While such abnormalities often occur at low frequencies, within the expected baseline levels, other refuges (such as those in Alaska) exhibit high frequencies (>10%) of abnormalities across multiple years. Thus far, however, the causes of these abnormalities remain unknown (see Reeves et al. 2008).

16.5 CONTINUED STUDY OF AMPHIBIAN ABNORMALITIES

Amphibian abnormalities have been the focus of extensive press and scientific debates since reports increased in the late 1990s. The confirmed accounts of abnormal amphibians in more than 30 states in the United States have created substantial concern that abnormalities indicate the presence of

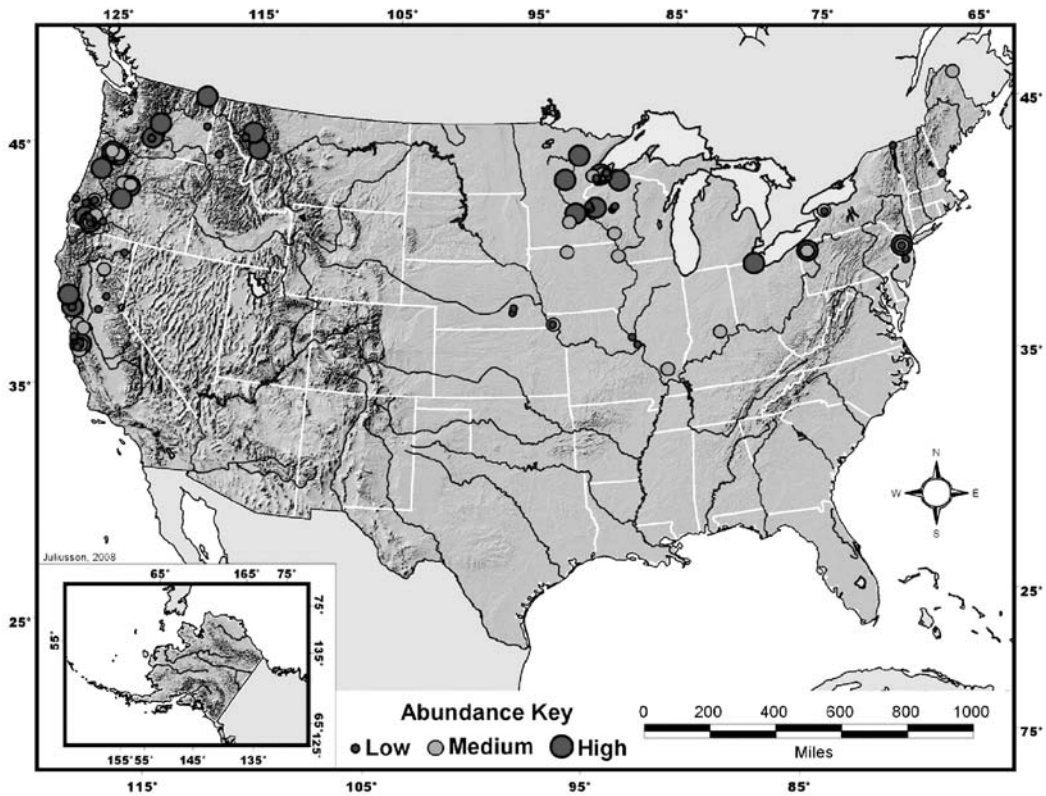

FIGURE 16.4 Geographic distribution of *Ribeiroia* from amphibians. Data represent a compilation of samples from US National Wildlife Refuges and additional sampling on private lands (sites that did not support *Ribeiroia* not depicted here). The size of each circle reflects the average infection abundance recorded in the sample (usually determined from a sample of 10 amphibians). In total, 16 amphibian species from 107 sites distributed across 20 states are included (1999 to 2007). Abundance values as follows: low (1 to 10 metacercariae per amphibian), medium (11 to 30 metacercariae), and high (31 to 135 metacercariae). Infection intensity for individual frogs ranged from 1 to 960. (Reprinted from Johnson and Mckenzie (2008), Effects of environmental change on helminth infections in amphibians: exploring the emergence of *Rebeiroia* and *Echinostoma* infections in North America. Chapter 11 (pp. 249–280) in fried, B. and R. Toledo, *The Biology of Echinostomes, from the Molecule to the Community.* Springer.)

harmful contaminants in the environment, which has kept the issue in the media spotlight. A careful review of the existing scientific literature reveals that malformations are not new to amphibians, and indeed have been recorded for over 300 years (see Ouellet 2000). However, emerging evidence suggests that, at least in some regions of the country, the prevalence of abnormal individuals is substantially greater than the expected baseline levels and may be increasing. A general lack of historical baseline data has often made interpretation of current patterns difficult.

Substantial progress has been made over the last decade in understanding amphibian malformations. Perhaps foremost among these advances is the growing recognition that multiple causes are involved, and that such causes may vary among wetlands and years but have a high potential for interaction. Nevertheless, it is imperative to recognize that simply because an agent can produce abnormalities in laboratory experiments does not mean that it actually is causing abnormalities in nature. Every agent proposed has the capacity to induce abnormalities, but without contextual field data on the concentration, intensity, or abundance of that agent in the environment, the significance of the agent's contribution to abnormalities in wild amphibian populations is difficult to determine. Because amphibian abnormalities are a series of phenomena, pursuit of a single "smoking gun" is

likely to hinder rather than help the investigation of cause-effect relationships, as it could prevent the recognition of different proximate causes for disparate sites or species. Large-scale and long-term regional surveys for amphibian abnormalities, such as those currently under way by the USFWS, are essential, particularly when accompanied by detailed data on the prevalence, composition, and extent of observed abnormalities.

Continued investigation of amphibian abnormalities demands an integrative approach, combining laboratory studies, field surveys, and large-scale experiments. Collaboration among scientists in varied fields — especially ecology, herpetology, developmental biology, and aquatic toxicology — is a necessary element in this approach. We emphasize the need for an ecologically relevant approach that follows a modified version of Koch's postulates, which are used to link an agent of disease to the pathology it causes by 1) isolating the agent from a diseased individual, 2) culturing it in vitro, and then 3) inducing illness by exposing a healthy individual to the agent. However, the narrow focus of these postulates requires that they be modified to a broader set of circumstances, as has been proposed in several other scientific fields (e.g., Glasgow et al. 2001; Grimes 2006; Plowright et al. 2008). With respect to amphibian malformations, field surveys should be used to identify malformation patterns and the environmental factors that correlate with their occurrence. Hypothesized causal factors should then be studied experimentally under ecologically relevant conditions, including experiments involving environmentally relevant dosages of the candidate agent(s) and the appropriate amphibian species. Results of such experiments can reveal whether the proposed factors are capable of inducing types and frequencies of malformations similar to those observed in nature. Ideally, experiments should begin with laboratory studies and develop into larger-scale, replicated field manipulations, including mesocosm studies, in situ enclosures/exclosures, and whole ecosystem manipulations. Field experiments offer the greatest potential for demonstrating causal control under realistic conditions (e.g., Vredenburg 2004), and may be instrumental in developing control strategies to reduce or eliminate malformations in amphibians undergoing declines. Given the potential ethical dilemmas intrinsic to adding teratogenic agents to field experiments or entire ecosystems, however, we advocate removal/elimination experiments, but acknowledge that this will be easier for some factors than others.

Finally, recognizing the potential for interactions among factors and the importance of indirect causes, research should return to the field to examine what underlying factors control or mediate the concentrations of a factor that is known to cause malformations. While this begins to deviate from Koch's postulates and the principle of parsimony, it is well founded upon the pillars of epidemiology (e.g., Lesser et al. 2007). Identifying the proximate cause of a disease (such as amphibian malformations) is only the first step; almost all emerging diseases involve environmental co-factors that influence the levels of pathology. Examples include introduced species, pollution, changes in community structure, land use change, and climate shifts (e.g., Ostfeld et al. 2008). This latter step is therefore essential in understanding amphibian malformations and mitigating or reversing their impacts on already declining amphibian populations. Thus far, none of the proposed causes of malformations have fully completed this series of causal demonstrations, and we recognize that the formula outlined here will not apply perfectly in all cases. We nevertheless suggest that it provides a useful guideline from which to develop an adaptive approach with the same general goals (Plowright et al. 2008).

Whatever causative agents are responsible, the study of amphibian abnormalities may aid the ongoing investigation of amphibian decline (e.g., Houlahan et al. 2000; Stuart et al. 2004). Amphibians have received international attention and funding as a result of abnormalities and disease, and broad-scale surveys have been initiated in many affected regions. Baseline data on the naturally occurring abnormality rates will help us to better understand the background fluctuations of amphibian population dynamics. Information on the role of pathogens and parasites in amphibians, not to mention increased attention to water quality and methods of assaying potentially harmful agents, is being generated in the ongoing study of amphibian abnormalities.

16.6 EPILOGUE

Today, more than 13 years after the original reports from this site elevated malformed frogs to national and international attention, the Ney Pond in Minnesota has been transformed into a nature center devoted to environmental education. The director of the center is one of the former students from the middle school class that first stumbled upon the deformed frogs in 1995. While *Ribeiroia* infection has been consistently documented in frogs from the pond, malformation levels have varied dramatically over the years, only rarely attaining the high frequency seen in 1995 (Vandenlangenberg et al. 2003; Johnson and Sutherland unpublished). In many respects, the patterns at this wetland illustrate both the progress we have made and the many questions that still surround this issue. Undoubtedly, parasite infection explains some fraction of the malformations observed at Ney, but how much remains unknown. Parasite infection is only part of the story. The ultimate factors that control variation in abnormalities among wetlands and among years are still at large, as are the factors that caused the "outbreak" of malformations at Ney in the 1990s. These same questions can be posed for sites that do not support *Ribeiroia*, often with even fewer answers, emphasizing the complexity of interactions among biotic and abiotic factors in controlling amphibian malformations.

The continued study of this issue requires more large-scale and long-term studies that not only document the location and frequency of abnormalities, but also examine other factors that may influence the number or type of abnormalities, such as hydrologic period (e.g., drought or excess precipitation), temperature, changes in landscape (e.g., development, agriculture), introduced predators (e.g., fish), water quality, and disease. Standard operating procedures that address collection techniques, frequency of sampling, shipping and handling of animals, and disinfecting equipment are required to limit or avoid detrimental impacts to amphibians and their habitats. Even today, variation in how abnormalities and "hot spots" are classified affects our ability to interpret large-scale patterns. Establishing standardized protocols and data sheets will help ensure that high-quality, objective data are gathered and analyzed to reduce subjectivity and variability. Importantly, however, the collection of additional data should be conducted in tandem with efforts to reduce or prevent the deleterious effects of malformations on amphibian populations. While no study has compellingly demonstrated a link between malformation and population losses in amphibians, growing evidence indicates that malformations occur alongside elevated levels of direct and indirect mortality, which becomes especially important in amphibian populations that are already declining due to habitat loss, introduced species, pollution, and other diseases. Thus, experimental efforts that strive not only to understand cause-effect relationships but also to apply such results toward lessening the impacts of malformations on amphibians are deemed particularly valuable.

GLOSSARY: MODIFIED LIST FROM OUELLET (2000) AND JOHNSON ET AL. (2001B)

Anophthalmia: Absence of one or both eyes.
Brachydactyly: Shorter digit or digits.
Brachymelia: Shorter limb or limbs.
Cutaneous fusion (skin webbing): A layer of skin connecting 2 or more limb segments.
Ectrodactyly (oligodactyly): Absence of one or more digits (adactyly) or parts of digits.
Ectromelia: Absence of one or more limbs or parts of limbs.
Edema: Fluid-filled swelling.
Hemimelia: Absence of all or part of the distal half of a limb.
Kyphosis: Abnormal backward curvature of the spine or "humpback."
Lordosis: Abnormal forward or concave curvature of the spine.

Mandibular hypoplasia: Underdeveloped mandible.
Micromelia: Shortened limb or parts of limb.
Microphthalmia: Eye smaller than normal.
Polydactyly: Supernumerary digit(s).
Polymelia: Supernumerary limb(s).
Polypody: A limb with 2 or more hands or feet.
Scoliosis: Abnormal lateral curvature of the spine.
Syndactyly: Fusion of 2 or more digits.
Synmelia: Fusion of a limb or parts of a limb to a body part.
Taumelia (bony triangle): Long bone bent at right angles to itself, often forming a triangle or pyramid.

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17 Population Estimation Methods for Amphibians and Reptiles

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CONTENTS

The topic of population estimation received only rudimentary coverage in past publications summarizing the ecotoxicology of amphibians and reptiles (e.g., Sparling et al. 2000; Linder et al. 2003). Studies designed to evaluate chemicals and chemical mixtures on amphibians and reptiles in natural or seminatural field settings were relatively new during the preparation of the first edition of this book, but their importance in the interpretation of laboratory results has resulted in an increase in field experiments and observational studies (e.g., Boone and Bridges 2003; Davidson 2004). Studies that integrate both field and laboratory components may provide the most powerful inference about the impacts of various stressors on reptile and amphibian populations. With this recent surge of field studies, there is a critical need for the development and application of rigorous population estimation methods. In this chapter, we review the latest methods for estimating populations and related parameters and provide relevant references in which these techniques have been successfully applied to amphibians and reptiles.

Part of the challenge of drawing inference about factors that may affect amphibian and reptile populations stems from the very definition of a "population." Green (1997) pointed out the importance of distinguishing between "changes in population size" and "changes in the number of populations" (Green 1997; Corn 2000). We agree, and we maintain this fundamental distinction throughout this chapter. Ultimately, which definition is appropriate depends on the study objectives that in turn determine appropriate estimation methods. For many studies whose focus is on the population level effect of a toxic spill or experimental application, measuring change in population size of a single to several local populations will be appropriate. In other contexts, a change in the

size of a single population (say, at a single breeding pond) may be meaningless in the context of the status of a metapopulation. Furthermore, the application or the area impacted by a potentially toxic agent may be widespread (e.g., pesticides, heavy metal deposition). For instance, interest can shift to the decline in the number of populations over a large geographic area (e.g., amphibians in the Sierra Nevada Mountains; Chapter 2, this volume). In these cases, it may be more appropriate to study changes in the number of populations over a wide geographic area, including impacted and nonimpacted locations.

Tremendous advances have been made in the development and application of model-based methods since the publication of Heyer et al.'s (1994) book on this subject. Fundamentally, these methods adjust for the proportion of the population(s) not seen during a given study or sampling period. Inherently, investigators acknowledge that in most field settings not all species, or individuals in a population, are detected under most study designs and sampling methods. The trick then consists in relating an observed count to the true state variable of interest, such as the number of individuals in a population or the number of populations across a landscape. Written as a mathematical expression, the expected count (C) is the result of the true state variable of interest (θ) multiplied by the probability of detection (p), $\mathrm{E}(C) = \theta p$. Rearranging the equation yields the basic, expression $\hat{\psi} = \frac{c}{p}$ which is the foundation of nearly all population estimators (Williams et al. 2002). For example, if abundance is the state variable of interest, then the number of individuals seen (C) is divided by the probability that an individual within the population is detected (here, p_{ind}) to obtain an estimate of population size: $\hat{N} = \frac{C}{\hat{p}_{\mathrm{ind}}}$. Alternatively, if the state variable of interest is the number or proportion of locations that are occupied by a target reptile or amphibian species, then the probability of occupancy (ψ) is a logical state variable. An estimator for the probability a location is occupied is obtained in a similar manner, by adjusting the observed proportion of locations where the species was detected by the probability of detecting species at an occupied location (here, p_{species}):

$$\hat{\psi} = \frac{\frac{c}{s}}{\hat{p}_{\mathrm{species}}}$$

where c is the number (or count) of sites where the species was detected out of s total sampled sites.

In the remaining part of this chapter, we present methods that are representative of these 2 classes of estimators. We do not present all available methods; rather, we focus on those that we believe are most relevant for field studies of reptiles and amphibians. Our aim is to provide a basic background in the application of these methods in recent herpetological studies and encourage investigators in making informed decisions about general methods that are appropriate in future studies focused on the potential impacts of ecotoxins on amphibian and reptile populations.

17.1 TECHNIQUES FOR ESTIMATING ABUNDANCE OF REPTILES AND AMPHIBIANS

A wide array of methods has been developed to estimate animal abundance or population size (see Williams et al. 2002; Mazerolle et al. 2007). A number of techniques are available for situations in which animals are captured, marked, and released to be recaptured at a later occasion. Here, we present those methods we believe to be most useful in the study of reptile and amphibian populations. We begin with classic closed population models (i.e., meaning no birth, deaths, immigration, or emigration) for studies conducted over very short periods. Next, we introduce open population models where population size changes between the beginning and the end of the study. We continue with robust design models, which are hybrids of closed and open models. Finally, we present techniques where individuals do not have to be marked uniquely, namely, removal methods, multiple observer sampling, distance sampling, as well as methods utilizing repeated counts and call indices.

17.1.1 Capture–Recapture or Resight Methods

17.1.1.1 Closed Populations

Several closed population models have been developed to estimate abundance in animal populations (reviewed by Otis et al. 1978; White et al. 1982; Williams et al. 2002). The term "closed" refers to demographic and geographic closure, where the population is assumed to be closed to births, deaths, immigration, and emigration for the duration of the study. On a given sampling occasion, animals are typically captured, individually marked, and released to be recaptured or resighted on subsequent occasions (relatively close in time). For each individual, a capture history is compiled, consisting of a series of 1s and 0s representing whether the individual was seen, or not seen, on each occasion. For instance, a history of 101 indicates that the individual was captured on the first and third occasions, but not on the second occasion. This type of data contains the information necessary to estimate population size, N, and the probability of capture, p (i.e., individual capture probability). Only 2 sampling occasions are required for the simplest models (i.e., Lincoln–Petersen estimator), but more sampling occasions increase flexibility in modeling and precision of the estimators (Williams et al. 2002). In addition to the closure assumption, closed population models also assume that all individuals have the same probability of being captured, that no individual on the site is impossible to capture, and that marks are neither lost nor overlooked by observers.

The relaxation of the assumption of equal capture probability among individuals has been the subject of many advances in closed models (Williams et al. 2002; Mazerolle et al. 2007). Some models allow the probability of capture to vary with time, behavior (trap shy or trap happy), or among individuals. One can also account for variation among sex and age classes or contaminant exposure with group variables. Alternative parameterizations of the models by Huggins (1991), conditional on the individuals observed, now enable the inclusion of individual covariates (e.g., weight) when estimating capture probability. Applications of closed population models in amphibian and reptile systems include Nelson et al. (2002), Funk et al. (2003), and Tyrrell et al. (2009). Several amphibian studies have compared various closed population estimators (Jung et al. 2000, 2002, 2005; Bailey et al. 2004a), and recent reviews provide practitioners with study design and analysis recommendations (Mazerolle et al. 2007; Bailey and Nichols 2009; Schmidt and Pellet 2009).

17.1.1.2 Open Populations

The best known capture–recapture model for open populations is the Jolly–Seber model (Jolly 1965; Seber 1965; Pollock et al. 1990). Here, the term "open" indicates that each capture occasion is separated by an interval during which the population may experience recruitment (births or immigrations) and deletions (deaths or emigration). Again, typical studies involve capturing, marking, and releasing individuals to be recaptured (or resighted) at a later occasion. In contrast to the closed models described above, the study must consist of at least 3 capture occasions. The Jolly–Seber model allows for the estimation of population size (N) and capture probability (p) at each time period, as well as recruitment numbers and apparent survival probabilities between time periods. Note that apparent survival denotes that mortality and emigration cannot be distinguished without additional information.

The Jolly–Seber model assumes that probabilities of survival and capture are homogeneous across individuals for a given occasion, all emigration from the study area is permanent, sampling occasions are instantaneous or at least short relative to the interval between occasions, and marks are neither lost nor overlooked by observers. As in closed models, heterogeneity in capture probabilities can severely bias estimates of population size under the Jolly–Seber model (Pollock et al. 1990). Temporary trap response and age effects can be accommodated in the model (Pollock 1975; Brownie and Robson 1983). One can stratify individuals into groups to account for the effects of certain variables (e.g., sex, size class). Examples of population size estimation with Jolly–Seber models include Weatherhead et al. (2002), Blackwell et al. (2004), and Bell et al. (2004).

17.1.2 Robust Design

Robust design models started as conceptual hybrids of open and closed population models and were developed as a solution to the sensitivity of Jolly–Seber abundance estimates to deviations from model assumptions (e.g., Carothers 1973; Pollock 1982; Pollock et al. 1990). In the robust design framework, sampling is conducted at 2 temporal scales. For example, Pollock's original robust design consists of multiple survey occasions conducted over short time periods during which the population is assumed closed. These closed "primary" periods are then separated by longer intervals over which the population is assumed open (see Kendall and Nichols 1995; Kendall et al. 1997 for joint likelihood approach).

Amphibian and reptile systems have motivated alternate versions of the robust design that relax the closure assumption within primary periods. Kendall (2004) provides a nice review of these models, including the open robust design (Schwarz and Stobo 1997; Kendall and Bjorkland 2001), where geographic closure is relaxed, allowing individuals to enter and exit the study area in a staggered fashion, but demographic closure is maintained (i.e., no births or deaths). Alternatively, the gateway robust design permits mortality within the primary period, while maintaining the geographic closure assumption (Bailey et al. 2004d). Pollock's original robust design has been utilized to estimate population size, temporary emigration, and survival probabilities for terrestrial and pond-breeding amphibians (Bailey et al. 2004b; Frétey et al. 2004; Muths et al. 2006), while open and gateway robust designs have focused on obtaining unbiased estimates of survival, movement, and breeding probabilities for sea turtles (Kendall and Bjorkland 2001; Mazerolle et al. 2007) and pond-breeding salamanders (Bailey et al. 2004d; Church et al. 2007).

17.1.3 Removal Methods

Removal methods are a particular case of the closed population behavioral response model, where there is a difference between initial and subsequent capture probabilities: more specifically, recapture probability equals zero (Otis et al. 1978; White et al. 1982). On each sampling occasion, captured individuals are removed from the population in some manner. Removal can be accomplished by either physically removing individuals from the site (e.g., retaining captured individuals until sampling is completed) or by marking individuals. The data then consist of the number of new individuals observed on each sampling occasion. In cases where sampling intensity is equal among occasions, one can use closed models that include a behavioral response in capture probability. When sampling effort is unequal across occasions, one can directly model the effect of sampling effort (e.g., hours of search) on the probability of capture. For herpetological applications of removal methods, see Bruce (1995), Petranka and Murray (2001), Jung et al. (2002), and Bailey et al. (2004a).

17.1.4 Incomplete Counts

17.1.4.1 Independent and Dependent Observers

Sampling with multiple independent or dependent observers can provide data amenable to estimating detectability and abundance. In the independent observer design, observers record animals independently within a very short time interval, often at the same time, such that the population is considered closed (Williams et al. 2002). The main caveat is that observers must be able to identify individuals so that for every observed individual, a record can be compiled denoting whether or not each observer saw the individual. The data are then analyzed using standard closed population models (Nichols et al. 1986). The dependent observer design involves 2 observers: a primary observer signals to the secondary observer when an animal is detected, whereas the secondary observer records all animals detected by the primary observer and any individual missed by the primary observer (Nichols et al. 2000). Observers should take turns as primary and secondary observers or switch roles halfway through a

survey. In herpetological settings, these methods have been used to estimate numbers of egg masses (e.g., Grant et al. 2005) or burrows/nests (e.g., Nomani et al. 2008).

17.1.4.2 Line Transect or Distance Sampling Methods

Several authors have developed a class of models where the goal is to estimate detectability and density using line transects (Buckland et al. 2001, 2004). In line transect sampling, the observer navigates along a line, records animals seen within a specified width on each side of the line, and measures the perpendicular distance from each individual to the center line (line transects are a type of distance sampling method). Then, detection probability is estimated using a function, $g(x)$, that describes the relationship between the probability of detection and the distance the individual is from the transect line. Detectability on the center line is usually assumed to be 1 (but see Buckland et al. 2004), and detection probability generally decreases with increasing distance from the line. In addition to the assumption that detection is perfect on the transect line, line transect models also assume that individuals are detected at their initial position before they attempt any evasive movement, and distances are measured accurately. Line transect methods have been used to estimate abundance or density of various reptile species (Freilich et al. 2000; Anderson et al. 2001; Grant and Doherty 2007; Nomani et al. 2008) and *Eleutherodactylus* frogs (Funk et al. 2003).

17.1.4.3 Multiple Repeated Counts

In studies conducted over large spatial scales and several sites, it becomes logistically impossible to mark and recapture or resight individuals. In these cases, multiple independent counts of individuals may be obtained at a collection of sites. Royle (2004a) developed the "point count model" to estimate parameters describing the distribution of abundance across sites. The simplest point count model yields 2 parameters: one for detectability of individuals, the other for the mean abundance across sites. Abundance is modeled using either the Poisson or negative binomial distribution, 2 distributions potentially useful for count data (Royle 2004a). Data amenable to this analysis consist of repeated counts of individuals at multiple sites from several visits conducted over a short time period (spatial and temporal replication). Alternatively, different observers can visit the sites and record counts independently.

The point count model assumes there is no change in abundance at the site between the first and last visit (i.e., closed population), the abundance follows the distribution specified in the model, and the probability of detecting individuals in a survey is the same for all individuals, sites, and surveys. The latter assumption can be relaxed by modeling the effects of covariates on detectability, such as habitat type, weather conditions, or sampling effort. One can also easily model the effects of covariates on abundance. To date, point count models have been used mostly in ornithological contexts (Kéry et al. 2005; Royle et al. 2005), but also have been used to estimate amphibian abundance (Dodd and Dorazio 2004; McKenny et al. 2006).

17.1.4.4 Call Indices

Call surveys, in which the investigator categorizes the level of calling intensity in 3 to 4 classes, are common in large-scale amphibian studies (e.g., Weir and Mossman 2005). For instance, the North American Amphibian Monitoring Program (NAAMP) uses values 0 to 3, where 0 is equivalent to not detected; 1 corresponds to discrete, nonoverlapping calls; 2 indicates discrete, overlapping calls; and 3 is for a full chorus. In such a framework, the goal is to estimate the true abundance level, also called the latent (i.e., unobserved) abundance index. In other words, the latent abundance index is the maximum index value that could be observed at a given site if a large number of visits were conducted. Royle (2004b), Royle and Link (2005), and MacKenzie et al. (2009) developed a class of models to estimate this latent abundance index at specific sites, given imperfect detection and index classification. Again, to apply these models, a series of repeated visits need to be performed at many sites where the call index is recorded at each survey. Call index models assume the true abundance index remains the same during the period over which surveys are conducted each year (i.e.,

closure assumption). Additionally, the probability of detecting the species and correctly classifying the abundance index is assumed to be similar for all surveys of occupied sites, unless modeled as a function of covariates (e.g., temperature). Application of these models has been limited due to their recent development, but examples are given in the original papers (Royle 2004b; Royle and Link 2005; MacKenzie et al. 2009).

17.2 TECHNIQUES FOR ESTIMATING ABUNDANCE OF LOCATIONS WITH POPULATIONS OF REPTILES AND AMPHIBIANS

Many large-scale studies do not have sufficient resources to estimate abundance of reptiles or amphibians at all study locations. Furthermore, densities for some species may be low, such that the objectives shift from determining local population size to simply determining whether the species is likely to exist at various sampling locations. In these cases, it is reasonable to use occupancy (the probability that a site is occupied) as a state variable. Quantitative development in this arena of estimation models has emerged recently, with a plethora of modeling options for single and multiple species over 1 or many seasons. All methods acknowledge the likely scenario that species are not always detected when present and emphasize that reliable inferences can still be made from detection/nondetection information (otherwise known as presence/absence data) if detection and occupancy probabilities are simultaneously estimated. MacKenzie and his colleagues offer an excellent review and development of these models and apply them to several herpetological examples (MacKenzie et al. 2006). Here, we introduce readers to these models and provide references for studies applying the models to reptile or amphibian species. We believe these models show great promise for a wide variety of ecotoxicological field studies, especially for highly susceptible species where the exposure and concentration of toxins may vary across the landscape (e.g., Davidson 2004).

17.2.1 Single-Season Models for Single Species

Envisioning a biological system where sampling units are chosen in some probabilistic manner and visited repeatedly within a single sampling season, MacKenzie et al. (2002, 2006) defined a probability-based model that consisted of 2 types of parameters: ψ represents the probability a site is occupied by the target species, and p_j is the probability of detecting the species at an occupied site during the jth independent survey of a site. Assuming the state of a site (i.e., occupied or unoccupied) is constant during the season and that detection histories from all sites are independent, maximum likelihood methods are used to estimate occupancy and detection probability. The same modeling procedure can also be used with a Bayesian philosophy to statistical inference and can be easily implemented using Markov chain Monte Carlo methods (Royle and Dorazio 2008). Using either procedure, it is possible to model occupancy and detection probability as functions of measured covariates, which usually represent multiple, competing hypotheses about factors believed to influence the distribution of amphibians or reptiles on the landscape. Occupancy probability may be modeled as a function of site-specific covariates that are representative of the entire season (e.g., habitat type, maximum or minimum metal or pesticide concentration), while detection probability may be modeled as a function of either site-specific or survey-specific covariates (e.g., weather conditions).

Single-season occupancy models have gained popularity in amphibian studies across the globe (e.g., Bailey et al. 2004c; Mazerolle et al. 2005; Muths et al. 2005; Pellet and Schmidt 2005) and the models are beginning to be applied to reptile species as well (Luiselli 2006; Roughton and Seddon 2006).

17.2.2 Multiseason Models for Single Species

The basic single-season model has been extended in many ways, including the case where data are collected over multiple seasons. When interest is focused on factors contributing to change in occupancy over time, MacKenzie et al. (2003, 2006) introduced 2 vital rate parameters that govern

changes in the occupancy state between successive seasons: ε_t represents the probability that an occupied site in season t becomes unoccupied in season $t + 1$ (i.e., the species goes locally extinct), and γ_t represents the probability that an unoccupied site in season t is occupied by the species in season $t + 1$ (colonization). The extinction and colonization processes are explicitly incorporated into a general model that also includes detection probability (MacKenzie et al. 2003, 2006). To utilize this model, data are collected on 2 timescales, similar to the robust design for population estimation (see previous section). The occupancy state is considered constant among surveys within a season, but may change (i.e., allow for local extinction or colonization) between seasons. An attractive property of both single- and multiple-season models is that they can accommodate "missing data," when some sites are not surveyed in each sampling occasion.

The development and advancement of many occupancy methods has moved beyond those mentioned here to include multiple species, occupancy states, and detection devices and to relax specific model assumptions (see MacKenzie et al. 2006, 2009). These advances are often motivated by herpetological systems, especially amphibian systems (MacKenzie et al. 2006, 2009). Organizations involved in large-scale, long-term monitoring (e.g., USGS Amphibian Research and Monitoring Initiative, Muths et al. 2005) have embraced occupancy as the primary state variable of interest and designed their studies to facilitate comparison among vastly different geographical regions. In cases where the effect of ecotoxins on target species is of particular interest (e.g., Davidson 2004), the development of new studies or modification of existing ones may allow comparison of the occupancy dynamics on impacted and nonimpacted sites.

17.3 CONCLUSIONS

In this chapter, we briefly reviewed the latest methods for estimating population size (abundance of individuals) or the probability that populations exist at various sites throughout a larger landscape (i.e., the number of populations). In each case, the possibility of missing organisms during a survey is accommodated by estimating a detection probability and adjusting observed counts accordingly. We strongly recommend these methods over unadjusted count indices because it is well known that not accounting for nondetection can lead to misleading inferences about factors affecting either population size or occupancy (Moilanen 2002; Williams et al. 2002; Conn et al. 2004; Gu and Swihart 2004). In addition to extensive written literature (see Mazerolle et al. 2007; Bailey and Nichols 2009; Schmidt and Pellet 2009 for herpetological reviews), numerous free software programs are available to assist investigators in both planning field studies and analyzing resulting data (Buckland et al. 2001; White et al. 1982; White and Burnham 1999; Williams et al. 2002; MacKenzie et al. 2006; Bailey et al. 2007).

To our knowledge, few of the methods we present have been used to explore impacts of ecotoxins in field studies; however, there is certainly opportunity to apply these methods to reptile and amphibian populations. Undoubtedly, experimental studies (both laboratory and field) provide the most powerful inference about the effect of toxins on amphibians and reptiles. Still, laboratory studies are limited in their ability to mimic the complex environments that amphibians and reptiles inhabit (Boone and Bridges 2003). Field experiments and observational studies with strong a priori hypotheses fill an important gap in the understanding of ecotoxicological effects on amphibian and reptile populations. In a reversal of traditional roles, laboratory results should aid in the formulation of the a priori hypotheses to test with field studies.

Population estimation methods presented here may be sufficient to answer some questions, but there may be situations where the ability to estimate population size is not currently possible. For example, the population size or density of larvae is expected to exhibit high variation from year to year (e.g., Van Buskirk 2005) and may provide little information about the overall amphibian population in an area. Even if population size is estimable and differences do exist, the next logical step is to explore why differences exist: What processes are responsible for the differences among populations? Inevitably, this will lead to shifting hypotheses from exploring ecotoxic effects on population

size to population vital rates (e.g., survival, breeding, and movement probabilities). Some of these issues can be addressed in a laboratory setting, but there may be a desire to address these questions in field settings. In some cases, fieldwork may be the only option given the long-lived, slow-maturing nature of many reptile and amphibian species. While it is beyond the scope of this chapter to address methods of estimating population vital rates, we encourage investigators to pursue methods that estimate and adjust for detection probabilities when designing their field studies (see Williams et al. 2002; Mazerolle et al. 2007; Bailey and Nichols 2009 for reviews of these methods).

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18 Epilogue
Ecotoxicology of Amphibians and Reptiles — Where Should We Be Going and How Do We Get There?

Greg Linder, Christine A. Bishop, Sherry K.Krest, and Donald Sparling

CONTENTS

As we opened this second edition, we observed that the nearly 10-year interval between editions displayed progress in our developing a knowledge base for evaluating effects of chemical stressors in amphibians and reptiles. The first edition, published in 2000, contained a literature synopsis that extended back to 1972. Over the course of the 10 years since the first edition, more papers had been published on the ecotoxicology of amphibians than in the 28-year period preceding 2000. Prior to 2000 the annual production rate for contaminant publications focused on amphibians was less than a dozen publications per year, but between 1996 and 2007 the annual rate increased to more than 60 papers. Productivity on reptiles also increased, going from 0 to 16 papers per year, but overall the focus on reptiles has been underwhelming. No doubt, the increased focus on amphibians related, in part, to the widely observed population declines and species extinctions and discoveries of dramatically deformed specimens, although similar observations subsequently expressed for reptiles have generated a much weaker response among the research community. While the increased focus on amphibians was commonly linked to their declining populations, population declines in reptiles have an equally compelling empirical basis that indicates that losses of reptile populations during the last 10 years may be greater than those observed for amphibians. Research in amphibians was also enhanced by a better understanding on how to raise tadpoles in captivity (although husbandry for adults is less well developed) and the availability of amphibians compared to reptiles. Research funding tracked these conservation and logistical concerns. Hopefully, a similar funding and research history will develop for reptiles during the next 10 years. Thus, progress in understanding the effects of contaminants on amphibians and reptiles has grown, but reptiles seriously lag amphibians, assuming that numbers of papers have something to do with increased understanding.

The past 10 years have seen several major developments in the study of amphibian and reptile ecotoxicology. Perhaps the most important development was been a switch from residue-only to effects-based studies in reptiles. Before 2000, there were extremely few experimental studies on reptiles, but since then attention has focused on effects. There is also progress in examining multiple stressors and

multiple chemicals on amphibians. The traditional single-chemical approach has been augmented with studies that expose amphibian larvae to multiple chemicals or to other factors, such as predators or competitors in addition to chemicals. Interesting and sometimes unpredictable results have occurred from these combinations. These multiple-factor studies, although often conducted in laboratories or outdoor mesocosms, add substantial reality to somewhat artificial, sterile conditions encountered in laboratory-only studies. Recent research has also expanded our knowledge of the effects of other chemicals, including polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs), perfluorooctanesulfonate (PFOS), and polybrominated diphenyl ethers (PBDEs); certain pesticides such as atrazine; and additional metals on amphibians and, to a lesser extent, on reptiles. Another area that has experienced noticeable improvement is the development of new or refined methods of testing, including advancements in mesocosm and microcosm experiments. Although the standard several-liter aquarium studies are still relied upon in laboratory studies, researchers are using their imaginations to improve controlled exposures to gain better perspectives on how contaminants may affect free-ranging populations and communities.

An important lesson that has been reinforced during this 10-year period is that while chemical contaminants may be a leading factor in population declines for many species of amphibians (and presumably reptiles), their effects are more likely to be chronic or sublethal rather than acute. Outright examples of massive die-offs are seldom reported in the literature. Rather, contaminants are more likely to affect herpetofauna by altering physiological states, impacting endocrine systems, inducing malformations, reducing food sources, or changing ecological relationships with predators or competitors. Subtle effects are less likely to draw the attention of the media or funding agencies when considered against large-scale die-offs due to disease or high frequencies of multillimbed frogs caused by parasitic infections. Nonetheless, subtle, oftentimes indirect effects or effects linked to long-term exposures to low-concentrations of chemicals in the field are no less a challenge to maintaining sustainable herpetofauna populations. The survival of a population or species is dependent on many interacting factors, any one of which may disrupt ecological structure and function and yield extirpation or extinction. Clearly, contaminants are major players in the conservation of reptiles and amphibians.

With these observations we close this second edition with reminders that much work remains to be completed and new, and perhaps as yet unasked, questions remain to be addressed before the "other vertebrates" gain parity with fish, wild birds, and mammals. In some ways it is inappropriate to lump amphibians and reptiles into common consideration. On one hand, they share a commonality in being less understood from ecological and toxicological bases than the other vertebrate classes. Also, they are poikliothermic, a trait that they share with fishes but not mammals or birds. On the other hand, amphibian exposures in the wild can differ markedly from those of reptiles, especially with respect to sensitive life stages, for example, exposure differences linked to anamniotic vs. amniotic eggs as well as anatomical and physiological changes during metamorphosis. Risks associated with exposures to bioaccumulative chemicals also differ between classes, for example, relative contributions of dietary exposures vs. those encountered via skin or gill absorption. Whereas amphibians are more likely to be exposed to the more commonly encountered water-soluble compounds through uptake via gills, jellied eggs, and permeable skin, reptiles may be exposed to chemical mixtures in the field that are predominantly of bioaccumulative concern. Whereas turtles, aquatic snakes, tadpoles, and breeding adult amphibians may be exposed through the water column, most species of reptiles are terrestrial and experience different exposure routes. Clearly, we must continue our efforts for amphibians, and accentuate work with reptiles. If we do not, we face a future of resource management decisions being made despite our ignorance regarding contaminant effects on an otherwise fascinating group of animals.

18.1 EVALUATION OF EXPOSURE

As noted in Chapter 5, although relatively poorly characterized, exposure of amphibians and reptiles to chemical stressors is no less significant than that for other wildlife. As with other terrestrial vertebrates, the herpetofauna present a wide range of daily, seasonal, and annual movements; hence,

exposure to mobile (e.g., through atmospheric exposures to gases and particulate materials such as dusts) and stationary (e.g., in soils and foods subsequently ingested) contaminant sources is inevitably experienced in the field. Indeed, exposures may be as complex as any envisioned for other vertebrates and may reflect mechanisms unique to life history attributes of amphibians and reptiles.

If we recognize that exposures occur across heterogeneous habitats and include a similarly heterogeneous collection of environmental chemicals and other stressors, future research must better characterize exposure and the links between these exposures and effects. Effects linked to chemical mixtures vs. individual chemicals will likely be related to the type of chemicals, but also be species specific. However, effects may be somewhat predictable based on taxon-specific or life history attributes. Clearly, regardless of the application to evaluations of risks, much work remains to be done, particularly for evaluating exposures to amphibians and reptiles in the field. Presently, there are no forecasting models that help risk assessors posit acceptable exposure limits for environmental chemicals for the herpetofauna, particularly reptiles. Although risk assessment tools are primitive for evaluating risks for wild birds and mammals, such tools applied to evaluating risks for herpetofauna are prehistoric at best. By analogy, our current set of tools for evaluating risks of chemical contaminants on reptiles could only strive to attain "Stone Age" status and would likely be characterized by wishful thinking in their early stages of development. For example, exposure models for bioaccumulation are dominated by dietary exposures (food and water consumption) in terrestrial vertebrates, yet many variables affect the magnitude of bioaccumulation in terrestrial exposures in adults or early developmental stages in the herpetofauna (e.g., in reptile eggs), not unlike those exceptions frequently dismissed in risk evaluations for wild birds and mammals. The transfer of chemicals within food chains may conveniently be described by transfer coefficients or functions that characterize the relationships among trophic levels (see Chapter 5, this volume), yet we have only a limited understanding of the interrelationships among other exposure matrices and critical life stages in the ontogeny of amphibians and reptiles.

Similarly, research focused on interactions between chemicals with other stressors is equally important. We are becoming increasingly aware that chemical stressors interact with other stressors in the field, such as disease, predation, and competition for limited resources (e.g., food or habitat). Even climate change may interact with chemical toxicity. Future research should include a focus on the interactions among multiple stressors coupled with shifting baselines of physical and biological habitat alterations. This challenge to ecotoxicology is not unique to amphibians and reptiles, yet the herpetofauna could serve as vertebrate models more tightly linked to environmental change because of their life history attributes that contrast sharply with those of birds and mammals. Furthermore, through research that improves our understanding of the nutritional, behavioral, and energetic interactions that influence exposure and sensitivity to chemicals in the wild vs. laboratory settings, we could develop tools for evaluating chemical contaminant effects on amphibians and reptiles. In turn, these tools might even benefit those charismatic endotherms that frequently dominate the risk assessment picture typical of today's resource management and regulatory practice.

A good example of this has been summarized in Chapter 16 on malformations. Here, the authors observe that further study of the abnormality phenomena requires more large-scale, multivariate, and long-term studies. These studies should document not only the location and number of abnormalities, but also other important factors, such as hydrologic period, temperature, changes in surrounding landscape (e.g., development, agriculture), introduced predators, water quality, and disease. This requires students and researchers to look beyond the immediate study area (e.g., pond) and a single variable (e.g., chemical or infectious agent) and consider broader external influences. For example, the realization by field biologists that the spread of disease may be linked to our becoming unintended vectors of infective agents represents a new and complex factor in conducting research on amphibians and reptiles. The use of standard operating procedures that employ proper collection techniques, disinfection of equipment, shipping and handling of animals, and the timing and frequency of collection is more closely scrutinized within the scientific community as factors

affecting study outcomes. The establishment of standardized protocols and data collection methods will ensure that higher-quality, objective data are gathered and analyzed, and reduce subjectivity and variability.

18.2 EVALUATION OF TOXICITY

For evaluating threshold effect levels for environmental chemicals, we presently observe an increasing, yet meager offering of data for amphibians. Data for reptiles remain conspicuous in their absence. We remind those who study amphibians and reptiles that toxicity test methods are available for both these vertebrates. Although not as widely applied to routine toxicity assessment practices, and while fewer species (especially for reptiles) serve as models for evaluating chemical effects for a given taxon (class, order, family, genus, species), toxicity test methods are available. Results from these tests could then be considered with extrapolation methods commonly applied to evaluating toxicity data for surrogate species, including applications linked to evaluation of special status species. Interspecies correlation estimation (ICE) models and species sensitivity distributions (SSDs) are commonly used in evaluating risks associated with chemical exposures to a wide range of species and can be used with toxicity data from multiple taxa levels (see Newman et al. 2000, 2002; Posthuma et al. 2002). SSDs, ICE, and development of acute-to-chronic ratios (ACRs) for representative species and for threatened and endangered species could serve as the primary tools for extrapolations of toxicity data from toxicity test species to other species presumptively protected by these surrogates (e.g., western fence lizard [*Sceloporus occidentalis*] if we consider toxicity testing tools available for evaluating chemical effects on reptiles).

SSDs are based on the assumption that available toxicity data for a group of taxonomically related species are representative of the responses of other species for which toxicity data are not available, and that a statistical description of the toxicity data can be generated that permits calculation of the proportion of all species that would be adversely affected by exposure to a chemical at a given concentration. As long as the toxicological endpoint for each species is the same (e.g., LC50, no observed effect concentration [NOEC]), an SSD can be generated for any toxicological endpoint and mix of species. This feature of SSDs is one of their great advantages relative to other endpoint estimation methodologies, in that they explicitly consider the results of all available toxicological information for a given assemblage of species (Dyer et al. 2006; Awkerman et al. 2008; Raimondo et al. 2008; see also Posthuma et al. 2002).

Similarly, ICE models consider extrapolation from literature-acquired toxicity data to representative species (Aslaw et al. 2003). It remains impractical for toxicologists to perform laboratory toxicity studies on all species of amphibians or reptiles throughout North America, much less the entire world. In the United States, the Environmental Protection Agency lists more than 62 000 chemicals under the Toxic Subtances Control Act (TSCA; http://www.epa.gov/oppt/newchems/pubs/invntory.htm); several thousand more are covered under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA; http://www.epa.gov/compliance/resources/faqs/monitoring/fifra/estab-general.html). With all chemicals to which they are exposed in the environment, and even more so for species whose rarity or limited distribution in the environment generally precludes their use as test organisms, ICE models are statistical regressions that permit estimations of LC50s to be made for a species or higher taxa (genus, family) having no measured acute toxicity information from a species for which 5 or more LC50s have been measured. ACRs are typically applied to acute toxicity data to estimate chronic values.

Beyond our opening salvo that much more work needs to be done to better understand contaminant effects in amphibians and reptiles, we close our decade update by identifying critical objectives to satisfy research goals laid out in Chapter 1. Clearly, we need to continue our work with amphibians, but more importantly, we need to increase our efforts on developing a knowledge base for reptiles. At best, we now depend on comparative analyses that rely on other vertebrates for evaluating risks of contaminants on reptiles. But given the scant data available for reptiles, our

interpretations of contaminant effects on them are more ephemerally derived than real. The leaps of faith that presently guide our evaluation of contaminant effects in reptiles are simply not justified. Instead of tackling problems head on, too often the technical community strives to answer the simple questions that serve as "low-hanging fruit," so often sought by policy makers and land managers to demonstrate results. To lay out critical objectives that address existing knowledge gaps for evaluating chemical stressors, we have offered these suggestions for future research along lines familiar to toxicologists and environmental scientists. Our "to do" list may only scratch the surface, but our intent is to remind readers that a wide range of research needs exist. Here we have simply categorized those low-hanging fruits as critical starting points in assessing toxicity or exposure. We hope that the scientific process will not end here, but will evolve and progress through the use of designed field and laboratory studies to further investigate the potential and frequently complex interactions among multiple stressors. This integrated approach affords the researcher the best of both worlds and a greater likelihood of reaching an optimum study design to address questions directly serving conservation and regulatory concerns.

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Appendix: Metal Contamination in Reptiles

An Appendix of Data Compiled from the Existing Literature

Britta Grillitsch and Luis Schiesari

Summary tables contained in Chapter 12 were developed from existing literature that was assembled, following a comprehensive library search as detailed below. The metadata and results of that literature search have been summarized in metal-specific tables included in this appendix. A complete bibliography and original sources for the data included in Chapter 12 are also included in this appendix. Please refer to Chapter 12 for interpretation of these data, as well as the authors' recommendations for future research focused on metals and their effects on reptiles.

LITERATURE SEARCH PROCESS

For selection of metallic elements included in this review, we followed the division of elements between metals, metalloids, and nonmetals of Manahan (2003, 2004) and the USEPA (2007) list of "Metals and Metalloids of Primary Interest" (see Figure 12 in Chapter 12). Information on radioactive elements was excluded, as were those alkali metals and alkaline earth metals that show generally low ecotoxicological relevance and tissue concentrations at "macro levels" (Ca, K, Na). If not stated otherwise, the term "metallic elements and their compounds" or, more briefly, "metals" has been used in Chapter 12 and this appendix as generic terms including both metals and semimetals.

For reptile taxonomy and for species biogeographic or ecosystem categorization, we followed Udvardy (1975), Zug et al. (2001), IUCN (2007), and Uetz et al. (2007). Because ecotoxicology is an interdisciplinary science and many terms are used in different contexts, we provide in the introduction of each section in Chapter 12 definitions for the terminology we use.

The body of literature upon which this review is based was derived from 1) structured literature searches in databases (BIOSIS, MEDLINE, ZOOLOGICAL RECORD), 2) review publications as listed in our introduction, and 3) primary publications. We included all published information available with the exception of conference abstracts and dissertations subsequently published in peer-reviewed journals. Most information included in this review was directly extracted from the primary literature; a few secondary sources that we were unable to obtain were also included (and so indicated) in the tables.

For presentation, the extracted information was first condensed into tables of results grouped by study type (i.e., kinetics vs. dynamics, experimental vs. observational) and subdivided by contaminant, reptile order or suborder, species, and year of publication. As available, each dataset contains key information on the biota and study design (e.g., species, sample sizes), symptoms (e.g., tissue residue levels, mass basis), exposure (e.g., location, type of contamination), and literature reference. Then, information was further condensed into summary tables and exemplary graphs that formed the basis for discussion and conclusions developed for this review.

For the results tables, quality assurance included checks for plausibility, completeness, correctness, and consistency. Data were first extracted into the results tables and subjected to plausibility

and completeness checks by one of the authors, followed by a cross-check by the other author. Cross-checking included direct comparison of the extracted information with that provided in the original primary literature, based on all publications of 2 key authors plus 10 further publications randomly selected among the remaining authors.

APPENDIX LEGEND
Concentrations (µg/g or ppm) of metals and metalloids in tissues of reptiles

Symbols and abbreviations

Columns

	—	No information given in the reference
	[ ]	Name in reference (for cases when species name in reference fell in synonymy after publication)
Species		
Specification		
	CCL	Curved carapace length
	TL	Total body length; carapace length for Testudines
	A	Age
	SCL	Standard carapace length; straight standard carapace length; straight-line carapace length
	MSCL	Minimum straight carapace length
	MCL	Midline carapace length
	SVL	Snout-vent length
	TM	Total weight; total mass
	M	Male
	F	Female
	U	Unknown sex
Concentration		
	ASH	Concentration based on presumed ash-free dry mass
	DRY	Concentration based on dry mass
	WET	Concentration based on wet mass
	DL	Detection limit
	ND	Not detected; below detection limit
	NA	Not analyzed
Statistics		
	GLSM	Geometric least squared mean
	GM	Geometric mean
	M	Mean; arithmetic mean
	MD	Median
	MAX	Maximum observed value
	MIN	Minimum observed value
	NC	Not calculated
	R	Range
	RM	Range of means
	RAM	Range of arithmetic means
	RGM	Range of geometric means
References, Locations, Remarks		
	asl	Above sea level

Note: Within the same study and row, specifications refer to all blank cells below. For body size (length and mass), information is often only available as the range across all specimens in the study; residue analysis information that we report may refer to only a subset of these individuals.

TABLE A.1
Aluminum (Al)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Testudines								
*Actinemys marmorata**								Henny et al. (2003); USA; western Oregon, Fern Ridge Reservoir; *[*Clemmys marmorata*]
	Egg		—	14*	Contents	ND (<4.0)	DRY	*14 nests, 1 egg per nest
Caretta caretta								Stoneburner et al. (1980); USA; 4 western Atlantic nesting beaches; Florida, Canaveral; Georgia, Cumberland Island; North Carolina, Cape Lookout; North Carolina, Cape Hatteras
	Egg		—	96	Yolk	3.56–6.30	—; RM*	*Combined by beach
*Caretta caretta**								Aguirre et al. (1994); USA; Hawaiian Islands; *[identification number 052, *Chelonia mydas* in the reference]
	"Pelagic"		—	1	Liver	ND (<1.0)	WET	
Caretta caretta								Torrent et al. (2004); Spain; Canary Islands; stranded turtles submitted to the Veterinary Faculty of the University of Las Palmas in Gran Canaria
	Juvenile and subadult	R	F/M	67/11	Kidney	0.72; 0.03–7.58	WET: M; R	*Values estimated from graph
	SCL = 15–65 cm*							
					Muscle	1.49; 0.06–8.32		
					Bone	30.49; 0.03–234.15		
					Liver	2.21; 0.12–31.08		
Chelonia sp.								Yoshinaga et al. (1992); Papua New Guinea
	—		—	—	Muscle	25.8	WET: M	
Chelonia mydas								Yoshinaga et al. (1992); Papua New Guinea
	—		—	—	Muscle	12.2	WET: M	

(*continued*)

TABLE A.1 (CONTINUED)
Aluminum (Al)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Chelonia mydas								Aguirre et al. (1994); USA; Hawaiian Islands
	L = 28.7–71.3 cm W = 3.2–43.6 kg	R	F/M	8/4	Liver	ND (< 1.0)–5.0	WET: R	
					Kidney	ND (<1.0)–2.0		
	Egg		—	3	Shell	3.0	WET: M	*Posthatch
	Hatchling				Whole body	ND (<1.0)		
Trachemys scripta elegans								Tryfonas et al. (2006); USA; Illinois, Lower Illinois River near Grafton; eggs laid in the lab from turtles collected in the field from 5 nesting areas
	Egg			—	Contents	2.2**	DRY: M*	*All sites combined; **values estimated from graphs
					Shell	ND(<—)**		**From graph
	Diet				*Lemna* sp.	5190		
	Environment			4–5*	Soil	14500–30700	RM	*2 sites, soil from nesting and lake bank areas
				3*	Sediment	15000–23000**		*2 sites, 3 sediment layers per site; **from graph
				3–4*	Water	4.4–5.0	WET: RM	*3 sites
Crocodylia								
Alligator mississippiensis								Heinz et al. (1991); USA; central and southern Florida; Lake Okeechobee, Lake Griffin, and Lake Apopka
	Egg		—	32*	Egg	1.3–2.0; 0.86–3.0	WET: RM; R**	*16 nests, 2 eggs per nest; **combined by lake
Alligator mississippiensis								Presley et al. (2005); USA; Louisiana, New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	—		—	1	Liver	0.37	WET	

					Kidney	3.17		
Crocodylus acutus								Stoneburner and Kushlan (1984); USA; Florida; Florida Bay, Everglades National Park
	Egg		—	9	Shell	52.36	DRY: M	
					Albumen-yolk	10.86		
Crocodylus niloticus								Swanepoel et al. (2000); South Africa; Kruger National Park (KNP) area
	TL = 1.40–4.15 m	R	F/M	6/9			DRY: M	
								Site 1: Shingwedzi River (Silwervis Dam), northern part of KNP; catchment area outside KNP is limited
					Muscle	367.8		Frozen tissues used for residue analyses
					Liver	487.6		
					Kidney	360.6		
					Fat	367.6		
								Site 2: Olifants River, central part of KNP; flows through areas of intense agricultural activity and passes mining areas and receives tributaries from Phalaborwa Mining Company before entering the KNP
					Muscle	147.2		Frozen tissues used for residue analyses
					Liver	363.9		
					Kidney	135.4		
					Fat	111.1		
								Site 3: Sabi River, southern part of KNP; flows through areas of intense agricultural activity before entering the KNP
					Muscle	73.5		Frozen tissues used for residue analyses
					Liver	175.2		
					Kidney	40.8		
					Fat	55.6		
Crocodylus porosus								Yoshinaga et al. (1992); Papua New Guinea
	—		—	—	Muscle	4.1	WET: M	

(*continued*)

TABLE A.1 (CONTINUED)
Aluminum (Al)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Crocodylus porosus								Jeffree et al. (2001); northern Australia; Alligator Rivers region, Kakadu National Park; samples from 3 river catchments; mining and hunting areas included
	Age = 5–40 years L = 168–499 cm	R	—	40			DRY: M; R	
			—	35	Muscle*	89.9; 26–132		*Tail
			—	40	Osteoderm*	ND (<0.05)		*Ventral pelvic region
Squamata: Sauria								
*Laudakia s. stellio**								Loumbourdis (1997); Greece, Thessaloniki region; *[*Agama s. stellio*]
	Adult		—	—	Liver	119.98	DRY: M	Urban area (500 m asl)
					Carcass	769.49		
					Liver	132.99		Agricultural area (50 m asl)
					Carcass	1601.70		
Tarentola mauritanica								Fletcher et al. (2006); southern Spain; Guadiamar River Valley; mine tailings release; Boliden-Apirsa mine at Aznalcóllar; 7 study sites spanning an expected contamination gradient; geckos collected from building walls
	Adult and juvenile			52	Whole body*		DRY: M; R	*Minus gut contents
			—	9		23.020; 10.857–46.876		Site 1: Rural not mine-affected site: near Guadalmellato (most pristine)
			—	13		23.012; 11.100–62.992		Site 2: Urban not mine-affected site: Villaviciosa de Cordoba (not contaminated by mining)
			—	8		21.238; 14.037-45.734		Site 3: Urban mine-affected site: Aznalcázar (contamination through aerosolized contaminants engulfed the town during cleanup)

			—	8		26.659; 11.250–46.182		Site 4: Urban mine-affected site: Aznalcóllar (contaminated by normal mine operations, or the disaster and subsequent remediation efforts)
			—	5		20.865; 14.369–35.299		Site 5: Floodplain mine-affected site: Guadiamar River floodplain near the Aznalcázar gauge station (24.8 km below the ruptured tailings dam)
			—	5		23.553; 10.349–54.887		Site 6: Floodplain mine-affected: Guadiamar River floodplain near the Guijo gauge station (7.4 km below the ruptured dam)
			—	4		37.997; 30.302–46.474		Site 7: Floodplain mine-affected site: Agrio River floodplain (4.4 km below and closest to the ruptured tailings dam)
								Statistical analysis: [Al] concentration was not significantly influenced by site
Varanus sp.								Yoshinaga et al. (1992); Papua New Guinea
	—		—	—	Muscle	6.1	WET: M	
Squamata: Serpentes								
Acrochordus javanicus								Yoshinaga et al. (1992); Papua New Guinea
	—		—	—	Muscle	3.0	WET: M	
Agkistrodon piscivorus								Presley et al. (2005); USA; Louisiana, New Orleans, near Maxent Canal site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 58.6 cm TM = 365 g	M	—	6	Liver	2.62	WET: M	
					Kidney	5.82		
Agkistrodon piscivorous								Burger et al. (2006); USA; South Carolina; 1 reference site, 1 polluted Savannah River site near Aiken
	SVL = 57 cm TM = 280 g	M	—	13	Muscle*	431	DRY: M**	*Tail; **both sites combined

(*continued*)

TABLE A.1 (CONTINUED)
Aluminum (Al)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
	SVL = 60 cm							
	TM = 270 g			5	Blood	3		
Coluber constrictor	SVL = 103.2 cm							Presley et al. (2005); USA; Louisiana, New Orleans, near Maxent Canal site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	TM = 300 g							
		M	—	1	Liver	12.70	WET	
					Kidney	2.34		
Nerodia cyclopion	SVL = 63.9 cm							Presley et al. (2005); USA; Louisiana, New Orleans, near Maxent Canal site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	TM = 191 g							
		M	—	1	Liver	6.65	WET	
					Kidney	12.20		
Nerodia fasciata								Burger et al. (2006); USA; South Carolina; 1 reference, 1 polluted Savannah River site, Aiken
	SVL = 51cm	M	—	47	Muscle*	93	DRY: M**	*Tail; **both sites combined
	TM = 130 g							
	SVL = 52 cm			34	Blood	2		
	TM = 140 g							
Nerodia taxispilota								Burger et al. (2006); USA; South Carolina; 2 sites: 1 reference and 1 polluted Savannah River site, Aiken
	SVL = 59 cm	M	—	10	Muscle*	169	DRY: M**	*Tail; **both sites combined
	TM = 170 g							
	SVL = 65 cm			9	Blood	2		
	TM = 230 g							

TABLE A.2
Antimony (Sb)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Testudines								
Caretta caretta								Kaska et al. (2004); Turkey; southwestern Mediterranean coast; stranded turtles
	—	—	—	32	Liver	ND (<—)	DRY: M; R	
				20	Bladder	0.96; ND (<—)–1.66		
				20	Kidney	2.35; 1.30–3.67		
				10	Lung	3.06; 0.97–5.43		
				32	Muscle	2.51; 1.21–3.84		
Chelonia mydas								Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 49.0; 40.0–63.5 cm	M; R	M	6			DRY: M; R	
	SCL = 52.2; 37.0–71.4 cm		F	20				
			M	6	Liver	0.22; 0.01–0.62		
				6	Kidney	0.08; ND (<0.01)–0.27		
				3	Muscle	ND (<0.01); ND (<0.01)		
			F	20	Liver	0.21; 0.01–0.85		
				19	Kidney	0.04; ND (<0.01)–0.17		
				9	Muscle	0.009; ND (<0.01)–0.01		
	Diet		—	8	Stomach content	0.07; 0.02–0.25		
Chelonia mydas								Kaska et al. (2004); Turkey; southwestern Mediterranean coast; stranded turtles
	—	—	—	22	Liver	ND (<—)		
				20	Bladder	0.74; ND (<—)–1.34		
				14	Kidney	1.46; 0.55–2.59		
				15	Lung	2.71; 2.06–3.12		
				22	Muscle	1.24; 0.36–1.94		

(*continued*)

TABLE A.2 (CONTINUED)
Antimony (Sb)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Chelonia mydas								Lam et al. (2004); South China
	Juvenile		—	2	Fat	0.029	DRY: M	Turtles stranded at Ham Tin Wan and Tung Pang Chau beaches (had started to decay upon collection)
					Kidney	0.015		
					Heart	0.009		
					Liver	0.103		
					Lung	0.019		
					Muscle	0.017		
					Stomach	0.035		
	Adult							Turtles believed to have been unintentionally caught by fishermen (were relatively fresh upon collection)
			—	3	Muscle	0.039		
				1	Liver	0.182		
Eretmochelys imbricata								Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 43.5; 33.5–48.9 cm	M; R	M	6	Liver	0.09; 0.02–0.27	DRY: M; R	
				6	Kidney	0.02; ND (<0.01)–0.04		
				1	Muscle	ND (<0.01)		
	SCL = 46.5; 43.8–67.9 cm		F	16	Liver	0.08; 0.01–0.24		
				13	Kidney	0.02; ND (<0.01)–0.04		
				8	Muscle	0.008; ND (<0.01)–0.02		
	Diet		—	6	Stomach content	0.02; ND (<0.01)–0.04		

Squamata: Sauria						
Tarentola mauritanica						Fletcher et al. (2006); Southern Spain; Guadiamar River Valley; mine tailings release: Boliden-Apirsa mine at Aznalcóllar; 7 study sites spanning an expected contamination gradient; geckos collected from building walls
Juvenile and adult		52	Whole body*		DRY: M; R	*Minus gut contents
	—	9		0.044; 0.044–0.044		Site 1: Rural not mine-affected site: near Guadalmellato (most pristine)
	—	13		0.044; 0.044–0.044		Site 2: Urban not mine-affected site: Villaviciosa de Cordoba (not contaminated by mining)
	—	8		0.044; 0.044–0.044		Site 3: Urban mine-affected site: Aznalcázar (contamination through aerosolized contaminants engulfed the town during cleanup)
	—	8		0.044; 0.044–0.044		Site 4: Urban mine-affected site: Aznalcóllar (contaminated by normal mine operations, or the disaster and subsequent remediation efforts)
	—	5		0.044; 0.044–0.044		Site 5: Floodplain mine-affected site: Guadiamar River floodplain near the Aznalcázar gauge station (24.8 km below the ruptured tailings dam)
	—	5		0.044; 0.044–0.044		Site 6: Floodplain mine-affected site: Guadiamar River floodplain near the Guijo gauge station (7.4 km below the ruptured dam)
	—	4		0.258; 0.209–0.336		Site 7: Floodplain mine-affected site: Agrio River floodplain (4.4 km below and closest to the ruptured tailings dam)
						Statistical analysis: [Sb] concentration was not significantly influenced by site

