Environment and Climate Change Canada
Environnement et Changement climatique Canada

Federal Contaminated Sites Action Plan (FCSAP)

Ecological Risk Assessment Guidance - Module 6: Ecological Risk Assessment for Amphibians on Federal Contaminated Sites

Version 1.0

Prepared by Environment and Climate Change Canada

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Acronyms

ASTM	American Society for Testing and Materials
CALA	Canadian Association for Laboratory Accreditation
CCME	Canadian Council of Ministers of the Environment
CHS	Canadian Herpetological Society
COC	Contaminant of Concern
COSEWIC	Committee on the Status of Endangered Wildlife in Canada
DELTS	Deformities, Erosion, Lesions, and Tumors
EC_x	Effect concentration
ECCC	Environment and Climate Change Canada
ERA	Ecological Risk Assessment
FETAX	Frog Embryo Teratogenesis Assay *Xenopus*
FCSAP	Federal Contaminated Sites Action Plan
HQ	Hazard Quotient
IC_x	Inhibitory concentration
LC_x	Lethal concentration
LOAEL	Lowest-observed-adverse-effect level
NOAEL	No-observed-adverse-effect level
OECD	Organization for Economic Co-operation and Development
PCB	Polychlorinated Biphenyls
ROC	Receptor of Concern
SETAC	Society of Environmental Toxicology and Chemistry
TRV	Toxicity Reference Value
USEPA	United States Environmental Protection Agency

Glossary

Acute – Although the definition of acute versus chronic vary widely by jurisdiction, for the purposes of this guide, acute is defined as relating to a small increment of time required to elicit an adverse environmental response. With respect to toxicity testing, the term describes tests applied over a short duration, typically less than 10% of an organism's lifespan. Note, however, that some short-term tests may be defined as chronic rather than acute if they are conducted using a sensitive life stage.

Assessment endpoint – An assessment endpoint is an explicit expression of the environmental value to be protected. An assessment endpoint must include an entity (typically a receptor or receptor group – i.e., a 'thing' to be protected) and a specific property of that receptor (an attribute). For example, if the entity is a fish community, attributes could include the number of species, the trophic structure, etc. An assessment endpoint may also have an explicit spatial or temporal component.

Background conditions – Conditions that are representative of naturally occurring concentrations in the environment primarily reflecting local geological variation and not influenced by human activity.

Bias – A systematic tendency that distorts the interpretation of results. In ERA, a bias occurs in two main forms. In the study design or interpretation, bias is a pejorative term that reflects the partiality of a practitioner that prevents objective consideration of an issue or situation. In statistical measurement, bias reflects a systematic under-or-over prediction of a true parameter of value. Both forms of bias introduce error into risk estimates.

Bioaccumulation – The process by which an organism absorbs a substance(s) at a rate faster than that at which the substance is lost by catabolism and excretion, thus causing an increase in the account of the substance in the tissues of living organisms. This occurs when the concentration of a contaminant of concern in an organism is higher than the concentration in the surrounding environment.

Chronic – Although the definition of chronic vary widely by jurisdiction, for the purposes of this guide, chronic is defined as relating to extended time duration. In the context of toxicity testing, the term is used to describe tests that expose organisms over a substantial portion of their life cycle, for example more than 10% of the life cycle or throughout a sensitive life stage.

Concentration-response – The relationship between an effects measure and exposure (measured as concentration) across a range of exposure concentrations.

Contaminants of concern – Contaminants that have been selected for evaluation in the ERA, usually based on a completed problem formulation.

Control – As a noun, an aspect of a controlled scientific experiment conducted for the purpose of determining the effect of a single variable of interest on a particular system, used to minimize the unintended influence of other variables on the same system. Negative controls confirm that the procedure is not causing an unrelated effect, and are intended to reduce incidence of false positives. The term control (as a verb) can also be used in experimental design to refer to manipulation of treatments intended to mitigate the confounding effect of external variables.

Ecological risk assessment (ERA) – The process of evaluating the potential adverse effects on non-human organisms, populations or communities in response to human-induced stressors. ERA entails the application of a formal framework, analytical process, or model to estimate the effects of human actions on natural organisms, populations or communities and interprets the significance of those effects in light of the uncertainties identified in each study component.

Effects assessment – For any line of evidence, the component of a risk assessment that characterizes the nature of effects elicited by each contaminant under an exposure condition that is relevant to each receptor of concern.

Effect concentration (EC) – The concentration at which a certain percentage of tested individuals experience a pre-defined, dichotomous effect. For example, if a study reports an EC_{50} for malformation as X mg/L of lead, it means that 50% of the test individuals exposed to X mg/L of lead exhibited some type of malformation.

Effect size – The absolute or relative magnitude of response to a stressor for a measurement endpoint.

Exposure assessment – For any line of evidence, the component of a risk assessment that quantifies the degree to which an organism encounters a stressor.

Exposure pathways – The routes through which a receptor of concern encounters a contaminant of concern in environmental media (e.g., soil, water, air, sediment). Examples of exposure pathways include ingestion and inhalation.

Gradient – A concept of experimental design in which treatments are planned to include a range of exposures from low to high, or a spatial range (e.g., near to far).

Guideline – A regulatory value that is recommended for the screening of environmental data, such as tissue residues or concentrations in abiotic media. A guideline usually differs from a standard in that a guideline does not convey a legal requirement or formal responsibility. Canadian Environmental Quality Guidelines are intended as nationally endorsed science-based goals for environmental quality. The term is also used to describe a technical practice that is recommended to facilitate consistency among practitioners, but that is not strictly required.

Hazard quotient (HQ) – A numerical ratio that divides an estimated environmental concentration or other exposure measure by a response benchmark. Typically the response benchmark is a value assumed to be protective of the receptor of concern. HQ values below one (1.0) indicate negligible potential for harm, whereas HQ values above one indicate that an adverse response is possible and must be addressed either through more precise or accurate evaluation of risks to address uncertainty or through risk remediation or risk management approaches.

Inhibitory concentration (IC) – A concentration at which a specific percentage of impairment occurs as a result of an exposure. For example, if an IC_{50} for growth is reported as X mg/L of mercury, this would mean that growth was impaired in the test organisms by 50% (on average, relative to controls) when the test individuals were exposed to X mg/L of mercury.

Lethal concentration (LC) – A concentration at which a specific percentage of mortalities occur as a result of an exposure. For example, if an LC_{50} is reported as X mg/L of cadmium, it would be estimated that X mg/L of cadmium would be lethal to 50% of the test organisms.

Line of evidence – Any pairing of exposure and effects measures that provides evidence for the evaluation of a specific assessment endpoint. Typically, a line of evidence requires the use of one or more measurement endpoints. If the focus of the line of evidence is an effects measure (e.g., a toxicity test), the paired exposure measure may be quantitative (e.g., contaminant concentrations) or categorical (e.g., on-site versus a reference condition).

Lowest-observed-adverse-effect level (LOAEL) – Lowest amount, dose, or concentration of an agent, found by experiment or observation, that causes an adverse alteration of morphology, functional capacity, growth, development or life span in an organism, system, or (sub)population. Methods vary for identifying a LOAEL, but often apply statistical significance as a criterion.

Measurement endpoint – A measurement endpoint is a parameter that measures or describes exposure of, or an effect on, a receptor of concern. Alternatively, the term describes a change in an attribute of an assessment endpoint (or its surrogate) in response to a stressor to which it is exposed. For example, length could be the measurement endpoint for the assessment endpoint “Growth”.

No-observed-adverse-effect level (NOAEL) – An exposure level at which there are no statistically or biologically significant increases in the frequency or severity of adverse effects between the exposed organisms or population and the appropriate control; some effects may be produced at this level, but they are not considered to be adverse. Methods for identifying a NOAEL vary, but often apply statistical significance as a criterion.

Point estimate – A single numerical value used to represent the state of a random variable. A point estimate collapses (or ignores) all of the variability and incertitude regarding a parameter or variable. The concentration that is lethal to 50% of test organisms (LC_{50}) is a common point estimate.

Receptor of concern – Any non-human individual organism, species, population, community, habitat or ecosystem that is potentially exposed to contaminants of concern and that is considered in the ERA. Identification of an organism as a receptor of concern does not mean that it is being harmed, only that a pathway exists such that there is potential for harm.

Reference (condition) – A location, group of locations, or experimental treatment designed to reflect the ambient physical and chemical conditions of a contaminated medium or location in the absence of the stressors of concern in the risk assessment. For example, in a study of soil contamination, the reference condition should reflect the climate, substrate, and habitat factors relevant to the site but with no incremental contamination relative to background conditions. In some cases, the term reference may be used in the context of an altered local background condition (i.e., where the local conditions surrounding a site are not pristine due to non-point sources of contaminants). In other cases, the term reference is used to refer to pristine conditions in the absence of both site-specific contamination and non-point sources of contaminants.

Surrogate receptor of concern – A surrogate receptor of concern is representative of a receptor type (e.g., a shrew may be used as a surrogate receptor of concern for insectivorous mammals). More than one surrogate receptor of concern may be used to represent a particular receptor type.

Threshold – Dividing line (in units of exposure concentration or dose) between a zone of potential response and a zone of negligible response. Thresholds may be estimated using theory, data, or a combination of both. In nature, thresholds generally do not occur as precise or static entities, due to the variations among individuals and environmental factors that influence responses. Therefore, a threshold is usually expressed as a best estimate considered protective of most of the population, and often includes a margin of safety in the derivation.

Toxicity – The observation of a chemically induced physiological or biological response that impairs the health of an organism.

Toxicity reference value (TRV) – An exposure concentration or dose that is not expected to cause an unacceptable level of effect in receptor(s) exposed to the contaminant of potential concern. A TRV is a specific type of *threshold*, as defined above.

Toxicology – The field of science that explores the relationship between substances of environmental concern and the responses elicited from organisms.

Uncertainty – Uncertainty is a term used in subtly different ways in a number of scientific fields. Generally, it refers to imperfect knowledge regarding a given parameter, process, or condition. In risk assessment, uncertainty is the state of having limited knowledge where it is impossible to exactly describe an existing state or future outcome. Uncertainties come in many forms, including measurement uncertainty, random variations, conceptual uncertainty, and ignorance.

Weight-of-evidence – A systematic procedure used to aggregate or synthesize a number of different types of evidence, with the objective of developing a single unified conclusion or explanation in an environmental characterization. Weight-of-evidence is one of the tools applied during the risk characterization stage of ERA.

Wetlands – Land that is saturated with water long enough to promote the formation of water altered soils, growth of water tolerant vegetation, and biological activity adapted to a wet environment. The Canadian Wetland Classification System breaks wetlands down into five classes:

> Bogs: peat-covered wetlands (aka peatlands), which is higher in elevation than the surrounding water table and fed in water by precipitations, occasioning a general lack of nutrients. The vegetation includes Sphagnum mosses, ericaceous shrubs, and black spruce trees.
>
> Fens: peatlands characterized by a high water table, and affected by its fluctuation, under with very slow internal drainage by seepage, and rich in dissolved minerals. The vegetation includes black spruce, tamarack, sedges, and various mosses.

Marshes: wetlands that are periodically inundated by standing or slow moving water. Generally nutrient rich, mineral-soil areas. The vegetation includes reeds, rushes or sedges, and no woody vegetation.

Swamps: wetlands where standing or slow moving water occurs seasonally or persist for long periods. The water may also be present as a subsurface flow of mineralized water. The vegetation, growing in a rich and organic soil, includes dense coniferous or deciduous forest, or tall shrub thickets.

Shallow waters: wetlands that are relatively small bodies of standing water (aka ponds or sloughs). The water depth is less than 2 m in mid-summer. The surface waters are free of emergent vegetation, but can contain floating, rooted, aquatic macrophytes.

1 Background

The Federal Contaminated Sites Action Plan (FCSAP) was developed to support federal departments, agencies and consolidated Crown corporations to reduce the risks to human health and the environment, as well as to reduce the financial liabilities associated with federal contaminated sites. Under FCSAP, ecological risk assessments (ERAs) are commonly used as a site management tool at federal contaminated sites. FCSAP is developing guidance documents for ERA supplemental to the existing Canadian Council of Ministers of the Environment (CCME 1996, 1997) guidance documents. The FCSAP ERA guidance documents consist of a main comprehensive guidance document, *Ecological Risk Assessment Guidance* (ERA Guidance, FCSAP 2012a), and several specific technical guidance modules, including this module:

- *Ecological Risk Assessment – Module 1: Toxicity Test Selection and Interpretation* (Module 1, FCSAP 2010a);
- *Ecological Risk Assessment – Module 2: Selection or Development of Site-specific Toxicity Reference Values* (Module 2, FCSAP 2010b);
- *Ecological Risk Assessment – Module 3: Standardization of Wildlife Receptor Characteristics* (Module 3, FCSAP 2012b);
- *FCSAP Supplemental Guidance for Ecological Risk Assessment – Module 4: Causality Assessment Module – Determining the Causes of Impairment at Contaminated Sites: Are Observed Effects due to Exposure to Site-Related Chemicals or due to Other Stressors* (Module 4, FCSAP 2013a);
- *Ecological Risk Assessment – Module 5: Defining Background Conditions and Using Background Concentrations* (Modules 5, FCSAP to be published); and
- *Ecological Risk Assessment – Module 7: Default Wildlife Toxicity Reference Values (TRVs) Recommended for Use at FCSAP Sites* (Module 7, FCSAP to be published).

1.1 Scope of Module

The potential for adverse effects on amphibians from exposure to anthropogenic contaminants has been receiving greater attention in recent years due to global declines in amphibian populations (Houlahan *et al.* 2000; IUCN 2014;Sparling *et al.* 2000; Stuart *et al.* 2005). Although comprehensive ecotoxicology reviews of amphibians have been published (e.g., Sparling *et al.* 2000, 2010), assessing risk to amphibians on contaminated sites remains a challenge, and technical guidance is limited. This FCSAP document is an ERA technical guidance module that provides information to help risk assessment practitioners assess amphibians at federal sites while identifying uncertainties that may be associated with an amphibian ERA.

In Canada, the FCSAP provides guidance for ecological risk assessment that recommends the use of a comprehensive weight-of-evidence approach to assess the risk that contaminants pose to all receptors, including amphibians. Risk at federal

contaminated sites can be assessed by using one or more of the following four different categories of lines of evidence (FCSAP 2012a):

1) indirect toxicological evidence (e.g., literature-based toxicity data);
2) indirect biological evidence (e.g., biological field studies reported in the scientific literature);
3) site-specific toxicological evidence (e.g., standard protocols for laboratory-based amphibian toxicity testing); and
4) site-specific biological evidence (e.g., field studies at the site of interest).

The main objectives of this guidance module are to provide amphibian-specific guidance for applying each of these four categories of lines of evidence, and to make amphibian biological and ecotoxicological information more accessible to ERA practitioners.

Sections 1.2 and 1.3 of this document provides general information on amphibian classification and biology. In Section 2, guidance is provided on when it is warranted to include amphibians in an ecological risk assessment. Section 3 provides detailed information on methodologies that can be used to assess effects on amphibians within a risk assessment. This document also includes conceptual diagrams for amphibian species in Canada and concentration-response profiles for several contaminants (cadmium, lead, inorganic mercury, and zinc), which are all available in the appendices.

1.2 Amphibian Classification

Taxonomically, amphibians belong to the class Amphibia. Frogs and toads (*Anura*) and salamanders (*Caudata*) make up over 95% of all amphibian species in the world (Hillman *et al.* 2009; McDiarmid and Mitchell 2000). The remaining amphibian species are caecilians (*Gymnophiona*), worm-like organisms that are found only in tropical regions (Hillman *et al.* 2009) and are therefore not discussed further in this module.

There are currently 25 species of frogs and toads and 25 species of salamanders native to Canada (CARCN 2010; Fisher *et al.* 2007). **Error! Reference source not found.** shows the taxonomy of amphibians in Canada according to the Canadian Amphibian and Reptile Conservation Network (CARCN 2010). **Appendix A** provides exposure pathways and media information relevant for each amphibian family.

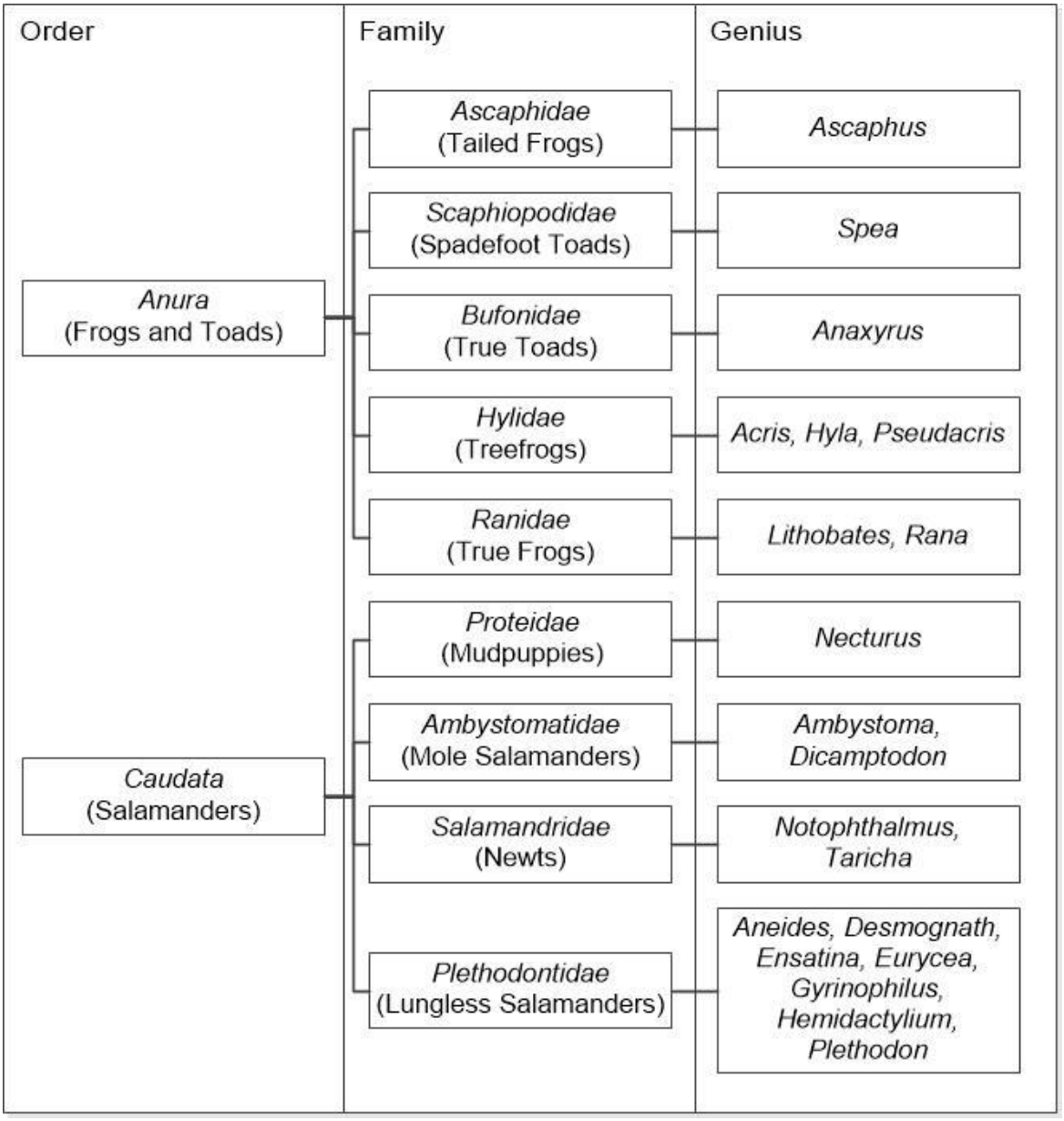
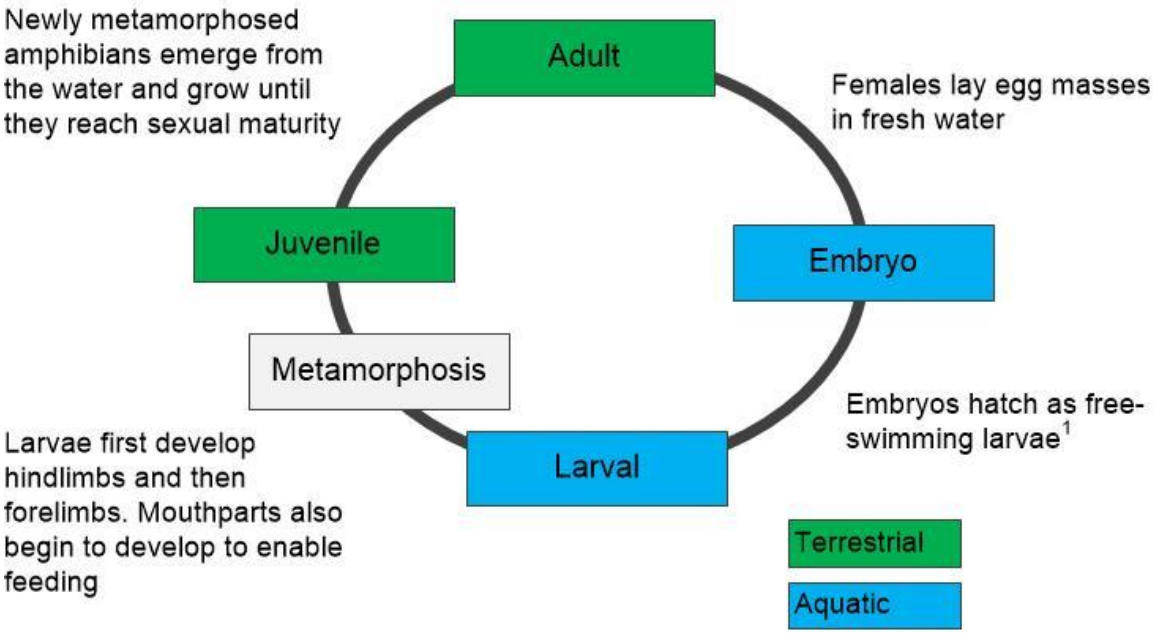


Figure 1: Amphibian classification in Canada (CARCN 2010)

1.3 Amphibian Biology

Most aquatic-breeding amphibians have a complex biphasic life cycle that involves transitioning from aquatic to terrestrial organisms (Figure 2). Frogs, toads, and salamanders undergo complex physiological and morphological changes which enable this transition. Frogs and toads (*Anurans*) have perhaps the most complex transition, as they undergo substantial changes in how they breathe (from external gills to internal gills to lungs) and feed (from filter-feeders as larvae to predators after metamorphosis). A detailed life stage classification is provided in Gosner (1960) for frogs and toads and Harrison (1969) for salamanders, which is summarized in Table 1. Figure 2 provides a summarized description of the aquatic-breeding amphibian life stage classification.



[1] Some amphibian species (e.g., members of the *Plethodontidae*) hatch as miniature adults (Wake and Hanken 1996). These species have been observed in Ontario and Quebec (CHS 2012).

Figure 2: Biphasic life cycle of aquatic-breeding amphibians (Murphy *et al.* 2000)

Table 1: Major life stages of amphibians.

Standard Larval Staging Key for Frogs and Toads (Gosner 1960)	Standard Larval Staging Key for Salamanders (Harrison 1969)
Gosner describes stages 1–19 as the embryo life stages, from egg fertilization to the first heartbeat.	Harrison describes stages 1–29 as the early embryo life stages, from egg fertilization to the development of the head and brain, as well as the tail bud.
Gosner describes stages 20–25 as covering the hatchling stages, culminating in stage 25, when the hatchling becomes an active, feeding tadpole.	Harrison describes stages 30–35 as the progressive straightening and elongation of the body. Slow pulsation of the heart begins between stages 34 and 35.
Gosner describes stages 26–41 as the larval stages covering the longest period of juvenile development, marked by the development of the hind limbs and a long, coiled intestine adapted to the digestion of plant material.	Harrison describes stages 36–40 as the start of differentiation of the anterior part of the trunk, with the liver and pancreas becoming defined posterior to the heart. Balancers are elongated and forelimb progresses to paddle form.
Gosner describes stages 42–46 as metamorphosis, involving development of the forelimb, resorption of the tail, and a reduction in intestine size along with alterations to mouthparts as the animal transitions to a carnivorous diet. By the end of Gosner stage 46, frogs and toads have attained their adult form, although they have not yet reached their adult size.	Harrison describes stages 41–46 as covering the development of mouth and forelimb. The digestive system is developed and feeding begins at stage 46. By the end of Harrison stage 46, the larvae are able to begin feeding. Metamorphosis is not covered in the Harrison staging key.

Frogs, toads and salamanders in Canada can be loosely categorized into pond breeders or stream breeders. Pond breeders attach their eggs to submerged vegetation or lay them on the pond bottom or water surface; ponds can be permanent or temporary. Stream breeders attach their eggs to the undersides of in-stream logs and rocks. The Northern Leopard Frog (*Lithobates pipiens*, formerly known as *Rana pipiens*), is an example of a pond breeder native to Canada, and the Coastal Tailed Frog (*Ascaphus truei*) is an example of a stream breeder. In the case of pond breeders, several species often breed in the same habitat. For example, Red-legged Frogs *(Rana aurora*), Pacific Tree Frogs (*Pseudacris regilla*), Long-toed Salamanders (*Ambystoma macrodactylum*) and Northwestern Salamanders (*Ambystoma gracile*) represent a common species assemblage in British Columbia. The larvae rear together, but the adults have less overlap in their habitat preferences.

