
11 Interdisciplinary and Hierarchical Approaches for Studying the Effects of Metals and Metalloids on Amphibians

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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 <i>entire</i> 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 <i>ad lib</i> ; 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 events (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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