TABLE A.3
Arsenic (As)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Testudines								
*Actinemys marmorata**								Henny et al. (2003); USA; Western Oregon, Fern Ridge Reservoir; *[*Clemmys marmorata*]
	Egg		—	14*	Contents	ND (<2.00)	DRY: R	*14 nests, 1 egg per nest
*Caretta caretta**								Aguirre et al. (1994); USA; Hawaiian Islands; *[identification number 052, *Chelonia mydas* in the reference]
	"Pelagic"		—	1	Liver	0.9	WET	
Caretta caretta								Gordon et al. (1998); Australia; southeastern Queensland, Moreton Bay region; stranded turtles
	—	—	—	6	Liver	0.46; 0.0–1.56	WET: M; R	
				3	Kidney	0.71;0.24–1.15		
Caretta caretta								Storelli et al. (1998a); Italy; Adriatic Sea, Apulian coasts; stranded turtles
	TM = 1.8–100 kg	R	F/M	9/3	Liver	21.67; 0.83–56.55	DRY: M; R	
					Lung	24.00; 10.62–44.93		
					Kidney	29.91; 6.09–139.60		
					Muscle	68.94; 11.21–139.60		
Caretta caretta								Storelli and Marcotrigiano (2000); Italy; Adriatic Sea; Apulian coast; stranded turtles; different sites
	TM = 1.8–90 kg	R		7	Muscle	15.47; 2.64–32.39	WET: M; R	Total As
						15.20; 2.38–31.11		Organic As
						0.18; 0.08–0.32		Inorganic As
					Liver	6.70; 2.68–13.07		Total As
						6.27; 2.17–12.66		Organic As
						0.63; 0.30–1.22		Inorganic As

Caretta caretta								Kubota et al. (2003); North Pacific Ocean; specimens were relatively fresh strandings along the coasts, accidential catches by fisherman, or caught for commercial and scientific purposes; samples were collected in 1990, stored at –20 °C, and freeze dried in 1999 and 2000
	SCL = 77; 46–92 cm	M; R	—	5	Liver	11.2	DRY: M	Total As
						6.15		Arsenobetaine
						0.44		Dimethylarsinic acid
						2.85		Arsenocholine
						0.04 ($n = 4$)		Tertramethylarsonium ion
						ND (<0.03) ($n = 1$)		
						ND (<0.03) ($n = 5$)		Methylarsonic acid
						0.04 ($n = 1$)		Arsenite
						ND (<0.03) ($n = 4$)		
Caretta caretta								Kaska et al. (2004); Turkey; southwestern Mediterranean coast; stranded turtles
	—		—	32	Liver	14.23; 2.19–24.32	DRY: M; R	
				20	Bladder	14.40; 1.48–34.08		
				20	Kidney	18.99; 0.98–35.37		
				10	Lung	8.66; 1.41–22.90		
				32	Muscle	20.80; 2.37–37.49		
Caretta caretta								Torrent et al. (2004); Spain; Canary Islands; stranded turtles submitted to the veterinary faculty of the University of Las Palmas in Gran Canaria; *values estimated from graph
	Juvenile and subadult	R	F/M	67/11				
	SCL = 15–65 cm*				Kidney	13.80; 1.16–122.12	WET: M; R	
					Muscle	7.35; 1.55–67.22		
					Bone	12.45; 9.93–150.44		
					Liver	17.07; 0.08–131.86		

(*continued*)

TABLE A.3 (CONTINUED)
Arsenic (As)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Chelonia mydas								Aguirre et al. (1994); USA; Hawaiian Islands
	L = 28.7–71.3 cm W = 3.2–43.6 kg		F/M	8/4			WET: R	
					Liver	ND (<0.6)–6.4		
					Kidney	ND (<0.6)–6.8		
	Egg*		—	3	Shell	ND (<0.6)		*Posthatch
	Hatchling				Whole body	ND (<0.6)		
Chelonia mydas								Gladstone (1996) from Gordon et al. (1998); Australia, Torres Strait
	—	—	—	7	Liver	1.5; 0.4–4.3	WET: M; R	
					Kidney	0.42; 0.07–1.2		
Chelonia mydas								Gordon et al. (1998); Australia; southeastern Queensland, Moreton Bay region; stranded turtles
	—	—	—	23	Liver	0.26; 0.04–0.74	WET: M; R	
				23	Kidney	0.19; 0.00–0.69		
Chelonia mydas								Kubota et al. (2003); North Pacific Ocean; specimens were relatively fresh strandings along the coasts, accidential catches by fisherman, or caught for commercial and scientific purposes; samples were collected in 1990, stored at –20 °C, and freeze dried in 1999 and 2000
	SCL = 48; 44–56 cm	M; R	—	5	Liver	3.65	DRY: M	Total As
						1.66		Arsenobetaine
						0.19		Dimethylarsinic acid
						0.24		Arsenocholine
						0.04 ($n = 4$) ND (<0.03) ($n = 1$)		Tertramethylarsonium ion

					ND (<0.03)		Methylarsonic acid
					0.07–0.05 (n = 2)		Arsenite
					ND (<0.03) (n = 3)		
Chelonia mydas							Fujihara et al. (2003); Japan; Ishigaki Island; fresh samples stored at –80 °C
	—	—	5	Liver	1.2; 0.80–2.3	WET: M; R	Total As
					0.10; 0.032–0.20		Dimethylarsenic acid
					0.79; 0.27–1.5		Arsenobetaine
					0.026; 0.011–0.049		Arsenocholine
					ND (<0.01)		Tetramethylarsonium ion
					0.065; 0.043–0.084		Methylarsonic acid
Chelonia mydas							Kaska et al. (2004); Turkey; southwestern Mediterranean coast; stranded turtles
	—	—	22	Liver	9.70; 1.43–16.89	DRY: M; R	
			20	Bladder	10.89; 1.83–18.22		
			14	Kidney	13.76; 5.16–20.69		
			15	Lung	7.51; 0.76–14.38		
			22	Muscle	15.46; 5.38–27.74		
Chelonia mydas							Lam et al. (2004); South China
	Juvenile					DRY: M	Turtles stranded on beaches at Ham Tin Wan and Tung Pang Chau; upon collection, turtles had started to decay
		—	2	Fat	1.273		
				Kidney	6.965		
				Heart	4.826		
				Liver	4.653		
				Lung	3.295		
				Muscle	14.45		
				Stomach	4.058		

(*continued*)

TABLE A.3 (CONTINUED)
Arsenic (As)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
	Adult							Turtles believed to have been unintentionally caught by fishermen; upon collection, turtles were relatively fresh
			—	3	Muscle	14.61		
				1	Liver	19.57		
Chelonia mydas								Celik et al. (2006); Turkey; Mediterranean Sea, Kazanli beach; stranded individuals
	Egg*			12**	Shell	ND (<0.19)	DRY	*Posthatch; **12 nests, 3 eggshells per nest
	Environment							Also available in this study: metal concentrations in sand, soil, plant, and water samples around the nesting environment
Dermochelys coriacea								Davenport and Wrench (1990), Davenport et al. (1990); Cardigan Bay, Irish Sea, Wales, Great Britain; Stranded animal
	L = 2.53 m W = 916 kg		M	1	Liver	0.58	DRY: M*	*4 replicate measures
					Muscle*	0.21		*Pectoral
					Blubber	1.28		
Dermochelys coriaca								Godley et al. (1998); Great Britain; Wales and Scotland, west coast; turtles died in fishing gear
	Adult CCL = 141–170 cm	R	M	3				
				1	Liver	8.2	DRY	
					Muscle	14.0		
					Liver	2.6	WET	
					Muscle	4.7		
Eretmochelys imbricata								Gordon et al. (1998); Australia; southeastern Queensland, Moreton Bay region; stranded turtles
	—	—	—	2	Liver	0.18–1.85	WET: R	

				2	Kidney	0.13–0.93		
Eretmochelys imbricata								Fujihara et al. (2003); Japan; Ishigaki Island
			—	5	Liver	4.4; 0.66–7.5	WET: M; R	Total As
				5		0.17; 0.048–0.23		Dimethylarsenic acid
				5		3.3; 0.37–5.9		Arsenobetaine
				5		0.18; 0.031–0.48		Arsenocholine
				2		ND (<0.01)–0.018		Tetramethylarsonium ion
				4		0.20; ND (<0.01)–0.44		Methylarsonic acid
Gopherus agassizii								Seltzer and Berry (2005); USA; California; preserved samples originating from tortoises (alive at necropsy) of previous studies (Homer et al. 1998; Berry et al. 2001) were analyzed using laser ablation inductively coupled plasma mass spectrometry (laser ablation ICP-MS)
	Adult				Dermal scutes		M*	*Concentration averaged over laser ablation transect profile
	MCL = 178 mm TM = 0.90 kg	R	M	1		0.2		Healthy individial
	MCL = 252 mm TM = 2.3		F	1		3.6		Diseased individial (mycoplasmosis)
	MCL = 271 mm TM = 3.73		M	1		1.4		Diseased individial (multiple inflammation)
	MCL = 210 mm TM = 1.75		F	1		5.3		Diseased individial (cutaneous dyskeratosis)
Malaclemys terrapin								Burger (2002); USA; Barnegat Bay, New Jersey
	Adult L = 14.3 cm	M	F	11	Liver	0.562	WET: M	
					Muscle	0.728		
	Egg		—	8	Egg *	0.012		* Ovarial

(*continued*)

TABLE A.3 (CONTINUED)
Arsenic (As)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Trachemys scripta								Nagle et al. (2001); USA; South Carolina, Aiken County, Savannah River site; in ovo exposure experiment; females collected in the field in 1993, 1994; oviposition induced in 1995; eggs from turtles collected in both polluted and unpolluted sites were incubated in coal ash-contaminated and uncontaminated soil in outdoor artificial nests
							DRY: M	Site 1: Coal ash-polluted Savannah River site
	Adult		F	4	Liver	9.56		
	Diet		—	12	Asiatic clam*	13.22		*Soft tissue
			—	3	Crayfish*	8.71		*Whole body
	Environment		—	—	Artifical nest*	25.08		*Incubation substrate
								Sites 2–3: Unpolluted control sites near Couchton
	Adult		F	3	Liver	0.26		
	Diet		—	10	Asiatic clam*	3.85		*Soft tissue
			—	3	Crayfish*	0.99		*Whole body
	Environment		—	—	Artifical nest*	0.93		*Incubation substrate
	Hatchling							Trial 1: Incubation substrate: coal ash contaminated
	CL = 30.34 mm	M	—	18 *				*18 hatchlings from 5 clutches
	TM = 8.20 g							
			—	6 **	Whole body	46		**3 clutches per site, 2 hatchlings per clutch
	Hatchling							Trial 2: Incubation substrate: uncontaminated
	TM = 30.36 g	M		18*				*18 hatchlings from 5 clutches
	CL = 8.02 mm							
				6**	Whole body	0.45		**3 clutches per site, 2 hatchlings per clutch
	Hatchling							Trial 3: Maternal residence: coal ash contaminated
	CL = 30.51 mm							
	TM = 8.02 g	M M	—	18*				*18 hatchlings from 4 clutches
			—	6**	Whole body	0.49		**3 clutches per site, 2 hatchlings per clutch
	Hatchling							Trial 4: Maternal residence: uncontaminated

	TM = 29.69							
	CL = 7.77 mm			18*				*18 hatchlings from 6 clutches
				6**	Whole body	0.43		**3 clutches per site, 2 hatchlings per clutch
								Statistical analysis: [As] concentrations were significantly higher in adult liver and prey from site 1; [As] concentrations in hatchlings were not significantly different between trials 1 and 2 or 3 and 4
Crocodylia								
Alligator mississippiensis								Ogden et al. (1974); USA; Florida; Everglades, Shark Valley, Central Shark Slough
	Egg	—	—	4	Contents	0.12; 0.05–0.2	WET: M; R	
Alligator mississippiensis								Delany et al. (1988); USA; Florida; 8 lakes, statewide
	TL = 2.9–3.8 m	—	F/M	1/23	Muscle*	ND (<0.02)	WET	*Tail
Alligator mississippiensis								Heinz et al. (1991); USA; central and southern Florida; Lake Okeechobee, Lake Griffin, and Lake Apopka
	Egg		—	32*	Egg	ND (<0.05)	WET	*16 nests, 2 eggs per nest
Alligator mississippiensis								Burger et al. (2000); USA; Florida; Lake Apoka (Lake and Orange Counties), Orange Lake (Alachua County), and Lake Woodruff (Volusia County)
	Yearling TL = 36–40 cm	R					WET: M*	*3 lakes combined
				30	Fat*	0.0176		*Abdominal
				31	Liver	0.0348		
				30	Muscle*	0.0112		*Abdominal
				29	Skin*	0.0194		*Abdominal
				29	Muscle*	0.0156		*Ventral, proximal tail
				22	Tail tip	0.0225		
				3	Tail*	0.00010		*Regenerated

(*continued*)

TABLE A.3 (CONTINUED)
Arsenic (As)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Alligator mississippiensis								Presley et al. (2005); USA; Louisiana, New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	—	—	—	1	Liver	0.06	WET	
					Kidney	0.16		
Alligator sinensis								Xu et al. (2006); China; Changxing County, Zhejiang Province; Changxing Nature Reserve and Breeding Research Center for Chinese alligator
	Adult		F/M	1/1	Heart	0.768/0.460	DRY: M	Alligators collected dead of unidentified causes
					Lung	0.639/0.441		
					Liver	0.676/0.497		
					Kidney	0.589/0.406		
					Intestine	0.327/0.250		
					Trachea	0.554/0.534		
					Pancreas	0.176/0.126		
					Gonad	0.595/0.078		
					Muscle	0.390/0.222		
					Stomach	0.525/0.356		
	Diet		—	—	Fish*	0.156		*Two fish prey species randomly sampled
	Excrements		—	6	Feces	1.515		
	Egg			10*			M; R	*10 eggs from 3 clutches
					Eggshells	0.262; 0.125–0.356		
					Shell membranes	0.167; 0.103–0.274		
					Egg contents	0.474; 0.446–0.501		
	Environment			10*				*Water and sediment (5 subsamples from different sites within a pond each) collected from 10 breeding ponds
					Sediment	4.96		

					Water	0.00077	WET: M	
								Statistical analysis: [As] concentrations were significantly higher in contents than in shells and membranes; differences between shell and membranes were not significant; significant positive correlations existed in the contents among concentrations of [As] and [Cu], [Pb] and [Cr]
Crocodylus acutus								Ogden et al. (1974); USA; Florida, Florida Bay, Everglades
	Egg	—	—	5	Contents	0.07; 0.06–0.08	WET: M; R	
Crocodylus acutus								Rainwater et al. (2007); Costa Rica; Rio Grande de Tarcoles; known to be polluted by several metals from various sources
	SVL = 155.7; 134.0–172.0 cm	M; R	F/M	1/5	Scute*	ND (<0.05)	WET: M	*1 whole caudal scute per crocodile
Crocodylus moreletii		M; R					WET	Rainwater et al. (2007); Belize
	SVL = 89.8; 65.0–129.5 cm		F/M	5/4				Site 1: Gold Buton Lagoon
					Scute*	ND (<0.05)		*1 whole caudal scute per crocodile
	SVL = 104.4; 59.5–156.7 cm		F/M	4/6				Site 2: New River Watershed
					Scute *	ND(<0.05)		*1 whole caudal scute per crocodile
Crocodylus niloticus								Almli et al. (2005); Zambia
	L = 2.7–3.4 m	R	F/M	2/2	Liver	0.008; 0.005–0.016	WET: MD; R	Site 1: Kafue River, Kafue National Park
					Kidney	0.009; 0.005–0.011		
	L = 2.0–4.0 m		F/M	4/1	Liver	0.049; 0.013–0.068		Site 2: Luangwa River, Luangwa National Park
					Kidney	0.020; 0.014–0.048		

(*continued*)

TABLE A.3 (CONTINUED)
Arsenic (As)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Squamata: Sauria							
"Lizard"							Hsu et al. (2006); South Taiwan; Kenting National Park; results indicated strong influence from industrial pollution
	—	—	—	1	Whole body	1.07	DRY
						0.15	Bioconcentration factor; reference media: soil and food items
Chamaeleo chamaeleon							Gomara et al. (2007); Southwest Spain; province of Cadiz; 3 sampling sites; 9 nests; eggs collected 0–15 days after oviposition
	Egg	—	—	2–4*	Contents	ND (<0.0004)–0.0019	WET: RM *number of eggs pooled per nest
					Shells	0.040–0.135	
*Iberolacerta monticola**							Marco et al. (2004); Spain; Avila, Gredos Mountains; gravid females collected in the field in 2001; freshly laid eggs incubated until hatching in As (arsenic acid in nitric acid)-loaded artificial breeding substrate (sterile As-free vermiculite) for 18 days; further elements (Cd, Cu, Pb, Zn), though not manipulated, were detected in eggshells and embryos; *[*Lacerta monticola cyreni*]
	Egg		—	4	Shell	As: 0.0147	WET: M Treatment: Control: no As addded to breeding substrate water
						Cd: 0.00348	
						Cu: 1.08	
						Pb: 0.1426	
						Zn: 9.19	
					Embryo	ND (<0.0002)	
						Cd: 0.00300	

	Cu: 0.79	
	Pb: 0.0317	
	Zn: 14.03	
Shell	As: 0.0296	Treatment: 0.050 µg As/ml substrate water
	Cd: 0.00414	
	Cu: 1.19	
	Pb: 0.0593	
	Zn: 7.59	
Embryo	As: 0.0160	
	Cd: 0.00300	
	Cu: 0.73	
	Pb: 0.612	
	Zn: 13.26	
Shell	As: 0.0574	Treatment: 0.100 µg/ml As substrate water
	Cd: 0.00710	
	Cu: 1.66	
	Pb: 0.819	
	Zn: 15.52	
Embryo	As: 0.0129	
	Cd: 0.00319	
	Cu: 0.49	
	Pb: 0.555	
	Zn: 13.82	
Shell	As: 0.0644	Treatment: 0.250 µg As/ml substrate water
	Cd: 0.00252	
	Cu: 0.74	
	Pb: 0.0482	
	Zn: 11.22	
Embryo	As: 0.0259	
	Cd: 0.00273	
	Cu: 0.71	
	Pb: 0.1409	
	Zn: 11.26	

(*continued*)

TABLE A.3 (CONTINUED)
Arsenic (As)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
					Shell	As: 0.0885 Cd: 0.002,85 Cu: 0.79 Pb: 0.0426 Zn: 9.30		Treatment: 0.500 µg As/ml substrate water
					Embryo	As: 0.0382 Cd: 0.00283 Cu: 0.54 Pb: 0.0966 Zn: 13.26		
								Statistical analyses: Positive relationship between As concentrations in substrate water, and both eggshells and embryos were highly significant. [As] concentrations were significantly higher in shells than in embryos. Also detected in eggshell and embryo where there was no relationship between As in the substrate and the concentrations of Cd, Cu, Pb, and Zn detected in eggshells or embryos.
*Norops sagrei**								Burger et al. (2004); USA; South Florida and Florida; [*Anolis sagrei*]
	Adult							
							WET: M; RM; R*	*6 sites combined
	SVL = 43.5; 35–50 mm	M; R	F	72	Whole body*	0.262; 0.0559–0.444; ND (<0.0002)–2.207		*Minus gut content
	SVL = 55.3; 45–67 mm		M	72		0.163; 0.0525–0.331; ND (<0.0002)–1.856		

Tarentola mauritanica							Fletcher et al. (2006); southern Spain; Guadiamar River Valley, Boliden-Apirsa mine at Aznalcóllar; mine tailings release event; 7 sites spanning an expected contamination gradient; geckos collected from building walls
	Adult and juvenile		52	Whole body*		DRY: M; R	*Minus gut contents
		—	9		0.066; 0.046–0.120		Site 1: Rural non-mine-affected site: near Guadalmellato (most pristine)
		—	13		0.186; 0.046–0.507		Site 2: Urban non-mine-affected site: Villaviciosa de Cordoba (not contaminated by mining)
		—	8		0.284; 0.112–0.854		Site 3: Urban mine-affected site: Aznalcázar (contamination through aerosolized contaminants engulfed the town during cleanup)
		—	8		0.542; 0.283–1.161		Site 4: Urban mine-affected site: Aznalcóllar (contaminated by normal mine operations, or the disaster and subsequent remediation efforts)
		—	5		0.493; 0.358–0.691		Site 5: Floodplain mine-affected site: Guadiamar River floodplain near the Aznalcázar gauge station (24.8 km below the ruptured tailings dam)
		—	5		0.928; 0.253–1.999		Site 6: Floodplain mine-affected site: Guadiamar River floodplain near the Guijo gauge station (7.4 km below the ruptured dam)
		—	4		3.436; 2.520–4.814		Site 7: Floodplain mine-affected site: Agrio River floodplain (4.4 km below, closest to the ruptured tailings dam)
							Statistical analysis: [As] concentration was significantly influenced by site
Varanus salvator							Boman et al. (2001); Vietnam; Dac Lac; seemingly unpolluted site
	Adult*, egg	—	1	Egg *	37	DRY	*Fully grown; *gonadal system

(*continued*)

TABLE A.3 (CONTINUED)
Arsenic (As)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Squamata: Serpentes								
"Snake"								Hsu et al. (2006); South Taiwan; Kenting National Park; site influenced from industrial pollution
	—		—	12	Whole body	3.08; 0.92–7.33	DRY: M; R	
						0.44		Bioconcentration factor (reference media: soil and food items)
Agkistrodon piscivorus								Presley et al. (2005); USA; Louisiana, New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 58.6 cm							
	TM = 365 g	M	—	6	Liver	0.17	WET	
					Kidney	0.11		
Agkistrodon piscivorous								Burger et al. (2006), USA; South Carolina; 1 reference site, 1 polluted Savannah River site, Aiken
	SVL = 57 cm							
	TM = 280 g	M	—	13	Muscle*	7.00	DRY: M**	*Tail; **both sites combined
	SVL = 60 cm							
	TM = 270 g			5	Blood	0.3		
Coluber constrictor								Presley et al. (2005); USA; Louisiana, New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 103.2 cm							
	TN = 300 g		—	1	Liver	0.58	WET	
					Kidney	0.15		
Lapemis hardwickii								Boman et al. (2001); Vietnam; Nha Trang

	Adult*		—	1	Muscle	27	DRY	*Fully grown
					Liver	24		
Nerodia sp.*								Winger et al. (1984); USA; Florida; Apalachicola River; [*Natrix* sp.]
	W = 226–544 g		—	15	Whole body	0.06; 0.05–0.07	WET: M; R	Upper reaches of river
	W = 272–725 g					0.05; 0.05–0.06		Lower reaches of river
Nerodia cyclopion								Presley et al. (2005); USA; Louisiana, New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 63.9 cm TM = 191 g		—	1	Liver	0.25	WET	
					Kidney	ND (<0.13)		
Nerodia fasciata								Hopkins et al. (1999); USA; South Carolina; Savannah River site, Aiken
							DRY: M*	*Values estimated from graph
	—			5	Liver	134		Site 1: Coal ash-contaminated site
				5		0.23		Site 2: Nearby reference site
								Statistical analysis: Differences in [As] concentrations between sites were statistically significant
	Diet							This study provides information on contamination levels of several prey species.
Nerodia fasciata								Hopkins et al. (2001); USA; South Carolina; Aquatic Ecology Laboratory near Aiken; lab feeding experiment; experimental animals were captured at uncontaminated reference site and fed 52 total meals of field-collected prey (primarily fish) for 13.5 months
	TM*							*Initial/final mass
	Juvenile and adult							
	TM = 61.9/169.6 g*	M	—	8			DRY	Treatment 1: Snakes fed prey items collected from coal ash-contaminated site: Savannah River site

(*continued*)

TABLE A.3 (CONTINUED)
Arsenic (As)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
				Gonad	0.15; ND (<0.009)–0.33	MD; R	
				Kidney	0.35; 0.25–0.74		
				Liver	0.86; 0.39–2.06		
				Shed skin	4.00; 1.88–6.25		
				Blood	0.03; 0.02–0.03		
				Tail clip	0.34; 0.22–0.58		
	Diet			Fish	1.281; 0.347–2.637	GLSM; R	
	Juvenile and adult						
	TM = 62.2/136.3 g*	M	—	7		MD; R	Treatment 2: Snakes fed prey items collected from uncontaminated reference site
				Gonad	ND (<0.009); ND (<0.009)–0.08		
				Kidney	0.03; ND(<0.007)–0.07		
				Liver	ND (<0.007)		
				Shed skin	0.28; 0.14–0.47		
				Blood	0.01; 0–0.01		
				Tail clip	ND (<0.006); ND (<0.006–0.04)		
	Diet			Fish	0.155; 0.115–0.332	GLSM; R	
							Statistical analysis: Treatment differences for [As] were significant for snake kidney, liver, and tail clip; and for fish
Nerodia fasciata							Hopkins et al. (2002); USA; South Carolina; Aquatic Ecology Laboratory near Aiken; 2-year feeding experiment; all snakes were lab reared and originated from a single gravid female that was collected from a reference site on the Savannah River site; exposure started after first hibernation

Diet	*		Fish		DRY	*3 prey species, 4 specimens per species
Juvenile**					DRY	**Morphometry in Figure 2 of Hopkins et al. (2002)
Diet	—	*	Fish	0.155	GLSM	Treatment 1: Control: snakes fed only prey items collected from uncontaminated reference site
Juvenile**	F	6	Gonad	0.182	M	
			Kidney	0.117		
			Liver	0.092		
	M	4	Gonad	0.325	M	
			Kidney	0.198		
			Liver	0.087		
Diet	—	*	Fish	0.693	GLSM	Treatment 2: Lower level exposure: snakes fed prey items collected from coal ash-contaminated site and from the reference site on alternating weeks
Juvenile**	F	6	Gonad	0.197	M	
			Kidney	0.615		
			Liver	0.623		
	M	4	Gonad	0.415	M	
			Kidney	0.401		
			Liver	0.585		
Diet	—	*	Fish	1.281	GLSM	Treatment 3: Higher-level exposure: snakes fed only prey items collected from coal ash-contaminated site
Juvenile**	F	6	Gonad	0.335	M	
			Kidney	1.055		
			Liver	1.851		
	M	3	Gonad	0.520	M	
			Kidney	0.817		
			Liver	2.010		
						Statistical analysis: Effects of organ, treatment, organ by treatment and organ by sex on [As] concentration were significant

(*continued*)

TABLE A.3 (CONTINUED)
Arsenic (As)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Nerodia fasciata								Burger et al. (2006); USA; South Carolina, 1 reference site, 1 polluted Savannah River site, Aiken
	SVL = 51 cm							
	TM = 130 g	M	—	47	Muscle*	0.4	DRY: M**	*Tail; **both sites combined
	SVL = 52 cm							
	TM = 140 g			34	Blood	0.3		
Nerodia fasciata								Burger et al. (2007); USA; South Carolina; relatively rural, Department of Energy site
	Adult	—	—	34	Blood	0.246	WET: M	
				47	Muscle	0.146		
				5	Liver	0.089		
Nerodia sipedon								Burger et al. (2005); USA; East Tennessee; reference site: Little River downstream from the Great Smoky Mountains National Park near Townsend; polluted superfund site: EFPC inside the US Department of Energy's (USDOE) Y-12 National Security Complex
	Adult		—	8	Egg*	0.022	WET: M**	*Gonadal; **both sites combined
				3	Testis	0.037		
				47	Kidney	0.060		
				47	Liver	0.094		
				47	Muscle	0.035		
				47	Skin	0.034		
				46	Blood	0.015		
								Statistical analysis: [As] concentrations were not significantly different between tissues
Nerodia sipedon								Campbell et al. (2005); USA; East Tennessee
	Adult	M; R					WET: M; R	

TM = 235; 53–464 g SVL = 65; 44.5–80 mm	F	21			
TM = 103; 74–146 g SVL = 53; 47–60 mm	M	26			
					Site 1: Reference site: Little River downstream from the Great Smoky Mountains National Park
	F/M	11/16	Blood	0.0184; 0.0011–0.076	
			Kidney	0.0669; 0.001–0.400	
			Liver	0.0369; 0.00098–0.0178	
			Muscle	0.0328; 0.0015–0.190	
			Skin	0.0323; 0.00015–0.140	
					Site 2: Polluted Superfund site: upper reach of East Fork Poplar Creek (EFPC) within the US Department of Energy's (USDOE) Y-12 National Security Complex
	F/M	10/10	Blood	0.0101; 0.0028–0.027	
			Kidney	0.0513; 0.0068–0.167	
			Liver	0.171; 0.000424–1.151	
			Muscle	0.0379; 0.0072–0.112	
			Skin	0.0369; 0.014–0.084	

(*continued*)

TABLE A.3 (CONTINUED)
Arsenic (As)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Nerodia sipedon								Burger et al. (2007); USA; New Jersey and Tennessee
	Adult TL = 72; 39.0–103.5 cm; TM = 159; 17.5–587.5 g	M; R	—	18			WET: M	
				18	Blood	0.020		Site 1: Urban/suburban; New Jersey
				18	Muscle	0.059		
				18	Liver	0.093		
					Kidney	0.089		
					Skin*	0.086		*Tail snips
				36	Blood	0.015		Site 2: Relatively rural; Tennessee; Department of Energy site
				46	Muscle	0.035		
				47	Liver	0.094		
Nerodia taxispilota								Burger et al. (2006); USA; South Carolina; 1 reference site, 1 polluted Savannah River site, Aiken
	SVL = 59 cm TM = 170 g	M	—	10	Muscle*	0.7	DRY: M**	*Tail; **both sites combined
	SVL = 65 cm TM = 230 g			9	Blood	0.4		

TABLE A.4
Barium (Ba)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Testudines								
Caretta caretta								Stoneburner et al. (1980); USA; 4 western Atlantic nesting beaches: Canaveral, Florida; Cumberland Island, Georgia; Cape Lookout, North Carolina; Cape Hatteras, North Carolina
							—: RM *	*Combined by beach
	Egg*		—	96	Yolk	2.09–6.87		*"Fresh"
*Caretta caretta**								Aguirre et al. (1994); USA; Hawaiian Islands; *[identification number 052, *Chelonia mydas* in the reference]
	"Pelagic"		—	1	Liver	0.83	WET	
Chelonia mydas								Aguirre et al. (1994); USA; Hawaiian Islands
	L = 28.7–71.3 cm W = 3.2–43.6 kg		F/M	8/4	Liver	0.68; 0.58–0.82	WET: M; R	
					Kidney	0.97; 0.76–1.58		
	Egg*		—	3	Shell	1.0		*Posthatch
	Hatchling				Whole body	0.35		
Chelonia mydas								Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 49.0; 40.0–63.5 cm CW = 40.5; 35.3–49.4	M; R	M	6			DRY: M; R	
	SCL = 52.2; 37.0–71.4 CW = 43.9; 31.7–58.5		F	20				
			M	6	Liver	0.68; 0.11–2.7		
				6	Kidney	0.59; 0.22–1.0		
				3	Muscle	0.30; 0.18–0.30		
			F	20	Liver	0.76; 0.12–2.3		
				19	Kidney	0.77; 0.27–2.5		
				9	Muscle	0.77; 0.20–2.2		

(*continued*)

TABLE A.4 (CONTINUED)
Barium (Ba)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
	Diet		—	8	Stomach content	3.6; 0.76–7.0		
Chelonia mydas								Lam et al. (2004); South China
	Juvenile						DRY: M	Turtles stranded on beaches at Ham Tin Wan and Tung Pang Chau; upon collection, turtles had started to decay
			—	2	Fat	0.080		
					Kidney	0.199		
					Heart	0.126		
					Liver	0.099		
					Lung	0.149		
					Muscle	0.073		
					Stomach	3.823		
	Adult							Turtles believed to have been unintentionally caught by fishermen; upon collection, turtles were relatively fresh
			—	3	Muscle	0.500		
				1	Liver	1.902		
Eretmochelys imbricata								Anan et al. (2001); Japan, Yaeyama Islands
	SCL = 43.5; 33.5–48.9 cm CW = 36.9; 32.6–39.6 g	M; R	M	6			DRY: M; R	
	SCL = 46.5; 43.8–67.9 cm CW = 32.8; 10.9–52.3 g		F	16				
			M	6	Liver	0.35; 0.14–0.61		
				6	Kidney	0.98; 0.17–2.9		
				1	Muscle	0.14		
			F	16	Liver	0.48; ND (<0.001)–2.6		
				13	Kidney	1.2; 0.049–7.9		

				8	Muscle	0.080; 0.061–0.13		
	Diet		—	6	Stomach content	110; 0.34–590		
Crocodylia								
Crocodylus johnstoni								Jeffree et al. (2001); northern Australia; north central Queensland; Lynd River; samples from a single population
	Age = 0.7–62.7 years L = 24.7–128.3 cm	R	F/M	9/21			DRY: M; R	
			—	30	Osteoderm *	35.3; 20.8-47.0		*Ventral pelvic region
Crocodylus porosus	Age = 5–40 years							
	L = 168499 cm	R	—	40			DRY: GM; R	Jeffree et al. (2001); northern Australia; Alligator Rivers region: Kakadu National Park; 3 river catchments; mining and hunting areas included
			—	35	Muscle*	0.554; 0.270–0.920		*Tail
			—	40	Osteoderm*	24.3; 1.90–143		*Ventral pelvic region
Squamata: Sauria								
*Laudakia s. stellio**								Loumbourdis (1997); Greece, Thessaloniki region; *[*Agama s. stellio*]
	Adult		—	—	Liver	13.04	DRY: M	Urban area (500 m asl)
					Carcass	49.76		
					Liver	22.77		Agricultural area (50 m asl)
					Carcass	122.88		
Tarentola mauritanica								Fletcher et al. (2006); southern Spain, Guadiamar River Valley; Boliden-Apirsa mine at Aznalcóllar; mine tailings release event; 7 sites spanning an expected contamination gradient; geckos collected from building walls

(*continued*)

TABLE A.4 (CONTINUED)
Barium (Ba)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
	Adult and juvenile		52	Whole body*		DRY: M; R	*Minus gut contents
		—	9		32.934; 14.005–58.444		Site 1: Rural non-mine-affected site: near Guadalmellato (most pristine)
		—	13		88.120; 37.152–197.913		Site 2: Urban non-mine-affected site: Villaviciosa de Cordoba (not contaminated by mining)
		—	8		20.712; 9.819–39.089		Site 3: Urban mine-affected site: Aznalcázar (contamination through aerosolized contaminants engulfed the town during cleanup)
		—	8		15.281; 8.878–26.762		Site 4: Urban mine-affected site: Aznalcóllar (contaminated by normal mine operations, or the disaster and subsequent remediation efforts)
		—	5		23.333; 10.669–39.102		Site 5: Floodplain mine-affected site: Guadiamar River floodplain near the Aznalcázar gauge station (24.8 km below the ruptured tailings dam)
		—	5		10.902; 4.120–19.104		Site 6: Floodplain mine-affected site: Guadiamar River floodplain near the Guijo gauge station (7.4 km below the ruptured dam)
		—	4		18.223; 5.637–30.501		Site 7: Floodplain mine-affected site: Agrio River floodplain (4.4 km below and closest to the ruptured dam)
							Statistical analysis: [Ba] concentration was significantly influenced by site
Squamata: Serpentes							
Agkistrodon piscivorous							Burger et al. (2006); USA; South Carolina; 1 reference site, 1 polluted Savannah River site, Aiken
	SVL = 57 cm TM = 280 g	—	13	Muscle*	43	DRY: M**	*Tail; **both sites combined
	SVL = 60 cm TM = 270 g		5	Blood	0.6		

Eunectes murinus								Calle et al. (1994); Venezuela
	L = 2.1–5.1 m M = 3.5–74.0 kg		F/M	7/5	Blood*	0.15; 0.1–0.2	WET: M; R	*Plasma
Nerodia fasciata								Burger et al. (2006); USA; South Carolina; 1 reference site, 1 polluted Savannah River site, Aiken
	SVL = 51 cm TM = 130 g		—	47	Muscle*	64	DRY: M**	*Tail; **both sites combined
	SVL = 52 cm TM = 140 g			34	Blood	0.4		
Nerodia taxispilota								Burger et al. (2006); USA; South Carolina; 1 reference site, 1 polluted Savannah River site, Aiken
	SVL = 59 cm TM = 170 g	M	—	10	Muscle*	28	DRY: M**	*Tail; **both sites combined
	SVL = 65 cm TM = 230 g			9	Blood	0.3		

TABLE A.5
Beryllium (Be)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Crocodylia							
Alligator mississippiensis							Heinz et al. (1991); USA; central and southern Florida; Lake Okeechobee, Lake Griffin, and Lake Apopka
	Egg	—	32*	Egg	ND (<0.004)	WET	*16 nests, 2 eggs per nest

TABLE A.6
Cadmium (Cd)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Testudines								
*Actinemys marmorata**								Henny et al. (2003); USA, Western Oregon, Fern Ridge Reservoir; *[*Clemmys marmorata*]
	Egg		—	14*	Contents	ND (<0.10)	DRY	*14 nests, 1 egg per nest
*Apalone spinifera**								
								Robinson and Wells (1975); lab experiment; turtles caught in the wild from an undisturbed site (USA; Tennessee; Cannon County) and administered a single oral dose of 2 mg Cd as cadmium acetate; *[*Trionyx spinifer*]
	TM = 127–277 g	R	—	—	"Tissues"	ND (<—)	WET: M	
	TM = 127–277 g	R	—	3*				*3 turtles per treatment
							48 hours/ 96 hours*	*Tissue concentrations determined 48 and 96 hours after administration
					Stomach*	6.24/1.23		*Contents discarded
					Small intestine	20.70/2.20		*Contents discarded
					Large intestine	12.48/5.12		*Contents discarded
					Liver	7.27/5.91		
					Kidney	3.45/2.60		
*Apalone spinifera**								Robinson and Wells (1975); USA; Tennessee; Rutherford County; site contaminated by sewage effluents from plating industries; *[*Trionyx spinifer*]
	Young		F	—	Kidney	9.87	WET: MAX	

(*continued*)

TABLE A.6 (CONTINUED)
Cadmium (Cd)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
	M = 76–90 g						
				Small intestine*	0.19	WET: MIN	*Contents discarded
Caretta caretta							Hillestad et al. (1974); USA; Georgia and South Carolina; 3 nesting beaches
	Egg	—	—	Yolk	0.17	—; M	
				Albumen	0.56		
Caretta caretta							Stoneburner et al. (1980); USA; 4 western Atlantic nesting beaches: Florida, Canaveral; Georgia, Cumberland Island; North Carolina, Cape Lookout; North Carolina, Cape Hatteras
	Egg	—	96	Yolk	0.03–0.19	—; RM	*Combined by beach
*Caretta caretta**							Aguirre et al. (1994); USA; Hawaiian Islands; *[identification number 052, *Chelonia mydas* in the reference]
	"Pelagic"	—	1	Liver	0.96	WET	
Caretta caretta							Sakai et al. (1995); Japan; Kochi Prefecture, Cape Ashizuri
	CL = 76–92 cm	F/M	6/1	Liver	9.29; 5.66–14.6	WET: M; R	
	TM = 75–108 kg						
				Kidney	39.4; 8.1–56.5		
				Muscle*	0.062; 0.041–0.117		*Pectoral
	Egg*	—	—	Shell	ND (<0.01)		*Oviductal eggs from 5 females
				Yolk	0.026; 0.019–0.035		
				Albumen	ND (<0.01)		
				Whole egg	0.013; 0.008–0.015*		*Calculated

Caretta caretta								Gordon et al. (1998); Australia; southeastern Queensland, Moreton Bay region; stranded turtles
	—	—	—	8	Liver	16.4; 7.3–35.1	WET: M; R	
				5	Kidney	28.3; 11.4–39.4		
Caretta caretta								Storelli et al. (1998a); Italy; Adriatic Sea, Apulian coasts; stranded turtles
	TM = 1.8–100 kg	R	F/M	9/3	Liver	7.60; 3.06–20.23	DRY: MD; R	
					Lung	2.15; 0.32–10.50		
					Kidney	24.23; 0.39–64.00		
					Muscle	0.55; 0.09–2.21		
Caretta caretta								Storelli et al. (1998a) from Storelli et al. (2005); dry mass based data from Storelli et al. (1998a) converted to wet mass
					Liver	2.24; 0.90–5.97	WET: M; R	
					Kidney	7.52; 0.12–19.9		
					Muscle	0.14; 0.02–0.55		
Caretta caretta								Caurant et al. (1999); France; Atlantic coasts, “Pertuis charentais” in the la Rochelle region; stranded turtles
	Juvenile SCL = 29.4; 21.3–34.5 cm TM = 2.8 0.3–7.5 kg	M; R	—	21				
				7	Liver	2.58; 0.30–11.8	WET: M; R	
				5	Kidney	13.3; 1.68–35.7		
				21	Muscle*	0.08; 0.004–0.18		*Pectoral

(*continued*)

TABLE A.6 (CONTINUED)
Cadmium (Cd)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Caretta caretta								Godley et al. (1999); northern Cyprus; eastern Mediterranean Sea, Alagadi beach
	CCL = 63.5; 56.0–79.0 cm	M; R		7*				*Stranded turtles
				4	Liver	8.64; 5.14–12.97	DRY: MD; R	
				2	Kidney	30.50; 18.80–42.20		
				4	Muscle	0.57; 0.30–1.43		
				48*				*Hatched nests; 1 sample (dead hatchling, dead embryo, or undeveloped egg taken per nest)
	Hatchling			16	Hatchling	0.34; ND (<0.01–1.45		
	Embryo			29	Embryo	0.21; ND (<0.01)–1.09		
	Egg			3	Contents*	0.23; 0.23–0.56		*Yolk and albumen
Caretta caretta								Sakai et al. (2000b); Japan; Kochi Prefecture, Cape Ashizuri; turtles accidentally caught in commercial fishing nets
	TM = 93/83 kg SCL = 83/85 cm	M	F/M	6/1	Liver	9.74/6.60	WET: M	
					Gullet	0.183/0.827		
					Stomach	0.451/0.149		
					Intestine	1.10/2.56		
					Pancreas	39.2/20.9		
					Heart	0.454/0.257		
					Trachea	0.035/0.154		
					Lung	0.371/0.254		
					Bladder	0.291/0.139		
					Spleen	1.19/0.516		
					Kidney	38.3/45.5		
					Salt gland	1.52/0.831		

					Brain	0.269/0.253		
					Testis	—/0.775		
					Oviduct	0.061		
					Ovary	0.037		
	Egg*				Whole egg	0.013		*Oviductal
					Shell	ND (<0.01)		
					Yolk	0.026		
					Albumen	—		
					Scale	0.085/0.028		
					Mesentary	0.413/0.211		
					Fat	0.066/0.093		
					Muscle	0.064/0.048		
					Bone	0.134/0.086		
					Carapace	0.129/0.072		
					Whole body*	0.810/0.800		*Calculated
Caretta caretta								Kaska and Furness (2001); southwestern Turkey; 4 beaches; samples collected either just before hatching or dead-in-shell 1 week after last hatching
	Embryo		—	22	Liver	1.26	DRY: M	
	Egg		—		Shell	0.649		
					Yolk	0.359		
								Statistical analysis: [Cd] liver concentrations were significantly different between nest regions (top vs. bottom)
Caretta caretta								Franzellitti et al. (2004); Italy; northwestern Adriatic Sea; coast from the Po delta to the Reno mouth; stranded turtles collected dead
	Juvenile to adult MSCL = 24.5–74 cm	R	—	*			WET: M	*16 victims of by-catch, 19 found dead at coast
				30	Liver	2.84		
				13	Lung	0.47		
				17	Muscle*	0.36		*Pectoral

(*continued*)

TABLE A.6 (CONTINUED)
Cadmium (Cd)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
				7	Fat*	2.33		*Abdominal
Caretta caretta								Kaska et al. (2004); Turkey; southwestern Mediterranean coast; stranded turtles
	—		—	32	Liver	10.84; 3.41–19.01	DRY: M; R	
				20	Bladder	11.16; 3.61–17.27		
				20	Kidney	16.96; 3.14–33.32		
				10	Lung	5.71; 1.24–12.20		
				32	Muscle	3.57; 0.58–14.96		
Caretta caretta								Torrent et al. (2004); Spain; Canary Islands; stranded turtles submitted to the veterinary faculty of the University of Las Palmas in Gran Canaria
	Juvenile to subadult SCL = 15–65 cm	R*	F/M	67/11				*Estimated from graph
					Kidney	5.01; 0.01–61.08	WET: M; R	
					Muscle	1.14; 0.15–12.48		
					Bone	1.36; 0.15–22.79		
					Liver	2.53; 0.04–21.98		
Caretta caretta								Maffucci et al. (2005); southern Italy; western Mediterranean Sea; South Tyrrhenian coasts; stranded turtles
	CCL = 37–82 cm	R	—	29			DRY: M; R	
				14	Liver	19.3; 1.6–114		
				19	Kidney	57.2; 10.9–158		
				26	Muscle	0.20; 0.06–0.78		
Caretta caretta								Storelli et al. (2005); Italy; Adriatic and Ionian Seas; stranded turtles

	SCL = 21–71 cm	R		19	Liver	3.36; 1.10–6.55	WET: M; R	
					Kidney	8.35; 1.26–16.4		
					Muscle	0.07; ND (<0.00002)–0.13		
					Spleen	0.90; 0.51–1.31		
					Heart	0.23; ND (<0.00002)–0.44		
					Lung	0.24; ND (<0.00002)–0.38		
					Fat	0.08; ND (<0.00002)–0.18		
Caretta caretta								Gardner et al. (2006); Mexico; Baja California peninsula; turtles died from fisheries capture
	MSCL = 57.0; 52.0–63.0	R		5	Liver	1.75; ND (<0.0009)–30.62	DRY: GM; R	
					Kidney	73.11; 13.72–140		
					Muscle*	0.1; ND (<0.0009)–1.45		*Pectoral
					Fat	0.5; 0.2–1.37		
Chelonia mydas								Aguirre et al. (1994); USA; Hawaiian Islands
	TL = 28.7–71.3 cm		F/M	8/4	Liver	9.30; 0.39–26.0	WET: M; R	
	TW = 3.2–43.6 kg							
					Kidney	26.0; 4.74–70.2		
	Egg*		—	3	Shell	0.2	M	*Posthatch
	Hatchling				Whole body	ND (<0.07)		
Chelonia mydas								Gladstone (1996) from Gordon et al. (1998); Australia, Torres Strait
	—	—	—	7	Liver	10.7; 6.0–17.0	WET: M; R	

(*continued*)

TABLE A.6 (CONTINUED)
Cadmium (Cd)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
				Kidney	26.0; 12.0–42.0		
Chelonia mydas							Gordon et al. (1998); Australia; southeastern Queensland, Moreton Bay region; stranded turtles
	—	—	—	38	Liver	12.5; 2.5–56.9	WET: M; R	
				38	Kidney	15.3; 1.7–75.9		
Chelonia mydas							Godley et al. (1999); northern Cyprus; eastern Mediterranean Sea, Alagadi beach
	CCL = 49.5; 27.5–56.0 cm	M; R		6*			DRY: MD; R	*Stranded turtles
				6	Liver	5.89; 2.53–10.73		
				1	Kidney	3.46		
				6	Muscle	0.37; 0.12–0.78		
				69*				*Hatched nests; 1 sample (dead hatchling, dead embryo, or undeveloped egg) taken per nest
	Hatchling			29	Hatchling	0.23; ND (<0.01)–0.94		
	Embryo			16	Embryo	0.33; ND (<0.01)–0.93		
	Egg			24	Contents*	0.27; 0.05–1.22		*Yolk and albumen
Chelonia mydas							Sakai et al. (2000a); Japan; Yaeyama Islands, Okinawa; turtles caught by fisherman for commercial use
	SCL = 51.0 cm	M	—	50			WET: M;R	
				50/50*	Liver	5.58; 0.30–18.6	WET: M; R	*Detected/analyzed
				23/23	Kidney	38.5; 7.31–80.7		
				45/47	Muscle	0.05; 0.01–0.54		

Chelonia mydas							Sakai et al. (2000b); Japan; Ogasawara Islands (Bonin Islands), Haha-Jima Island; turtles collected in coastal waters
TM = 124/117 kg SCL = 93/97 cm	M	F/M	1/1	Liver	3.90/12.1	WET: M	CW = 72/73 cm
				Gullet	0.389/0.164		
				Stomach	0.089/0.196		
				Intestine	2.85/5.91		
				Pancreas	7.16/12.6		
				Heart	0.066/0.234		
				Trachea	0.084/0.182		
				Lung	0.132/0.224		
				Bladder	0.091/0.161		
				Spleen	0.994/0.414		
				Kidney	37.0/45.5		
				Salt gland	0.581/0.903		
				Brain	0.103/0.164		
				Reproductive tissues	Testis	1.190	
					Oviduct	0.026	
					Ovary	ND (<0.03)	
				Egg	Whole egg	ND (<0.03)	
					Shell	ND (<0.03)	
					Yolk	ND (<0.03)	
					Albumen	ND (<0.03)	
				Scale	0.024/0.036		
				Mesentary	0.053/0.258		
				Fat	0.063/0.060		
				Muscle	0.011/0.034		
				Bone	0.025/0.042		
				Carapace	0.054/0.033		
				Whole body*	0.390/0.710		*Calculated

(*continued*)

TABLE A.6 (CONTINUED)
Cadmium (Cd)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Chelonia mydas								Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 49.0; 40.0–63.5 cm	M; R	M	6				
	SCL = 52.2; 37.0–71.4		F	20				
			M	6	Liver	12.2; 3.58–27.1	DRY: M; R	
			M	6	Kidney	113; 46.7–214		
			M	3	Muscle	0.203; 0.111–0.231		
			F	20	Liver	19.9; 6.89–38.3		
			F	19	Kidney	153; 20.3–285		
			F	9	Muscle	0.250; 0.070–0.635		
	Diet		—	8	Stomach content	0.367; 0.047–1.03		
Chelonia mydas								Anan et al. (2002); Japan; Okinawa Prefecture, Yaeyama Islands; tissues provided by fishermen
	—	—	—	9	Liver	8.96; 3.21–21.6	WET: M; R	
Chelonia mydas								Kaska et al. (2004); Turkey; southwestern Mediterranean coast; stranded turtles
	—		—	22	Liver	7.38; 3.75–15.86	DRY: M; R	
				20	Bladder	9.34; 2.75–17.02		
				14	Kidney	15.83; 5.49–23.73		
				15	Lung	1.84; 0.41–8.59		
				22	Muscle	1.46; 0.64–2.38		
Chelonia mydas								Lam et al. (2004); South China

	Juvenile		—	2	Fat	ND (<—)	DRY: M	Turtles stranded on beaches at Ham Tin Wan and Tung Pang Chau; upon collection, turtles had started to decay
					Kidney	2.488		
					Heart	ND (<—)–0.977		
					Liver	1.098		
					Lung	ND (<—)–0.011		
					Muscle	ND (<—)–0.096		
					Stomach	175.5		
	Adult		—	3	Muscle	0.172		Specimens believed to have been unintentionally caught by fishermen; upon collection, turtles were relatively fresh
				1	Liver	1.445		
Chelonia mydas								Celik et al. (2006); Turkey; Mediterranean Sea, Kazanli beach
	Egg			12*	Shell	0.58	DRY: M	*12 nests, 3 eggshells per nest collected after hatching
	Environment*							*In this study, information is available on metal concentrations in sand, soil, plant, and water samples around the turtle nesting environment
Chelonia mydas								Gardner et al. (2006); Mexico; Baja California peninsula; turtles died from fisheries capture
	MSCL = 62.13; 48.5–76.9 cm	M; R		11	Liver	3.30; ND (<0.0009)-102	DRY: GM; R	
					Kidney	121; 6.09–653		
					Muscle*	0.01; ND (<0.0009)–39.24		*Pectoral
					Fat	0.002; ND (<0.0009)–1.47		
Chelydra serpentina								Stone et al. (1980); USA; New York
	TM = 2.0–4.2 kg		F/M	1/1	Liver	ND (<0.06)	WET: M; R	Site 1: North Bay at Tivoli on Hudson River

(*continued*)

TABLE A.6 (CONTINUED)
Cadmium (Cd)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
				Muscle*	ND (<0.06)		*Skeletal
	TM = 1.0–8.6 kg	F/M	2/2	Liver	16.9; 8.58–26.20		Site 2: Constitution Island Marsh, Hudson River
				Muscle*	0.77; 0.12–1.41		*Skeletal
Chelydra serpentina							Helwig and Hora (1983); USA; Minnesota; 3 river sites
	TL = 14–38 cm						
	TM = 0.8–9.9 kg	F/M/U	5/10/2	Muscle*	0.010; 0.002–0.025	—; M; R	*Leg
Chelydra serpentina							Albers et al. (1986); USA; Maryland and New Jersey
	Adult						
	TL = 20–40 cm	M	7	Liver	0.07	WET: M	Site 1: Undisturbed freshwater site: Patuxent Wildlife Research Center, Maryland
		F	6	Liver	0.06		
		M	7	Kidney	0.07		
		F	6	Kidney	0.07		
		M	8	Liver	0.10		Site 2: Contaminated brackish water site: Hackensack Meadowlands, New Jersey
		F	3	Liver	0.08		
		M	8	Kidney	0.24		
		F	3	Kidney	0.30		
		M	8	Liver	0.08		Site 3: Contaminated freshwater site: Hackensack Meadowlands, New Jersey
				Kidney	0.09		

Chrysemys picta							Rie et al. (2001); USA; lab experiment; turtles were obtained from the wild by a commercial supplier in Oshkosh, WI; distribution of Cadmium-109 radioisotope (single dose of 5.10 µCi of ^{109}Cd applied via intravascular injection) in the turtles was analyzed 6, 24, and 192 hours postinjection
	Adult						
	TM = 607–1000 g	R				WET; M**	6/24/192 hours postinjection
			F	2/2/1*	Brain	1.97/1.53/1.95	*Sex not known; **cpm/mg ^{109}Cd
					Skin	2.48/4.24/2.36	
					Fat	1.71/1.86/2.08	
					Muscle	1.65/4.43/1.75	
					Liver	1.42/18.3/63.01	
					Heart	3.28/7.84/3.29	
					Gut	0.67/2.81/4.27	
					Spleen	0.74/3.02/2.39	
					Kidney	1.78/9/15.27	
					Bone	1.26/3.28/1.8	
					Pancreas	1.66/10.23/18.19	
	Gonadal system				Ovarial follicle wall	5.18/6.52/3.67	
					Ovarial follicle yolk	0.37/0.265/0.34	
					Corpus luteum	3.44/9.24/6.14	
	Egg				Oviductal egg yolk	NA/0.27/0.31	
					Oviductal egg albumen	NA/0.11/0.32	
					Oviductal eggshell	0.68/0.37/2.35	
					Oviduct wall	1.9/5.66/2.7	

(*continued*)

TABLE A.6 (CONTINUED)
Cadmium (Cd)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
					Adrenal-interrenal	9.03/10.06/6.68		
					Blood	87/19.3/9	%*	*Percent of total injected dose
					Bile	0.007/0.006/0.006		
					Holding water	NA/5/28		
Dermochelys coriacea								Davenport and Wrench (1990); Davenport et al. (1990); Great Britain; Wales, Irish Sea, Cardigan Bay; stranded turtle
	Adult L = 2.53 m TM = 916 kg		M	1			DRY: m*	*4 replicate measures
					Liver	0.22		
					Muscle*	0.06		*Pectoral
					Blubber	ND (<0.01)		
Dermochelys coriacea								Vazquez et al. (1997); Mexico; Pacific coast
	—		—	—	Seawater	0.03	DRY: M	
					Sand	23.2		
	Egg*		—		Shell	0.9		*Posthatch
Dermochelys coriaca								Godley et al. (1998); Great Britain; Wales and Scotland, west coast; turtles died in fishing gear
	Adult	R	M	3				
	CCL = 141–170 cm				Liver	5–88	DRY: R	
					Muscle	1.4–7.5		
				1	Liver	28	WET	
					Muscle	2.5		
Dermochelys coriacea								Caurant et al. (1999); France; Atlantic coasts, "Pertuis charentais" in the la Rochelle region; stranded turtles

	Juvenile SCL = 145.7; 115–188 cm	M; R	—	16				
			—	18	Liver	6.84; 0.60–14.7	DRY: M; R	
				5	Kidney	30.3; 8.47–62.0		
				16	Muscle*	0.35; 0.16–1.00		*Pectoral
Eretmochelys imbricata								Gordon et al. (1998); Australia; southeastern Queensland, Moreton Bay region; stranded turtles
	—	—	—	2	Liver	2.4–6.2	WET: R	
				2	Kidney	3.6–12.7		
Eretmochelys imbricata								Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 43.5; 33.5–48.9 cm	M; R	M	6	Liver	11.4; 3.77–33.6	DRY: M; R	
				6	Kidney	146; 44.2–310		
				1	Muscle	0.080		
	SCL = 46.5; 43.8–67.9 cm		F	16	Liver	5.40; 1.80–9.41		
				13	Kidney	69.7; 19.1–191		
				8	Muscle	0.067; 0.020–0.145		
	Diet		—	6	Stomach content	0.591; 0.346–0.852		
Eretmochelys imbricata								Anan et al. (2002); Japan; Okinawa Prefecture, Yaeyama Islands; tissues provided by fishermen
	—	—	—	7	Liver	1.23; 0.634–1.82	DRY: M; R	
Eretmochelys imbricata								Gardner et al. (2006); Mexico; Baja California peninsula; turtles died from fisheries capture
	MSCL = 48.4 cm			1	Liver	0.49	DRY: GM; R	
					Kidney	4.20		
					Muscle*	1.02		*Pectoral

(*continued*)

TABLE A.6 (CONTINUED)
Cadmium (Cd)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
					Fat	0.43		
*Gopherus agassizii**								Jacobson et al. (1991); USA; California; [*Xerobates agassizii*]
	TL = 133–306 mm TM = 390–4900 g		F/M	1/11	Liver	0.51	—; M	Kern County; with clinical signs of upper respiratory tract disease
	TL = 199–280 mm TM = 1180–3887 g		M	4	Liver	0.41		San Bernardino County; clinically healthy
Lepidochelys kempii								Caurant et al. (1999); France; Atlantic coasts, "Pertuis charentais" in the la Rochelle region; stranded turtles
	Adult SCL = 25.8; 21.3–34.5 cm TM = 2.5; 1.4–5.2 kg	M; R	—	6			WET: M; R	
				6	Muscle*	0.09; 0.01–0.26		*Pectoral
				2	Pancreas	68.8		
Lepidochelys olivacea								Sahoo et al. (1996); India; Gahirmatha, Orissa
	Environment		—	8 24*	Beach sand	1–3	DRY: R*	*mg/g^{D} *8 nests, 3 eggs per nest
	Egg*		—		Shell Albumen-yolk	1.3 ND (<1.0)		*Fresh
	Egg*				Shell	ND (<1.0)		*Posthatch
	Hatchling				Whole body	2.0		*Fresh
Lepidochelys olivacea								Gordon et al. (1998); Australia; southeastern Queensland, Moreton Bay region; stranded turtles

	—	—	—	1	Liver	6.4	WET: M; R	
				1	Kidney	29.8		
Lepidochelys olivacea								Gardner et al. (2006); Mexico; Baja California peninsula; turtles died from fisheries capture
	MSCL = 60.1; 53.0–66.0	M; R		6	Liver	17.89; 4.98–148	DRY: GM; R	
					Kidney	60.03; 0.81–274		
					Muscle*	0.48; ND (<0.0009)–8.85		*Pectoral
					Fat	0.69; 0.33–2.54		
Malaclemys terrapin								Burger (2002); USA; New Jersey; Barnegat Bay
	Adult L = 14.3 cm	M	F	11	Liver	0.066	WET: M	
					Muscle	0.018		
	Egg		—	8	Egg*	0.00026; 0.00001–0.00074	M; R	*Ovarial
Trachemys sripta								Thomas et al. (1994); lab experiment; intraperitoneal injection of 10 mg Cd/kg body weight per day for 6 days as cadmium chloride
	Juvenile TM = 20–25 g		F	6	Liver	42	WET: %*	*Percentage of total body burden
					Kidney	20		
					Spleen	14		
					Heart	8		
					Lung	2		
					Muscle	3		
					Carapace	4		
					Brain	1		
					Blood	1		
					Ovary	9		

(*continued*)

TABLE A.6 (CONTINUED)
Cadmium (Cd)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Trachemys scripta								Burger and Gibbons (1998); USA; South Carolina; Savannah River site, Aiken, clutches laid in the lab 2–3 days after collection of females in the field
	Egg		—	16*	Contents	0.067; 0.444	DRY: M; MAX	*16 clutches, 1 egg per clutch
					Shells	0.013; 0.029		
								Statistical analysis: [Cd] concentrations were not significantly different between egg compartments
Trachemys scripta								Nagle et al. (2001); USA; South Carolina, Aiken County, Savannah River site; in ovo exposure experiment; females collected in the field in 1993, 1994; oviposition induced in 1995; eggs from turtles collected in both polluted and unpolluted sites were incubated in coal ash-contaminated and uncontaminated soil in outdoor artificial nests
							DRY: M	Site 1: Coal ash-polluted Savannah River site
	Adult		F	4	Liver	3.57		
	Diet		—	12	Asiatic clam*	4.02		*Soft tissue
			—	3	Crayfish*	2.78		*Whole body
	Environment		—	—	Artifical nest*	0.04		*Incubation substrate
								Sites 2 to 3: Unpolluted control sites near Couchton
	Adult		F	3	Liver	0.17		
	Diet		—	10	Asiatic clam*	1.39		*Soft tissue
			—	3	Crayfish*	0.02		*Whole body
	Environment		—	—	Artifical nest*	0.03		*Incubation substrate
	Hatchling							Trial 1: Incubation substrate: coal ash contaminated
	CL = 30.34 mm TM = 8.20 g	M	—	18*				*18 hatchlings from 5 clutches
			—	6*	Whole body	0.03		*3 clutches per site, 2 hatchlings per clutch

	Hatchling						Trial 2: Incubation substrate: uncontaminated
	TM = 30.36 g	M		18*			*18 hatchlings from 5 clutches
	CL = 8.02 mm						
				6*	Whole body	0.03	*3 clutches per site, 2 hatchlings per clutch
	Hatchling						Trial 3: Maternal residence: coal ash contaminated
	CL = 30.51 mm	M	—	18*			*18 hatchlings from 4 clutches
	TM = 8.02 g						
			—	6*	Whole body	0.03	*3 clutches per site, 2 hatchlings per clutch
	Hatchling						Trial 4: Maternal residence: uncontaminated
	TM = 29.69 g	M		18*			*18 hatchlings from 6 clutches
	CL = 7.77 mm						
				6*	Whole body	0.03	*3 clutches per site, 2 hatchlings per clutch
							Statistical analysis: [Cd] concentrations were significantly higher in adult liver and prey from site 1; [Cd] concentrations in hatchlings were not significantly different between trials 1 and 2 or 3 and 4
Trachemys scripta elegans							Tryfonas et al. (2006); USA; Illinois; Lower Illinois River near Grafton; eggs laid in the lab from turtles collected in the field from 5 nesting areas
						DRY: M*	*All sites combined
	Egg			—	Contents	ND (<—)	**	**Values estimated from graphs
					Shell	0.16	**	**From graph
	Diet				*Lemna* sp.	ND (<0.02)		
	Environment			4–5*	Soil	0.5–1.1	RM	*From 2 sites, soil from nesting and lake bank areas
				3*	Sediment	0.19–0.61	**	*From 2 sites, 3 layers per site; **from graph
				3–4*	Water	ND (<0.02)	WET	*From 3 sites
Trachemys scripta								Hays and McBee (2007); USA; Oklahoma
			—	34*	Blood*	Whole blood	WET: R	Sample size was 3 individuals for all sex (2), site (2), season (3) combinations except site 2 spring ($n = 2$ for males) and site 1 summer ($n = 2$ for females)

(*continued*)

TABLE A.6 (CONTINUED)
Cadmium (Cd)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
						ND (<—)–0.45		Site 1: Heavily mined from the 1890s to 1970 and currently contaminated with lead, zinc, and cadmium: Tar Creek Superfund site
						ND (<—)*		Site 2: Unmined reference site: Sequoyah National Wildlife Refuge; *except one male
Crocodylia								
Alligator mississippiensis								Bell and Lopez (1985); lab experiment; single intracardiac injection of 1.0 mg Cd/kg body mass as $CdCl_2$; tissue levels determined 10 days after injection
	—		—	7	Liver	22.5	WET: M	
					Kidney	10.0		
					Heart	6.6		
					Muscle*	0.18		*Tail
Alligator mississippiensis								Delany et al. (1988); USA; Florida; 8 lakes, statewide
	TL = 2.9–3.8 m	R	F/M	1/31			WET: M;RM	*Combined by lake
			*	24	Muscle*	0.03; 0.01–0.06		*Tail
Alligator mississippiensis								Heinz et al. (1991); USA; central and southern Florida; Lake Okeechobee, Lake Griffin, and Lake Apopka
	Egg		—	32*	Egg	ND (<0.01)	WET	*16 nests, 2 eggs per nest
Alligator mississippiensis								Burger et al. (2000); USA; Florida; Lake Apoka (Lake and Orange Counties), Orange Lake (Alachua County), and Lake Woodruff (Volusia County)
	Yearling	R					WET; M*	*3 lakes combined