Frog and toad embryos hatch in about three to four days and are relatively immobile for one to two days post-hatch. Salamander embryos take longer to hatch, and they are more fully developed and more closely resemble their adult form upon hatching. Once hatched, frogs, toads and salamanders begin to develop mouthparts that enable them to begin feeding (Duellman and Trueb 1994). Most frog and toad larvae are herbivorous while most salamander larvae are carnivorous. Frogs and toads undergo dramatic changes in their digestive structure during metamorphosis as larvae transition from filter-feeding (primarily eating algae and decayed plant matter) to predacious feeding (Henry 2000). Salamanders do not undergo such an extreme transition and remain carnivorous. The complete larval transformation can take from several weeks to a couple of years depending on the species (Fisher *et al.* 2007). Ephemeral pond breeders, such as many treefrog species, must emerge before their natal habitats dry out, while some stream breeders (e.g., tailed frogs) do not mature into adults for more than two years (Fisher *et al.* 2007).

Newly metamorphosed amphibians, also known as juveniles, expend most of their resources and energy on growth until they reach sexual maturity. Both juveniles and adults are considered terrestrial, although many species spend a significant amount of time in or near freshwater environments (Figure 2).

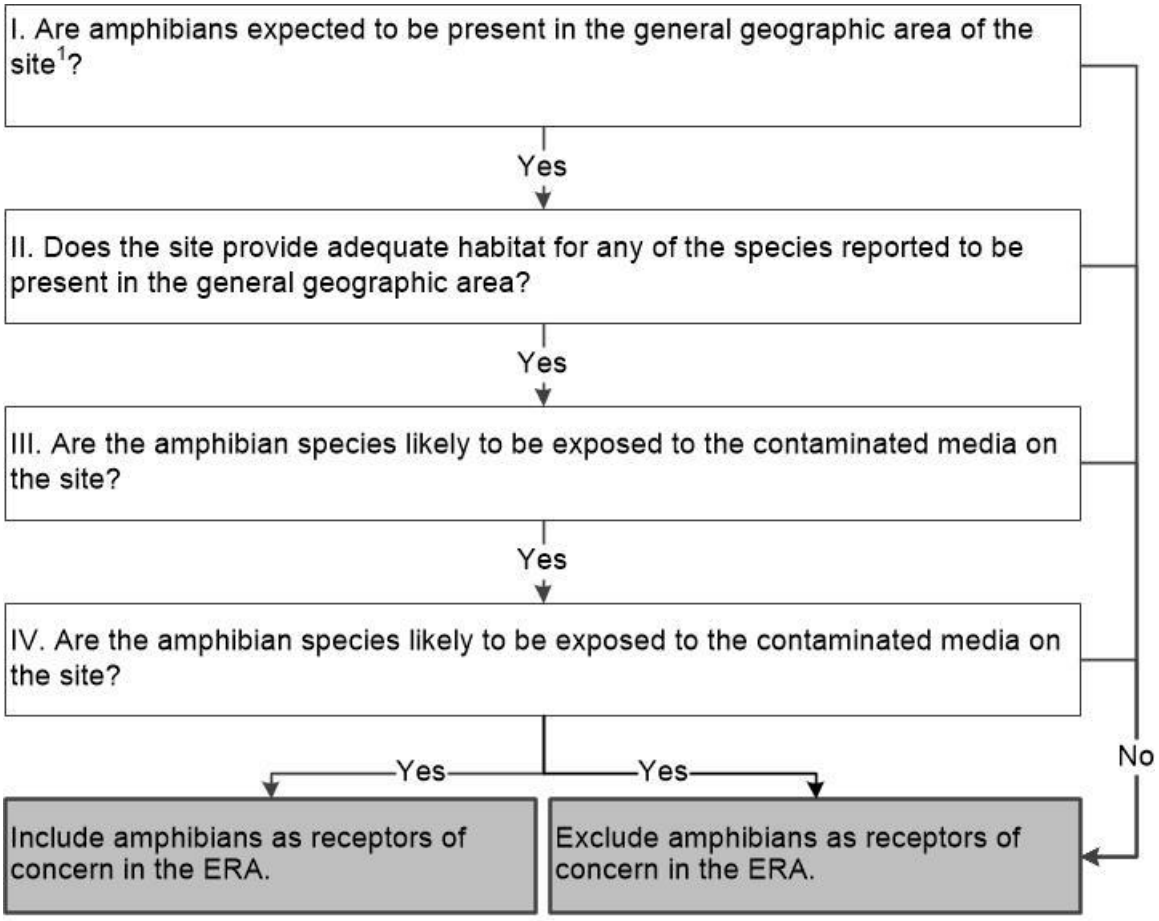
Some species of salamanders, including several species native to Canada, exhibit a life history called neoteny. The Mudpuppy (*Necturus maculosus*), Cope's Giant Salamander (*Dicamptodon copei*),) and Northwestern Salamander (*Ambystoma gracile*) are examples of neotenic species found in Canada. Their life history does not correspond to Figure 2, as they never leave the water. These species reach reproductive maturity while retaining the larval external morphology (i.e., gills). Adults remain in the natal water body.

2 Amphibians as receptors of concern

Amphibians live in a variety of aquatic and terrestrial habitats, including some habitats that are commonly found on contaminated sites on federal land. FCSAP ERA guidance states that all receptors, including amphibians, should be considered as possible receptors of concern. The guidance also states that ERA practitioners need to provide the rationale for including particular receptor types in an ERA or excluding them. A receptor of concern is defined as “any non-human individual organism, species, population, community, habitat or ecosystem that is potentially exposed to contaminants of concern” (FCSAP 2012a). Therefore, if amphibians are currently present on a site, or are likely to be present in the future, they should be included as receptors of concern unless it is evident that they will not be exposed to contamination on the site. ERA practitioners should also consider whether amphibians have been extirpated from the site and were therefore present in the past and could potentially be present in the future.

2.1 Determining Whether Amphibians are Receptors of Concern

The following decision tree (Figure 3**Error! Reference source not found.**) can be used to determine whether or not amphibians should be included as receptors of concern in an ecological risk assessment. Further guidance and resources for answering questions in the decision tree are provided following **Error! Reference source not found.**.



[1] If water bodies are not present on the site, but there is moist coniferous and/or deciduous forested habitat with an abundance of decayed logs and wood cavities, it is possible that fully terrestrial amphibian species are present on or near the site. In this case, answer "Yes" to question I

Figure 3: Decision tree for determining whether amphibians should be included as receptors of concern in a site-specific risk assessment

I. Are amphibians expected to be present in the general geographic area of the site?

Table 2 lists general sources of information on amphibians. These sources include information on the distribution of amphibians in general geographic areas. Province-specific resources are preferable because species identification and distribution is typically provided at a more detailed spatial resolution.

It is recommended to identify amphibians at the species level if amphibian presence on site is probable. Track the scientific names of species (genus and species) reported in the resources in Table 2. The genus will help to answer question II.

Example of how to summarize the information:

I. Amphibians present?	Species name	II. Habitat	III. Contaminated media?	IV. Other findings
Yes	*Lithobates pipiens*			

Table 2: List of sources of information on amphibians for selected provinces. If information on amphibians for the province is not available, refer to Canadian and global resources.

Area	Distribution	Sighting	Habitat	Diet	Reproduction	Reference	Websites
Alberta	●	N.A.	●	●	●	Russell and Bauer 1993	N.A.
	N.A.	N.A.	●	●	●	AEP 2014	N.A.
British Columbia	●	N.A.	●	●	●	BCMOE 2015	http://www.env.gov.bc.ca/wld/frogwatch/whoswho
	N.A.	●	●	●	●	E-Fauna BC 2015	http://ibis.geog.ubc.ca/biodiversity/efauna/
	●	N.A.	●	●	●	Matsuda *et al.* 2006	N.A.
	●	●	●	N.A.	●	Corkran and Thoms 1996	N.A.
Labrador	●	N.A.	N.A.	N.A.	N.A.	Newfoundland Labrador 2018	https://www.flr.gov.nl.ca/wildlife/all_species/amphibians.html
Manitoba	●	●	●	●	●	Nature North 2018	http://naturenorth.com/Herps/Manitoba_Herps_Atlas.html
New Brunswick	●	N.A.	●	●	●	Gorham 1970	N.A.
Northwest Territories	●	N.A.	●	●	●	Government of Northwest Territories 2018	https://www.enr.gov.nt.ca/en/services/amphibians-and-reptiles
Nova Scotia	●	N.A.	N.A.	●	N.A.	Nova Scotia Museum 2015	http://novascotia.ca/museum/amphibians/en/frogs/
Ontario	N.A.	●	N.A.	N.A.	N.A.	Ontario Nature 2015	http://www.ontarioinsects.org/herpatlas/herp_online.html
	●	N.A.	●	●	●	MacCulloch 2002	N.A.
Prince Edward Island	N.A.	●	N.A.	N.A.	N.A.	PEI Nature Tracker 2018	http://www.peinaturetracker.ca/filter/reptiles-and-amphibian

Area	Distribution	Sighting	Habitat	Diet	Reproduction	Reference	Websites
Quebec	●	●	●	N.A	N.A.	AARQ 2019	http://www.atlasamphibiensreptiles.qc.ca/
	N.A.	●	N.A.	N.A.	N.A.	Bider and Matte 1996	N.A.
Saskatchewan	N.A.	N.A.	●	N.A.	N.A.	Saskatchewan Fish and Wildlife Branch 2014a, 2014b	http://www.environment.gov.sk.ca/adx/aspx/adxGetMedia.aspx?DocID=df579dc1-5ed4-43fa-ba4d-7d4ef60b5fc4
Yukon	●	N.A.	●	N.A.	N.A.	Government of Yukon 2019	http://www.env.gov.yk.ca/animals-habitat/amphibians.php
Canada	●	N.A.	●	N.A.	●	NatureWatch 2019	https://www.naturewatch.ca/frogwatch/
	●	N.A.	●	●	●	COSEWIC 2014	https://wildlife-species.canada.ca/species-risk-registry/sar/index/default_e.cfm
	●	N.A.	●	●	●	CHS 2012	http://canadianherpetology.ca/species/index.html
	●	N.A.	●	●	●	Fisher et al. 2007	N.A.
	●	N.A.	●	N.A.	●	Cook 1984	N.A.
	●	N.A.	●	N.A.	N.A.	Conant and Collins 1998	N.A.
Global	●	N.A.	●	●	●	IUCN 2014	http://www.iucnredlist.org/initiatives/amphibians

● = Information exists
N.A. = Information not available

II. Does the site provide adequate habitat for any of the species reported to be present in the general geographic area?

The general habitat requirements for each family are provided in Table 3. This information can be used to determine the habitat needed for species identified in the general geographic area. The wetlands can be discribed using the Canadian Wetland Classification system nomenclature (Warner and Rubec 1997).

It is also important to review references provided in Table 2 for species-specific information.

Example of how to summarize the information (continued):

I. Amphibians present?	Species name	II. Habitat	III. Contaminated media?	IV. Other findings
Yes	*Lithobates pipiens*	Yes, there is a permanent pond on or near the contaminated site.		

Table 3: General habitat requirements for each family of amphibians native to Canada (CHS 2012; Cook 1984; Fisher *et al.* 2007).

Family	Genus	Embryo	Larva	Adult
Ambystomatidae (Mole Salamanders)	*Ambystoma, Dicamptodon*	Inhabit vegetation at the bottom and margins of ponds with adequate riparian zones.	Remains in the water.	Inhabit forests, parklands, grasslands, sub-alpine meadows and semi-deserts. Neotenic adults remain in permanent water bodies.
Ascaphidae (Tailed Frogs)	*Ascaphus*	Inhabit the underside of rocks in cold, high-gradient, fast-moving mountain streams in forested areas.	Remains in streams and overwinter under rocks or stream substrate.	Inhabit forested areas around breeding stream. May overwinter under stream substrates.
Bufonidae (True Toads)	*Anaxyrus*	Inhabit shallow temporary or permanent still ponds with aquatic or emergent vegetation.	Remains in the ponds.	Migrate from breeding ponds to grasslands, forests, lawns, and gardens. Spend most of the time underground.
Hylidae (Treefrogs)	*Acris, Hyla, Pseudacris*	Inhabit vegetation at the bottom and margins of ponds with adequate riparian zones.	Remains in the pond.	Most are tree-dwelling but some reside in grasslands (*Pseudacris*) and along water bodies (*Acris*).
Plethodontidae (Lungless Salamanders)	*Aneides, Desmognath, Ensatina, Eurycea, Gyrinophilus,*	*Eurycea* and *Gyrinophilus*: water. *Ensatina*, *Aneides*, and *Plethodon*: rotting wood or other moist habitats. *Desmognathus*: near water.	Some species remain in the water, and some develop and emerge in terrestrial habitats.	Inhabit underground in forested areas. *Ensatina*, *Aneides*, and *Plethodon* do not have a larval stage and develop directly into adults. *Gyrinophilus* and *Dicamptodon*

Family	Genus	Embryo	Larva	Adult
	Hemidactylium, Plethodon	*Hemidactylium:* damp moss over bog pools.		may stay in aquatic habitats at adult age.
Proteidae (Mudpuppies)	*Necturus*	Inhabit areas under rocks in permanent water that does not freeze to the bottom.	Remains in the water.	Remains in the water.
Ranidae (True Frogs)	*Lithobates, Rana*	Inhabit vegetation at the bottom and margins of ponds with adequate riparian zones.	Remains in the pond.	Inhabits areas near or on the edge of permanent or ephemeral water bodies. However, some frog species from this family (e.g. northern leopard frog, wood frog) have a large foraging range during summer after breeding, and can be encountered long distances from the breeding wetland.
Salamandridae (Newts)[1]	*Notophthalmus, Taricha*	Inhabit vegetation or logs at the bottom of swamps in forested areas.	Remains in the water.	Eft[1]: Under logs, bark and other forest debris near water. Adult: Move back to breeding water.
Scaphiopodidae (Spadefoot Toads)	*Spea*	Inhabit shallow temporary or permanent wetland ponds within semi-arid grasslands.	Remains in the water and develop rapidly.	Inhabit semi-arid grasslands with loose soil. Spend prolonged periods underground.

[1] Newts are almost completely aquatic except for a period during the juvenile stage when they are completely terrestrial. This life stage, referred to as the “eft”, lasts approximately two years (Fisher *et al.* 2007).

III. Are the amphibian species likely to be exposed to the contaminated media on the site?

It is important to determine which exposure media are contaminated (e.g., food items), as this enables identification of potential exposure pathways. Table 4 lists general exposure pathways for pond-breeding frogs and toads. Detailed exposure pathway conceptual diagrams for all native amphibian families, including stream-breeding varieties, are included in Appendix A. Note that the maternal transfer pathway is relevant for contaminated sites where bioaccumulative substances are present. Hopkins *et al.* (2006) reported that *Gastrophryne carolinensis* females can transfer up to 70% of their contaminant body burden to their embryos.

Where exposure pathways for amphibian species are identified, differentiation of the major and minor pathways can help ERA practitioners determine which life stage to assess on a priority basis. For example, if the water is contaminated and it is a major exposure pathway for the larval stage but a minor pathway for other life stages, then the risk assessment should focus on the larval stage. FCSAP recognizes that all the pathways cannot be assessed with the resources currently available; however, ERA practitioners should acknowledge all pathways, and those that cannot be assessed should be described as sources of uncertainty in the ERA.

For the purpose of this guidance module, major pathways provide substantial exposure to contaminants. Minor pathways provide limited exposure. For example, exposure to contaminants through direct contact with contaminated water is considered a major pathway for the aquatic embryonic and larval life stages, but only a minor pathway for adult life stages, because adults spend most of their time in the terrestrial environment.

Example of how to summarize the information (continued):

I. Amphibians present?	Species name	II. Habitat	III. Contaminated media?	IV. Other findings
Yes	*Lithobates pipiens*	Yes, there is a pond on or near the contaminated site.	Sediment and water are contaminated, but soil and other media listed in Table 4 are not contaminated.	

Table 4: General exposure pathways for pond-breeding frogs and toads (CHS 2012; Cook 1984; Fisher *et al.* 2007).

Exposure Media	Exposure Pathways	Life Stage		
		Embryo	Larva	Adult
Surface Water	Direct contact [1]	●	●	○
	Respiration[2]	—	●	○
Sediment and Sediment Porewater	Direct contact[1]	●	●	○
	Respiration[2]	—	●	○
	Incidental ingestion	—	●	○
Soil	Direct contact	—	—	●
	Incidental ingestion	—	—	○
Air	Direct contact[1]	—	○	●
	Respiration[2]	—	○	●
Aquatic Food Items	Algae / aquatic plants	—	●	—
	Small fish, amphibians and invertebrates	—	○	●
Terrestrial Food Items	Invertebrates	—	—	●
	Small mammals	—	—	●
● = Major Pathway ○ = Minor Pathway — = Not a pathway				

[1] Direct contact includes cutaneous respiration.

[2] Respiration refers to respiration through the lungs or gills.

IV. Is there documented proof or some other rationale (other than contamination) of why amphibians are unlikely to be present on the site, now or in the near future?

If amphibian species in the geographic area have the potential of being impacted by contamination, they should be included in the ecological risk assessment, unless the ERA practitioners can demonstrate that amphibians ***are not*** present on the site and are not likely to be present in the future (for reasons other than contamination). ERA practitioners will need to provide a strong rational for the exclusion of amphibians. Detailed site surveys at appropriate times of the year may be necessary to adequately answer this question. A biologist with experience in amphibian biology and field survey techniques should be included in the ERA team.

The resources listed in Table 2 may provide some information to help answer this question concerning the potential absence of amphibian species at or near the site. However, if suitable habitats exist at or near the contaminated site that could support amphibian species, the ERA practitioner must exercise care in concluding that other site factors are sufficient to exclude the presence of amphibians.

Example of how to summarize the information (continued):

I. Amphibians present?	Species name	II. Habitat	III. Contaminated media?	IV. Other findings
Yes	*Lithobates pipiens*	Yes, there is a pond on or near the contaminated site.	Sediment and water are contaminated, but soil and other media listed in Table 4 are not contaminated.	Detailed biological analyses demonstrate that aquatic habitats contain predatory fish species with which amphibians cannot co-exist. Site survey confirms the absence of *Lithobates pipiens*.

3 Amphibian effects assessment

The ERA guidance (FCSAP 2012a) emphasizes a weight-of-evidence framework approach to risk assessment. This approach involves using a number of different lines of evidence to evaluate whether or not receptors are at risk from exposure to contaminants at federal contaminated sites. Each line of evidence assesses whether a receptor currently experiences or will in the future experience an effect due to contaminant exposure. Ideally, the various lines of evidence in a weight-of-evidence risk assessment include different methods of effects assessment. The ERA guidance (FCSAP 2012a) describes four broad categories of effects assessment methods:

- Indirect (literature-based) toxicity information
- Indirect (literature-based) biological information
- Site-specific toxicity studies
- Site-specific biological studies

When many lines of evidence are used, the conclusions of an ERA will be robust and uncertainties will be reduced, especially if the lines of evidence include effects assessment measures from all four categories and the results from individual lines of evidence are congruent. It is not always possible to assess the risk to all receptors using several lines of evidence from all four categories. Some lines of evidence are more suitable than others, depending on the receptors involved, the site, the availability of relevant information, and the type of contamination. The advantages and disadvantages associated with lines of evidence from the four broad categories are discussed in detail in the FCSAP ERA Guidance (2012a).

The following sections provide information on how to assess risk to amphibians using approaches that fall within the four effects assessment categories listed above. In this document, a hierarchical and step-wise approach is recommended for using these four categories, beginning with a review of the available literature-based toxicity. Does-response profiles could also be used using a similar approach, where the information was available.

Note that this module does not address the topic of effect size used to determine the existence or absence of a risk. This topic is rather addressed in the ERA Guidance (FCSAP 2012a).

3.1 Indirect (Literature-Based) Toxicity Information

Literature-based toxicity refers to data from published literature studies (FCSAP 2012a). These studies can be a source of relevant data for developing concentration-response profiles to illustrate effects for a range of concentrations of a given contaminant. This section will focus on how to develop and use concentration-response profiles for amphibian ecotoxicology data. Section 3.1.1 describes how to compile data from multiple studies in order to generate concentration-response profiles. ERA practitioners have the option of using all available data (Section 3.1.2) or refining the profile based on site-specific parameters and data quality requirements in order to improve its relevance (Section 3.1.3). ERA practitioners also have the option of fitting a statistical model to the data when appropriate. Statistical evaluation of the data is not discussed in this module but information is provided in Module 2 (FCSAP 2010b).

Comparing site-specific concentrations to concentration-response profiles that summarize results from numerous relevant published toxicity studies is an effective option for estimating effects (FCSAP 2010b, 2012a). Even when toxicity data are limited, all the available data can be combined in concentration-response profiles to allow for a more comprehensive assessment (FCSAP 2010b).

3.1.1 Developing concentration-response profiles

Developing concentration-response profiles involves collecting published studies and recording relevant data in a consistent manner. The general process is outlined in **Error! Reference source not found.** and is based on guidance provided in CCME (2007), Dillon *et al.* (2010), FCSAP (2010b) and Hill *et al.* (2013). Using this process, examples of concentration-response profiles for four metals (cadmium, lead, mercury, zinc) have been created as part of this guidance module (Appendix B). These profiles summarize available toxicity data on amphibian exposure to the four metals in water and sediment. Limited information is also provided on amphibian exposure to contaminants in soil. Ideally, a concentration-response profile will be developed for all relevant contaminants as part of the ERA. Alternatively, the profiles included in this module may be used and updated with the most current data.

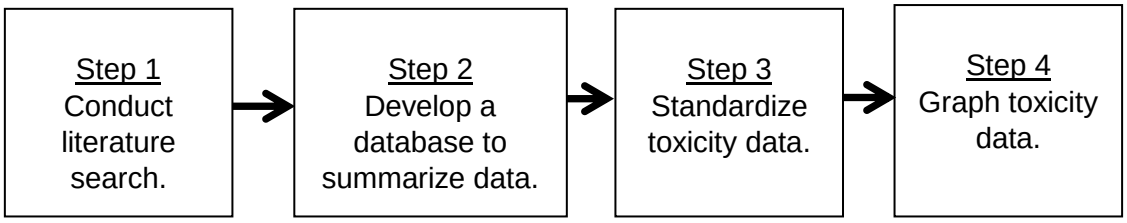


Figure 3: The general process for developing multi-study concentration-response profiles

3.1.1.1 Step 1: Conduct literature search

A search for published toxicity data should begin with existing databases and data compilations. Prior to using data that are referenced in these published data compilations, the original sources should be checked whenever possible to ensure that the data were interpreted appropriately (FCSAP 2010b). In addition to consulting published data compilations, ERA practitioners should conduct a general search to find the most up-to-date published studies.

Main secondary sources: The main databases and data compilation sources for amphibian ecotoxicology data are listed below. These sources mostly contain point estimates of amphibian toxicity (e.g., LC_{50}, EC_{50}, NOAEL, and LOAEL) from many individual studies[1]:

1. Bleiler, J., D. Pillard, D. Barclift, A. Hawkins, and J. Speicher. 2004. *Development of a standardized approach for assessing potential risks to amphibians exposed to sediment and hydric soils*. Technical Report TR-2245-ENV, prepared for Naval Facilities Engineering Service Center (NAVFAC), Port Hueneme, CA. Westford (MA): ENSR International.

2. Linder, G., and G. Grillitsch. 2000. Ecotoxicology of Metals. In: *Ecotoxicology of Amphibians and Reptiles*. Sparling, D.W., G. Linder and C.A. Bishop, eds, pp. 325-459. SETAC Press, Pensacola, FL, USA.

3. Pauli, B.D., J.A. Perrault, and S.L. Money. 2000. RATL: A Database of Reptile and Amphibian Toxicology Literature. Technical Report Series No. 357. Canadian Wildlife Service, Headquarters, Hull, Quebec, Canada.

[1] The original studies, obtained from citations in these summary reports and additional recently published works, were consulted to compile concentration-response data for the multi-study concentration-response profiles included in Appendix B. If the published works did not include detailed concentration-response data (i.e., only point estimates were published), the authors were contacted to obtain the complete concentration-response datasets.

4. Schuytema, G.S., and A.V. Nebeker. 1996. *Amphibian toxicity data for water quality criteria chemicals*. US Environmental Protection Agency, Office of Research and Development, Corvallis OR. USEPA/600/R-96/124.

5. USEPA. 2015. ECOTOX User Guide: ECOTOXicology Database System. Version 4.0. Available at: https://cfpub.epa.gov/ecotox/

3.1.1.2 Step 2: Develop a database to summarize data

All relevant information from toxicity studies (e.g., species, life stage, exposure period) can be consolidated in a database. It is necessary to ensure that the information is recorded in the database in a consistent manner. The database used to construct the concentration-response profiles provided in Appendix B includes the following categories:

- Species (scientific name)
- Life stage
- Exposure time
- Concentration (formulated or measured)
- Treatment response (effect level)
- Control response
- Measurement endpoint
- Normalized response
- Contaminant (chemical form)
- Modifying factors (e.g., temperature, pH, water hardness)
- References

Effect concentration (EC) and inhibitory concentration (IC) toxicity data should be recorded separately. EC measures the percentage of test individuals that experience a certain effect, and is used for a dichotomous effect. While IC measures the extent to which a concentration can inhibit certain biological activities in test individuals, as a result of the exposure.

3.1.1.3 Step 3: Standardize toxicity data

To compare data from different studies, the contaminant concentrations and effect levels need to be normalized. The data in the concentration-response profiles in Appendix B have been normalized using the method recommended by Dillon *et al.* (2010) and Hill *et al.* (2013), which is described below.

- **Normalizing chemical concentration (mg/L)**: The chemical concentration refers to the original concentration of the chemical added to the exposure media. The general process is outlined in Figure 5. Ideally, the concentration of the simplest form of the contaminant will be recorded. For example, metal compounds, e.g., Pb $(CH_3COO)_2$, can be converted to the elemental form (e.g., Pb^{2+}) because it is lead that is likely causing adverse effects[1] as opposed to other elements in the tested compound.

For example, 10 mg/L of lead acetate used in toxicity experiments by Kamimura and Tanimura (1986) can be converted to elemental lead as follows:

[lead acetate] = 10 mg/L
Molar weight of Pb = 207.2 g/mole
Molar weight of lead acetate (Pb $(CH_3COO)_2$) = 325.29 g/mole

[Pb^{2+}] = (207.2 g/mole ÷ 325.29 g/mole) x 10 mg/L = 6.37 mg/L

Figure 4: Normalizing chemical concentrations (mg/L)

- **Normalizing response levels:** Responses can be recorded as EC or IC values. The general process is outlined in Figure 6. Both types of responses should be normalized in relation to the control so that effect levels account for responses directly attributable to the tested contaminant, not those caused by activities unrelated to the chemical exposure (i.e., animal husbandry). If the reported effect has already been normalized, the data can be directly recorded in the database. If the results reported in a study are not normalized, the response levels should be normalized using the study-specific control response.

[1] Metal concentrations in Appendix B represent the elemental form.

Treatment group: The group exposed to the contaminant.
Control group: The group not exposed to the contaminant.
Response level (%) = treatment response ÷ control response

Example of EC normalization (from Hill et al. 2013):

Treatment = 80% survival;
Control = 95% survival;
Normalized response % = 80% ÷ 95% x 100 = 84.2% survival

Example of IC endpoint normalization (Manson and O'Flaherty, 1978):

Treatment = 48 mm (average larval length);
Control = 57.1 mm (average larval length);
Normalized response = ((57.1 mm – 48mm) ÷ 57.1 mm) x 100 = 15.9% reduced length in treatment group

Figure 5: Normalizing response levels

3.1.1.4 Step 4: Graph toxicity data

All available toxicity data can be plotted on a graph to show the effects at different exposure concentrations. Figure 7 shows an example of a multi-study concentration-response profile for amphibians exposed to cadmium in water. Appendix B contains similar concentration-response profiles for four contaminants (cadmium, lead, mercury and zinc) in water and sediment. ERA practitioners can use these profiles directly (Section 3.1.2) or refine the profiles (Section 3.1.3) as applicable. Statistical analysis and models can also be applied to the dataset. Information on statistical approaches is available in ERA Module 2 (FCSAP 2010b).

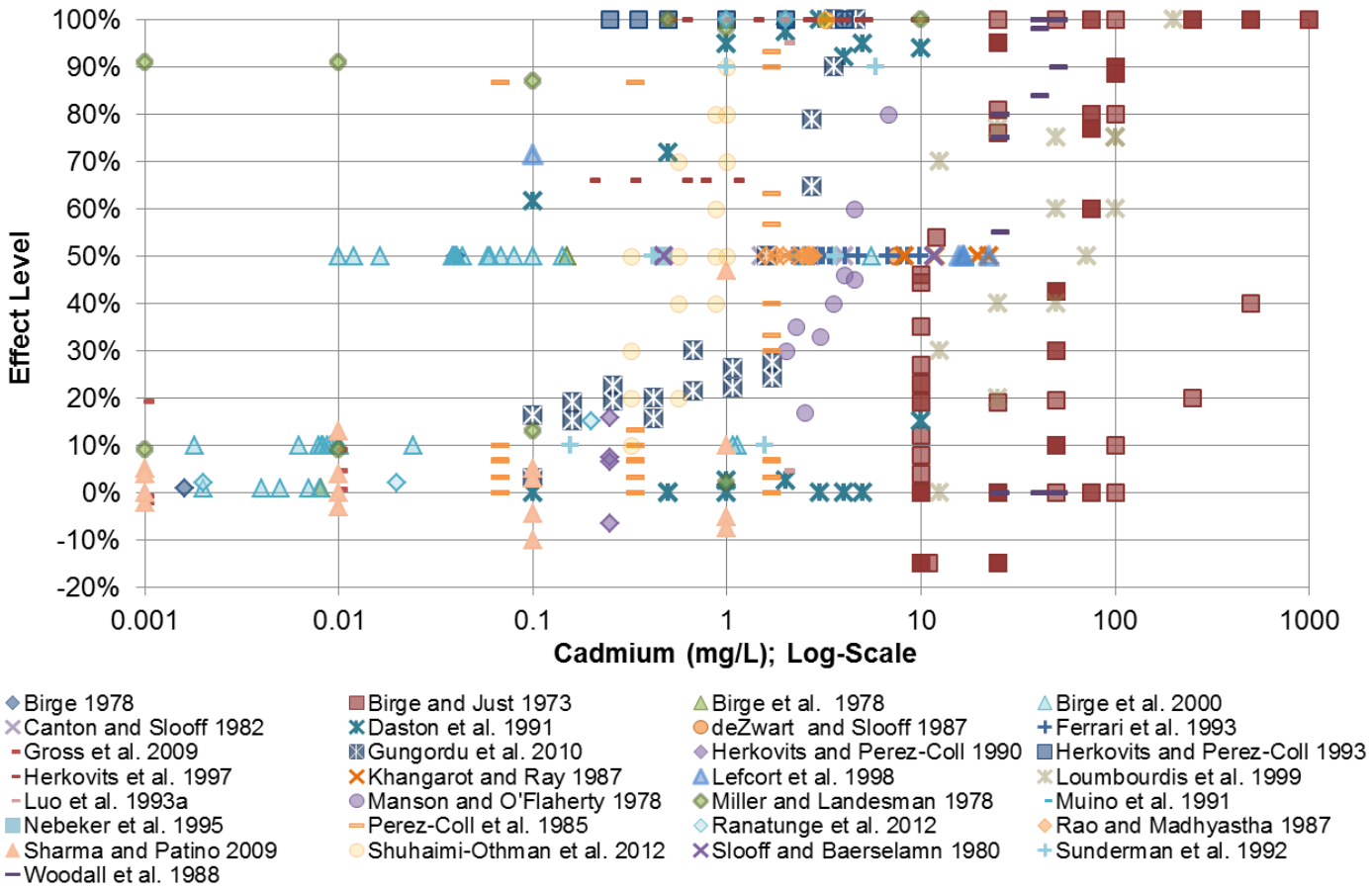


Figure 6 **Multi-study concentration-response profile for cadmium in water, showing EC endpoints. Darker shaded symbols indicate several overlapping data points and negative effects levels occur when the treatment outperforms the control.**

3.1.2 Using Multi-Study Concentration-Response profiles

The multi-study concentration-response profiles are intended to aid ERA practitioners in determining potential site-specific adverse effects on amphibians. While the profiles capture many of the available literature-based toxicity values for amphibians, the data available for amphibians is much sparser than that for other receptors (e.g., invertebrates and fish). Toxicity studies focus primarily on amphibian toxicity from exposure to contaminated water. Studies on exposure to contaminated sediment and soil are limited. These data limitations can present a significant source of uncertainty in amphibian ERAs.

ERA practitioners can assess the potential effects of contaminants present at the site by comparing site concentrations to the multi-study concentration-response profile[1,2]. If exposure concentrations are higher than the level of effect corresponding to the protection objectives established for amphibians at the site (e.g., level of effect <10% for species at risk) in the concentration-response profiles, adverse effects on amphibians may be unacceptable.

In such circumstances, two site management options are available:

1. Clean-up of contamination to levels that provide general protection (e.g., water and sediment quality guidelines of the applicable jurisdiction); or,

2. Conduct more detailed ERA work to demonstrate that site-specific protection of amphibians is provided, using the following approaches:

    a. *Adapt concentration-response data to site-specific conditions* - For example, if a certain species or life stages are not relevant to a specific site, the effects data for this species or life stage may be excluded from consideration.
    b. *Conduct weight-of-evidence risk assessment* - Assess the risk to amphibians using additional lines of evidence, such as site-specific toxicity or indirect biological studies.

Most of the published toxicity information has been generated in controlled laboratory settings which cannot fully represent the dynamic conditions found at contaminated sites. For example, site-specific modifying factors (e.g., hardness, alkalinity, pH, and dissolved organic carbon) often play a major role in determining metal bioavailability in aqueous exposures. Toxicity studies can also be limited by animal husbandry factors (e.g., lighting may impact physiology and behaviors) and by the availability of amphibian test species.

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[1] This module is not intended to define effect sizes that are indicative of an acceptable or unacceptable risk, since a thorough consideration of critical effect sizes is beyond the scope of the module. The effect size should be determined by, among other things, considering the possible presence of species at risk.

[2] **Appendix C** compares guidelines from several jurisdictions to the multi-study concentration-response profiles.

These sources of uncertainty can be reduced by using multiple lines of evidence, to the extent practicable.

3.1.3 Refining Concentration-Response Profiles

The multi-study concentration-response profiles may be the best starting point for determining whether contaminants have adverse effects on amphibians. If contaminant concentrations at the site fall within the range of reported effects, it is difficult to make definitive inferences about risk based on the profiles directly. To reduce variability, the ERA practitioner may choose to refine the concentration-response profile by considering study quality and site-specific parameters. If enough toxicity data are available, the data should be filtered to increase the relevance of the toxicological information for the site (FCSAP 2010b).

For example, the concentration-response profiles provided in **Appendix B** represent all available published toxicity data for cadmium, lead, mercury, and zinc. These profiles can be refined by filtering the complete database (available upon request from FCSAP; see contact information at the end of this document) based on specific study parameters and data quality.

3.1.3.1 Data quality

A critical evaluation of study design and data quality is recommended when refining the concentration-response profiles. Based on data quality, ERA practitioners can determine whether a study should be excluded from the concentration-response profile, or should be given more or less weight relative to other studies. CCME sets out data quality requirements which are applied in the process of establishing guidelines (e.g., 2007 CCME protocol for the derivation of water quality guidelines for the protection of aquatic life). The data quality requirements that are outlined in this section have been adapted from the CCME approach (Table 5). Studies can be ranked based on the number of data quality requirements that have been fulfilled (Table 6): studies ranked 1 fulfill the largest number of requirements, and studies ranked 4 fulfill the least number[1]. Figure 8 shows the concentration-response profiles for cadmium, with the data grouped according to the rank. Similar profiles for other contaminants can be found in **Appendix B**. It is important to note that studies ranked 1 or 2 may meet more data quality requirements but may be less relevant (e.g., test species not present on the site). ERA practitioners should consider both data quality and data relevance when selecting toxicity data for use in an ERA. Data relevance is discussed in the following sections.

[1] Published toxicity studies used for the multi-study concentration-response profiles in **Appendix B** were assessed using methods adapted from the CCME protocol for the derivation of water quality guidelines for the protection of aquatic life (CCME 2007). **Appendix D** presents the data quality evaluation for each study used in the multi-study concentration-response profiles.

Table 5: Data quality requirements considered in this module (CCME 2007).

Data Quality Requirements	Description
Appropriate Study Design	Published toxicity studies should describe the method used, preferably referencing one of the existing amphibian toxicity testing protocols (see Section 3.3). In cases where an existing toxicity testing protocol was used, the study was assumed to have an appropriate study design. Studies that used other methods were assessed on a case-by-case basis to determine whether the study design was scientifically defensible.
Replication	Studies should include replication in each treatment whenever possible (and avoid pseudo-replication). Replicates should yield similar results for any given treatment.
Data Analysis	Depending on the study design, published studies should include statistical analysis of the results (e.g., hypothesis testing, p-values, etc.). If a study includes appropriate statistical analysis, the data quality criteria are assumed to be met.
Modifying Factors	Studies should report modifying factors (e.g., water hardness, dissolved oxygen, temperature). Some studies may report more modifying factors than others but this may not impact the ranking.
Use of Control and Control Response	Published toxicity studies should use control(s) during the experiment to determine whether the test procedure is causing effects that are likely unrelated to the tested contaminant. Acceptable control responses should be considered for comparison to the treatment responses. The various amphibian test protocols cited in this module use a range of control acceptability criteria of ≥ 80–90% (for the survival endpoint). A control that meets these criteria is considered acceptable (the test passes), which indicates that the test procedure itself is not causing an unrelated effect. In instances where the treatment(s) outperform the control (e.g., control survival is lower than treatment survival), it is possible that the test should be considered a failure and the results discarded. ERA practitioners can also evaluate elements of the study that may point to a cause. For example, there may be evidence of hormesis, i.e., the treatment outperforms the control at low concentrations due to the test organisms' adaptation to moderate stress. In such cases, inclusion of the study in the profile is justified.

Table 6. Descriptions of ranking of toxicity studies.

Rank	Description
1	These studies meet all data quality requirements. The toxicity tests are scientifically defensible. ERA practitioners may want to give more weight to these studies than to others.
2	These studies meet the majority of data quality requirements. ERA practitioners may want to give more weight to these studies than to other studies.
3	These studies meet some of the data quality requirements. ERA practitioners may want to give less weight to these studies than to those ranked 1 or 2. If all studies are ranked 3 or higher, ERA practitioners should consider including other lines of evidence.
4	The studies that fall in this category contain substantial uncertainty in study design, data analysis, etc. and do not meet most of the data quality requirements. It is recommended that these studies be given a lower weighting, or that they be excluded. If all studies are ranked 4, ERA practitioners should consider including other lines of evidence.

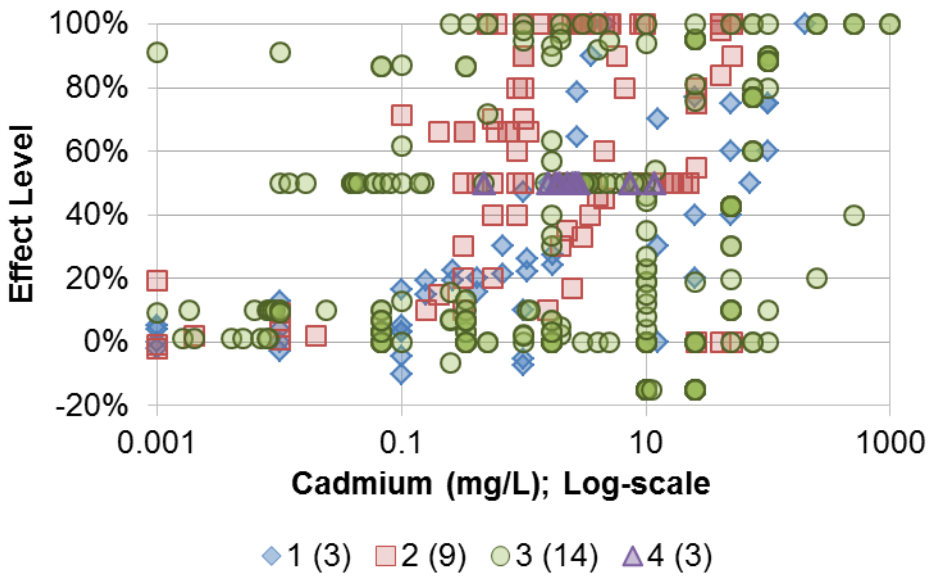

Figure 7 Multi-study concentration-response profile for cadmium (water) by data quality ranking, with the number of studies shown in parentheses. Darker shaded symbols indicate several overlapping data points and negative effects levels occur when the treatment outperforms the control.

3.1.3.2 Modifying factors

Modifying factors, such as hardness and pH, may affect contaminant bioavailability and/or effect level. Modifying factors should be considered in the process of refining multi-study concentration-response profiles. ERA practitioners have the option of selecting studies with modifying factors that are more applicable to site conditions. More information on modifying factors is included in **Appendix D** and in the ERA Guidance (FCSAP 2012a).

3.1.3.3 Life stage

ERA practitioners can investigate whether use of the habitat at a contaminated site is limited to certain life stages, based on the conceptual exposure models provided in **Appendix A** or based on a site-specific field investigation. If the contaminated medium is only used by certain life stages, the database can be filtered accordingly. For example, if a pond is used for breeding only, toxicity data on adult amphibians may be excluded from the profiles. However, this level of detail may not be available for many federal sites, in which case, the risk to amphibians should be assessed comprehensively for all life stages.

It is important to consider this factor because different life stages tend to have varying levels of sensitivity to contaminants. Figure 9 shows an example of a multi-study concentration-response profile filtered by life stage[1]. In general, embryo and larval stages appear to be more susceptible to contaminants than the adult stage; however, this is based on the limited toxicological information on adult amphibians available in the literature. Even less toxicological information is available for the metamorphosis stage. This is a data gap and source of uncertainty in an amphibian ERA.

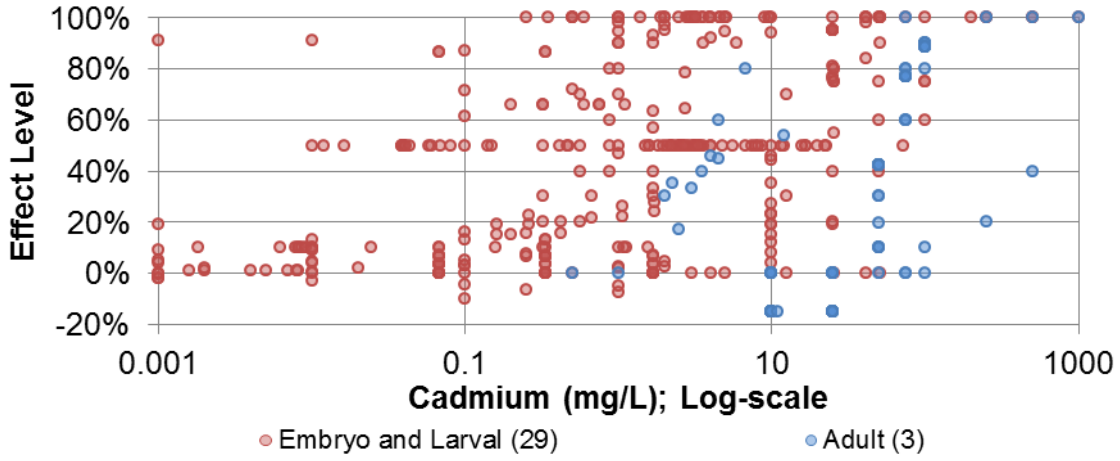

Figure 9: Multi-study concentration-response profile for cadmium (water) by life stage, with the number of studies shown in parentheses. Darker shaded symbols indicate several overlapping data points and negative effects levels occur when the treatment outperforms the control.

[1] Similar graphs for other contaminants are available in **Appendix E**.

3.1.3.4 Exposure time

Longer exposures are often more relevant to contaminated site scenarios since the duration of exposure at contaminated sites is typically much longer than the exposure periods used in laboratory testing. However, most toxicity test data for amphibians are from short-term or acute exposure experiments because chronic exposure tests are more expensive and difficult to conduct in a laboratory setting and standard protocols are limited. In such cases, ERA practitioners need to carefully consider the acute data in conjunction with any available chronic data.

Several published studies reported a direct relationship between exposure time and effect levels when all other parameters (e.g., species and life stage) are constant (Birge and Just 1973; Brodeur *et al.* 2009; Shuhaimi-Othman *et al.* 2012; Sobotka and Rahwan 1995). These studies generally observed effects at lower concentrations for longer exposure compared with shorter exposure. The impacts of exposure time on effects levels are not as evident in the concentration-response profiles when other parameters vary. Figure 10 shows the concentration-response profile for cadmium with the data categorized according to the different exposure times. This observation highlights the importance of considering all parameters that may impact effect levels to reduce biased results.

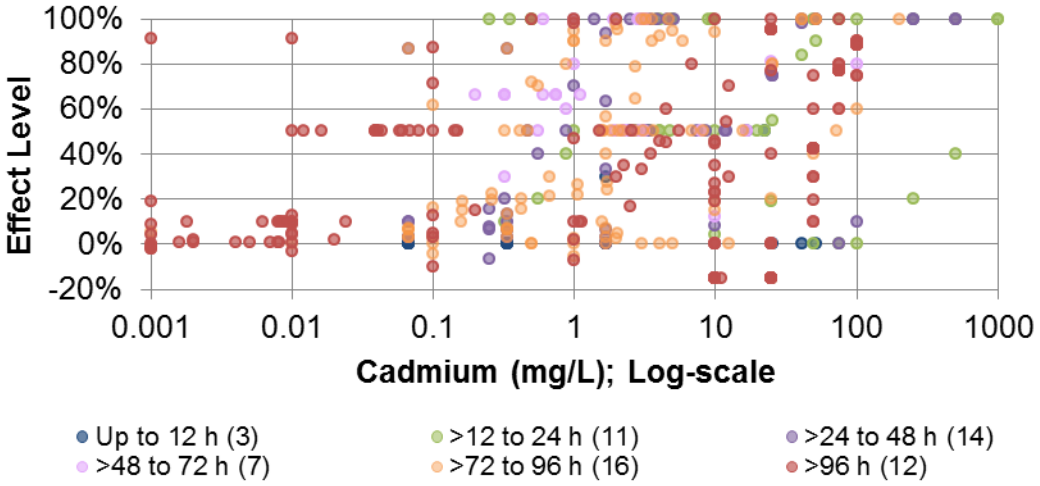

Figure 8 Multi-study concentration-response profile for cadmium (water), by exposure time, with the number of studies shown in parentheses. Darker shaded symbols indicate several overlapping data points and negative effects levels occur when the treatment outperforms the control.

3.1.3.5 Species Sensitivity

Sensitivity to environmental contaminants can vary considerably among species. For federal contaminated sites, it may be difficult to filter data by species native to Canada because most laboratory toxicity testing is based on the use of *Xenopus laevis* (African Clawed Frog), which is not native to North America. If toxicity data for species potentially

present at the site are not available, it is recommended that ERA practitioners use pooled toxicity data from a number of different species (FCSAP 2010b).

It is important for ERA practitioners to acknowledge the uncertainties associated with using common laboratory toxicity test species (e.g., *X. laevis*) to represent species relevant to the site. Figure 11 shows an example of multi-study concentration-response data comparing embryonic toxicity data for *X. laevis* to embryonic toxicity data for native Canadian species. Similar graphs for cadmium, lead, mercury, and zinc are provided in **Appendix G**. Native species for which data are available appear to be more sensitive than *X. laevis*. Birge *et al.* (2000) reported that *X. laevis* was generally less sensitive to contaminants than other species.

Birge *et al.* (2000) conducted a comprehensive analysis of species sensitivity by comparing the LC_{50} values for various amphibian species. The most sensitive species found in Canada that were included in the Birge study appear to be *Lithobates catesbeiana* and *Lithobates pipiens*.

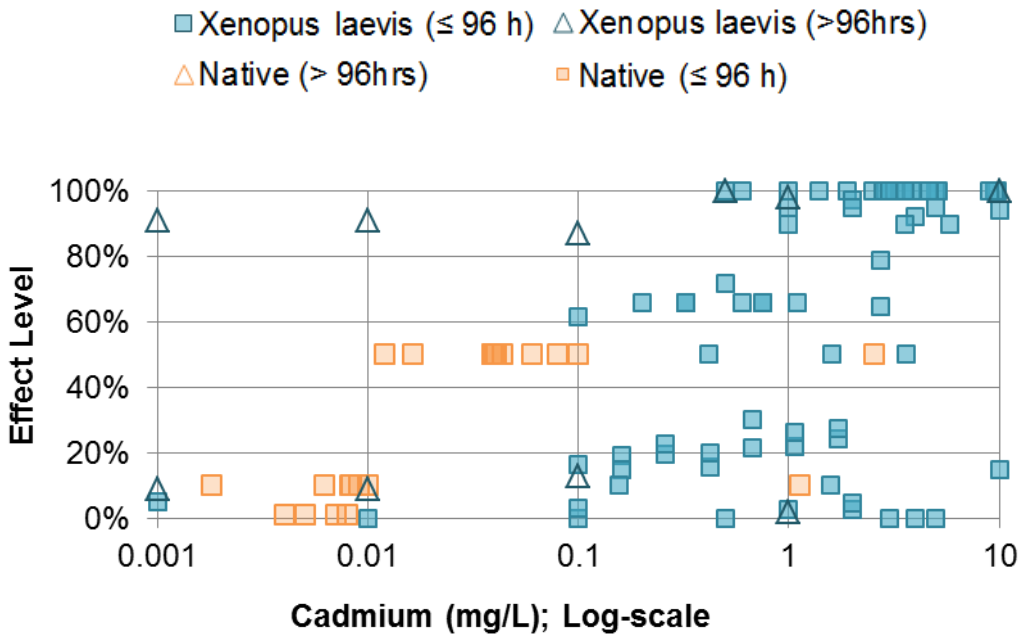

Figure 9 Multi-study concentration-response profile cadmium (water) for embryos, by species and exposure time. Darker shaded symbols indicate several overlapping data points and negative effects levels occur when the treatment outperforms the control.

3.1.4 Use of literature-based point estimates as a line of evidence in the ERA

Multi-study concentration-response profiles as presented in this module provide a comprehensive overview of literature-based toxicity information. Multi-study concentration-response profiles are the recommended methodology for comparing site-specific contaminant levels to literature-based toxicity data. More commonly, though, individual reports present point estimates (e.g. LC_{50}, $EC_{50,}$ NOAEL, LOAEL). Literature-based point estimates are not typically the best line of evidence for the ERA. Yet, point estimates are often used as a toxicity reference value (TRV) in the calculation of a hazard quotient (HQ = exposure estimate/toxicity estimate). HQs are in turn used to make inferences about the risk that a given contaminant poses to a given receptor. In general, an HQ < 1 is considered indicative of unlikely adverse effects. HQs based on point-estimate TRVs are a fairly simplistic way of considering risk and are only useful as a preliminary screening tool. The limitations of point estimates are discussed in detail in the comprehensive FCSAP ERA Guidance (2012a) and in Module 2 (2010b).