	TL = 36–40 cm							
				30	Fat	0.0570		*Abdominal
				31	Liver	0.123		
				30	Muscle	0.0315		*Abdominal
				29	Skin	0.0540		*Abdominal
				29	Muscle	0.0628		*Ventral proximal tail
				22	Tail tip	0.0998		
				3	Tail*	0.0113		*Regenerated
Alligator mississippiensis								Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal Site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	—		—	1	Liver	ND (<0.02)	WET	
					Kidney	ND (<0.01)		
Alligator mississippiensis							WET: M; R	Lance et al. (2006); USA; Louisiana
	TM = 34; 21–50 kg	M; R	M	8				Wild alligators, trapped in aquaculture ponds
	TL = 214; 161–279 cm							
				3	Liver	0.046; 0.014–0.064		
				7	Kidney	0.024; 0.006–0.067		
	TM = 29;19–42 kg		F	7				
	TL = 207; 85–226 cm							
				2	Liver	0.017; 0.016–0.018		
				6	Kidney	0.030; 0.012–0.103		
	TM = 217; 54–279 kg		M	16				Captive alligators, raised at the experimental alligator breeding facility (Louisiana Department of Wildlife and Fisheries, USA); fed nutria (*Myocastor copus*) meat
	TL = 353; 246–404 cm							

(*continued*)

TABLE A.6 (CONTINUED)
Cadmium (Cd)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
			7	Liver	0.034; 0.012–0.110		
			8	Kidney	0.016; 0.003–0.029		
	TM = 85; 73–132 kg TL = 279; 187–295 cm	F	28				
			20	Liver	0.023; 0.013–0.060		
			19	Kidney	0.033; 0.012–0.086		
Alligator sinensis							Xu et al. (2006); China; Changxing County, Zhejiang Province; Changxing Nature Reserve and Breeding Research Center for Chinese alligator
	Adult					DRY	Alligators died of unidentified causes
		F/M	1/1	Heart	0.460/0.421		
				Lung	0.391/0.842		
				Liver	0.275/0.530		
				Stomach	0.222/0.321		
				Kidney	0.162/0.320		
				Intestine	0.177/0.086		
				Trachea	0.141/0.120		
				Pancreas	0.038/0.036		
				Gonad	0.101/0.038		
				Muscle	0.109/0.201		
	Diet	—	—	Fish*	0.496	DRY: M; R	*2 fish prey species randomly sampled
	Excrements	—	6	Feces	3.269		
	Egg		10*				*10 eggs from 3 clutches; year: 2003
				Eggshells	0.230; 0.150–0.347		
				Shell membranes	0.088; 0.070–0.107		
				Egg contents	0.172; 0.122–0.261		

	Environment			10*				*Water and sediment from 10 breeding ponds (5 subsamples from different sites within a pond each)
					Sediment	0.27		
					Water	0.0045	WET: M	
								Statistical analysis: [Cd] concentrations were not significantly different between contents and shells; those in contents were higher than in membranes
Crocodylus acutus								Ogden et al. (1974); USA; Florida; Everglades, Florida Bay
	Egg		—	5	Contents	0.05; 0.05–0.05	WET: M; R	
Crocodylus acutus								Stoneburner and Kushlan (1984); USA; Florida; Florida Bay, Everglades National Park
	Egg		—	9	Shell	1.36	DRY: M	
					Albumen-yolk	0.13		
					Albumen-yolk	0.01	WET: M	Albumen-yolk dry mass-based concentrations transformed to wet mass
Crocodylus acutus								Rainwater et al. (2007); Costa Rica; Rio Grande de Tarcoles polluted by serveral metals from various sources
	SVL = 155.7; 134.0–172.0 cm	M; R	1F:5M	6**	Caudal scute	0.3375	WET: M	
			F	1		1.900		
Crocodylus johnstoni								Jeffree et al. (2001); northern Australia; north central Queensland, Lynd River; samples from a single population
	Age = 0.7–62.7 years		F/M	9/21	Osteoderm*	0.102; 0.061–0.130	DRY: M; R	*Ventral pelvic region
	TL = 24.7–128.3 cm							
Crocodylus moreletii								Rainwater et al. (2007); Belize

(*continued*)

TABLE A.6 (CONTINUED)
Cadmium (Cd)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
	SVL = 89.8; 65.0–129.5 cm							Site 1: Gold Buton Lagoon
			F/M	5/4*	Caudal scutes	ND (<0.05)	WET: M	*1 whole scute per crocodile
	SVL = 104.4; 59.5–156.7 cm							Site 2: New River Watershed
			F/M	4/6		0.0707		*1 whole scute per crocodile
			F	1		0.482		
Crocodylus niloticus								Phelps et al. (1986); Zimbabwe; 10 samples from 8 sites
	Egg		—	26	Contents	0.051; ND (<0.030) –0.168	DRY: M; R	
Crocodilus niloticus								Almli et al. (2005); Zambia
	TL = 2.7–3.4 m	R	F	2	Liver	0.04; 0.03–0.07	WET: MD; R	Kafue River, Kafue National Park
			M	2	Kidney	0.15; 0.04–0.22		
	TL = 2.0–4.0 m		F	4	Liver	0.04; 0.03–0.08		Luangwa River, Luangwa National Park
			M	1	Kidney	0.16; 0.14–0.45		
Squamata: Sauria								
"Lizard"								Hsu et al. (2006); South Taiwan; Kenting National Park; strong influence from industrial pollution
	—		—	1	Whole body	0.16	DRY	
						6.75		Bioconcentration factor (reference media: soil and food items)
Chamaeleo chamaeleon								Diaz-Paniagua et al. (2002); southern Spain; Province of Cadiz; 2 sampling sites; 9 nests in agricultural lands and coastal urban areas; eggs collected immediately after oviposition
	Egg			4*	Contents	ND (<0.008)**	WET	*Number of eggs pooled per nest; **ng/ml

Chamaeleo chamaeleon								Gomara et al. (2007); southwestern Spain; Province of Cadiz; 3 sampling sites; 9 nests; eggs collected 0 to15 days after oviposition
			—	2–4*	Contents	0.0046–0.0170	WET: RM	*Number of eggs pooled per nest
					Eggshells	0.0065–0.120		
Hemidactylus mabouia								Schmidt (1984b); Brazil; Rio Grande do Sul; Porto Alegre; urban area
	TL = 35–70 mm	R	—	154	Whole body	0.011–0.493; 0.045–0.090	DRY: R; RM	9 sites combined
				21	Liver	0.132–4.007; 0.402–1.299		3 sites combined
Hemidactylus mabouia								Schmidt (1986); Brazil; Rio Grande do Sul, Porto Alegre; urban area; 1 site
	—		—	20	Whole body	0.048; 0.011–0.101	DRY: M; R	
				8	Liver	0.402; 0.132–0.676		
				6	Excrements	0.838; 0.560–1.464		
*Iberolacerta monticola**						See As for details		Marco et al. (2004); Spain; Avila, Gredos Mountains; gravid females collected in the field in 2001; freshly laid eggs incubated until hatching in As (arsenic acid in nitric acid)-loaded artificial breeding substrate (sterile As-free vermiculite) for 18 days; further elements (Cd, Cu, Pb, Zn), though not manipulated, were detected in eggshells and embryos; *[*Lacerta monticola cyreni*]
Lacerta agilis								Schmidt (1984a, 1988); Germany; Saarbrücken; urban area; 1 site
	—		M	4	Whole body	0.90	DRY: M	
			F	4		0.52		

(*continued*)

TABLE A.6 (CONTINUED)
Cadmium (Cd)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
*Laudakia s. stellio**			—	—				Loumbourdis (1997); Greece, Thessaloniki region; *[*Agama s. stellio*]
	Adult				Liver	9.87	DRY: M	Urban area (500 m asl)
					Carcass	1.01		
					Liver	27.18		Agricultural area (50 m asl)
					Carcass	1.36		
*Norops sagrei**								Burger et al. (2004); USA; South Florida and Florida Keys; *[*Anolis sagrei*]
	Adult							
							WET: M; RM; R*	*6 sites combined
	SVL = 43.5;35–50 mm	M; R	F	72	Whole body*	0.0730; 0.0142–0.146; 0.0042–0.396		*Minus gut contents
	SVL = 55.3;45–67 mm		M	72		0.0439; 0.00743–0.0810; 0.003–0.447		
Podarcis carbonelli	Adult		—	—				Mann et al. (2006, 2007); lab feeding experiment; experimental animals caught at the coastal dune systems of Sao Jacinto and Torreira on the north central coast of Portugal; feeding experiment started after first overwintering in the laboratory; lizards fed Cd-contaminated crickets for 20 days and sacrificed 8 days after their final meal
							WET	Treatment 1: Lizards consumed a mean total of 8.85 µg Cd; Cd administered to prey crickets via diet
					Whole body	0.37	M*	µg total Cd burdon
					Whole body	0.11	M*	*Values (µg/g) estimated from graph
					Gut	3.0		
					Liver	0.44		
					Kidney	0.31		
					Carcass	0.01		

						WET	Treatment 2: Lizards consumed a mean total of 16.90 µg Cd; Cd administered to crickets superficially
				Whole body	1.03	M*	*µg total Cd burdon
				Whole body	0.25	M*	*Values (µg/g) estimated from graph
				Gut	6.0		
				Liver	1.25		
				Kidney	0.45		
				Carcass	0.05		
				Carcass	0.006		Treatment 3: Control
*Podarcis muralis**							Schmidt (1980); Germany; Saarbrücken; urban area; 2 sites; *[*Lacerta muralis*]
	—		—	—	Whole body	1.5–2	DRY: RM*	*Estimated from graph
Podarcis muralis							Schmidt (1984a, 1988); Germany; Saarbrücken; urban area; 4 sites
			M	45	Whole body	0.8–1.7	DRY: RM	*Phases combined
			F	30		0.5–0.6		
	Adult		*	34		0.7–2.6		*Same sample, sex combined
	Semiadult			19		0.6–0.9		
	Juvenile			22		0.4–0.6		
Sceloporus undulatus								Brasfield et al. (2004); USA; Arkansas; Clark County; in ovo exposure lab experiment; lab laid eggs from acclimated field collected lizards; eggs exposed via breeding substrate (perlite)
			—	*				*Eggs from 10 clutches
								Nominal exposure concentrations: µg Cd/g perlite (from $CdCl_2$):
	Egg*	_		*	Contents*		DRY: M	*Failed hatches (embryos and yolk)
		4				ND (<—)		Treatment: 0 µg Cd/g
		1				ND (<—)		Treatment: 1.48 µg Cd/g
		2				ND (<—)		Treatment: 14.8 µg Cd/g

(*continued*)

TABLE A.6 (CONTINUED)
Cadmium (Cd)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
		2				7.9		Treatment: 148 µg Cd/g (highest dose at which hatching still occurred)
		9				406.2		Treatment: 1480 µg Cd/g
		11				626.6		Treatment: 14 800 µg Cd/g
	Hatchling*							*Sucessfully hatched
		11						Treatment: 0 µg Cd/g
		10			Whole body	ND (<—)		Treatment: 1.48 µg Cd/g
		7				ND (<—)		Treatment: 4.8 µg Cd/g
		7				21.0		Treatment: 148 (highest dose at which hatching still occurred) µg Cd/g
								Statistical analysis: [Cd] concentrations in hatchlings exposed to 148 µg Cd/g perlite were not significantly different from failed hatches at the same Cd exposure concentration; [Cd] concentrations were similar in failed hatches at the two highest exposure concentrations
Tarentola mauritanica								Fletcher et al. (2006); southern Spain; Guadiamar River Valley; mine tailings release event; Boliden-Apirsa mine at Aznalcóllar; 7 study sites spanning an expected contamination gradient; geckos collected from building walls
	Juvenile and adult			52	Whole body*		DRY: M; R	*Minus gut contents
			—	9		0.062; 0.038–0.133		Site 1: Rural non-mine-affected site: near Guadalmellato (most pristine)
			—	13		0.081; 0.023–0.186		Site 2: Urban non-mine-affected site: Villaviciosa de Cordoba (not contaminated by mining)
			—	8		0.069; 0.035–0.145		Site 3: Urban mine-affected site: Aznalcázar (contamination through aerosolized contaminants engulfed the town during cleanup)

		—	8		0.114; 0.032–0.239		Site 4: Urban mine-affected site: Aznalcóllar (contaminated by normal mine operations, or the disaster and subsequent remediation efforts)
		—	5		0.313; 0.106–0.488		Site 5: Floodplain mine-affected site: Guadiamar River floodplain near the Aznalcázar gauge station (24.8 km below the ruptured tailings dam)
		—	5		0.179; 0.079–0.262		Site 6: Floodplain mine-affected site: Guadiamar River floodplain near the Guijo gauge station (7.4 km below the ruptured dam)
		—	4		0.311; 0.074–0.435		Site 7: Floodplain mine-affected site: Agrio River floodplain (4.4 km below and closest to the ruptured tailings dam)
							Statistical analysis: [Cd] concentration was significantly influenced by site
*Zootoca vivipara**							Schmidt (1980); Germany; Saarbrücken; urban area; 3 sites; *[*Lacerta vivipara*]
	—	M	15	Whole body	1.0–3.0	DRY: RM*	*Estimated from graph
*Zootoca vivipara**		—					Avery et al. (1983); Great Britain; England; *[*Lacerta vivipara*]
	Juvenile and adult		11*				Site 1: Little used road; *8 juveniles, 3 adults
				Bone*	0.3	DRY: M	*Femur
				Liver	0.5		
				Lung	ND (<0.16)		
				Kidney	ND (<0.25)		
				Remainder	0.6		
	Juvenile and adult		19 *				Site 2: Busy road; *12 juveniles, 7 adults
				Bone*	0.4		*Femur
				Liver	0.6		
				Lung	<0.16		
				Kidney	0.5		
				Remainder	0.2		

(*continued*)

TABLE A.6 (CONTINUED)
Cadmium (Cd)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
	Juvenile and adult		14				Site 3: Former lead mine; *9 juveniles, 5 adults
				Bone*	0.9		*Femur
				Liver	3.5		
				Lung	1.4		
				Kidney	7.5		
				Remainder	11.0		
*Zootoca vivipara**							Schmidt (1984a, 1988); Germany; Saarbrücken; urban area; 1 site; *[*Lacerta vivipara*]
		M	15	Whole body	1.99	DRY: M	*Combined by sex
		F	5		0.58		
	Adult	*	4		3.1	DRY: M	*Same sample combined by phase
	Semiadult		12		1.4		
	Juvenile		4		0.7		
*Zootoca vivipara**	—	M	2				Gutleb and Gutleb (1991); Austria; Carinthia; remote area; *[*Lacerta vivipara*]
				Liver	0.560–0.616	DRY: R	
				Kidney	0.133–0.367		
				Muscle	0.060–0.080		
Squamata: Serpentes							
"Snake"							Hsu et al. (2006); South Taiwan; Kenting National Park; strong influence from industrial pollution
	—	—	12	Whole body	0.04; 0.02–0.07	DRY: M; R	
					1.73		Bioconcentration factor (reference media: soil and food items)
Agkistrodon piscivorus							Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event

	SVL = 58.6 cm TM = 365 g	M	—	6	Liver Kidney	0.01 0.03	WET: M	
Coluber constrictor								Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 103.2 cm TN = 300 g	M	—	1	Liver Kidney	0.29 0.07	WET	
Nerodia sp.*								Winger et al. (1984); USA; Florida; Apalachicola River; *[*Natrix* sp.]
	TM = 226–544 g	R	—	15	Whole body	0.01; 0.01–0.01	WET: M; R	Site 1: Upper reaches of river
	TM = 272–725 g	R				0.02; 0.01–0.03		Site 2: Lower reaches of river
Nerodia cyclopion								Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 63.9 cm TM = 191 g		—	1	Liver Kidney	ND (<0.01) ND (<0.04)	WET	
Nerodia fasciata								Hopkins et al. (1999); USA; South Carolina; Savannah River site, Aiken
							DRY: M*	*Values estimated from graph
	—			5 5	Liver	0.5 0.12		Site 1: Coal ash-polluted site Site 2: Nearby reference site
								Statistical analysis: Differences in [Cd] concentrations between sites were statistically significant
	Diet							This study provides information on contamination levels of several prey species.

(*continued*)

TABLE A.6 (CONTINUED)
Cadmium (Cd)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Nerodia fasciata							Hopkins et al. (2001); USA; South Carolina; Aquatic Ecology Laboratory near Aiken; lab feeding experiment; experimental animals were captured at uncontaminated reference site and fed 52 total meals of field-collected prey (primarily fish) for 13.5 months
	TM*					DRY	*Initial/final mass
	Juvenile and adult	M	—	8	Gonad	ND (<0.005)	MD; R	Treatment 1: Snakes fed prey items collected from coal ash-contaminated site: Savannah River site
	TM = 61.91/169.6 g*						
					Kidney	0.44; 0.14–0.57		
					Liver	1.07; 0.70–2.11		
					Shed skin	0.28; 0.10–0.76		
					Blood	ND (<0.01)		
					Tail clip	0.04; 0.02–0.74		
	Diet				Fish	0.227; 0.103–0.697	GLSM; R	
	Juvenile and adult	M	—	7			MD; R	Treatment 2: Snakes fed prey items collected from uncontaminated reference site
	TM = 62.2/136.3 g*						
					Gonad	ND (<0.005); ND (<0.005)–0.04		
					Kidney	0.06; 0.02–0.15		
					Liver	0.18; 0.09–0.80		
					Shed skin	0.17; 0.11–0.31		
					Blood	ND (<0.001)		
					Tail clip	0.02; 0.01–0.04		
	Diet				Fish	0.051; 0.019–0.129	GLSM; R	

							Statistical analysis: Treatment differences for [Cd] were significant for snake kidney and for fish
Nerodia fasciata							Hopkins et al. (2002); USA; South Carolina; Aquatic Ecology Laboratory near Aiken; 2-year feeding experiment; all snakes were lab reared and originated from a single gravid female that was collected from a reference site on the Savannah River site; exposure started after first hibernation
	Diet		*	Fish		DRY	*3 prey species, 4 specimens per species
	Juvenile**						**Morphometry in Figure 2 of Hopkins et al. (2002)
	Diet	—	*	Fish	0.051	GLSM	Treatment 1: Control: snakes fed only prey items collected from uncontaminated reference site
	Juvenile**	F	6	Gonad	0.012	M	
				Kidney	0.082		
				Liver	0.148		
		M	4	Gonad	0.122	M	
				Kidney	0.090		
				Liver	0.128		
	Diet	—	*	Fish	0.135	GLSM	Treatment 2: Lower-level exposure: snakes fed prey items collected from coal ash-contaminated site and from the reference site on alternating weeks
	Juvenile**	F	6	Gonad	0.026	M	
				Kidney	0.398		
				Liver	0.695		
		M	4	Gonad	0.041	M	
				Kidney	0.169		
				Liver	0.723		
	Diet	—	*	Fish	0.227	GLSM	Treatment 3: Higher-level exposure: snakes fed only prey items collected from coal ash-contaminated site
	Juvenile**	F	6	Gonad	0.055	M	
				Kidney	0.573		
				Liver	1.718		
		M	3	Gonad	0.059	M	

(*continued*)

TABLE A.6 (CONTINUED)
Cadmium (Cd)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
				Kidney	0.234		
				Liver	1.625		
							Statistical analysis: Effects of organ, treatment, organ by treatment and organ by sex on [Cd] concentration were significant
Nerodia sipedon							Niethammer et al. (1985); USA, Missouri; lead belt
	Juvenile and adult	—	15	Carcass	ND (<0.1)	WET: R	Site 1: Upstream formerly mined area
			15	Carcass	ND (<0.1)–0.12		Site 2: Downstream formerly mined area
			50	Carcass	ND (<0.1)		Site 3: Currently mined area
Nerodia sipedon							Burger et al. (2005); USA; eastern Tennessee; 1 reference site: Little River downstream from the Great Smoky Mountains National Park near Townsend; 1 polluted Superfund site: EFPC inside the the US Department of Energy's (USDOE) Y-12 National Security Complex
	Adult	—	—			WET; M*	*Both sites combined
			47	Kidney	0.035		
			47	Liver	0.074		
			47	Muscle	0.015		
			47	Skin	0.020		
			46	Blood	0.007		
			3	Testis	0.016		
	Egg		8	Egg*	0.006		*Gonadal
							Statistic analysis: [Cd] concentrations were significantly highest in the liver
Nerodia sipedon							Campbell et al. (2005); USA; eastern Tennessee

	Adult TM = 235; 53–464 g SVL = 65; 44.5–80 mm	M; R	F	21				
	TM = 103; 74–146 g SVL = 53; 47–60 mm		M	26				
			F/M	11/16	Blood	0.0095; 0.00001–0.094	WET: M; R	Site 1: Reference site: Little River downstream from the Great Smoky Mountains National Park
					Kidney	0.0273; 0.0018–0.0816		
					Liver	0.0411; 0.0059–0.123		
					Muscle	0.0143; 0.0003–0.096		
					Skin	0.0149; 0.0039–0.048		
			F/M	10/10	Blood	0.00417; 0.00001–0.011		Site 2: Polluted Superfund site: upper reach of East Fork Poplar Creek (EFPC) within the US Department of Energy's (USDOE) Y-12 National Security Complex
					Kidney	0.0453; 0.0037–0.177		
					Liver	0.118; 0.0059–0.247		
					Muscle	0.0162; 0.00044–0.054		
					Skin	0.0261; 0.0033–0.0623		
Nerodia sipedon								Burger et al. (2007); USA; New Jersey and Tennessee
	Adult TL = 72; 39.0–103.5 cm TM = 159; 17.5–587.5 g	M; R	—				WET: M	Site 1: Urban/suburban; New Jersey
				18	Blood	0.003		
					Kidney	0.040		
					Liver	0.066		
					Muscle	0.011		
					Skin	0.032		
				36	Blood	0.008		Site 2: Relatively rural; Tennessee, Department of Energy site

(*continued*)

TABLE A.6 (CONTINUED)
Cadmium (Cd)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
				46	Muscle	0.015		
				47	Liver	0.074		
*Pantherophis guttatus**								Jones and Holladay (2006); USA; Florida; lab feeding experiment; experimental animals obtained from commercial provider; fed dead mice for 34 weeks; *[*Elaphe guttata*]
	TM* = 44.6; 52.0–260.3/211.1; 107.9–353.8 g	M; R*						*Initial mass/final mass
			—	3	Shed skins	0.006; 0.005–0.025	DRY: MD; R	Treatment 1: Control: fed only not contaminated mice
			—	10		1.0311; 0.182–1.470		Treatment 2: Fed metal-injected mice enriched with a mixture of three metals (Cd, Hg, Pb) at a dose of 2mg/kg per snake, metal, and month
Pituophis melanoleucus								Burger (1992); USA, New Jersey; 4 sites, 4 years
	Hatchling TM = 24.65g			46	Skin	0.115; 0.058–0.195	DRY: M; RM	*Combined by year
				16	Whole body*	0.115; 0.055–0.178		*Saggital section from the center of the body, including bone
Vipera berus								Gutleb and Gutleb (1991); Austria; Carinthia; remote area
	—		M	1	Liver	0.173	DRY	
					Kidney	0.032		

TABLE A.7
Caesium (Cs)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Testudines							
Chelonia mydas							Lam et al. (2004); south China
	Juvenile	—	2	Fat	0.142	WET: M	Specimens stranded on beaches at Ham Tin Wan and Tung Pang Chau; upon collection, turtles had started to decay; years: 2001 and 2003
				Kidney	0.123		
				Heart	0.115		
				Liver	0.036		
				Lung	0.149		
				Muscle	0.157		
				Stomach	0.462		
	Adult	—	3	Muscle	0.048		Specimens believed to have been unintentionally caught by fishermen; upon collection, turtles were relatively fresh; year: —
			1	Liver	0.061		
Squamata: Sauria							
*Laudakia s. stellio**							Loumbourdis (1997); Greece, Thessaloniki region; *[*Agama s. stellio*]
	Adult	—	—	Liver	0.48	DRY: M	Site 1: Urban area (500 m asl)
				Carcass	0.26		
				Liver	0.98		
				Carcass	0.16		Site 2: Agricultural area (50 m asl)
Tarentola mauritanica							Fletcher et al. (2006); southern Spain; Guadiamar River Valley; mine tailings release event; Boliden-Apirsa mine at Aznalcóllar; 7 study sites spanning an expected contamination gradient; geckos collected from building walls

(*continued*)

TABLE A.7 (CONTINUED)
Caesium (Cs)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
	Juvenile and adult		52	Whole body*		DRY: M; R	*Minus gut contents
		—	9		0.017; 0.005–0.025		Site 1: Rural non-mine-affected site: near Guadalmellato (most pristine)
		—	13		0.022; 0.012–0.040		Site 2: Urban non-mine-affected site: Villaviciosa de Cordoba (not contaminated by mining)
		—	8		0.029; 0.016–0.042		Site 3: Urban mine-affected site: Aznalcázar (contamination through aerosolized contaminants engulfed the town during cleanup)
		—	8		0.054; 0.026–0.098		Site 4: Urban mine-affected site: Aznalcóllar (contaminated by normal mine operations, or the disaster and subsequent remediation efforts)
		—	5		0.023; 0.021–0.026		Site 5: Floodplain mine-affected site: Guadiamar River floodplain near the Aznalcázar gauge station (24.8 km below the ruptured tailings dam)
		—	5		0.032; 0.014–0.057		Site 6: Floodplain mine-affected site: Guadiamar River floodplain near the Guijo gauge station (7.4 km below the ruptured dam)
		—	4		0.035; 0.029–0.038		Site 7: Floodplain mine-affected site: Agrio River floodplain (4.4 km below and closest to the ruptured tailings dam). Statistical analysis: [Cs] concentration was significantly influenced by site
Squamata: Serpentes							
Agkistrodon piscivorous							Burger et al. (2006); USA, South Carolina; 1 reference site, 1 polluted Savannah River site, Aiken
	SVL(M) = 57 cm TM(M) = 280 g SVL(M) = 60 cm TM(M) = 270 g	M	— 5	13 5	Muscle* Blood	0.3 0.05	DRY: M **	*Tail; **both sites combined

Nerodia fasciata			—					Burger et al. (2006); USA, South Carolina; 1 reference site, 1 polluted Savannah River site, Aiken
	SVL(M) = 51 cm TM(M) = 130 g	M		47	Muscle*	0.3	DRY: **	*Tail; **both sites combined
	SVL(M) = 52 cm TM(M) = 140 g			34	Blood	0.03		
Nerodia taxispilota								Burger et al. (2006); USA, South Carolina; 1 reference site, 1 polluted Savannah River Site, Aiken
	SVL(M) = 59 cm TM(M) = 170 g	M	—	10	Muscle*	0.2	DRY: **	*Tail; **both sites combined
	SVL(M) = 65 cm TM(M) = 230 g			9	Blood	0.04		

TABLE A.8
Chromium (Cr)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Testudines								
*Actinemys marmorata**								Henny et al. (2003); USA; Western Oregon; Fern Ridge Reservoir; *[*Clemmys marmorata*]
	Egg		—	14*	Contents	ND (<2.00)–44.9	DRY: R*	*14 nests, 1 egg per nest; **Cr above detection limit in 6 eggs Statistical analysis: No significant differences in contaminant concentrations related to hatching rate
Caretta caretta								Stoneburner et al. (1980); USA; 4 western Atlantic nesting beaches: Canaveral, Florida; Cumberland Island, Georgia; Cape Lookout, North Carolina; Cape Hatteras, North Carolina
	Egg*		—	96	Yolk	1.04–1.71	—; RM*	* Fresh; **combined by beach
*Caretta caretta**								Aguirre et al. (1994); USA; Hawaiian Islands; *[identification number 052, *Chelonia mydas* in the reference]
	"Pelagic"		—	1	Liver	ND (<0.2)	WET	
Caretta caretta							DRY: M; R	Storelli et al. (1998a); Italy; Adriatic Sea, Apulian coasts; stranded turtles
	TM = 1.8–100 kg	R	F/M	9/3	Liver	1.05; 0.20–2.07		
					Lung	2.29; 0.38–5.41		
					Kidney	1.57; 0.20–6.80		
					Muscle	1.43; 0.30–2.89		
Caretta caretta								Kaska et al. (2004); Turkey; southwestern Mediterranean coast; stranded turtles
	—		—	32	Liver	2.77; 1.34–4.54	DRY: M; R	
				20	Bladder	1.96; 0.75–2.93		
				20	Kidney	2.06; 1.13–2.87		
				10	Lung	2.54; 0.61–3.46		
				32	Muscle	1.51; 0.41–3.79		

Chelonia sp.								Yoshinaga et al. (1992); Papua New Guinea
	—		—	—	Muscle	0.50	WET: M	
Chelonia mydas								Yoshinaga et al. (1992); Papua New Guinea
	—		—	—	Muscle	0.25	WET: M	
Chelonia mydas								Aguirre et al. (1994); USA; Hawaiian Islands
	TL = 28.7–71.3 cm	R	F/M	8/4	Liver	ND (<0.2)–0.5 ND (<0.2)–0.4	WET: R	
	TM = 3.2–43.6 kg							
					Kidney			
	Egg		—	3	Shell	0.4		*Posthatch
	Hatchling				Whole body	ND (<0.2)		
Chelonia mydas							DRY: M; R	Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 49.0; 40.0–63.5 cm	M; R	M	6				
	SCL = 52.2; 37.0–71.4		F	20				
			M	6	Liver	2.2; 1.6–3.1		
				6	Kidney	2.1; 1.6–2.9		
				3	Muscle	1.4; 1.3–1.4		
			F	20	Liver	2.3; 1.5–3.5		
				19	Kidney	2.2; 1.4–3.8		
				9	Muscle	1.4; 1.2–1.8		
	Diet		—	8	Stomach*	1.3; 0.53–2.8		*Contents
Chelonia mydas	—		—					Kaska et al. (2004); Turkey; southwestern Mediterranean coast; stranded turtles
				22	Liver	2.46; 0.96–6.12	DRY: M; R	
				20	Bladder	2.18; 0.96–9.05		
				14	Kidney	2.60; 0.66–11.36		
				15	Lung	2.48; 0.31–4.02		
				22	Muscle	2.22; 0.12–8.73		

(*continued*)

TABLE A.8 (CONTINUED)
Chromium (Cr)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Chelonia mydas						DRY: M	Lam et al. (2004); South China
	Juvenile	—	2	Fat	0.906		Turtles stranded on beaches at Ham Tin Wan and Tung Pang Chau; upon collection, turtles had started to decay
				Kidney	1.066		
				Heart	2.582		
				Liver	ND (<—)–1.105		
				Lung	0.730		
				Muscle	2.706		
				Stomach	ND (<—)–1.185		
	Adult	—	3	Muscle	2.184		Turtles believed to have been unintentionally caught by fishermen; upon collection, turtles were relatively fresh
			1	Liver	0.847		
Chelonia mydas							Celik et al. (2006); Turkey, Mediterranean Sea, Kazanli beach; stranded individuals
	Egg		12*	Shell**	0.555	DRY: M	*12 nests, 3 eggshells per nest; **collected after hatching
	Environment						In this study, information is available on metal levels in sand, soil, plant, and water samples around the nesting environment.
Chelydra serpentina							Albers et al. (1986); USA; Maryland and New Jersey
	Adult	M	7	Liver	1.00	WET: M	Site 1: Undisturbed freshwater site: Patuxent Wildlife Research Center, Maryland
	L = 20–40 cm						
		F	6		1.97		
		M	7	Kidney	0.93		
		F	6		1.26		

			M	8	Liver	0.60		Site 2: Contaminated brackish water site: Hackensack Meadowlands, New Jersey
			F	3		0.60		
			M	8	Kidney	2.97		
			F	3		2.70		
			M	8	Liver	0.36		Site 3: Contaminated freshwater site: Hackensack Meadowlands, New Jersey
					Kidney	1.13		
Dermochelys coriaca								Godley et al. (1998); Great Britain; Wales and Scotland, west coast; turtles died in fishing gear
	Adult	R	M	3				
	CCL = 141–170 cm							
				1	Liver	ND (<0.018)	WET	
					Muscle	0.097		
Eretmochelys imbricata								Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 43.5; 33.5–48.9	M; R	M	6	Liver	1.1; 0.43–2.1	DRY: M; R	
				6	Kidney	2.1; 1.8–2.9		
				1	Muscle	3.0		
	SCL = 46.5; 43.8–67.9		F	16	Liver	0.76; 0.21–2.0		
				13	Kidney	1.3; 0.3–2.2		
				8	Muscle	0.89; 0.12–2.2		
	Diet		—	6	Stomach*	0.61; 0.4–0.89		*Contents
Lepidochelys olivacea								Sahoo et al. (1996); India; Gahirmatha, Orissa
	—		—	8	Beach sand	28–49	DRY: R*	*mg/g
	Egg*		—	24**	Shell	10.0		*Fresh; **8 nests, 3 eggs per nest
					Albumen-yolk	2.6		
	Egg*				Shell	11.6		*Posthatch
	Hatchling*				Whole body	10.3		*Fresh

(*continued*)

TABLE A.8 (CONTINUED)
Chromium (Cr)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Malaclemys terrapin								Burger (2002); USA; New Jersey; Barnegat Bay
	Adult TL = 14.3 cm	M	F	11	Liver	0.069	WET: M	
					Muscle	0.297		
	Egg		—	8	Egg	0.390; 0.120–0.899	M; R	*Ovarial
Trachemys scripta								Burger and Gibbons (1998); USA; Savannah River site near Aiken, South Carolina; females laid eggs in the lab 2–3 days after collection in the field
	Egg		—	16*	Contents	0.139; 0.353	DRY: M; MAX	*16 clutches, 1 egg per clutch
					Shell	0.383; 0.611		
								Statistical analysis: [Cr] concentrations were significantly higher in the shells than in contents
Trachemys scripta								Nagle et al. (2001); USA; South Carolina, Aiken County, Savannah River site; in ovo exposure experiment; females collected in the field in 1993, 1994; oviposition induced in 1995; eggs from turtles collected in both polluted and unpolluted sites were incubated in coal ash-contaminated and uncontaminated soil in outdoor artificial nests
							DRY: M	Site 1: Coal ash-polluted Savannah River site
	Adult		F	4	Liver	6.19		
	Diet		—	12	Asiatic clam*	5.63		*Soft tissue
			—	3	Crayfish*	2.46		*Whole body
	Environment		—	—	Artifical nest*	6.92		*Incubation substrate
								Sites 2–3: Unpolluted control sites near Couchton
	Adult		F	3	Liver	1.16		
	Diet		—	10	Asiatic clam*	2.71		*Soft tissue
	Diet		—	3	Crayfish*	1.59		*Whole body

	Environment		—	—	Artifical nest*	0.05		*Incubation substrate
	Hatchling							Trial 1: Incubation substrate: coal ash contaminated
	CL = 30.34 mm	M	—	18*				*18 hatchlings from 5 clutches
	TM = 8.20 g							
			—	6*	Whole body	1.05		*3 clutches per site, 2 hatchlings per clutch
	Hatchling							Trial 2: Incubation substrate: uncontaminated
	TM = 30.36 g	M		18*				*18 hatchlings from 5 clutches
	CL = 8.02 mm							
				6*	Whole body	0.75		*3 clutches per site, 2 hatchlings per clutch
	Hatchling							Trial 3: Maternal residence: coal ash contaminated
	CL = 30.51 mm	M	—	18*				*18 hatchlings from 4 clutches
	TM = 8.02 g							
			—	6*	Whole body	0.98		*3 clutches per site, 2 hatchlings per clutch
	Hatchling							Trial 4: Maternal residence: uncontaminated
	TM = 29.69 ± 0.61 g *	M		18*				*18 hatchlings from 6 clutches
	CL = 7.77 ± 0.38 mm *							
				6*	Whole body	0.85		*3 clutches per site, 2 hatchlings per clutch
								Statistical analysis: [Cr] concentrations were significantly higher in adult liver but not consistently in prey from site 1; [Cr] concentrations in hatchlings were not significantly different between trials 1 and 2 or 3 and 4
Trachemys scripta elegans								Tryfonas et al. (2006); USA; Illinois; Lower Illinois River near Grafton; eggs laid in the lab from turtles collected in the field from 5 nesting areas
	Egg			—	Contents	ND (<—)	DRY: M*	*All sites combined; values estimated from graph
					Shell	1.6		
	Diet				*Lemna sp.*	0.8		
	Environment			4–5*	Soil	7–18	RM*	*Samples (soil from nesting and lake bank areas) from 2 sites
				3*	Sediment	5–9	RM*	*Samples (3 layers per site) from 2 sites; values estimated from graph
				3–4*	Water	ND (<0.02)	WET: RM*	*Samples from 3 sites; values estimated from graph

(*continued*)

TABLE A.8 (CONTINUED)
Chromium (Cr)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Crocodylia								
Alligator mississippiensis								Delany et al. (1988); USA; Florida; 8 lakes, statewide
	L = 2.9–3.8 m	R	F/M	1/31				
			*	24	Muscle*	0.06; 0.03–0.11	WET: M; RM**	*Tail; **combined by lake
Alligator mississippiensis								Heinz et al. (1991); USA; central and southern Florida; Lake Okeechobee, Lake Griffin, and Lake Apopka
	Egg		—	32*	Egg	0.08–0.09; ND (<0.04)–1.2	WET: RM; R**	*16 nests, 2 eggs per nest; **combined by lake
Alligator mississippiensis								Burger et al. (2000); USA; Florida; Lake Apoka (Lake and Orange Counties), Orange Lake (Alachua County), and Lake Woodruff (Volusia County)
	Yearling						WET: M*	*3 lakes combined
	TL = 36–40 cm							
				30	Fat*	0.0860		*Abdominal
				31	Liver	0.0909		
				30	Muscle*	0.0597		*Abdominal
				29	Skin*	0.111		*Abdominal
				29	Muscle*	0.237		*Ventral proximal tail
				22	Tail tip	0.192		
				3	Tail*	0.0623		*Regenerated
Alligator mississippiensis								Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	—		—	1	Liver	0.01	WET	

				Kidney	0.06		
Alligator sinensis							Xu et al. (2006); China; Changxing County, Zhejiang Province; Changxing Nature Reserve and Breeding Research Center for Chinese alligator; alligators collected dead of unidentified causes
	Adult					DRY	
		F/M	1/1	Heart	0.841/0.689		
				Lung	0.242/0.251		
				Liver	0.395/0.333		
				Stomach	0.271/0.194		
				Kidney	0.268/0.169		
				Intestine	0.102/0.095		
				Trachea	0.112/0.178		
				Pancreas	0.147/0.142		
				Gonad	0.142/0.135		
				Muscle	0.130/0.180		
	Diet	—	—	Fish*	0.067	DRY: M; R	*2 fish prey species randomly sampled
	Excrements	—	6	Feces	3.937		
	Eggs		10*				*10 eggs from 3 clutches
				Eggshells	0.314; 0.256–0.370		
				Shell membranes	0.319; 0.175–0.387		
				Egg contents	0.098; 0.091–0.104		
	Environment		10*				*Water and sediment collected from 10 breeding ponds (5 subsamples from different sites within a pond each)
				Sediment	55.46		
				Water	0.00593	WET: M	
							Statistical analysis: [Cr] concentrations were significantly higher in shells and membranes than in contents; significant positive correlations existed in the contents among concentrations of [As] and [Cu], [Pb] and [Cr]

(*continued*)

TABLE A.8 (CONTINUED)
Chromium (Cr)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Crocodylus acutus								Stoneburner and Kushlan (1984); USA; Florida; Florida Bay, Everglades
	Egg		—	9	Shell	20.46	DRY: M	
					Albumen-yolk	2.64		
Crocodylus niloticus								Swanepoel et al. (2000); South Africa; Kruger National Park (KNP) area
	TL = 1.40–4.15 m	R	F/M	6/9			DRY: M	
								Site 1: Shingwedzi River (Silwervis Dam), northern part of KNP; catchment area outside KNP is limited
					Muscle	90.5		Frozen tissues used for residue analysis
					Liver	69.0		
					Kidney	82.0		
					Fat	105.4		
								Site 2: Olifants River, central part of KNP; flows through areas of intense agricultural activity and passes mining areas and receives tributaries from Phalaborwa Mining Company before entering the KNP
					Muscle	9.8		Frozen tissues used for residue analysis
					Liver	5.1		
					Kidney	5.1		
					Fat	14.6		
								Site 3: Sabi River, southern part of KNP; flows through areas of intense agricultural activity before entering the KNP
					Muscle	18.4		
					Liver	5.5		Frozen tissues used for residue analysis
					Kidney	0.7		
					Fat	30.3		

Crocodylus porosus								Yoshinaga et al. (1992); Papua New Guinea
	—		—	—	Muscle	0.16	WET: M	
Crocodylus porosus								Jeffree et al. (2001); northern Australia; Alligator Rivers region, Kakadu National Park; samples from 3 river catchments, mining and hunting areas included
	A = 5–40 years L = 168–499 cm	R	—	40				
			—	35	Muscle*	0.413; 0.120–1.02	DRY: GM; R	*Tail
			—	40	Osteoderms	0.219; 0.07–0.39	DRY: M; R	*Ventral pelvic region
Squamata: Sauria								
*Laudakia s. stellio**								Loumbourdis (1997); Greece; Thessaloniki region; [*Agama s. stellio*]
	Adult		—	—	Liver	2.37	DRY: M	Site 1: Urban area (500 m asl)
					Carcass	4.16		
					Liver	1.35		Site 2: Agricultural area (50 m asl)
					Carcass	5.57		
*Norops sagrei**								Burger et al. (2004); USA; South Florida and Florida Keys; [*Anolis sagrei*]
	Adult				Whole body		WET: M; RM; R**	*Minus gut content; **6 sites combined
	SVL = 43.5; 35–50 mm	M; R	F	72		0.197; 0.114–0.268; 0.048–0.850		
	SVL = 55.3; 45–67 mm		M	72		0.0854; 0.00285–0.155; ND (<0.0010)–0.484		
Varanus sp.								Yoshinaga et al. (1992); Papua New Guinea
	—		—	—	Muscle	0.11	WET: M	

(*continued*)

TABLE A.8 (CONTINUED)
Chromium (Cr)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Squamata: Serpentes								
Acrochordus javanicus								Yoshinaga et al. (1992); Papua New Guinea
	—		—	—	Muscle	0.06	WET: M	
Agkistrodon piscivorus							WET: M	Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 58.6 cm TM = 365 g	M	—	6	Liver	0.08		
					Kidney	0.14		
Agkistrodon piscivorous								Burger et al. (2006); USA; South Carolina; 1 reference site, 1 polluted Savannah River Site, Aiken
	SVL = 57 cm TM = 280 g	M	—	13	Muscle*	1	DRY: M**	*Tail; **both sites combined
	SVL = 60 cm TM = 270 g			5	Blood	0.1		
Coluber constrictor								Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 103.2 cm TN = 300 g		—	1	Liver	0.09	WET	
					Kidney	0.10		
Nerodia cyclopion								Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event

	SVL = 63.9 cm TM = 191 g		—	1	Liver	0.10	WET	
					Kidney	0.17		
Nerodia fasciata								Hopkins et al. (1999); USA; South Carolina; Savannah River site, Aiken
							DRY: M*	*Values estimated from graph
	—	—	—	5	Liver	2		Site 1: Contaminated by coal combustion wastes
				5		0.8		Site 2: Nearby reference site
								Statistical analysis: Differences in [Cr] concentrations between sites were not significant
	Diet							This study provides information on contamination levels of several prey species
Nerodia fasciata								Burger et al. (2006); USA; eastern Tennessee, 1 reference site: Little River downstream from the Great Smoky Mountains National Park near Townsend; 1 polluted Superfund site: EFPC inside the USA Department of Energy's (USDOE) Y-12 National Security Complex
	SVL = 51 cm TM = 130 g	M	—	47	Muscle*	0.9	DRY: M**	*Tail; **both sites combined
	SVL = 52 cm TM = 140 g			34	Blood	0.6		
Nerodia fasciata								Burger et al. (2007); USA; South Carolina; 1 relatively rural site
	Adult	—	—	34	Blood	0.160	WET: M	
				47	Muscle	0.263		
				5	Liver	0.343		
Nerodia sipedon								Burger et al. (2005); USA; eastern Tennessee; 1 reference site, 1 polluted Superfund site
	Adult	—	—				WET: M*	*Both sites combined
				47	Kidney	0.062		
				47	Liver	0.042		
				47	Muscle	0.051		
				47	Skin	0.098		

(*continued*)

TABLE A.8 (CONTINUED)
Chromium (Cr)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
				46	Blood	0.013		
				3	Testis	0.031		
	Egg			8	Egg*	0.012		*Ovarial Statistic analysis: [Cr] concentrations were significantly highest in the skin
Nerodia sipedon								Campbell et al. (2005); USA; eastern Tennessee
	Adult						WET: M; R	
	TM = 235; 53–464 g SVL = 65; 44.5–80 mm	M; R	21 F					
	TM = 103; 74–146 g VL = 53; 47–60 mm	26 M						
			F/M	11/16	Blood	0.0131; 0.00004–0.091		Site 1: Reference site: Little River downstream from the Great Smoky Mountains National Park
					Kidney	0.0843; 0.00237–0.654		
					Liver	0.0530; 0.00017–0.696		
					Muscle	0.0555; 0.0068–0.151		
					Skin	0.0932; 0.0146–0.294		
			F/M	10/10	Blood	0.0131; 0.00098–0.074		Site 2: Polluted Superfund site: upper reach of East Fork Poplar Creek (EFPC) within the USA Department of Energy's (USDOE) Y-12 National Security Complex
					Kidney	0.0314; 0.00004–0.230		

					Liver	0.0273; 0.00004–0.130		
					Muscle	0.0464; 0.00004–0.270		
					Skin	0.105; 0.00004–0.367		
Nerodia sipedon								Burger et al. (2007); USA, New Jersey and Tennessee
	Adult							
	TL = 72; 39.0–103.5 cm TM = 159; 17.5–587.5 g	M; R	—	18				Site 1: Urban/suburban: New Jersey
					Blood	0.052	WET: M	
					Kidney	0.187		
					Liver	0.058		
					Muscle	0.110		
					Skin	0.274		
								Site 2: Relatively rural: Tennessee
				36	Blood	0.015		
				46	Muscle	0.051		
				47	Liver	0.042		
Nerodia taxispilota								Burger et al. (2006); USA; South Carolina; 1 reference site, 1 polluted Savannah River site, Aiken
	SVL = 59 cm TM = 170 g	M	—	10	Muscle*	1.0	DRY: M**	*Tail; **both sites combined
	SVL = 65 cm TM = 230 g			9	Blood	0.1		
Pituophis melanoleucus								Burger (1992); USA; New Jersey; 4 years
	Hatchling TM = 24.65 g		—	46	Skin	5.047; 4.178–6.823	DRY: M; RM*	*Years combined
				16	Whole body*	3.479; 1.354–6.717		*Sagittal section from the center of the body, including bone

(*continued*)

TABLE A.9
Cobalt (Co)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Testudines								
*Actinemys marmorata**								Henny et al. (2003); USA; western Oregon; Fern Ridge Reservoir; *[*Clemmys marmorata*]
	Egg		—	14*	Contents	ND (<0.30)–0.60	DRY: R*	*14 nests, 1 egg per nest; above detection limit in only 1 egg
Caretta caretta								Stoneburner et al. (1980); USA; 4 western Atlantic nesting beaches: Florida, Canaveral; Georgia, Cumberland Island; North Carolina, Cape Lookout; North Carolina, Cape Hatteras
	Egg*		—	96	Yolk	ND (<—)–0.0729	—; RM**	*Fresh; **combined by beach
Caretta caretta								Sakai et al. (1995); Japan; Kochi Prefecture, Cape Ashizuri
	L = 76–92 cm W = 75–108 kg		F/M	6/1	Liver	ND (<0.03)	WET: R	
					Kidney	ND (<0.03)–0.257		
					Muscle	ND (<0.03)		*Pectoral
	Egg*		—	*	Shell	ND (<0.03)		*Oviductal eggs from 5 females
					Yolk	ND (<0.03)		
					Albumen	ND (<0.03)		
Chelonia mydas								Sakai et al. (2000a); Japan; Yaeyama Islands, Okinawa; turtles caught by fishermen for commercial use
	SCL = 51.0 cm	M	—	50			WET: M; R	
				1/50*	Liver	0.067		*Detected/analyzed
				23/23	Kidney	0.808; 0.063–2.50		
				0/47	Muscle	ND (<0.03)		

Chelonia mydas							Sakai et al. (2000b); Japan; Ogasawara Islands (Bonin Islands), Haha-Jima Island; turtles collected in coastal waters
	TM = 124/117 kg SCL = 93/97 cm	M	F/M	1/1	Liver	ND (<0.03)	WET: M	CW = 72/73 cm
					Gullet	ND (<0.03)		
					Stomach	ND (<0.03)		
					Intestine	ND (<0.03)		
					Pancreas	ND (<0.03)		
					Heart	ND (<0.03)		
					Trachea	ND (<0.03)		
					Lung	ND (<0.03)		
					Bladder	ND (<0.03)		
					Spleen	ND (<0.03)		
					Kidney	0.571/ND (<0.03)		
					Salt gland	ND (<0.03)		
					Brain	ND (<0.03)		
	Reproductive tissues				Testis	ND (<0.03)		
					Oviduct	ND (<0.03)		
					Ovary	ND (<0.03)		
	Egg				Whole egg	ND (<0.03)		
					Shell	ND (<0.03)		
					Yolk	ND (<0.03)		
					Albumen	ND (<0.03)		
					Scale	ND (<0.03)		
					Mesentary	ND (<0.03)		
					Fat	ND (<0.03)		
					Muscle	ND (<0.03)		
					Bone	ND (<0.03)		

(*continued*)

TABLE A.9 (CONTINUED)
Cobalt (Co)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
					Carapace	ND (<0.03)		
					Whole body	*		*Not calculated
Chelonia mydas							DRY: M; R	Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 49.0; 40.0–63.5 cm	M; R	M	6				
	CW = 40.5; 35.3–49.4							
	SCL = 52.2; 37.0–71.4		F	20				
	CW = 43.9; 31.7–58.5							
			M	6	Liver	0.12; 0.084–0.17		
				6	Kidney	1.6; 0.19–4.5		
				3	Muscle	0.007; 0.006–0.008		
			F	20	Liver	0.29; 0.074–1.3		
				19	Kidney	3.0; 0.23–10.0		
				9	Muscle	0.01; 0.005–0.020		
	Diet		—	8	Stomach*	0.18; 0.025–0.64		*Contents
Chelonia mydas							DRY: M	Lam et al. (2004); South China
	Juvenile		—	2	Fat	0.052		Turtles stranded on beaches at Ham Tin Wan and Tung Pang Chau; upon collection, turtles had started to decay
					Kidney	11.39		
					Heart	0.349		
					Liver	0.585		
					Lung	0.732		
					Muscle	0.083		
					Stomach	0.344		

	Adult		—	3	Muscle	0.109		Turtles believed to have been unintentionally caught by fishermen; upon collection, turtles were relatively fresh
				1	Liver	0.304		
Chelydra serpentina								Meyers-Schöne et al. (1993); USA; Tennessee
	Adult		M	12	Liver	5.17×10^{-2}	WET: M ^{60}Co Bq/g	Site 1: Contaminated site: White Oak Lake
			F/M	3/6	Liver	ND ($<3.7 \times 10^{-3}$)		Site 2: Reference site: Bearden Creek embayment
Eretmochelys imbricata								Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 43.5; 33.5–48.9 CW = 36.9; 32.6–39.6	M; R	M	6	Liver	0.90; 0.34–1.5	DRY: M; R	
				6	Kidney	3.6; 1.7–6.2		
				1	Muscle	0.036		
	SCL = 46.5; 43.8–67.9 CW = 32.8; 10.9–52.3		F	16	Liver	0.62; 0.17–2.1		
				13	Kidney	2.9; 0.9–9.6		
				8	Muscle	0.047; 0.012–0.091		
	Diet		—	6	Stomach*	0.60; 0.057–1.4		*Contents
Lepidochelys olivacea								Sahoo et al. (1996); India; Gahirmatha, Orissa
	—		—	8	Beach sand	11–32	DRY: R*	*mg/g
	Egg*		—	24**	Shell	7.6		*Fresh; **8 nests, 3 eggs per nest
					Albumen-yolk	2.3		
	Egg*				Shell	4.6		*Posthatch
	Hatchling*				Whole body	12.3		*Fresh

(*continued*)

TABLE A.9 (CONTINUED)
Cobalt (Co)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Trachemys scripta								Meyers-Schöne et al. (1993); USA; Tennessee
	Adult		F/M	6/6	Liver	6.03×10^{-2}	WET: M	Site 1: Contaminated site: White Oak Lake
			F/M	6/6	Liver	ND ($<3.7 \times 10^{-3}$)	^{60}Co Bq/g	Site 2: Reference site: Bearden Creek embayment
Crocodylia								
Alligator mississippiensis	—		—				WET	Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
				1	Liver	ND (<0.01)		
					Kidney	0.02		
Crocodylus acutus			—					Stoneburner and Kushlan (1984); USA; Florida; Florida Bay, Everglades
	Egg			9	Shell	1.70	DRY: M	
					Albumen-yolk	1.12		
Crocodylus johnstoni							DRY: M; R	Jeffree et al. (2001); northern Australia, north central Queensland, Lynd River; samples from a single population
	A = 0.7–62.7 years L = 24.7–128.3 cm	R	F/M	9/21	Osteoderm*	0.100; 0.068–0.122		*Ventral pelvic region
Crocodylus niloticus								Swanepoel (1998, 2000), S: Campbell (2003); South Africa Site 1: Olifants River in central part of Kruger National Park
					Liver	2.20; 1.8–2.6	DRY: M; R	Formalinized tissues analyzed
					Kidney	2.3; 1.5–3.1		

								Site 2: Sabi River in southern part of Kruger National Park
					Liver	2.70		Formalinized tissues analyzed
					Kidney	2.40		
Crocodylus niloticus								Almli et al. (2005); Zambia
	L = 2.7–3.4 m	R	F	2	Liver	0.02; 0.01–0.03	WET: MD; R	Site 1: Kafue River, Kafue National Park
			M	2	Kidney	0.08; 0.08–0.35		
	L = 2.0–4.0 m		F	4	Liver	0.05; 0.04–0.07		Site 2: Luangwa River, Luangwa National Park
			M	1	Kidney	0.31; 0.21–0.46		
Crocodylus porosus			—	40				Jeffree et al. (2001); northern Australia, Alligator Rivers region: Kakadu National Park; samples from 3 river catchments, mining and hunting areas included
	A = 5–40 years L = 168–499 cm	R	—	35	Muscle	ND (<0.01)	DRY: M; R	*Tail
			—	40	Osteoderms	0.344; 0.220–0.478		*Ventral pelvic region
Squamata: Sauria								
*Laudakia s. stellio**			—	—				Loumbourdis (1997); Greece; Thessaloniki region; *[*Agama s. stellio*]
	Adult				Liver	3.50	DRY: M	Urban area (500 m asl)
					Carcass	2.53		
					Liver	5.08		Agricultural area (50 m asl)
					Carcass	3.62		
Tarentola mauritanica								Fletcher et al. (2006); southern Spain; Guadiamar River Valley; mine tailings release event; Boliden-Apirsa mine at Aznalcóllar; 7 study sites spanning an expected contamination gradient; geckos collected from building walls
	Juvenile and adult			52	Whole body*		DRY: M; R	*Minus gut contents

(*continued*)

TABLE A.9 (CONTINUED)
Cobalt (Co)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
			—	9		0.284; 0.183–0.401		Site 1: Rural non-mine-affected site: near Guadalmellato (most pristine)
			—	13		0.271; 0.179–0.446		Site 2: Urban non-mine-affected site: Villaviciosa de Cordoba (not contaminated by mining)
			—	8		0.238; 0.170–0.286		Site 3: Urban mine-affected site: Aznalcázar (contamination through aerosolized contaminants engulfed the town during cleanup)
			—	8		0.190; 0.119–0.290		Site 4: Urban mine-affected site: Aznalcóllar (contaminated by normal mine operations, or the disaster and subsequent remediation efforts)
			—	5		0.298; 0.158–0.418		Site 5: Floodplain mine-affected site: Guadiamar River floodplain near the Aznalcázar gauge station (24.8 km below the ruptured tailings dam)
			—	5		0.197; 0.151–0.254		Site 6: Floodplain mine-affected site: Guadiamar River floodplain near the Guijo gauge station (7.4 km below the ruptured dam)
			—	4		0.234; 0.160–0.336		Site 7: Floodplain mine-affected site: Agrio River floodplain (4.4 km below and closest to the ruptured tailings dam) Statistical analysis: [Co] concentration was significantly influenced by site
Squamata: Serpentes								
Agkistrodon piscivorus								Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 58.6 cm TM = 365 g	M	—	6	Liver	0.05	WET	
					Kidney	0.07		

Agkistrodon piscivorous			—					Burger et al. (2006); USA; South Carolina; 1 reference site, 1 polluted Savannah River site, Aiken
	SVL = 57 cm TM = 280 g	M		13	Muscle*	0.31	DRY: M**	*Tail; **both sites combined
	SVL = 60 cm TM = 270 g			5	Blood	0.1		
Coluber constrictor			—					Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 103.2 cm TN = 300 g			1	Liver	0.16	WET	
					Kidney	ND (<0.07)		
Nerodia cyclopion			—					Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 63.9 cm TM = 191 g			1	Liver	ND (<0.03)	WET	
					Kidney	0.09		
Nerodia fasciata								Burger et al. (2006); USA; South Carolina; 1 reference site, 1 polluted Savannah River site, Aiken
	SVL = 51 cm TM = 130 g	M	—	47	Muscle*	0.24	DRY: M**	*Tail; **both sites combined
	SVL = 52 cm TM = 140 g			34	Blood	0.1		
Nerodia taxispilota								Burger et al. (2006); USA; South Carolina; 1 reference site 1 polluted Savannah River site, Aiken
	SVL = 59 cm TM = 170 g	M	—	10	Muscle*	0.2	DRY: M**	*Tail; **both sites combined
				9	Blood	0.08		

TABLE A.10
Copper (Cu)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Testudines								
*Actinemys marmorata**								Henny et al. (2003); USA; Western Oregon; Fern Ridge Reservoir; *[*Clemmys marmorata*]
	Egg		—	14*	Contents	8.05; 4.39–77.0	DRY: GM; R	*14 nests, 1 egg per nest
								Statistical analysis: No significant differences in contaminant concentrations related to hatching rate
Caretta caretta			—	—				Hillestad et al. (1974); USA; Georgia and South Carolina; 3 nesting beaches
	Egg				Yolk Albumen	2.08 6.0	—: M	
Caretta caretta								Musquera et al. (1976); —
	—		—	1	Blood*	0.68	WET	*Plasma
Caretta caretta								Stoneburner et al. (1980); USA; 4 western Atlantic nesting beaches: Florida, Canaveral; Georgia, Cumberland Island; North Carolina, Cape Lookout; North Carolina, Cape Hatteras
	Egg*		—	96	Yolk	4.97–6.61	—: RM*	*Fresh; **combined by beach
*Caretta caretta**								Aguirre et al. (1994); USA; Hawaiian Islands; *[identification number 052, *Chelonia mydas* in the reference]
	"Pelagic"		—	1	Liver	2.8	WET	
Caretta caretta								Sakai et al. (1995); Japan; Kochi Prefecture, Cape Ashizuri
	TL = 76–92 cm TM = 75–108 kg	R	F/M	6/1	Liver	17.9; 6.47–33.9	WET: M; R	

					Kidney	1.30; 0.988–1.56		
					Muscle*	0.830; 0.531–1.28		*Pectoral
	Egg*		—	*	Shell*	5.57; 4.25–6.18		*Oviductal eggs from 5 females
					Yolk	1.57; 1.48–1.68		
					Albumen	0.129; 0.034–0.235		
					Whole egg	1.05; 0.772–1.31		Whole egg concentrations calculated as a summation of the products of concentrations and weights of each part
Caretta caretta								Caurant et al. (1999); France; Atlantic coasts, "Pertuis charentais" in the la Rochelle region; stranded turtles
	Juvenile	M; R	—	21			WET: M; R	
	SCL = 29.4; 21.3–34.5 cm							
	TM = 2.8; 0.3–7.5 kg							
			—	7	Liver	8.25; 2.32–20.9		
				5	Kidney	2.21; 1.76–2.83		
				21	Muscle*	0.73; 0.34–2.23		*Pectoral
Caretta caretta								Sakai et al. (2000b); Japan; Kochi Prefecture, Cape Ashizuri; turtles accidentally caught in commercial fishing nets
	TM = 93/83 kg	M	F/M	6/1				
	SCL = 83/85 cm				Liver	17.7/19.4	WET: M	
					Gullet	0.406/0.279		
					Stomach	0.624/0.493		
					Intestine	0.712/0.688		
					Pancreas	1.02/0.951		
					Heart	1.23/1.06		
					Trachea	0.322/0.258		
					Lung	0.545/0.508		
					Bladder	0.669/0.641		
					Spleen	0.696/0.744		
					Kidney	1.30/1.29		

(*continued*)

TABLE A.10 (CONTINUED)
Copper (Cu)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
					Salt gland	2.83/2.04		
					Brain	2.05/2.00		
	Reproductive tissues				Testis	0.867		
					Oviduct	1.73		
					Ovary	1.60		
	Egg				Whole egg	1.05		
					Shell	5.57		
					Yolk	1.57		
					Albumen	0.129		
					Scale	0.398/0.372		
					Mesentary	0.395/0.278		
					Fat	0.109/0.237		
					Muscle	0.810/0.948		
					Bone	0.200/0.384		
					Carapace	0.251/0.298		
					Whole body*	1.21/1.13		*Calculated
Caretta caretta								Kaska and Furness (2001), southwestern Turkey; 4 beaches; collected either just before hatching or dead in shell 1 week after last hatching
	Embryo		—	22	Liver	21.21	DRY: M	
	Egg				Eggshell	5.29		
			—		Yolk	0.928		
								Statistical analysis: No significant differences were observed
Caretta caretta								Franzellitti et al. (2004); Italy; northwestern Adriatic Sea; turtles collected dead along the Adriatic Sea coast from the Po delta to the Reno mouth
	Juvenile to adult	R	—	35*			WET: M*	16 from fishery by-catch, 19 found dead along the coast

	MSCL = 24.5–74 cm							
				30	Liver	7.4		
				13	Lung	1.8		
				17	Muscle*	1.5		*Pectoral
				7	Fat*	3.4		*Abdominal
Caretta caretta							DRY: M	Kaska et al. (2004); Turkey; southwestern Mediterranean coast; stranded turtles
	—		—	32	Liver	2.98; 0.27–4.18		
				20	Bladder	1.48; 0.33–2.68		
				20	Kidney	2.08; 1.18–3.08		
				10	Lung	2.76; 0.12–15.30		
				32	Muscle	1.55; 0.86–5.45		
Caretta caretta								Torrent et al. (2004); Spain; Canary Islands; stranded turtles submitted to the veterinary faculty of the University of Las Palmas in Gran Canaria
	Juvenile and subadult	R*						*Values estimated from graph
	SCL = 15–65 cm		F/M	67/11				
					Kidney	4.60; 0.13–49.06	WET: M; R	
					Muscle	2.85; 0.01–27.25		
					Bone	3.81; 0.09–24.49		
					Liver	15.02; 0.01–65.57		
Caretta caretta								Maffucci et al. (2005); southern Italy; western Mediterranean Sea; South Tyrrhenian coasts; stranded turtles
	CCL = 37–82 cm	R	—	29				
				14	Liver	37.3; 9.4–41.8	DRY: M; R	
				19	Kidney	2.6; 1.7–4.7		
				26	Muscle	2.7; 0.8–7.0		
Caretta caretta								Storelli et al. (2005); Italy; Adriatic and Ionian Seas; stranded turtles