It is acknowledged that, despite the inherent limitations of point estimates, they continue to serve a purpose under FCSAP, albeit a limited one. Point estimates for amphibian effects from exposure to a variety of contaminants are available from various sources including those listed in Section 3.1.1. If single point estimates are to be used in amphibian risk assessments as a basis for inferences about risk, a detailed rationale should be included in the ERA, and the following should be carefully considered:

- Is there a range of appropriate point estimates (i.e., more than one)? Are appropriate species, life stages and exposure times considered?

- What is the effect size of the point estimate? An LC_{50} is not a good point estimate for establishing a TRV, unless the 50% mortality effect size is considered acceptable *a priori*.

- Was the threshold response level bounded in the study design used to derive the threshold? A threshold derived from a study that tested concentrations above and below the threshold (bounded) is more reliable than a threshold derived by statistical extrapolation beyond the range of concentrations tested (unbounded).

- Are safety or uncertainty factors used when a TRV is derived from the point estimate? If so, they should be used with great caution, as the available amphibian toxicity data likely does not provide a sound technical basis for the use of these factors.

- NOAEL- and LOAEL-based TRVs have limited use in ERA, because the type and magnitude of effects is typically not identified. Without additional information, NOAEL and LOAEL have a limited ability to express ecological significance or biological relevance (FCSAP 2012a). NOAEL and LOAEL values are also subject to bias based on the statistical treatment of the data. NOAEL and LOAEL should only be used if no other toxicological data are available, or if additional information (e.g., type and

magnitude of effect) is provided. If only NOAEL- and LOAEL-based TRVs are available, they should generally be supported by other lines of evidence in an ERA.

3.2 Indirect Biological Information

Indirect biological information refers to toxicity information from field studies (e.g., population study) that are reported in the literature. A comparison of site-specific exposure data to published field studies can serve as a separate line of evidence. FCSAP emphasizes the need to utilize different lines of evidence from the four general categories of effects assessment approaches.

When contamination-related effects are reported in field studies, the published information can be used to assess possible effects on amphibians at a different site. This is particularly valuable if the type of contamination, habitat, and receptors are comparable. For more information on using indirect biological information as a line of evidence, see the ERA Guidance (FCSAP 2012a).

Unfortunately, indirect biological information is not easily accessible. Ecological risk assessments using amphibian biological studies as a line of evidence are rarely published. One of the more comprehensive amphibian risk assessments that include biological studies is from the Housatonic River (Massachusetts) project, which is discussed in more detail in Section 3.4.2. For federal sites contaminated with polychlorinated biphenyls (PCBs) or other organic contaminants, ERA practitioners may find the results from the Housatonic River useful with respect to potential amphibian responses (USEPA 2003, 2004). For example, reduced species richness (number of species entering and leaving different pools) and increased malformations (based on procedures developed by the North American Reporting Center for Amphibian Malformations) were reported in vernal pools with PCB-impacted sediments (>24 mg/kg) compared to reference vernal pools (sediment PCB concentration = 0.72 mg/kg).

3.3 Site-Specific Toxicity Studies

3.3.1 Laboratory Toxicity Studies

Lines of evidence from the third effects assessment category, site-specific toxicity studies (toxicity tests and bioassays), involve direct assessment of whether amphibians are affected by contaminants in site media (e.g., water, sediment, and soil). Instead of using literature studies to make inferences about possible effects on amphibians, this approach consists of testing the site media under controlled laboratory conditions following established toxicity testing protocols.

Standard protocols (e.g., ASTM, OECD, and USEPA) for laboratory-based amphibian toxicity testing are available for water and sediment (Tables 7 and 8). When selecting toxicity tests for an amphibian ERA, the following should be considered:

- project requirements, scope and budget;
- test duration, keeping in mind that longer durations are typically more representative of on-site exposure scenarios;
- exposure type (e.g., lethal, sub-lethal);
- relevant species; and,
- test species availability.

The ERA Guidance (FCSAP 2010a) provides detailed guidance on the selection of appropriate toxicity tests and the interpretation of test results.

Standardized toxicity testing protocols are designed and verified for a particular species, but can be adapted to reflect site-specific parameters and locally relevant species where warranted in light of the complexity of the ERA (Marlatt 2015, pers. comm.). When adjusting standardized methods, alterations to exposure duration and other test conditions (e.g., temperature, photoperiod) may be required to accommodate the physiology of a different test species. For example, understanding the duration of exposure and the environmental conditions that the test species requires for metamorphosis is essential for obtaining reliable and accurate control animal data if the exposure period extends to or includes metamorphosis. In addition, the time required to reach each developmental stage and/or at which the exposure is initiated is likely different for a non-standard test species, and test methodology should be adapted accordingly. Laboratory should first be contacted to discuss the possibility of modifying testing protocols.

Environment and Climate Change Canada (ECCC) is currently developing a standardized toxicity test method for *L. pipiens*. ECCC's protocol will focus on the sub-lethal effects, such as on growth and developmental progress, of chronic exposure to contaminated water. Exposure of two distinct developmental stages will be highlighted: the early larval stage and the metamorphosis stage. Development of the method is currently focused on the supply of test organisms, the test duration, and animal husbandry. Questions about ECCC's test method should be addressed to the Ecotoxicity and Wildlife Health Division in the Science and Technology Branch of Environment and Climate Change Canada.

Commercial toxicity testing laboratories in Canada typically do not perform routine amphibian toxicity testing, but some of them will collaborate with federal custodians and/or their consultants on a site-specific basis using the methods listed in Tables 7 and 8. A general list of laboratories in Canada is available from the Canadian Association for Laboratory Accreditation (CALA) directory of laboratories (www.cala.ca). Some laboratories may provide amphibian testing upon request. It is recommended that ERA practitioners discuss site-specific testing needs with applicable laboratories prior to designing a risk assessment strategy that includes site-specific amphibian toxicity testing.

FCSAP recognizes that some jurisdictions (e.g., USEPA 2004) allow the use of fish toxicity data as a surrogate for aquatic-phase amphibians and bird toxicity data as a surrogate for terrestrial-phase amphibians. The current literature on the use of birds as surrogates for amphibians is limited. Reports in the literature on using fish as a surrogate for amphibians are inconclusive. Some studies support the use of fish as surrogate receptors for amphibians based on the LC_{50} values for several contaminants (Kerby *et al.* 2010; Weltje

et al. 2013). Other studies discourage the use of fish as surrogate. For example, Birge *et al.* (2000) compared LC_{10} and LC_{50} data for various amphibian species to LC_{10} and LC_{50} data for several fish species. Many of the amphibian species were more sensitive to metal exposure compared to the fish. There was no apparent relationship between LC_{50} data for fish and LC_{50} data for amphibians that would justify using fish toxicity data instead of amphibian toxicity data. Other researchers have likewise discouraged the use of fish as a surrogate (Fort and McLaughlin 2003; Johnson *et al.* 2016). Given the life history difference between amphibians and non-amphibian surrogate species, FCSAP does not recommend the use of fish or bird toxicity data as a surrogate for amphibian data unless amphibian toxicity data are not available. If toxicity data from fish or bird are used, FCSAP recommends including additional lines of evidence that assess risk to amphibians more directly, as well as a comprehensive uncertainty analysis pertaining to the use of the selected non-amphibian surrogate species data.

Water and sediment toxicity testing protocols focus on aquatic life stages of amphibians (embryo, larval). During the terrestrial life stages (juvenile and adult), most amphibians are also susceptible to exposure to soil contaminants. Toxicity testing protocols for soil exposures are not available and only a limited number of published studies on soil exposure toxicity testing are available to help address this data gap. Table 9 provides examples of recent studies that assessed the effects of contaminated soil on amphibians. The USEPA has developed a tool for calculating dietary exposure and the risk to terrestrial-phase amphibians and reptiles from pesticides (USEPA 2008); it may be used as a reference. Assessing the risk to amphibians from contaminated soil exposure remains a challenge. Where soil exposure is likely to be the risk driver, a site-specific testing program may need to be developed like those used in the publications listed in Table 10 or *in situ* toxicity testing should be done as discussed in Section 3.3.2. Although for most federal sites, such testing may be beyond the scope of the risk assessment, it should be considered when amphibians are likely to be the most sensitive receptor. Until the data gap related to amphibian soil exposure has been addressed, combining the scarce information available from a number of sub-optimal lines of evidence with a detailed characterization of the associated uncertainty may be the only way to assess risk to amphibians from soil exposure. Lines of evidence that can be used in this context include biological field testing, inferred toxicity information from aquatic exposure data or inferred toxicity information from surrogate species provided that the uncertainty is thoroughly characterized.

Table 7. Standard protocols for toxicity testing of amphibians exposed to contaminated water.

Method	Species	Life Stage and Exposure Time	Endpoint[1,2]	Reference
ASTM-E729-96 (chemical specific)	General guide for acute toxicity tests, not specific to amphibians	96 h - Exposed from the young larval stage	EC: Mortality, malformation	ASTM International 2014a
ASTM-E1192-97 (aqueous effluents)	General guide for acute toxicity tests, not specific to amphibians	96 h - Exposed from the young larval stage	EC: Mortality, malformation	ASTM International 2014b
ASTM-1439-12: FETAX	*Xenopus laevis*	96 h - Exposed from the embryo to larval stage	EC: Morality, malformation IC: Growth	ASTM International 2012
AMPHIEMB	*Rhinella arenarum*	96 h - Exposed from the embryo stage	EC: Mortality, malformation	Herkovits and Pérez-Coll 2003: Available from ASTM as AMPHITOX
AMPHIACUT	*Rhinella arenarum*	96 h - Exposed from end of embryo development	EC: Mortality, malformation	
AMPHISHORT	*Rhinella arenarum*	7 days - Exposed from end of embryo development	EC: Mortality, malformation	
AMPHICHRO	*Rhinella arenarum*	14 days - Exposed from end of embryo development	EC: Mortality, malformation	

Method	Species	Life Stage and Exposure Time	Endpoint[1,2]	Reference
EPA 890-2300 (Draft): see OECD – 241	*Xenopus laevis*	16 weeks - Exposed from embryo to 10 weeks after metamorphosis	EC: Mortality IC: Developmental stage, growth, thyroid histology	OECD 2009
OECD – 241: The Larval Amphibian Growth and Development Assay (LAGDA)				OECD 2015
EPA OPPTS 890-1100: see OECD-231 (The Amphibian Metamorphosis Assay)	*Xenopus laevis*	21 days - Exposed from the larval stage	EC: Mortality, malformation IC: Developmental stage, growth, thyroid histology	OECD 2009
Northern Leopard Frog Assay	*Lithobates pipiens*	Test protocol is in development		ECCC (to be published)

[1] Effect concentration (EC) refers to the percentage of tested individuals that experienced a certain effect when exposed to a certain concentration.
[2] Inhibitory concentration (IC) refers to the percentage of impairment that occurs as a result of the exposure.

Table 8. Standard protocols for toxicity testing of amphibians exposed to contaminated sediment.

Method	Species	Life Stage and Exposure Time	Endpoint[1,2]	Reference
ASTM-2591-07	*Lithobates pipiens*	10 days - Exposed from the larval stage	EC: Mortality, malformation IC: Growth	ASTM International 2013
EPA 850-1800	*Lithobates catesbeianus*	30 days - Exposed from the larval stage	EC: Mortality, malformation	USEPA 1996
NAVFAC Sediment Toxicity Tests	Various	10 days - Exposed from the larval stage	EC: Mortality, malformation IC: Growth	Bleiler *et al.* 2004

[1] Effect concentration (EC) refers to the percentage of tested individuals that experienced a certain effect when exposed to a certain concentration.
[2] Inhibitory concentration (IC) refers to the percentage of impairment that occurs as a result of the exposure.

Table 9 Literature studies that assessed the toxicity of contaminated soil to amphibians.

Study	Species	Exposure Period	Endpoint[1,2]	Reference
The effect of soil composition and hydration on the bioavailability and toxicity of cadmium to hibernating juvenile American toads (*Bufo americanus*)	*Anaxyrus americanus* (formerly *Bufo americanus*)	From the juvenile stage to the end of hibernation	EC: Survival IC: Development, mobility	James *et al.* 2004
Sensitivity and behaviour of the Iberian newt, *Triturus boscai*, under terrestrial exposure to ammonium nitrate	*Triturus boscai*	Initiated at the adult stage	EC: Mortality IC: Movement	Ortiz-Santaliestra *et al.* 2006
Toxicological responses of red-backed salamanders (*Plethodon cinereus)* to subchronic soil exposures of 2,4-dinitrotoluene	*Plethodon cinereus*	28 days - Initiated at the adult stage	EC: Mortality IC: Development	Johnson *et al.* 2007

[1] Effect concentration (EC) refers to the percentage of tested individuals that experienced a certain effect when exposed to a certain concentration.
[2] Inhibitory concentration (IC) refers to the percentage of impairment that occurs as a result of the exposure.

Table 10 Examples of amphibian *in situ* methods (enclosures and mesocosm).

Study	Species	Exposure Period	Endpoint[1,2]	Reference
In situ effects of pesticides on amphibians in the Sierra Nevada.	*Pseudacris regilla*	Exposed from early larval stage until metamorphosis	EC: Malformation, mortality IC: Development rate	Sparling *et al.* 2015
Effect of herbicide release on mortality, avoidance response, and growth of amphibian larvae in two forest wetlands.	*Lithobates clamitans*[3] and *Lithobates pipiens*[4]	Larval exposure for a total of 77 days after herbicide application. Assessment conducted continuously during exposure.	EC: Mortality, avoidance response, and growth	Wojtaszek *et al.* 2004, 2005
Establishing cause-effect relationships for chemical stressors in amphibians: Providing adequate data for the ERA.	*Lithobates sphenocephala*	Exposed from embryo/ larval stages through metamorphosis	EC: Malformation, mortality	Fort and McLaughlin 2003
Low levels of the herbicide atrazine alter sex ratios and reduce metamorphic success in *Rana pipiens* tadpoles raised in outdoor *mesocosms*.	*Lithobates pipiens*[4]	Exposed from early larval stage (Gosner stage 27) until metamorphosis climax (Gosner stage 42)	IC: Developmental rate	Langlois *et al.* 2010

Study	Species	Exposure Period	Endpoint[1,2]	Reference
Field exposure of frog embryos and tadpoles along a pollution gradient in the Fox River and Green Bay ecosystem in Wisconsin, USA.	*Lithobates clamitans*[3] and *Lithobates pipiens*[4]	Exposed from embryo through tadpole stage and early metamorphosis	EC: Malformation, mortality IC: Development, growth	Karasov *et al.* 2005
Development and survivorship of Northern Leopard Frogs (*Rana pipiens*) and Green Frogs (*Rana clamitans*) exposed to contaminants in the water and sediments of the St. Lawrence River near Cornwall, Ontario.	*Lithobates clamitans*[3] and *Lithobates pipiens*[4]	Exposed from early larval stage to metamorphosis	EC: Malformation, mortality IC: Development, growth	McDaniel *et al.* 2004
Deformities in Cane Toad (*Bufo marinus*) populations in Bermuda.	*Rhinella marina*	90 days: from embryo until the last organism in a given treatment completed metamorphosis	EC: Mortality, malformation (internal and external), metamorphic completion frequencies IC: Growth (weight), and sex ratio	Fort *et al.* 2006

1 Effect concentration (EC) refers to the percentage of tested individuals that experienced a certain effect when exposed to a certain concentration.

2 Inhibitory concentration (IC) refers to the percentage of impairment that occurs as a result of the exposure.

3 Formally known as *Rana pipiens*

4 Formally known as *Rana clamitans*

3.3.2 In situ Toxicity Studies

In situ toxicity studies which include enclosure and mesocosm set-ups at the actual site are ideal for determining the effects of contaminants because uncertainties (i.e., potential confounding effects) are reduced (FCSAP 2012a). However, they are more complex than laboratory toxicity studies, and their cost may put them beyond the scope of most amphibian ERAs. These studies are typically designed on a site-specific basis, and are relevant for large and complex sites where amphibians are key receptors of concern. Standard protocols for *in situ* testing of amphibians are not available, but Table 10 provides examples of published studies that have conducted amphibian *in situ* toxicity tests.

When *in situ* toxicity testing is beyond the scope of the ERA, conducting toxicity testing in the laboratory with media collected from the site should be considered. If ERA practitioners are interested in designing an *in situ* study, a discussion with any of the laboratories mentioned in the previous section would be beneficial during the study design phase.

An example of *in situ* exposure: collecting leopard frog eggs from reference sites, and from water bodies located on the contaminated site and use them in *in situ* toxicity tests. Precautions need to be taken to ensure the eggs are healthy and disease free in order to avoid infecting the local population (e.g., use laboratory control treatment to ensure quality). Sediment, water and organism tissue chemistry data would be collected prior to commencement of the test and at regular intervals during testing. Embryos would be placed in mesh enclosures, with an equal number of organisms per enclosure to allow for replication. To ensure organism quality, laboratory control treatment should be carried out simultaneously. Test endpoints may include hatching success, followed by larval mortality, growth, and malformations. *In situ* tests should also consider effects caused by disease/parasites. Test duration could be limited to time of hatching, with percent hatch as an endpoint, or test duration could include the larval stages and extend to metamorphosis, with malformations, growth and percent metamorphosis as test endpoints.

3.4 Site-Specific Biological Studies

Site-specific biological studies, or field studies, are considered a separate category of effect assessment methods. They are intended to provide biological data (e.g., presence/absence surveys) rather than toxicological data. These lines of evidence can add valuable information within a weight-of-evidence framework. The ERA Guidance (FCSAP 2012a) provides a general overview of how to conduct site-specific biological studies. These studies are most effective when paired with relevant literature toxicity data and/or site-specific toxicity studies.

Amphibian biological field studies can include community (e.g., species diversity, and richness) and individual endpoints (e.g., amphibian body size and reproductive indicators). Biological studies usually involve a control-impact design (comparison with reference site) or a gradient design. To design biological studies, two different components are needed: 1) selection of relevant reference site(s) with which to compare impacted site(s), and 2) determination of monitoring methods. The selection of relevant reference sites is discussed in Module 5 (FCSAP to be published). General methods for monitoring amphibians are discussed below (Section 3.4.1). An example of a contaminated site where biological/field studies were used to assess risk to amphibians is discussed in Section 3.4.2.

3.4.1 General Methods – Amphibian Field Studies

The general protocols for monitoring amphibians in the field are listed in Table 11. These resources should be used in conjunction with guidance for selecting proper sites for either control/impact or gradient-based studies (FCSAP 2012a; FCSAP Module 5 to be published). As well, it is critical to have an amphibian biologist as part of the ERA team. Field identification of egg masses and larval amphibians is very difficult, and the time of year for assessment of these life stages can be very limited and specific. Field studies may include:

- Egg mass surveys: for identification of species presence and habitat preferences for breeding.
- Larval surveys: dip nets for pond-breeding amphibians can be used to determine species presence and general species assemblages.
- Adult surveys: trapping or calling surveys can be used to ascertain species presence and breeding and foraging habitat preferences.

Table 11 General methods for amphibian field studies

Title	Habitat Types	Notes	Reference	Link
A Standardized Protocol for Surveying Aquatic Amphibians	Aquatic	Various endpoints tested (e.g., reproduction, presence-absence). Developed for California.	Fellers and Freel 1995	http://www.werc.usgs.gov/ProductDetails.aspx?ID=1032
Anuran Inventory Method in Quebec	Aquatic	Standardized method for auditory survey. Also provide references for other survey technics.	Bouthillier *et al.* 2015	ftp://ftp.mrn.gouv.qc.ca/Public/Reg06/Monteregie/Protocoles_standardises/MFFP_Mars_2015_Protocole_inventaire_anoures.pdf
Survey Protocol for the Northern Leopard Frog	Aquatic	Tailored to Northern Leopard Frog. The protocol could be adapted for other species.	Kendell 2002	https://www.ab-conservation.com/downloads/report_series/nlfr_survey_protocol_2002.pdf
Various	Aquatic	Methods and forms for measuring amphibian biodiversity	BCMOE 1998-2009	https://www2.gov.bc.ca/gov/content/environment/natural-resource-stewardship/laws-policies-standards-guidance/inventory-standards/aquatic-ecosystems

Title	Habitat Types	Notes	Reference	Link
Methods for Evaluating Wetland Condition: #12: Using Amphibians in Bioassessments of Wetlands	Aquatic (wetland)	The USEPA has incorporated amphibians into the monitoring approach for wetland biodiversity.	Sparling *et al.* 2001	https://pubs.er.usgs.gov/publication/5200254
Wetland Amphibian Monitoring Protocol	Aquatic (Wetland)	This protocol focuses on monitoring amphibians in Toronto marsh habitat.	Toronto and Region Conservation Authority (TRCA) 2011	http://trca.on.ca/dotAsset/185467.pdf
Measuring and Monitoring Biological Diversity: Standard methods for amphibians	Aquatic and Terrestrial	Various endpoints tested (e.g., species diversity and richness).	Heyer *et al.* 1994	N/A
Species Detection Survey Protocols: Amphibian Auditory Survey	Aquatic and Terrestrial	Call descriptions only available for frogs and toads in Saskatchewan.	Saskatchewan Fish and Wildlife Branch 2014a	www.environment.gov.sk.ca/adx/aspx/adxGetMedia.aspx?DocID=8def8861-4e48-45e6-b397-7e4ec860bf19

Title	Habitat Types	Notes	Reference	Link
Species Detection Survey Protocols: Amphibian Visual Surveys	Aquatic and Terrestrial	For frogs and toads that cannot be detected through an auditory survey, visual detection can be used.	Saskatchewan Fish and Wildlife Branch 2014b	www.environment.gov.sk.ca/adx/aspx/adxGetMedia.aspx?DocID=df579dc1-5ed4-43fa-ba4d-7d4ef60b5fc4
Multiple Species Inventory and Monitoring Technical Guide	Terrestrial	Chapter 8: Amphibian and Reptile monitoring. The rest of the document contains monitoring methods for other organisms.	Manley *et al.* 2006	https://www.fs.fed.us/psw/publications/wo/wo_gtr073.pdf
Sampling Methods for Terrestrial Amphibians and Reptiles	Terrestrial	Various methods described (e.g., experimental design, field methods). Method based on sampling work on herpetofauna found in the forests of Oregon and Washington.	Corn and Bury 1990	https://www.hathitrust.org/
Support Manuals	Terrestrial	Methods and forms for measuring amphibian biodiversity	BCMOE 1998-2009	https://www2.gov.bc.ca/gov/content/environment/natural-resource-stewardship/laws-policies-standards-guidance/inventory-standards/terrestrial-ecosystems-biodiversity

3.4.2 Case Study: Housatonic River

The Housatonic River, located in Massachusetts, is a USEPA Superfund site contaminated with PCBs. The Housatonic River site provided breeding habitat for several amphibian species. As part of the ERA, the effects of PCBs on amphibian reproduction were studied using both site-specific biological and toxicity studies. A gradient study design was used to compare both the PCB concentrations and biological endpoints. In the reports (USEPA 2003, 2004), the assessment endpoints (e.g., completion of breeding, species richness and diversity of breeding populations) and methods are recorded. Table 12 summarizes the biological field study for the Housatonic ERA.

The Housatonic project represents perhaps the largest amphibian ERA ever conducted in North America. The typical FCSAP amphibian ERA is not expected to have the scope and magnitude of the Housatonic assessment, but this case study provides valuable information on amphibian field study design and relevant biological endpoints, as well as toxicology endpoints for laboratory studies.

Table 12. The biological studies used in the Housatonic River ERA to determine amphibian reproductive success in vernal pools (USEPA 2003).

Study	Methods	Endpoints
Amphibian presence	Count species and sex	Species richness, diversity, and biomass Sex ratio of breeding population
	Measure body metrics by species and sex	Body size by species and sex
	Count deformities, erosion, lesions, and tumors (DELTs)	Rate of DELTS
	Marking and recapturing organisms	Length of time spent in the pool
Courtship and breeding	Audio survey of chorusing	Breeding behaviours
	Record breeding activities	Breeding activities
	Presence of egg mass and spermatophores	Completion of breeding
Embryos and larvae	Record egg hatching in an enclosed area	Hatching success
	Measure length of larvae in an enclosed area	Growth and development Early survival rates
	Measure larvae in funnel traps	Growth and development Rates of DELTs
Metamorphosis and emergence	Count metamorphs leaving the area by species	Metamorphs per breeding female

4 Conclusion

Ecological risk assessments conducted on federal contaminated sites are intended to assess the risk to all relevant receptor groups, including amphibians (FCSAP 2012a). Amphibians have a complex life history, often involving both an aquatic and terrestrial component, thereby complicating the evaluation of contaminant exposure pathways. The toxicity literature for amphibians is sparse, particularly relative to other aquatic organisms (e.g., fish and aquatic invertebrates). There are fewer standard toxicity testing protocols for amphibians relative to fish and invertebrates, and not all bioassay laboratories have sufficient expertise to conduct toxicity testing on amphibians. The number of available TRVs are thus smaller. In the past, these limitations have been presented as a rationale for excluding amphibians from ERAs even when amphibians were present at and relevant to contaminated sites.

FCSAP developed this module to facilitate the inclusion of amphibians in ecological risk assessments, taking into account the existing gaps in published toxicity data and testing methodologies. The tools and resources presented in this module are intended to guide efforts to include amphibians as receptors of concern. Limitations and knowledge gaps remain a challenge, but with the increasing recognition of amphibians as a relevant receptor group in ecological risk assessment, the body of work related to amphibian ERA is expected to continually grow and the current limitations and uncertainties are expected to be reduced. Future work by academia, government, private laboratories and scientific organizations such as the Society of Environmental Toxicology and Chemistry (SETAC) Ecotoxicology of Amphibian and Reptiles Advisory Group (SETAC 2015) will provide valuable resources that can be used in conjunction with this module.

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Appendix A. Exposure Pathways for Amphibians

If amphibians are included in the ERA as a receptor of concern, the potential exposure media need to be determined. This section provides a brief description of each exposure medium (Section A1) followed by conceptual diagrams outlining exposure pathways for each of the nine different amphibian families (Section A2). In addition to these pathways, maternal transfer should be considered for contaminants that are bioaccumulative. Hopkins *et al.* (2006) reported that *Gastrophryne carolinensis* females can transfer up to 70% of contaminants to embryos.

A1. Exposure Media Relevant to Amphibians

Surface Water

Most amphibians in Canada require water for survival and reproduction. Early life stages of most amphibians (except for some species of lungless salamanders) live completely in permanent or temporary water bodies. Although most amphibian species breed in permanent water bodies, temporary water bodies (e.g., such as ditches and ephemeral ponds/streams) are breeding habitat for some species. Species that can breed in these environments tend to develop much faster. Direct contact with surface water is one of the most important exposure pathways for early life stages because the skin of amphibians can absorb waterborne contaminants (Birge *et al.* 2000; Duellman and Trueb 1994; Henry 2000; Hillman *et al.* 2009). Direct contact includes respiration (gas exchange through the skin or gills), which is common for amphibians. When amphibians complete metamorphosis, they reside mostly in the terrestrial environments. Adult amphibians rarely consume water, except under extremely dry conditions (Henry 2000).

Sediment and Sediment Porewater

Sediment and sediment porewater are major exposure pathways for early amphibian life stages as well as for mature life stages. Some amphibian species deposit their embryos directly in sediment. Once hatched, the larvae can forage in sediment and are thus exposed to contaminants through direct contact and incidental ingestion. The same exposure pathways apply to some adults, such as species belonging to the family *Ascaphidae* and the *Ranidae Lithobates*, which hibernate in sediments. Whether sediments provide adequate habitat and subsequently contaminant exposure pathways depends on the composition and properties of the sediment and the chemical and physical characteristics of the contaminant. It is recommended that sediment sampling in potentially contaminated amphibian habitats also include measurement of total organic carbon, grain size, acid volatile sulfides, and simultaneously extracted metals. These parameters can influence the bioavailability of contaminants.

Soil

Similar to sediments, the importance of soil as an exposure medium depends on its composition and properties (James *et al.* 2004). Soil can be an important exposure medium for adults because some amphibians bury themselves in soil to stay moist during dry seasons or hibernate in the soil during winter. Amphibians also absorb much of the water in the soil as a way to remain hydrated in the terrestrial environment; thus dermal uptake of dissolved contaminants from soil pore water is a significant exposure pathway (Birge *et al.* 2000). Knowledge gaps remain regarding amphibian exposure to contaminants in soil, primarily due to a lack of published toxicity data. The USEPA has developed a tool for calculating dietary exposure and risk to terrestrial-phase amphibians and reptiles from pesticides (USEPA 2008).

Air

During the embryo and larval life stages, gas exchange in the aquatic environment occurs predominately through skin and gills (Henry 2000). At the end of the larval stage, the gills are resorbed and the lungs are developed. Adult amphibians use lungs for gas exchange in the terrestrial environment in conjunction with cutaneous respiration, except for species belonging to the family *Plethodontidae*, which retain their gills (Duellman and Trueb 1994; Henry 2000; Linder *et al.* 2010). Gas exchange through the skin is covered under “direct contact” while “respiration” refers to gas exchange through the lungs.

Food

Embryos are dependent on the yolk sac as a food source. It provides all the nutrients needed for development until they hatch (Henry 2000); it may also contain contaminants from maternal transfer. Most frog and toad larvae are herbivorous but a few species become omnivorous. Salamander larvae are generally carnivorous (Cook 1984; Duellman and Trueb 1994). The plains spadefoot and the western tiger salamander have had occurrences of cannibalistic larvae, which can happen in high density conditions in the Northern Great Plains, often linked to drying of ponds. This could have an impact in situations where bioaccumulative substances are present. Most amphibian adults are carnivorous, feeding on invertebrates such as worms and beetles, and occasionally on small mammals, such as mice and birds (Cook 1984). Dietary information on amphibians is extremely limited. Module 3 (FCSAP 2012b) provides dietary information on the Wood Frog (*Lithobates sylvatic*). Information sources on that matter are also available in Table 2 of the present document.

A2. Exposure Pathways and Media Relevant to Amphibian Families

For each family, general contaminant exposure pathways are shown in the conceptual diagrams below (Table A1 to Table A9). These conceptual diagrams are based on information from three main resources: The Canadian Herpetological Society (CHS 2012), Cook (1984), and Fisher *et al.* (2007).

The conceptual diagrams serve two main purposes: 1) Determine what exposure pathways are applicable for amphibian species at the site; 2) Prioritize the type of assessment to include when resources are limited. For example, if surface water is a major pathway for larval exposure but a minor pathway for embryo and adult exposure, ERA practitioners may assess the risk to amphibian larvae exposed to contaminated water on a priority basis relative to exposure of embryos or adults.

For the purpose of this guidance module, major pathways involve substantial (e.g., continuous) exposure to contaminants and minor pathways involve limited exposure (e.g., infrequent). For example, exposure to contaminants present in water through direct contact is considered a major pathway for the aquatic embryonic and larval life stages, but a minor pathway for terrestrial adult life stages.

A2.1. *Ascaphidae* (tailed frogs)

The family *Ascaphidaeis* represented in Canada by two species (CHS 2012): *Ascaphus montanus* (Rocky Mountain Tailed Frog) and *Ascaphus truei* (Coastal Tailed Frog). The female lays eggs under rocks in high-gradient, permanent mountain streams in forested areas (Fisher *et al.* 2007). The larvae remain in the stream for several years until they reach sexual maturity. The larvae are herbivorous; they feed on algae and periphyton that they scrape from rocks. Adults are active at night, and remain under rocks in the stream during the cold season. Adults of *A. montanus* forage for invertebrates close to streams and adults and a of *A. truei* feed on invertebrates in upland areas (Fisher *et al.* 2007).

Table A1. **Exposure media and pathways for members of the family *Ascaphidae* (tailed frogs).**

Exposure Media	Exposure Pathways	Life Stage		
		Embryo	Larva	Adult
Surface Water	Direct contact[1]	●	●	○
	Respiration[2]	—	●	○
Sediment and Sediment Porewater	Direct contact[1]	●	●	●
	Respiration[2]	—	●	●
	Incidental ingestion	—	●	○
Soil	Direct contact	—	—	○
	Incidental ingestion	—	—	○
Air	Direct contact[1]	—	○	○
	Respiration[2]	—	○	●
Aquatic Food Items	Algae / aquatic plants	—	●	—
	Small fish, amphibians and invertebrates	—	○	●
Terrestrial Food Items	Invertebrates	—	—	●
	Small mammals	—	—	—

● = Major Pathway ○ = Minor Pathway — = Not a pathway

[1] Direct contact includes cutaneous respiration.
[2] Respiration refers to respiration through the lungs or gills.

A2.2. *Scaphiopodidae* (spadefoot toads)

The family *Scaphiopodidae* is represented in Canada by two species (CHS 2012): *Spea bombifrons* (Plains Spadefoot) and *Spea intermontana* (Great Basin Spadefoot). *Scaphiopodidae* are mostly terrestrial but breed in permanent or temporary ponds. The female lays eggs on vegetation or on the bottom of the pool (Fisher *et al.* 2007). The larvae remain in their natal pond until they transform into adults, which takes about three to six months (Fisher *et al.* 2007). The embryos and larvae are mostly herbivorous, feeding on algae and aquatic plants. Adults of both *S. bombifrons* and *S. intermontana* live in grasslands or dry valleys in areas with loose soil. They spend most of the year below ground (Fisher *et al.* 2007). Adults are mostly active at night and they feed on a variety of terrestrial invertebrates, such as insects (Cook 1984).

Table A2. **Exposure media and pathways for the family *Scaphiopodidae* (spadefoot toads).**

Exposure Media	Exposure Pathways	Life Stage		
		Embryo	Larva	Adult
Surface Water	Direct contact[1]	●	●	—
	Respiration[2]	—	●	—
Sediment and Sediment Porewater	Direct contact[1]	○	●	—
	Respiration[2]	—	●	—
	Incidental ingestion	—	●	—
Soil	Direct contact	—	—	●
	Incidental ingestion	—	—	○
Air	Direct contact[1]	—	○	●
	Respiration[2]	—	○	●
Aquatic Food Items	Algae / aquatic plants	—	●	—
	Small fish, amphibians and invertebrates	—	—	—
Terrestrial Food Items	Invertebrates	—	—	●
	Small mammals	—	—	—

● = Major Pathway ○ = Minor Pathway — Not a pathway

[1] Direct contact includes cutaneous respiration.
[2] Respiration refers to respiration through the lungs or gills.

A2.3. *Bufonidae* (true toads)

The family *Bufonidae* is represented in Canada by five species (CHS 2012): *Anaxyrus americanus* (Eastern American Toad), *A. boreas (*Western Toad), *A. cognatus* (Great Plains Toad), *A. fowleri (*Fowler's Toad), and *A. hemiophrys* (Canadian Toad). These species are almost completely terrestrial, except during spring when they breed in temporary pools of standing water that remain after heavy rains (Cook 1984). True toads tend to submerge their eggs in vegetation under water (CHS 2012; Fisher *et al.* 2007). The larvae have adapted to develop relatively quickly since early life stages depend on the moisture from a temporary pool (CHS 2012; Cook 1984; Fisher *et al.* 2007). Embryos and larvae are mostly herbivorous, feeding on plants. Adults tend to remain underground, except when feeding (CHS 2012; Fisher *et al.* 2007). They are voracious feeders of terrestrial invertebrates and small animals (e.g., birds) (Cook 1984). During colder seasons, they hibernate underground (Cook 1984).

Table A3. **Exposure media and pathways for the family *Bufonidae* (true toads).**

Exposure Media	Exposure Pathways	Life Stage		
		Embryo	**Larva**	**Adult**
Surface Water	Direct contact[1]	●	●	—
	Respiration[2]	—	●	—
Sediment and Sediment Porewater	Direct contact[1]	○	●	—
	Respiration[2]	—	●	—
	Incidental ingestion	—	●	—
Soil	Direct contact	—	—	●
	Incidental ingestion	—	—	○
Air	Direct contact[1]	—	○	●
	Respiration[2]	—	○	●
Aquatic Food Items	Algae / aquatic plants	—	●	—
	Small fish, amphibians and invertebrates	—	—	—
Terrestrial Food Items	Invertebrates	—	—	●
	Small Mammals	—	—	●

● = Major Pathway ○ = Minor Pathway — = Not a pathway

[1] Direct contact includes cutaneous respiration.
[2] Respiration refers to respiration through the lungs or gills.

A2.4. *Hylidae* (treefrogs)

The family *Hylidae* is represented in Canada by seven species (CHS 2012): *Acris blanchardi* (Blanchard's Cricket Frog), *Hyla chrysoscelis* (Cope's Gray Treefrog), *H. versicolor* (Gray Treefrog), *Pseudacris crucifer* (Spring Peeper), *P. maculata* (Boreal Chorus Frog), *P. regilla* (Pacific Treefrog), and *P. triseriata* (Western Chorus Frog). These species are mostly terrestrial but lay eggs in permanent or ephemeral water bodies near trees and in riparian or wetland shrub habitat (CHS 2012). Clusters of embryos are attached to submerged vegetation (Fisher *et al.* 2007). The larvae remain in natal pools until they metamorphose, which takes a few weeks or months. The embryos and larvae are herbivorous, feeding on algae and aquatic plants. The adults are mostly tree-dwelling although some genera also live in grasslands (*Pseudacris*) or near water bodies used for breeding (*Acris;* Cook 1984). The adults feed on invertebrates found in shrubs and trees (Fisher *et al.* 2007).

Table A4. **Exposure media and pathways for the family Hylidae (treefrogs).**

Exposure Media	Exposure Pathways	Life Stage		
		Embryo	Larva	Adult
Surface Water	Direct contact[1]	●	●	—
	Respiration[2]	—	●	—
Sediment and Sediment Porewater	Direct contact[1]	○	●	—
	Respiration[2]	—	●	—
	Incidental ingestion	—	●	—
Soil	Direct contact	—	—	●
	Incidental ingestion	—	—	○
Air	Direct contact[1]	—	○	●
	Respiration[2]	—	○	●
Aquatic Food Items	Algae / aquatic plants	—	●	—
	Small fish, amphibians and invertebrates	—	—	—
Terrestrial Food Items	Invertebrates	—	—	●
	Small mammals	—	—	—

● = Major Pathway ○ = Minor Pathway — = Not a pathway

[1] Direct contact includes cutaneous respiration.
[2] Respiration refers to respiration through the lungs or gills.

A2.5. *Ranidae* (true frogs)

The family *Ranidae* is represented in Canada by ten species (CHS 2012):

- *Lithobates catesbeianus* (American Bullfrog),
- *L. clamitans melanota* (Green Frog),
- *L. palustris* (Pickerel Frog),
- *L. pipiens (*Northern Leopard Frog),
- *L. septentrionalis* (Mink Frog),
- *L. sylvaticus (*Wood Frog),
- *Rana aurora (*Northern Red-legged Frog),
- *R. pretiosa (*Oregon Spotted Frog), and
- *R. luteiventris* (Columbia Spotted Frog).

Species classified as true frogs tend to follow the typical frog life history. Early breeders (e.g., *R. aurora*) attach their eggs to aquatic/submerged vegetation, while late breeders (e.g., *L. catesbeianus*) lay their eggs on the surface of permanent bodies of water (Cook 1984; Fisher *et al.* 2007). Larvae tend to stay in the breeding pool until they metamorphose. A few species may take more than a year to metamorphose (e.g., bullfrogs), but many reach metamorphosis within one growing season. Larvae are herbivorous, feeding on aquatic plants and algae (CHS 2012; Fisher *et al.* 2007). Adults tend to live in well-vegetated areas (Cook 1984; Fisher *et al.* 2007); some live in forested areas (e.g., *R. aurora*) while others live along the riparian margins of breeding streams (e.g., *L. c. melanota*). Most adults are carnivorous (CHS 2012; Fisher *et al.* 2007), feeding on a variety of aquatic and terrestrial invertebrates as well as fish and other amphibians. Larger *Ranidae* (e.g., American Bullfrog) have been reported to feed on small mammals such as mice (Cook 1984).

Table A5. **Exposure media and pathways for the family *Ranidae* (truefrogs).**

Exposure Media	Exposure Pathways	Life Stage		
		Embryo	**Larva**	**Adult**
Surface Water	Direct contact[1]	●	●	○
	Respiration[2]	—	●	○
Sediment and Sediment Porewater	Direct contact[1]	○	●	○
	Respiration[2]	—	●	○
	Incidental ingestion	—	●	○
Soil	Direct contact	—	—	●
	Incidental ingestion	—	—	○
Air	Direct contact[1]	—	○	●
	Respiration[2]	—	○	●
Aquatic Food Items	Algae / Aquatic Plants	—	●	—
	Small fish, amphibians and invertebrates	—	—	●
Terrestrial Food Items	Invertebrates	—	—	●
	Small mammals	—	—	●

● = Major Pathway ○ = Minor Pathway — = Not a pathway

[1] Direct contact includes cutaneous respiration.
[2] Respiration refers to respiration through the lungs or gills.

A2.6. *Proteidae* (mudpuppies)

The family *Proteidae* is represented in Canada by *Necturus maculosus* (CHS 2012*)*, a salamander which is fully aquatic. This species requires permanent water that does not freeze to the bottom (Fisher *et al.* 2007). Females lay eggs under submerged rocks and guard the eggs until they hatch (CHS 2012; Cook 1984; Fisher *et al.* 2007). Larvae are omnivorous, feeding on a combination of algae, plants and small insects. Adults feed on a variety of aquatic invertebrates, including crayfish, insects, fish, and snails. During the day, adults are rarely seen because they usually hide under rocks or in muddy, weed-choked water. *Necturus maculosus* adults have few natural competitors and can live up to 30 years (CHS 2012).

Table A6. **Exposure media and pathways for the family *Proteidae* (Mudpuppy).**

Exposure Media	Exposure Pathways	Life Stage		
		Embryo	**Larva**	**Adult**
Surface Water	Direct contact[1]	●	●	●
	Respiration[2]	—	●	●
Sediment and Sediment Porewater	Direct contact[1]	●	●	●
	Respiration[2]	—	●	○
	Incidental ingestion	—	●	○
Soil	Direct contact	—	—	—
	Incidental ingestion	—	—	—
Air	Direct contact[1]	—	—	—
	Respiration[2]	—	—	—
Aquatic Food Items	Algae / aquatic plants	—	●	—
	Small fish, amphibians and invertebrates	—	○	●
Terrestrial Food Items	Invertebrates	—	—	—
	Small mammals	—	—	—

● = Major Pathway ○ = Minor Pathway — = Not a pathway

[1] Direct contact includes cutaneous respiration.
[2] Respiration refers to respiration through the lungs (for terrestrial) or gills (aquatic).

A2.7. *Ambystomatidae* (mole salamanders)

The mole salamanders are represented in Canada by twelve species/sub-species (CHS 2012):

- *Ambystoma gracile* (Northwestern Salamander);
- *A. jeffersonianum* (Jefferson Salamander);
- *A. laterale* (Blue-spotted Salamander);
- *A. macrodactylum columbianum* (Eastern Long-toed Salamander);
- *A. macrodactylum krausei* (Northern Long-toed Salamander);
- *A. maculatum* (Yellow-Spotted Salamander);
- *A. mavortium diabolic* (Gray Tiger Salamander);
- *A. mavortium melanostictum* (Blotched Western Tiger Salamander);
- *A. texanum* (Small-mouthed Salamander);
- *A. tigrinum* (Eastern Tiger Salamander);
- *A. macrodactylum (Western-long toed salamander);* and
- *Dicamptodon tenebrosus* (Coastal Giant Salamander).

This family is largely terrestrial but requires water for reproduction (Cook 1984). Females lay eggs on vegetation or logs at the bottom of shallow ephemeral or permanent water bodies that are often near permanent watercourses in forested areas (CHS 2012; Fisher *et al.* 2007). Once hatched, the larvae will remain in the pond and feed on invertebrates that are small enough for them to consume and also smaller larvae of other amphibian species (Cook 1984; Fisher *et al.* 2007). Adults spend most of their time underground in forested areas and feed on a variety of terrestrial invertebrates and small mammals (e.g., mice and shrews). Some *A. gracile* and *D. tenebrosus* individuals are neotenic and remain in aquatic habitat (Fisher *et al.* 2007). Neotenic adults feed only on aquatic biota.

Table A7. **Exposure media and pathways for the family *Ambystomatidae* (mole salamanders).**

Exposure Media	Exposure Pathways	Life Stage		
		Embryo	Larva	Adult
Surface Water	Direct contact[1]	●	●	▬ /●[3]
	Respiration [2]	▬	●	▬ /●[3]
Sediment and Sediment Porewater	Direct contact[2]	○	●	▬ /●[3]
	Respiration[3]	▬	●	▬
	Incidental ingestion	▬	○	▬ /●[3]
Soil	Direct contact	▬	▬	●
	Incidental ingestion	▬	▬	○
Air	Direct contact[1]	▬	▬	●
	Respiration[2,3]	▬	○	●
Aquatic Food Items	Algae / aquatic plants	▬	○	▬
	Small fish, amphibians and invertebrates	▬	●	▬
Terrestrial Food Items	Invertebrates	▬	▬	●
	Small mammals	▬	▬	●

● = Major Pathway ○ = Minor Pathway ▬ = Not a pathway

1 Direct contact includes cutaneous respiration.

2 Respiration refers to respiration through the lungs or gills.

3 Neotonic adults remain in water due to the retention of their gills and therefore lungs are not a relevant exposure pathway for these species.

A2.8. *Salamandridae* (newts)

The family *Salamandridae* is represented in Canada by two species (CHS 2012): *Notophthalmus viridescens* (Eastern Newt) and *Taricha granulosa* (Rough-skinned Newt). Newts lay eggs on vegetation in slow-moving streams in forested areas (Fisher *et al.* 2007). The larvae remain in the water for a couple of months. Larvae are herbivorous, feeding mainly on algae and aquatic plants. Once larval development is complete, the newts transform into efts. The eft stage, which can last up to five years, is the only stage

during which newts are terrestrial, living under forest debris and feeding on terrestrial invertebrates. After the eft stage, the newts return to the aquatic environment and change into adults. Adults feed on a variety of aquatic invertebrates such as insects and mollusks. Some adults hibernate on land while others hibernate in the water, depending on the species' preferences.

Table A8. **Exposure media and pathways for the family *Salamandridae* (newts).**

Exposure Media	Exposure Pathways	Life Stage			
		Embryo	**Larva**	**Eft**	**Adult**
Surface Water	Direct contact[1]	●	●	—	●
	Respiration[2]	—	●	—	—
Sediment and Sediment Porewater	Direct contact[1]	○	●	—	●
	Respiration[2]	—	●	—	—
	Incidental ingestion	—	○	—	○
Soil	Direct contact	—	—	●	○
	Incidental ingestion	—	—	○	○
Air	Direct contact[1]	—	—	●	●
	Respiration[2]	—	○	●	○
Aquatic Food Items	Algae / aquatic plants	—	●	—	—
	Small fish, amphibians and invertebrates	—	—	—	●
Terrestrial Food Items	Invertebrates	—	—	●	—
	Small mammals	—	—	—	—

● = Major Pathway ○ = Minor Pathway — = Not a pathway

[1] Direct contact includes cutaneous respiration.
[2] Respiration refers to respiration through the lungs or gills.

A2.9. *Plethodontidae* (lungless salamanders)

The family *Plethodontidae* is represented in Canada by ten species (CHS 2012):

- *Aneides vagrans* (Wandering Salamander);
- *Desmognathus fuscus* (Northern Dusky Salamander);
- *D. ochrophaeus* (Allegheny Mountain Dusky Salamander);
- *Ensatina eschscholtzii oregonensis* (Oregon Ensatina);
- *Eurycea bislineata* (Northern Two-lined Salamander);
- *Gyrinophilus porphyriticus* (Spring Salamander);
- *Hemidactylium scutatum* (Four-toed Salamander);
- *Plethodon cinereus* (Eastern Red-backed Salamander);
- *P. idahoensis* (Coeur d'Alene Salamander); and
- *P. vehiculum* (Western Red-backed Salamander).

Members of the *Plethodontidae* lack lungs and breathe primarily through their skin. The females lay their eggs in moist environments in forested areas. Species of this family that are native to Canada do not require water for breeding or rearing (Cook 1984). *A. vagrans*, *E. eschscholtzii*, *P. idahoensis*, *P. cinereus*, and *P. vehiculum* do not have a larval stage. The hatchlings are a miniature version of the terrestrial adults. *Plethodontidae* adults inhabit forested areas, either near streams (e.g., *E. bislineata*) or in forest debris (e.g., *P. cinereus*).

Table A9. **Exposure media and pathways for members of the family *Plethodontidae* (lungless salamanders).**

Exposure Media	Exposure Pathways	Life Stage		
		Embryo	Larva	Adult
Surface Water	Direct contact[1]	—	○	○
	Respiration[2]	—	—	—
Sediment and Sediment Porewater	Direct contact[1]	—	○	○
	Respiration[2]	—	—	—
	Incidental ingestion	—	—	—
Soil	Direct contact	●	●	●
	Incidental ingestion	—	○	○
Air	Direct contact[1]	—	●	●
	Respiration[2]	—	—	—
Aquatic Food Items	Algae / aquatic plants	—	—	—
	Small fish, amphibians and invertebrates	—	—	—
Terrestrial Food Items	Invertebrates	—	●	●
	Small mammals	—	—	—

● = Major Pathway ○ = Minor Pathway — = Not a pathway

[1] Direct contact includes cutaneous respiration.
[2] Respiration refers to respiration through the lungs or gills.