(*continued*)

TABLE A.10 (CONTINUED)
Copper (Cu)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
	SCL = 21–71 cm	R		19	Liver	7.69; 1.43–17.8	WET: M; R	
					Kidney	1.21; 0.36–2.12		
					Muscle	0.59; 0.19–1.35		
					Spleen	1.27; 0.95–1.69		
					Heart	2.02; 0.77–4.18		
					Lung	0.76; 0.32–1.50		
					Fat	0.19; 0.10–0.51		
Caretta caretta								Gardner et al. (2006); Mexico; Baja California peninsula; turtles died from fisheries capture
	MSCL = 57.0; 52.0–63.0	M; R		5	Liver	33.94; 16.6–58.98	DRY: GM; R	
					Kidney	4.35; 1.39–8.23		
					Muscle*	0.41; ND (<0.0025)–3.44		*Pectoral
					Fat	0.69; 0.53–1.15		
Chelonia sp.								Yoshinaga et al. (1992); Papua New Guinea
	—		—	—	Muscle	0.57	WET: M	
Chelonia mydas								Yoshinaga et al. (1992); Papua New Guinea
	—		—	—	Muscle	0.73	WET: M	
Chelonia mydas								Aguirre et al. (1994); USA; Hawaiian Islands
	L = 28.7–71.3 cm W = 3.2–43.6 kg	R	F/M	8/4	Liver	94.67; 1.3–189.0	WET: M; R	
					Kidney	3.633; 1.1–10.5		
	Egg*		—	3	Shell	14.3		*Posthatch
	Hatchling				Whole body	2.2		
Chelonia mydas							WET: M; R	Sakai et al. (2000a); Japan; Yaeyama Islands, Okinawa; turtles caught by fisherman for commercial use

	SCL = 51.0 cm	M	—	50			WET: M; R	
				50/50*	Liver	50.2; 4.27–113		*Detected/analyzed
				23/23	Kidney	2.15; 0.950–3.94		
				47/47	Muscle	0.353; 0.076–2.65		
Chelonia mydas								Sakai et al. (2000b); Japan; Ogasawara Islands (Bonin Islands), Haha-Jima Island; turtles collected in coastal waters
	TM = 124/117 kg	M	F/M	1/1	Liver	8.73/13.5	WET: M	CW = 72/73 cm
	SCL = 93/97 cm							
					Gullet	1.55/0.337		
					Stomach	0.327/0.412		
					Intestine	0.463/0.519		
					Pancreas	1.04/0.970		
					Heart	0.945/0.950		
					Trachea	0.271/0.243		
					Lung	0.299/0.356		
					Bladder	0.454/0.462		
					Spleen	0.519/0.455		
					Kidney	1.71/1.33		
					Salt gland	3.26/2.61		
					Brain	0.652/1.07		
	Reproductive tissues				Testis	0.502		
					Oviduct	2.17		
					Ovary	0.456		
	Egg				Whole egg	0.781		
					Shell	4.74		
					Yolk	0.634		
					Albumen	0.157		
					Scale	0.133/0.182		
					Mesentary	0.349/0.253		
					Fat	0.216/0.407		
					Muscle	0.270/0.240		

(*continued*)

TABLE A.10 (CONTINUED)
Copper (Cu)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
					Bone	0.187/0.320		
					Carapace	0.350/0.240		
					Whole body*	1.52/0.590		*Calculated
Chelonia mydas								Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 49.0; 40.0–63.5 cm	M; R	M	6				
	SCL = 52.2; 37.0–71.4 cm		F	20				
			M	6	Liver	120; 75.9–161	DRY: M; R	
				6	Kidney	10.1; 3.38–18.9		
				3	Muscle	0.730; 0.660–0.853		
			F	20	Liver	145; 36.4–340		
				19	Kidney	7.63; 2.41–12.8		
				9	Muscle	0.928; 0.541–2.06		
Chelonia mydas								Anan et al. (2002); Japan; Okinawa Prefecture, Yaeyama Islands; tissues provided by fishermen
	—	—	—	9	Liver	49.9; 18.4–130	WET: M; R	
Chelonia mydas							DRY: M	Kaska et al. (2004); Turkey; southwestern Mediterranean coast; stranded turtles
	—		—	22	Liver	1.71; 0.56–3.0		
				20	Bladder	1.92; 0.27–3.08		
				14	Kidney	1.75; 0.70–2.56		
				15	Lung	1.55; 0.65–2.57		
				22	Muscle	2.10; 0.94–3.54		
Chelonia mydas								Lam et al. (2004); South China

	Juvenile		—	2	Fat	0.989	DRY: M	Turtles stranded on beaches at Ham Tin Wan and Tung Pang Chau; upon collection, turtles had started to decay
					Kidney	15.20		
					Heart	14.43		
					Liver	133.0		
					Lung	8.693		
					Muscle	3.735		
					Stomach	10.42		
	Adult		—	3	Muscle	1.562		Turtles believed to have been unintentionally caught by fishermen; upon collection; turtles were relatively fresh
				1	Liver	9.168		
Chelonia mydas								Celik et al. (2006); Turkey; Mediterranean Sea, Kazanli beach; standed turtles
	Egg			12*	Shell	18.435	DRY: M	*12 nests, 3 eggshells per nest collected after hatching
	Environment							In this study, information is available on metal concentrations in sand, soil, plant, and water samples around the nesting environment
Chelonia mydas								Gardner et al. (2006); Mexico; Baja California peninsula; turtles died from fisheries capture
	MSCL = 62.13; 48.5–76.9	M; R		11	Liver	60.04; 6.79–133	DRY: GM; R	
					Kidney	5.67; 1.59–20.36		
					Muscle*	0.03; ND (<0.0025)–13.76		*Pectoral
					Fat	0.01; ND (<0.0025)–9.48		
Chelydra serpentina								Albers et al. (1986); USA; Maryland and New Jersey
	L = 20–40 cm	R						Site 1: Undisturbed freshwater site: Patuxent Wildlife Research Center, Maryland
			M	7	Liver	1.28	WET: M	

(*continued*)

TABLE A.10 (CONTINUED)
Copper (Cu)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
		F	6		1.57		
		M	7	Kidney	0.82		
		F	6		1.07		
		M	8	Liver	9.72		Site 2: Contaminated brackish water site: Hackensack Meadowlands, New Jersey
		F	3		5.17		
		M	8	Kidney	1.81		
		F	3		1.27		
		M	8	Liver	2.08		Site 3: Contaminated freshwater site: Hackensack Meadowlands, New Jersey
				Kidney	1.73		
Cuora amboinensis							Boman et al. (2001); Vietnam; Dac Lac
	Adult*	—	1	Muscle	6.8	DRY	*Fully grown
				Liver	20		
	Egg			Egg*	5.5		*Gonadal system
Cyclemys dentata							Boman et al. (2001); Vietnam; Dac Lac
	Adult*	—	1	Testicle	7.4	DRY	*Fully grown
	Egg*			Egg	5.5		*Gonadal system
Dermochelys coriacea							
							Davenport and Wrench (1990), Davenport et al. (1990); Great Britain; Wales, Irish Sea, Cardigan Bay; stranded turtle
	TL = 2.53 m TM = 916 kg	M	1	Liver	0.15	DRY: M*	*4 replicate measures
				Muscle*	0.26		*Pectoral

					Blubber	0.06		
Dermochelys coriacea								Vazquez et al. (1997); Mexico; Pacific coast
	Environment				Seawater	0.06	DRY: M	
			—	—	Sand	10.8		
	Egg*		—	—	Shell	8.9		*Posthatch
Dermochelys coriaca								Godley et al. (1998); Great Britain; Wales and Scotland, west coast; turtles died in fishing gear
	Adult							
	CCL = 141–170 cm	R	M	3				
				1	Liver	31.0	DRY	
					Muscle	2.3		
			M	1	Liver	9.7	WET	
					Muscle	0.76		
Dermochelys coriacea								Caurant et al. (1999); France; Atlantic coasts, "Pertuis charentais" in the la Rochelle region; stranded turtles
	Juvenile		—	16			WET: M; R	
	SCL = 145.7; 115–188 cm							
			—	18	Liver	8.61; 1.05–19.7		
				5	Kidney	2.68; 2.36–3.02		
				16	Muscle*	0.95		*Pectoral
Emys orbicularis								Musquera et al. (1976); —
	—		—	2	Blood*	2.10	WET: M	*Plasma
Eretmochelys imbricata								Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 43.5; 33.5–48.9		M	6	Liver	129; 17.3–570	DRY: M; R	
				6	Kidney	8.93; 6.51–17.9		
				1	Muscle	0.673		

(*continued*)

TABLE A.10 (CONTINUED)
Copper (Cu)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
	SCL = 46.5; 43.8–67.9		F	16	Liver	27.1; 8.28–47.7		
				13	Kidney	6.17; 4.89–7.61		
				8	Muscle	0.998; 0.612–1.46		
	Diet		—	6	Stomach*	5.02; 3.22–7.10		*Contents
Eretmochelys imbricata								Anan et al. (2002); Japan; Okinawa Prefecture, Yaeyama Islands; tissues provided by fishermen
	—	—	—	7	Liver	8.62: 4.69–18.9	DRY: M; R	
Eretmochelys imbricata								Gardner et al. (2006); Mexico; Baja California peninsula; turtles died from fisheries capture
	MSCL = 48.4			1	Liver	2.47	DRY: GM; R	
					Kidney	3.89		
					Muscle*	3.68		*Pectoral
					Fat	0.72		
Gopherus agassizii								Jacobson et al. (1991); USA; California [*Xerobates agassizii*]
			F/M	1/11				
	TL = 133–306 mm			10	Liver	8.67	—: M	Kern County; turtles with clinical signs of upper respiratory tract disease
	TM = 390–4900 g							
	TL = 199–280 mm		M	4	Liver	9.16		San Bernardino County; clinically healthy turtles
	TM = 1180–3887 g							
Lepidochelys kempii								Caurant et al. (1999); France; Atlantic coasts, “Pertuis charentais” in the la Rochelle region; stranded turtles
	Adult	M; R	—	6	Muscle*	0.98; 0.44–1.85	WET: M; R	*Pectoral
	SCL = 25.8; 21.3–34.5 cm							

	TM = 2.5; 1.4–5.2 kg			2	Pancreas	23.2		
Lepidochelys kempii								Kenyon et al. (2001); USA; Texas and Louisiana; turtles captured alive in nets at 4 beachfront sites
	SCL = 38.3; 21.6–65.8 cm	M; R	F/M/U	46/38/ 22*			WET: M; R	*99 wild grown plus 7 head-start female turtles
					Blood*	0.524; 0.215–1.300		*Whole blood
Lepidochelys olivacea								Witkowski and Frazier (1982); Ecuador; Manta
	Adult		—	3	Bone*	7.2–11.0; 8.6–9.1	ASH: M; RM**	*Humerus; **3 replicate measures
Lepidochelys olivacea								Sahoo et al. (1996); India; Gahirmatha, Orissa
	Environment		—	8	Beach sand	7–17	DRY: R*	*mg/g
	Egg*		—	24**	Shell	7.6	DRY: M	*Fresh; **8 nests, 3 eggs per nest
					Albumen-yolk	3.6		
	Egg				Shell	18.0		*Posthatch
	Hatchling				Whole body	9.3		*Fresh
Lepidochelys olivacea								Gardner et al. (2006); Mexico; Baja California peninsula; turtles died from fisheries capture
	MSCL = 60.1; 53.0–66.0	M; R		6	Liver	36.73; 16.99–100	DRY: GM; R	
					Kidney	4.86; 0.81–53.40		
					Muscle*	1.28; 0.7–4.37		*Pectoral
					Fat	0.83; 0.47–2.54		
*Macrochelodina rugosa**								Beck (1956); Western Australia; *[*Chelodina oblonga*]
	Adult		*	1	Liver	34	DRY	*Nonpregnant

(*continued*)

TABLE A.10 (CONTINUED)
Copper (Cu)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
*Pelodiscus sinensis**								Boman et al. (2001); Vietnam; Hay Tay; *[*Tryonyx sinensis*]
	Egg		—	1	Egg	3.6	DRY	
Testudo hermanni								Musquera et al. (1976); —
	—		—	9	Blood	1.47	WET: M	*Plasma
Trachemys sripta								Thomas et al. (1994); lab experiment; intraperitoneal administration at a dose of 10 mg Cd/kg/day for 6 days as $CdCl_2$
	Juvenile	R	F	6	Liver	33	WET: %*	*Percentage of total body burden
	TM = 20–25 g							
					Kidney	23		
					Spleen	—		
					Heart	10		
					Lung	2		
					Muscle	—		
					Carapace	—		
					Brain	10		
					Blood	2		
					Ovary	15		
Trachemys scripta							WET: GM	Clark et al. (2000); USA; Texas
	—		—	14	Whole blood	0.418	WET: GM	Site 1: Contaminated; Municipal Lake (chemical manufacturing plant)
						0.502		Site 2: Apparently uncontaminated; Research Park Lake (parkland)
						0.736		Site 3: Contaminated; Old River Slough (cotton and corn cultivation with intensive chemical application)

Trachemys scripta							Nagle et al. (2001); USA; South Carolina, Aiken County, Savannah River site; in ovo exposure experiment; females collected in the field in 1993, 1994; oviposition induced in 1995; eggs from turtles collected in both polluted and unpolluted sites were incubated in coal ash-contaminated and uncontaminated soil in outdoor artificial nests
							Site 1: Coal ash-polluted Savannah River site
	Adult		F	4	Liver	102.23	DRY: M
	Diet		—	12	Asiatic clam*	64.87	*Soft tissue
			—	3	Crayfish*	158.52	*Whole body
	Environment		—	—	Artificial nest*	10.57	*Incubation substrate
							Sites 2–3: Unpolluted control sites near Couchton
	Adult		F	3	Liver	52.73	
	Diet		—	10	Asiatic clam*	55.61	*Soft tissue
			—	3	Crayfish*	46.42	*Whole body
	Environment		—	—	Artificial nest*	2.23	*Incubation substrate
	Hatchling						Trial 1: Incubation substrate: coal ash contaminated
	CL = 30.34 mm	M	—	18*			*18 hatchlings from 5 clutches
	TM = 8.20 g						
			—	6*	Whole body	5.14	*3 clutches per site, 2 hatchlings per clutch
	Hatchling						Trial 2: Incubation substrate: uncontaminated
	TM = 30.36 g	M		18*			*18 hatchlings from 5 clutches
	CL = 8.02 mm						
				6*	Whole body	4.86	*3 clutches per site, 2 hatchlings per clutch
	Hatchling						Trial 3: Maternal residence: coal ash contaminated
	CL = 30.51 mm	M	—	18*			*18 hatchlings from 4 clutches
	TM = 8.02 g						
			—	6*	Whole body	5.58	*3 clutches per site, 2 hatchlings per clutch
	Hatchling						Trial 4: Maternal residence: uncontaminated
	TM = 29.69 g	M		18*			*18 hatchlings from 6 clutches
	CL = 7.77 mm						

(*continued*)

TABLE A.10 (CONTINUED)
Copper (Cu)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
			6*	Whole body	4.42		*3 clutches per site, 2 hatchlings per clutch Statistical analysis: [Cu] concentrations were not significantly higher in adult liver and prey from site 1; [Cu] concentrations in hatchlings were not significantly different between trials 1 and 2 or 3 and 4
Trachemys scripta elegans							Tryfonas et al. (2006); USA; Illinois, Lower Illinois River near Grafton; eggs laid in the lab from turtles collected in the field from 5 nesting areas
	Eggs		—	Contents	0.9	DRY: M**	*All sites combined; **values estimated from graphs
				Shell	1.9	**	**From graph
	Diet			*Lemna* sp.	18.8		
	Environment		4–5*	Soil	20–35	RM	*From 2 sites, soil from nesting and lake bank areas
			3*	Sediment	17–27*	**	*From 2 sites, 3 layers per site; **from graph
			3–4*	Water	0.02–0.1	WET	*From 3 sites
Crocodylia							
Alligator mississippiensis							Lance et al. (1983); farm feeding experiment; 4 months of reproductive cycle (April–July)
	Adult	F		Blood*		WET: RM*	*Plasma; **combined by month
	TL = 190–243 cm	R	25		0.55–0.70		Farm-reared animals, fed fish (*Micropogon undulatus*)
	TL = 189–326 cm		38		0.52–0.66		Farm-reared animals, fed nutria (*Myocastor coypus*)
	TL = 163–325 cm		51		0.55–0.66		USA; Louisiana; Rockefeller Refuge, Grand Chenier; wild animals (nutria constituted 70% of the diet)
	TM = 15.9–146 kg						
Alligator mississippiensis							Delany et al. (1988); USA; Florida; 8 lakes, statewide

	TL = 2.9–3.8 m	R	F/M	1/31	Muscle	1.17; 0.28–6.03	WET: M; RM**	*Tail; **combined by lake
Alligator mississippiensis								Heinz et al. (1991); USA; central and southern Florida; Lake Okeechobee, Lake Griffin, and Lake Apopka
	Egg		—	32*	Egg	0.32–0.78; 0.12–0.86	WET: RM; R**	*16 nests, 2 eggs per nest; **combined by lake
Alligator mississippiensis								Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	—		—	1	Liver	0.34	WET	
					Kidney	1.58		
Alligator sinensis							DRY	Ding et al. (2001) from Xu et al. (2006); China; Anhui Province; Anhui Captive Breeding Center
	Egg			1/1*	Shell	3.67/2.21		*1 infertile egg from captive breeding center/1 from the wild
					Shell membranes	20.03/7.88		
					Albumen	2.24/1.38		
					Yolk	2.54/2.22		
Alligator sinensis								Xu et al. (2006); China; Changxing County, Zhejiang Province; Changxing, Nature Reserve and Breeding Research Center for Chinese alligator; alligators collected dead of unidentified causes
	Adult		F/M	1/1	Heart	15.03/13.87	DRY	
					Lung	8.22/14.02		
					Liver	26.67/35.54		
					Stomach	11.32/15.67		
					Kidney	17.23/14.89		
					Intestine	5.12/7.45		
					Trachea	4.23/5.65		
					Pancreas	2.74/2.91		

(*continued*)

TABLE A.10 (CONTINUED)
Copper (Cu)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
				Gonad	7.81/2.65		
				Muscle	8.10/4.69		
	Diet	—	—	Fish*	6.15	DRY: M; R	*2 fish prey species randomly sampled
	Excrements	—	6	Feces	15.15		
	Egg		10*				*10 eggs collected from 3 clutches
				Shell	43.46; 32.26–50.82		
				Shell membranes	32.71; 24.69–38.80		
				Contents	33.33; 29.56–35.21		
	Environment		10*				*Water and sediment from 10 breeding ponds (5 subsamples from different sites within a pond each)
				Sediment	16.15		
				Water	0.00634	WET: M	
							Statistical analysis: [Cu] concentrations were not significantly different between contents, shells, and membranes; significant positive correlation was found between [Cu] and [Pb] in membranes; significant positive correlations existed in the contents among concentrations of [As] and [Cu], [Pb] and [Cr]
Crocodylus acutus							Ogden et al. (1974); USA; Florida; Florida Bay, Everglades
	Egg	—	5	Contents	3.74; 0.90–15.0	WET: M; R	
Crocodylus acutus							Stoneburner and Kushlan (1984); USA; Florida; Florida Bay, Everglades, National Park
	Egg	—	9	Shell	17.17	DRY: M	
				Albumen-yolk	6.21		
				Albumen-yolk	5.59	WET: M	Albumen-yolk dry mass-based concentrations transformed to wet mass

Crocodylus acutus								Rainwater et al. (2007); Costa Rica; Rio Grande de Tarcoles; polluted by serveral metals from various sources
	SVL = 155.7; 134.0–172.0 cm	M; R	F/M	1/5	Scute*	0.125	WET: M	*Caudal
			M	1		0.420	WET: MAX	
Crocodylus johnstoni								Beck (1956); Western Australia
	Adult		*	4	Liver	17.7; 11–23	DRY: M; R	*Nonpregnant
Crocodylus johnstoni								Jeffree et al. (2001); northern Australia; north central Queensland, Lynd River; samples from a single population
	A = 0.7–62.7 years L = 24.7–128.3 cm	R	F/M	9/21	Osteoderm*	3.36; 2.83–3.85	DRY: M; R	*Ventral pelvic region
Crocodylus moreletii								Rainwater et al. (2007); Belize; 2 sampling sites
					Scute*		WET: M	*1 whole caudal scute per crocodile
	SVL = 89.8; 65.0–129.5 cm	M; R	F/M	5/4		0.346		Site 1: Gold Buton Lagoon
	SVL = 104.4; 59.5–156.7 cm		F/M	4/6		0.4518		Site 2: New River Watershed
			F	1		0.650	WET: MAX	
Crocodylus niloticus								Swanepoel et al. (2000); South Africa; Kruger National Park (KNP) area
	TL = 1.40–4.15 m	R	F/M	6/9			DRY: M	
								Site 1: Shingwedzi River (Silwervis Dam), northern part of KNP; catchment area outside KNP is limited

(*continued*)

TABLE A.10 (CONTINUED)
Copper (Cu)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
					Muscle	7.9		Frozen tissues used for residue analyses
					Liver	23.2		
					Kidney	13.9		
					Fat	7.6		
								Site 2: Olifants River, central part of KNP; flows through areas of intense agricultural activity and passes mining areas and receives tributaries from Phalaborwa Mining Company before entering the KNP
					Muscle	10.5		Frozen tissues used for residue analyses
					Liver	27.1		
					Kidney	5.3		
					Fat	6.1		
					Liver	15.90		Formalinized tissues used for residue analyses
					Kidney	3.60		
								Site 3: Sabi River, southern part of KNP; flows through areas of intense agricultural activity before entering the KNP
					Muscle	12.6		Frozen tissues used for residue analyses
					Liver	30.6		
					Kidney	3.5		
					Fat	6.5		
					Liver	12.70		Formalinized tissues used for residue analyses
					Kidney	4.050		
Crocodilus niloticus								Almli et al. (2005); Zambia
	L = 2.7–3.4 m	R	F	2	Liver	5.70; 2.00–10.00	WET: MD; R	Site 1: Kafue National Park, Kafue River
			M	2	Kidney	2.20; 1.80–3.10		
	L = 2.0–4.0 m	R	F	4	Liver	4.00; 3.20–9.30		Site 2: Luangwa River, Luangwa National Park

			M	1	Kidney	2.00; 1.60–2.20		
Crocodylus porosus								Yoshinaga et al. (1992); Papua New Guinea
	—		—	—	Muscle	0.17	WET: M	
Crocodyylus porosus								Jeffree et al. (2001); Northern Australia; Kakadu National Park, Alligator Rivers region; samples from 3 river catchments; mining and hunting areas included
	A = 5–40 years L = 168–499 cm	R	—	40				
			—	35	Muscle*	1.14; 0.340–2.02	DRY: M; R	*Tail
			—	40	Osteoderm*	4.68, 2.20–13.1	DRY: GM; R	*Ventral pelvic region
Squamata: Sauria								
"Blue-tongued lizard"								Beck (1956); Western Australia
	Adult		*	1	Liver**	19	DRY***	*Nonpregnant; **liver fatty; ***concentration on fat-free basis
"Lizard"								Hsu et al. (2006); southern Taiwan; Kenting National Park; strong influence from industrial pollution
	—		—	1	Whole body	3.8	DRY	
						0.5		Bioconcentration factor (reference media: soil and food items)
Chamaeleo chamaeleon								Diaz-Paniagua et al. (2002); southern Spain; Province of Cadiz; 2 sampling sites; nests located in agricultural lands and coastal urban areas; eggs collected immediately after oviposition
	Egg		—	9*	Contents	0.26; 0.15–0.39	WET: M; R	*9 nests, 4 eggs pooled per nest

(*continued*)

TABLE A.10 (CONTINUED)
Copper (Cu)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Chamaeleo chamaeleon							Gomara et al. (2007); southwestern Spain; Province of Cadiz; 3 sampling sites; eggs collected 0 to 15 days after oviposition
	Egg	—	9*	Contents	0.567–0.706	WET: RM	*9 nests; 2 to 4 eggs pooled per nest
				Shell	1.487–4.361		
Egernia napoleonis							Beck (1956); Western Australia
	Adult	*	1	Liver	10	DRY	*Nonpregnant
Heloderma horridum							Zarafonetis and Kalas (1960); kept in captivity
	Adult TL = 34.6 cm	F	1	Blood*	1.96	WET	*Plasma
*Iberolacerta monticola**					See As for details		Marco et al. (2004); Spain; Avila, Gredos Mountains; gravid females collected in the field in 2001; freshly laid eggs incubated until hatching in As (arsenic acid in nitric acid)-loaded artificial breeding substrate (sterile As-free vermiculite) for 18 days; further elements (Cd, Cu, Pb, Zn), though not manipulated, were detected in eggshells and embryos; *[*Lacerta monticola cyreni*]
*Laudakia s. stellio**							Loumbourdis (1997); Greece; Thessaloniki region; *[*Agama s. stellio*]
	Adult	—	—	Liver	139.67	DRY: M	Site 1: Urban area (500 m asl)
				Carcass	27.44		
				Liver	209.09		Site 2: Agricultural area (50 m asl)
				Carcass	27.14		
*Lerista microtis**							Beck (1956); Western Australia; *[*Lygosoma microtis*]
	Adult	*	1	Liver	10	DRY	*Nonpregnant

*Podarcis taurica**							Sharygin et al. (1979/80); Crimean mountains; between Yalta and Alushta (400–900 m asl); *[*Lacerta taurica*]
	—	—	—	Whole body	200	ASH: M	
*Pseudemoia trilineatum**							Beck (1956); Western Australia; *[*Lygosoma trilineatum*]
	Adult	*	1	Liver	10	DRY	*Nonpregnant
Tarentola mauritanica							Fletcher et al. (2006); southern Spain; Guadiamar River Valley, Mine tailings release: Boliden-Apirsa mine at Aznalcóllar; 7 study sites spanning an expected contamination gradient; geckos collected from building walls
	Juvenile and adult		52	Whole body*		DRY: M; R	*Minus gut contents
		—	9		2.757; 1.943–4.300		Site 1: Rural not mine-affected site: near Guadalmellato (most pristine)
		—	13		3.731; 2.016–7.226		Site 2: Urban not mine-affected site: Villaviciosa de Córdoba (not contaminated by mining)
		—	8		2.732; 2.106–3.304		Site 3: Urban mine-affected site: Aznalcázar (contamination through aerosolized contaminants engulfed the town during cleanup)
		—	8		4.072; 1.948–6.599		Site 4: Urban mine-affected site: Aznalcóllar (contaminated by normal mine operations, or the disaster and subsequent remediation efforts)
		—	5		4.522; 3.443–6.682		Site 5: Floodplain mine-affected site: Guadiamar River floodplain near the Aznalcázar gauge station (24.8 km below the ruptured tailings dam)
		—	5		4.372; 2.417–6.873		Site 6: Floodplain mine-affected: Guadiamar River floodplain near the Guijo gauge station (7.4 km below the ruptured dam)

(*continued*)

TABLE A.10 (CONTINUED)
Copper (Cu)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
		—	4		3.879; 2.510–5.321		Site 7: Floodplain mine-affected site: Agrio River floodplain (4.4 km below and closest to the ruptured tailings dam) Statistical analysis: [Co] concentration was not significantly influenced by site
*Tiliqua rugosa**							Beck (1956); Western Australia; *[*Trachysaurus rugosus*]
	Adult	*	9	Liver**	14; 10–20	DRY: M; R***	*Nonpregnant; **liver fatty in 7 animals; ***concentrations on fat-free basis
			5	Blood*	0.78; 0.75–0.82	WET: M; R	*Total
Varanus salvator							Boman et al. (2001); Vietnam; Dac Lac
	—	—	1	Muscle	4.7	DRY	
				Liver	10		
				Egg	5.6		
Varanus sp.							Yoshinaga et al. (1992); Papua New Guinea
	—	—	—	Muscle	0.17	WET: M	
*Zootoca vivipara**							Avery et al. (1983); Great Britain, England; *[*Lacerta vivipara*]
	Juvenile and adult	—	—				Site 1: Little used road
			11*	Bone**	ND (<0.7)	DRY: M	**8 juveniles, 3 adults; **femur
				Liver	7.1		
				Lung	ND (<0.8)		
				Kidney	ND (<1.25)		
				Remainder	2.9		
							Site 2: Busy road
			19*	Bone**	ND (<0.7)		**12 juveniles, 7 adults; **femur
				Liver	18.7		
				Lung	ND (<0.8)		

				Kidney	ND (<1.25)		
				Remainder	3.5		
							Site 3: Lead mine
			14*	Bone**	ND (<0.7)		**9 juveniles, 5 adults; **femur
				Liver	13.6		
				Lung	ND (<0.8)		
				Kidney	ND (<1.25)		
				Remainder	3.8		
*Zootoca vivipara**							Gutleb et al. (1992); Austria, Carinthia; remote area; *[*Lacerta vivipara*]
	—	—	2	Liver	9.7–41.6	DRY: R	
				Kidney	28.9–41.6		
Squamata: Serpentes							
"Brown snake" ("Linga")							Beck (1956); Western Australia
	Adult	*	1	Liver	45	DRY	*Nonpregnant
"Snake"							Hsu et al. (2006); southern Taiwan; Kenting National Park; strong influence from industrial pollution
	—	—	12	Whole body	2.70; 1.60–4.80	DRY: M; R	
					0.35		Bioconcentration factor (reference media: soil and food items)
Acrochordus javanicus							Yoshinaga et al. (1992); Papua New Guinea
	—	—	—	Muscle	0.13	WET: M	
Agkistrodon piscivorus							Clark et al. (2000); USA; Texas; 1 contaminated site: Old River Slough (cotton and corn cultivation with intensive chemical application)

(*continued*)

TABLE A.10 (CONTINUED)
Copper (Cu)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
	—		—	—	Whole blood	0.899	WET: GM	
Agkistrodon piscivorus								Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 58.6 cm TM = 365 g	M	—	6	Liver	5.35	WET	
					Kidney	2.36		
Agkistrodon piscivorous								Burger et al. (2006); USA; South Carolina; 1 reference site, 1 polluted Savannah River site, Aiken
	SVL = 57 cm TM = 280 g	M	—	13	Tail muscle	3	DRY: M*	*Both sites combined
	SVL = 60 cm TM = 270 g			5	Blood	0.6		
Coluber constrictor								Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 103.2 cm TN = 300 g		—	1	Liver	4.43	WET	
					Kidney	1.72		
Eunectes murinus								Calle et al. (1994); Venezuela
	TL = 2.1–5.1 m TM = 3.5–74.0 kg	R	F/M	7/5	Blood*	1.1; 0.4–1.7	WET: M; R	*Plasma

Lapemis hardwickii								Boman et al. (2001); Vietnam; Nha Trang
	—		—	1	Muscle	12	DRY	
					Liver	17		
Nerodia cyclopion								Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 63.9 cm		—	1	Liver	2.99	WET	
	TM = 191 g							
					Kidney	2.46		
Nerodia fasciata								Hopkins et al. (1999); USA; South Carolina; Savannah River site, Aiken
							DRY: M*	*Values estimated from graph
	—			5	Liver	82*		Site 1: Coal ash-contaminated site
				5		36*		Site 2: Nearby reference site
								Statistical analysis: Differences in [Cu] concentrations between sites were not significant
	Diet							This study provides information on contamination levels of several prey species.
Nerodia fasciata								Hopkins et al. (2001); USA; South Carolina; Aquatic Ecology Laboratory near Aiken; lab feeding experiment; experimental animals were captured at uncontaminated reference site and fed 52 total meals of field-collected prey (primarily fish) for 13.5 months
	TM*						DRY	*Initial/final mass
	Juvenile and adult	M	—	8	Gonad	7.55; ND (<0.017)–8.58	MD; R	Treatment 1: Snakes fed prey items collected from coal ash-contaminated site: Savannah River site
	TM = 61.91/169.6 g*							
					Kidney	7.78; 5.72–11.85		
					Liver	35.07; 19.00–54.44		
					Shed skin	82.48; 7.88–172.90		

(*continued*)

TABLE A.10 (CONTINUED)
Copper (Cu)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
					Blood	0.54; 0.42–0.68		
					Tail clip	2.27; 1.06–6.79		
	Diet				Fish	2.398; 1.240–6.169	GLSM; R	
	Juvenile and adult TM = 62.2/136.3 g*	M	—	7	Gonad	6.28; 4.31–7.63	MD; R	Treatment 2: Snakes fed prey items collected from uncontaminated reference site
					Kidney	7.45; 5.03–26.07		* = initial/final mass
					Liver	17.73; 14.68–65.90		
					Shed skin	103.78; 9.44–246.46		
					Blood	0.65; 0.53–5.22		
					Tail clip	1.95; 1.42–3.08		
	Diet				Fish	1.738; 1.208–2.454	GLSM; R	
								Statistical analysis: Treatment differences for [Cu] were significant for fish only
Nerodia fasciata								Hopkins et al. (2002); USA; South Carolina; Aquatic Ecology Laboratory near Aiken; 2-year feeding experiment; all snakes were lab reared and originated from a single gravid female that was collected from a reference site on the Savannah River site; exposure started after first hibernation
	Diet			*	Fish*			*3 prey species, 4 specimens per species
	Juvenile**						DRY	**Morphometry in Figure 2 of Hopkins et al. (2002)
	Diet		—	*	Fish	1.738	GLSM	Treatment 1: Control: snakes fed only prey items collected from uncontaminated reference site
	Juvenile**		F	6	Gonad	5.613	M	
					Kidney	6.009		
					Liver	46.237		
			M	4	Gonad	5.699	M	
					Kidney	7.124		

					Liver	26.356		
	Diet		—	*	Fish	2.053	GLSM	Treatment 2: Lower-level exposure: snakes fed prey items collected from coal ash-contaminated site and from the reference site on alternating weeks
	Juvenile**		F	6	Gonad	5.400	M	
					Kidney	7.768		
					Liver	39.164		
			M	4	Gonad	4.695	M	
					Kidney	7.269		
					Liver	29.567		
	Diet		—	*	Fish	2.398	GLSM	Treatment 3: Higher-level exposure: snakes fed only prey items collected from coal ash-contaminated site
	Juvenile**		F	6	Gonad	5.570	M	
					Kidney	6.475		
					Liver	60.475		
			M	3	Gonad	5.299	M	
					Kidney	6.777		
					Liver	27.822		
								Statistical analysis: Effects of organ on [Cu] concentration were significant
Nerodia fasciata								Burger et al. (2006); USA; South Carolina; 1 reference site, 1 polluted Savannah River site, Aiken
	SVL = 51 cm	M	—	47	Muscle*	2	DRY: M**	*Tail; **both sites combined
	TM = 130 g							
	SVL = 52 cm			34	Blood	0.6		
	TM = 140 g							
Nerodia rhombifer								Clark et al. (2000); USA; Texas
	—		—	10	Whole blood	0.648	WET: GM	Site 1: Contaminated site: Old River Slough (cotton and corn cultivation with intensive chemical application)

(*continued*)

TABLE A.10 (CONTINUED)
Copper (Cu)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
						0.586		Site 2: Reference site: private lake (pasture)
Nerodia taxispilota								Burger et al. (2006); USA; South Carolina; 1 reference site, 1 polluted Savannah River site, Aiken
	SVL = 59 cm TM = 170 g	M	—	10	Muscle*	3	DRY: M**	*Tail; **both sites combined
	SVL = 65 cm TM = 230 g			9	Blood	0.3		
Pseudonaja nuchalis								Beck (1956); Western Australia; **[*Demansia nuchalis*]
	Adult*		**	1	Liver	40	DRY	**Nonpregnant animal, just emerged from hibernation
Python molurus								Boman et al. (2001); Vietnam; Dac Lac
	—		—	1	Muscle	4.4	DRY	
					Testicle	11		
Vipera berus								Gutleb et al. (1992); Austria; Carinthia; remote area
	—		—	1	Liver	76.9	DRY	
					Kidney	13.9		
*Waglerophis merremii**								De Jorge et al. (1971); Brazil; *[*Xenodon merremii*]
	Egg* TM = 2.569 g	M	—	10	Whole egg	2.969	WET: M	*Oviductal
				5	Contents	5.626		
	Adult					0.96–3.00	WET: RM	Organs listed in the order of increasing concentration

TL = 86–100 cm TM = 272–302 g	F	6	Ovary, abdominal fat, dorsal scales, head plates, ventral scales, cloaca, spinal cord, stomach, tongue, heart, supralabial gland, infralabial gland, gall bladder, lung, Harderian gland, and subcaudal scales			
			Kidney, small intestine, oviduct, dorsal tail scales, pancreas, trunk muscles, glottis, adanal gland, head muscles, esophagus, and trachea	3.00–4.00		
			Rectum, large intestine, bile, spleen, eyes, parotid gland, and thyroid gland	4.00–8.00		
			Bone, brain, anal plates, and liver	8.00–26.72		
			Liver	26.718	WET: M	
			Kidney	3.016		
			Blood*	1.055		*Serum
			Blood*	1.214		*Total

TABLE A.11
Iron (Fe)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Testudines								
11 chelonian species								Dessauer et al. (1962) from Musquera et al. (1976)
	—		—	—	Blood*	0.36	WET: M	*Plasma
*Actinemys marmorata**								Henny et al. (2003); USA; western Oregon, Fern Ridge Reservoir; *[*Clemmys marmorata*]
	Egg		—	14*	Contents	89.3; 57.0–325	DRY: GM; R	*14 nests, 1 egg per nest
								Statistical analysis: No significant differences in contaminant concentrations related to hatching rate
Caretta caretta								Musquera et al. (1976); —
	—		—	1	Blood*	0.75	WET	*Plasma
Caretta caretta								Stoneburner et al. (1980); USA; Florida; 4 western Atlantic nesting beaches: Canaveral; Georgia, Cumberland Island; North Carolina, Cape Lookout; North Carolina, Cape Hatteras
	Egg*		—	96	Yolk	71.27–74.67	—: RM**	*Fresh; **combined by beach
*Caretta caretta**								Aguirre et al. (1994); USA; Hawaiian Islands; *[identification number 052, *Chelonia mydas* in the reference]
	"Pelagic"		—	1	Liver	92.8	WET	
Caretta caretta								Sakai et al. (1995); Japan; Kochi Prefecture, Cape Ashizuri
	L = 76–92 cm W = 75–108 kg	R	F/M	6/1	Liver	649; 226–1260	WET: M; R	
					Kidney	35.9; 11.4–110		
					Muscle	20.1; 11.3–35.2		*Pectoral

	Egg*		—	*	Shell	10.6; 7.23–13.3	*Oviductal eggs from 5 females
					Yolk	25.1; 22.9–28.3	
					Albumen	0.870; 0.499–1.30	
	Egg				Whole egg	11.5; 10.5–13.7*	*Calculated
Caretta caretta							Sakai et al. (2000b); Japan; Kochi Prefecture, Cape Ashizuri; turtles accidentally caught in commercial fishing nets
	TM = 93/83 kg	M	F/M	6/1	Liver	604/917	WET: M
	SCL = 83/85 cm				Gullet	8.48/4.31	
					Stomach	15.2/3.96	
					Intestine	9.72/3.16	
					Pancreas	34.1/14.6	
					Heart	153/47.4	
					Trachea	16.5/10.1	
					Lung	134/50.6	
					Bladder	16.8/7.15	
					Spleen	65.1/44.8	
					Kidney	30.0/11.4	
					Salt gland	47.0/31.8	
					Brain	21.2/12.1	
	Reproductive system				Testis	24.1	
					Oviduct	17.4	
					Ovary	33.0	
	Egg				Whole egg	11.5	
					Shell	10.6	
					Yolk	25.1	
					Albumen	0.870	
					Scale	16.7/8.31	
					Mesentary	45.0/15.2	
					Fat	9.92/18.9	
					Muscle	19.8/22.2	

(*continued*)

TABLE A.11 (CONTINUED)
Iron (Fe)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
					Bone	6.70/8.49		
					Carapace	26.2/9.77		
					Whole body*	62.2/58.4		*Calculated
Caretta caretta								Kaska and Furness (2001); southwesternTurkey; 4 beaches; collected just before hatching or dead-in shell 1 week after last hatching
	Embryo		—	22	Liver	35.83	DRY: M	
	Egg		—		Shell	17.75		
					Yolk	15.79		
								Statistical analysis: No significant differences were observed
Caretta caretta								Franzellitti et al. (2004); Italy; northwestern Adriatic Sea; Adriatic Sea coast, from the Po delta to the Reno mouth
	Juvenile to adult		—	*				16 from fishery by-catch, 19 dead on coast
	MSCL = 24.5–74 cm	R		30	Liver	377.4	WET: M	
				13	Lung	184.1		
				17	Muscle*	60.9		*Pectoral
				7	Fat*	132.3		*Abdominal
Caretta caretta								Kaska et al. (2004); Turkey; southwestern Mediterranean coast; stranded turtles
	—		—	32	Liver	15.74; 7.07–23.01	DRY: M; R	
				20	Bladder	12.48; 1.29–17.00		
				20	Kidney	15.31; 9.47–20.68		
				10	Lung	15.61; 7.70–20.42		
				32	Muscle	20.62; 15.42–23.89		

Caretta caretta								Torrent et al. (2004); Spain; Canary Islands; stranded turtles submitted to the veterinary faculty of the University of Las Palmas in Gran Canaria
	Juvenile and subadult SCL = 15–65 cm	R*	F/M	67/11	Kidney	37.96; 0.29–273.14	WET: M; R	*Values estimated from graph
					Muscle	8.65; 0.16–98.37		
					Bone	9.47; 0.06–189.46		
					Liver	342.73; 0.35–2180.36		
Caretta caretta								Storelli et al. (2005); Italy; Adriatic and Ionian Seas; stranded turtles
	SCL = 21–71 cm	R		19	Liver	456; 210–789	WET: M; R	
					Kidney	48.2; 17.4–122		
					Muscle	31.9; 21.6–51.8		
					Spleen	221; 121–283		
					Heart	154.4; 77.7–222		
					Lung	107; 32.8–196		
					Fat	11.4; 7.5–22.3		
Caretta caretta								Gardner et al. (2006); Mexico; Baja California peninsula; turtles died from fisheries capture
	MSCL = 57.0; 52.0–63.0	M; R		5	Liver	301; 71.88–2042	DRY: GM; R	
					Kidney	237; 94.59–660		
					Muscle*	77.44; 52.48–97.12		*Pectoral
					Fat	1.33; ND (<0.005)–13.61		
Chelonia sp.								Yoshinaga et al. (1992); Papua New Guinea
	—		—	—	Muscle	34.7	WET: M	
Chelonia mydas								Yoshinaga et al. (1992); Papua New Guinea
	—		—	—	Muscle	48.9	WET: M	

(*continued*)

TABLE A.11 (CONTINUED)
Iron (Fe)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Chelonia mydas								Aguirre et al. (1994); USA; Hawaiian Islands
	L = 28.7–71.3 cm W = 3.2–43.6 kg	R	F/M	8/4			WET: M; R	
				12	Liver	1261.4; 101.0–2450.0		
					Kidney	43.3; 8.8–179.0		
	Egg*		—	3	Shell	14.1		*Posthatch
	Hatchling				Whole body	128.0		
Chelonia mydas								Sakai et al. (2000a); Japan; Yaeyama Islands, Okinawa; turtles caught by fisherman for commercial use
	SCL = 51.0 cm	M	—	50			WET: M;R	
				50/50	Liver	461; 32.7–1270		*Detected/analyzed
				23/23	Kidney	22.8; 11.4–59.2		
				47/47	Muscle	5.28; 0.97–29.6		
Chelonia mydas								Sakai et al. (2000b); Japan; Ogasawara Islands (Bonin Islands), Haha-Jima Island; turtles collected in coastal waters
	TM = 124/117 kg SCL = 93/97 cm	M	F/M	1/1	Liver	126/145	WET: M	CW = 72/73 cm
					Gullet	4.00/3.89		
					Stomach	2.93/3.94		
					Intestine	8.57/2.87		
					Pancreas	13.2/12.8		
					Heart	43.2/34.5		
					Trachea	14.3/9.59		
					Lung	38.2/57.6		
					Bladder	6.52/6.12		

				Spleen	44.1/48.2		
				Kidney	12.9/14.9		
				Salt gland	33.2/19.9		
				Brain	10.9/13.2		
	Reproductive tissues			Testis	27.3		
				Oviduct	6.69		
				Ovary	28.0		
	Egg			Whole egg	10.9		
				Shell	1.98		
				Yolk	24.4		
				Albumen	1.07		
				Scale	7.23/8.64		
				Mesentary	19.8/11.9		
				Fat	23.0/14.8		
				Muscle	9.07/13.7		
				Bone	7.78/4.20		
				Carapace	13.0/6.48		
				Whole body*	26.4/23.1		*Calculated
Chelonia mydas							Kaska et al. (2004); Turkey; southwestern Mediterranean coast; stranded turtles
	—	—	22	Liver	12.48; 3.74–17.30	DRY: M; R	
			20	Bladder	13.04; 6.65–19.87		
			14	Kidney	12.61; 8.36–18.87		
			15	Lung	13.59; 0.67–17.96		
			22	Muscle	17.48; 0.75–23.52		
Chelonia mydas							Celik et al. (2006); Turkey; Mediterranean Sea, Kazanli beach; stranded individuals
	Egg		12*	Shell	49.155	DRY: M	*12 nests, 3 egg shells per nest collected after hatching
	Environment						In this study, information is available on metal concentrations in sand, soil, plant, and water samples around the nesting environment

(*continued*)

TABLE A.11 (CONTINUED)
Iron (Fe)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Chelonia mydas				11				Gardner et al. (2006); Mexico; Baja California peninsula; turtles died from fisheries capture
	MSCL = 62.13; 48.5–76.9	M; R			Liver	14.35; ND (<0.005)–1765	DRY: GM; R	
					Kidney	44.09; ND (<0.005)–516		
					Muscle*	20.99; ND (<0.005)–225		*Pectoral
					Fat	2.63; ND (<0.005)–154		
Cuora amboinensis								Boman et al. (2001); Vietnam; Dac Lac; seemingly unpolluted site
	Adult*		—	1	Muscle	200	DRY	*Fully grown
					Liver	3300		
	Egg*				Egg	66		*Gonadal system
Cyclemys dentata							DRY	Boman et al. (2001); Vietnam; Dac Lac; seemingly unpolluted site
	Adult*		—	1	Testicle	83		*Fully grown
	Egg*				Egg	66		*Gonadal system
Dermochelys coriaca								Godley et al. (1998); Great Britain; Wales and Scotland, West Coast; turtles died in fishing gear
	Adult CCL = 141–170 cm	R	M	3				
				1	Liver	5770	WET	
					Muscle	38		
Emys orbicularis								Musquera et al. (1976); —
	—		—	10	Blood*	1.39 ± 0.46	WET: M	*Plasma

Eretmochelys imbricata								
								Gardner et al. (2006); Mexico; Baja California peninsula; turtles died from fisheries capture
	MSCL = 48.4		—	1	Liver	71.88	DRY: GM; R	
					Kidney	362		
					Muscle*	258		*Pectoral
					Fat	11.14		
Gopherus agassizii								Jacobson et al. (1991); USA; California; *[*Xerobates agassizii*]
	L = 133–306 mm	R	F/M	1/11			—: M	Kern County; turtles with clinical signs of upper respiratory tract disease
	W = 390–4900 g							
			*	10	Liver	1526		
	L = 199–280 mm W = 1180–3887		M	4	Liver	361		San Bernardino County; clinically healthy turtles
Lepidochelys olivacea								Witkowski and Frazier (1982); Ecuador; Manta
	Adult		—	3	Bone*	57.5–380.0; 78.5–309.0	ASH: M; RM**	*Humerus; **3 replicate measures
Lepidochelys olivacea								Sahoo et al. (1996); India; Orissa, Gahirmatha
	Environment		—	8	Beach sand	785–1656	DRY: R	
	Egg*		—	24**	Shell	47.3	M	*Fresh; **8 nests, 3 eggs per nest
					Albumen-yolk	19.3		
	Egg*				Shell	41.6		*Posthatch
	Hatchling*				Whole body	65.6		*Fresh
Lepidochelys olivacea								Gardner et al. (2006); Mexico; Baja California peninsula; turtles died from fisheries capture
	MSCL = 60.1; 53.0–66.0 cm	M; R		6	Liver	731; 119–9201	DRY: GM; R	

(*continued*)

TABLE A.11 (CONTINUED)
Iron (Fe)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
				Kidney	193; 40.37–667		
				Muscle*	93.09; 57.35–319		*Pectoral
				Fat	27.91; 6.37–236		
*Pelodiscus sinensis**							Boman et al. (2001); Vietnam; Hay Tay; seemingly unpolluted site; *[*Tryonyx sinensis*]
	Egg*	—	1	Egg	91	DRY	*Gonadal system
Testudo hermanni							Musquera et al. (1976); —
	—	—	8	Blood*	1.05	WET: M	*Plasma
Crocodylia							
Alligator mississippiensis							Lance et al. (1983); studied for 4 months of reproductive cycle (April-July)
	Adult	F		Blood*		WET: RM**	*Plasma; **combined by month
	L = 190–243 cm		24		0.32–2.17		Farm reared, fed fish (*Micropogon undulatus*)
	L = 189–326 cm		39		0.58–2.30		Farm reared, fed nutria (*Myocastor coypus*)
	L = 163–325 cm W = 15.9–146 kg		37		0.43–2.35		USA; Louisiana; Rockefeller Refuge, Grand Chenier; wild animals (nutria constituted 70% of the diet)
Alligator mississippiensis							Delany et al. (1988); USA; Florida; 8 lakes, statewide
	L = 2.9–3.8 m	F/M	1/31				
		*	24	Muscle*	11.91; 4.56–22.76	WET: M; RM**	*Tail; **combined by lake
Alligator mississippiensis							Heinz et al. (1991); USA; central and southern Florida; Lake Okeechobee, Lake Griffin, and Lake Apopka

	Egg	—	32*	Egg	11–13; 9.0–22	WET: RM; R**	*16 nests, 2 eggs per nest; **combined by lake
Alligator mississippiensis							Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	—	—	1	Liver	142.00	WET	
				Kidney	48.50		
Alligator sinensis							Xu et al. (2006); China; Changxing County, Zhejiang Province; Changxing Nature Reserve and Breeding Research Center for Chinese alligator; alligators collected dead of unidentified causes
	Adult	F/M	1/1	Heart	78.5/56.0	DRY	
				Lung	81.2/47.8		
				Liver	407.5/352.4		
				Stomach	48.6/57.5		
				Kidney	72.8/109.2		
				Intestine	37.5/52.5		
				Trachea	55.0/35.4		
				Pancreas	8.7/12.8		
				Gonad	12.1/13.1		
				Muscle	78.0/55.9		
	Diet	—	—	Fish*	19.45	DRY: M; R	*2 fish prey species randomly sampled
	Excrements	—	6	Feces	940.9		
	Egg		10*				*10 eggs from 3 clutches
				Shell	45.88; 39.1–52.3		
				Shell membranes	117.95; 93.8–133.9		
				Egg contents	63.58; 53.7–70.4		
	Environment		10*				*Water and sediment collected from 10 breeding ponds (5 subsamples from different sites within a pond each)

(*continued*)

TABLE A.11 (CONTINUED)
Iron (Fe)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
					Sediment	10700		
					Water	0.126	WET: M	
								Statistical analysis: [Fe] concentrations were significantly higher in contents than in shells; those in membranes higher than in contents; significant negative correlation was found between [Fe] and [Pb] in the egg shells
Crocodylus johnstoni								Jeffree et al. (2001); northern Australia; north central Queensland, Lynd River; samples from a single population
	A = 0.7–62.7 years L = 24.7–128.3 cm	R	F/M	9/21			DRY: M; R	
			—	30	Osteoderm*	320; 175–432		*Ventral pelvic region
Crocodylus niloticus								Swanepoel et al. (2000); South Africa; Kruger National Park (KNP) area
	TL = 1.40–4.15 m	R	F/M	6/9			DRY: M	
								Site 1: Shingwedzi River (Silwervis Dam), northern part of KNP; catchment area outside KNP is limited
					Muscle	156.0		Frozen tissues used for residue analyses
					Liver	690.8		
					Kidney	438.3		
					Fat	188.1		
								Site 2: Olifants River, central part of KNP; flows through areas of intense agricultural activity and passes mining areas and receives tributaries from Phalaborwa Mining Company before entering the KNP
					Muscle	399.6		Frozen tissues used for residue analysis
					Liver	12851.3		

					Kidney	520.0		
					Fat	297.7		
					Liver	5264.20		Formalinized tissues used for residue analysis
					Kidney	80.00		
								Site 3: Sabi River, southern part of KNP; flows through areas of intense agricultural activity before entering the KNP
					Muscle	615.0		Frozen tissues used for residue analysis
					Liver	9427.5		
					Kidney	131.3		
					Fat	292.0		
					Liver	2747.20		Formalinized tissues used for residue analysis
					Kidney	82.90		
Crocodilus porosus								Yoshinaga et al. (1992); Papua New Guinea
	—		—	—	Muscle	8.8	WET: M	
Crocodylus porosus								Jeffree et al. (2001); northern Australia; Kakadu National Park, Alligator Rivers region; 3 river catchments, mining and hunting areas included
	A = 5–40 years L = 168–499 cm	R	—	40			DRY: GM; R	
			—	35	Muscle*	88.7; 13–303		*Tail
			—	40	Osteoderm*	6.52; 1.33–21.2		*Ventral pelvic region
Squamata: Sauria								
"Lizard"								Hsu et al. (2006); South Taiwan; Kenting National Park; results indicated strong influence from industrial pollution
	—		—	1	Whole body	430	DRY	
						0.03		Bioconcentration factor (reference media: soil and food items)
Tarentola mauritanica								

(*continued*)

TABLE A.11 (CONTINUED)
Iron (Fe)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
							Fletcher et al. (2006); southern Spain; Guadiamar River Valley; mine tailings release: Boliden-Apirsa mine at Aznalcollar; 7 study sites spanning an expected contamination gradient; geckos collected from building walls
	Adults and juveniles		52	Whole body*		DRY: M; R	*Minus gut contents
		—	9		442.934; 336.941–588.338		Site 1: Rural not mine-affected site: near Guadalmellato (most pristine)
		—	13		550.867; 360.589–233.659		Site 2: Urban not mine-affected site: Villaviciosa de Cordoba (not contaminated by mining)
		—	8		562.816; 378.012–808.278		Site 3: Urban mine-affected site: Aznalcázar (contamination through aerosolized contaminants engulfed the town during cleanup)
		—	8		418.296; 283.870–527.399		Site 4: Urban mine-affected site: Aznalcollar (contaminated by normal mine operations, or the disaster and subsequent remediation efforts)
		—	5		563.918; 365.317–747.608		Site 5: Floodplain mine-affected site: Guadiamar River floodplain near the Aznalcázar gauge station (24.8 km below the ruptured tailings dam)
		—	5		437.151; 353.842–553.346		Site 6: Floodplain mine-affected site: Guadiamar River floodplain near the Guijo gauge station (7.4 km below the ruptured dam)
		—	4		474.287; 390.687–540.161		Site 7: Floodplain mine-affected site: Agrio River floodplain (4.4 km below and closest to the ruptured tailings dam)
							Statistical analysis: [Fe] concentration was significantly influenced by site
Varanus salvator							Boman et al. (2001); Vietnam; Dac Lac; seemingly unpolluted site

	Adult*		—	1	Muscle	380	DRY	*Fully grown
					Liver	1900		
	Egg*				Egg	74		*Gonadal system
Varanus sp.								Yoshinaga et al. (1992); Papua New Guinea
	—		—	—	Muscle	22.7	WET: M	
Squamata: Serpentes								
"Snake"								Hsu et al. (2006); South Taiwan; Kenting National Park; strong influence from industrial pollution
	—		—	12	Whole body	785; 24.7–2516	DRY: M; R	
						0.06		Bioconcentration factor (reference media: soil and food items)
Acrochordus javanicus								Yoshinaga et al. (1992); Papua New Guinea
	—		—	—	Muscle	7.1	WET: M	
Agkistrodon piscivorus								Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 58.6 cm TM = 365 g	M	—	6	Liver	458.50	WET	
					Kidney	65.85		
Agkistrodon piscivorous								Burger et al. (2006); USA; South Carolina; 1 reference site, 1 polluted Savannah River site, Aiken
	SVL = 57 cm TM = 280 g	M	—	13	Muscle*	1527	DRY: M**	*Tail; **both sites combined
	SVL = 60 cm TM = 270 g			5	Blood	190		

(*continued*)

TABLE A.11 (CONTINUED)
Iron (Fe)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Coluber constrictor							Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 103.2 cm TN = 300 g	M	—	1	Liver	983.00	WET	
					Kidney	67.10		
Eunectes murinus								Calle et al. (1994); Venezuela
	L = 2.1–5.1 m W = 3.5–74.0 kg	R	F/M	7/5	Blood*	1.4; 0.7–3.4	WET: M; R	*Plasma
Lapemis hardwickii								Boman et al. (2001); Vietnam; Nha Trang; seemingly unpolluted site
	Adult*		—	1	Muscle	65	DRY	*Fully grown
					Liver	1100		
Nerodia cyclopion							WET	Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 63.9 cm TM = 191 g		—	1	Liver	170.00		
					Kidney	51.00		

Nerodia fasciata								Burger et al. (2006); USA; South Carolina; 1 reference and 1 polluted Savannah River site, Aiken
	SVL = 51 cm TM = 130 g	M	—	47	Muscle*	663	DRY: M**	*Tail; **both sites combined
	SVL = 52 cm TM = 140 g			34	Blood	236		
Nerodia taxispilota								Burger et al. (2006); USA; South Carolina; 1 reference site, 1 polluted Savannah River site, Aiken
	SVL = 59 cm TM = 170 g	M	—	10	Muscle*	1779	DRY: M**	*Tail; **both sites combined
	SVL = 65 cm TM = 230 g			9	Blood	220		
Python molurus								Boman et al. (2001); Vietnam; Dac Lac; seemingly unpolluted site
	Adult		—	1	Muscle Testicle	210 67	DRY	*Fully grown
*Waglerophis merremii**								De Jorge et al. (1971); Brazil; *[*Xenodon merremii*]
	Egg TM = 2.569 g	M	—	10	Whole egg	31.0	WET: M	*Oviductal
				5	Contents	33.2		
	Adult TL = 86–100 cm TM = 272–302 g	R	F	6	Glottis, spinal cord, eyes, trachea, rectum, fat, oviduct, ovary, supralabial gland, and gall bladder	12.4–20.0	RM	

(*continued*)

TABLE A.11 (CONTINUED)
Iron (Fe)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
				Small intestine, infralabial gland, stomach, bone, head muscles, trunk muscles, cloaca, large intestine, brain, head plates, and dorsal scales	20.0–30.0		
				Pancreas, tongue, adanal gland, esophagus, anal plates, ventral scales, and subcaudal scales	30.0–50.0		
				Dorsal tail scales, heart, kidney, thyroid gland, and lung	50.0–78.2		
				Spleen	296.9	M	
				Liver	561.8		
				Blood*	0.902		*Serum

TABLE A.12
Tin (Sn)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Testudines							
Caretta caretta							Iwata et al. (1997); Japan; Tosashimizu, Pacific coast of Shikoku Island
	—	—	—	Liver	0.068	WET	Mono-butyltin
					0.060		Di-butyltin
					0.0024		Tri-butyltin
					0.13		Butyltin compounds
Trachemys scripta elegans							Tryfonas et al. (2006); USA; Illinois; Lower Illinois River near Grafton; eggs laid in the lab from turtles collected in the field from 5 nesting areas
	Egg		—	Contents	1.8	DRY: M*	*All sites combined; values estimated from graph
				Shell	3.7		
	Diet			*Lemna* sp.	377		
	Environment		4–5*	Soil	1190–1910	RM*	*Samples (soil from nesting and lake bank areas) from 2 sites
			3*	Sediment	1100–1600	RM*	*Samples (3 layers per site) from 2 sites; values estimated from graph
			3–4*	Water	0.20–0.27	WET; RM*	*Samples from 3 sites; values estimated from graph
Crocodylia							
Alligator mississippiensis							Burger et al. (2000); USA; Florida; Lake Apoka (Lake and Orange Counties), Orange Lake (Alachua County), and Lake Woodruff (Volusia County)
	Yearling R L = 36–40 cm					WET: M*	*3 lakes combined
		—	30	Fat*	0.130		*Abdominal
			31	Liver	0.183		
			30	Muscle*	0.0609		*Abdominal

(*continued*)

TABLE A.12 (CONTINUED)
Tin (Sn)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
				29	Skin*	0.0877		*Abdominal
				29	Muscle*	0.0991		*Ventral proximal tail
				22	Tail tip	0.254		
				3	Tail regenerate	0.651		
Alligator mississippiensis								Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	—		—	1	Liver	0.31	WET	
					Kidney	0.07		
Squamata: Sauria								
"Lizard"								Hsu et al. (2006); South Taiwan; Kenting National Park; strong influence from industrial pollution
	—		—	1	Whole body	7.07	DRY	
						8.19		Bioconcentration factor (reference media: soil and food items)
Squamata: Serpentes								
"Snake"								Hsu et al. (2006); South Taiwan; Kenting National Park; strong influence from industrial pollution
	—		—	12	Whole body	9.64; 2.52–27.1	DRY: M; R	
						11.17	BCF	Bioconcentration factor (reference media: soil and food items)
Agkistrodon piscivorus								Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 58.6 cm TM = 365 g	M	—	6	Liver	0.12	WET: M	

					Kidney	1.41		
Coluber constrictor								Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 103.2 cm TN = 300 g	M	—	1	Liver	0.24	WET	
					Kidney	0.66		
Nerodia cyclopion								Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 63.9 cm TM = 191 g	M	—	1	Liver	ND (<0.10)	WET	
					Kidney	0.84		

TABLE A.13
Lead (Pb)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Testudines								
*Actinemys marmorata**								Henny et al. (2003); USA; western Oregon; Fern Ridge Reservoir; *[*Clemmys marmorata*]
	Egg		—	14*	Contents	ND (<1.50)	DRY	*14 nests, 1 egg per nest
Caretta caretta								Hillestad et al. (1974); USA; Georgia and South Carolina; 3 nesting beaches
	Egg		—	—	Yolk	2.87	—: M	
					Albumen	12.0		
Caretta caretta								Stoneburner et al. (1980); USA; 4 western Atlantic nesting beaches: Florida, Canaveral; Georgia, Cumberland Island; North Carolina, Cape Lookout; North Carolina, Cape Hatteras
	Egg*		—	96	Yolk	1.13–2.18	—: RM**	*Fresh; **combined by beach
Caretta caretta								Sakai et al. (1995); Japan; Kochi Prefecture, Cape Ashizuri
	L = 76–92 cm W = 75–108 kg		F/M	6/1			WET	
					Liver	ND (<—)		
					Kidney	ND (<—)		
					Muscle*	ND (<—)		*Pectoral
	Egg*		*	—	Shell	ND (<—)		*Oviductal eggs from 5 females
					Yolk	ND (<—)		
					Albumen	ND (<—)		
Caretta caretta								Storelli et al. (1998a); Italy; Adriatic Sea, Apulian coasts; stranded turtles
	TM = 1.8–100 kg	R	F/M	9/3	Liver	1.23; ND (<0.002)–3.38	DRY: M; R	

					Lung	0.60; ND (<0.002)–1.10		
					Kidney	0.70; ND (<0.002)–1.35		
					Muscle	0.54; ND (<0.002)–0.74		
Caretta caretta							WET: M; R	Storelli et al. (1998a), from Storelli et al. (2005); dry mass-based data from Storelli et al. (1998a) converted to wet mass
					Liver	0.36; ND (<—)–0.99		
					Kidney	0.21; ND (<—)–0.42		
					Muscle	0.12; ND (<—)–0.18		
Caretta caretta								Godley et al. (1999); northern Cyprus; eastern Mediterranean Sea, Alagadi beach; stranded turtles
	CCL = 63.5; 56.0–79.0 cm	M; R		7			DRY: MD; R	
				4	Liver	ND (<0.01); ND (<0.01)–4.90		
				2	Kidney	2.45; ND (<0.01)–4.90		
				4	Muscle	2.46; ND (<0.01)–5.53		
				48*				*1 sample (a dead hatchling, dead embryo, or undeveloped egg) taken per hatched nest
	Hatchling			16	Hatchling	0.13; <0.01–10.56		
	Embryo			—	Embryo	ND (<0.01); ND (<0.01)–6.48		
	Egg			3	Yolk and albumen	0.19; ND (<0.01)–3.93		
Caretta caretta								Sakai et al. (2000b); Japan; Kochi Prefecture, Cape Ashizuri; turtles accidentally caught in commercial fishing nets
	TM = 93/83 kg SCL = 83/85 cm	M	F/M	6/1	Liver	0.08/0.21	WET: M	

(*continued*)

TABLE A.13 (CONTINUED)
Lead (Pb)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations	References, Locations, Remarks
				Gullet	ND (<0.03)	
				Stomach	ND (<0.03)	
				Intestine	ND (<0.03)	
				Pancreas	ND (<0.03)	
				Heart	ND (<0.03)	
				Trachea	ND (<0.03)	
				Lung	ND (<0.03)	
				Bladder	ND (<0.03)	
				Spleen	ND (<0.03)	
				Kidney	0.16/ND (<0.03)	
				Salt gland	ND (<0.03)	
				Brain	ND (<0.03)	
	Reproductive tissues			Testis	ND (<0.03)	
				Oviduct	ND (<0.03)	
				Ovary	ND (<0.03)	
	Egg			Whole egg	ND (<0.03)	
				Shell	ND (<0.03)	
				Yolk	ND (<0.03)	
				Albumen	ND (<0.03)	
				Scale	0.08/0.07	
				Mesentary	ND (<0.03)	
				Fat	ND (<0.03)	
				Muscle	0.02/ND (<0.03)	
				Bone	3.53/1.82	
				Carapace	2.42/1.56	
				Whole body	*	*Not calculated

Caretta caretta								Kaska and Furness (2001); southwestern Turkey; 4 beaches; collected just before hatching or dead in shell embryos 1 week after last hatching
	Embryo		—	22	Liver	2.48	DRY: M	
	Egg		—		Shell	0.633		
					Yolk	1.307		
								Statistical analysis: [Pb] eggshell concentrations were significantly different between beaches
Caretta caretta								Kaska et al. (2004); Turkey; southwestern Mediterranean coast; stranded turtles
	—		—	32	Liver	3.55; 1.18–5.38	DRY: M; R	
				20	Bladder	3.96; 1.18–5.38		
				20	Kidney	3.99; 0.89–11.05		
				10	Lung	3.95; 0.9–16.32		
				32	Muscle	2.42; 0.76–19.78		
Caretta caretta								Torrent et al. (2004); Spain; Canary Islands; stranded turtles submitted to the veterinary faculty of the University of Las Palmas in Gran Canaria
	Juvenile and subadult SCL = 15–65 cm	R*	F/M	67/11	Kidney	2.44; 0.02–17.29	WET: M; R	*Values estimated from graph
					Muscle	2.26; 0.22–21.07		
					Bone	2.36; 0.08–19.92		
					Liver	2.94; 0.05–33.09		
Caretta caretta								Storelli et al. (2005); Italy; Adriatic and Ionian Seas; stranded turtles
	SCL = 21–71 cm	R		19	Liver	0.16; ND (<0.00015)–0.29	WET: M; R	
					Kidney	0.12; ND (<0.00015)–0.21		
					Muscle	0.04; ND (<0.00015)–0.09		
					Spleen	0.12; 0.05–0.18		
					Heart	0.07; 0.03–0.14		

(*continued*)

TABLE A.13 (CONTINUED)
Lead (Pb)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
				Lung	0.03; ND (<0.00015)–0.07		
				Fat	0.09; 0.06–0.14		
Caretta caretta						DRY: GM; R	Gardner et al. (2006); Mexico; Baja California peninsula; turtles died from fisheries capture
	MSCL = 57.0; 52.0–63.0	M; R	5	Liver	ND (<0.006)		
				Kidney	0.03; ND (<0.006)–69.89		
				Muscle*	0.01; ND (<0.006)–1.57		*Pectoral
				Fat	ND (<0.006)		
Chelonia sp.							Yoshinaga et al. (1992); Papua New Guinea
	—	—	—	Muscle	0.062	WET: M	
Chelonia mydas							Yoshinaga et al. (1992); Papua New Guinea
	—	—	—	Muscle	0.033	WET: M	
Chelonia mydas							Godley et al. (1999); northern Cyprus; eastern Mediterranean Sea, Alagadi beach; stranded turtles
	CCL = 49.5; 27.5–56.0 cm	M; R	6			DRY: MD; R	
			6	Liver	ND (<0.01); ND (<0.01)–1.84		
			1	Kidney	1.81		
			6	Muscle	ND (<0.01); ND (<0.01)–2.45		
			69*				*1 sample (a dead hatchling, dead embryo, or undeveloped egg) taken per hatched nest
	Hatchling		29	Hatchling	ND (<0.01); ND (<0.01)–3.86		

	Embryo			16	Embryo	0.66; ND (<0.01)–3.41		
	Egg			24	Yolk and albumen	ND (<0.01); ND (<0.01)–1.61		
Chelonia mydas								Sakai et al. (2000a); Japan; Yaeyama Islands, Okinawa; turtles caught by fishermen for commercial use
	SCL = 51.0 cm	M	—	50			WET: M; R	
				0/50*	Liver	ND (<0.03)		*Detected/analyzed
				18/23	Kidney	0.18; 0.05–0.28		
				0/47	Muscle	ND (<0.03)		
Chelonia mydas								Sakai et al. (2000b); Japan; Ogasawara Islands (Bonin Islands), Haha-Jima Island; turtles collected in coastal waters
	TM = 124/117 kg SCL = 93/97 cm	M	F/M	1/1	Liver	0.12/ND (<0.03)	WET: M	CW = 72/73 cm
					Gullet	1.20/ND (<0.03)		
					Stomach	ND (<0.03)		
					Intestine	ND (<0.03)		
					Pancreas	0.03/ND (<0.03)		
					Heart	ND (<0.03)		
					Trachea	ND (<0.03)		
					Lung	ND (<0.03)		
					Bladder	ND (<0.03)		
					Spleen	0.06/0.05		
					Kidney	ND (<0.03)/0.14		
					Salt gland	ND (<0.03)		
					Brain	ND (<0.03)		
	Reproductive tissues				Testis	ND (<0.03)		
					Oviduct	ND (<0.03)		
					Ovary	ND (<0.03)		
	Egg*				Whole egg	ND (<0.03)		Oviductal

(*continued*)

TABLE A.13 (CONTINUED)
Lead (Pb)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
					Shell	ND (<0.03)		
					Yolk	ND (<0.03)		
					Albumen	ND (<0.03)		
					Scale	0.09/0.14		
					Mesentary	ND (<0.03)		
					Fat	ND (<0.03)		
					Muscle	ND (<0.03)		
					Bone	2.40/2.30		
					Carapace	2.30/3.10		
					Whole body	*		*Not calculated
Chelonia mydas							DRY: M; R	Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 49.0; 40.0–63.5 cm	M; R	M	6				
	SCL = 52.2; 37.0–71.4 cm		F	20				
			M	6	Liver	0.511; 0.096–1.55		
				6	Kidney	0.510; 0.190–1.37		
				3	Muscle	0.043; 0.035–0.043		
			F	20	Liver	0.505; 0.059–1.49		
				19	Kidney	0.919; 0.160–2.38		
				9	Muscle	0.110; 0.030–0.211		
	Diet		—	8	Stomach	0.198; 0.126–0.278		*Content
Chelonia mydas								Anan et al. (2002); Japan; Okinawa Prefecture, Yaeyama Islands; tissues provided by fishermen
	—	—	—	9	Liver	0.140; 0.031–0.361	WET: M; R	
Chelonia mydas								Kaska et al. (2004); Turkey; southwestern Mediterranean coast; stranded turtles
	—		—	22	Liver	2.47; 1.18–13.52	DRY: M	

				20	Bladder	1.52; 0.52–5.01		
				14	Kidney	1.96; 0.63–6.79		
				15	Lung	1.93; 0.40–9.80		
				22	Muscle	1.44; 1.07–2.63		
Chelonia mydas							DRY: M	Lam et al. (2004); southern China
	Juvenile		—	2	Fat	0.085		Turtles stranded on beaches at Ham Tin Wan and Tung Pang Chau; upon collection, turtles had started to decay
					Kidney	0.311		
					Heart	0.209		
					Liver	0.152		
					Lung	0.172		
					Muscle	0.082		
					Stomach	0.600		
	Adult		—	3	Muscle	0.264		Turtles believed to have been unintentionally caught by fishermen; upon collection, turtles were relatively fresh
				1	Liver	0.826		
Chelonia mydas								Celik et al. (2006); Turkey; Mediterranean Sea, Kazanli beach; standed turtles
	Egg			12*	Shell	0.04	DRY: M	*12 nests, 3 eggshells per nest collected after hatching
	Environment							In this study, information is available on metal concentrations in sand, soil, plant, and water samples around the nesting environment
Chelonia mydas								Gardner et al. (2006); Mexico; Baja California peninsula; turtles died from fisheries capture
	MSCL = 62.13; 48.5–76.9	M; R		11	Liver	ND (<0.006)	DRY: GM; R	
					Kidney	0.01; ND (<0.006)–0.36		
					Muscle*	0.01; ND (<0.006)–1.23		*Pectoral

(*continued*)

TABLE A.13 (CONTINUED)
Lead (Pb)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
					Fat	0.03; ND (<0.006)–1.11		
Chelydra serpentina								Albers et al. (1986); USA; Maryland and New Jersey
	Adult	R					WET: M	*Values estimated from graph
	TL = 20–40 cm*							
			M	7	Liver	0.07		Site 1: Undisturbed freshwater site: Patuxent Wildlife Research Center, Maryland
			F	6		ND (<—)		
			M	7	Kidney	0.07		
			F	6		0.16		
			M	8	Liver	ND (<—)		Site 2: Contaminated brackish-water site: Hackensack Meadowlands, New Jersey
			F	3		ND (<—)		
			M	8	Kidney	0.19		
			F	3		ND (<—)		
			M	8	Liver	0.12		Site 3: Contaminated freshwater site: Hackensack Meadowlands, New Jersey
					Kidney	0.10		
Chelydra serpentina								Overmann and Krajicek (1995); USA; Missouri; Old Led Belt region
	TL = 0.200 m	M	F/M	7/8	Muscle	0.126	WET: M	Site 1: Outside Lead mining belt; from Meyers-Schöne and Walton (1994)
	TM = 2.18 kg							
					Brain	0.166		
					Liver	0.177		
					Blood	0.280		
					Carapace	0.977		

					Bone	1.015		
	TL = 0.203 m	M	F/M	5/9	Muscle	0.264		Site 2: Within lead mining belt, upstream of tailings pile
	TM = 2.35 kg							
					Brain	0.190		
					Liver	0.300		
					Blood	0.806		
					Carapace	12.334		
					Bone	37.560		
	TL = 0.204 m	M	F/M	4/4	Muscle	0.201		Site 3: Within lead mining belt, downstream of tailings pile
	TM = 2.17 kg							
					Brain	0.292		
					Liver	0.490		
					Blood	2.514		
					Carapace	33.013		
					Bone	114.563		
Cuora amboinensis								Boman et al. (2001); Vietnam; Dac Lac; seemingly unpolluted site
	Adult		—	1	Muscle	12	DRY	*Fully grown
Dermochelys coriacea								Davenport and Wrench (1990), Davenport et al. (1990); Great Britain; Wales, Irish Sea, Cardigan Bay; stranded turtle
	L = 2.53 m		M	1	Liver	0.12	DRY: M*	*4 replicate measures
	W = 916 kg							
					Muscle*	0.31		*Pectoral
					Blubber	0.04		
Dermochelys coriaca								Vazquez et al. (1997); Mexico; Pacific coast
	Environment		—	—	Seawater	0.35	DRY: M	
					Sand	23.2		
	Egg				Shell	11.6		*Posthatch

(*continued*)

TABLE A.13 (CONTINUED)
Lead (Pb)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Dermochelys coriaca								Godley et al. (1998); Great Britain; Wales and Scotland, west coast; turtles died in fishing gear
	Adult	R	M	3	Liver	0.02–14.0	DRY: R	
	CCL = 141–170 cm							
					Muscle	ND (<0.01–<0.09)		
				1	Liver	4.3	WET	
					Muscle	ND (<0.031)		
Eretmochelys imbricata							DRY: M; R	Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 43.5; 33.5–48.9	M; R	M	6	Liver	0.287; 0.02–0.534		
				6	Kidney	0.433; 0.087–1.12		
				1	Muscle	0.030		
	SCL = 46.5; 43.8–67.9		F	16	Liver	0.125; 0.064–0.215		
				13	Kidney	0.195; 0.094–0.408		
				8	Muscle	0.045; 0.003–0.146		
	Diet		—	6	Stomach*	0.212; 0.061–0.306		*Content
Eretmochelys imbricata								Anan et al. (2002); Japan; Okinawa Prefecture, Yaeyama Islands; tissues provided by fishermen
	—	—	—	7	Liver	0.033; 0.007–0.056	DRY: M; R	
Eretmochelys imbricata								Gardner et al. (2006); Mexico; Baja California peninsula; turtles died from fisheries capture
	MSCL = 48.4			1	Liver	ND (<0.006)	DRY	
					Kidney	ND (<0.006)		
					Muscle	0.38		
					Fat	ND (<0.006)		
*Gopherus agassizii**							—: M	Jacobson et al. (1991); USA; California; *[*Xerobates agassizii*]

	L = 133–306 mm W = 390–4900 g		FM	1/11				
			—	10	Liver	0.035		Site 1: Kern County; turtles with clinical signs of upper respiratory tract disease
	L = 199–280 mm W = 1180–3887		M	4	Liver	0.012		San Bernardino County; clinically healthy turtles
Lepidochelys empii								Kenyon et al. (2001); USA; Texas and Louisiana; turtles captured alive in nets at 4 beachfront sites
	SCL = 38.3; 21.6–65.8 cm	M; R	F/M/U	46/38/22*			WET: M; R	*99 wild grown plus 7 head-start female turtles
					Blood*	0.011; 0.00–0.0343		*Whole blood
Lepidochelys olivacea								Witkowski and Frazier (1982); Ecuador; Manta
	Adult		—	3	Bone*	39.0–110.0; 41.5–97.2	ASH: M; RM**	*Humerus; **3 replicate measures
Lepidochelys olivacea	—		—					Sahoo et al. (1996); India; Orissa, Gahirmatha
				8	Beach sand	24–86	DRY: R*	*mg/g
	Egg*		—	24**	Shell	11.0		*Fresh; **8 nests, 3 eggs per nest
					Albumen-yolk	3.6		
	Egg*				Shell	15.6		*Posthatch
	Hatchling*				Whole body	20.0		*Fresh
Lepidochelys olivacea							DRY: GM; R	Gardner et al. (2006); Mexico; Baja California peninsula; turtles died from fisheries capture
	MSCL = 60.1; 53.0–66.0	M; R		6	Liver	ND (<0.006)		
					Kidney	0.03; ND (<0.006)–2.63		
					Muscle*	ND (<0.006)		*Pectoral
					Fat	ND (<0.006)		

(*continued*)

TABLE A.13 (CONTINUED)
Lead (Pb)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Malaclemys terrapin								Burger (2002); USA; New Jersey; Barnegat Bay
	Adult	M	F	11	Liver	0.090	WET: M	
	TL = 14.3 cm							
					Muscle	0.062		
	Egg		—	8	Egg*	0.040; 0.006–0.099	M; R	*Ovarial
Terrapene carolina triunguis								Beresford et al. (1981); USA; Missouri and West Virginia
	W = 390–456 g	R	M	4			WET: M; R	Site 1: Contaminated area near lead smelters: Glover and Bixby, Missouri
					Bone*	51.8; 25.78–113.04		*Humerus
					Bone*	64.50; 21.73–135.86		*Femur
					Liver	21.60; 9.12–46.95		
					Kidney	24.31; 8.45–52.24		
					Blood	6.00; 2.17–11.59		
					Skin	0.35; 0.24–0.44		
					Lung	0.20; 0.10–0.34		
								Site 2: Less contaminated area distant from industry and main roads: Morgantown, West Virginia
	W = 362–475 g	R	F/M	3/1	Bone*	3.69; 2.54–4.51		*Humerus
					Bone*	3.80; 2.23–5.55		*Femur
					Liver	1.15; 0.63–2.21		
					Kidney	1.79; 0.55–4.83		
					Blood	0.11; ND (<—)–0.22		
					Skin	0.07; ND (<—)–0.16		
					Lung	ND (<—)		

Trachemys scripta						DRY: M; MAX	Burger and Gibbons (1998); USA; South Carolina; Savannah River site, Aiken, clutches laid in the lab 2–3 days after collection of females in the field
	Egg	—	16*	Contents	0.687; 1.852		*16 clutches, 1 egg per clutch
				Shell	0.219; 1.369		
							Statistical analysis: [Pb] concentrations were significantly higher in egg contents than in shells
Trachemys scripta							Hays and McBee (2007); USA; Oklahoma
	SCL	—	—	Blood	ND (<—)–0.09	WET: R	Site 1: Heavily mined from the 1890s to 1970 and currently contaminated with lead, zinc, and cadmium: Tar Creek Superfund site
		—	16	Carapace	ND (<—)–63.18		
		—	—	Blood	ND (<—)–0.09		Site 2: Unmined reference site: Sequoyah National Wildlife Refuge
		—	15	Carapace	0.94–25.28		
Trachemys scripta elegans	Eggs						Tryfonas et al. (2006); USA; Illinois; Lower Illinois River near Grafton; eggs laid in the lab from turtles collected in the field from 5 nesting areas
		—		Contents	ND (<—)	DRY: M**	*All sites combined; **values estimated from graphs
				Shell	1.3	**	**From graph
	Diet			*Lemna* sp.	4.1		
	Environment		4–5*	Soil	16–20	RM	*From 2 sites, soil from nesting and lake bank areas
			3*	Sediment	8–13	**	*From 2 sites, 3 layers per site; **from graph
		—	3–4*	Water	ND (<0.06)	WET: RM	*From 3 sites
Crocodylia							
"Crocodilian"							M. Watanabe (unpublished) from Brazaitis et al. (1996a); Brazil; Amazonian region
	—	—	—	Tissues including skin and meat	>0.5	—: MIN	

(*continued*)

TABLE A.13 (CONTINUED)
Lead (Pb)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Alligator mississippiensis								Cook et al. (1988) from Campbell (2003); zoo animals; not fed urban dwelling pigeons
	Diet		—	—	Blood	0.77; 0.45–1.08	WET; M; R	
Alligator mississippiensis								Delany et al. (1988); USA; Florida; 8 lakes, statewide
	L = 2.9–3.8 m	R	F/M	1/31				
				24	Muscle*	0.08; 0.04–0.12	WET: R; RM**	*Tail; **combined by lake
Alligator mississippiensis								Heinz et al. (1991); USA; central and southern Florida; Lake Okeechobee, Lake Griffin, and Lake Apopka
	Egg		—	32*	Egg	ND (<0.20)–0.22; ND (<0.20)–0.9	WET: RM; R**	*3 lakes, 16 nests, 2 eggs per nest; **combined by lake
Alligator mississippiensis								Camus et al. (1998); USA; Louisiana; 2 commercial alligator farms feeding ground nutria (*Mycocastor coypus*) carcasses contaminated by lead bullet fragments from gunshot
	Diet			3	Blood	0.03–0.04	WET R	Control (age-matched individuals that had never been fed nutria meat)
				2	Kidney	0.04–0.13		
				2	Liver	0.03–0.06		
				2	Muscle	ND (<0.1)		
				13	Blood	0.07–2.80		Nutria fed, Farm A
				4	Kidney	0.4–5.6		
				4	Muscle	ND (<0.1)		
				2	Blood	1.20–1.70		Nutria fed, Farm B
				2	Kidney	1.20–1.30		
				2	Liver	0.37–0.46		
				2	Muscle	ND (<0.1)		

Alligator mississippiensis								Burger et al. (2000); USA; Florida; Lake Apoka (Lake and Orange Counties), Orange Lake (Alachua County), and Lake Woodruff (Volusia County)
	Yearling L = 36–40 cm	R		30	Fat	0.0219	WET: M*	*Abdominal; **3 lakes combined
				31	Liver	0.0277		
				30	Muscle	0.0152		*Abdominal
				29	Skin	0.0197		*Abdominal
				29	Tail muscle	0.0408		*Ventral proximal tail
				22	Tail tip	0.101		
				3	Tail*	0.0770		*Regenerated
Alligator mississippiensis								Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	—		—	1	Liver	0.11	WET	
					Kidney	0.23		
Alligator mississippiensis								Lance et al. (2006); USA; Louisiana
	TL = 214; 161–279 cm TM = 34; 21–50 kg	M; R	M	8			M; R*	Wild alligators, trapped in aquaculture ponds; no Pb pellets found in stomach content; *ppm in table
	TL = 207; 185–226 cm TM = 29; 19–42 kg		F	7				
			—	11	Bone	7.98; 0–31.22	WET	
				5	Liver	0.54; 0.04–1.68		
				14	Kidney	0.60; 0.03–to 0.89		
	Egg		F	5	Yolk*	0.74; 0–1,97	DRY	*From ovarian follicles
	TL = 353; 246–404 cm TM = 217; 54–279 kg		M	16				Captive alligators, raised at the experimental alligator breeding facility (Louisiana Department of Wildlife and Fisheries, USA); fed nutria (*Myocastor copus*) meat; lead pellets found in stomach contents

(*continued*)

TABLE A.13 (CONTINUED)
Lead (Pb)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
	TL = 279; 187–295 cm TM = 85; 73–132 kg		F	28				
	Diet		—	30	Bone	252.43; 95.96–530.80	WET	
				26	Liver	12.50; 2.12–31.88		
				27	Kidney	4.97; 1.91–7.06		
	Egg		F	11	Yolk*	17.33 ± 2.75; 3.37–26.43	DRY	*From ovarian follicles
Alligator mississippiensis								Seltzer et al. (2006); USA; Louisiana; preserved samples originated from alligators subject to previous studies (Lance et al. 2006) and were analyzed using laser ablation inductively coupled plasma mass spectrometry (laser ablation ICP-MS)
	Adult A = ca. 27 years				Bone*		DRY: R**	*Femur transects; **bulk concentration; ppm
	TL = 2.69–3.82 m	R	F/M	1/3		105–386		Captive alligators
	TL = 2.24–2.49 m		—	3		ND (<1)–3.57		Wild alligators
Alligator sinensis								Cook et al. (1988) from Campbell (2003); zoo animals; not fed urban dwelling pigeons
	A = 7 years Diet		F	—	Blood	0.58; 0.43–0.86	WET: M; R	
Alligator sinensis								Ding et al. (2001) from Xu et al. (2006); China; Anhui Province; Anhui Captive Breeding Center
	Egg			1/1*			DRY	*1 infertile egg from captive breeding center/1 from the wild
					Eggshells	26.41/14.69		
					Shell membranes	3.33/1.813		
					Albumen	1.17/0.204		

				Yolk	1.02/0.730		
Alligator sinensis							Xu et al. (2006); China; Changxing County, Zhejiang Province; Changxing Nature Reserve and Breeding Research Center for Chinese alligator
	Adult	F/M	1/1	Heart	0.98/0.63	DRY	Alligators collected dead of unidentified causes
				Lung	0.61/0.90		
				Liver	0.54/0.85		
				Stomach	0.73/0.75		
				Kidney	0.34/0.41		
				Intestine	0.51/0.47		
				Trachea	0.52/0.42		
				Pancreas	0.23/0.26		
				Gonad	0.37/0.21		
				Muscle	0.75/0.71		
	Diet	—	—	Fish*	1.05	M; R	*2 fish prey species randomly sampled
	Excrements	—	6	Feces	6.10		
	Eggs		10*				*10 eggs from 3 clutches
				Shell	1.16; 0.94–1.52		
				Shell membranes	1.27; 0.94–1.62		
				Contents	0.80; 0.71–0.98		
	Environment		10*				*Water and sediment (5 subsamples from different sites within a pond each) from 10 breeding ponds
				Sediment	34.99		
				Water	0.00445	WET: M	
							Statistical analysis: [Pb] concentrations were significantly higher in shells and membranes than in contents; significant negative correlation was found between [Fe] and [Pb] in the eggshells; significant positive correlation was found between [Cu] and [Pb] in membranes; significant positive correlations existed in the contents among concentrations of [As] and [Cu], [Pb] and [Cr]

(*continued*)

TABLE A.13 (CONTINUED)
Lead (Pb)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Caiman crocodilus							Cook et al. (1988) from Campbell (2003); USA; zoo animals; not fed urban dwelling pigeons
	A = 3 years Diet	F	2	Blood	ND (<0.25)–0.49	WET: R	
Caiman yacare							Cook et al. (1988) from Campbell (2003); USA; New York, Bronx, New York Zoological Park; not fed urban dwelling pigeons
	3 years old Diet	M	—	Blood	ND (<0.25)		
"Caiman"							Odierna (undated) from Brazaitis et al. (1996b); Brazil; intensive gold mining regions
	—	—	—	Skin and liver	84.0	—: MAX	
Caiman c. crocodilus and *Caiman yacare*							Brazaitis et al. (1996b); Brazil; intensive gold mining regions
	—	—	227*	—	ND (<0.01) 0.01–0.50 0.50–2.00 >2.0	—: R*	17.6% of the specimens; *for both species 33.5% 34.4% 14.5%
Crocodylus acutus							Ogden et al. (1974); USA; Florida, Everglades, Florida Bay
	Egg	—	5	Contents	0.34; 0.2–0.5	WET: M; R	
Crocodylus acutus							Stoneburner and Kushlan (1984); USA; Florida; Everglades, Florida Bay
	Egg	—	9	Shell Albumen-yolk Albumen-yolk	16.42 3.35 0.64	DRY: M WET: M*	 *Albumen-yolk dry mass-based concentrations transformed to wet mass

Crocodylus acutus								Rainwater et al. (2007); Costa Rica; Rio Grande de Tarcoles; polluted by several metals from various sources
	SVL = 155.7; 134.0–172.0 cm	M; R	F/M	1/5	Scute*	0.4915	WET: M	*Caudal
			M	1		2.590	WET: MAX	
Crocodylus johnstoni								Jeffree et al. (2001); northern Australia; north central Queensland, Lynd River; samples from a single population
	A = 0.7–62.7 years L = 24.7–128.3 cm	R	F/M	9/21	Osteoderm*	2.22; 1.09–3.05	DRY: M; R	*Ventral pelvic region
Crocodylus moreletii								Rainwater et al. (2007); Belize
					Scute*		WET: M*	*1 whole caudal scute per crocodile
	SVL = 89.8; 65.0–129.5 cm		F/M	5/4		ND<(0.05)		Site 1: Gold Buton Lagoon
	SVL = 104.4; 59.5–156.7 cm		F/M	4/6		1.097		Site 2: New River Watershed
			F	1		0.808		The most highly contaminated *C. moreletii* individual
Crocodylus niloticus								Phelps et al. (1986); Zimbabwe, 10 samples from 8 sites
	Egg		—	26	Contents	4.844; 1.250–10.211	DRY: M; R	
Crocodylus niloticus								Swanepoel et al. (2000); South Africa; Kruger National Park (KNP) area
	TL = 1.40–4.15 m	R	F/M	6/9			DRY: M	
								Site 1: Shingwedzi River (Silwervis Dam), northern part of Kruger National Park; catchment area outside KNP is limited
					Muscle	3.7		Frozen tissues used for residue analysis
					Liver	14.1		

(*continued*)

TABLE A.13 (CONTINUED)
Lead (Pb)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
					Kidney	9.7		
					Fat	8.5		
								Site 2: Olifants River, central part of KNP; flows through areas of intense agricultural activity and passes mining areas and receives tributaries from Phalaborwa Mining Company before entering the KNP
					Muscle	20.3		Frozen tissues used for residue analysis
					Liver	19.85		
					Fat	1.9		
								Site 3: Sabi River, southern part of KNP; flows through areas of intense agricultural activity before entering the KNP
					Muscle	ND (<—)		
					Liver	ND (<—)		Frozen tissues used for residue analysis
					Kidney	NG(<—)		
					Fat	ND (<—)		
Crocodylus niloticus								Almli et al. (2005); Zambia
	L = 2.7–3.4 m	R	F/M	2/2	Liver	8.70; 6.60–17.00	WET: MD; R	Site 1: Kafue River, Kafue National Park
					Kidney	1.60; 0.47–2.20		
	L = 2.0–4.0 m		F/M	4/1	Liver	3.30; 0.71–11.00		Site 2: Luangwa River, Luangwa National Park
					Kidney	0.28; 0.07–2.00		
Crocodylus porosus								Yoshinaga et al. (1992); Papua New Guinea
	—		—	—	Muscle	0.036	WET: M	

Crocodylus porosus								Twining et al. (1999); northern Australia; Alligator Rivers region; Kakadu National Park; 4 major catchments; wild crocodiles, live or recently dead at collection
	A = 5–40 years* TL = ca. 1.7–5 m	R		40	Osteoderm	25.2; 18.8–44.2	DRY: M; R	Sites hunted using Pb ammunition; estimated age
					Muscle*	0.54		*Tail "flesh"
					Osteoderm	3.4; 1.2–9.7		Sites not hunted using Pb ammunition
					Muscle*	0.33		*Tail "flesh"
Crocodylus porosus			—	40				Jeffree et al. (2001); northern Australia; Alligator Rivers region: Kakadu National Park; 3 river catchments, mining and hunting areas included
								Site 1: Area not hunted using Pb ammunition
	A = 5–40 years	R	—	31	Muscle*	0.308; 0.120–0.450	DRY: GM; R	*Tail
	L = 168–499 cm							
				40	Osteoderm*	3.001.2–9.4		*Ventral pelvic region
								Site 2: Area hunted using Pb ammunition
				4	Muscle*	0.54	M	*Tail
				4	Osteoderm*	24.0		*Ventral pelvic region
Crocodylus porosus								Hammerton et al. (2003); Australia; farm feeding experiment; animals reared and housed at *Crocodylus* Park, near Darwin, Northern Territory
	Juvenile A = 2.3 years L = 1.7–2.0 m	R	—		Blood*		WET	*Whole blood
				4		1.93*	M	Preexposure; *background level
	Diet			3		27.8–36.3*	RM	Treatment 1: Single-dose exposure: fed 5 lead shot; *steady-state equilibrium
				1		51.4*	M	Treatment 2: Single-dose exposure: fed 10 lead shot; *upper plateau (after 85–140 days of exposure)

(*continued*)

TABLE A.13 (CONTINUED)
Lead (Pb)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
			2		3.08–1.47	RM	Treatment 3: Control
Crocodylus rhombifer							Cook et al. (1989); zoo animal
	Adult A = 18 years						Without clinical signs of lead toxicosis, fed wild caught urban dwelling pigeons with elevated bone Pb levels
		F	1	Blood	0.247	—	247 µg/kg
	Diet	—	10	Pigeon	124–544	—	124–544 ppm; bone Pb levels of wild caught urban dwelling pigeons (range detected in 8 out of 10 pigeons)
Paleosuchus palpebrosus							Cook et al. (1988) from Campbell (2003); wild caught animal, not fed urban dwelling pigeons
	A = 3 years Diet	M	1	Blood	0.035	WET	
Paleosuchus trigonatus							Cook et al. (1988) from Campbell (2003); wild caught animal, not fed urban dwelling pigeons
	A = 3 years Diet	M	1	Blood	ND (<0.035)	WET	
Tomistoma schlegelii							Cook et al. (1989); zoo animal; without clinical signs of lead toxicosis, fed wild caught urban dwelling pigeons with elevated bone Pb levels
	Adult A = 25 years TM = 93.22 kg	F	1	Blood	14.7	—	147 µg/dl
	Adult	F	1		17.8		178 µg/dl
	Diet	—	10	Pigeon	124–544	—	124–544 ppm; bone Pb levels of wild caught urban dwelling pigeons (range detected in 8 out of 10 pigeons)

Squamata: Sauria								
"Lizard"								Hsu et al. (2006); South Taiwan; Kenting National Park; strong influence from industrial pollution
	—		—	1	Whole body	6.01 0.77	DRY	Bioconcentration factor (reference media: soil and food items)
"Wall lizard"								Kaur (1988); India; Punjab
	—		—	20	Scales	7.00 150.53	DRY: M	Remote rural sites Urban sites
*Norops sagrei**								Burger et al. (2004) USA; Florida; southern Florida and Florida Keys; *[*Anolis sagrei*]
	Adult							
	SVL = 43.5; 35–50 mm	M; R	F	72	Whole body	0.277; 0.0239–0.522; 0.0020–1.557	WET: M; RM; R**	*Minus gut content; **6 sites combined
	SVL = 55.3; 45–67 mm		M	72		0.192; 0.0174–0.385; 0.00028–1.334		
Chamaeleo chamaeleon								Diaz-Paniagua et al. (2001); southern Spain; Province of Cadiz; 2 sampling sites; nests located in agricultural lands and coastal urban areas; eggs collected immediately after oviposition
	Egg			9*	Contents	14.20; 4.00–22.10	WET: M; R	*9 nests, 4 eggs pooled per nest
Chamaeleo chamaeleon								Gomara et al. (2007); southern Spain; Province of Cadiz; 3 sampling sites; eggs collected 0–15 days after oviposition
	Egg			9*	Contents Shells	ND (<0.0012)–0.020 0.175–1.454	WET: RM	*9 nests, 2–4 eggs pooled per nest
Hemidactylus mabouia			—				DRY: R; RM	Schmidt (1984b); Brazil; Rio Grande do Sul; Porto Alegre; urban area
	TL = 35–70 mm*							*Values estimated from graph

(*continued*)

TABLE A.13 (CONTINUED)
Lead (Pb)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
			154	Whole body	0.2–30.3; 0.8–13.0		9 sites
			21	Liver	0.7–12.4; 1.6–5.1		3 sites
Hemidactylus mabouia							Schmidt (1986); Brazil; Rio Grande do Sul; Porto Alegre; urban area
	—	—	20	Whole body	1.8; 0.5–3.2	DRY: M; R	
			8	Liver	2.0; 1.3–2.6		
	Excrements		6	Excrements	15.3; 9.9–22.7		
*Iberolacerta monticola**					See As for details		Marco et al. (2004); Spain; Avila, Gredos Mountains; gravid females collected in the field in 2001; freshly laid eggs incubated until hatching in As (arsenic acid in nitric acid)-loaded artificial breeding substrate (sterile As-free vermiculite) for 18 days; further elements (Cd, Cu, Pb, Zn), though not manipulated, were detected in eggshells and embryos; *[*Lacerta monticola cyrenni]*
Lacerta agilis	—					DRY: M	Schmidt (1984a); Germany, Saarbrücken; urban area
		F	4	Whole body	3.24		
		M	4		6.07		
*Laudakia s. stellio**							Loumbourdis (1997); Greece, Thessaloniki region; *[*Agama s. stellio*]
	Adult	—	—	Liver	6.79	DRY; M	Site 1: Urban area (500 m asl)
				Carcass	12.81		
				Liver	13.32		Site 2: Agricultural area (50 m asl)
				Carcass	15.65		
*Podarcis muralis**							Schmidt (1980); Germany; Saarbrücken; 2 urban area sites: *[*Lacerta muralis*]
	—	—	—	Whole body	7.5–10.0	DRY: M	
Podarcis muralis							Schmidt (1984a, 1988); Germany; Saarbrücken; 4 urban sites

		M	45	Whole body	3.9–23.0	DRY: RM*	*Combined by sex
		F	30		2.8–12.8		
	Adult		34		3.9–29.5	DRY: RM*	*Same sample combined by phase
	Semiadult		19		3.7–15.5		
	Juvenile		22		2.8–8.5		
Tarentola mauritanica							Fletcher et al. (2006); southern Spain; Guadiamar River Valley; mine tailings release: Boliden-Apirsa mine at Aznalcóllar; 7 study sites spanning an expected contamination gradient; geckos collected from building walls
	Adults and juveniles		52	Whole body*		DRY: M; R	*Minus gut contents
		—	9		2.036; 0.782–3.145		Site 1: Rural not mine-affected site: near Guadalmellato (most pristine)
		—	13		17.316; 1.489–50.521		Site 2: Urban not mine-affected site: Villaviciosa de Córdoba (not contaminated by mining)
		—	8		9.712; 4.474–23.378		Site 3: Urban mine-affected site: Aznalcázar (contamination through aerosolized contaminants engulfed the town during cleanup)
		—	8		8.376; 4.852–19.829		Site 4: Urban mine-affected site: Aznalcóllar (contaminated by normal mine operations, or the disaster and subsequent remediation efforts)
		—	5		7.936; 1.866–20.217		Site 5: Floodplain mine-affected site: Guadiamar River floodplain near the Aznalcázar gauge station (24.8 km below the ruptured tailings dam)
		—	5		3.840; 1.80–5.666		Site 6: Floodplain mine-affected site: Guadiamar River floodplain near the Guijo gauge station (7.4 km below the ruptured dam)
		—	4		19.199; 8.863–27.431		Site 7: Floodplain mine-affected site: Agrio River floodplain (4.4 km below and closest to the ruptured tailings dam)

(*continued*)

TABLE A.13 (CONTINUED)
Lead (Pb)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
							Statistical analysis: [Pb] concentration was significantly influenced by site
Varanus salvator							Boman et al. (2001); Vietnam; Dac Lac; seemingly unpolluted site
	Adult*	—	1	Muscle	14	DRY	*Fully grown
Varanus sp.							Yoshinaga et al. (1992); Papua New Guinea
	—	—	—	Muscle	0.007	WET: M	
*Zootoca vivipara**							Schmidt (1980); Germany; Saarbrücken; urban area, 3 sites; *[*Lacerta vivipara*]
	—	—	15	Whole body	22.5–68.8	DRY: RM	
*Zootoca vivipara**							Avery et al. (1983); Great Britain; England; *[*Lacerta vivipara*]
	Juvenile and adult	—	11*			DRY: M	Site 1: Little used road; *8 juveniles, 3 adults
				Bone*	0.9		*Femur
				Liver	2.7		
				Lung	ND (<0.8)		
				Kidney	ND (<1.25)		
				Remainder	3.6		
			19*				Site 2: Busy road; *12 juveniles, 7 adults
				Bone*	9.5		*Femur
				Liver	3.5		
				Lung	1.1		
				Kidney	7.5		
				Remainder	11.0		
			14*				Site 3: Lead mine; *9 juveniles, 5 adults
				Bone*	62.3		*Femur
				Liver	1.2		
				Lung	ND (<0.8)		
				Kidney	18.0		

					Remainder	56.1		
*Zootoca vivipara**								Schmidt (1984b, 1988); Germany; Saarbrücken; urban area, 1 site; [*Lacerta vivipara*]
			M	15	Whole body	45.9	DRY: M*	*Combined by sex
			F	5		11.9		
	Adult			4		65.2	DRY: M*	*Same sample combined by phase
	Semi-adult			12		35.4		
	Juvenile			4		15.6		
*Zootoca vivipara**	—		—	2				Gutleb et al. (1992); Austria; Carinthia; remote area; *[*Lacerta vivipara*]
					Liver	1.5–1.7	DRY: R	
					Kidney	1.6–4.9		
Squamata: Serpentes								
"Cobra"								Kaur (1988); India; Punjab
	—		—	20	Shed skins	5.00	DRY: M	Remote rural sites
						200.53		Urban sites
"Snake"								Hsu et al. (2006); South Taiwan; Kenting National Park; strong influence from industrial pollution
	—		—	12	Whole body	8.44; 2.77–20.6	DRY: M; R	
						1.07		Bioconcentration factor (reference media: soil and food items)
Acrochordus javanicus								Yoshinaga et al. (1992); Papua New Guinea
	—		—	—	Muscle	ND (<0.005)	WET	
Agkistrodon piscivorus							WET	Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 58.6 cm TM = 365 g	M	—	6	Liver	0.14		
					Kidney	0.97		

(*continued*)

TABLE A.13 (CONTINUED)
Lead (Pb)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Akistrodon piscivorous			—					Burger et al. (2006); USA; South Carolina; 1 reference site, 1 polluted Savannah River site, Aiken
	SVL = 57 cm TM = 280 g	M		13	Muscle*	3	DRY: M**	*Tail; **both sites combined
	SVL = 60 cm TM = 270 g			5	Blood	0.04		
Coluber constrictor							WET	Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 103.2 cm TN = 300 g		—	1	Liver	0.14		
					Kidney	0.39		
Crotalus viridis								Bauerle et al. (1975); USA; Colorado; Pawnee National Grassland
	—		F/M	1/5	Liver	0.003–0.659	—: R	
*Pantherophis guttatus**								Jones and Holladay (2006); USA; Florida; experimental animals obtained from commercial provider; fed dead mice for 34 weeks; *[*Elaphe guttata*]
	TM = 44.6; 52.0–260.3/211.1; 107.9–353.8 g*	M; R						*Initial mass/final mass
			—	3	Shed skins	0.0095; 0.0050–0.0140	DRY: MD; R	Treatment 1: Control: fed only not contaminated mice
	Die		—	10		0.1730; 0.0540–0.7830		Treatment 2: Fed metal injected mice enriched with a mixture of three metals (Cd, Hg, Pb) at a dose of 2 mg/kg per snake, metal, and month

Lapemis hardwickii								Boman et al. (2001); Vietnam; Nha Trang; seemingly unpolluted site
	Adult*		—	1	Liver	16	DRY	*Fully grown
Nerodia sp.*								Winger et al. (1984); USA; Florida; Apalachicola River; *[*Natrix* sp.]
	W = 226–544 g	R	—	15	Whole body	0.48; 0.22–0.87	WET: M; R	Site 1: Upper reaches of river
	W = 272–725 g					0.10; 0.10–0.10		Site 2: Lower reaches of river
Nerodia cyclopion								Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 63.9 cm TM = 191 g		—	1	Liver	ND (<0.11)	WET	
					Kidney	0.73		
Nerodia fasciata								Burger et al. (2006); USA; South Carolina; 1 reference site, 1 polluted Savannah River site, Aiken
	SVL = 51 cm TM = 130 g	M		47	Muscle*	2.0	DRY: M**	*Tail; **both sites combined
	SVL = 52 cm TM = 140 g			34	Blood	0.1		
Nerodia fasciata								Burger et al. (2007); USA; South Carolina; relatively rural site
	Adult		—	34	Blood	0.056	WET: M	
				47	Muscle	0.656		
				5	Liver	0.133		
Nerodia sipedon								Niethammer et al. (1985); USA; Missouri, Lead Belt region
	Adult and juvenile		—	15	Carcass	ND (<0.1)–0.64	WET: R	Upstream formerly mined area
				15		1.60 yo –41.1		Downstream formerly mined area
				50		ND (<0.1)–3.90		Currently mined area

(*continued*)

TABLE A.13 (CONTINUED)
Lead (Pb)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Nerodia sipedon								Burger et al. (2005); USA; eastern Tennessee; 1 reference site: Little River downstream from the Great Smoky Mountains National Park near Townsend; and 1 polluted Superfund site: EFPC inside the US Department of Energy's (USDOE) Y-12 National Security Complex
	Adult	—	—				WET: M*	*Both sites combined
				47	Kidney	0.061		
				47	Liver	0.036		
				47	Muscle	0.060		
				47	Skin	0.106		
				46	Blood	0.049		
				3	Testis	0.051		
	Egg		—	8	Egg*	0.025		*Ovarial Statistical analysis: [Pb] concentrations were significantly highest in the skin
Nerodia sipedon								Campbell et al. (2005); USA; eastern Tennessee; 2 sites
	Adult		—				WET: M; R	
	TM = 235; 53–464 g SVL = 65; 44.5–80 mm	M; R	21 F					
	TM = 103; 74–146 g SVL = 53; 47–60 mm		26 M					
			F/M	11/16	Blood	0.0551; 0.016–0.252		Site 1: Reference site: Little River downstream from the Great Smoky Mountains National Park
					Kidney	0.0384; 0.00008–0.118		

					Liver	0.0260; 0.00008–0.0913		
					Muscle	0.0585; 0.00008–0.418		
					Skin	0.0619; 0.0048–0.190		
			F/M	10/10	Blood	0.0418; 0.012–0.137		Site 2: Polluted Superfund site: upper reach of East Fork Poplar Creek (EFPC) within the US Department of Energy's (USDOE) Y-12 National Security Complex
					Kidney	0.0921; 0.031–0.173		
					Liver	0.0504; 0.015–to 109		
					Muscle	0.0602; 0.0097–0.118		
					Skin	0.161; 0.022–0.398		
Nerodia sipedon								Burger et al. (2007); USA; New Jersey and Tennessee
	Adult TL = 72; 39.0–103.5 cm TM = 159; 17.5–587.5 g	M; R	—	18	Blood	0.108	WET: M	
					Kidney	0.343		Site 1: Urban/suburban; New Jersey
					Liver	0.063		
					Muscle	0.103		
					Skin	0.467		
				36	Blood	0.049		Site 2: Relatively rural; Tennessee
				46	Muscle	0.060		
				47	Liver	0.036		

(*continued*)

TABLE A.13 (CONTINUED)
Lead (Pb)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Nerodia taxispilota								Burger et al. (2006); USA; South Carolina; 1 reference site, 1 polluted Savannah River site, Aiken
	SVL = 59 cm TM = 170 g	M	—	10	Muscle*	32	DRY; M**	*Tail; **both sites combined
	SVL = 65 cm TM = 230 g			9	Blood	0.04		
Pituophis melanoleucus			—					Burger (1992); USA; New Jersey; 4 sites, 4 years
	Hatchling	M		46	Skin	1.331; 0.630–2.706	DRY: M; RM*	*All sites combined by year
	TM = 24.65 g			16	Whole body*	0.607; 0.521–0.856		*Saggital section from the center of the body, including bone
Python molurus								Boman et al. (2001); Vietnam; Dac Lac; seemingly unpolluted site
	Adult*		—	1	Testicle	11	DRY	*Fully grown
Thamnophis sirtalis								Rolfe et al. (1977); USA; Illinois; Champaign and Urbana region; environmental Pb levels low
	—		—	13	Whole body	9.6	DRY: M	
Thamnophis radix								Rolfe et al. (1977); USA; Illinois; Champaign and Urbana region; environmental Pb levels high
	—		—	13	Whole body	69.7	DRY: M	
Vipera berus								Gutleb et al. (1992); Austria; Carinthia; remote area
	—		—	1	Liver Kidney	0.9 0,8	DRY	

TABLE A.14
Manganese (Mn)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Testudines								
Actinemys marmorata *								Henny et al. (2003); USA; western Oregon; Fern Ridge Reservoir; *[*Clemmys marmorata*]
	Egg		—	14*	Contents	2.22; 0.81–5.25	DRY: GM; R	*14 nests, 1 egg per nest Statistical analysis: No significant differences in contaminant concentrations related to hatching rate
*Caretta aretta**								Aguirre et al. (1994); USA; Hawaiian Islands; *[identification number 052, *Chelonia mydas* in the reference]
	"Pelagic"		—	1	Liver	1.59	WET	
Caretta caretta								Sakai et al. (1995); Japan; Cape Ashizuri, Kochi Prefecture
	L = 76–92 cm W = 75–108 kg	R	F/M	6/1	Liver	2.07; 1.44–2.94	WET: M; R	
					Kidney	1.57; 0.808–1.97		
					Muscle*	0.300; 0.129–0.446		*Pectoral
	Egg*		—	*	Shell	0.68; ND (< —)–1.2		*Oviductal eggs from 5 females
					Yolk	0.91; 0.52–1.4		
					Albumen	0.17; ND (< —)-–.71		
					Whole egg*	0.52; 0.30–0.90		*Calculated
Caretta caretta								Sakai et al. (2000b); Japan; Kochi Prefecture, Cape Ashizuri; turtles accidentally caught in commercial fishing nets
	TM = 93/83 kg SCL = 83/85 cm	M	F/M	6/1	Liver	2.18/1.44	WET: M	
					Gullet	0.12/0.24		

(*continued*)

TABLE A.14 (CONTINUED)
Manganese (Mn)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations	References, Locations, Remarks
				Stomach	0.43/0.42	
				Intestine	0.51/0.83	
				Pancreas	26.4/2.89	
				Heart	0.32/0.57	
				Trachea	0.13/0.24	
				Lung	0.12/0.25	
				Bladder	0.16/0.32	
				Spleen	0.37/0.37	
				Kidney	1.50/1.97	
				Salt gland	0.75/0.80	
				Brain	0.35/0.63	
	Reproductive tissues			Testis	0.46	
				Oviduct	0.54	
				Ovary	0.70	
	Egg			Whole egg	0.52	
				Shell	0.68	
				Yolk	0.91	
				Albumen	0.17	
				Scale	0.14/0.27	
				Mesentary	0.14/0.19	
				Fat	0.12/0.18	
				Muscle	0.28/0.43	
				Bone	10.8/18.0	
				Carapace	7.01/8.94	
				Whole body*	2.31/3.44	*Calculated
Caretta caretta						Franzellitti et al. (2004); Italy; northwestern Adriatic Sea; Adriatic Sea coast from the Po delta to the Reno mouth; turtles collected dead along the coast
	Juvenile to adult	R				16 from fishery by-catch, 19 dead on coast

	MSCL = 24.5–74 cm							
			—	30	Liver	6.23	WET: M	
				13	Lung	5.3		
				17	Muscle*	2.7		*Pectoral
				7	Fat*	8.4		*Abdominal
Caretta caretta								Gardner et al. (2006); Mexico; Baja California peninsula; turtles died from fisheries capture
	MSCL = 57.0; 52.0–63.0	M; R		5	Liver	1.29; 0.11–8.60	DRY: GM; R	
					Kidney	6.0; 2.37–9.97		
					Muscle*	0.84; ND (<0.002)–5.4		*Pectoral
					Fat	1.82; 0.8–3.2		
Chelonia sp.								Yoshinaga et al. (1992); Papua New Guinea
	—		—	—	Muscle	0.38	WET: M	
Chelonia mydas								Yoshinaga et al. (1992); Papua New Guinea
	—		—	—	Muscle	0.34	WET: M	
Chelonia mydas			F/M	8/4			WET: M; R	Aguirre et al. (1994); USA; Hawaiian Islands
	L = 28.7–71.3 cm W = 3.2–43.6 kg	R			Liver	1.60; 0.15–2.79		
					Kidney	0.96; 0.48–1.39		
	Egg*		—	3	Shell	0.31		*Posthatch
	Hatchling				Whole body	1.12		
Chelonia mydas								Sakai et al. (2000a); Japan; Yaeyama Islands, Okinawa; caught by fisherman for commercial use
	SCL = 51.0 cm	M	—	50			WET: M; R	
				50/50*	Liver	1.86; 0.70–5.41		*Detected/analyzed
				23/23	Kidney	1.21; 0.72–2.30		
				45/47	Muscle	0.11; 0.05–0.36		

(*continued*)

TABLE A.14 (CONTINUED)
Manganese (Mn)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Chelonia mydas								Sakai et al. (2000b); Japan; Ogasawara Islands (Bonin Islands), Haha-Jima Island; turtles collected in coastal waters
	TM = 124/117 kg SCL = 93/97 cm	M	F/M	1/1	Liver	1.86/1.91	WET: M	CW = 72/73 cm
					Gullet	0.21/0.20		
					Stomach	0.27/0.34		
					Intestine	0.36/0.38		
					Pancreas	2.66/7.31		
					Heart	0.28/0.36		
					Trachea	0.21/0.26		
					Lung	0.25/0.25		
					Bladder	0.35/0.32		
					Spleen	0.30/0.35		
					Kidney	1.09/1.55		
					Salt gland	1.09/0.94		
					Brain	0.40/0.55		
	Reproductive tissues				Testis	0.38		
					Oviduct	0.33		
					Ovary	0.81		
	Egg				Whole egg	0.38		
					Shell	1.33		
					Yolk	0.57		
					Albumen	ND (<0.03)		
					Scale	0.29/0.34		
					Mesentary	0.14/0.12		
					Fat	0.20/0.19		
					Muscle	0.24/0.29		

					Bone	5.67/7.46		
					Carapace	3.92/5.04		
					Whole body*	1.22/1.62		*Calculated
Chelonia mydas								Anan et al. (2001); Japan, Yaeyama Islands
	SCL = 49.0; 40.0–63.5 cm	M; R	M	6			DRY: M; R	
	SCL = 52.2; 37.0–71.4 cm		F	20				
			M	6	Liver	4.25; 2.72–8.24		
				6	Kidney	4.87; 3.67–6.97		
				3	Muscle	0.408; 0.327–0.475		
			F	20	Liver	4.89; 2.28–11.1		
				19	Kidney	5.86; 2.78–8.36		
				9	Muscle	0.474; 0.322–0.609		
	Diet		—	8	Stomach*	7.63; 2.45–18.9		*Contents
Chelonia mydas								Lam et al. (2004); South China
	Juvenile		—	2	Fat	0.188	DRY: M	Turtles stranded on beaches at Ham Tin Wan and Tung Pang Chau; upon collection, turtles had started to decay
					Kidney	11.59		
					Heart	2.494		
					Liver	16.27		
					Lung	1.983		
					Muscle	1.278		
					Stomach	28.82		
	Adult		—	3	Muscle	5.037		Turtles believed to have been unintentionally caught by fishermen; upon collection, turtles were relatively fresh
				1	Liver	10.50		
Chelonia mydas							DRY: GM; R	Gardner et al. (2006); Mexico; Baja California peninsula; turtles died from fisheries capture

(*continued*)

TABLE A.14 (CONTINUED)
Manganese (Mn)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
	MSCL = 62.13; 48.5–76.9	M; R		11	Liver	0.06; ND (<0.002)–6.74		
					Kidney	0.31; ND (<0.002)–8.12		
					Muscle*	0.003; ND (<0.002)–7.75		*Pectoral
					Fat	0.003; ND (<0.002)–0.79		
Eretmochelys imbricata								Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 43.5; 33.5–48.9	M; R	M	6	Liver	10.1; 4.36–17.9	DRY: M; R	
				6	Kidney	12.4; 6.09–16.4		
				1	Muscle	0.573		
	SCL = 46.5; 43.8–67.9		F	16	Liver	7.6; 3.76–15.8		
				13	Kidney	13.6; 10.8–17.7		
				8	Muscle	0.714; 0.416–2.08		
	Diet		—	6	Stomach*	3.55; 0.935–6.30		*Contents
Eretmochelys imbricata								Gardner et al. (2006); Mexico, Baja California peninsula; turtles died from fisheries capture
	MSCL = 48.4		—	1	Liver	0.74	DRY	
					Kidney	7.62		
					Muscle*	1.78		*Pectoral
					Fat	2.53		
Lepidochelys olivacea								Witkowski and Frazier (1982); Ecuador; Manta

	Adult		—	3	Bone*	7.2–38.0; 8.4–35.67	ASH: M; RM; **	*Humerus; **3 replicate measures
Lepidochelys olivacea	—							Sahoo et al. (1996); India; Gahirmatha, Orissa
	Environment			8	Beach sand	158–325	DRY: R*	*mg/g
	Egg*		—	24**	Shell	3.6		*Fresh; *8 nests, 3 eggs per nest
					Albumen-yolk	4.3		
	Egg*				Shell	5.3		*Posthatch
	Hatchling*				Whole body	23.6		*Fresh
Lepidochelys olivacea								Gardner et al. (2006); Mexico; Baja California peninsula; turtles died from fisheries capture
	MSCL = 60.1; 53.0–66.0 cm	M; R		6	Liver	0.1; ND (<0.002)–9.2	DRY: GM; R	
					Kidney	5.31; 3.93–7.52		
					Muscle*	0.77; ND (<0.002)–4.34		*Pectoral
					Fat	2.1; 0.88–3.65		
Malaclemys terrapin							WET: M	Burger (2002); USA; New Jersey; Barnegat Bay
	Adult L = 14.3 cm	M	F	11				
					Liver	2.750		
					Muscle	0.665		
			—	8	Egg*	0.248; 0.014–0.502	M; R	*Ovarial
Trachemys scripta								Burger and Gibbons (1998); USA; South Carolina; Savannah River site near Aiken; year: 1996; *eggs laid in the laboratory 2 to 3 days after females caught in the field
	Egg		—	16*	Contents	4.477; 8.224	DRY: M; MAX	*16 clutches, 1 egg per clutch
					Shells	3.490; 4.989		

(*continued*)

TABLE A.14 (CONTINUED)
Manganese (Mn)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
							Statistical analysis: [Mn] concentrations were not significantly different between egg compartments
Trachemys scripta elegans							Tryfonas et al. (2006); USA; Illinois; Lower Illinois River near Grafton; eggs laid in the lab from turtles collected in the field from 5 nesting areas
						DRY: M*	*All sites combined
	Egg		—	Contents	1.1	**	**Values estimated from graphs
				Shell	1.0	**	**From graph
	Diet			*Lemna* sp.	3100		
	Environment		4–5*	Soil	420–481*	RM	*From 2 sites, soil from nesting and lake bank areas
			3*	Sediment	200–510*	**	*From 2 sites, 3 sediment layers per site; **from graph
			3–4*	Water	0.10–0.56*	WET: RM	*From 3 sites
Crocodylia							
Alligator mississippiensis							Heinz et al. (1991); USA; Florida; central and southern Florida; Lake Okeechobee, Lake Griffin, and Lake Apopka
	Egg	—	32*	Egg	0.14–0.15; 0.10–0.26	WET: RM; R**	*16 nests, 2 eggs per nest; **combined by lake
Alligator mississippiensis							Burger et al. (2000); USA; Florida; Lake Apoka (Lake and Orange Counties), Orange Lake (Alachua County), and Lake Woodruff (Volusia County)
	Yearling R L = 36–40 cm					WET: M*	*3 lakes combined
			30	Fat*	0.320		*Abdominal
			31	Liver	1.220		
			30	Muscle*	0.300		*Abdominal
			29	Skin*	0.383		*Abdominal

			29	Muscle*	0.706		*Proximal ventral tail
			22	Tail tip	0.918		
			3	Tail*	3.120		*Regenerated
Alligator mississippiensis							Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	—	—	1	Liver	0.10	WET	
				Kidney	2.83		
Alligator sinensis							Xu et al. (2006); China; Changxing County, Zhejiang Province; Changxing Nature Reserve and Breeding Research Center for Chinese alligator
						DRY	
	Adult	F/M	1/1	Heart	1.84/1.42		Alligators collected dead of unidentified causes
				Lung	0.82/1.66		
				Liver	5.40/4.74		
				Stomach	0.96/1.28		
				Kidney	5.10/4.66		
				Intestine	1.67/1.52		
				Trachea	2.05/1.94		
				Pancreas	0.58/0.67		
				Gonad	1.86/0.42		
				Muscle	3.60/1.76		
	Diet	—	—	Fish	8.26	DRY M; R	Two fish prey species randomly sampled
	Excrements	—	6	Feces	157.1		
	Egg		10*				*10 eggs from 3 clutches
				Shell	14.21; 9.28–16.73		
				Membranes	10.32; 8.80–12.61		
				Contents	1.68; 1.22–2.05		
	Environment		10*				*Water and sediment (5 subsamples from different sites within a pond each) collected from 10 breeding ponds
				Sediment	385.9		

(*continued*)

TABLE A.14 (CONTINUED)
Manganese (Mn)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
					Water	0.01214	WET: M	
								Statistical analysis: [Mn] concentrations were significantly higher in shells and membranes than in contents; concentrations in membranes were significantly higher
Crocodylus johnstoni							DRY: M; R	Jeffree et al. (2001); northern Australia; northcentral Queensland, Lynd River; samples from a single population
	A = 0.7–62.7 years L = 24.7–128.3 cm	R	F/M	9/21	Osteoderm*	17.1; 8.6–23.5		*Ventral pelvic region
Crocodylus niloticus								Swanepoel et al. (2000); South Africa; Kruger National Park (KNP) area
	TL = 1.40–4.15 m	R	F/M	6/9			DRY: M	
								Site 1: Shingwedzi River (Silwervis Dam), northern part of KNP; catchment area outside KNP is limited
					Muscle	17.8		Frozen tissues used for residue analysis
					Liver	15.5		
					Kidney	17.6		
					Fat	18.7		
								Site 2: Olifants River, central part of KNP; flows through areas of intense agricultural activity and passes mining areas and receives tributaries from Phalaborwa Mining Company before entering the KNP
					Muscle	0.1		Frozen tissues used for residue analysis
					Liver	0.1		

					Kidney	0.12		
					Fat	0.1		
					Liver	2.20		Formalinized tissues used for residue analysis
					Kidney	2.20		
								Site 3: Sabi River, southern part of KNP; flows through areas of intense agricultural activity before entering the KNP
					Muscle	0.1		Frozen tissues used for residue analysis
					Liver	0.1		
					Kidney	0.2		
					Fat	0.2		
					Liver	2.10		Formalinized tissues used for residue analysis
					Kidney	2.5		
Crocodylus niloticus								Almli et al. (2005); Zambia
	L = 2.7–3.4 m	R	F/M	2/2	Liver	1.40; 1.10–2.90	WET: MD; R	Site 1: Kafue River, Kafue National Park
					Kidney	2.10; 2.00–2.20		
	L = 2.0–4.0 m		F/M	4/1	Liver	1.10; 0.70–1.40		Site 2: Luangwa River, Luangwa National Park
					Kidney	1.10; 0.84–1.60		
Crocodylus porosus								Yoshinaga et al. (1992); Papua New Guinea
	—		—	—	Muscle	0.77	WET: M	
Crocodylus porosus							DRY: GM; R	Jeffree et al. (2001); northern Australia; Alligator Rivers region; Kakadu National Park; 3 river catchments, mining and hunting areas included
	A = 5–40 years							
	L = 168–499 cm	R	—	40				
			—	35	Muscle*	0.706; 0.350–1.30		*Tail
			—	40	Osteoderm*	2.63; 0.190–13.4		*Ventral pelvic region

(*continued*)

TABLE A.14 (CONTINUED)
Manganese (Mn)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Squamata: Sauria								
"Lizard"								Hsu et al. (2006); southern Taiwan; Kenting National Park; strong influence from industrial pollution
	—		—	1	Whole body	319	DRY	
						7.41		Bioconcentration factor (reference media: soil and food items)
*Norops sagrei**								Burger et al. (2004); USA; Florida; southern Florida and Florida Keys; *[*Anolis sagrei*]
	Adult							
							WET: M; RM; R*	*6 sites combined
	SVL = 43.5; 35–50 mm	M; R	F	72	Whole body	1.943; 0.874–5.762; 0.507–33.792		*Minus gut content
	SVL = 55.3; 45–67 mm		M	72		0.922; 0.466–2.549; 0.203–5.839		
*Laudakia s. stellio**								Loumbourdis (1997); Greece; Thessaloniki region; *[*Agama s. stellio*]
	Adult		—	—	Liver	41.02	DRY: M	Urban area (500 m asl)
					Carcass	40.71		
					Liver	52.03		Agricultural area (50 m asl)
					Carcass	61.09		
*Podarcis taurica**								Sharygin et al. (1979/80); Crimean mountains between Yalta and Alushta (400 and 900 m); * [*Lacerta taurica*]
	—		—	—	Whole body	900	MAX	

Tarentola mauritanica							Fletcher et al. (2006); southern Spain; Guadiamar River Valley; mine tailings release event; Boliden-Apirsa mine at Aznalcóllar; 7 study sites spanning an expected contamination gradient; geckos collected from building walls
	Juvenile and adult		52	Whole body*		DRY: M; R	*Minus gut contents
		—	9		1.299; 0.872–2.000		Site 1: Rural not mine-affected site: near Guadalmellato (most pristine)
		—	13		1.770; 0.840–3.561		Site 2: Urban not mine-affected site: Villaviciosa de Cordoba (not contaminated by mining)
		—	8		1.057; 0.750–1.676		Site 3: Urban mine-affected site: Aznalcázar (contamination through aerosolized contaminants engulfed the town during cleanup)
		—	8		1.679; 0.672–2.761		Site 4: Urban mine-affected site: Aznalcóllar (contaminated by normal mine operations, or the disaster and subsequent remediation efforts)
		—	5		1.549; 0.761–2.567		Site 5: Floodplain mine-affected site: Guadiamar River floodplain near the Aznalcázar gauge station (24.8 km below the ruptured tailings dam)
		—	5		1.423; 0.946–2.206		Site 6: Floodplain mine-affected site: Guadiamar River floodplain near the Guijo gauge station (7.4 km below the ruptured dam)
		—	4		2.466; 1.837–2.889		Site 7: Floodplain mine-affected site: Agrio River floodplain (4.4 km below and closest to the ruptured tailings dam)
							Statistical analysis: [Mn] concentration was not significantly influenced by site
Varanus sp.							Yoshinaga et al. (1992); Papua New Guinea
	—	—	—	Muscle	0.56	WET: M	