Appendix B. Multi-Study Concentration-Response Profiles

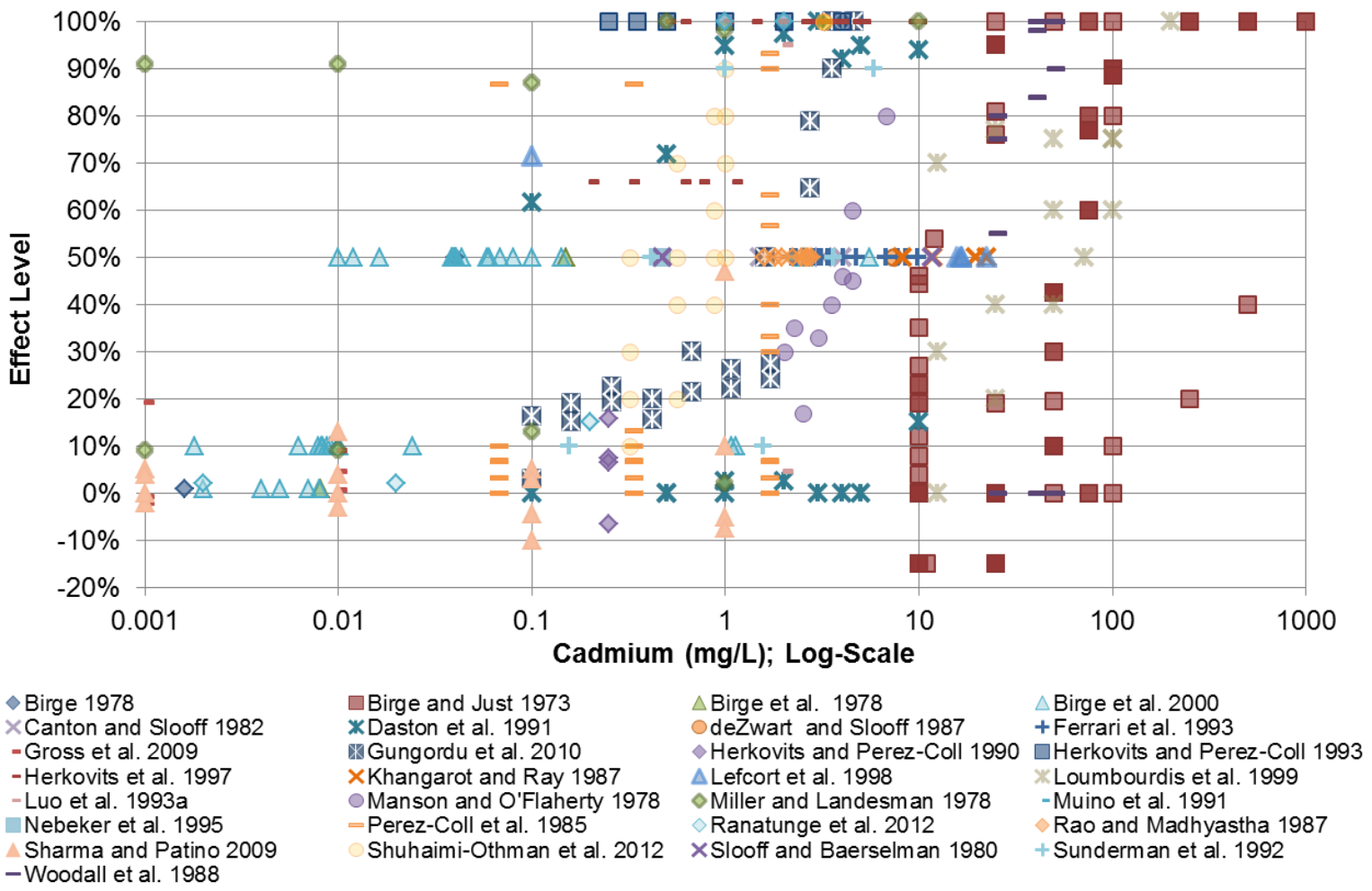
FCSAP has collected published toxicological data for four contaminants that are commonly found at federal contaminated sites (cadmium, lead, mercury, zinc), using the methodology described in Section 3.1.1. These data are summarized in the concentration-response profiles presented below (Sections B1 to B3). ERA practitioners can refine these profiles by filtering for site-specific parameters or data quality (Section 3.1.3) using the complete database, which is available upon request from FCSAP (see contact information at the end of this document).

For the multi-study response profiles provided in this section, effect concentration (EC) and inhibitory concentration (IC) data are plotted separately when sufficient data are available (surface water data), but plotted together when data are limited (sediment and sediment porewater data). Studies on soil are extremely scarce and therefore descriptions of individual studies are presented instead of plotted data.

B1. Surface Water

The profiles below are based on published EC toxicity data from 26 studies for cadmium, 14 studies for lead, 16 studies for mercury, and 18 studies for zinc (Figures B1, B3, B5 and B6). Together, these studies cover a range of exposure times, species and life stages. Toxicity studies reporting IC endpoints are much more limited and are presented separately for surface water (Figures B2 and B4; Table B1).

Mortality and malformations were the most common EC endpoints. Malformations in these studies refer to physical abnormalities that appear during development, including neural tube defects, eye abnormalities, tail curvature, underdeveloped gills, and reduced body size. These commonly available EC endpoints are presented for each metal (cadmium, lead, mercury, and zinc) in a multi-study concentration-response profile. The less common IC endpoints (e.g., behavioural, limb regeneration, growth) are presented following the EC multi-study concentration-response profiles for each metal (Figures B2 and B4; Table B1).

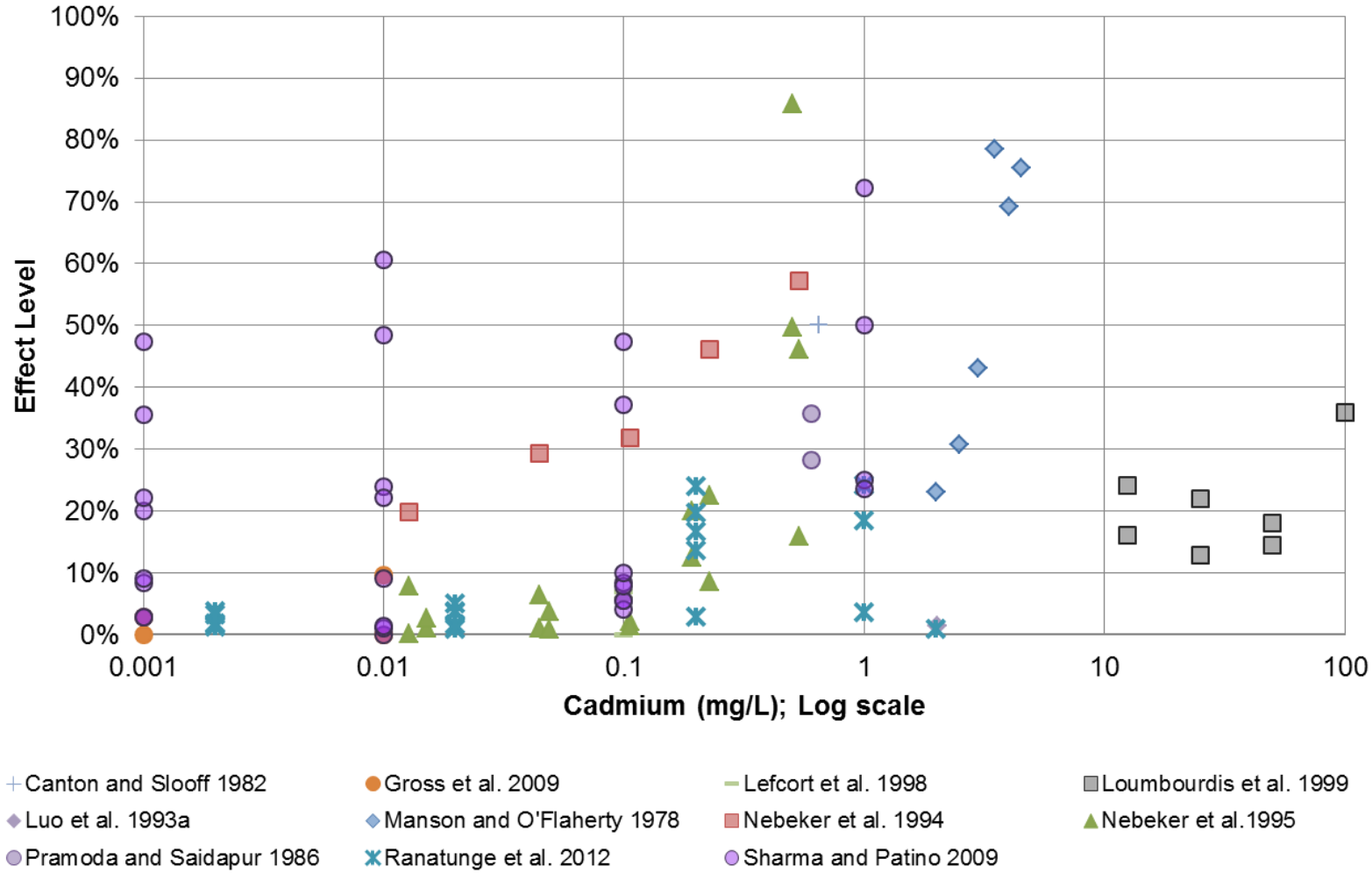


1 Darker shaded symbols indicate several overlapping data points.

2 Negative effects levels occur when the treatment outperforms the control.

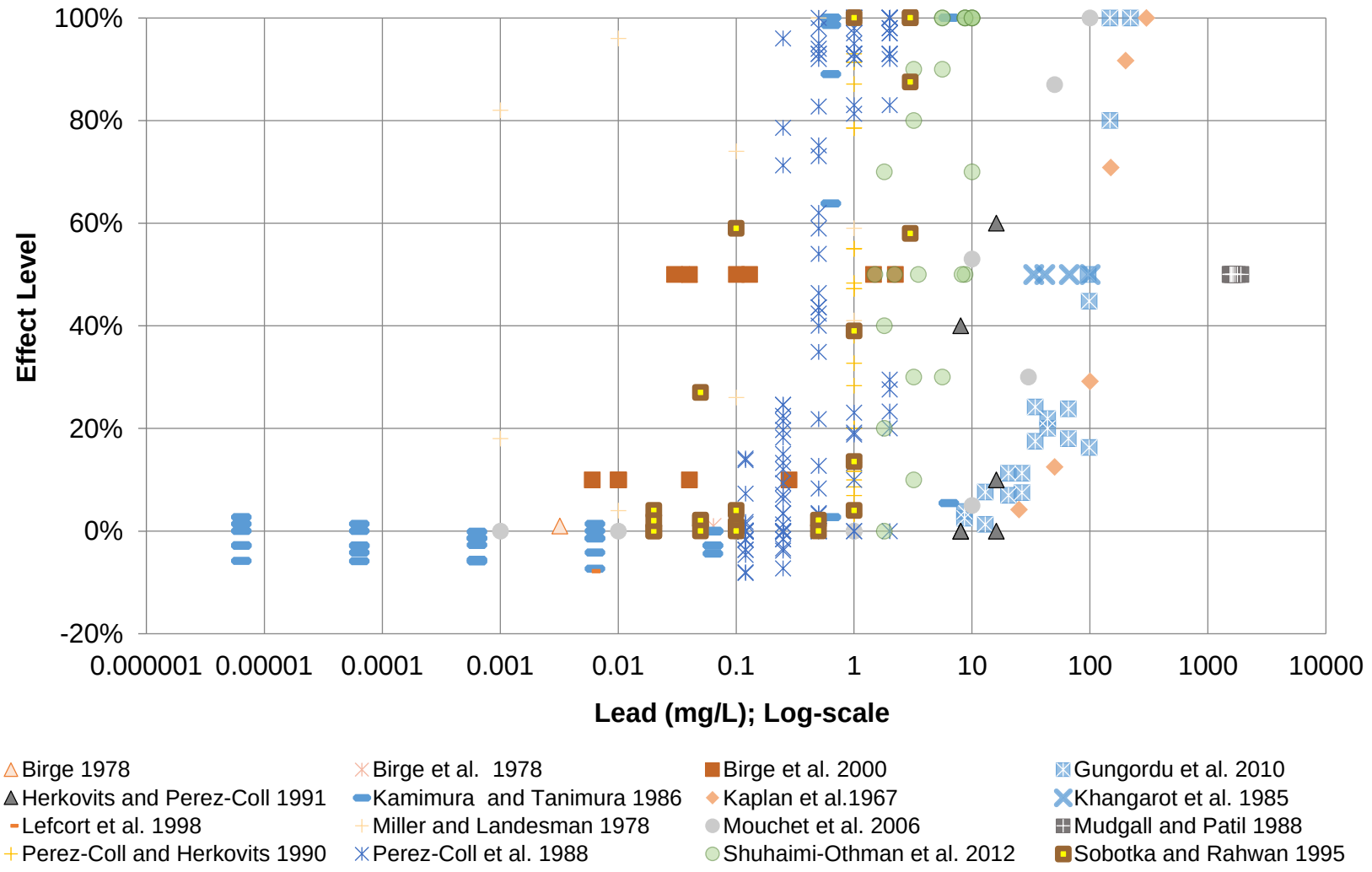
3 References included in these profiles are listed in Section 5 (References) of the main document.

Figure B1. Cadmium multi-study concentration-response profile for water, showing EC endpoints [1,2,3].



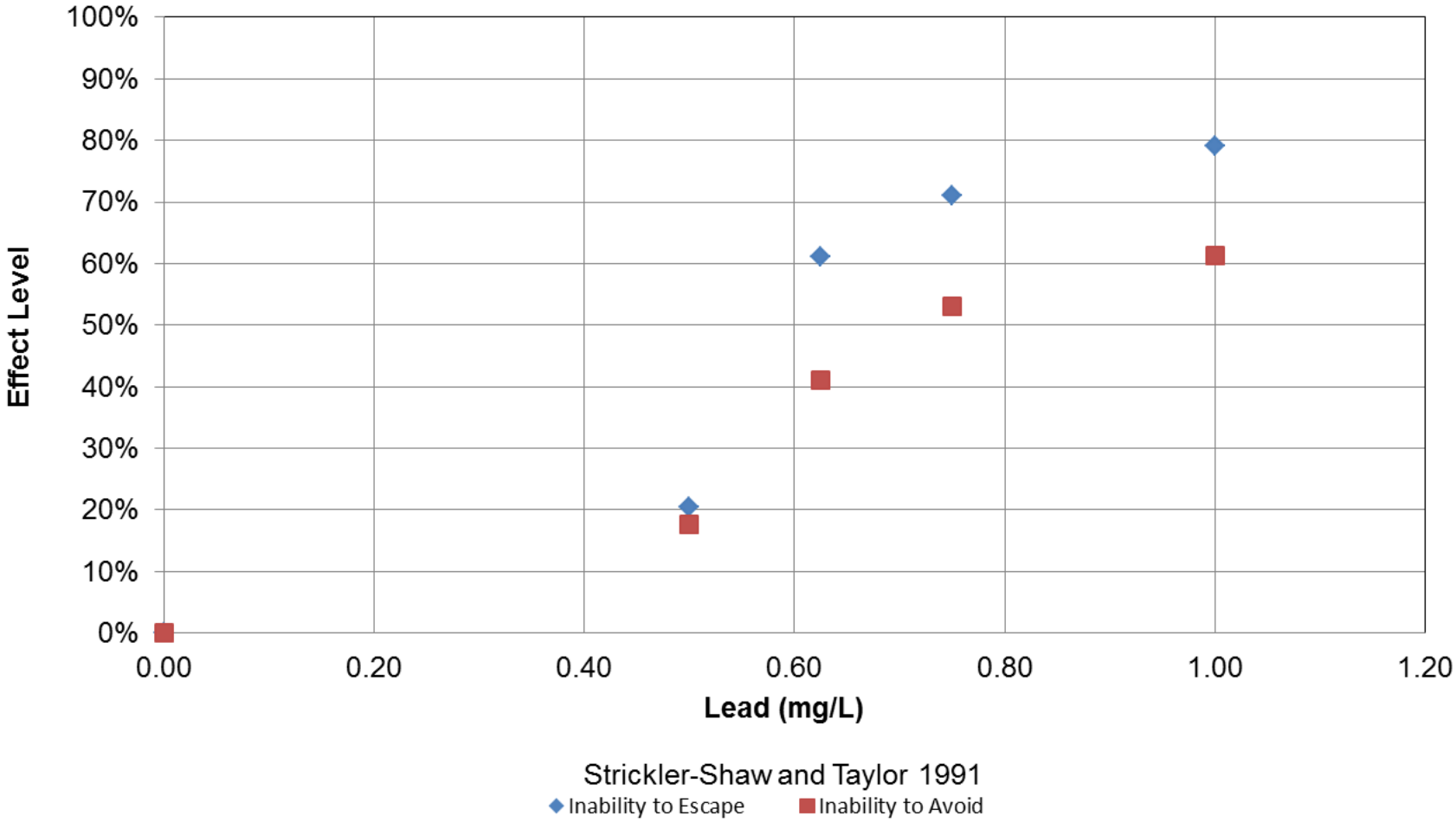
[1] Darker shaded symbols indicate several overlapping data points.
[2] IC endpoints included forelimb regeneration, growth (length and weight), developmental stage, and female gonad weight.
[3] References included in these profiles are listed in Section 5 (References) of the main document.\

Figure B2. Cadmium multi-study concentration-response profile for water, showing IC endpoints [1,2,3].



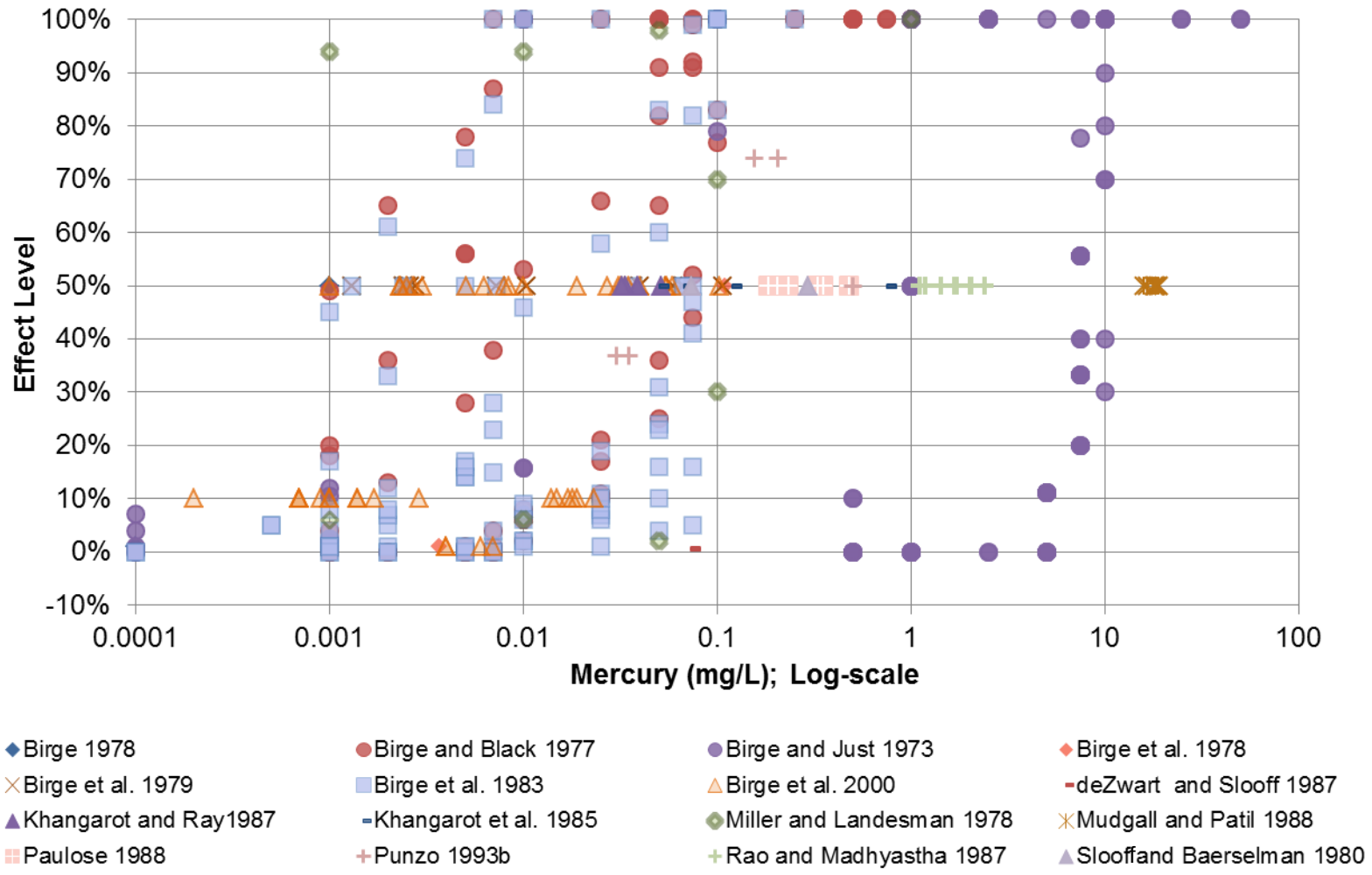
1 Darker shaded symbols indicate several overlapping data points.
2 Negative effect levels occur when the treatment outperforms the control.
3 References included in these profiles are listed in Section 5 (References) of the main document.

Figure B3. Lead multi-study concentration-response profile for water, showing EC endpoints [1,2,3].



[1] Darker shaded symbols indicate several overlapping data points.
[2] References included in these profiles are listed in Section 5 (References) of the main document.

Figure B4. Lead concentration-response profile for water, showing IC endpoints [1,2].



1 Darker shaded symbols indicate several overlapping data points.
2 Negative effects levels occur when the treatment outperforms the control.
3 References included in these profiles are listed in Section 5 (References) of the main document.

Figure B5. Inorganic mercury multi-study concentration-response profile for water, showing EC endpoints [1,2,3].

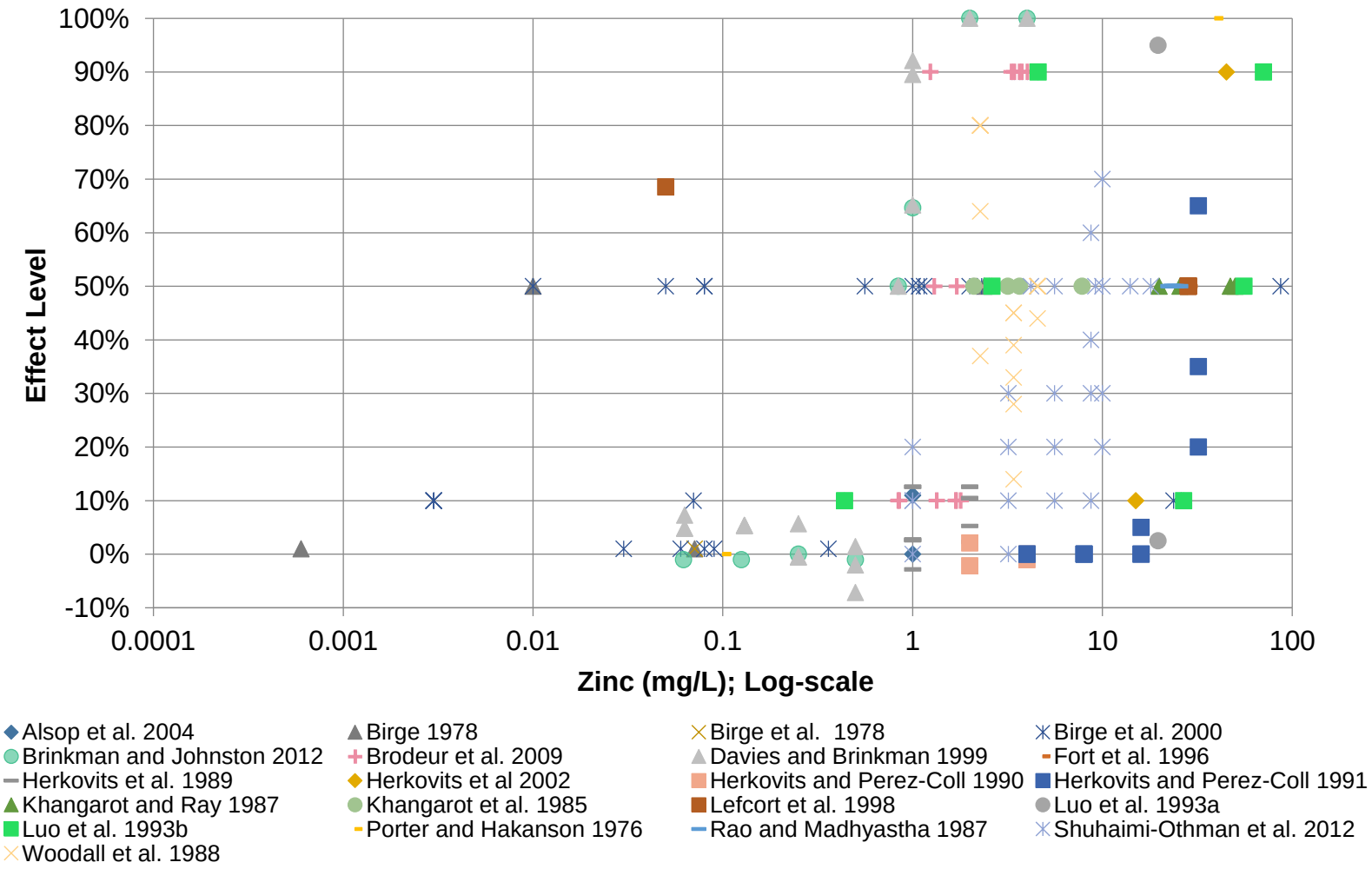
Table B1. **Inorganic mercury two-study concentration-response profile for water, showing IC endpoints[1,2,3].**

Endpoint	Effect Size at 0.65 mg/L of Hg (Punzo 1993a)	Effect Size at 0.71 mg/L of Hg (Kanamadi and Saidapur 1991)
Experiment 1: 30-day exposure during post-breeding period		
Ovary mass (g/100 g body mass)	↓ 39%	↓ 61%
Oviduct mass (g/100 g body mass)	↓ 26%	↓ 40%
Number of oocytes per female (mean)	↓ 45%	↓ 49%
Experiment 2: 30-day exposure during pre-breeding / breeding period		
Ovary mass (g/100 g body mass)	↓ 38%	↓ 25%
Oviduct mass (g/100 g body mass)	No significant difference	No significant difference
Number of oocytes per female (mean)	No significant difference	No significant difference
Experiment 3: 60-day exposure during pre-breeding / breeding period		
	60-day period	**65-day period**
Ovary mass (g/100 g body mass)	↓ 40%	↓ 52%
Oviduct mass (g/100 g body mass)	↓ 33%	↓ 37%
Number of oocytes per female (mean)	No significant difference	No significant difference

1 Effect size represents the reduction relative to the control. The arrow (↓) indicates that the effect size is significantly decreasing $p < 0.01$ or $p < 0.05$.

2 There was a 65-day exposure period in Kanamadi and Saidapur (1991), and a 60-day exposure period in Punzo (1993a). Both studies targeted the pre-breeding / breeding phase.

3 All references for studies included in these profiles are listed in Section 5 (References) of the main document.



1 Darker shaded symbols indicate several overlapping data points.

2 Negative effect levels occur when the treatment outperforms the control.

3 IC endpoints are not included in a separate figure because only one study was available and no effects were reported.

4 References included in these profiles are listed in Section 5 (References) of the main document.

Figure B6. Zinc multi-study concentration-response profile for water, showing EC endpoints [1,2,3,4].

B2. Sediment and Sediment Porewater

Toxicity data on amphibian exposure to contaminated sediments are much more limited than data on exposure to surface water. For these profiles, effect concentration (EC) and inhibitory concentration (IC) toxicity data are plotted on the same graph. The profiles presented below are based on published toxicity data from 3 studies for cadmium, 2 studies for lead, 1 study for mercury, and 2 studies for zinc.

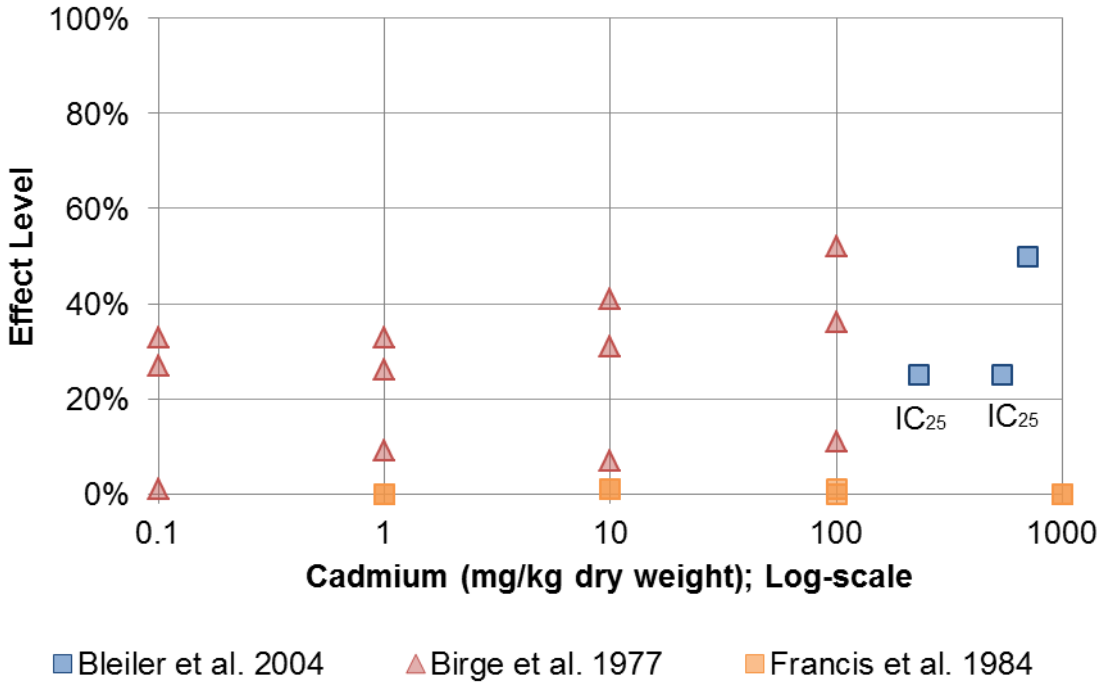
B2.1. Cadmium

For cadmium, concentration-response data from three studies are summarized in Figure B7 (Birge *et al.* 1977; Bleiler *et al.* 2004; Francis *et al.* 1984). In addition to the spiked and measured cadmium concentrations in sediment, some of these studies also reported cadmium concentrations in the overlying water (dissolved concentration) and in the organism's tissue (Table B2).

Birge *et al.* (1977) measured mortality at hatch and 4 days post-hatch for *Gastrophryne carolinensis*. Teratogenesis among the surviving population was also measured at hatch but not 4 days post-hatch. Effect levels for the same concentration vary due to the difference in exposure duration (at hatch vs. 4 days post-hatch) and endpoints (mortality and teratogenesis). When *G. carolinensis* was exposed to 0.1 mg/kg of cadmium (lowest concentration measured), mortality was as high as 33%. Mortality increased by 20% when the spiked concentration increased from 0.1 to 100 mg/kg (Birge *et al.* 1977). Cadmium concentrations in the overlying water remained relatively constant at different spiked concentrations: 0.0047 mg/L (for 0.1 mg Cd/kg spiked) to 0.0072 mg/L (for 100 mg/kg of cadmium added).

Bleiler et al. (2004) studied the effects on larvae of both *Lithobates pipiens* and *Anaxyrus americanus* after exposure to cadmium for 240 h. When the IC_{25} values were compared, *L. pipiens* was found to be more sensitive than *A. americanus*. The IC_{25} value (larval body length) was 230 mg/kg sediment (0.57 mg/L in the overlying water) for *L. pipiens* and 540 mg/kg sediment (1 mg/L in the overlying water) for *A. americanus*. The LC_{50} for *L. pipiens* was 700 mg/kg sediment (2.9 mg/L of cadmium in the overlying water); the LC_{50} was not calculated for *A. americanus*.

Francis *et al.* (1984) reported no effects on *Lithobates pipiens* embryos exposed to sediments spiked with cadmium at concentrations ranging from 1 to 1,000 mg/kg for 168 h. Measured concentrations in sediment were similar to the spiked concentrations and the average cadmium concentration in the overlying water was reported to range from 0.0011 mg/L (for 1 mg/kg of cadmium added) to 0.0765 mg/L (for 1,000 mg/kg of cadmium added). There was a strong correlation between the cadmium concentrations in the water, sediment, and tissues ($r^2 > 0.99$) but no effects were observed across the range of exposure concentrations.



1 Unless specified as an IC_{25} on the graph, all data points represent EC.

2 References included in these profiles are listed in Section 5 (References) of the main document.

Figure B7. Cadmium contaminated sediment multi-study concentration-response profile[1,2].

Table B2. **Cadmium concentration in sediment and in associated media[1].**

Study	Sediment Concentration (mg/kg dry weight)		Measured Overlying Water Concentration (mg/L)	Measured Tissue Concentration (mg/kg)
	Spiked	Measured[1]		
Birge *et al.* 1977	0.1	1.34	0.0047 ±0.0024	NR
	1	2.18	0.0068 ±0.0017	NR
	10	14.8	0.0075 ±0.0019	NR
	100	122.8	0.0072 ±0.0015	NR
Bleiler *et al.* 2004	230 (IC_{25}; *Lithobates pipiens*)		0.57 (IC_{25})	51
	540 (IC_{25}; *Anaxyrus americanus*)		1(IC_{25})	170
	700 (LC_{50}; *Lithobates pipiens*)		2.9 (LC_{50})	NR
Francis *et al.* 1984	1	2.28 ±0.14	0.0011 ±0.0008	0.08
	10	11.48 ±0.21	0.0021 ±0.0044	0.34
	100	96.8 ±2.4	0.0044 ±0.0018	3.08
	1000	1074 ±14	0.0765 ±0.0171	12.55

NR = not reported/available.

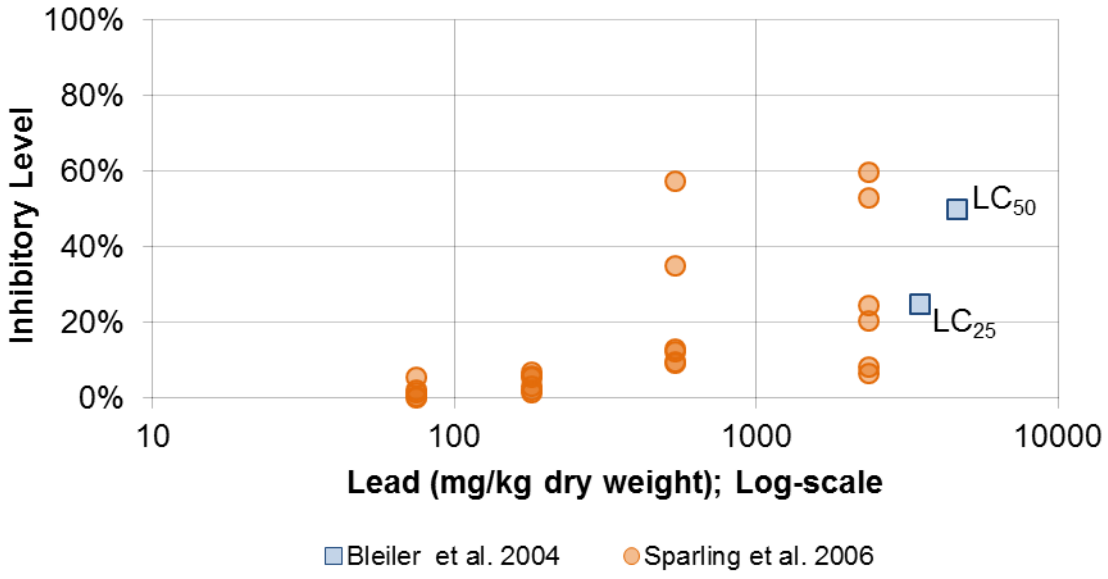
[1] The difference between the spiked and measured concentration is attributable to the variation of the baseline element concentration in the respective media.

B2.2. Lead

Two studies (Bleiler *et al.* 2004; Sparling *et al.* 2006) provide concentration-response data for lead in sediment (Figure B8). Both studies report the spiked concentration of lead and the resulting measured lead concentrations in overlying water and amphibian tissue (Table B3).

Bleiler *et al.* (2004) reported effects on *Lithobates pipiens* larvae after 240 h of exposure to lead. The LC_{25}, calculated using the measured contaminant concentration in sediment, was 3,550 mg/kg of lead (0.43 mg/L in the overlying water). The LC_{50} was 4,662 mg/kg of lead (0.58 mg/L in the overlying water).

Sparling *et al.* (2006) provided inhibitory concentrations for *Lithobates sphenocephalus* exposed to lead from the larval stage to the onset of metamorphosis and from the onset of metamorphosis to its completion. Inhibitory effects included days to metamorphosis and duration of metamorphosis. Snout vent length and mass at onset and completion of metamorphosis were also measured for each concentration. The difference in endpoints resulted in varying inhibitory effects for the same concentration. For example, inhibitory effects were first observed when the lead concentration reached 540 mg/kg. At this concentration, the body mass at completion of metamorphosis was reduced by 57% compared to the control, while snout vent length was reduced by 10%.



[1] Unless specified, data points represent effects reported as IC.

[2] References included in these profiles are listed in Section 5 (References) of the main document.

Figure B8. Lead contaminated sediment multi-study concentration-response profile[1].

Table B3. **Lead concentration in sediment and associated media[1].**

Study	Sediment Concentration (mg/kg dry weight)		Measured Concentration in Overlying Water (mg/L)	Measured Concentration in Tissue (mg/kg)[1]
	Spiked	Measured		
Bleiler *et al.* 2004	3,550 ($LC_{25;}$ *Lithobates pipiens)*		0.43	NR
	4,662 (LC_{50}; *Lithobates pipiens)*		0.58	1308
Sparling *et al.* 2006	75	N.A.	0.227	35.6 ± 11.4
	180	N.A.	0.589	73.1 ± 36.9
	540	N.A.	1.833	166 ± 146
	2,360	N.A.	8.121	568 ± 456

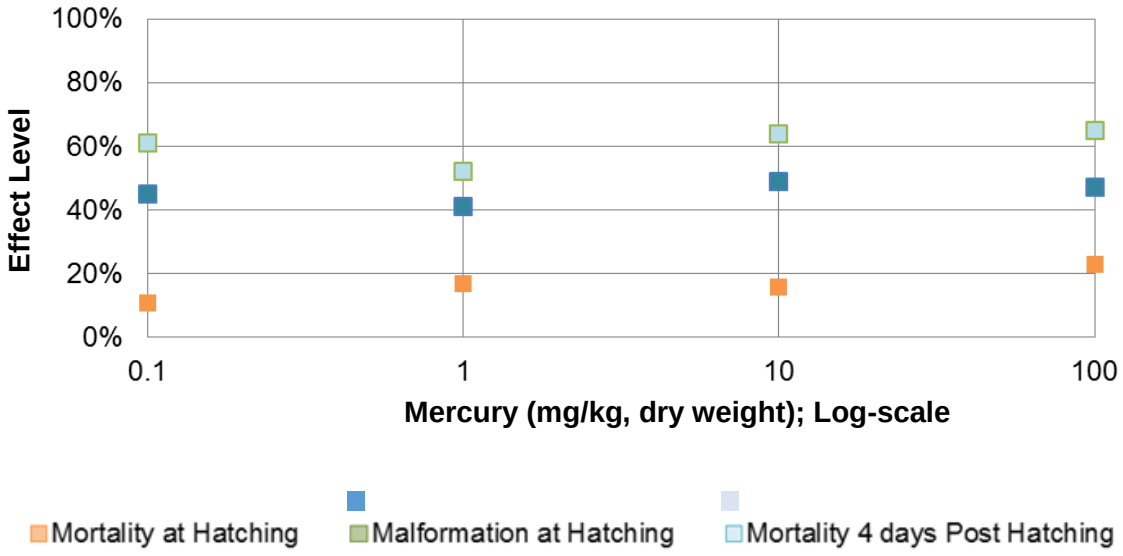
N.A. = Not available

NR = Not reported

[1] The tissue concentration refers to the contaminant concentration in the whole body.

B2.3. Inorganic Mercury

Only one study was found in the literature on the effects of inorganic mercury in sediments. The concentration-response data for mercury are presented in Figure B9 (Birge *et al.* 1977). Effect levels for *Gastrophryne carolinensis* exposed to 0.1 mg/kg of mercury (lowest spiked sediment concentration) were similar to those for exposure to 100 mg/kg of mercury (highest spiked sediment concentration). The measured concentration of mercury was similar to the spiked concentration, and mercury concentrations in overlying water ranged from 0.00025 mg/L (at 0.1 mg/kg) to 0.0064 mg/L (at 100 mg/kg). Measured concentrations in tissue were not reported (Table B4). The authors acknowledged that the effects were consistent for all tested concentrations and suggested that this could be related to the test species' short and sensitive embryonic period.



[1] Mortality at hatching refers to the number of embryos that died. Mortality 4-days post-hatch includes both embryos and larvae.

[2] References included in these profiles are listed in Section 5 (References) of the main document.

Figure B9. Inorganic mercury contaminated sediment concentration-response profile (Birge *et al.* 1977) [1, 2].

Table B4. **Inorganic mercury concentration in sediment and associated media (Birge *et al.* 1977)[1,2].**

Sediment Concentration (mg/kg dry weight)[1]		Measured Concentration in Overlying Water (mg/L)	Measured Concentration in Tissue (mg/kg)[2]
Spiked	Measured		
0.1	0.146	0.00025 ± 0.00009	NR
1	1.188	0.00015 ± 0.00006	NR
10	12.08	0.00183 ± 0.00149	NR
100	122.83	0.00640 ± 0.00366	NR

NR = Not reported / available.

[1] The difference between the spiked and measured concentration is attributable to the variation of the baseline element concentration in the respective media.

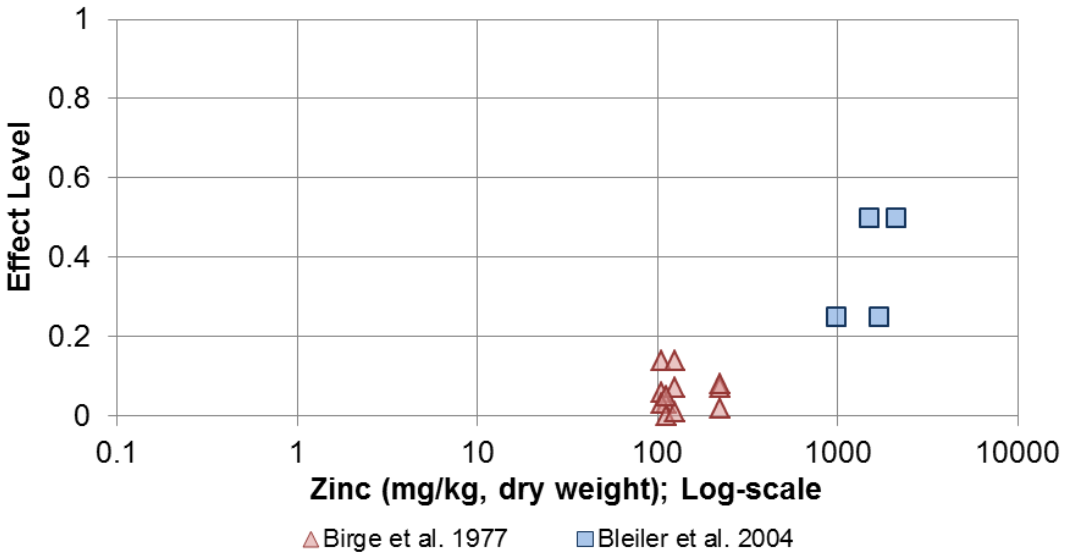
[2] The tissue concentration refers to the contaminant concentration in the whole body.

B2.4. Zinc

Zinc concentration-response data from two studies (Birge *et al.* 1977; Bleiler *et al.* 2004) are summarized in Table B5. The corresponding measured zinc concentrations in the overlying water are shown in Figure B10.

Birge *et al.* (1977) reported low mortality in *Gastrophryne carolinensis* embryos regardless of zinc concentrations. With exposure to zinc levels in sediments ranging from 0.1 to 100 mg/kg, mortality rates varied little (0% to 14%). Measured concentrations in the overlying water ranged from 0.017 mg/L (at 0.1 mg Zn/kg sediment) to 0.1228 mg/L (at 100 mg/kg of zinc in the sediment). The author noted that the measured concentration of zinc in sediment did not correlate strongly with the spiked sediment concentration but does not indicate whether this could explain the minimal changes in effect level.

Bleiler *et al.* (2004) studied the effects of zinc exposure on both *Lithobates pipiens* and *Anaxyrus americanus* larvae exposed to contaminated sediment for 240 h. *L. pipiens* appeared to be more sensitive than *A*. *americanus*. The LC_{25} for *L. pipiens* was 980 mg/kg (7.2 mg/L for overlying water) compared to 1,700 mg/kg (19 mg/L for overlying water) for *A. americanus*. The LC_{50} was reported to be 1,500 mg/kg (28 mg/L for overlying water) for *L. pipiens* and 2,100 mg/kg (35 mg/L for overlying water) for *A. americanus*.



[1] References included in these profiles are listed in Section 5 (References) of the main document.

Figure B10. Zinc contaminated sediment multi-study concentration-response profile, for mortality endpoint[1].

Table B5. **Zinc concentration in sediment and associated media.**

Study	Sediment Concentration		Measured Concentration in Overlying Water (mg/L)	Measured Concentration in Tissue (mg/kg)
	Spiked	Measured[1]		
Birge *et al.* 1977	0.1	104.6	0.017 ± 0.0046	NR
	1	112.6	0.0212 ± 0.004	NR
	10	124.5	0.0323 ± 0.0072	NR
	100	222.7	0.1228 ± 0.0153	NR
Bleiler *et al.* 2004	980 (LC_{25}; *Lithobates*		7.2	NR
	1,500 (LC_{50}; *Lithobates*		19	NR
	1,700 (LC_{25}; *Anaxyrus*		28	NR
	2,100 (LC_{50}; *Anaxyrus*		35	NR

[1] The difference between the spiked and measured concentration is attributable to the variation of the baseline element concentration in the respective media.
NR = not reported / available.

B3. Soil

Soil can be a major exposure pathway for post-larval amphibians; juvenile and adult amphibians can be exposed to soil contamination through dermal absorption and incidental soil ingestion (see Appendix A). Information on amphibian effects from exposure to contaminated soil is very limited. This represents a large knowledge gap in amphibian toxicology and ERA. According to Birge *et al.* (2000), amphibians are often exposed to dissolve contaminants in the soil porewater because amphibians must absorb water from the environment to stay hydrated. James *et al.* (2004) investigated the effects of cadmium-contaminated soil and food on the toad *Anaxyrus americanus* (formerly known as *Bufo americanus*), which is known to stay buried in soil for most of the year. These toads did not experience any effects after exposure to up to 120 mg/kg of cadmium in soil (measured dry weight). However, mortality did increase to 44% when the same species were fed with crickets that contained 15 mg/kg of cadmium dry weight.

Appendix C. Comparing Contaminant Effect Concentrations to Guidelines

In Canada, environmental quality guidelines published by the Canadian Council of Ministers of the Environment (CCME) are used when assessing and managing federal contaminated sites (CCME 1999a, 1999b). If CCME guidelines are not available for specific contaminants, guidelines from other jurisdictions may be substituted (FCSAP 2013b) in the ERA. For cadmium, lead, mercury, and zinc, several jurisdictions, including the CCME, have developed risk-based water and sediment quality guidelines for the protection of aquatic life. The guidelines are developed to protect all aquatic receptors, but in most cases amphibians were not specifically considered when the guidelines were derived. In this section, the amphibian multi-study concentration-response data are compared to environmental quality guidelines for purposes of consideration in the preliminary stages of an amphibian ERA.

C1. Water

Water quality guidelines for the protection of freshwater aquatic life from various jurisdictions are listed in Table C1. The amphibian toxicity data from the multi-study concentration-response profiles (EC endpoints) are compared to the guidelines in Figure C1**Error! Reference source not found.**. Where guidelines are hardness dependent, guidelines for both soft water (30 mg/L of $CaCO_3$ or minimum hardness allowed for the equation to be applicable) and hard water (360 mg/L of $CaCO_3$ or maximum hardness allowed for the equation to be applicable) are displayed. If guidelines for chronic and acute exposure are provided, the guideline for chronic exposure is displayed. Most water quality guidelines, including those provided by CCME, appear to provide adequate protection of amphibians against exposure to lead, mercury, cadmium, and zinc, based on currently available amphibian toxicity data for these metals.

In some cases, the multi-study concentration-response profiles show amphibian effects at concentrations below guidelines. This does not necessarily mean that the guidelines do not protect amphibians. Such a situation simply warrants further investigation, as no amphibian toxicity data were used in guideline derivation.

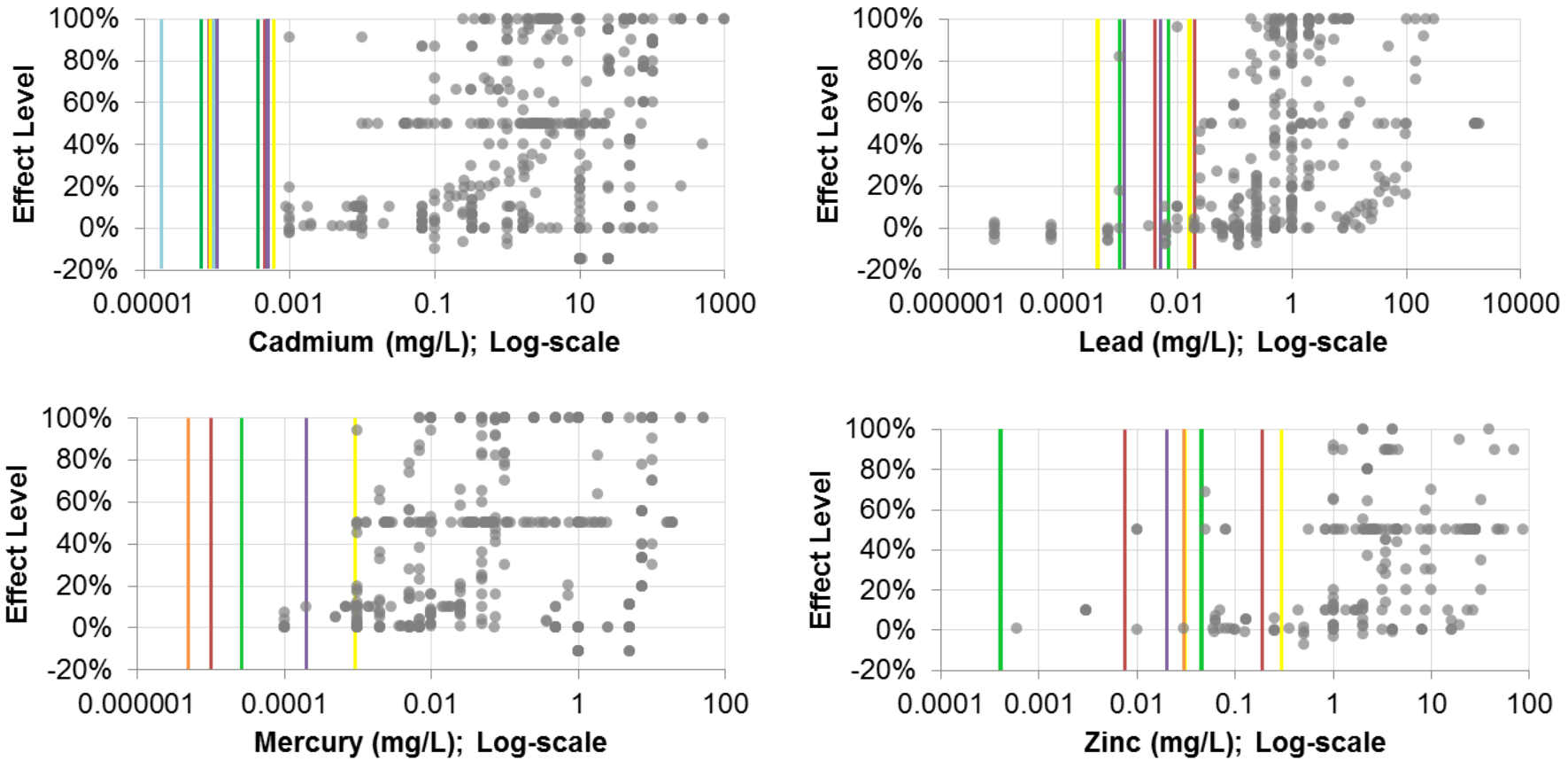
Table C1. **Water quality guidelines for freshwater (mg/L) for chronic exposure, by jurisdiction[1,2,3].**

	Jurisdiction	Cd	Pb	Hg	Zn
	Canada (CCME 2018)	0.00006 -0.00037	0.001-0.007	0.000026	0.0004- 0.045
	Alberta (Government of Alberta 2018)	CCME	CCME	0.000005	0.03
	British Columbia (Government of British Columbia 2018)	0.000087– 0.00046	0.004-0.02	0.00001	0.0075- 0.1875
	Ontario (Government of Ontario 1994)	0.0001 - 0.0005	0.001-0.005	0.0002	0.02
	Saskatchewan (Government of Saskatchewan 2006)	0.000017 - 0.00009	CCME	CCME	CCME
	Quebec (Government of Quebec 2018)	0.000082 – 0.00061	0.00041 – 0.016	0.00091	0.031 – 0.30

[1] This table is intended to permit comparison with the amphibian multi-study concentration-response profiles. Consult the original source before applying any of the guidelines.