(*continued*)

TABLE A.14 (CONTINUED)
Manganese (Mn)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Squamata: Serpentes								
"Snake"								Hsu et al. (2006); southern Taiwan; Kenting National Park; strong influence from industrial pollution
	—		—	12	Whole body	83.2; 1.30–287	DRY: M; R	
						1.93		Bioconcentration factor (reference media: soil and food items)
Acrochordus javanicus	—		—	—	Muscle	0.57	WET: M	Yoshinaga et al. (1992); Papua New Guinea
Agkistrodon piscivorus								Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 58.6 cm TM = 365 g	M	—	6	Liver	0.62	WET	
					Kidney	1.61		
Agkistrodon piscivorous			—					Burger et al. (2006); USA; South Carolina; 1 reference site; 1 polluted Savannah River site, Aiken
	SVL = 57cm TM = 280 g	M		13	Muscle*	19	DRY: M**	*Tail; **both sites combined
	SVL = 60 cm TM = 270 g	M		5	Blood	0.12		
Coluber constrictor								Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 103.2 cm TM = 300 g		—	1	Liver	1.00	WET	
					Kidney	1.78		

Eunectes murinus								Calle et al. (1994); Venezuela
	L = 2.1–5.1 m W = 3.5–74.0 kg	R	F/M	7/5	Blood*	0.07; 0.06–0.08	WET: M; R	*Plasma
Nerodia cyclopion								Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 63.9 cm TM = 191 g		—	1	Liver	0.95	WET	
					Kidney	2.01		
Nerodia fasciata			—					Burger et al. (2006); USA; South Carolina; 1 reference site, 1 polluted Savannah River site, Aiken
	SVL = 51 cm TM = 130±16; 100 g	M		47	Muscle*	66	DRY: M**	*Tail; **both sites combined
	SVL = 52 cm TM = 140 g			34	Blood	0.17		
Nerodia fasciata								Burger et al. (2007); USA; South Carolina; relatively rural site
	Adult	—	—	34	Blood	0.170	WET : M	
				47	Muscle	20.442		
				5	Liver	2.173		
Nerodia sipedon								Burger et al. (2005); USA; eastern Tennessee; 1 reference site: Little River downstream from the Great Smoky Mountains National Park near Townsend; 1 polluted Superfund site: EFPC inside the US Department of Energy's (USDOE) Y-12 National Security Complex
	Adult		—				WET: M*	*Both sites combined
				47	Kidney	0.786		
				47	Liver	1.304		

(*continued*)

TABLE A.14 (CONTINUED)
Manganese (Mn)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
				47	Muscle	4.286		
				47	Skin	2.197		
				46	Blood	0.135		
				3	Testis	0.706		
	Egg			8	Egg*	4.941		*Ovarial Statistic analysis: [Mn] concentrations were significantly highest in the eggs and muscle
Nerodia sipedon								Campbell et al. (2005); USA; eastern Tennessee
	Adult						WET: M; R	
	SVL = 65; 44.5–80 mm TM = 235; 53–464 g TM = 103; 74–146 g SVL = 53; 47–60 mm	M;R	21 F 26 M					
			F/M	11/16	Blood	0.120; 0.026–0.385		Site 1: Reference site: Little River downstream from the Great Smoky Mountains National Park
					Kidney	0.628; 0.201–1.178		
					Liver	1.008; 0.252–2.116		
					Muscle	4.473; 1.107–13.649		
					Skin	1.134; 0.258–2.467		
			F/M	10/10	Blood	0.155; 0.039–0.546		Site 2: Polluted Superfund site: upper reach of East Fork Poplar Creek (EFPC) within the US Department of Energy's (USDOE) Y-12 National Security Complex
					Kidney	1.000; 0.053–3.729		

					Liver	1.703; 0.630–3.098		
					Muscle	4.560; 0.982–14.602		
					Skin	3.557; 0.350–8.553		
Nerodia sipedon								Burger et al. (2007); USA; New Jersey and Tennessee
	Adult TL = 72; 39.0–103.5 cm TM = 159; 17.5–587.5 g	M; R	—	18	Blood	0.104	WET: M	Site 1: Urban/suburban; New Jersey
					Kidney	1.025		
					Liver	1.897		
					Muscle	7.010		
					Skin	2.232		
				36	Blood	0.135		Site 2: Relatively rural; Tennessee
				46	Muscle	4.286		
				47	Liver	1.304		
Nerodia taxispilota								Burger et al. (2006); USA; South Carolina; 1 reference site; 1 polluted Savannah River site, Aiken
	SVL = 59 cm TM = 170 g	M	—	10	Muscle*	88	DRY: M**	*Tail; **both sites combined
	SVL = 65 cm TM = 230 g			9	Blood	0.2		
Pituophis melanoleucus							DRY: M; RM*	Burger (1992); USA; New Jersey; 4 sites, 4 years
	Hatchling TM = 24.65 g	M		46	Skin	3.840; 1.440–8.342		*Combined by year
				16	Whole body*	15.039; 11.921–17.083		*Saggital section from the center of the body, including bone

TABLE A.15
Mercury (Hg)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Testudines								
*Actinemys marmorata**								Henny et al. (2003); USA; western Oregon; Fern Ridge Reservoir; *[*Clemmys marmorata*]
	Egg		—	14*	Contents	ND (<0.25)–0.54	DRY: R**	*14 nests, 1 egg per nest; **Hg found above detection limit in only 4 eggs Statistical analysis: No significant differences in contaminant concentrations related to hatching rate
Caretta caretta			—	—				Hillestad et al. (1974); USA; Georgia and South Carolina; 3 nesting beaches
	Egg				Yolk	0.02–0.09	—: R	
					Albumen	0.01–0.03		
Caretta caretta								Stoneburner et al. (1980); USA; 4 western Atlantic nesting beaches: Florida, Canaveral; Georgia, Cumberland Island; North Carolina, Cape Lookout; North Carolina, Cape Hatteras; *combined by beach
	Egg*		—	96	Yolk	0.4123–1.3912	—: RM*	*Fresh
Caretta caretta								Sakai et al. (1995); Japan; Kochi Prefecture, Cape Ashizuri
	L = 76–92 cm W = 75–108kg	R	F/M	6/1	Liver	1.51; 0.253–8.15	WET: M; R	
					Kidney	0.247; 0.040–0.441		
					Muscle*	0.108; 0.053–0.189		*Pectoral
	Egg*		F	5	Shell	0.0040; 0.0020–0.0054		*Oviductal
					Yolk	0.0121; 0.0080–0.0158		
					Albumen	0.0005; 0.0001–0.0008		

					Whole egg	0.005; 0.0038–0.0074		Whole egg concentrations calculated as a summation of the products of concentrations and weights of each part
Caretta caretta								Gordon et al. (1998); Australia; southeastern Queensland, Moreton Bay region; stranded turtles
	—	—	—	6	Liver	0.015; 0.00–0.032	WET: M; R	
				3	Kidney	0.045; 0.033–0.067		
Caretta caretta								Storelli et al. (1998a); Italy; Adriatic Sea, Apulian coasts; stranded turtles
							DRY: M; R	
	TM = 1.8–100 kg	R	F/M	9/3	Liver	1.68; 0.35–3.72	Total Hg	
					Lung	0.45; 0.12–0.97		
					Kidney	0.65; 0.30–1.53		
					Muscle	0.69; 0.17–1.81		
Caretta caretta								Torrent et al. (2004); Spain; Canary Islands; stranded turtles submitted to the veterinary faculty of the University of Las Palmas in Gran Canaria
	Juvenile and subadult SCL = 15–65 cm	R*	F/M	67/11	Kidney	0.04; 0.01–0.33	WET: M; R	*Values estimated from graph
					Liver	0.04; 0.001–0.47		
Caretta caretta								Storelli et al. (1998a) from Storelli et al. (2005); dry mass-based data from Storelli et al. (1998a) converted to wet mass
					Liver	0.49; 0.10–1.09	WET: M; R	
					Kidney	0.20; 0.09–0.47		
					Muscle	0.17; 0.04–0.45		
Caretta caretta								Storelli et al. (1998b); Italy; Adriatic Sea, Apulian coasts; stranded turtles
	TM = 6.7–18 kg	R		7			DRY: M; R	

(*continued*)

TABLE A.15 (CONTINUED)
Mercury (Hg)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
				Muscle	0.06; 0.02–0.15	Total Hg	
				Liver	0.70; 0.37–1.10		
						Methylated Hg	
				Muscle	0.23; ND (<0.02)–0.41		
				Liver	0.28; 0.24–0.33		
				Muscle	79.75; 55.00–95.00	% methylated Hg	
				Liver	46.45; 27.27–64.86		
Caretta caretta							Storelli et al. (1998b) from Storelli et al. (2005); dry mass-based data from Storelli et al. (1998b) converted to wet mass
			7	Liver	0.70; 0.37–1.10	WET: M; R	
				Muscle	0.21; 0.07–0.43		
Caretta caretta			7				Godley et al. (1999); northern Cyprus; eastern Mediterranean Sea, Alagadi beach; stranded turtles
	CCL = 63.5; 56.0–79.0 cm	M; R	5	Liver	2.41; 0.82–7.50	DRY: MD; R	
			2	Kidney	0.47; 0.13–0.80		
			7	Muscle	0.48; ND (<0.01)–1.78		
	Hatched nests*		48				*1 sample (a dead hatchling, dead embryo. or undeveloped egg) taken per nest
			16	Hatchling	0.02; ND (<0.01)–0.75		
			27	Embryo	0.01; ND (<0.01)–0.22		
	Egg		3	Yolk and albumen	0.19; 0.16–0.57		

Caretta caretta							Sakai et al. (2000b); Japan; Kochi Prefecture, Cape Ashizuri; turtles accidentally caught in commercial fishing nets
	TM = 93/83 kg						
	SCL = 83/85 cm	M	F/M	6/1	Liver	0.400/8.150	WET: M
					Gullet	0.0491/0.193	
					Stomach	0.0362/—	
					Intestine	0.0491/0.102	
					Pancreas	0.102/1.990	
					Heart	0.0922/—	
					Trachea	0.0103/0.0337	
					Lung	0.0397/0.127	
					Bladder	0.0370/0.0821	
					Spleen	0.0557/0.289	
					Kidney	0.237/0.304	
					Salt gland	0.0533/0.216	
					Brain	0.0387	
	Reproductive tissues				Testis	—	
					Oviduct	0.0220	
					Ovary	0.0175	
	Egg*				Whole egg	0.00554	*Oviductal
					Shell	0.00405	
					Yolk	0.0121	
					Albumen	0.00049	
					Scale	0.0352/0.279	
					Mesentary	0.0430/0.0342	
					Fat	0.00559/0.0372	
					Muscle	0.0944/0.189	
					Bone	0.00727/0.0140	
					Carapace	0.0432/0.159*	*Hg values taken as ng/g in Table 2 as in Table 3 of original publication.

(*continued*)

TABLE A.15 (CONTINUED)
Mercury (Hg)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
					Whole body*	0.0555/0.359		*Calculated
Caretta caretta								Kaska and Furness (2001); southwestern Turkey; 4 beaches; collected just before hatching or dead in shell 1 week after last hatching
	Embryo		—	22	Liver	0.51	DRY: M	
	Egg		—		Shell	ND (<—)		
					Yolk	ND (<—)		
								Statistical analysis: [Hg] liver concentrations were significantly different between beaches and nest regions (top vs. bottom)
Caretta caretta								Maffucci et al. (2005); southern Italy; western Mediterranean Sea; South Tyrrhenian coasts; stranded turtles
	CCL = 37–82 cm	R	—	29				
				22	Liver	1.10; 0.42–8.76	DRY: M; R	
				20	Kidney	0.90; 0.37–3.41		
				26	Muscle	0.40; 0.14–1.92		
Caretta caretta								Day et al. (2005) from Bergeron et al. (2007); USA; coastal waters between South Carolina and Florida
					Blood	0.057–0.141	WET: R Total Hg	
Caretta caretta								Storelli et al. (2005); Italy; Adriatic and Ionian Seas; stranded turtles
	SCL = 21–71 cm	R		19	Liver	0.43; 0.13–1.26	WET: M; R	
					Kidney	0.16; 0.06–0.31		
					Muscle	0.18; 0.03–0.66		

					Spleen	0.11; ND (<0.00002)–0.19		
					Heart	0.12; 0.04–0.26		
					Lung	0.06; ND (<0.00002)–0.15		
					Fat	0.04; ND (<0.00002)–0.09		
Chelonia sp.								Yoshinaga et al. (1992); Papua New Guinea
	—		—	—			WET: M	
					Muscle	0.038	Total Hg	
						0.023	Organic Hg	
						0.015	Inorganic Hg	
Chelonia mydas								Yoshinaga et al. (1992); Papua New Guinea
							WET: M	
	—		—	—	Muscle	0.002	Total Hg	
						0.002	Organic Hg	
						ND (<0.0005)	Inorganic Hg	
Chelonia mydas								Gladstone (1996) from Gordon et al. (1998); Australia, Torres Strait
	—	—	—	7	Liver	0.08; 0.02–0.17	WET: M; R	
					Kidney	0.02; 0.01–0.04		
Chelonia mydas								Gordon et al. (1998); Australia; southeastern Queensland, Moreton Bay region; stranded turtles
	—	—	—	23	Liver	0.021; 0.00–0.052	WET: M; R	
				23	Kidney	0.020; 0.00–0.049		
Chelonia mydas								Godley et al. (1999); northern Cyprus; eastern Mediterranean Sea, Alagadi beach; stranded turtles
	CCL = 49.5; 27.5–56.0 cm			6	Liver	0.55; 0.27–1.37	DRY: MD; R	

(*continued*)

TABLE A.15 (CONTINUED)
Mercury (Hg)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
				1	Kidney	ND (<0.01)		
				5	Muscle	0.09; ND (<0.01)–0.37		
	Hatched nests*			69				*1 sample (a dead hatchling, dead embryo, or undeveloped egg) taken per nest
				24	Hatchling	ND (<0.01); ND (<0.01)–0.24		
				18	Embryo	ND (<0.01); ND (<0.01)–0.12		
	Egg			17	Yolk and albumen	ND (<0.01); ND (<0.01)–0.19		
Chelonia mydas								Sakai et al. (2000a); Japan; Yaeyama Islands, Okinawa; turtles caught by fishermen for commercial use
	SCL = 51.0 cm	M	—	50			WET: M; R	
				46/46*	Liver	0.287; 0.053–0.64		*Detected/analyzed
				21/21	Kidney	0.132; 0.029–0.248		
				46/46	Muscle	0.019; 0.001–0.119		
Chelonia mydas								Sakai et al. (2000b); Japan; Ogasawara Islands (Bonin Islands), Haha-Jima Island; turtles collected in coastal waters
	TM = 124/117 kg							
	SCL = 93/97 cm	M	F/M	1/1	Liver	0.301/0.0767	WET: M	CW = 72/73 cm
					Gullet	0.0491/0.00577		
					Stomach	—		
					Intestine	0.00754/0.00739		
					Pancreas	—/0.0256		
					Heart	—		
					Trachea	0.00245/0.00182		

					Lung	0.00271/0.00225		
					Bladder	0.00416/0.00377		
					Spleen	0.00689/0.00585		
					Kidney	0.0422/0.0478		
					Salt gland	0.00671/0.00638		
					Brain	—		
	Reproductive tissues				Testis	0.00950		
					Oviduct	0.00461		
					Ovary	0.00472		
	Egg				Whole egg	0.00135		
					Shell	0.00120		
					Yolk	0.00251		
					Albumen	0.00005		
					Scale	0.00511/0.00281		
					Mesentary	0.00265/0.00149		
					Fat	0.00242/0.00281		
					Muscle	0.00694/0.00213		
					Bone	0.00201/0.00215		
					Carapace	0.00279/0.00203		
					Whole body*	0.0124/0.00402		*Calculated
Chelonia mydas								Anan et al. (2001); Japan, Yaeyama Islands
	SCL = 49.0; 40.0–63.5 cm	M; R	M	6				
	SCL = 52.2; 37.0–71.4		F	20				
			M	6	Liver	0.51; 0.31–0.67	DRY: M; R	
				6	Kidney	0.38; 0.33–0.43		
				3	Muscle	0.11; ND (<0.05)–0.23		
			F	20	Liver	0.40; 0.23–0.88		
				19	Kidney	0.27; 0.11–0.55		

(*continued*)

TABLE A.15 (CONTINUED)
Mercury (Hg)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
			9	Muscle	0.01; ND (<0.05)–0.11		
	Diet	—	8	Stomach*	0.03; ND (<0.05)–0.05		*Contents
Chelonia mydas							Lam et al. (2004); southern China
	Juvenile	—	2	Fat	0.004776	DRY: M	Turtles stranded on beaches at Ham Tin Wan and Tung Pang Chau; upon collection, turtles had started to decay
				Kidney	0.3417		
				Heart	0.2164		
				Liver	0.7806		
				Lung	0.2002		
				Muscle	0.4256		
				Stomach	0.2990		
	Adult	—	3	Muscle	0.05252		Turtles believed to have been unintentionally caught by fishermen; upon collection, turtles were relatively fresh
			1	Liver	0.1257		
Chelydra serpentina							Helwig and Hora (1983); USA; Minnesota; 4 river sites, 2 lake sites
	TL = 14–38 cm	F/M/U	4/10/2				
	TM = 0.8–9.9 kg						
			17	Muscle*	0.145; 0.05–0.30	—: M; R	*Leg
			15	Fat body	0.024; ND (<0.02)–0.04		
Chelydra serpentina							Albers et al. (1986); USA; Maryland; Patuxent Wildlife Research Center; undisturbed freshwater site

	Adult TL = 20–40 cm	R*	M	7	Liver	0.90	WET: M	*Values estimated from graph
			F	6		0.46		
			M	7	Kidney	0.44		
			F	6		0.56		
			M	8	Liver	1.28		USA; New Jersey, Hackensack Meadowlands; contaminated brackish water site
			F	3		1.27		
			M	8	Kidney	0.55		
			F	3		0.41		
			M	8	Liver	0.60		USA; New Jersey, Hackensack Meadowlands; contaminated freshwater site
					Kidney	0.39		
Chelydra serpentina								Meyers-Schöne (1989) from Meyers-Schöne and Walton (1994); USA; Tennessee; contaminated lake
	Adult		M	12	Kidney	1.30	WET: M	
					Muscle	0.17		
Chelydra serpentina								Meyers-Schöne et al. (1993); USA; Tennessee
	Adult		M	12	Kidney	1.30	WET: M	Site 1: Contaminated site (3–5.9 µg Hg/g dw in sediment): White Oak Lake
					Muscle	0.17		
			F/M	3/6	Kidney	0.34		Site 2: Reference site: Bearden Creek embayment
					Muscle	0.10		
Chelydra serpentina								Meyers-Schöne et al. (1993) from Meyers-Schöne and Walton (1994); USA, Tennessee; reference wetland
	Adult		M	6	Kidney	0.41	WET: M	
			F	3		0.21		
			M	6	Muscle	0.12		
			F	3		0.06		

(*continued*)

TABLE A.15 (CONTINUED)
Mercury (Hg)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
*Chelydra serpentina**							Bonin et al. (1995); Canada; St. Lawrence River (highly polluted) and Ottawa River (much less polluted); *[*Chelydra s. serpentina*]; 10 locations
	Egg	—	39	Contents	ND (<0.02)–0.445	WET: R	*39 clutches, 5 eggs per clutch pooled
*Chelydra serpentina**							Bishop et al. (1998); Canada; Great Lakes–St. Lawrence River Basin; *[*Chelydra s. serpentina*]
			39*			—: RM** Total Hg	*Overall number of clutches from 5 sites; **clutches combined by site
	Egg			Contents	ND (<0.05)–0.14		
							Statistical analysis: No significant differences in contaminant concentrations related to hatching rate
Chelydra serpentina							Golet and Haines (2001); USA; southeastern Connecticut; 5 lakes
	Adult	—	25	Muscle*	0.050–0.500	WET: R Total Hg	*Shoulder, hind leg, tail
				Blood	0.050 – 0.500		
				Liver	0.500–3.300		
				Scute*	0.500–3.300		*Marginal carapax
*Chelydra serpentina**							Ashpole et al. (2004); Canada; Great Lakes–St. Lawrence River Basin; *[*Chelydra s. serpentina*]; eggs collected within 5 hours of oviposition
	Egg		*	Contents		DRY: M Total Hg	*Number of clutches pooled per site, 5 eggs pooled per clutch
		—	10		0.110		Lake St. Clair (1 site)
		—	9		0.050		Lake Ontario (1 site)
		—	9		0.090		St. Lawrence River north shore (2 sites)
		—	4		0.250		
		—	9		0.090		St. Lawrence River south shore (3 sites)
		—	5		0.720		

Chelydra serpentina	—						Bergeron et al. (2007); USA; Virginia
				Blood		WET: M*	*Values taken from graph
						Total Hg	Hg contaminated site (7 subsites); South River reference sites; South River and Middle River
		—	99		~ 0.975		
	—	—	53		~ <0.050		
Chelydra serpentina *Chrysemys picta* *Sternotherus odoratus*							Bergeron et al. (2007); USA; Virginia; South River (Hg contaminated site with 7 subsites; 1 reference site), Middle River (1 reference site)
	—	—	—	Blood		WET	
					3.600	MAX Total Hg	Contaminated reaches of South River
Chrysemys picta							Bergeron et al. (2007); USA; Virginia
				Blood		WET: M* Total Hg	*Values taken from graph
	—	—	170		~ 0.450		Hg contaminated site (7 subsites); South River
	—	—	106		~ <0.050		Reference sites; South River and Middle River
Dermochelys coriacea		M	1				Davenport and Wrench (1990), Davenport et al. (1990); Great Britain; Wales, Cardigan Bay, Irish Sea; stranded turtle
	TL = 2.53 m						
	TM = 916 kg			Liver	0.39	DRY: M*	*4 replicate measures
				Muscle*	0.12		*Pectoral
				Blubber	0.11		
Dermochelys coriaca							Godley et al. (1998); Great Britain; Wales and Scotland, west coast; turtles died in fishing gear
	Adult CCL = 141–170 cm	M	3	Liver	0.29–1.2	DRY: R	
				Muscle	0.04–0.29		

(*continued*)

TABLE A.15 (CONTINUED)
Mercury (Hg)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
			1	Liver	0.37	WET	
				Muscle	0.013		
Emys orbicularis	—	—					Srebocan et al. (1981); Yugoslavia; Crna Mlaka, bird reserve
			8	Mud	0.564; 0.479–0.693	DRY: M; R	
			25	Liver	3.493; 0.273–8.765	WET: M; R	
			25	Muscle	0.163; 0.055–0.360		
Emys orbicularis							Swartz et al. (2003); Azerbaijan; Apsheron Peninsula
	—	—	5	Liver	19.9	DRY: MD Total Hg	Site 1: Heavily contaminated wetland receiving runoff from nearby industrial wastewater treatment plant and cooling towers of the city of Sumgayit
	Environment	—	—	Sediment	2.7		
	—						Site 2: Uncontaminated ponds at a fish hatchery near Ali Bairamly (about 150 km south of Sumgayit)
		—	8	Liver	1.59		
Eretmochelys imbricata							Gordon et al. (1998); Australia; southeastern Queensland, Moreton Bay region; stranded turtles
	— —	—	2	Liver	0.036–0.048	WET: R	
			2	Kidney	0.034–0.038		
Eretmochelys imbricata							Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 43.5; 33.5–48.9 cm	M	6	Liver	0.58; 0.29–1.2	DRY: M; R	
			6	Kidney	0.91; 0.66–1.2		
			1	Muscle	ND (<0.05)		
	SCL = 46.5; 43.8–67.9 cm	F	16	Liver	0.94; 0.05–8.7		
			13	Kidney	1.4; 0.08–5.0		

				8	Muscle	0.04; ND (<0.05)–0.09		
	Diet		—	6	Stomach*	0.04; ND (<0.05)–0.09		*Contents
*Gopherus agassizii**								Jacobson et al. (1991); USA; California; *[*Xerobates agassizii*]
	TL = 133–306 mm							
	TM = 390–4900 g		F/M		1/11			
			—	10	Liver	0.326	—: M	Site 1: Kern County, turtles with clinical signs of upper respiratory tract disease
	TL = 199–280 mm							
	TM = 1180–3887 g		M	4	Liver	0.0287		Site 2: San Bernardino County; clinically healthy turtles
Kinosternon flavescens								Flickinger and King (1972); USA; Texas; Warton County; Texas rice belt
	—		—	3	Whole body*	0.12	WET: M	*Minus carapace
Lepidochelys kempii								Kenyon et al. (2001); USA; Texas and Louisiana; turtles captured alive in nets at 4 beachfront sites
	SCL = 38.3; 21.6–65.8 cm	M; R	F/M/U	46/38/22*			WET: M;R	*99 wild grown plus 7 head-start female turtles
					Blood*	0.0180; 0.00050–0.0673		*Whole blood
Malaclemys terrapin								Burger (2002); USA; New Jersey; Barnegat Bay
	Adult		F	11	Liver	1.139	WET: M	
	L = 14.3 ± 0.7 cm							
					Muscle	0.172		

(*continued*)

TABLE A.15 (CONTINUED)
Mercury (Hg)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
	Egg*	—	8	Egg*	0.035; 0.021–0.053	M; R	*Ovarial
Malaclemys terrapin							Galluzzi (1981) from Albers et al. (1986); USA; New Jersey; contaminated site
	—	—	2	Liver	3.6–7.6	—: R	
				Muscle	1.1–2.4		
Malaclemys terrapin							Blanvillain et al. (2007); USA; South Carolina and Georgia; estuaries
	Adult					WET: M; R*	*2 sites combined
	CL = 15–23 cm	F					
	CL = 10–14	M					
		F/M	32/32	Blood*	0.0453; 0.0192–1.377	Total Hg	*Whole blood
				Blood*	0.0410		*Red blood cell fraction
				Blood*	0.0013		*Plasma fraction
		—	8	Scute*	0.2515; 0.1451–8.2027		*Scrapes
				BMF*	11.3	Total Hg	*Overall biomagnification factor from snails to female terrapins
					173.5	Methyl-Hg	
							Site 1: South Carolina, Ashley River; 4 areas
		—	3	Scute*	0.339; 0.202–0.433	Total Hg	*Scrapes
					0.307; 0.182–0.392	Methyl-Hg	
	Diet		20	Snail*	0.339	Total Hg	*Body tissues of salt marsh periwinkles, *Littoria irrorata*
					0.307	Methyl-Hg	
				BMF*	24.2	Total Hg	*Biomagnification factor from snails to female terrapins
					351.1	Methyl-Hg	

							Site 2: Purvis Creek, Brunswick, Georgia; near highly polluted Superfund site
		—	5	Scute*	3.863; 2.432–5.701	Total Hg	*Scrapes
					3.465; 2.259–5.067	Methyl-Hg	
	Diet		20	Snail*	0.563	Total Hg	*Body tissues of salt marsh periwinkles, *Littoria irrorata*
					0.035	Methyl-Hg	
				BMF*	5–8.8	Total Hg	*Biomagnification factor from snails to female terrapins
					82.3–148.1	Methyl-Hg	
							Statistical analyses: Site location had significant effect on [Hg] concentrations in both blood and scute; differences between biomagnification factors not analyzed
Podocnemis unifilis							Aula et al. (1994) from Eisler (2004); Brazil; Tucurui reservoir and its surrounding area in Para; animals from near active Brazilian gold mining and refining sites
	Egg	—	—	Egg	0.01; 0.007–0.02	WET: M; R*	*Total Hg
Pseudemys rubriventris							Bergeron et al. (2007); USA; Virginia
						WET: M*	*Values taken from graph; total Hg
	—	—	36	Blood	~0.050		Site 1: Hg contaminated site (7 subsites): South River
Sternotherus odoratus							Bergeron et al. (2007); USA; Virginia
				Blood		WET: M*	*Values taken from graph
	—	—	78		~0.900	Total Hg	Site 1: Hg contaminated site (7 subsites): South River
	—	—	11		~0.050		Site 2: Reference sites; South River and Middle River
Trachemys scripta							Meyers-Schöne (1989) from Meyers-Schöne and Walton (1994); USA; Tennessee; contaminated lake
	Adult	M	6	Kidney	1.11	WET: M	

(*continued*)

TABLE A.15 (CONTINUED)
Mercury (Hg)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
		F	6		0.18		
		M	6	Muscle	0.15		
		F	6		0.06		
Trachemys scripta							Meyers-Schöne et al. (1993); USA; Tennessee
	Adult	F/M	6/6	Kidney	0.64	WET: M	Site 1: Contaminated site (3–5.9 µg Hg/g dry mass in the sediment): White Oak Lake
				Muscle	0.10		
		F/M	6/6	Kidney	0.12		Site 2: Reference site: Bearden Creek embayment
				Muscle	0.03		
Trachemys scripta							Meyers-Schöne et al. (1993) from Meyers-Schöne and Walton (1994); USA; Tennessee; reference wetland
	Adult	M	6	Kidney	0.15	WET: M	
		F	6		0.09		
		M	6	Muscle	0.03		
		F	6		0.03		
Trachemys scripta							Burger and Gibbons (1998); USA; South Carolina; Savannah River site near Aiken; eggs laid in the lab 2–3 days after females caught in the field
	Egg	—	16*	Contents	0.040; 0.240	DRY: M; MAX	*16 clutches, 1 egg per clutch
				Shell	ND (<0.002)		
							Statistical analysis: [Hg] concentrations were significantly higher in egg contents than in shells
Trachemys scripta							Clark et al. (2000) USA; Texas
	—	—	14	Whole blood	0.0168	WET: GM	Site 1: Contaminated: Municipal Lake (chemical manufacturing plant)

					0.0126		Site 2: Apparently uncontaminated; Research Park Lake (parkland)
					0.0257		Site 3: Contaminated: Old River Slough (cotton and corn cultivation with intensive chemical application)
*Trachemys scripta elegans**							Flickinger and King (1972); USA; Texas; Warton County; Texas rice belt; *[*Pseudemys scripta elegans*]
	—	—	2	Whole body*	0.08	WET: M	*Minus carapace
	Egg	—	4	Egg*	ND (<—)		*Oviductal
Crocodylia							
Alligator mississippiensis							Ogden et al. (1974); USA; Florida; Shark Valley
	Egg	—	4	Contents	0.54; 0.41–0.71	WET: M; R	
Alligator mississippiensis							Delany et al. (1988); USA; Florida; 8 lakes, statewide
	L = 2.9–3.8 m	*	24	Muscle*	0.31; 0.04–0.61	WET: M; RM**	*Tail; **combined by lake
Alligator mississippiensis							Hand and Friedman (1990) from Campbell (2003); USA; Louisiana (1 site), Florida (45 sites)
				Muscle*		—: M; R	*Tail
					0.025–3.126	R	Range of M values
					0.020–2.370		Range of MIN values
					0.030–3.880		Range of MAX values
					0.030–0.920		Range of individual values (n = 1 per site)
Alligator mississippiensis							Hord et al. (1990); USA; Florida
	L = 2.0; 1.2–2.8 m	—	18	Muscle*	2.29; 0.46–3.88	—: M; R	Everglades, Water Conservation Areas; *Tail
	L = 2.0; 1.5–3.0 m		19		0.74; 0.17–2.52		Urban canals, southeast Florida

(*continued*)

TABLE A.15 (CONTINUED)
Mercury (Hg)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
	—		58		0.36; 0.13–0.90		North, central, and south Florida; meat from meat processors
Alligator mississippiensis							Heinz et al. (1991); USA; central and southern Florida; Lake Okeechobee, Lake Griffin, and Lake Apopka
	Egg	—	32*	Egg	ND (<0.03)	WET	*16 nests, 2 eggs per nest
Alligator mississippiensis							Roelke et al. (1991) from Campbell (2003); USA; southern Florida; 4 sites
				Muscle	0.32–2.96; 0.27–3.55	WET: RM; R	
Alligator mississippiensis							Roelke et al. (1991) from Rumbold et al. (2002); USA; southern Florida; Shark River Slough
	—		5	Muscle	2.96	WET: GM	
Alligator mississippiensis							Ruckel (1993); USA; Georgia; 10 collection areas
	L = 1.9–3.2 m		22	Muscle*	0.48; 0.1–1.4	WET: M; R	*Tail
Alligator mississippiensis							Facemire et al. (1995); USA; South Florida, Everglades National Park, Shark Slough
	—	—	5	Muscle	3.57	—: M	
Alligator mississippiensis							Bowles (1996) from Rainwater et al. (2002); USA; South Carolina
	Egg		10	Egg	0.01–0.02	WET: R	
Alligator mississippiensis							Heaton-Jones et al. (1997); USA; Florida
	Adult	F/M	4/8	Liver	39.99; 8.86–99.48	WET: M; R	Shark Valley Slough, Everglades
			7/5		2.52; 0.14–16.01		Alachua, Brevard, and Collier Counties, outside Everglades
			2/4		0.10; 0.06–0.16		Farm raised

	4/8	Kidney	25.85; 5.37–65.33	Shark Valley Slough, Everglades
	7/5		1.58; 0.15–9.56	Alachua, Brevard, and Collier Counties, outside Everglades
	2/4		0.09; 0.03–0.20	Farm raised
	4/8	Spleen	3.70; 1.04–13.10	Shark Valley Slough, Everglades
	7/5		0.45; 0.09–1.31	Alachua, Brevard, and Collier Counties, outside Everglades
	2/4		0.09; 0.04–0.15	Farm raised
	4/8	Muscle*	2.61; 1.11–4.28	Shark Valley Slough, Everglades; *Tail
	7/5		0.33; 0.04–1.00	Alachua, Brevard, and Collier Counties, outside Everglades
	2/4		0.10; 0.04–0.19	Farm raised
	4/8	Muscle*	2.70; 0.61–6.05	Shark Valley Slough, Everglades; *Leg
	7/5		0.28; 0.05–0.60	Alachua, Brevard, and Collier Counties, outside Everglades
	2/4		0.11; 0.03–0.20	Farm raised
	4/8	Heart	2.31; 1.21–4.62	Shark Valley Slough, Everglades
	7/5		0.30; 0.08–0.85	Alachua, Brevard, and Collier Counties, outside Everglades
	2/4		0.11; 0.07–0.16	Farm raised
	4/8	Brain	1.37; 0.52–2.50	Shark Valley Slough, Everglades
	7/5		0.16; 0.03–0.31	Alachua, Brevard, and Collier Counties, outside Everglades
	2/4		0.08; 0.03–0.11	Farm raised
	4/8	Spinal cord	1.34; 0.45–2.55	Shark Valley Slough, Everglades
	7/5		0.97; 0.06–4.98	Alachua, Brevard, and Collier Counties, outside Everglades
	2/4		0.16; 0.04–0.39	Farm raised
F	4	Ovary	0.70; 0.39–1.34	Shark Valley Slough, Everglades
F	7		1.30; 0.03–5.91	Alachua, Brevard, and Collier Counties, outside Everglades
F	2		0.12; 0.11–0.13	Farm raised
F	4	Oviduct	1.19; 0.89–1.59	Shark Valley Slough, Everglades

(*continued*)

TABLE A.15 (CONTINUED)
Mercury (Hg)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
			F	7		1.20; 0.06–5.42		Alachua, Brevard, and Collier Counties, outside Everglades
			F	2		0.20; 0.19–0.20		Farm raised
			M	8	Testis	1.17; 0.31–2.35		Shark Valley Slough, Everglades
			M	5		0.19; 0.01–0.48		Alachua, Brevard, and Collier Counties, outside Everglades
			M	4		0.18; 0.03–0.39		Farm raised
			F/M	4/8	Scales*	1.03; 0.40–1.86		Shark Valley Slough, Everglades;* Tail
				7/5		0.34; 0.04–1.10		Alachua, Brevard, and Collier Counties, outside Everglades
				2/4		0.08; 0.04–0.16		Farm raised
				4/8	Lung	0.98; 0.39–1.76		Shark Valley Slough, Everglades
				12		0.27; 0.08–0.63		Alachua, Brevard, and Collier Counties, outside Everglades
				2/4		0.10; 0.05–0.16		Farm raised
				4/8	Scales*	0.82; 0.40–1.73		Shark Valley Slough, Everglades; *Leg
				7/5		0.35; 0.06–1.04		Alachua, Brevard, and Collier Counties, outside Everglades
				2/4		0.08; 0.04–0.16		Farm raised
				4/8	Bile	0.17; ND (<0.01)–0.53		Shark Valley Slough, Everglades
				7/5		0.23; 0.03–0.59		Alachua, Brevard, and Collier Counties, outside Everglades
				2/4		0.10; ND (<0.01)–0.25		Farm raised
Alligator mississippiensis								Yanochko et al. (1997); USA; Florida and South Carolina
	L = 121–196 cm	R	F/M	8/10			DRY: M	Site 1: Everglades, Florida; 2 locations
				17	Kidney	36.42		
				18	Liver	41.09		

				18	Muscle	5.57		
				17	Dermal scute*	5.83		*Tail
	L = 81–392 cm	R	F/M/U	15/25/9				Site 2: South Carolina, Savannah River site, Par Pond; 1 location
				17	Liver	17.73		
				21	Muscle	4.08		
				39	Dermal scute*	4.58		*Tail
Alligator mississippiensis				12	Blood*	2.20	WET: M	*Whole blood
Alligator mississippiensis								Jagoe et al. (1998); southeastern USA
							DRY: M	
				18	Muscle	5.57	Total Hg	Site 1: Florida, Everglades
				18	Liver	41.03		
				17	Kidney	36.42		
				17	Scute	5.83		
				—	Claw	—		
				21	Muscle	1.58		Site 2: Central Florida, various locations
				21	Liver	14.61		
				21	Kidney	12.59		
				20	Scute	0.52		
				21	Claw	2.69		
				9	Muscle	0.80		Site 3: Georgia, Okefenokee National Wildlife Refuge
					Liver	4.30		
					Kidney	4.82		
					Scute	0.29		
					Claw	1.67		
					Bone	0.16		
					Fat	0.19		
					Spleen	0.63		
					Brain	0.46		
				17	Muscle	4.83		Site 4: South Carolina, Savannah River site
				14	Liver	14.90		

(*continued*)

TABLE A.15 (CONTINUED)
Mercury (Hg)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
				—	Kidney	—		
				39	Scute	5.14		
				—	Claw	—		
				11	Whole blood	2.19	WET	
Alligator mississippiensis								Elsey et al. (1999); USA; Louisiana; 3 regions
	L = 124–368 cm	R		42	Muscle*	0.131; 0.047–0.386	—: M; R	*Tail
	L = 124–368 cm		M	25		0.143		
	L = 142–264 cm		F	10		0.117		
Alligator mississippiensis								Burger et al. (2000); USA; Florida; Lake Apopka (Lake and Orange Counties); Orange Lake (Alachua County), and Lake Woodruff (Volusia County)
	Yearlings		—	30	Fat*	0.0487	WET : M**	*Abdominal; **3 lakes combined
	L = 36–40 cm							
				31	Liver	0.403		
				30	Muscle*	0.0756		*Abdominal
				29	Skin*	0.0558		*Abdominal
				29	Muscle*	0.0625		*Proximal ventral tail
				22	Tail tip	0.0514		
				3	Tail*	0.0580		*Regenerated
Alligator mississippiensis								Khan and Tansel (2000); USA; Florida; Everglades
								Bioconcentration factors (BCFs): Ratios (tissue concentrations/exposure concentrations) based on data from the literature; tissue concentrations from Heaton-Jones et al. (1997), Yanochko et al. (1997), Jagoe et al. (1998); exposure concentrations (Hg in the water column) from Stober et al. (1995)

	Juvenile				Liver	10.5×10^7		
	A < 4 years							
					Kidney	9.34×10^7		
					Muscle	1.43×10^7		
					Scute*	1.5×10^7		*Tail
	Adult	R			Liver	39.9×10^7		
	A = 7–14 years							
					Kidney	32.9×10^7		
					Muscle	2.87×10^7		
					Scute*	0.62×10^7		*Tail
Alligator mississippiensis								Presley et al. (2005); USA; Louisiana, New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	—		—	1	Liver	0.01	WET	
					Kidney	0.00		
Alligator mississippiensis								Rumbold et al. (2002); USA; southern Florida; Everglades
	Juvenile to subadult							Transsect through the Everglades along 5 sites (Water Conservation Areas 1, 2, 3, Everglades National Park, Big Cypress National Preserve); year of collection: 1999
	A = 6.9 years							
	TL = 154.8 cm							
	TM = 10.4 kg	M	F/M	16/12	Liver	0.6–17	WET: R	
					Muscle*	0.1–1.8	Total Hg	*Tail
								Florida Fish and Wildlife Conservation Commission (FFWCC, unpublished data); years of collection: 1992–1994
	TL = 162–221 cm	R		12	Muscle*	0.23		Lake Okeechobee
	TL = 124–193 cm			11		1.62		WCA 3A north

(*continued*)

TABLE A.15 (CONTINUED)
Mercury (Hg)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
	TL = 122–289 cm		11		1.89		WCA 3A north
Alligator sinensis							Xu et al. (2006); China; Changxing County, Zhejiang Province; Changxing Nature Reserve and Breeding Research Center for Chinese alligator; alligators collected dead of unidentified causes
	Adult	F/M	1/1	Heart	0.343/0.350	DRY	
				Lung	0.405/0.248		
				Liver	0.405/0.248		
				Stomach	0.349/0.232		
				Kidney	0.935/0.869		
				Intestine	0.409/0.389		
				Trachea	0.147/0.092		
				Pancreas	0.080/0.042		
				Gonad	0.085/0.032		
				Muscle	0.281/0.105		
	Diet	—	—	Fish*	0.102	M; R	*2 fish prey species randomly sampled
	Excrements	—	6	Feces	0.456		
	Egg		10*				*10 eggs from 3 clutches
				Shell	0.057; 0.043–0.083		
				Shell membranes	0.175; 0.145–0.213		
				Contents	0.111; 0.098–0.124		
	Environment		10*				*Water and sediment (5 subsamples from different sites within a pond each) from 10 breeding ponds
				Sediment	0.084		
				Water	0.00104	WET	
							Statistical analysis: [Hg] concentrations were significantly higher in contents than in shells; those in membranes were higher than in those in contents

Caiman crocodilus								Vermeer et al. (1974); western and coastal part of Surinam; rice-growing project
	—		—	10	Brain	0.04	WET: M	
					Liver	0.41		
Crocodylus acutus								Ogden et al. (1974); USA; Florida, Everglades, Florida Bay
	Egg		—	5	Contents	0.09; 0.07–0.14	WET: R	
Crocodylus acutus								Stoneburner and Kushlan (1984); USA; Florida; Everglades National Park, Florida Bay
	Egg		—	9	Shell	0.21	DRY: M	
					Albumen-yolk	0.66		
					Albumen-yolk	0.13	WET: M*	*Albumen-yolk dw-based concentrations transformed to ww
Crocodylus acutus								Rainwater et al. (2007); Costa Rica; Rio Grande de Tarcoles; polluted by several metals from various sources
	SVL = 155.7; 134.0–172.0 cm	M; R	F/M	1/5	Scute*	0.0935	WET: M	*1 whole caudal scute per crocodile
Crocodylus niloticus								Almli et al. (2005); Zambia
	L = 2.7–3.4 m	R	F/M	2/2			WET: MD; R	Kafue River, Kafue National Park
					Liver	3.50; 0.97–20.00		
					Kidney	0.76; 0.60–15.00		
	L = 2.0–4.0 m		F/M	4/1				Luangwa River, Luangwa National Park
					Liver	3.70; 2.20–16.00		
					Kidney	2.70; 1.30–8.70		
Crocodylus moreletii								Rainwater et al. (2002); northern Belize; eggs from undisturbed, naturally incubating nests, 8 nests from 3 locations (nonalluvial lagoons)
	Egg*						WET: M; R	*Not viable eggs

(*continued*)

TABLE A.15 (CONTINUED)
Mercury (Hg)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
			31**	Contents	0.07; ND (<0.02)–0.23*	Total Hg	*Locations combined; **31 eggs from 8 nests
					0.11; 0.02–0.21*		*Nest combined
Crocodylus moreletii							Rainwater et al. (2007); Belize; 2 sites
				Scute*		WET: M	*1 whole caudal scute per crocodile
	SVL = 89.8 ± 6.7; 65.0–129.5 cm*	F/M	5/4		0.0987		Gold Buton Lagoon
	SVL = 104.4 ± 9.6; 59.5–156.7 cm*		4/6		0.0727		New River Watershed
Crocodylus niloticus							Phelps et al. (1986); Zimbabwe; 10 samples from 8 sites
	Egg	—	26	Contents	0.226; 0.020–0.535	DRY: M; R	
Crocodylus porosus							Yoshinaga et al. (1992); Papua New Guinea
						WET: M	
	—	—	—	Muscle	0.131	Total Hg	
					0.111	Organic Hg	
					0.020	Inorganic Hg	
Paleosuchus sp.							Aula et al. (1994) from Eisler (2004); Brazil; Tucurui reservoir and its surrounding area in Para; animals from near active Brazilian gold mining and refining sites
	—	—	—	Muscle	1.9; 1.2–3.6	WET: M; R	
	—	—	—	Liver	19.0; 11.0–30.0	Total Hg	

Squamata: Sauria								
"Lizard"								Hsu et al. (2006); southern Taiwan; Kenting National Park; strong influence from industrial pollution
	—		—	1	Whole body	37.06 317.86	DRY	Bioconcentration factor (reference media: soil and food items)
Ameiva exsul								Burger et al. (1992); Puerto Rico; east and southwest coast, 3 estuaries
	—		—	—	Whole body	ND (<0.08)	WET	
*Norops sagrei**								Burger et al. (2004); USA; South Florida and Florida Keys; *[*Anolis sagrei*]
	Adult						WET: M; RM; R*	*6 sites combined
	SVL = 43.5; 35–50 mm	M; R	F	72	Whole body*	0.0988; 0.0318–0.198; 0.0025–0.695		*Minus gut content
	SVL = 55.3; 45–67 mm		M	72		0.0587; 0.0238–0.105; 0.0034–0.262		
Varanus sp.								Yoshinaga et al. (1992); Papua New Guinea
	—		—	—	Muscle	0.175 0.157 0.018	WET: M Total Hg Organic Hg Inorganic Hg	
Squamata: Serpentes								
"Snake"								Hsu et al. (2006); southern Taiwan, Kenting National Park; results indicated strong influence from industrial pollution
	—		—	12	Whole body	5.41; 0.16–23.9	DRY: M; R	

(*continued*)

TABLE A.15 (CONTINUED)
Mercury (Hg)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
						46.38		Bioconcentration factor (reference media: soil and food items)
Acrochordus javanicus								Yoshinaga et al. (1992); Papua New Guinea
							WET: M	
	—		—	—	Muscle	1.306	Total Hg	
						1.135	Organic Hg	
						0.171	Inorganic Hg	
Agkistrodon piscivorus								EAES&T (1992) from Facemire et al. (1995); USA; Alabama
	—		—	—	—	1.6	WET: MAX	
Agkistrodon piscivorus								Clark et al. (2000); USA; Texas; contaminated site: Old River Slough (cotton and corn cultivation with intensive chemical application)
	—		—	—	Blood*	0.0135	WET: GM	*Whole blood
Agkistrodon piscivorus								Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 58.6 cm							
	TM = 365 g	M	—	6	Liver	0.13	WET: M	
					Kidney	0.05		
Agkistrodon piscivorus								Rainwater et al. (2005); USA; northeastern Texas, Longhorn Army Ammunitions Plant, Superfund site; 3 contaminated drainages
	SVL = 47.5–92.5 cm	R	M	11				

	TM = 123.95–936.04 g							
	SVL = 42.6–67.2 cm		F	8				
	TM = 110.35–290.15 g							
			—	19	Liver	0.7394	WET: M	
					Kidney	0.2114		
					Tail clip	0.1631		
	Environment		—	8	Sediment	0.14321		Sediment cores collected from 2 of the drainages
				9		0.55959		
Agkistrodon piscivorous								Burger et al. (2006); USA; South Carolina; 1 reference site, 1 polluted Savannah River site, Aiken;
	SVL = 57 cm							
	TM = 280 g	M	—	13	Muscle*	0.9	DRY: M**	*Tail; **both sites combined
	SVL = 60 cm							
	TM = 270 g			5	Blood	0.1		
Coluber constrictor							WET	Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 103.2 cm		—	1	Liver	0.15		
	TN = 300 g							
					Kidney	0.11		
Natrix natrix	—		—					Srebocan et al. (1981); Yugoslavia; Crna Mlaka, Bird reserve
				8	Mud	0.564; 0.479–0.693	DRY: M; R	
				5	Muscle	0.423; 0.253–0.532		
					Liver	0.546; 0.308–0.835		
Nerodia sp.*								Winger et al. (1984); USA; Florida; Apalachicola River; *[*Natrix* sp.]
	W = 226–544 g		—	15	Whole body	0.18; 0.13–0.21	WET: M; R	Upper reaches of river

(*continued*)

TABLE A.15 (CONTINUED)
Mercury (Hg)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
	W = 272–725 g					0.29; 0.17–0.38		Lower reaches of river
Nerodia cyclopion							WET	Presley et al. (2005); USA; Louisiana, New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 63.9 cm		—	1	Liver	0.04		
	TM = 191 g							
					Kidney	0.07		
Nerodia fasciata								Burger et al. (2006); USA; South Carolina; 1 reference site, 1 polluted Savannah River site, Aiken;
	SVL = 51cm							
	TM = 130 g	M	—	47	Muscle*	0.6	DRY: M**	*Tail; **both sites combined
	SVL = 52 cm							
	M = 140 g			34	Blood	0.4		
Nerodia fasciata								Burger et al. (2007); USA; South Carolina; relatively rural site
	Adult		—	34	Blood	0.400	WET: M	
				47	Muscle	0.192		
				5	Liver	1.857		
Nerodia rhombifer								Clark et al. (2000); USA; Texas
	—		—	10	Whole blood	0.146	WET: GM	Site 1: Contaminated site: Old River Slough (cotton and corn cultivation with intensive chemical application)
						0.0613		Site 2: Reference site: private lake (pasture)
Nerodia sipedon								Heinz et al. (1980); USA; Wisconsin; Lake Michigan, Pilot Island
	SVL = 855 mm		F	1	Whole body	0.45	WET	

Nerodia sipedon								Burger et al. (2005); USA; eastern Tennessee; 1 reference site: Little River downstream from the Great Smoky Mountains National Park near Townsend; 1 polluted Superfund site: EFPC inside the the USA Department of Energy's (USDOE) Y-12 National Security Complex
	Adult		—	47	Kidney	0.696	WET: M*	*Both sites combined
				47	Liver	1.027		
				47	Muscle	0.673		
				47	Skin	0.423		
				46	Blood	0.407		
				3	Testis	0.289		
	Egg			8	Egg*	0.061		*Ovarial Statistic analysis: [Hg] concentrations were significantly highest in the liver followed by the kidney and muscle
Nerodia sipedon								Campbell et al. (2005); USA; East Tennessee
	Adult		—					
	TM = 235; 53–464 g SVL = 65 ± 2; 44.5–80 mm	M; R	21 F					
	TM = 103; 74–146 g SVL = 53; 47–60 mm		26 M					
			F/M	11/16	Blood	0.436; 0.0098–1.420	WET: M; R	Site 1: Reference site: Little River downstream from the Great Smoky Mountains National Park
					Kidney	0.382; 0.0425–0.784		
					Liver	0.750; 0.090–1.615		
					Muscle	0.741; 0.224–1.630		

(*continued*)

TABLE A.15 (CONTINUED)
Mercury (Hg)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
					Skin	0.365; 0.0905–0.917		
			F/M	10/10	Blood	0.372; 0.141–0.816		Site 2: Polluted Superfund site: upper reach of East Fork Poplar Creek (EFPC) within the USA Department of Energy's (USDOEs) Y-12 National Security Complex
					Kidney	1.121; 0.209–3.505		
					Liver	1.403; 0.220–3.795		
					Muscle	0.582; 0.0515–1.015		
					Skin	0.500; 0.0505–0.902		
Nerodia sipedon								Burger et al. (2007); USA; New Jersey and Tennessee
	Adult TL = 72; 39.0–103.5 cm TM = 159; 17.5–587.5 g	M; R						
			—	18	Blood	0.128	WET: M	Site 1: Urban/suburban; New Jersey
					Kidney	0.136		
					Liver	0.303		
					Muscle	0.357		
					Skin	0.159		
				36	Blood	0.417		Site 2: Relatively rural; Tennessee, Department of Energy site
				46	Muscle	0.671		
				47	Liver	1.024		
Nerodia taxispilota								Burger et al. (2006); USA; South Carolina; 1 reference site; 1 polluted Savannah River site, Aiken
	SVL = 59 cm TM = 170 g	M	—	10	Muscle*	0.7	DRY: M**	*Tail; **both sites combined

	SVL = 65 cm TM = 230 g		9	Blood	0.7		
*Pantherophis uttatus**							Jones and Holladay (2006); lab feeding experiment; experimental animals obtained from commercial provider (Florida, USA); fed dead mice for 34 weeks; *[*Elaphe guttata*]
	TM* = 44.6; 52.0–260.3/211.1; 107.9–353.8 g	M; R				DRY: MD; R	*Initial mass/final mass
		—	3	Shed skin	0.0287; 0.0007–0.0320		Treatment 1: Control: fed only not contaminated mice
		—	10		0.3356; 0.1122–0.8870		Treatment 2: Fed metal-injected mice enriched with a mixture of three metals (Cd, Hg, Pb) at a dose of 2 mg/kg per snake, metal, and month
Pituophis melanoleucus							Burger (1992); USA; New Jersey; 4 sites, 4 years
	Hatchling TM = 24.65g	—	46	Skin	0.280; 0.050–0.633	DRY: M; RM*	*Combined by year
			16	Whole body*	0.130; 0.050–0.272		*Saggital section from the center of the body, including bone
Thamnophis sirtalis							Dustman et al. (1972); Canada, Lake St. Clair
	—	F	3	Carcass Liver	ND (<0.10)–0.2 0.45–0.6	WET: R	
Thamnophis sirtalis							Heinz et al. (1980); USA; Wisconsin; Lake Michigan, Spider Island
	SVL = 646–752 mm	F/M	4/2	Whole body	0.14–0.41	WET: R	

TABLE A.16
Molybdenum (Mo)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Testudines								
Caretta caretta								Stoneburner et al. (1980); USA; 4 western Atlantic nesting beaches: Florida, Canaveral; Georgia, Cumberland Island; North Carolina, Cape Lookout; North Carolina, Cape Hatteras
	Egg*		—	96	Yolk	2.66–17.93	—: RM**	*Fresh; **combined by beach
*Caretta caretta**								Aguirre et al. (1994); USA; Hawaiian Islands; *[identification number 052, *Chelonia mydas* in the reference]
	"Pelagic"		—	1	Liver	0.2	WET	
Chelonia mydas								Aguirre et al. (1994); USA; Hawaiian Islands
	L = 28.7–71.3 cm							
	W = 3.2–43.6 kg		F/M	8/4	Liver	ND (<0.1)–0.6	WET: R	
					Kidney	ND (<0.1)–0.3		
	Egg*		—	3	Shell	ND (<0.1)		*Posthatch
	Hatchling				Whole body	ND (<0.1)		
Chelonia mydas								Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 49.0; 40.0–63.5 cm	M; R	M	6			DRY: M; R	
	CW = 40.5; 35.3–49.4							
	SCL = 52.2; 37.0–71.4							
	CW = 43.9; 31.7–58.5		F	20				
			M	6	Liver	0.472; 0.077–0.965		
				6	Kidney	0.499; 0.271–0.782		
				3	Muscle	0.012; 0.008–0.012		

			F	20	Liver	0.577; 0.261–1.21		
				19	Kidney	0.739; 0.301–1.53		
				9	Muscle*	0.013; 0.008–0.025		
	Diet		—	8	Stomach*	0.789; 0.262–2.25		*Contents
Chelonia mydas								Anan et al. (2002); Japan; Okinawa Prefecture, Yaeyama Islands; tissues provided by fishermen
	—	—	—	9	Liver	0.209; 0.087–0.383	WET: M; R	
Chelonia mydas								Lam et al. (2004); South China
	Juvenile		—	2	Fat	0.020	DRY: M	Specimens stranded on beaches at Ham Tin Wan and Tung Pang Chau; upon collection, turtles had started to decay
					Kidney	0.786		
					Heart	0.169		
					Liver	1.212		
					Lung	0.144		
					Muscle	0.033		
					Stomach	0.592		
	Adult		—	3	Muscle	0.096		Specimens believed to have been unintentionally caught by fishermen; upon collection, turtles were relatively fresh
				1	Liver	0.088		
Eretmochelys imbricata								Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 43.5; 33.5–48.9 CW = 36.9; 32.6–39.6	M; R	M	6	Liver	1.47; 0.663–3.02	DRY: M; R	
				6	Kidney	1.67; 0.756–2.99		
				1	Muscle	0.016		
	SCL = 46.5; 43.8–67.9		F	16	Liver	1.07; 0.196–4.09		

(*continued*)

TABLE A.16 (CONTINUED)
Molybdenum (Mo)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
	CW = 32.8; 10.9–52.3							
				13	Kidney	1.33; 0.432–2.66		
				8	Muscle	0.024; 0.011–0.052		
	Diet		—	6	Stomach*	42.7; 0.252–128		*Contents
Eretmochelys imbricata								Anan et al. (2002); Japan; Okinawa Prefecture, Yaeyama Islands; tissues provided by fishermen
	—	—	—	7	Liver	0.416; 0.227–0.664	DRY: M; R	
Crocodylia								
Alligator mississippiensis								Heinz et al. (1991); USA; central and southern Florida; Lake Okeechobee, Lake Griffin, and Lake Apopka
	Egg		—	32*	Egg	ND (<0.03)	WET	*16 nests, 2 eggs per nest
Crocodylus acutus								Stoneburner and Kushlan (1984); USA; Florida; Florida Bay, Everglades National Park
	Egg		—	9	Shell	25.43	DRY: M	
					Albumen-yolk	2.37		
Crocodilus niloticus								Almli et al. (2005); Zambia
	L = 2.7–3.4 m	R	F/M	2/2			WET: MD; R	Site 1: Kafue River, Kafue National Park
					Liver	0.15; 0.10–0.17		
					Kidney	0.21; 0.18–0.27		
	L = 2.0–4.0 m		F/M	4/1				Site 2: Luangwa River, Luangwa National Park
					Liver	0.17; 0.11–0.20		
					Kidney	0.16; 0.12–0.24		

Squamata: Sauria							
*Laudakia s. stellio**							Loumbourdis (1997); Greece; Thessaloniki region; *[*Agama s. stellio*]
	Adult	—	—	Liver	8.32	DRY: M	Site 1: Urban area (500 m asl)
				Carcass	1.24		
				Liver	7.51		Site 2: Agricultural area (50 m asl)
				Carcass	1.39		
Tarentola mauritanica							Fletcher et al. (2006); Southern Spain, Guadiamar River Valley, mine tailings release event; Boliden-Apirsa mine at Aznalcóllar; 7 study sites spanning an expected contamination gradient; geckos collected from building walls
	Juvenile and adult		52	Whole body*		DRY: M; R	*Minus gut contents
		—	9		0.132; 0.071–0.217		Site 1: Rural not mine-affected site: near Guadalmellato (most pristine)
		—	13		0.142; 0.071–0.258		Site 2: Urban not mine-affected site: Villaviciosa de Cordoba (not contaminated by mining)
		—	8		0.152; 0.071–0.288		Site 3: Urban mine-affected site: Aznalcázar (contamination through aerosolized contaminants engulfed the town during cleanup)
		—	8		0.184; 0.071–0.229		Site 4: Urban mine-affected site: Aznalcóllar (contaminated by normal mine operations, or the disaster and subsequent remediation efforts)
		—	5		0.161; 0.101–0.208		Site 5: Floodplain mine-affected site: Guadiamar River floodplain near the Aznalcázar gauge station (24.8 km below the ruptured tailings dam)
		—	5		0.137; 0.071–0.205		Site 6: Floodplain mine-affected site: Guadiamar River floodplain near the Guijo gauge station (7.4 km below the ruptured dam)
		—	4		0.114; 0.071–0.171		Site 7: Floodplain mine-affected site: Agrio River floodplain (4.4 km below and closest to the ruptured tailings dam)
							Statistical analysis: [Mo] concentration was not significantly influenced by site

TABLE A.17
Nickel (Ni)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Testudines								
*Actinemys marmorata**								Henny et al. (2003); USA; western Oregon; Fern Ridge Reservoir; *[*Clemmys marmorata*]
	Egg		—	14*	Contents	2.41; 0.56–27.7	DRY: GM; R	*14 nests, 1 egg per nest
								Statistical analysis: No significant differences in contaminant concentrations related to hatching rate
Caretta caretta								Stoneburn et al. (1980); USA; 4 western Atlantic nesting beaches: Florida, Canaveral; Georgia, Cumberland Island; North Carolina, Cape Lookout; North Carolina, Cape Hatteras
	Egg*		—	96	Yolk	ND (<—)–2.28	—: RM**	*Fresh; **combined by beach
*Caretta caretta**								Aguirre et al. (1994); USA; Hawaiian Islands; *[identification number 052, *Chelonia mydas* in the reference]
	"Pelagic"		—	1	Liver	ND (<0.4)	WET	
Caretta caretta								Sakai et al. (1995); Japan; Kochi Prefecture, Cape Ashizuri
	L = 76–92 cm W = 75–108 kg	R	F/M	6/1	Liver	ND (<0.03)	WET: R	
					Kidney	ND (<0.03)–0.27		
					Muscle*	ND (<0.03)		*Pectoral
	Egg*		—	*	Shell	ND (<0.03)		*Oviductal eggs from 5 females
					Yolk	ND (<0.03)		
					Albumen	ND (<0.03)		
Caretta caretta								Sakai et al. (2000b); Japan; Kochi Prefecture, Cape Ashizuri; turtles accidentally caught in commercial fishing nets

TM = 93/83 kg SCL = 83/85 cm	M	F/M	6/1	Liver	ND (<0.03)	WET: M	
				Gullet	ND (<0.03)		
				Stomach	ND (<0.03)/0.086		
				Intestine	ND (<0.03)		
				Pancreas	0.084/0.045		
				Heart	ND (<0.03)		
				Trachea	0.041/0.115		
				Lung	ND (<0.03)		
				Bladder	ND (<0.03)/0.030		
				Spleen	0.022/0.037		
				Kidney	0.217/0.053		
				Salt gland	ND (<0.03)		
				Brain	ND (<0.03)/0.050		
Reproductive tissues				Testis	ND (<0.03)		
				Oviduct	ND (<0.03)		
				Ovary	ND (<0.03)		
Egg				Whole egg	ND (<0.03)		
				Shell	ND (<0.03)		
				Yolk	ND (<0.03)		
				Albumen	ND (<0.03)		
				Scale	0.081/0.048		
				Mesentary	ND (<0.03)		
				Fat	ND (<0.03)		
				Muscle	0.083/0.056		
				Bone	0.140/0.086		
				Carapace	0.094/0.063		
				Whole body	*		*Not calculated

(*continued*)

TABLE A.17 (CONTINUED)
Nickel (Ni)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Caretta caretta			—					Franzellitti et al. (2004); Italy, northwestern Adriatic Sea coast; Po delta to the Reno mouth
	Juvenile to adult MSCL = 24.5–74 cm			*			WET: M	*16 from fisheries by-catch, 19 dead on coast
				30	Liver	4.38		
				13	Lung	1.80		
				17	Muscle*	2.76		*Pectoral
				7	Fat*	18.77		*Abdominal
Caretta caretta								Kaska et al. (2004); Turkey; southwestern Mediterranean coast; stranded turtles
	—		—	32	Liver	11.56; 5.65–16.68	DRY: M; R	
				20	Bladder	7.99; 4.07–11.57		
				20	Kidney	9.63; 4.32–12.90		
				10	Lung	10.08; 2.08–14.13		
				32	Muscle	10.02; 2.27–15.78		
Caretta caretta							WET: M; R	Torrent et al.(2004); Spain, Canary Islands; stranded turtles submitted to the veterinary faculty of the University of Las Palmas in Gran Canaria
	Juvenile and subadult SCL = 15–65 cm	R*	F/M	67/11	Kidney	5.81; 0.04–48.13		*Estimated from graph
					Muscle	1.74; 0.03–13.15		
					Bone	1.00; 0.02–11.60		
					Liver	2.88; 0.01–13.81		
Caretta caretta								Gardner et al. (2006); Mexico; Baja California peninsula; turtles died from fisheries capture
	MSCL = 57.0; 52.0–63.0	M; R		5	Liver	0.35; ND (<0.004)–3.26	DRY: GM; R	

					Kidney	0.04; ND (<0.004)–3.38		
					Muscle*	0.01; ND (<0.004)–0.65		*Pectoral
					Fat	0.17; ND (<0.004)–1.63		
Chelonia mydas								Aguirre et al. (1994); USA; Hawaiian Islands
	L = 28.7–71.3 cm W = 3.2–43.6 kg		F/M	8/4	Liver	ND (<0.4)	WET: R	
					Kidney	ND (<0.4)–1.0		
	Egg*		—	3	Shell	ND (<0.4)	WET: M	*Posthatch
	Hatchling				Whole body	ND (<0.4)		
Chelonia mydas							WET: M; R	Sakai et al. (2000a); Japan; Yaeyama Islands; Okinawa; turtles caught by fishermen for commercial use
	SCL = 51.0 cm	M	—	50			WET: M;R	
				2/50*	Liver	0.06–0.31		*Detected/analyzed
				23/23	Kidney	0.615; 0.124–1.33		
				0/47	Muscle	ND (<0.03)		
Chelonia mydas								Sakai et al. (2000b); Japan; Ogasawara Islands (Bonin Islands), Haha-Jima Island; turtles collected in coastal waters
	TM = 124/117 kg SCL = 93/97 cm	M	F/M	1/1	Liver	0.059/0.071	WET: M	CW = 72/73 cm
					Gullet	ND (<0.03)/0.219		
					Stomach	ND (<0.03)/0.068		
					Intestine	0.043/ND (<0.03)		
					Pancreas	0.075/0.158		
					Heart	ND (<0.03)		
					Trachea	ND (<0.03)/0.331		
					Lung	ND (<0.03)/0.187		

(*continued*)

TABLE A.17 (CONTINUED)
Nickel (Ni)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
				Bladder	ND (<0.03)/0.034		
				Spleen	0.072/0.077		
				Kidney	0.561/0.463		
				Salt gland	ND (<0.03)/0.049		
				Brain	ND (<0.03)/0.118		
	Reproductive tissues			Testis	0.072		
				Oviduct	ND (<0.03)		
				Ovary	ND (<0.03)		
	Egg			Whole egg	ND (<0.03)		
				Shell	ND (<0.03)		
				Yolk	ND (<0.03)		
				Albumen	ND (<0.03)		
				Scale	ND (<0.03)/0.888		
				Mesentary	ND (<0.03)/0.111		
				Fat	ND (<0.03)/0.059		
				Muscle	0.059/ND (<0.03)		
				Bone	0.058/ND (<0.03)		
				Carapace	0.191/ND (<0.03)		
				Whole body	*		*Not calculated
Chelonia mydas							Kaska et al. (2004); Turkey; southwestern Mediterranean coast; stranded turtles
	—	—	22	Liver	9.25; 5.94–12.69	DRY: M; R	
			20	Bladder	7.32; 4.31–12.77		
			14	Kidney	10.41; 4.29–14.06		
			15	Lung	6.40; 3.22–17.68		
			22	Muscle	9.71; 4.63–19.93		
Chelonia mydas							Lam et al. (2004); South China

	Juvenile		—	2	Fat	0.15	DRY: M	Specimens stranded on beaches at Ham Tin Wan and Tung Pang Chau; upon collection, turtles had started to decay
					Kidney	0.204		
					Heart	0.282		
					Liver	0.270		
					Lung	0.214		
					Muscle	0.217		
					Stomach	0.386		
	Adult		—	3	Muscle	1.070		Specimens believed to have been unintentionally caught by fishermen; upon collection, turtles were relatively fresh
				1	Liver	0.712		
Chelonia mydas								Celik et al. (2006); Turkey; Mediterranean Sea, Kazanli beach; stranded turtles
	Egg			12*	Shell	3.645	DRY: M	*12 nests, 3 eggshells per nest collected after hatching
	Environment							In this study, information is available on metal levels in sand, soil, plant, and water samples around the nesting environment.
Chelonia mydas								Gardner et al. (2006); Mexico; Baja California peninsula; turtles died from fisheries capture
	MSCL = 62.13; 48.5–76.9	M; R		11	Liver	0.01; ND (<0.004)–7.40	DRY: GM; R	
					Kidney	1.15; ND (<0.004)–26.43		
					Muscle*	0.03; ND (<0.004)–4.0		*Pectoral
					Fat	0.02; ND (<0.004)–13.42		
Chelydra serpentina								Albers et al. (1986); USA; Maryland and New Jersey
	Adult	R	M	7	Liver	0.44	WET: M	Site 1: Undisturbed freshwater site: Patuxent Wildlife Research Center, Maryland

(*continued*)

TABLE A.17 (CONTINUED)
Nickel (Ni)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
	TL = 20–40 cm							
			F	6		0.99		
			M	7	Kidney	0.35		
			F	6		0.43		
			M	8	Liver	0.24		Site 2: Contaminated brackish water site: Hackensack Meadowlands, New Jersey
			F	3		0.27		
			M	8	Kidney	1.24		
			F	3		1.07		
			M	8	Liver	0.13		Site 3: Contaminated freshwater site: Hackensack Meadowlands, New Jersey
					Kidney	0.45		
Dermochelys coriacea								Davenport and Wrench (1990), Davenport et al. (1990); Great Britain; Wales, Irish Sea, Cardigan Bay; stranded turtle
	TL = 2.53 m TM = 916 kg		M	1	Liver	2.13	DRY: M*	*4 replicate measures
					Muscle*	1.62		*Pectoral
					Blubber	0.07		
Dermochelys coriaca								Vazquez et al. (1997); Mexico; Pacific coast
	Environment				Seawater	0.54	DRY: M	
					Sand	63.6		
	Egg*		—	—	Shell	7.90		*Posthatch
Dermochelys coriaca								Godley et al. (1998); Great Britain; Wales and Scotland, west coast; turtles died in fishing gear
Dermochelys coriaca								Godley et al. (1998); Great Britain; Wales and Scotland, west coast; turtles died in fishing gear
	Adult	R	M	3				

	CCL = 141–170 cm						
		M	1	Liver	ND (<0.19)	DRY	
				Muscle	1.6		
		M	1	Liver	ND (<0.062)	WET	
				Muscle	0.53		
Eretmochelys imbricata						WET	Sadiq and Zaidi (1984); Saudi Arabia; collected from shoreline, freshly dead or near dying (April 1983) after oil spill (February 1983)
	—	—	1	Muscle	2.59		
				Liver	4.93		
Eretmochelys imbricata							Gardner et al. (2006); Mexico; Baja California peninsula; turtles died from fisheries capture
	MSCL = 48.4		1	Liver	2.48	DRY: GM; R	
				Kidney	1.61		
				Muscle*	ND (<0.004)		*Pectoral
				Fat	ND (<0.004)		
Lepidochelys olivacea	—	—					Sahoo et al. (1996); India; Gahirmatha, Orissa
	Environment		8	Beach sand	12–34	DRY: R	
	Egg*	—	24**	Shell	13.0		*Fresh; **8 nests, 3 eggs per nest
				Albumen-yolk	5.0		
	Egg*			Shell	12.6		*Posthatch
	Hatchling*			Whole body	25.0		*Fresh
Lepidochelys olivacea							Gardner et al. (2006); Mexico; Baja California peninsula; turtles died from fisheries capture
	MSCL = 60.1; 53.0–66.0	M; R	6	Liver	0.58; ND (<0.004)–3.88	DRY: GM; R	
				Kidney	0.02; ND (<0.004)–2.46		
				Muscle*	0.01; ND (<0.004)–0.41		*Pectoral

(*continued*)

TABLE A.17 (CONTINUED)
Nickel (Ni)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
				Fat	0.03; ND (<0.004)–0.51		
Trachemys scripta elegans							Tryfonas et al. (2006); USA; Illinois; lower Illinois River near Grafton; eggs laid in the lab from turtles collected in the field from 5 nesting areas
	Egg		—	Contents	ND (<—)	DRY: M*	*All sites combined; values estimated from graph
				Shell	1.3		
	Diet			*Lemna* sp.	21.8		
	Environment		4–5*	Soil	117–140	RM*	*Samples (soil from nesting and lake bank areas) from 2 sites
			3*	Sediment	66–95	RM*	*Samples (3 layers per site) from 2 sites; values estimated from graph
			3–4*	Water	0.08–0.11	WET; RM*	* Samples from 3 sites; values estimated from graph
Crocodylia							
Alligator mississippiensis							Heinz et al. (1991); USA; central and southern Florida; Lake Okeechobee, Lake Griffin, and Lake Apopka
	Egg	—	32	Egg	0.05–0.09; 0.02–0.64	WET: RM; R**	*16 nests, 2 eggs per nest; **combined by lake
Alligator mississippiensis							Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	—	—	1	Liver	ND (<0.01)	WET	
				Kidney	0.04		
Crocodylus acutus							Stoneburner and Kushlan (1984); USA; Florida; Florida Bay, Everglades National Park
	Egg	—	9	Shell	22.04	DRY: M	
				Albumen-yolk	2.35		

Crocodylus johnstoni							Jeffree et al. (2001); northern Australia, north central Queensland; Lynd River; samples from a single population
	A = 0.7–62.7 years L = 24.7–128.3 cm	R	F/M	9/21		DRY: M; R	
			—	30	Osteoderm*	3.67; 3.29–4.02	*Ventral pelvic region
Crocodylus niloticus							Swanepoel et al. (2000); South Africa; Kruger National Park (KNP) area
	TL = 1.40–4.15 m	R	F/M	6/9		DRY: M	
							Site 1: Shingwedzi River (Silwervis Dam), northern part of KNP; catchment area outside KNP is limited
					Muscle	24.9	Frozen tissues used for residue analysis
					Liver	23.0	
					Kidney	28.9	
					Fat	31.2	
							Site 2: Olifants River, central part of KNP; flows through areas of intense agricultural activity and passes mining areas and receives tributaries from Phalaborwa Mining Company before entering the KNP
					Muscle	10.3	Frozen tissues used for residue analysis
					Liver	8.25	
					Kidney	8.1	
					Fat	12.8	
							Site 3: Sabi River, southern part of KNP; flows through areas of intense agricultural activity before entering the KNP
					Muscle	9.1	Frozen tissues used for residue analysis
					Liver	7.3	

(*continued*)

TABLE A.17 (CONTINUED)
Nickel (Ni)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
					Kidney	ND (<—)		
					Fat	20.6		
Crocodylus porosus								Jeffree et al. (2001); northern Australia; Alligator Rivers region: Kakadu National Park; samples from 3 river catchments, mining and hunting areas included
	A = 5–40 years	R	—	40			DRY: GM; R	
	L = 168–499 cm							
			—	35	Muscle*	0.507; 0.140–0.850		*Tail
			—	40	Osteoderm*	4.20; 1.16–7.22		*Ventral pelvic region
Squamata: Sauria								
*Laudakia s. stellio**								Loumbourdis (1997); Greece; Thessaloniki region; *[*Agama s. stellio*]
	Adult		—	—	Liver	3.60	DRY: M	Site 1: Urban area (500 m asl)
					Carcass	33.83		
					Liver	7.33		Site 2: Agricultural area (50 m asl)
					Carcass	47.52		
*Podarcis taurica**								Sharygin et al. (1979/80); Crimean Mountains between Yalta and Alushta (400–900 m asl); *[*Lacerta taurica*]
	—		—	—	Whole body	400	ASH: MAX	
Tarentola mauritanica								Fletcher et al. (2006); southern Spain; Guadiamar River Valley, mine tailings release event; Boliden-Apirsa mine at Aznalcóllar; 7 study sites spanning an expected contamination gradient; geckos collected from building walls

Juvenile and adult		52	Whole body*		DRY: M; R	*Minus gut contents
	—	9		0.370; 0.236–0.630		Site 1: Rural not mine-affected site: near Guadalmellato (most pristine)
	—	13		0.371; 0.214–0.555		Site 2: Urban not mine-affected site: Villaviciosa de Cordoba (not contaminated by mining)
	—	8		0.367; 0.261–0.465		Site 3: Urban mine-affected site: Aznalcázar (contamination through aerosolized contaminants engulfed the town during cleanup)
	—	8		0.353; 0.226–0.663		Site 4: Urban mine-affected site: Aznalcóllar (contaminated by normal mine operations, or the disaster and subsequent remediation efforts)
	—	5		0.512; 0.265–0.685		Site 5: Floodplain mine-affected site: Guadiamar River floodplain near the Aznalcázar gauge station (24.8 km below the ruptured tailings dam)
	—	5		0.317; 0.228–0.394		Site 6: Floodplain mine-affected site: Guadiamar River floodplain near the Guijo gauge station (7.4 km below the ruptured dam)
	—	4		0.330; 0.288–0.397		Site 7: Floodplain mine-affected site: Agrio River floodplain (4.4 km below and closest to the ruptured tailings dam)
						Statistical analysis: [Ni] body concentration was significantly influenced by site
Squamata: Serpentes						
"Sea snake"						Sadiq and Zaidi (1984); Saudi Arabia; collected from shoreline, freshly dead or near dying (April 1983) after oil spill (February 1983)
—	—	7	Muscle	1.70–5.38	WET: R	
			Liver	1.87–4.73		

(*continued*)

TABLE A.17 (CONTINUED)
Nickel (Ni)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Agkistrodon piscivorus								Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 58.6 cm TM = 365 g	M	—	6	Liver	0.03	WET	
					Kidney	0.14		
Agkistrodon piscivorous								Burger et al. (2006); USA; South Carolina; 1 reference site; 1 polluted Savannah River site, Aiken
	SVL = 57 cm TM = 280 g	M	—	13	Muscle*	5	DRY: M**	*Tail; **both sites combined
	SVL = 60 cm TM = 270 g			5	Blood	0.2		
Coluber constrictor								Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 103.2 cm TN = 300 g	M	—	1	Liver	0.14	WET	
					Kidney	ND (<0.09)		
Nerodia cyclopion			—				WET	Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 63.9 cm TM = 191 g		—	1	Liver	ND (<0.39)		
					Kidney	ND (<0.13)		

Nerodia fasciata								Burger et al. (2006); USA; South Carolina; 1 reference site; 1 polluted Savannah River site, Aiken
	SVL = 51 cm TM = 130 g	M	—	47	Muscle*	5	DRY: M**	*Tail; **both sites combined
	SVL = 52 cm TM = 140 g			34	Blood	0.2		
Nerodia taxispilota			—					Burger et al. (2006); USA; South Carolina; 1 reference site; 1 polluted Savannah River site, Aiken
	SVL = 59 cm TM = 170 g	M		10	Muscle*	5	DRY: M**	*Tail; **both sites combined
	SVL = 65 cm TM = 230 g			9	Blood	0.1		

TABLE A.18
Platinum (Pt)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Crocodylia								
Alligator mississippiensis								Delany et al. (1988); USA; Florida; 8 lakes, statewide
	L = 2.9–3.8 m	R	F/M	1/32	Muscle*	ND (<0.50)	WET	*Tail

TABLE A.19
Rubidium (Rb)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Testudines								
Chelonia mydas								Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 49.0; 40.0–6 3.5 cm	M; R	M	6			DRY: M; R	
	CW = 40.5; 35.3–4 9.4							
	SCL = 52.2; 37.0–7 1.4		F	20				
	CW = 43.9; 31.7–5 8.5							
			M	6	Liver	5.53; 2.95–10.7		
				6	Kidney	9.00; 6.04–12.1		
				3	Muscle	9.03; 6.57–9.32		
			F	20	Liver	7.56; 4.56–17.1		
				19	Kidney	11.5; 4.29–18.5		
				9	Muscle	8.98; 5.64–16.1		
	Diet		—	8	Stomach*	6.03; 2.06–15.5		*Contents
Chelonia mydas								Anan et al. (2002); Japan; Okinawa Prefecture, Yaeyama Islands; tissues provided by fishermen
	—	—	—	9	Liver	1.92; 1.00–2.71	WET: M; R	
Cuora amboinensis								Boman et al. (2001); Vietnam; Dac Lac; seemingly unpolluted site
	Adult*		—	1	Muscle	99	DRY	*Fully grown
					Liver	60		
	Egg*				Egg	35		*Gonadal system
Cyclemys dentata								Boman et al. (2001); Vietnam; Dac Lac
	Adult*		—	1	Testicle	67	DRY	*Fully grown

(*continued*)

TABLE A.19 (CONTINUED)
Rubidium (Rb)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
	Egg*			Egg	35		*Gonadal system
Eretmochelys imbricata							Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 43.5; 33.5–4 8.9 cm	M	6	Liver	9.01; 4.19–15.6	DRY: M; R	
			6	Kidney	8.63; 6.88–12.2		
			1	Muscle	13.0		
	SCL = 46.5; 43.8–6 7.9 cm	F	16	Liver	8.32; 3.86–13.4		
			13	Kidney	8.26; 6.13–12.4		
			8	Muscle	8.80; 6.86–12.4		
	Diet	—	6	Stomach*	3.68; 0.619–7.04		*Contents
Eretmochelys imbricata							Anan et al. (2002); Japan; Okinawa Prefecture, Yaeyama Islands; tissues provided by fishermen
	— —	—	7	Liver	2.23; 1.55–3.76	DRY: M; R	
*Pelodiscus sinensis**							Boman et al. (2001); Vietnam; Hay Tay; *[*Trionyx sinensis*]
	Egg*	—	1	Egg	30	DRY	*Gonadal system
Squamata: Sauria							
*Laudakia s. stellio**							Loumbourdis (1997); Greece; Thessaloniki region; *[*Agama s. stellio*]
	Adult	—	—	Liver	33.92	DRY: M	Site 1: Urban area (500 m asl)
				Carcass	30.96		
				Liver	35.05		Site 2: Agricultural area (50 m asl)
				Carcass	33.73		

Tarentola mauritanica							Fletcher et al. (2006); southern Spain, Guadiamar River Valley; mine tailings release event; Boliden-Apirsa mine at Aznalcóllar; 7 study sites spanning an expected contamination gradient; geckos collected from building walls
	Juvenile and adult		52	Whole body*		DRY: M; R	*Minus gut contents
		—	9		5.104; 3.794–7.963		Site 1: Rural not mine-affected site: near Guadalmellato (most pristine)
		—	13		8.645; 5.000–24.053		Site 2: Urban not mine-affected site: Villaviciosa de Cordoba (not contaminated by mining)
		—	8		7.853; 5.131–9.461		Site 3: Urban mine-affected site: Aznalcázar (contamination through aerosolized contaminants engulfed the town during cleanup)
		—	8		8.899; 5.186–16.034		Site 4: Urban mine-affected site: Aznalcóllar (contaminated by normal mine operations, or the disaster and subsequent remediation efforts)
		—	5		7.395; 6.078–9.119		Site 5: Floodplain mine-affected site: Guadiamar River floodplain near the Aznalcázar gauge station (24.8 km below the ruptured tailings dam)
		—	5		7.199; 4.661–9.680		Site 6: Floodplain mine-affected site: Guadiamar River floodplain near the Guijo gauge station (7.4 km below the ruptured dam)
		—	4		6.299; 4.121–8.927		Site 7: Floodplain mine-affected site: Agrio River floodplain (4.4 km below and closest to the ruptured tailings dam)
							Statistical analysis: [Rb] concentration was significantly influenced by site
Varanus salvator							Boman et al. (2001); Vietnam; Dac Lac
	Adult*	—	1	Muscle	63	DRY	*Fully grown
				Liver	49		
	Egg*			Egg	6.3		*Gonadal system

(*continued*)

TABLE A.19 (CONTINUED)
Rubidium (Rb)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Squamata: Serpentes								
Agkistrodon piscivorous								Burger et al. (2006); USA; South Carolina; 1 reference site; 1 polluted Savannah River site, Aiken
	SVL = 57 cm TM = 280 g	M	—	13	Muscle*	21	DRY: M**	*Tail; **both sites combined
	SVL = 60 cm TM = 270 g			5	Blood	3		
Lapemis hardwickii								Boman et al. (2001); Vietnam; Nha Trang
	Adult*		—	1	Muscle Liver	5.0 9.2	DRY	*Fully grown
Nerodia fasciata								Burger et al. (2006); USA; South Carolina; 1 reference site; 1 polluted Savannah River site, Aiken
	SVL = 51 cm TM = 130 g	M	—	47	Muscle*	26	DRY: M**	*Tail; **both sites combined
	SVL = 52 cm TM = 140 g			34	Blood	5		
Nerodia taxispilota								Burger et al. (2006); USA; South Carolina; 1 reference site; 1 polluted Savannah River site, Aiken; *both sites combined
	SVL = 59 cm TM = 170 g	M	—	10	Muscle*	21	DRY: M**	*Tail; **both sites combined
	SVL = 65 cm TM = 230 g			9	Blood	4		
Python molurus								Boman et al. (2001); Vietnam; Dac Lac
	Adult*		—	1	Muscle Testicle	94 470	DRY	*Fully grown

TABLE A.20
Selenium (Se)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Testudines								
Caretta caretta *	Pelagic		—	1	Liver	3.39	WET	Aguirre et al. (1994); USA.; Hawaiian Islands; *[identification number 052, *Chelonia mydas* in the reference]
Caretta caretta	Se							Gordon et al. (1998); Australia; south-eastern Queensland, Moreton Bay region; stranded turtles
	—	—	—	6	Liver	2.21; 1.42–2.70	WET: M; R	
				3	Kidney	1.52; 1.28–1.78		
Caretta caretta							DRY: M; R	Storelli et al. (1998a); Italy; Adriatic Sea, Apulian coasts; stranded turtles
	TM = 1.8–100 kg	R	F/M	9/3	Liver	15.88; 2.12–27.44		
					Lung	10.77; 4.12–30.52		
					Kidney	10.33; 5.73–15.57		
					Muscle	10.81; 6.51–15.45		
Caretta caretta								Storelli et al. (1998a) from Storelli et al. (2005); dry mass based data from Storelli et al. (1998a) converted to wet mass
					Liver	4.68; 0.62–8.09	WET: M; R	
					Kidney	3.20; 1.77–4.83		
					Muscle	2.70; 1.63–3.87		
Caretta caretta								Storelli et al. (1998b); Italy; Adriatic Sea, Apulian coasts; stranded turtles
	TM = 6.7–18 kg	R		7	Muscle	2.33; 1.19–3.24	DRY: M;R	
					Liver	4.86; 4.00–6.11		
Caretta caretta								Storelli et al. (1998a) from Storelli et al. (2005); dry mass based data from Storelli et al. (1998b) converted to wet mass

(*continued*)

TABLE A.20 (CONTINUED)
Selenium (Se)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
					Liver	4.86; 4.00–6.11	WET: M; R	
					Muscle	2.33; 1.19–3.24		
Caretta caretta								Kaska et al. (2004); Turkey; southwestern Mediterranean coast; stranded turtles
	—		—	32	Liver	12.88; 3.17–22.33	DRY: M; R	
				20	Bladder	8.82; 2.33–13.42		
				20	Kidney	7.77; 2.57–13.98		
				10	Lung	7.44; 1.01–17.28		
				32	Muscle	7.05; 2.35–13.43		
Caretta caretta								Maffucci et al. (2005); southern Italy; western Mediterranean Sea; turtles stranded along the South Tyrrhenian coasts
	CCL = 37–82 cm	R	—	29			DRY: M;R	
				22	Liver	9.8; 1.0–24.9		
				21	Kidney	15.5; 4.5–41.8		
				26	Muscle	11.2; 4.0–24.1		
Caretta caretta								Storelli et al. (2005); Italy; Adriatic and Ionian Sea; stranded turtles
	SCL = 21–71 cm	R		19	Liver	3.54; 1.01–5.48	WET: M; R	
					Kidney	2.20; 0.66–2.78		
					Muscle	1.65; 0.68–2.30		
					Spleen	1.45; 0.75–2.85		
					Heart	1.40; 0.77–1.79		
					Lung	1.19; 0.68–2.13		
					Fat	1.84; 1.20–3.03		
Chelonia mydas								Aguirre et al. (1994); USA; Hawaiian Islands

	L = 28.7–71.3 cm W = 3.2–43.6 kg	R	F/M	8/4	Liver	0.79; 0.136–2.53	WET: M; R	
					Kidney	0.46; 0.159–1.58		
Chelonia mydas								Gladstone (1996) from Gordon et al. (1998); Australia, Torres Strait
	—	—	—	7	Liver	1.06; 0.34–3.4	WET: M; R	
					Kidney	0.45; 0.16–1.3		
Chelonia mydas								Gordon et al. (1998); Australia; south-eastern Queensland, Moreton Bay region; stranded turtles
	—	—	—	23	Liver	1.18; 0.07–2.68	WET: M; R	
				23	Kidney	0.59; 0.09–1.85		
Chelonia mydas								Anan et al. (2001); Japan, Yaeyama Islands
	SCL = 49.0; 40.0–63.5 cm	M; R	M	6			DRY: M; R	
	SCL = 52.2; 37.0–71.4		F	20				
			M	6	Liver	4.9; 2.9–9.3		
				6	Kidney	5.1; 2.3–11.0		
				3	Muscle	3.3; 2.8–3.3		
			F	20	Liver	5.2; 2.0–9.9		
				19	Kidney	5.4; 2.1–10		
				9	Muscle	3.2; 1.0–4.8		
	Diet		—	8	Stomach*	1.0; 0.37–1.8		*Contents
Chelonia mydas								Anan et al. (2002); Japan; Okinawa Prefecture, Yaeyama Islands; tissues provided by fishermen
	—	—	—	9	Liver	3.3; 0.87–7.5	WET: M; R	
Chelonia mydas								Kaska et al. (2004); Turkey; southwestern Mediterranean coast; stranded turtles
	—		—	22	Liver	10.67; 0.98–19.06	DRY: M; R	
				20	Bladder	5.94; 1.36–9.97		

(*continued*)

TABLE A.20 (CONTINUED)
Selenium (Se)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
			14	Kidney	7.83; 2.60–11.55		
			15	Lung	9.38; 1.94–16.69		
			22	Muscle	6.30; 2.15–9.93		
Chelonia mydas							Lam et al. (2004); South China
	Juvenile	—	2	Fat	0.721	DRY: M	Turtles stranded on beaches at Ham Tin Wan and Tung Pang Chau; upon collection, turtles had started to decay
				Kidney	5.893		
				Heart	4.992		
				Liver	25.65		
				Lung	7.517		
				Muscle	4.491		
				Stomach	8.070		
	Adult	—	3	Muscle	17.02		Turtles believed to have been unintentionally caught by fishermen; upon collection, turtles were relatively fresh
			1	Liver	12.43		
Chelydra s. serpentina, Chrysemys picta, Pseudemys rubriventris, Sternotherus odoratus						WET: RM	Bergeron et al. (2007); USA; Virginia; South River (Hg contaminated site with 7 sub-sites; 1 reference site); Middle River (1 reference site)
		—	138	Blood	0.160–0.339		Statistical analysis: Differences were significant for species but not for sites
Cuora amboinensis							Boman et al. (2001); Vietnam; Dac Lac; seemingly unpolluted site
	Adult*	—	1	Muscle	1.9	DRY	*Fully grown up

					Liver	9.5		
	Egg*				Egg	1.5		*Gonadal system
Cyclemys dentata								Boman et al. (2001); Vietnam, Site: Dac Lac; seemingly unpolluted site
	Adult*		—	1	Testicle	2.3	DRY	*Fully grown up
	Egg*				Egg	1.5		*Gonadal system
Dermochelys coriaca								Godley et al. (1998); Great Britain, Wales and Scotland, West Coast; Turtles died in fishing gear
	Adult CCL = 141–170 cm	R	M	3				
					Liver	20	DRY	
					Muscle	13		
					Liver	6.5	WET	
					Muscle	4.3		
Dermochelys coriacea								Davenport and Wrench (1990), Davenport et al. (1990); Great Britain; Wales, Irish Sea, Cardigan Bay; stranded animal
	L = 2.53 m W = 916 kg	R	M	1	Liver	1.41	DRY: M*	*4 replicate measures
					Muscle*	3.61		*Pectoral
					Blubber	ND(<0.05)		
Emys orbicularis								Swartz et al. (2003); Azerbaijan; Apsheron Peninsula; heavily contaminated wetland receiving runoff from nearby industrial wastewater treatment plant and cooling towers of the city of Sumgayit
	—		—	5	Liver	7.4	DRY: MD	
Eretmochelys imbricata								Gordon et al. (1998); Australia; south-eastern Queensland, Moreton Bay region; stranded turtles
	—	—	—	2	Liver	2.68–3.65	WET: R	

(*continued*)

TABLE A.20 (CONTINUED)
Selenium (Se)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
				2	Kidney	2.22–2.49		
Eretmochelys imbricata								Anan et al. (2001); Japan, Yaeyama Islands
	SCL = 43.5; 33.5–48.9	M; R	M	6	Liver	47; 14–89	DRY: M; R	
				6	Kidney	43; 12–76		
				1	Muscle	4.7		
	SCL = 46.5; 43.8–67.9		F	16	Liver	49; 12–150		
				13	Kidney	22; 9.0–39		
				8	Muscle	12; 2.6–33		
	Diet		—	6	Stomach*	14; 3.6–29		*Contents
Eretmochelys imbricata								Anan et al. (2002); Japan; Okinawa Prefecture, Yaeyama Islands; tissues provided by fishermen
	—	—	—	7	Liver	19; 2.9–32	DRY: M; R	
*Gopherus agassizii**							—: M	Jacobson et al. (1991); USA.; California; *[*Xerobates agassizii*]
	L = 133–306 mm W = 390–4900 g		*	10	Liver	0.383		Kern County; turtles with clinical signs of Upper Respiratory Tract Disease; *total sample: 11 M, 1 F
	L = 199–280 mm W = 1180–3887 g		M	4	Liver	0.212		San Bernardino County; clinically healthy turtles
Malaclemys terrapin								Burger (2002); USA, New Jersey, Barnegat Bay
	Adult L = 14.3 cm	M	F	11	Liver	1.621	WET: M	
					Muscle	0.507		

		—	8	Egg *	0.498; 0.469–0.547	M; R	*Ovarial
Mauremys caspica							Swartz et al. (2003); Azerbaijan; Apsheron Peninsula; heavily contaminated wetland receiving runoff from nearby industrial wastewater treatment plant and cooling towers of the city of Sumgayit
	—		6	Liver	4.2	DRY: MD	
*Pelodiscus sinensis**							Boman et al. (2001); Vietnam; Hay Tay; seemingly unpolluted site; *[*Trionyx sinensis*]
	Adult*, egg**	—	1	Egg	1.5	DRY	*Fully grown up; **gonadal system
Trachemys scripta						DRY: M; MAX	Burger and Gibbons (1998); USA; South Carolina; Savannah River Site, Aiken; eggs laid in the lab by turtles collected in the field 2–3 days before
	Egg	—	16*	Contents	0.417; 1.041		*16 clutches, 1 egg per clutch
				Shell	0.036; 0.208		
							Statistical analysis: [Se] concentrations were significantly higher in egg contents than in shells
Trachemys scripta							Clark et al. (2000); USA; Texas
	—	—	14	Whole blood	0.170	WET: GM	Site 1: Contaminated; Municipial Lake (chemical manufacturing plant)
					0.241		Site 2: Apparently uncontaminated; Research Park Lake (parkland)
					0.261		Site 3: Contaminated; Old River Slough (cotton and corn cultivation with intensive chemical application)

(*continued*)

TABLE A.20 (CONTINUED)
Selenium (Se)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Trachemys scripta							Nagle et al. (2001); USA; South Carolina, Aiken County, Savannah River Site; in ovo exposure experiment; females collected in the field in 1993, 1994; oviposition induced in 1995; eggs from turtles collected in both polluted and unpolluted sites were incubated in coal ash-contaminated and uncontaminated soil in outdoor artificial nests
							Site 1: Coal ash-polluted Savannah River Site
	Adult	F	4	Liver	37.18	DRY: M	
	Diet	—	12	Asiatic clam*	8.69		*Soft tissue
		—	3	Crayfish*	14.92		*Whole body
	Environment	—	—	Artificial nest*	2.56		*Incubation substrate
							Sites 2–3: Unpolluted control sites near Couchton
	Adult	F	3	Liver	3.41		
	Diet	—	10	Asiatic clam*	2.01		*Soft tissue
		—	3	Crayfish*	1.50		*Whole body
	Environment	—	—	Artificial nest*	0.20		*Incubation substrate
	Hatchling						Trial 1: Incubation substrate: coal ash-contaminated
	CL = 30.34 mm TM = 8.20 g	—	18 *				*18 hatchlings from 5 clutches
		—	6 *	Whole body	4.45		*3 clutches per site, 2 hatchlings per clutch
	Hatchling						Trial 2: Incubation substrate: uncontaminated

	TM = 30.36 g CL = 8.02 mm		18*				*18 hatchlings from 5 clutches
			6*	Whole body	4.45		*3 clutches per site, 2 hatchlings per clutch
	Hatchling						Trial 3: Maternal residence: coal ash-contaminated
	CL = 30.51 mm TM = 8.02 g	—	18*				*18 hatchlings from 4 clutches
		—	6*	Whole body	7.36		*3 clutches per site, 2 hatchlings per clutch
	Hatchling						Trial 4: Maternal residence: uncontaminated
	TM = 29.69 g CL= 7.77 mm		18*				*18 hatchlings from 6 clutches
			6*	Whole body	1.63		*3 clutches per site, 2 hatchlings per clutch
							Statistical analysis: [Se] concentrations were significantly higher in adult liver and prey from site 1; [Se] concentrations in hatchlings were not significantly different between trials 1 and 2 but significantly higher in trial 3 than 4.
Crocodylia							
Alligator mississippiensis							Lance et al. (1983); studied reproductive cycle (April-July) for 4 months
	Adult	F		Blood*		WET: RM *	*Plasma; **combined by month
	TL = 190–243 cm	R	24		0.23–0.27		Farm-reared, fed fish (*Micropogon undulatus*) containing 2.8 mg Se/kg (DRY)
	TL = 189–326 cm		29		0.16–0.23		Farm-reared, fed (nutria *Myocastor coypus*) containing 0.04 mg Se/kg (WET)

(*continued*)

TABLE A.20 (CONTINUED)
Selenium (Se)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
	TL = 163–325 cm TM = 15.9–146 kg		48		0.15–0.20		USA; Lousiana; Rockefeller Refuge, Grand Chenier, Wild animals (nutria constituted 70% of the diet)
Alligator mississippiensis							Heinz et al. (1991); USA; central and southern Florida; Lake Okeechobee, Lake Griffin, and Lake Apopka
	Egg	—	32*	Egg	0.30–0.37; 0.24–0.53	WET:RM; R**	*16 nests, 2 eggs per nest; **combined by lake
Alligator mississippiensis							Burger et al. (2000); USA; Florida; Lake Apoka (Lake and Orange Counties), Orange Lake (Alachua County), and Lake Woodruff (Volusia County)
	L = 36–40 cm	R				WET: M	*3 lakes combined
	Yearlings		30	Fat*	0.153		*Abdominal
			31	Liver	0.641		
			30	Muscle*	0.119		*Abdominal
			29	Skin*	0.131		*Abdominal
			29	Muscle*	0.153		*Ventral proximal tail
			22	Tail tip	0.160		
			3	Tail*	0.102		*Regenerated
Alligator mississippiensis							Roe et al. (2004); USA; South Carolina; 3 sites downstream a coal-burning electric power plant located on the U.S. Department of Energy Savannah River Site
	Egg*			Egg*		DRY	*Field-collected, recently laid eggs prepared for Se analysis immediately after collection

Posthatch**		Chorioallantoic membrane and hatchling**			**Field-collected, recently laid eggs incubated until hatching in the lab in artificial substrate (vermiculate)
Egg	5	Egg	7.30–7.44	RM	Site 1: Highly coal ash-polluted site: Drainage swamp receiving effluent from the power plant
Posthatch	3	Hatchling	7.20–7.75		
		Chorioallantoic membrane	27.2	M	
Egg	5	Egg	2.21–3.00	RM	Site 2: Creek 2.0–2.5 km downstream from the power plant
Posthatch	3	Hatchling	2.11–2.36		
		Chorioallantoic membrane	6.7	M	
Egg	5	Egg	1.76–2.34	RM	Site 3: Reference site: unpolluted pond
Posthatcch	3	Hatchling	1.41–1.95		
		Chorioallantoic membrane	5.0	M	
Environment					High Se pollution of water, sediment, and biota from sites 1 and 2 documented in previous publications Statistical analysis: not given; [Se] concentrations were generally highest in all comparttments from site 1; mean [Se] concentrations in CAMs exceeded those in eggs and hatchlings

(*continued*)

TABLE A.20 (CONTINUED)
Selenium (Se)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Alligator mississippiensis							Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; Site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	—	—	1	Liver	ND(<0.02)	WET	
				Kidney	0.39		
Alligator mississippiensis							Lance et al. (2006); USA; Louisiana
	TM = 34; 21–50 kg M; R TL = 214; 161–279 cm	M	8			WET: M; R	Wild alligators, trapped in aquaculture ponds.
	TM = 29; 19–42 kg TL = 207; 185–226 cm	F	7				
		—	5	Liver	1.39; 1.18–1.65		
			14	Kidney	2.00; 1.13–3.65		
	TM = 217 ± 21; 54–279 kg TL = 353 ± 11; 246–404 cm	M	16				Captive alligators, raised at the experimental alligator breeding facility (Louisiana Department of Wildlife and Fisheries, USA); fed nutria (Myocastor copus) meat
	TM = 85; 73–132 kg TL = 279; 187–295 cm	F	28				
		—	27	Liver	0.92; 0.63–1.47		
			27	Kidney	1.44; 0.77–2.22		
Crocodylus niloticus							Phelps et al. (1986); Zimbabwe; 10 samples from 8 sites
	Egg	—	26	Contents	0.873; 0.485–1.206	DRY: M; R	

Crocodylus niloticus							DRY: M; R	Swanepoel (1998), Swanepoel et al. (2000) from Campbell (2003); South Africa Site 1: Olifants River in central part of Kruger National Park
					Liver	3.10; 2.7–3.5		Formalinized tissues analyzed
					Kidney	2.90; 2.7–3.1		
					Liver	3.10; 2.7–3.5		Site 2: Sabi River in southern part of Kruger National Park
					Kidney	2.90; 2.8–3.0		Formalinized tissues analyzed
Crocodylus niloticus								Almli et al. (2005); Zambia
	L = 2.7–3.4 m	R	F/M	2/2			WET: MD; R	Site 1: Kafue River, Kafue National Park
					Liver	1.80; 1.00–4.80		
					Kidney	0.94; 0.74–4.10		
	L = 2.0–4.0 m		F/M	4/1				Site 2: Luangwa River, Luangwa National Park
					Liver	2.30; 2.00–4.40		
					Kidney	2.20; 1.10–5.60		
Crocodylus porosus								Jeffree et al. (2001); Northern Australia; Alligator Rivers region: Kakadu National Park; samples from 3 river catchments, mining and hunting areas included
	Age = 5–40 years L = 168–499 cm		—	40			DRY: M; R	
			—	35	Muscle*	0.993; 0.440–2.00		*Tail
			—	40	Osteoderm*	ND(<0.01)		*Ventral pelvic region
Squamata: Sauria								
*Norops sagrei**	Adult							Burger et al. (2004); USA; South Florida and Florida Keys; *[*Anolis sagrei*]
	SVL = 43.5; 35–50 mm	M; R	F	72	Whole body*	0.376; 0.166–1.209; 0.059–2.112	WET: M; RM; R*	*Minus gut contents; **6 sites combined
	SVL = 55.3; 45–67 mm		M	72		0.269; 0.114–0.786; 0.023–1.029		

(*continued*)

TABLE A.20 (CONTINUED)
Selenium (Se)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Sceloporus occidentalis							Hopkins (2005b); lab feeding experiment simulating terrestrial food chain. Lizards were fed crickets (*Acheta domestica*), and crickets were fed commercial feed laden with seleno-d, l-methionine for 98 days. Experimental animals (original parental stock originated from San Joaquin Valley, California, USA) were obtained as hatchlings from a breeding colony at Oklahoma State University in 2002.
	Juvenile at start of experiment		60			DRY; M*	*values estimated from graph
							Treatment 1: control (background level): lizards fed prey containing < 1 µg/g Se dry mass (0.55 µg/g Se DRY overall actual mean in cricket subsamples).
		F	—	Carcass	0.7		
				Liver	1.4		
				Gonad	1.1		
		M	—	Carcass	0.7		
				Liver	1.5		
				Gonad	1.0		
		—	—	Tail	0.5		
							Treatment 2: lizards fed prey containing 15 µg/g Se DRY (14.7 µg/g Se dry mass overall actual mean in cricket subsamples)
		F	—	Carcass	9.1		
				Liver	11.7		
				Gonad	13.7		

		M	—	Carcass	11.5		
				Liver	12.3		
				Gonad	11.5		
		—	—	Tail	7.6		
							Statistical analysis: Effects of tissue, treatment, tissue-by-treatment and tissue-by-sex on [Se] concentration were significant; regardless of dietary treatment, proportion of body Se burden partitioned into gonads was higher in females than in males
Tarentola mauritanica							Fletcher et al. (2006); southern Spain; Guadiamar River valley, mine tailings release: Boliden-Apirsa mine at Aznalcollar; 7 study sites spanning an expected contamination gradient; geckos collected from building walls
	Juvenile and adult		52	Whole body*		DRY: M; R	*Minus gut contents
		—	9		0.583; 0.419–0.791		Site 1: Rural not mine-affected site: near Guadalmellato (most pristine)
		—	13		0.820; 0.573–1.010		Site 2: Urban not mine-affected site: Villaviciosa de Cordoba (not contaminated by mining)
		—	8		0.789; 0.656–0.958		Site 3: Urban mine-affected site: Aznalcazar (contamination through aerosolized contaminants engulfed the town during cleanup)
		—	8		0.912; 0.525–1.242		Site 4: Urban mine-affected site: Aznalcollar (contaminated by normal mine operations, or the disaster and subsequent remediation efforts)

(*continued*)

TABLE A.20 (CONTINUED)
Selenium (Se)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
			—	5		0.919; 0.504–1.474		Site 5: Floodplain mine-affected site: Guadiamar River floodplain near the Aznalca´zar gauge station (24.8 km below the ruptured tailings dam)
			—	5		1.465; 0.940–1.761		Site 6: Floodplain mine-affected: Guadiamar River floodplain near the Guijo gauge station (7.4 km below the ruptured dam)
			—	4		1.003; 0.830–1.252		Site 7: Floodplain mine-affected site: Agrio River floodplain (4.4 km below and closest to the ruptured tailings dam). Statistical analysis: [Se] concentration was significantly influenced by site
Varanus salvator								Boman et al. (2001); Vietnam; Dac Lac; seemingly unpolluted site
	Adult*		—	1	Liver	1.5	DRY	*Fully grown
	Egg*				Egg	7.0		*Gonadal system
Squamata: Serpentes								
Agkistrodon piscivorus								Clark et al. (2000); USA; Texas
	—	M	—	—	Whole blood	0.277	WET: GM	1 contaminated site: Old River Slough (cotton and corn cultivation with intensive chemical application)
Agkistrodon piscivorus								Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event

	SVL = 58.6 cm TM = 365 g	M	—	6	Liver Kidney	0.10 0.57		
Agkistrodon piscivorous								Burger et al. (2006); USA; South Carolina; 1 reference site; 1 polluted Savannah River Site, Aiken
	SVL = 57 cm TM = 280 g	M	—	13	Muscle*	1.0	DRY: M**	*Tail; **both sites combined
	SVL = 60 cm TM = 270 g			5	Blood	0.6		
Coluber constrictor								Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 103.2 cm TN = 300 g	M	—	1	Liver	ND(<0.10)	WET	
					Kidney	0.78		
Lamprophis fuliginosus			F				DRY	Hopkins et al. (2004); 10 months lab feeding experiment; experimental animals (natural distribution: South Africa) bred and reared under controlled laboratory conditions in 2000 (parents obtained from Ophidian Research Colony at the University of Texas, USA in 1999), and fed Se dosed prey items (rodents, injected seleno-D,L-methionine) in overall 26 meals
	Juvenile*							*At start of exposure
	SVL = 40.90 cm M = 25.93 g		F	11	Liver	2	M**	Treatment 1: control (background level): snakes fed 1 µg/g Se (nominal, dry mass) per meal; **estimated from graph

(*continued*)

TABLE A.20 (CONTINUED)
Selenium (Se)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
				Kidney	3		
				Ovary	4		
					0.88		
	Egg	—	—	Viable egg		M	
				Inviable egg	0.94		
	Juvenile*	F	11	Liver	11.5	M**	Treatment 2: snakes fed 10 µg/g Se (nominal, 12.52 ± 0.32 actual, dry mass); ** estimated from graph
				Kidney	12.5		
				Ovary	20		
	Egg	—	—	Viable egg	—	M	
				Inviable egg	12.03		
	Juvenile*	F	11	Liver	20	M**	Treatment 3: snakes fed 20 µg/g Se (nominal, 22.95±0.37 actual, dry mass); ** estimated from graph
				Kidney	21.5		
				Ovary	31.5		
	Egg	—	—	Viable egg	22.57	M	
				Inviable egg	22.70		
							Statistical analysis: Effects of tissue and treatment on [Se] concentration were significant
Lapemis hardwickii							Boman et al. (2001); Vietnam; Nha Trang; seemingly unpolluted site
	Adult*	—	1	Muscle	2.1	DRY	*Fully grown
				Liver	4.1		
*Nerodia sp**							Winger and al. (1984); USA; Florida; Apalachicola River; *[*Natrix* sp.]
	TM = 226–544 g	—	15	Whole body	0.30; 0.28–0.33	WET: M; R	Site 1: Upper reaches of river
	TM = 272–725 g				0.44; 0.42–0.48		Site 2: Lower reaches of river

Nerodia cyclopion							Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 63.9 cm TM = 191 g		—	1	Liver	ND(<0.13)	WET	
					Kidney	0.47		
Nerodia fasciata								Hopkins et al. (1999); USA; South Carolina; Savannah River Site; Aiken
							DRY: M*	*Values estimated from graph
	—			5	Liver	142		Site 1: Coal ash-polluted site
				5		3.62		Site 2: Nearby reference site
								Statistical analysis: Differences in [Se] concentrations between sites were significant
	Diet							This study provides information on contamination levels of several prey species
Nerodia fasciata								Hopkins et al. (2001); USA; South Carolina; Aquatic Ecology Laboratory near Aiken; lab feeding experiment; experimental animals were captured at uncontaminated reference site, and fed 52 total meals of field collected prey (primarily fish) for 13.5 months
	TM*						DRY	*Initial /final mass
	Juvenile and adult TM = 61.91 / 169.6 g*	M	—	8	Gonad	15.34; 11.09–18.33	MD; R	Treatment 1: Snakes fed prey items collected from coal ash-contaminated site: Savannah River Site
					Kidney	23.20; 14.92–26.84		
					Liver	22.63; 13.46–27.53		

(*continued*)

TABLE A.20 (CONTINUED)
Selenium (Se)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
				Shed skin	26.14; 22.85–34.31		
				Blood	1.61; 1.14–2.42		
				Tail clip	7.74; 6.84–9.07		
	Prey			Fish	22.700; 15.960–40.801	GLSM; R	
	Juvenile and adult TM = 62.2/ 136.3 g*	M	—	7 Gonad	3.40; 2.47–3.97	MD; R	Treatment 2: Snakes fed prey items collected from uncontaminated reference site
				Kidney	3.41; 2.14–4.24		
				Liver	2.37; 2.04–2.95		
				Shed skin	2.11; 1.41–3.21		
				Blood	0.35; 0.27–0.38		
				Tail clip	0.04; ND(<0.088)-0.64		
	Prey			Fish	0.994; 0.622–1.321	GLSM; R	
							Treatment differences for [Se] were significant for snake gonad, kidney, liver, shed skin, blood, and tail clip, and for fish
Nerodia fasciata							Hopkins et al. (2002); USA; South Carolina; Aquatic Ecology Laboratory near Aiken; 2 year feeding experiment; all snakes were lab reared and originated from a single gravid female which was collected from a reference site on the Savannah River Site; exposure started after first hibernation
	Prey		*	Fish			*3 prey species, 4 specimens per species
	Juvenile**					DRY	**Morphometry in Figure 2 (Hopkins et al. 2002)

Prey	—	*	Fish	0.994	GLSM	Treatment 1: Control (background level): snakes fed only prey items collected from uncontaminated reference site
Juvenile**	F	6	Gonad	3.562	M	
			Kidney	4.119		
			Liver	2.628		
	M	4	Gonad	3.048	M	
			Kidney	4.146		
			Liver	2.288		
Prey	—	*	Fish	11.361	GLSM	Treatment 2: Lower level exposure: snakes fed prey items collected from coal ash-contaminated site and from the reference site on alternating weeks
Juvenile**	F	6	Gonad	9.972	M	
			Kidney	16.006		
			Liver	11.630		
	M	4	Gonad	9.534	M	
			Kidney	21.055		
			Liver	10.798		
Prey	—	*	Fish	22.700	GLSM	Treatment 3: Higher level exposure: snakes fed only prey items collected from coal ash-contaminated site
Juvenile**	F	6	Gonad	17.642	M	
			Kidney	25.379		
			Liver	24.076		
	M	3	Gonad	19.060	M	
			Kidney	32.036		
			Liver	24.220		
						Statistical analysis: Effects of organ, treatment, organ-by-treatment and organ-by-sex on [Se] concentration were significant

(*continued*)

TABLE A.20 (CONTINUED)
Selenium (Se)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Nerodia fasciata								Burger et al. (2006); USA; South Carolina; 1 reference site; 1 polluted Savannah River Site, Aiken
	SVL = 51cm TM = 130 g	M	—	47	Muscle*	1	DRY: M**	*Tail; **both sites combined
	SVL = 52 cm TM = 140 g	M		34	Blood	0.4		
Nerodia fasciata								Burger et al. (2007); USA; South Carolina; 1 relatively rural site
	Adult		—	34	Blood	0.378	WET: M	
				47	Muscle	0.361		
				5	Liver	1.629		
Nerodia rhombifer								Clark et al. (2000); USA; Texas
	—		—	10	Whole blood	0.352; 0.264–0.470;	WET: GM	Site 1: Contaminated site: Old River Slough (cotton and corn cultivation with intensive chemical application)
						0.318; 0.271–0.373		Site 2: Reference site: Private Lake (pasture)
Nerodia sipedon								Burger et al. (2005); USA; East Tennessee; 1 reference site: Little River downstream from the Great Smoky Mountains National Park near Townsend; 1 polluted superfund site: EFPC inside the the USA Department of Energy's (USDOE's) Y-12 National Security Complex;
	Adult		—				WET: M*	*Both sites combined
				47	Kidney	1.516		
				47	Liver	1.815		

				47	Muscle	0.700		
				47	Skin	0.887		
				46	Blood	1.090		
				3	Testis	1.710		
	Egg			8	Egg*	1.465		*Ovarial Statistic analysis: [Se] concentrations were significantly highest in the eggs and testes, kidney, and liver
Nerodia sipedon	Adult		—					Campbell et al. (2005); USA; eastern Tennessee
	TM = 235; 53–464 g SVL = 65; 44.5–80 mm	M; R	F	21				
	TM = 103; 74–146 g SVL = 53; 47–60 mm		M	26				
			F/M	11/16	Blood	1.085; 0.370–1.899	WET: M; R	
					Kidney	1.217; 0.245–3.227		
					Liver	1.305; 0.440–2.587		
					Muscle	0.554; 0.188–2.330		
					Skin	0.848; 0.236–2.498		Site 1: Reference site: Little River downstream from the Great Smoky Mountains National Park
			F/M	10/10	Blood	1.097; 0.561–2.279		
					Kidney	1.921; 0.586–2.797		
					Liver	2.503; 0.688–5.004		
					Muscle	0.898; 0.317–1.528		

(*continued*)

TABLE A.20 (CONTINUED)
Selenium (Se)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
					Skin	0.942; 0.088–1.535		Site 2: Polluted superfund site: upper reach of East Fork Poplar Creek (EFPC) within the USA Department of Energy's (USDOE's) Y-12 National Security Complex
Nerodia sipedon							WET: M	Burger et al. (2007); USA; New Jersey and Tennessee
	Adult TL = 72; 39.0–103.5 cm TM = 159; 17.5–587.5 g	M; R	—	18				
					Blood	0.560		Site 1: Urban/suburban; New Jersey
					Kidney	1.077		
					Liver	1.184		
					Muscle	0.565		
					Skin	0.725		
				36	Blood	1.090		Site 2: Relatively rural; Tennessee, Department of Energy site
				46	Muscle	0.694		
				47	Liver	1.815		
Nerodia taxispilota								Burger et al. (2006); USA; South Carolina; 1 reference site; 1 polluted Savannah River Site, Aiken
	SVL = 59 cm TM = 170 g	M	—	10	Muscle *	2	DRY: M**	*Tail; **Both sites combined
	SVL = 65 cm TM = 230 g			9	Blood	1		

Pituophis melanoleucus							Ohlendorf et al. (1988); USA; Merced County, California
	—	—	18	Liver	11.1; 4.7–32.0	DRY: M; RM	Kesterson Reservoir; area contaminated by subsurface agricultural drainage water 4 sites, 2 years
			9		2.05; 1.4–3.6		Volta Wildlife Area and Grassland Water District.; 2 nearby reference sites, 2 years
					2.14; 1.3–3.6		
			1		1.6		USA, California, Yolo County, University of California at Davis
Pituophis melanoleucus							Burger (1992); USA; New Jersey
	Hatchling TM = 24.65 g	—	46	Skin	1.947; 1.210–2.556	DRY: M; RM	
			16	Whole body*	2.745; 1.612–3.451		*Saggital section from the center of the body, including bone
Python molurus							Boman et al. (2001); Vietnam; Dac Lac; seemingly unpolluted site
	Adult*	—	1	Muscle	0.9	DRY	* Fully grown
				Testis	2.1		

TABLE A.21
Silver (Ag)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Testudines								
Chelonia mydas								Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 49.0; 40.0–63.5 cm	M; R	M	6			DRY: M; R	
	SCL = 52.2; 37.0–71.4		F	20				
			M	6	Liver	1.7; 0.68–3.7		
				6	Kidney	0.021; 0.003–0.043		
				3	Muscle	0.003; 0.001–0.003		
			F	20	Liver	3.7; 1.1–9.3		
				19	Kidney	0.032; 0.008–0.120		
				9	Muscle	0.009; 0.002–0.021		
	Diet		—	8	Stomach*	0.027; 0.003–0.057		*Contents
Chelonia mydas								Anan et al. (2002); Japan; Okinawa Prefecture, Yaeyama Islands; tissues provided by fishermen
	—	—	—	9	Liver	0.89: 0.67–1.2	WET: M; R	
Chelonia mydas								Lam et al. (2004); South China
	Juvenile		—	2	Fat	0.007	DRY: M; R	Turtles stranded on beaches at Ham Tin Wan and Tung Pang Chau; upon collection, turtles had started to decay
					Kidney	0.058		
					Heart	ND (<—)–0.032		
					Liver	3.540		
					Lung	0.026		
					Muscle	ND (<—)–0.007		
					Stomach	ND (<—)–2.666		
	Adult		—	3	Muscle	ND (<—)–0.006		Specimens believed to have been unintentionally caught by fishermen; upon collection, turtles were relatively fresh

				1	Liver	0.150		
Dermochelys coriaca								Godley et al. (1998); Great Britain; Wales and Scotland, west coast; turtles died in fishing gear
	Adult CCL = 141–170 cm	R	M	3				
				1	Liver Muscle	0.17 ND (<0.003)	WET	
Eretmochelys imbricata								Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 43.5; 33.5–48.9	M; R	M	6	Liver	1.9; 0.80–3.3	DRY: M; R	
				6	Kidney	0.020; 0.011–0.032		
				1	Muscle	0.001		
	SCL = 46.5; 43.8–67.9		F	16	Liver	1.2; 0.17–2.8		
				13	Kidney	0.019; 0.009–0.038		
				8	Muscle	0.002; 0.001–0.003		
	Diet		—	6	Stomach* Contents	0.063; 0.024–0.16		
Eretmochelys imbricata								Anan et al. (2002); Japan; Okinawa Prefecture, Yaeyama Islands; tissues provided by fishermen
	—	—	—	7	Liver	0.38; 0.18–0.70	DRY: M; R	
Lepidochelys kempii								Kenyon et al. (2001); USA; Texas and Louisiana; turtles captured alive in nets at 4 beachfront sites
	SCL = 38.3; 21.6–65.8 cm	M; R	F/M/U	46/38/ 22*			WET: M; R	*99 wild grown plus 7 head-start female turtles
					Blood*	0.00094; 0.000042–0.00274		*Whole blood

TABLE A.22
Strontium (Sr)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Testudines								
Caretta caretta								Stoneburner et al. (1980); USA; 4 western Atlantic nesting beaches: Canaveral, Florida; Cumberland Island, Georgia; Cape Lookout, North Carolina; Cape Hatteras, North Carolina
	Egg*		—	96	Yolk	66.13–74.03	—: RM**	*Fresh; **combined by beach
Chelonia mydas								Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 49.0; 40.0–63.5 cm	M; R	M	6	Liver	1.35; 0.491–2.29	DRY: M; R	
				6	Kidney	5.97; 4.47–9.55		
				3	Muscle	2.22; 1.91–2.39		
	SCL = 52.2; 37.0–71.4 cm		F	20	Liver	1.46; 0.633–3.30		
				19	Kidney	5.96; 2.28–13.4		
				9	Muscle	4.16; 1.64–10.4		
	Diet		—	8	Stomach*	158; 49.4–326		*Contents
Chelonia mydas								Lam et al. (2004); South China
	Juvenile		—	2	Fat	1.121	DRY: M	Turtles stranded on beaches at Ham Tin Wan and Tung Pang Chau; upon collection, turtles had started to decay
					Kidney	6.553		
					Heart	5.760		
					Liver	2.236		
					Lung	12.90		
					Muscle	2.052		
					Stomach	12.86		

	Adult		—	3	Muscle	102.0		Turtles believed to have been unintentionally caught by fishermen; upon collection, turtles were relatively fresh
				1	Liver	114.4		
Chelydra serpentina								Meyers-Schöne et al. (1993); USA; Tennessee
	Adult		M	12	Bone	16.5	WET: M ^{90}Sr Bq/g	Site 1: White Oak Lake; contaminated site (3–5.9 µg Hg/g dry mass in sediment)
					Carapace	16.6		
			F/M	3/6	Bone	ND (<0.18)		Site 2: Bearden Creek embayment; reference site
					Carapace	ND (<0.18)		
Cuora amboinensis								Boman et al. (2001); Vietnam; Dac Lac; seemingly unpolluted site
	Adult*		—	1	Muscle	2.4	DRY	*Fully grown
					Liver	—		
	Egg*				Egg	69		*Gonadal system
Cyclemys dentata								Boman et al. (2001); Vietnam; Dac Lac; seemingly unpolluted site
	Adult*		—	1	Testis	11	DRY	*Fully grown
	Egg*				Egg	69		*Gonadal system
Eretmochelys imbricata								Anan et al. (2001); Japan; Yaeyama Islands; seemingly unpolluted site
	SCL = 43.5; 33.5–48.9 cm	M; R	M	6	Liver	1.65; 0.813–2.61	DRY: M; R	
				6	Kidney	10.7; 2.90–30.7		
				1	Muscle	1.12		
	SCL = 46.5; 43.8–67.9		F	16	Liver	3.59; 0.506–18.2		
				13	Kidney	18.5; 2.90–59.0		
				8	Muscle	4.29; 0.681–14.6		
	Diet		—	6	Stomach*	1.130; 22.9–5160		*Contents

(*continued*)

TABLE A.22 (CONTINUED)
Strontium (Sr)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
*Pelodiscus sinensis**							Boman et al. (2001); Vietnam; site: Hay Tay; seemingly unpolluted site; *[*Trionyx sinensis*]
	Egg*	—	1	Egg	9.3	DRY	*Gonadal system
Trachemys scripta							Meyers-Schöne et al. (1993); USA; Tennessee
	Adult	F/M	6/6	Bone	4.26×10^2	WET: M ^{90}Sr Bq/g	Site 1: Contaminated site: White Oak Lake (3–5.9 µg Hg/g dry mass in the sediment)
				Carapace	3.66×10^2		
		F/M	6/6	Bone	<0.28		Site 2: Reference site: Bearden Creek embayment
				Carapace	0.16		
Crocodylia							
Alligator mississippiensis							Seltzer et al. (2006); USA; Louisiana; preserved samples originated from alligators subject to previous studies (Lance et al. 2006) and analyzed using laser ablation inductively coupled plasma mass spectrometry (laser ablation ICP-MS)
	Adult			Bone*		R**	*Femur; **bulk concentration
	A = ca. 27 years						
	TL = 2.69–3.82 m	F/M	1/3		329–377		Captive alligators
	TL = 2.24–2.49 m	—	3		898–1065		Wild alligators
Crocodylus acutus							Stoneburner and Kushlan (1984); USA; Florida, Florida Bay, Everglades, National Park
	Egg	—	9	Shell	529.50	DRY: M	
				Albumen-yolk	45.65		

Crocodylus johnstoni								Jeffree et al. (2001); northern Australia; north central Queensland, Lynd River; samples from a single population
	A = 0.7–62.7 years L = 24.7–128.3 cm		F/M	9/21			DRY: M; R	
			—	30	Osteoderm*	512; 341–645		*Ventral pelvic region
Crocodylus niloticus								Swanepoel et al. (2000); South Africa; Kruger National Park (KNP) area
	TL = 1.40–4.15 m	R	F/M	6/9			DRY: M	
								Site 1: Shingwedzi River (Silwervis Dam), northern part of KNP; catchment area outside KNP is limited
					Muscle	26.7		Frozen tissues used for residue analysis
					Liver	24.4		
					Kidney	32.5		
					Fat	23.2		
								Site 2: Olifants River, central part of KNP; flows through areas of intense agricultural activity and passes mining areas and receives tributaries from Phalaborwa Mining Company before entering the KNP
					Muscle	6.7		Frozen tissues used for residue analysis
					Liver	6.6		
					Kidney	8.3		
					Fat	6.9		
								Site 3: Sabi River, southern part of KNP; flows through areas of intense agricultural activity before entering the KNP
					Muscle	8.2		Frozen tissues used for residue analysis
					Liver	8.5		

(*continued*)

TABLE A.22 (CONTINUED)
Strontium (Sr)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
				Kidney	8.0		
				Fat	8.2		
Crocodylus porosus							Yoshinaga et al. (1992); Papua New Guinea
	—	—	—	Muscle	0.17	WET: M	
Crocodylus porosus							Jeffree et al. (2001); northern Australia; Alligator Rivers region, Kakadu National Park; 3 river catchments, mining and hunting areas included
	A = 5–40 years L = 168–499 cm	—	40			DRY: M; R	
		—	35	Muscle*	ND (<0.05)		*Tail
		—	40	Osteoderm*	318; 121–923		*Ventral pelvic region
Squamata: Sauria							
*Laudakia s. stellio**							Loumbourdis (1997); Greece; Thessaloniki region; *[*Agama s. stellio*]
	Adult		—	Liver	16.48	DRY: M	Site 1: Urban area (500 m asl)
				Carcass*	163.23		*Gut removed
				Liver	40.24		Site 2: Agricultural area (50 m asl)
				Carcass*	396.12		*Gut removed
Tarentola mauritanica							Fletcher et al. (2006); southern Spain; Guadiamar River Valley; mine tailings release event; Boliden-Apirsa mine at Aznalcóllar; 7 study sites spanning an expected contamination gradient; geckos collected from building walls
	Adults and juveniles		52	Whole body*		DRY: M; R	*Minus gut contents

		—	9		97.764; 52.363–145.582		Site 1: Rural not mine-affected site: near Guadalmellato (most pristine)
		—	13		103.923; 58.008–174.609		Site 2: Urban not mine-affected site: Villaviciosa de Cordoba (not contaminated by mining)
		—	8		77.397; 45.724–120.103		Site 3: Urban mine-affected site: Aznalcázar (contamination through aerosolized contaminants engulfed the town during cleanup)
		—	8		59.640; 39.080–93.112		Site 4: Urban mine-affected site: Aznalcóllar (contaminated by normal mine operations, or the disaster and subsequent remediation efforts)
		—	5		94.039; 56.376–141.967		Site 5: Floodplain mine-affected site: Guadiamar River floodplain near the Aznalcázar gauge station (24.8 km below the ruptured tailings dam)
		—	5		66.995; 26.554–99.786		Site 6: Floodplain mine-affected site: Guadiamar River floodplain near the Guijo gauge station (7.4 km below the ruptured dam)
		—	4		71.092; 31.063–113.816		Site 7: Floodplain mine-affected site: Agrio River floodplain (4.4 km below and closest to the ruptured tailings dam)
							Statistical analysis: [Sr] concentration was significantly influenced by site
Varanus salvator							Boman et al. (2001); Vietnam; Dac Lac; seemingly unpolluted site
	Adult*	—	1	Muscle	3.3	DRY	*Fully grown
				Liver	1.7		
	Egg*			Egg	72		*Gonadal system
Varanus sp.							Yoshinaga et al. (1992); Papua New Guinea
	—	—	—	Muscle	0.11	WET: M	

(*continued*)

TABLE A.22 (CONTINUED)
Strontium (Sr)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Squamata: Serpentes							
Acrochordus javanicus							Yoshinaga et al. (1992); Papua New Guinea
	—	—	—	Muscle	0.15	WET: M	
Agkistrodon piscivorus							Burger et al. (2006); USA; South Carolina; 1 reference site; 1 polluted Savannah River site, Aiken
	SVL = 57 cm TM = 280 g	—	13	Muscle*	152	DRY: M**	*Tail; **both sites combined
	SVL = 60 cm TM = 270 g		5	Blood	0.2		
Lapemis hardwickii							Boman et al. (2001); Vietnam; Nha Trang; seemingly unpolluted site
	Adult*	—	1	Muscle Liver	3.1 2.0	DRY	*Fully grown
Nerodia fasciata							Hopkins et al. (2001); USA; South Carolina; Aquatic Ecology Laboratory near Aiken; lab feeding experiment; experimental animals were captured at uncontaminated reference site and fed 52 total meals of field-collected prey (primarily fish) for 13.5 months
	TM*					DRY	*Initial/final mass
	Juvenile and adult TM = 61.91/169.6 g*	M	—	8	Gonad	1.98; 1.13–3.27	MD; R	Treatment 1: Snakes fed prey items collected from coal ash-contaminated site: Savannah River site
				Kidney	2.10; 1.72–2.82		
				Liver	0.90; 0.68–1.79		
				Shed skin	6.51; 4.21–10.25		
				Blood	0.42; 0.34–0.51		

				Tail clip	238.0; 184.9–320.73		
	Diet			Fish	274.992; 220.727–362.650	GLSM; R	
							Treatment 2: Snakes fed prey items collected from uncontaminated reference site
	Juvenile and adult	M	—	7	Gonad	0.88; 0.35–1.95	
	TM = 62.2/136.3 g*						
					Kidney	1.00: 0.81–2.18	MD; R
					Liver	0.46; 0.29–1.02	
					Shed skin	5.14; 1.57–19.64	
					Blood	0.2; 0.12–0.25	
					Tail clip	102.64; 69.85–149.2	
	Diet				Fish	128.085; 104.038–184.044	GLSM; R
							Statistical analysis: Treatment differences for [Sr] were significant for snake blood, and fish
Nerodia fasciata							Hopkins et al. (2002); USA; South Carolina; Aquatic Ecology Laboratory near Aiken; 2-year feeding experiment; all snakes were lab reared and originated from a single gravid female that was collected from a reference site on the Savannah River site; exposure started after first hibernation
	Diet			*	Fish		DRY	*3 prey species, 4 specimens per species
	Juvenile**						DRY	**Morphometry in Figure 2 in Hopkins et al. (2002)
	Diet		—	*	Fish	128.085	GLSM	Treatment 1: Control: snakes fed only prey items collected from uncontaminated reference site
	Juvenile**		F	6	Gonad	1.217	M	
					Kidney	1.059		
					Liver	0.584		
			M	4	Gonad	1.504	M	
					Kidney	1.401		

(*continued*)

TABLE A.22 (CONTINUED)
Strontium (Sr)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
					Liver	0.338		
	Diet		—	*	Fish	198.250	GLSM	Treatment 2: Lower-level exposure: snakes fed prey items collected from coal ash-contaminated site and from the reference site on alternating weeks
	Juvenile**		F	6	Gonad	1.715	M	
					Kidney	2.023		
					Liver	0.765		
			M	4	Gonad	1.839	M	
					Kidney	2.914		
					Liver	0.521		
	Diet		—	*	Fish	274.992	GLSM	Treatment 3: Higher-level exposure: snakes fed only prey items collected from coal ash-contaminated site
	Juvenile**		F	6	Gonad	2.435	M	
					Kidney	2.279		
					Liver	0.836		
			M	3	Gonad	2.177	M	
					Kidney	3.412		
					Liver	0.730		
								Statistical analysis: Effects of organ, treatment, and organ by sex on [Sr] concentration were significant
Nerodia fasciata								Burger et al. (2006); USA; South Carolina; 1 reference site; 1 polluted Savannah River site, Aiken
	SVL = 51 cm TM = 130 g	M	—	47	Muscle*	94	DRY: M**	*Tail; **both sites combined
	SVL = 52 cm TM = 140 g			34	Blood	0.14		

Nerodia taxispilota							Burger et al. (2006); USA; South Carolina; 1 reference site; 1 polluted Savannah River site, Aiken
	SVL = 59 cm TM = 170 g	—	10	Muscle*	156	DRY: M**	*Tail; **both sites combined
	SVL = 65 cm TM = 230 g		9	Blood	0.2		
Python molurus						DRY	Boman et al. (2001); Vietnam; Dac Lac; seemingly unpolluted site
	Adult*	—	1	Muscle Testis	2.2 2.8		*Fully grown

TABLE A.23
Thallium (Tl)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Testudines								
*Caretta caretta**								Aguirre et al. (1994); USA; Hawaiian Islands; *[identification number 052, *Chelonia mydas* in the reference]
	"Pelagic"		—	1	Liver	1.1	WET	
Chelonia mydas								Aguirre et al. (1994); USA; Hawaiian Islands
	L = 28.7–71.3 cm W = 3.2–43.6 kg	R	F/M	8/4	Liver	ND (<0.7)–1.0	WET: R	
					Kidney	ND (<0.7)		
	Egg*		—	3	Shell	ND (<0.7)		*Posthatch
	Hatchling				Whole body	ND (<0.7)		
Chelonia mydas								Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 49.0; 40.0–63.5 cm	M; R	M	6			DRY: M; R	
	SCL = 52.2; 37.0–71.4 cm		F	20				
			M	6	Liver	0.002; <0.001–0.003		
				6	Kidney	0.014; 0.005–0.023		
				3	Muscle	0.004; 0.003–0.004		
			F	20	Liver	0.002; <0.001–0.003		
				19	Kidney	0.018; 0.007–0.044		
				9	Muscle	0.002; 0.001–0.004		
	Diet		—	8	Stomach*	0.006; 0.003–0.015		*Contents
Chelonia mydas								Lam et al. (2004); South China

	Juvenile		—	2	Fat	0.001	DRY: M	Tutles stranded on beaches at Ham Tin Wan and Tung Pang Chau; upon collection, turtles had started to decay
					Kidney	0.014		
					Heart	0.007		
					Liver	0.002		
					Lung	0.002		
					Muscle	0.002		
					Stomach	0.020		
	Adult		—	3	Muscle	0.004		Turtles believed to have been unintentionally caught by fishermen; upon collection, turtles were relatively fresh
				1	Liver	0.003		
Eretmochelys imbricata								Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 43.5; 33.5–48.9 cm	M; R	M	6	Liver	0.008; 0.004–0.014	DRY: M; R	
				6	Kidney	0.015		
				1	Muscle	0.005; 0.020–0.004		
	SCL = 46.5; 43.8–67.9 cm		F	16	Liver	0.006; 0.002–0.015		
				13	Kidney	0.013; 0.004–0.033		
				8	Muscle	0.005; 0.001–0.008		
	Diet		—	6	Stomach*	0.004; 0.001–0.006		*Contents
Crocodylia								
Alligator mississippiensis								Heinz et al. (1991); USA; central and southern Florida; Lake Okeechobee, Lake Griffin, and Lake Apopka
	Egg		—	32*	Egg	ND (<0.80)	WET	*16 nests, 2 eggs per nest

(*Continued*)

TABLE A.23 (CONTINUED)
Thallium (Tl)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Squamata: Sauria							
Tarentola mauritanica							Fletcher et al. (2006); southern Spain; Guadiamar River Valley, Boliden-Apirsa mine at Aznalcóllar; mine tailings release event; 7 study sites spanning an expected contamination gradient; geckos collected from building walls
	Juvenile and adult		52	Whole body*		DRY: M; R	*Minus gut contents
		—	9		0.004; 0.004–0.004		Site 1: Rural not mine-affected site: near Guadalmellato (most pristine)
		—	13		0.004; 0.004–0.010		Site 2: Urban not mine-affected site: Villaviciosa de Cordoba (not contaminated by mining)
		—	8		0.009; 0.004–0.043		Site 3: Urban mine-affected site: Aznalcázar (contamination through aerosolized contaminants engulfed the town during cleanup)
		—	8		0.006; 0.004–0.012		Site 4: Urban mine-affected site: Aznalcóllar (contaminated by normal mine operations, or the disaster and subsequent remediation efforts)
		—	5		0.017; 0.011–0.021		Site 5: Floodplain mine-affected site: Guadiamar River floodplain near the Aznalcázar gauge station (24.8 km below the ruptured tailings dam)
		—	5		0.041; 0.004–0.076		Site 6: Floodplain mine-affected site: Guadiamar River floodplain near the Guijo gauge station (7.4 km below the ruptured dam)
		—	4		0.048; 0.018–0.060		Site 7: Floodplain mine-affected site: Agrio River floodplain (4.4 km below and closest to the ruptured tailings dam)
							Statistical analysis: [Tl] concentration was not significantly influenced by site

TABLE A.24
Titanium (Ti)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Crocodylia								
Crocodylus porosus								
								Jeffree et al. (2001); northern Australia, Kakadu National Park, Alligator Rivers region; samples from 3 river catchments including mining and hunting areas
	Age = 5–40 years L = 168–499 cm	R	—	40				
							DRY: GM; R	
			—	35	Muscle*	6.22; 1.80–10.2		*Tail
			—	40	Osteoderm*	<DL (0.01)		*Ventral pelvic region

TABLE A.25
Vanadium (V)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Testudines								
*Actinemys marmorata**								Henny et al. (2003); USA; Western Oregon, Fern Ridge Reservoir; *[*Clemmys marmorata*]
	Egg		—	14*	Egg contents	ND (<1.50)	DRY: GM; R	*14 nests, 1 egg per nest
*Caretta caretta**								Aguirre et al. (1994); USA; Hawaiian Islands; *[identification number 052, *Chelonia mydas* in the reference]
	"Pelagic"		—	1	Liver	ND (<0.2)	WET	
Chelonia mydas								Aguirre et al. (1994); USA; Hawaiian Islands
	L = 28.7–71.3 cm W = 3.2–43.6 kg		F/M	8/4	Liver	ND (<0.2)–1.5	WET: R	
					Kidney	ND (<0.2)–2.5		
	Egg*		—	3	Shell	ND (<0.2)		*Posthatch
	Hatchling				Whole body	ND (<0.2)		
Chelonia mydas								Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 49.0; 40.0–63.5 cm	M; R	M	6			DRY: M; R	
	SCL = 52.2; 37.0–71.4		F	20				
			M	6	Liver	0.58; 0.065–1.1		
				6	Kidney	0.59; 0.23–1.2		
				3	Muscle	0.14; 0.1–0.17		
			F	20	Liver	1.1; 0.15–2.4		
				19	Kidney	1.9; 0.29–3.6		
				9	Muscle	0.16; 0.12–0.21		
	Diet		—	8	Stomach*	13; 2.0–49		*Contents
Chelonia mydas								Lam et al. (2004); South China

	Juvenile		—	2	Fat	1.393	DRY: M	Turtles stranded on beaches at Ham Tin Wan and Tung Pang Chau; upon collection, turtles had started to decay
					Kidney	0.481		
					Heart	0.483		
					Liver	0.577		
					Lung	0.849		
					Muscle	0.284		
					Stomach	0.889		
	Adult		—	3	Muscle	0.525		Turtles believed to have been unintentionally caught by fishermen; upon collection, turtles were relatively fresh
				1	Liver	1.237		
Eretmochelys imbricata								Sadiq and Zaidi (1984); Saudi Arabia; turtle collected from shoreline, freshly dead or near dying (April 1983) after oil spill (February 1983)
	—		—	1	Muscle	ND (<1)	WET	
					Liver	6.50		
Eretmochelys imbricata								Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 43.5; 33.5–48.9	M; R	M	6	Liver	0.43; 0.064–0.79	DRY: M; R	
				6	Kidney	0.46; 0.21–0.62		
				1	Muscle	0.28		
	SCL = 46.5; 43.8–67.9		F	16	Liver	0.32; 0.05–1.1		
				13	Kidney	0.46; 0.098–1.2		
				8	Muscle	0.036; 0.005–0.088		
	Diet		—	6	Stomach*	0.51; 0.21–0.79		*Contents
Trachemys scripta elegans								Tryfonas et al. (2006); USA; Illinois; Lower Illinois River near Grafton; eggs laid in the lab from turtles collected in the field from 5 nesting areas

(*continued*)

TABLE A.25 (CONTINUED)
Vanadium (V)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
	Egg		—	Contents	ND (<—)	DRY: M*	*All sites combined; values estimated from graph
				Shell	1.6		
	Diet			*Lemna* sp.	47.9		
	Environment		4–5*	Soil	141–333	RM*	*Samples (soil from nesting and lake bank areas) from 2 sites
			3*	Sediment	180–220	RM*	*Samples (3 layers per site) from 2 sites; values estimated from graph
			3–4*	Water	ND (<0.01)	WET; RM*	*Samples from 3 sites; values estimated from graph
Crocodylia							
Alligator mississippiensis							Heinz et al. (1991); USA; central and southern Florida; Lake Okeechobee, Lake Griffin, and Lake Apopka
	Egg	—	32*	Egg	ND (<0.01)	WET	*16 nests, 2 eggs per nest
Squamata: Sauria							
Tarentola mauritanica							Fletcher et al. (2006); southern Spain; Guadiamar River Valley; mine tailings release event; Boliden-Apirsa mine at Aznalcóllar; 7 study sites spanning an expected contamination gradient; geckos collected from building walls
	Juvenile and adult	—	52	Whole body*		DRY: M; R	*Minus gut contents
		—	9		0.360; 0.283–0.439		Site 1: Rural not mine-affected site: near Guadalmellato (most pristine)
		—	13		0.514; 0.277–0.959		Site 2: Urban not mine-affected site: Villaviciosa de Cordoba (not contaminated by mining)
		—	8		0.532; 0.268–0.796		Site 3: Urban mine-affected site: Aznalcázar (contamination through aerosolized contaminants engulfed the town during cleanup)

			—	8		0.586; 0.353–1.068		Site 4: Urban mine-affected site: Aznalcóllar (contaminated by normal mine operations, or the disaster and subsequent remediation efforts)
			—	5		0.415; 0.316–0.588		Site 5: Floodplain mine-affected site: Guadiamar River floodplain near the Aznalcázar gauge station (24.8 km below the ruptured tailings dam)
			—	5		0.574; 0.331–1.355		Site 6: Floodplain mine-affected site: Guadiamar River floodplain near the Guijo gauge station (7.4 km below the ruptured dam)
			—	4		0.530; 0.417–0.763		Site 7: Floodplain mine-affected site: Agrio River floodplain (4.4 km below and closest the ruptured tailings dam) Statistical analysis: [Al] concentration was significantly influenced by site
Squamata: Serpentes								
"Sea snake"	—		—					Sadiq and Zaidi (1984); Saudi Arabia; snakes collected from shoreline, freshly dead or near dying (April 1983) after oil spill (February 1983)
				7	Muscle Liver	ND (<1)–2.36 1–6.64	WET: R	
Agkistrodon piscivorus								Burger et al. (2006); USA; South Carolina; 1 reference site; 1 polluted Savannah River site, Aiken
	SVL = 57 cm TM = 280 g	M	—	13	Muscle*	2	DRY: M**	*Tail; **both sites combined
	SVL = 60 cm TM = 270 g			5	Blood	0.1		
Nerodia fasciata								Hopkins et al. (2001); USA; South Carolina; Aquatic Ecology Laboratory near Aiken; lab feeding experiment; experimental animals were captured at uncontaminated reference site and fed 52 total meals of field-collected prey (primarily fish) for 13.5 months

(*continued*)

TABLE A.25 (CONTINUED)
Vanadium (V)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
	TM*					DRY	*Initial/final mass
	Juvenile and adult	M					Treatment 1: Snakes fed prey items collected from coal ash-contaminated site: Savannah River site
	TM = 61.91/169.6 g*						
		—	8	Gonads	ND (<0.011); ND (<0.011)–1.87	MD; R	
				Kidney	0.25; 0.06–0.90		
				Liver	1.38; 0.99–3.29		
				Shed skin	0.47; 0.12–1.35		
				Blood	0.01; ND (<0.003)–0.02*		*WET
				Tail clip	ND (<0.007)		
	Diet			Fish	1.786; 0.347–7.281	GLSM; R	
	Juvenile and adult	M				MD; R	Treatment 2: Snakes fed prey items collected from uncontaminated reference site
	TM = 62.2/136.3 g*						
		—	7	Gonads	ND (<0.011); ND (<0.011)–0.99		
				Kidney	ND (<0.009); ND (<0.009)–0.25		
				Liver	ND (<0.009); ND (<0.009)–1.5		
				Shed skin	0.05; ND (<0.008)–0.32		
				Blood	ND (<0.01); ND (<0.003)–0.02*		*WET
				Tail clip	ND (<0.007)		

	Diet			Fish	0.163; 0.045–0.463	GLSM; R	
							Statistical analysis: Treatment differences for [V] were significant for fish only
Nerodia fasciata							
							Hopkins et al. (2002); USA; South Carolina; Aquatic Ecology Laboratory near Aiken; 2-year feeding experiment; all snakes were lab reared and originated from a single gravid female that was collected from a reference site on the Savannah River site; exposure started after first hibernation
	Diet		*	Fish			*3 prey species, 4 specimens per species
	Juvenile**					DRY	**Morphometry in Figure 2 in Hopkins et al. (2002)
	Diet	—	12*	Fish	0.163	GLSM	Treatment 1: Control: snakes fed only prey items collected from uncontaminated reference site
	Juvenile**	F	6	Gonad	0.179	M	
				Kidney	0.184		
				Liver	0.392		
		M	4	Gonad	0.214	M	
				Kidney	0.249		
				Liver	0.366		
	Diet	—	12*	Fish	0.938	GLSM	Treatment 2: Lower-level exposure: snakes fed prey items collected from coal ash-contaminated site and from the reference site on alternating weeks
	Juvenile**	F	6	Gonad	0.123	M	
				Kidney	0.483		
				Liver	0.914		
		M	4	Gonad	0.482	M	
				Kidney	0.310		
				Liver	0.939		
	Diet	—	12*	Fish	1.786	GLSM	Treatment 3: Higher-level exposure: snakes fed only prey items collected from coal ash-contaminated site
	Juvenile**	F	6	Gonad	0.179	M	

(*continued*)

TABLE A.25 (CONTINUED)
Vanadium (V)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
					Kidney	0.613		
					Liver	1.693		
			M	3	Gonad	0.410	M	
					Kidney	0.494		
					Liver	1.558		
								Statistical analysis: Effects of organ, treatment, sex, organ by treatment, organ by sex, and organ by treatment by sex on [V] concentration were significant
Nerodia fasciata			—					Burger et al. (2006); USA; South Carolina; 1 reference site; 1 polluted Savannah River site, Aiken
	SVL = 51 cm TM = 130 g	M		47	Muscle*	0.6	DRY: M**	*Tail; **both sites combined
	SVL = 52 cm TM = 140 g			34	Blood	0.04		
Nerodia taxispilota			—					Burger et al. (2006); USA; South Carolina; 1 reference site; 1 polluted Savannah River site, Aiken
	SVL = 59 cm TM = 170 g	M		10	Muscle*	0.9	DRY: M**	*Tail; **both sites combined
	SVL = 65 cm TM = 230 g			9	Blood	0.04		

TABLE A.26
Zinc (Zn)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Testudines							
*Actinemys marmorata**							Henny et al. (2003); USA; western Oregon, Fern Ridge Reservoir; *[*Clemmys marmorata*]
	Egg	—	14*	Contents	65.1; 43.5–100	DRY: GM; R	*14 nests, 1 egg per nest
							Statistical analysis: No significant differences in contaminant concentrations related to hatching rate
*Aldabrachelys gigantea**							Lance et al. (1995); various sources (zoos to wild caught); *[*Geochelone gigantea*]
	—	—	3	Blood*	6.87; 1.81–10.7	WET: M; R	*Plasma
Caretta caretta							Hillestad et al. (1974); USA; Georgia and South Carolina; 3 nesting beaches
	Egg	—	—	Yolk Albumen	32.25 26.0	—: M	
Caretta caretta							Stoneburner et al. (1980); USA; 4 western Atlantic nesting beaches: Florida, Canaveral; Georgia, Cumberland Island; North Carolina, Cape Lookout; North Carolina, Cape Hatteras
	Egg*	—	96	Yolk	73.54–80.50	—: RM**	*Fresh; **combined by beach
*Caretta caretta**							Aguirre et al. (1994); USA; Hawaiian Islands; *[identification number 052, *Chelonia mydas* in the reference]
	"Pelagic"	—	1	Liver	15.1	WET	
Caretta caretta							Sakai et al. (1995); Japan; Kochi Prefecture, Cape Ashizuri

(*continued*)

TABLE A.26 (CONTINUED)
Zinc (Zn)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
	L = 76–92 cm W = 75–108 kg		F/M	6/1	Liver	27.9; 23.2–35.1	WET: M; R	
					Kidney	25.8; 19.2–30.4		
					Muscle*	24.2; 19.5–31.0		*Pectoral
	Egg*		—	*	Shell	2.17; 1.66–2.87		*Oviductal eggs from 5 females
					Yolk	34.4; 30.5–38.0		
					Albumen	0.594; 0.058–1.54		
					Whole egg	14.7; 13.2–16.5*		*Calculated
Caretta caretta								Gordon et al. (1998); Australia; southeastern Queensland, Moreton Bay region; stranded turtles
	—	—	—	5	Liver	22.8; 13.7–32.6	WET: M; R	
				5	Kidney	18.4; 16.7–21.3		
Caretta caretta								Caurant et al. (1999); France; Atlantic coasts; "Pertuis charentais" in the la Rochelle region; stranded turtles
	Juvenile SCL = 29.4; 21.3–34.5 cm TM = 2.8; 0.3–7.5 kg	M; R	—	21				
				7	Liver	25.0; 14.5–38.4	WET: M; R	
				5	Kidney	23.6; 16.5–33.8		
				21	Muscle*	19.6; 12.2–36.3		*Pectoral
Caretta caretta								Sakai et al. (2000b); Japan; Kochi Prefecture, Cape Ashizuri; turtles accidentally caught in commercial fishing nets
	TM = 93/83 kg SCL = 83/85 cm	M	F/M	6/1	Liver	28.1/26.6	WET: M	
					Gullet	12.5/10.5		
					Stomach	13.0/3.22		

				Intestine	30.4/20.1		
				Pancreas	117/86.5		
				Heart	36.2/32.5		
				Trachea	4.56/2.93		
				Lung	16.7/12.1		
				Bladder	14.4/12.6		
				Spleen	21.9/20.3		
				Kidney	25.4/28.4		
				Salt gland	14.5/11.1		
				Brain	8.78/5.64		
	Reproductive tissues			Testis	26.0		
				Oviduct	7.48		
				Ovary	37.7		
	Egg			Whole egg	14.7		
				Shell	2.17		
				Yolk	34.4		
				Albumen	0.59		
				Scale	12.6/7.05		
				Mesentary	7.92/5.65		
				Fat	96.1/85.6		
				Muscle	25.0/19.5		
				Bone	197/139		
				Carapace	198/142		
				Whole body*	73.8/56.7		*Calculated
Caretta caretta							Kaska and Furness (2001); southwestern Turkey; 4 beaches; collected either just before hatching or dead in shell 1 week after last hatching
	Embryo	—	22	Liver	23.84	DRY: M	
	Egg	—		Eggshell	5.00		
				Yolk	57.21		
							Statistical analysis: No significant differences were observed

(*continued*)

TABLE A.26 (CONTINUED)
Zinc (Zn)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Caretta caretta								Franzellitti et al. (2004); Italy; northwestern Adriatic Sea; turtles collected dead along the Adriatic Sea coast from the Po delta to the Reno mouth
	Juvenile to adult MSCL = 24.5–74 cm	R	—	*			WET: M	*16 from fisheries by-catch, 19 dead on coast
				30	Liver	27.9		
				13	Lung	17.0		
				17	Muscle*	30.9		*Pectoral
				7	Fat*	68.2		*Abdominal
Caretta caretta								Torrent et al. (2004); Spain; Canary Islands; stranded turtles submitted to the veterinary faculty of the University of Las Palmas in Gran Canaria
	Juvenile and subadult SCL = 15–65 cm	R*	F/M	67/11	Kidney	9.09; 0.07–38.53	WET: M; R	*Values estimated from graph
					Muscle	6.70; 0.05–32.37		
					Bone	51.28; 0.46–216.25		
					Liver	13.48; 0.09–91.38		
Caretta caretta								Maffucci et al. (2005); southern Italy; western Mediterranean Sea; turtles stranded along the South Tyrrhenian coasts
	CCL = 37–82 cm	R	—	29			DRY: M; R	
				14	Liver	66.0; 23.8–178		
				21	Kidney	97.0; 62.4–206		
				24	Muscle	107.0; 76.4–177		
Caretta caretta								Storelli et al. (2005); Italy; Adriatic and Ionian Seas; stranded turtles
	SCL = 21–71 cm	R		19	Liver	29.3; 18.8–46.5	WET: M; R	

					Kidney	23.1; 16.6–27.9		
					Muscle	27.9; 19.8–35.1		
					Spleen	39.9; 19.4–53.7		
					Heart	30.3; 21.4–42.2		
					Lung	22.5; 13.0–35.9		
					Fat	64.7; 37.6–94.9		
Caretta caretta								Gardner et al. (2006); Mexico; Baja California peninsula; turtles died from fisheries capture
	MSCL = 57.0; 52.0–63.0	M; R		5	Liver	69.14; 42.45–91.87	DRY: GM; R	
					Kidney	32.47; 2.68–130		
					Muscle*	31.11; 0.63–100		*Pectoral
					Fat	12.66; 0.53–44.76		
Chelonia sp.								Yoshinaga et al. (1992); Papua New Guinea
	—		—	—	Muscle	15.7	WET: M	
Chelonia mydas								Yoshinaga et al. (1992); Papua New Guinea
	—		—	—	Muscle	20.4	WET: M	
Chelonia mydas								Aguirre et al. (1994); USA; Hawaiian Islands
	L = 28.7–71.3 cm W = 3.2–43.6 kg		F/M	8/4			WET: M; R	
				12	Liver	31.87; 18.1–45.8		
					Kidney	22.29; 12.5–38.1		
	Egg*		—	3	Shell	6.25		*Posthatch
	Hatchling				Whole body	12.1		
Chelonia mydas								Lance et al. (1995); Costa Rica; *Nesting
	Adult		F*	5	Blood*	1.00; 0.67–1.29	WET: M; R	*Plasma
Chelonia mydas								Gladstone (1996) from Gordon et al. (1998); Australia, Torres Strait
	—	—	—	7	Liver	38.6; 24.0–52.0	WET: M; R	
					Kidney	23.8; 19.0–29.0		

(*continued*)

TABLE A.26 (CONTINUED)
Zinc (Zn)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Chelonia mydas								Gordon et al. (1998); Australia; south-eastern Queensland, Moreton Bay region; stranded turtles
	—	—	—	30	Liver	39.7; 16.7–92.7	WET: M; R	
				30	Kidney	21.3; 15.4–31.8		
Chelonia mydas								Sakai et al. (2000a); Japan; Yaeyama Islands, Okinawa; caught by fishermen for commercial use
	SCL = 51.0 cm	M	—	50			WET: M; R	
				50*	Liver	30.3; 17.5–47.1		*All samples above detection limit
				23*	Kidney	29.6; 17.5–44.7		
				47*	Muscle	8.79; 3.30–33.4		
Chelonia mydas								Sakai et al. (2000b); Japan; Ogasawara Islands (Bonin Islands), Haha-Jima Island; turtles collected in coastal waters
	TM = 124/117 kg SCL = 93/97 cm	M	F/M	1/1	Liver	59.5/57.1	WET: M	CW = 72/73 cm
					Gullet	40.8/15.9		
					Stomach	11.9/15.9		
					Intestine	31.6/24.0		
					Pancreas	1120/347		
					Heart	24.4/31.5		
					Trachea	7.03/9.82		
					Lung	11.9/14.9		
					Bladder	16.7/13.3		
					Spleen	20.6/18.4		
					Kidney	32.8/35.2		
					Salt gland	17.9/11.5		
					Brain	8.19/9.38		
	Reproductive tissues				Testis	11.0		
					Oviduct	7.63		

					Ovary	50.5		
	Egg				Whole egg	20.3		
					Shell	0.555		
					Yolk	47.2		
					Albumen	1.29		
					Scale	26.7/13.4		
					Mesentary	5.87/6.52		
					Fat	56.4/46.2		
					Muscle	10.5/8.84		
					Bone	247/106		
					Carapace	347/292		
					Whole body*	91.2/74.0		*Calculated
Chelonia mydas								Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 49.0; 40.0–63.5 cm	M; R	M	6				
	CW = 40.5; 35.3–49.4							
	SCL = 52.2; 37.0–71.4		F	20			DRY: M; R	
	CW = 43.9; 31.7–58.5							
			M	6	Liver	76.4; 42.6–116		
				6	Kidney	142; 97.0–189		
				3	Muscle	58.0; 39.2–61.0		
			F	20	Liver	90.4; 51.2; 166		
				19	Kidney	179; 85.5–352		
				9	Muscle	44.3; 24.4–69.2		
	Diet		—	8	Stomach*	6.42; 3.79–9.53		*Contents
Chelonia mydas								Anan et al. (2002); Japan; Okinawa Prefecture, Yaeyama Islands; tissues provided by fishermen
	—	—	—	9	Liver	33.3; 22.2–56.6	WET: M; R	

(*continued*)

TABLE A.26 (CONTINUED)
Zinc (Zn)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Chelonia mydas								Lam et al. (2004); south China
	Juvenile		—	2	Fat	105.0	DRY: M	Turtles stranded on beaches at Ham Tin Wan and Tung Pang Chau; upon collection, turtles had started to decay
					Kidney	143.1		
					Heart	232.6		
					Liver	128.9		
					Lung	145.8		
					Muscle	238.7		
					Stomach	211.6		
	Adult		—	3	Muscle	147.7		Turtles believed to have been unintentionally caught by fishermen; upon collection, turtles were relatively fresh
				1	Liver	254.6		
Chelonia mydas								Gardner et al. (2006); Mexico; Baja California peninsula; turtles died from fisheries capture
	MSCL = 62.13; 48.5–76.9	M; R		11	Liver	62.91; 1.32–166	DRY: GM; R	
					Kidney	128; 1.59–330		
					Muscle*	38.26; 10.44–134		*Pectoral
					Fat	49.82; 19.51–163		
*Chelonoidis nigra**								Lance et al. (1995); various sources (zoos to wild caught); *[*Geochelone elephantopus*]
	—		—	5	Blood*	3.07; 2.39–3.53	WET: M; R	*Plasma
Chelydra serpentina								Albers et al. (1986); USA; Maryland and New Jersey
	L = 20–40 cm	R	M	7	Liver	27.72	WET: M	Site 1: Undisturbed freshwater site: Maryland, Patuxent Wildlife Research Center
			F	6		29.29		

		M	7	Kidney	8.80		
		F	6		9.60		
		M	8	Liver	50.38		Site 2: Contaminated brackish water site: New Jersey, Hackensack Meadowlands
		F	3		38.95		
		M	8	Kidney	9.93		
		F	3		9.79		
		M	8	Liver	30.68		Site 3: Contaminated freshwater site: New Jersey Hackensack Meadowlands
				Kidney	10.51		
Chelydra serpentina							Lance et al. (1995); various sources (zoos to wild caught)
	—	—	22	Blood*	2.70; 1.02–4.62	WET: M; R	*Plasma
Chrysemys picta							Lance et al. (1995); various sources (zoos to wild caught)
	—	—	2	Blood*	1.56–2.34	WET: R	*Plasma
Cuora amboinensis							Boman et al. (2001); Vietnam; Dac Lac; seemingly unpolluted site
	Adult*	—	1	Muscle	280	DRY	*Fully grown
				Liver	100		
	Egg*			Egg	100		*Gonadal system
Cyclemys dentata							Boman et al. (2001); Vietnam; Dac Lac; seemingly unpolluted site
	Adult*	—	1	Testicle	74	DRY	*Fully grown
	Egg*			Egg	100		*Gonadal system
Dermochelys coriacea							Davenport and Wrench (1990), Davenport et al. (1990); Great Britain; Wales, Irish Sea, Cardigan Bay; stranded turtle
	L = 2.53 m W = 916 kg	M	1	Liver	2.62	DRY: M*	*4 replicate measures
				Muscle*	1.89		*Pectoral
				Blubber	0.08		

(*continued*)

TABLE A.26 (CONTINUED)
Zinc (Zn)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Dermochelys coriacea								Lance et al. (1995); Costa Rica
	Adult		F*	5	Blood**	1.73; 1.27–2.22	WET: M; R	*Nesting female; **plasma
Dermochelys coriacea								Vazquez et al. (1997); Mexico; Pacific coast
	—		—	—	Seawater	0.26	DRY: M	
					Sand	58.9		
	Egg*				Shell	11.9		*Posthatch
Dermochelys coriaca								Godley et al. (1998); Great Britain; Wales and Scotland, west coast; turtles died in fishing gear
	Adult		M	3				
	CCL = 141–170 cm							
				1	Liver	132	DRY	
					Muscle	153		
					Liver	42	WET	
					Muscle	51		
Dermochelys coriacea								Caurant et al. (1999); France; Atlantic coasts; "Pertuis charentais" in the la Rochelle region; stranded turtles
	Juvenile	M; R	—	16			WET: M; R	
	SCL = 145.7; 115–188 cm							
			—	18	Liver	29.2; 21.9–36.5		
				5	Kidney	25.7; 18.5–33.8		
				16	Muscle*	25.9; 18.3–37.3		*Pectoral
Eretmochelys imbricata								Gordon et al. (1998); Australia; southeastern Queensland, Moreton Bay region; stranded turtles
	—	—	—	2	Liver	17.7–30.3	WET: R	
				2	Kidney	13.2–20.9		

Eretmochelys imbricata								Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 43.5; 33.5–48.9 CW = 36.9; 32.6–39.6	M; R	M	6	Liver	142; 63–306	DRY: M; R	
				6	Kidney	146; 97.0–200		
				1	Muscle	55.2		
	SCL = 46.5; 43.8–67.9 CW = 32.8; 10.9–52.3		F	16	Liver	97.4; 52.3–143		
				13	Kidney	108; 81.2–148		
				8	Muscle	47.7; 25.8–111		
	Diet		—	6	Stomach*	24.2; 6.84–50.8		*Contents
Eretmochelys imbricata								Anan et al. (2002); Japan; Okinawa Prefecture, Yaeyama Islands; tissues provided by fishermen
	—	—	—	7	Liver	25.5; 22.2–29.5	DRY: M; R	
Eretmochelys imbricata								Gardner et al. (2006); Mexico; Baja California peninsula; turtles died from fisheries capture
	MSCL = 48.4			1	Liver	25.89	DRY: GM; R	
					Kidney	82.45		
					Muscle* Pectoral	102		
					Fat	42.39		
Gopherus agassizii								Lance et al. (1995); various sources (zoos to wild caught)
	—		—	4	Blood*	2.10; 1.51–2.67	WET: M	*Plasma

(*continued*)

TABLE A.26 (CONTINUED)
Zinc (Zn)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Gopherus agassizii							Seltzer and Berry (2005); USA; California; preserved samples originated from tortoises (alive at necropsy) and subject to previous studies (Homer et al. 1998; Berry et al. 2001) and were analyzed using laser ablation inductively coupled plasma mass spectrometry (laser ablation ICP-MS)
	Adult			Dermal scute		—: M*	*Concentration averaged over laser ablation transect profile
	MCL = 178 mm TM = 0.90 kg	1	M		59.9		Healthy individual
	MCL = 252 mm TM = 2.3	1	F		51.3		Ill individual (mycoplasmosis)
	MCL = 271 mm TM = 3.73	1	M		46.4		Ill individual (multiple inflammation)
	MCL = 210 mm TM = 1.75	1	F		30.6		Ill individual (cutaneous dyskeratosis)
Lepidochelys kempii							Lance et al. (1995); Costa Rica
	Adult	F*	1	Blood**	0.84	WET: M; R	*Nesting; **plasma
Lepidochelys kempii							Caurant et al. (1999); France; Atlantic coasts; "Pertuis charentais" in the la Rochelle region; stranded turtles
	Adult						
	SCL = 25.8; 21.3–34.5 cm TM = 2.5; 1.4–5.2 kg	M; R —	6	Muscle*	16.4; 13.3–20.5	WET: M; R	*Pectoral
			2	Pancreas	142.3		

Lepidochelys kempii								Kenyon et al. (2001); USA; Texas and Louisiana; turtles captured alive in nets at 4 beachfront sites
	SCL = 38.3; 21.6–65.8 cm	M; R	F/M/U	46/38/22*			WET: M; R	*99 wild grown plus 7 head-start female turtles
					Blood*	7.500; 3.280–18.900		*Whole blood
Lepidochelys olivacea								Witkowski and Frazier (1982); Ecuador; Manta
	Adult		—	3	Bone*	575.0–955.0	ASH: R	*Humerus
Lepidochelys olivacea								Lance et al. (1995); Costa Rica
	Adult		F*	5	Blood**	0.64; 0.37–0.89	WET: M	*Nesting; **plasma
Lepidochelys olivacea								Sahoo et al. (1996); India; Orissa, Gahirmatha
	—		—				DRY: R*	*mg/g^{D}
				8	Beach sand	54–132		
	Egg*		—	24**	Shell	13.0	M	*Fresh; **8 nests, 3 eggs per nest
					Albumen-yolk	4.3		
	Egg*				Shell	16.6		*Posthatch
	Hatchling*				Whole body	17.3		*Fresh
Lepidochelys olivacea								Gordon et al. (1998); Australia; southeastern Queensland, Moreton Bay region; stranded turtles
	—	—	—	1	Liver	14.8	WET: M; R	
				1	Kidney	18.8		
Lepidochelys olivacea								Gardner et al. (2006); Mexico; Baja California peninsula; turtles died from fisheries capture
	MSCL = 60.1; 53.0–66.0	M; R	—	6	Liver	47.14; 18.66–85.75	DRY: GM; R	
					Kidney	6.68; 0.43–114		
					Muscle*	85.78; 49.89–107		*Pectoral
					Fat	3.7; 0.41–16.65		

(*continued*)

TABLE A.26 (CONTINUED)
Zinc (Zn)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Pelodiscus sinensis								Boman et al. (2001); Vietnam; Hay Tay; seemingly unpolluted site
	Egg*		—	1	Eggs	81	DRY	*Gonadal system
*Sternotherus doratus**								Lance et al. (1995); various sources (zoos to wild caught); *[*Kinosternon odoratus*]
	—		—	2	Blood*	4.14–6.20	WET: R	*Plasma
*Terrapene carolina**								Lance et al. (1995); various sources (zoos to wild caught); *[*Terrapene carolinensis*]
	—		—	2	Blood*	4.70–4.80	WET: R	*Plasma
Testudo horsfieldii								Lance et al. (1995); various sources (zoos to wild caught)
	—		—	1	Blood*	2.78	WET	*Plasma
Trachemys sripta								Thomas et al. (1994); intraperitoneal administration as $CdCl_2$ at a dose of 10 mg Cd/kg/day for 6 days
	Juvenile TM = 20–25 g	R	F	6	Liver	13	WET: %*	*Percentage of total body burden
					Kidney	11		
					Spleen	8		
					Heart	5		
					Lung	6		
					Muscle	10		
					Carapace	22		
					Brain	5		
					Blood	1		
					Ovary	19		
Trachemys scripta								Lance et al. (1995); various sources (zoos to wild caught)
	—		—	12	Blood*	3.88; 2.46–7.64	WET: M; R	*Plasma

Trachemys scripta							Clark et al. (2000); USA; Texas
	—	—	14	Blood*		WET: GM	*Whole blood
					4.87		Site 1: Contaminated site: Municipal Lake (chemical manufacturing plant)
					4.79		Site 2: Apparently uncontaminated site: Research Park Lake (parkland)
					5.48		Site 3: Contaminated site: Old River Slough (cotton and corn cultivation with intensive chemical application)
Trachemys scripta elegans							Tryfonas et al. (2006); USA; Illinois; Lower Illinois River near Grafton; eggs laid in the lab from turtles collected in the field from 5 nesting areas
	Egg		42	Contents	24.2	DRY: M*	*All sites combined
				Shell	6.8		
	Diet			*Lemna* sp.	44		
	Environment		4–5*	Soil	40–95		*From 2 sites, soil from nesting and lake bank areas
			3*	Sediment	50*	RM**	*From 2 sites, 3 layers per site; **values estimated from graph
			3–4*	Water	ND (<0.006)–0.007	WET: RM	*From 3 sites
Crocodylia							
Alligator mississippiensis							Lance et al. (1983); studied reproductive cycle for 4 months (April–July)
	Adult	F		Blood*		WET: RM**	*Plasma; **combined by month
	L = 190–243 cm		29		0.68–1.84		Farm reared, fed fish (*Micropogon undulatus*)
	L = 189–326 cm		41		0.46–1.81		Farm reared, nutria fed (*Myocastor coypus*)
	L = 163–325 cm W = 15.9–146 kg		34		0.49–1.42		USA; Lousiana, Grand Chenier, Rockefeller Refuge; wild animals (nutria constituted 70% of the diet)
Alligator mississippiensis							Delany et al. (1988); USA; Florida; 8 lakes, statewide

(*continued*)

TABLE A.26 (CONTINUED)
Zinc (Zn)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
	L = 2.9–3.8 m	*	24	Muscle*	23.72; 14.20–36.00	WET: M; RM**	*Tail; **combined by lake
Alligator mississippiensis							Heinz et al. (1991); USA; central and southern Florida; Lake Okeechobee, Lake Griffin, and Lake Apopka
	Egg		32*	Egg	5.6–7.6; 4.9–9.2	WET: RM; R**	*16 nests, 2 eggs per nest; **combined by lake
*Alligator mississippiensis**							Cort et al. (1995); USA; Louisiana; San Diego Zoo and Rockefeller Wildlife Refuge; *["Alligator"]
	—	—	5	Blood*	0.27	WET: M	*Plasma
Alligator mississippiensis						WET: M; R	Lance et al. (1995); USA; Louisiana; Rockefeller Wildlife Refuge
	Adult	M	24	Blood*	0.44; 0.18–0.70		*Plasma
		F*	18		0.50; 0.32–0.90		*Nonbreeding
		F**	22		1.83; 1.28–3.48		**Breeding
Alligator mississippiensis							Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	—	—	1	Liver	1.10	WET	
				Kidney	11.00		
Alligator sinensis							Ding et al. (2001) from Xu et al. (2006); China; Anhui Province; Anhui Captive Breeding Center
	Egg		1/1*	Shell	37.55/24.45	DRY	*1 infertile egg from captive breeding center/1 from the wild
				Shell membranes	45.47/10.70		
				Albumen	10.27/4.53		
				Yolk	7.08/6.22		

Alligator sinensis							Xu et al. (2006); China; Changxing County, Zhejiang Province; Changxing Nature Reserve and Breeding Research Center for Chinese alligator; alligators collected dead of unidentified causes
	Adult	F/M	1/1	Heart	143.21/142.64	DRY	
				Lung	59.25/37.64		
				Liver	151.22/94.33		
				Stomach	61.85/67.35		
				Kidney	70.96/119.69		
				Intestine	115.54/70.37		
				Trachea	51.85/81.31		
				Pancreas	7.46/8.94		
				Gonad	2.11/1.42		
				Muscle	128.30/120.97		
	Diet	—	—	Fish*	33.33	M; R	*2 fish prey species randomly sampled
	Excrements	—	6	Feces	263.70		
	Egg		10*				*10 eggs from 3 clutches
				Eggshell	8.66; 6.22–11.56		
				Shell membranes	93.96; 67.25–128.0		
				Egg contents	58.87; 53.20–72.72		
	Environment		10*				*Water and sediment collected from 10 breeding ponds (5 subsamples from different sites within a pond each)
				Sediment	48.1		
				Water	0.01278	WET: M	
							Statistical analysis: [Zn] concentrations were significantly higher in contents than in shells; those in membranes were higher than those in contents

(*continued*)

TABLE A.26 (CONTINUED)
Zinc (Zn)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Crocodylus acutus								Ogden et al. (1974); USA; Florida; Florida Bay, Everglades
	Egg		—	5	Contents	8.76; 7.2–11.0	WET: M; R	
Crocodylus acutus								Rainwater et al. (2007); Costa Rica; 1 sampling site
	SVL = 155.7; 134.0–172.0 cm	M; R						Rio Grande de Tarcoles; polluted by several metals from various sources
			F/M	1/5	Scute*	4.1402	WET: M	*Caudal
Crocodylus johnstoni								Jeffree et al. (2001); northern Australia; north central Queensland, Lynd River; samples from a single population
	Age = 0.7–62.7 years L = 24.7–128.3 cm		F/M	9/21	Osteoderm*	57.9; 41.3–70.1	DRY M; R	*Ventral pelvic region
Crocodylus moreletii								Rainwater et al. (2007); Belize; 2 sampling sites
					Scute*		WET: M	*1 whole caudal scute per crocodile
	SVL = 89.8; 65.0–129.5 cm	M; R	F/M	5/4		ND (<0.05)		Site 1: Gold Buton Lagoon
	SVL = 104.4; 59.5–156.7 cm		F/M	4/6		ND (<0.05)		Site 2: New River Watershed
Crocodylus niloticus								Phelps et al. (1986); Zimbabwe
	Egg		—	26*	Contents	33.838; 22.54–47.51	DRY: M; R	*10 samples from 8 sites
Crocodylus niloticus								Swanepoel et al. (2000); South Africa; Kruger National Park (KNP) area
	TL = 1.40–4.15 m	R	F/M	6/9			DRY: M	

								Site 1: Shingwedzi River (Silwervis Dam), northern part of KNP; catchment area outside KNP is limited
					Muscle	109.7; 65.9–153.5		Frozen tissues used for residue analysis
					Liver	61.5; 46.5–76.5		
					Kidney	54.2; 35.7–72.7		
					Fat	11.2; 8.2–14.2		
								Site 2: Olifants River, central part of KNP; flows through areas of intense agricultural activity and passes mining areas and receives tributaries from Phalaborwa Mining Company before entering the KNP
					Muscle	39.4; 36.2–42.6		Frozen tissues used for residue analysis
					Liver	100.7;76.8–124.6		
					Kidney	94.4; 38.2–150.6		
					Fat	27.5; ND (<0.001)–57.3		
					Liver	64.80; 45.3–84.3		Formalinized tissues used for residue analysis
					Kidney	51.40; 42.0–60.8		
								Site 3: Sabi River, southern part of KNP; flows through areas of intense agricultural activity before entering the KNP
					Muscle	44.7; 42.9–46.5		Frozen tissues used for residue analysis
					Liver	122.5; 46.1–198.9		
					Kidney	66.4; 28.1–104.7		
					Fat	7.6		
					Liver	61.20; 55.1–67.3		Formalinized tissues used for residue analysis
					Kidney	58.40; 40.4–76.4		
Crocodylus niloticus								Almli et al. (2005); Zambia
	L = 2.7–3.4 m	R	F/M	2/2			WET: MD; R	Site 1: Kafue River, Kafue National Park
					Liver	18; 14–28		
					Kidney	14; 13–15		

(*continued*)

TABLE A.26 (CONTINUED)
Zinc (Zn)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
	L = 2.0–4.0 m	R	F/M	4/1				Site 2: Luangwa River, Luangwa National Park
					Liver	31; 29–40		
					Kidney	12; 10–13		
Crocodylus porosus								Yoshinaga et al. (1992); Papua New Guinea
	—		—	—	Muscle	5.8	WET: M	
Crocodylus porosus								Jeffree et al. (2001); northern Australia; Alligator Rivers region, Kakadu National Park; samples from 3 river catchments, mining and hunting areas included
	Age = 5–40 years	R	—	40			DRY: GM; R	
	L = 168–499 cm							
			—	35	Muscle*	81.4; 45.0–192		*Tail
			—	40	Osteoderm*	5.23; 1.73–9.57		*Ventral pelvic region
Crocodylus rhombifer								Cook et al. (1989); zoo animal; with a 12-day history of anorexia, depression, and weight loss, and stomach containing metallic foreign bodies (10 coins a.o.)
	3.75 years old		M	1	Blood*	45.3	WET	*Serum
	TM = 10 kg							
	Dietary uptake					30.7		Same animal 18 days after removal of the foreign bodies and CaEDTA treatment
						4.88		Same animal 39 days after removal of the foreign bodies and CaEDTA treatment (feeding resumed at 24 days post-op)

Squamata: Sauria								
"Lizard"								Hsu et al. (2006); South Taiwan; Kenting National Park; strong influence from industrial pollution
	—		—	1	Whole body	142	DRY	
						2.23		Bioconcentration factor (reference media: soil and food items)
Helodermatidae 2 species	—		—	19		0.02–1.62	WET: RM	Lance et al. (1995); various sources (zoos to wild caught)
					Blood*			*Plasma
Iguanidae 2 species	—		—	16		1.1–4.1	WET: RM	Lance et al. (1995); various sources (zoos to wild caught)
					Blood*			*Plasma
Scincidae 4 species								Lance et al. (1995); various sources (zoos to wild caught)
	—		—	7	Blood*	4.9–9.8	WET: RM	*Plasma
Varanidae 8 species								Lance et al. (1995); various sources (zoos to wild caught)
	—		—	43	Blood*	0.31–24.38	WET: RM	*Plasma
Chamaeleo chamaeleon								Diaz-Paniagua et al. (2002); southern Spain; Province of Cadiz; 2 sampling sites; 9 nests located in agricultural lands and coastal urban areas; eggs collected immediately after oviposition
	Egg			4*	Egg contents	11.05; 8.23–13.23	WET: M; R	*Number of eggs pooled per nest
Chamaeleo chamaeleon							WET: RM	Gomara et al. (2007); southern Spain; Province of Cadiz; 3 sampling sites; 9 nests; eggs collected 0–15 days after oviposition
	Egg		—	2–4*	Egg contents	10.100–12.950		*Number of eggs pooled per nest
					Eggshells	4.878–13.290		
Hemidactylus mabouia								Schmidt (1984b); Brazil; Rio Grande do Sul, Porto Alegre; urban area
	L = 35–70 mm	R	—	154	Whole body	27–303; 96–128	DRY: R; RM	9 sites
				21	Liver	61–544; 125–226		3 sites

(*continued*)

TABLE A.26 (CONTINUED)
Zinc (Zn)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Hemidactylus mabouia							Schmidt (1986); Brazil; Rio Grande do Sul, Porto Alegre; urban area; 1 site
	—	—	20	Whole body	112; 84–142	DRY: M; R	
			8	Liver	226; 69–544		
			6	Excrements	465; 310–620		
*Iberolacerta monticola**					See As for details		Marco et al. (2004); Spain; Avila, Gredos Mountains; gravid females collected in the field in 2001; freshly laid eggs incubated until hatching in As (arsenic acid in nitric acid)-loaded artificial breeding substrate (sterile As-free vermiculite) for 18 days; further elements (Cd, Cu, Pb, Zn), though not manipulated, were detected in eggshells and embryos; *[*Lacerta monticola cyreni*]
*Laudakia s. stellio**							Loumbourdis (1997); Greece; Thessaloniki region; *[*Agama s. stellio*]
	Adult	—	—	Liver	614.55	DRY: M	Site 1: Urbal area (500 m asl)
				Carcass	608.99		
				Liver	794.14		Site 2: Agricultural area (50 m asl)
				Carcass	643.47		
Tarentola mauritanica							Fletcher et al. (2006); southern Spain; Guadiamar River Valley; mine tailings release event; Boliden-Apirsa mine at Aznalcóllar; 7 study sites spanning an expected contamination gradient; geckos collected from building walls
	Juvenile and adult			Whole body*		DRY: M; R	*Minus gut contents
		—	9		114.408; 91.161–158.649		Site 1: Rural non-mine-affected site: near Guadalmellato (most pristine)
		—	13		127.192; 90.991–191.892		Site 2: Urban non-mine-affected site: Villaviciosa de Cordoba (not contaminated by mining)

		—	8		127.552; 106.272–161.173		Site 3: Urban mine-affected site: Aznalcázar (contamination through aerosolized contaminants engulfed the town during cleanup)
		—	8		108.479; 83.605–126.117		Site 4: Urban mine-affected site: Aznalcóllar (contaminated by normal mine operations, or the disaster and subsequent remediation efforts)
		—	5		109.689; 71.364–150.149		Site 5: Floodplain mine-affected site: Guadiamar River floodplain near the Aznalcázar gauge station (24.8 km below the ruptured tailings dam)
		—	5		106.352; 91.681–126.341		Site 6: Floodplain mine-affected site: Guadiamar River floodplain near the Guijo gauge station (7.4 km below the ruptured dam)
		—	4		153.008; 141.309–166.442		Site 7: Floodplain mine-affected site: Agrio River floodplain (4.4 km below and closest to the ruptured tailings dam)
							Statistical analysis: [Zn] concentration was significantly influenced by site
Tiliqua rugosa							Lance et al. (1995); various sources (zoos to wild caught)
	—	—	1	Blood*	5.0	WET	*Plasma
Varanus salvator							Boman et al. (2001); Vietnam; Dac Lac; seemingly unpolluted site
	Adult*	—	1	Muscle	180	DRY	*Fully grown
				Liver	65		
	Egg*			Egg	220		*Gonadal system
Varanus sp.							Yoshinaga et al. (1992); Papua New Guinea
	—	—	—	Muscle	16.9	WET: M	
*Zootoca vivipara**		—					Avery et al. (1983); Great Britain; England; *[*Lacerta vivipara*]
							Site 1: Little used road
	Juvenile and adult		11 *	Bone**	249.7	DRY: M	*8 juveniles, 3 adults; **femur
				Liver	95.0		

(*continued*)

TABLE A.26 (CONTINUED)
Zinc (Zn)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
				Lung	42.9		
				Kidney	21.7		
				Remainder	90.2		
							Site 2: Busy road
			19*	Bone**	204.4		*12 juveniles, 7 adults; **femur
				Liver	106.0		
				Lung	41.8		
				Kidney	12.1		
				Remainder	87.6		
							Site 3: Lead mine
			14*	Bone**	245.5		*9 juveniles, 5 adults; **femur
				Liver	93.2		
				Lung	58.7		
				Kidney	41.8		
				Remainder	42.9		
*Zootoca vivipara**	—	—				DRY: R	Gutleb et al. (1992); Austria; Carinthia, remote area; *[*Lacerta vivipara*]
			2	Liver	46.5–85.7		
			1	Kidney	398.8		
Squamata: Serpentes							
"Snake"							Hsu et al. (2006); South Taiwan; Kenting National Park; strong influence from industrial pollution
	—	—	12	Whole body	248; 145–538	DRY: M; R	
					3.90		Bioconcentration factor (reference media: soil and food items)
"Sea snake"							Lance et al. (1995); various sources (zoos to wild caught)
	—	—	1	Blood*	25.3	WET	*Plasma

Boidae 6 species								Lance et al. (1995); various sources (zoos to wild caught)
	—		—	10	Blood*	6.1–45.0	WET: RM	*Plasma
Colubridae 41 species								Lance et al. (1995); various sources (zoos to wild caught)
	—		—	115	Blood*	5.5–116.5	WET: RM	*Plasma
Elapidae 7 species								Lance et al. (1995); various sources (zoos to wild caught)
	—		—	19	Blood*	9.6–41.9	WET: RM	*Plasma
Viperidae 20 species								Lance et al. (1995); various sources (zoos to wild caught)
	—		—	35	Blood*	2.4–19.0	WET: RM	*Plasma
Acrochordus javanicus								Yoshinaga et al. (1992); Papua New Guinea
	—		—	—	Muscle	17.6	WET: M	
Acrochordus javanicus								Lance et al. (1995); various sources (zoos to wild caught)
	—		—	1	Blood*	20.5	WET	*Plasma
Agkistrodon piscivorus								Clark et al. (2000); USA; Texas; 1 contaminated site: Old River Slough (cotton and corn cultivation with intensive chemical application)
	—		—	—	Blood*	8.88	WET: GM	*Whole blood
Agkistrodon piscivorus	SVL = 58.6 cm	M	—				WET	Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	TM = 365 g							
				6	Liver	19.85		
					Kidney	13.73		

(*continued*)

TABLE A.26 (CONTINUED)
Zinc (Zn)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Agkistrodon piscivorous							Burger et al. (2006); USA; South Carolina; 1 reference site; 1 polluted Savannah River site, Aiken
	SVL = 57 cm TM = 280 g	M	—	13	Muscle*	141	DRY: M**	*Tail; **both sites combined
	SVL = 60 cm TM = 270 g			5	Blood	8		
Coluber constrictor							WET	Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 103.2 cm TN = 300 g		—	1	Liver	30.40		
					Kidney	24.10		
Cylindrophis rufus								Lance et al. (1995); various sources (zoos to wild caught)
	—		—	2	Blood*	9.3–17.4	WET: R	*Plasma
Eunectes murinus								Calle et al. (1994); Venezuela
	L = 2.1–5.1 m W = 3.5–74.0 kg		F/M	7/5	Blood*	13.8; 10–18.4	WET: M; R	*Plasma
Lapemis hardwickii								Boman et al. (2001); Vietnam; Nha Trang; seemingly unpolluted site
	Adult*		—	1	Muscle	170	DRY	*Fully grown
					Liver	130		
Leptotyphlops humilis								Lance et al. (1995); various sources (zoos to wild caught)
	—		—	1	Blood*	12.5	WET	*Plasma
Natrix natrix								Lance et al. (1995); various sources (zoos to wild caught)

	—		—	2	Blood*	24.0–50.0	WET: R	*Plasma
Nerodia cyclopion			—				WET	Presley et al. (2005); USA; Louisiana; New Orleans, near Maxent Canal; site contaminated by floodwaters from Lake Pontchartrain during Hurricane Katrina event
	SVL = 63.9 cm TM = 191 g			1	Liver	9.40		
					Kidney	18.40		
Nerodia fasciata								Burger et al. (2006); USA; South Carolina; 1 reference site; 1 polluted Savannah River site, Aiken
	SVL = 51	M	—	47	Muscle*	150	DRY: M**	*Tail; **both sites combined
	SVL = 52			34	Blood	11		
Nerodia rhombifer								Clark et al. (2000); USA; Texas
	—		—	10	Whole blood	13.6	WET: GM	Site 1: Contaminated site: Old River Slough (cotton and corn cultivation with intensive chemical application)
						13.9		Site 2: Reference site: private lake (pasture)
Nerodia sipedon								Niethammer et al. (1985); USA; Missouri; Lead Belt
	Juvenile and adult		—	15	Carcass	18.4–42.6	WET: R	Site 1: Upstream formerly mined area
				15		23.9–63.2		Site 2: Downstream formerly mined area
				50		19.7–51.3		Site 3: Currently mined area
Nerodia taxispilota								Burger et al. (2006); USA; South Carolina; 1 reference site; 1 polluted Savannah River site, Aiken
	SVL = 59 cm TM = 170 g	M	—	10	Muscle*	151	WET: M**	*Tail; **both sites combined
	SVL = 65 cm TM = 230 g			9	Blood	13		
Pituophis melanoleucus								Lance et al. (1995); various sources (zoos to wild caught)
	—		—	5	Blood*	40.2; 32.4–54.0	WET: M; R	*Plasma

(*continued*)

TABLE A.26 (CONTINUED)
Zinc (Zn)

Taxa	Specifications	Sex	*n*	Compartments	Concentrations		References, Locations, Remarks
Python molurus							Boman et al. (2001); Vietnam; Dac Lac; seemingly unpolluted site
	Adult*	—	1	Muscle	270	DRY	*Fully grown
				Testis	110		
Ramphotyphlops sp.							Lance et al. (1995); various sources (zoos to wild caught)
	—	—	1	Blood*	5.6	WET	*Plasma
Thamnophis sirtalis							Lance et al. (1995); various sources (zoos to wild caught)
	—	—	4	Blood*	30.3; 23.5–35.0	WET: M; R	*Plasma
Vipera berus							Gutleb et al. (1992); Austria; Carinthia; remote area
	—	—	1	Liver	34.1	DRY	
				Kidney	271.7		
Xenopeltis unicolor							Lance et al. (1995); various sources (zoos to wild caught)
	—	—	1	Blood*	30.5	WET	*Plasma

TABLE A.27
Zirconium (Zr)

Taxa	Specifications		Sex	*n*	Compartments	Concentrations		References, Locations, Comments
Testudines								
Chelonia mydas								Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 49.0; 40.0–63.5 cm	M; R	M	6			DRY: M; R	
				6	Liver	0.042; 0.014–0.143		
				6	Kidney	0.03; 0.011–0.045		
				3	Muscle	0.187; 0.026–0.439		
	SCL = 52.2; 37.0–71.4 cm		F	20				
				20	Liver	0.040; 0.004–0.175		
				19	Kidney	0.046; 0.006–0.418		
				9	Muscle	0.073; 0.006–0.409		
	Diet		—	8	Stomach*	0.060; 0.027–0.114		*Contents
Eretmochelys imbricata								Anan et al. (2001); Japan; Yaeyama Islands
	SCL = 43.5; 33.5–48.9 cm	M; R	M	6			DRY: M; R	
				6	Liver	0.017; 0.003–0.067		
				6	Kidney	0.007; 0.004–0.016		
				1	Muscle	0.008		
	SCL = 46.5; 43.8–67.9 cm		F	16				
				16	Liver	0.026; 0.005–0.130		
				13	Kidney	0.007; 0.004–0.018		
				8	Muscle	0.006; 0.004–0.013		
	Diet		—	6	Stomach*	0.056; 0.011–0.110		*Contents

Data encountered across the range of available literature were very heterogeneous in terms of completeness and appropriateness; it is beyond the purpose of this study to systematically evaluate the reliability of the reported datasets and conclusions. Hence, statistical treatment was limited, and only descriptive statistics were included in summary tables as means, ranges of means, and ranges of variation. Wherever significance of results in inferential statistics is stated, it is simply a report of the analysis performed in the original publication.

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Index

B

C

D

E

F

G

H

I

P

Q

R

U

V

W

X

Z

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