[2] Provinces and territories that are not listed apply CCME guidelines, except the Yukon, which has adopted B.C. Contaminated Sites Regulation guidelines.

[3] Hardness-dependent guidelines are presented for a hardness range of 30 to 360 $CaCO_3$/L or allowable limited as specified for the equation.



[1] The coloured lines reference the guidelines corresponding to each coloured box in Table C1 above.

[2] Negative effects levels occur when the treatment outperforms the control.

Figure C1. Comparison of CCME and provincial/territorial water quality guidelines with the multi-study concentration-response profiles [1,2].

C2. Sediment

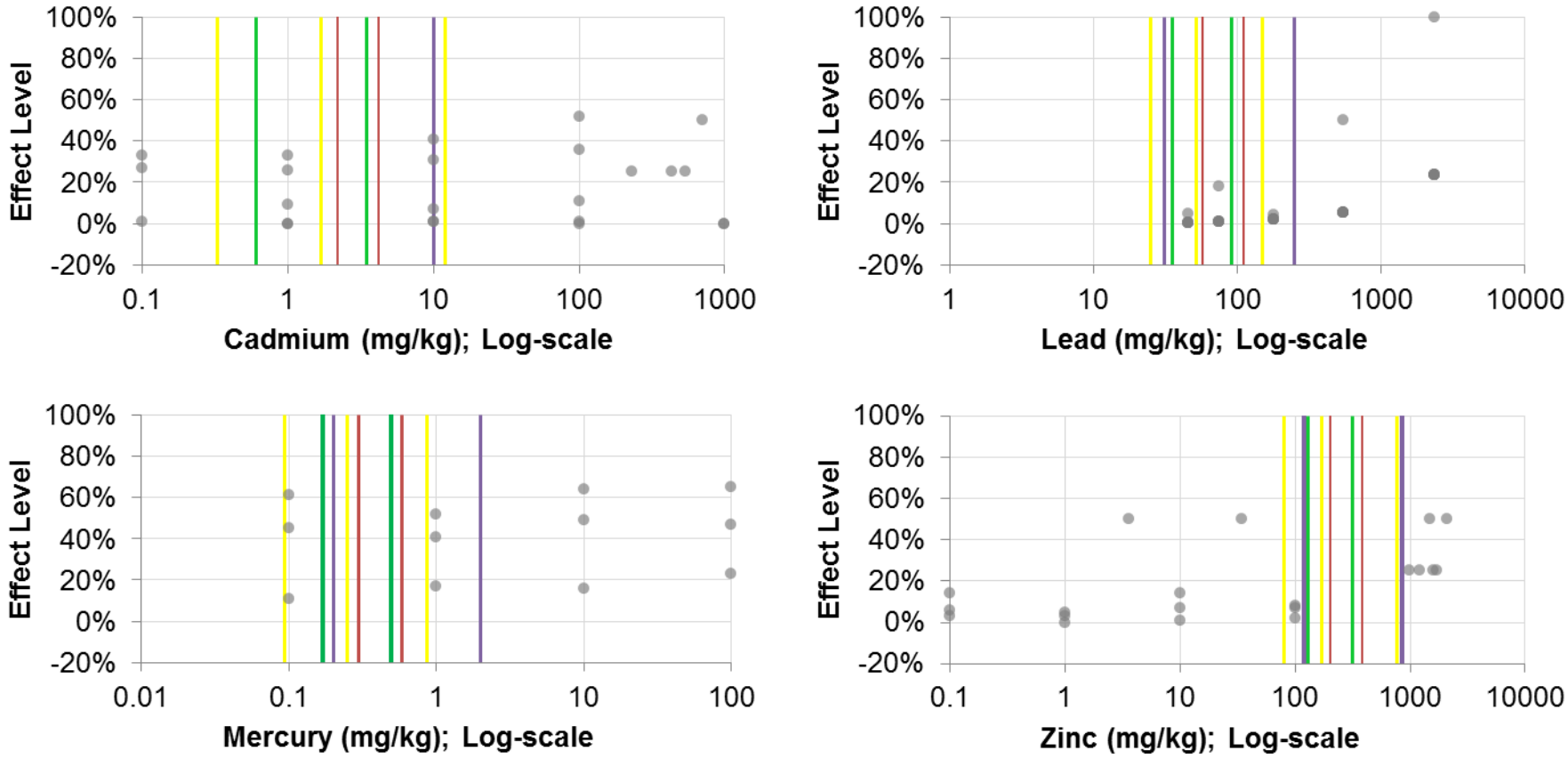
Freshwater sediment quality guidelines for various jurisdictions are listed in Table C2. These sediment quality guidelines were developed for the protection of benthic invertebrates only. Comparing guidelines with sediment toxicity data is a much less reliable method for making inferences about ecological risk because the amphibian toxicity data for sediment exposures is so limited. For each of the four metals, most amphibian toxicity data originates from one study per metal (Birge *et al.* 1977, for Cd, Hg, and Zn; Sparling *et al.* 2006, for Pb). The limitations of the available data increase the uncertainty associated with the analysis of a single study (FCSAP 2012a).

Using the available information, Figure C2 compares the amphibian toxicity data to guidelines from different jurisdictions. The lack of data precludes the possibility of making any definitive inferences regarding the protection that existing sediment quality guidelines provide to amphibians, but highlights the need for further research.

Table C2. **Freshwater sediment quality guidelines, by jurisdiction (mg/kg dry weight)[1].**

	Jurisdiction	Type	Cd	Pb	Hg	Zn
	Canada (CCME 2018)	Interim Sediment Quality Guideline	0.6	35	0.17	123
		Probable Effect Level	3.5	91.3	0.86	315
	British Columbia Schedule 9 (Government of British Columbia 2018)	Sensitive	2.2	57	0.3	200
		Typical	4.2	110	0.58	380
	Quebec (Environment Canada and MDDEPQ 2007)	Rare Effects Concentration	0.33	25	0.094	80
		Occasional Effects Concentration	1.7	52	0.25	170
		Frequent Effects Concentration	12	150	0.87	770
	Ontario (Government of Ontario 1996)	Lowest Effect Level	CCME	31	0.2	120
		Severe Effect Level	10	250	2	820

[1] The table is intended to permit comparison of the amphibian data presented in this module with the guidelines. Consult the original source before applying any of the guidelines.



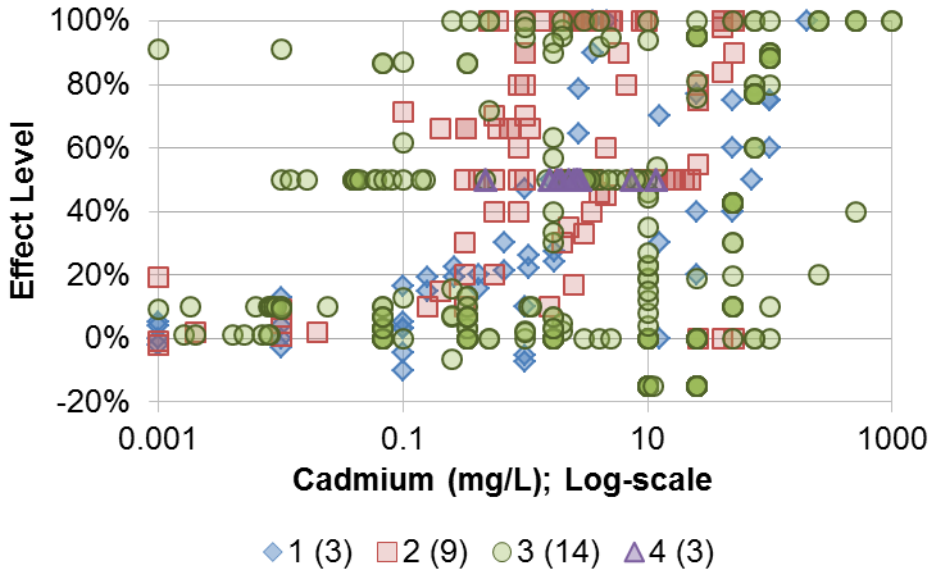
[1] The coloured lines reference the guidelines corresponding to each coloured box in the Table C2 above.

Figure C2. Comparison of CCME and provincial/territorial sediment quality guidelines with the multi-study concentration-response profiles[1].

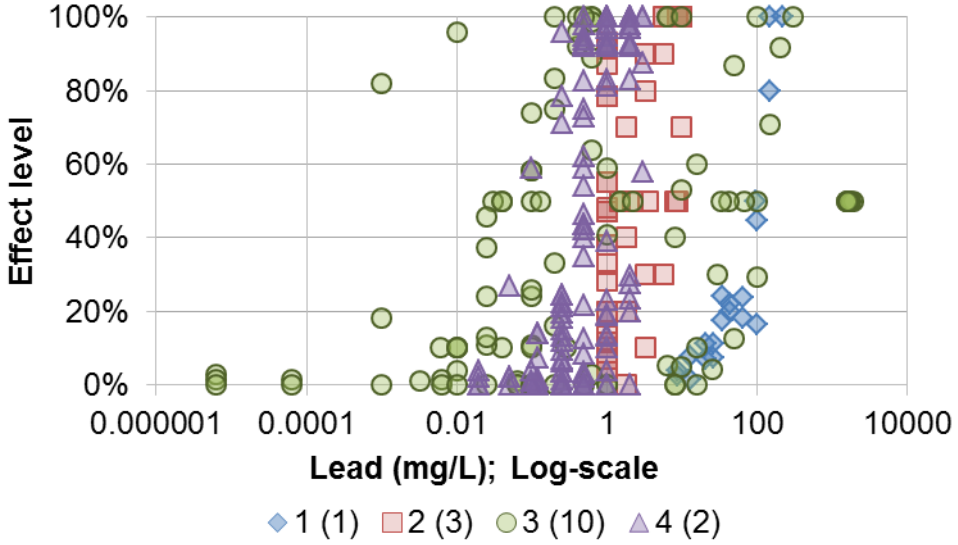
Appendix D. Data Quality and Modifying Factors

An independent QA/QC was conducted to evaluate the data quality of studies used in the concentration-response profiles developed for this module. Each study has been ranked loosely based on the data quality requirements considered in the CCME protocol for the derivation of water quality guidelines for the protection of aquatic life (2007): appropriate study design, replication, control response, data analysis, and modifying factors. Studies were then assigned a data quality ranking from 1 to 4. A rank of 1 is assigned to studies that meet all data quality requirements, a rank of 2 to studies that meet the majority of data quality requirements, a rank of 3 to studies that meet some of the data quality requirements, and a rank of 4 to studies that fail to meet most of the data quality requirements. Details on how data quality was evaluated are provided in Section 3.1.3.1. Figure D1 presents the concentration-response profiles according to rankings from the QA/QC process. Table D1 presents QA/QC and modifying factors from the primary studies used for the concentration-response profiles.

(a)[1]

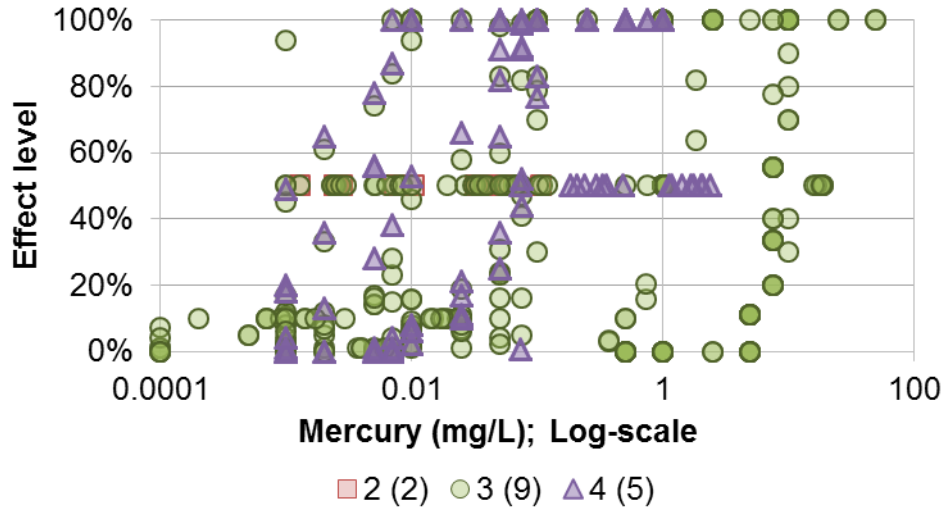


(b)



[1] Negative effects levels occur when the treatment outperforms the control.

(c)



(d)

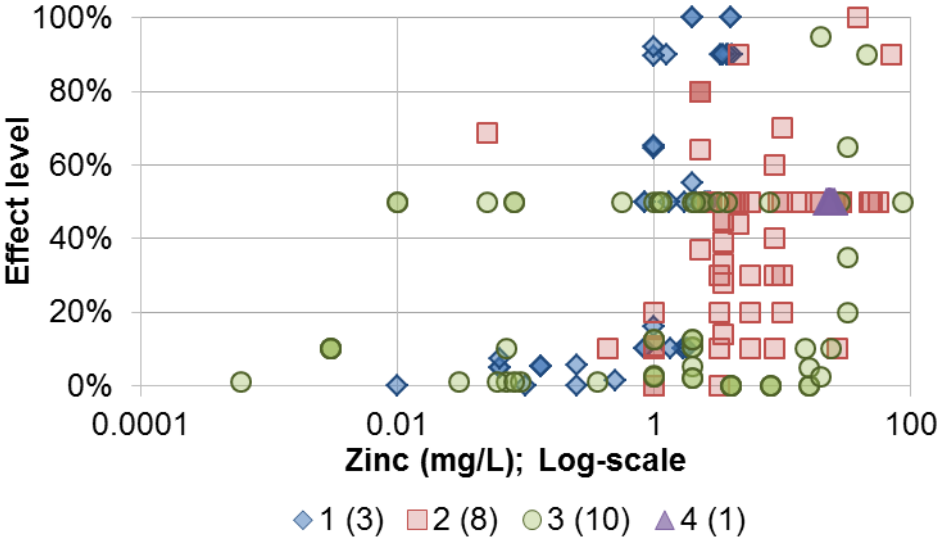


Figure D1. Multi-study concentration-response profile (water) filtered by data quality, with the number of studies shown in parentheses.

Table D1. QA/QC and modifying factors from the primary studies used for the concentration-response profiles.

Reference	QA/QC						Modifying Factors				
	Control	Replication	Study design suitable	Data analysis	Treatment statistically significant relative to control	Rank	Temp. (°C)	Hardness (ppm $CaCO_3$)	pH	Sample type	Other
Alsop *et al.* 2004	Y	N.A.	Y	Y	N.A.	**2**	N.A.	410	8.2	stock solution	Ca^{2+}: 105 mg/L; Mg^{2+}: 36 mg/L; Na^{+}: 24 mg/L; Cl: 52 mg/L; Alkalinity: 250 mg/L of $CaCO_3$
Birge and Just 1973	Y	N.A.	Y	N.A.	N.A.	**3**	13.3	N.A.	7.6-8.0	stock solution	N.A.
Birge and Black 1977	N.A.	N.A.	N.A.	N.A.	N.A.	**4**	22	200	7.9	stock solution	Total Alkalinity: 82 mg/L $CaCO_3$
Birge *et al.* 1977	Y	N.A.	Y	N.A.	N.A.	**2**	20-21	200	7.9	spiked	N.A.
Birge 1978	Y	N.A.	Y	Y	Y	**3**	21-23	189.6-200.4	7.3-7.5	assume stock solution	N.A.
Birge *et al.* 1978	Y	N.A.	Y	Y	Y	**3**	19-22	93-105	7.2-7.8	assume stock solution	N.A.
Birge *et al.* 1979	Y	N.A.	Y	Y	Y	**2**	20.5-21.5	90-105	7-7.8	stock solution	N.A.
Birge *et al.* 1983	Y	N.A.	Y	Y	N.A.	**3**	19-22	90-105	7-7.8	stock solution	N.A.
Birge *et al.* 2000	N.A.	Y	Y	Y	N.A.	**3**	N.A.	N.A.	N.A.	assume stock solution	N.A.
Bleiler *et al.* 2004	Y	Y	Y	Y	Y	**1**	23	N.A.	N.A.	spiked	N.A.
Brinkman and Johnston 2012	Y	Y	Y	Y	Y	**1**	18.1-20.7	59.4-64.4	7-7.44	stock solution	Alkalinity: 35.9 mg/L as $CaCO_3$; DO: 8.1-9.1 mg/L
Brodeur *et al.* 2009	Y	Y	Y	Y	Y	**1**	18-22	N.A.	N.A.	stock solution	N.A.

Reference	QA/QC						Modifying Factors				
	Control	Replication	Study design suitable	Data analysis	Treatment statistically significant relative to control	Rank	Temp. (°C)	Hardness (ppm $CaCO_3$)	pH	Sample type	Other
Canton and Slooff 1982	N.A.	Y	Y	N.A.	N.A.	**3**	19-22	N.A.	N.A.	stock solution	N.A.
Daston *et al.* 1991	Y	N.A.	Y	Y	Y	**3**	N.A.	N.A.	N.A.	assume stock solution	N.A.
Davies and Brinkman 1999	Y	Y	Y	Y	Y	**1**	20	57	7.2	stock solution	Alkalinity: 36 mg/L $CaCO_3$; DO: 8.6
deZwart and Slooff 1987	N.A.	N.A.	Y	Y	N.A.	**4**	19-21	N.A.	N.A.	stock solution	N.A.
Ferrari *et al.* 1993	Y	N.A.	Y	Y	Y	**3**	20 and 25	N.A.	N.A.	stock solution	N.A.
Fort *et al.* 1996	Y	Y	Y	Y	Y	**1**	23-25	N.A.	7.2-7.5	spiked	N.A.
Francis *et al.* 1984	Y	Y	Y	Y	N.A.	**2**	22.1-22.5	Water: 101.6 ± 9.8	Sediment: 7.6-7.7; Water: 7.9-8.2	spiked	Sediment Composition: 52.6 ± 3.4 Sand; 35.4 ± 4.7% Silt; 12 ± 1.3% Clay
Gross *et al.* 2009	Y	Y	Y	Y	Y for some data only	**3**	23	280	7.5-7.8	stock solution	N.A.
Gungordu *et al.* 2010	Y	Y	Y	Y	Y	**1**	22-24	N.A.	N.A.	stock solution	N.A.
Herkovits and Pérez-Coll 1990	Y	Y	Y	Y	Y	**3**	25	N.A.	N.A.	stock solution	N.A.
Herkovits and Pérez-Coll 1991	Y	N.A.	Y	Y	Y	**3**	20	N.A.	N.A.	stock solution	N.A.
Herkovits and Pérez-Coll 1993	Y	Y	Y	Y	Y	**3**	18-21	N.A.	N.A.	stock solution	N.A.

Reference	QA/QC						Modifying Factors				
	Control	Replication	Study design suitable	Data analysis	Treatment statistically significant relative to control	Rank	Temp. (°C)	Hardness (ppm $CaCO_3$)	pH	Sample type	Other
Herkovits *et al.* 1997	N.A.	Y	Y	Y	N.A.	**2**	25	N.A.	N.A.	stock solution	N.A.
Kamimura and Tanimura 1986	Y	N.A.	Y	N.A.	N.A.	**3**	N.A.	N.A.	N.A.	stock solution	N.A.
Kaplan *et al.*1967	Y	N.A.	Y	N.A.	N.A.	**3**	8	N.A.	N.A.	stock solution	N.A.
Khangarot *et al.* 1985	Y	Y	Y	Y	Y	**3**	13-16	13-80	6.2-6.7	stock solution	Air Temp: =14-16 °C; Acidity: = 13-25 ppm $CaCO_3$; Alkalinity: = 24-40 ppm $CaCO_3$; DO: = 6.2-7.0 ppm; Calcium: = 6.4-6.5 ppm; Magnesium: = 0.6-0.85 ppm
Khangarot and Ray 1987	Y	Y	Y	Y	Y	**2**	29-34	165-215	7.1-7.6	stock solution	Air Temp: 31-36 °C; Alkalinity: 120-160 ppm $CaCO_3$; DO: 5.8-7.8 ppm; Conductivity: 750-1100 µS/cm; Total Solids: 650-1250 mg/L; Dissolved Solids: 390-630 mg/L
Lefcort *et al.* 1998	Y	Y	Y	Y	Y for some data only.	**2**	N.A.	N.A.	N.A.	stock solution	N.A.
Loumbourdis *et al.* 1999	Y	Y	Y	Y	Y	**1**	20-25	288	7.4	stock solution, dilution series	Conductivity: 650 ± 700 µS/cm; Nitrites: <0.025 mg/L; Phosphates: <0.10 mg/L; Ammonium: <0.05 mg/L
Luo *et al.* 1993a	Y	N.A.	Y	Y	Y	**3**	23-24	N.A.	N.A.	stock solution	N.A.

Reference	QA/QC						Modifying Factors				
	Control	Replication	Study design suitable	Data analysis	Treatment statistically significant relative to control	Rank	Temp. (°C)	Hardness (ppm $CaCO_3$)	pH	Sample type	Other
Manson and O'Flaherty 1978	Y	Y	Y	Y	Y	**2**	N.A.	N.A.	N.A.	stock solution	N.A.
Miller and Landesman 1978	Y	N.A.	Y	N.A.	N.A.	**3**	18	N.A.	N.A.	stock solution	N.A.
Mudgall and Patil 1988	Y	Y	Y	N.A.	Y	**3**	22-25	60-70	7.38-7.8	stock solution	DO: 6.7-7.9 mg/L
Muino *et al.* 1991	Y	Y	Y	Y	Y	**2**	N.A.	N.A.	N.A.	stock solution	N.A.
Nebeker *et al.* 1994	Y	Y	Y	Y	Y for some data only	**3**	19-21	45	6.8	stock solution	Alkalinity: 39 mg/L; Conductivity: 145 µS/cm
Nebeker *et al.*1995	Y	Y	Y	Y	Y for some data only	**3**	19-21	45	6.8	stock solution	Alkalinity: 39 mg/L; Conductivity: 145 µS/cm
Paulose 1988	N.A.	N.A.	Y	Y	N.A.	**4**	22-24	220-240	7.4-7.6	stock solution	Total Alkalinity: 110-125 ppm; Chloride: 76-82 ppm; DO: 7.2-8 ppm;
Pérez-Coll *et al.*1985	Y	N.A.	Y	N.A.	N.A.	**3**	20 and 30	N.A.	N.A.	stock solution	N.A.
Pérez-Coll *et al.* 1988	Y	Y	N.A.	N.A.	N.A.	**4**	20-21	N.A.	N.A.	stock solution	N.A.
Pérez-Coll and Herkovits 1990	Y	Y	Y	Y	Y	**2**	19-22	N.A.	N.A.	Pb added to Holtfreter	N.A.
Pramoda and Saidapur 1986	Y	N.A.	Y	Y	Y	**3**	N.A.	N.A.	N.A.	adults injected with CD	Cadmium was injected into the frogs

Reference	QA/QC						Modifying Factors				
	Control	Replication	Study design suitable	Data analysis	Treatment statistically significant relative to control	Rank	Temp. (°C)	Hardness (ppm $CaCO_3$)	pH	Sample type	Other
Punzo 1993b	Y	Y	Y	Y	Y	**3**	20.5-21.5	336.6-366	7.07-7.39	stock solution	Conductivity: 721 ± 30.4 µΩ/cm; Total Alkalinity: 280.3 ± 20.1 mg/L as $CaCO_3$; Nitrate: 0.77 ± 0.12 mg/L; Nitrite: 0.009 ± 0.001mg/L; Ammonia: 0.37 ± 0.02mg/L; Calcium: 84.8 ± 4.8 mg/L; Magnesium: 31.2 ± 1.8 mg/L; and Copper: 0.003 ± 0.0001 mg/L
Ranatunge *et al.* 2012	N.A.	Y	Y	Y	Y	**2**	27.36-27.44	N.A.	N.A.	stock solution	N.A.
Rao and Madhyastha 1987	Y	N.A.	Y	Y	Y	**4**	25.5-26	142-145.5	6.86-6.94	assume stock solution	Conductivity: 12.88 - 12.96 µΩ/cm; DO 8.2 - 8.4 ppm; Total Alkalinity: 97-98 ppm; Total EDTA precipitation
Sharma and Patiño 2009	Y	Y	Y	Y	Y	**1**	20.6-21.7	N.A.	7.5-8.3	stock solution	Standard WQ parameters are included in the paper
Shuhaimi-Othman *et al.* 2012	Y	Y	Y	Y	Y	**2**	28-30	16.8-20.4	6.4-6.6	stock solution	DO: 6.3 ± 0.1mg/L; Conductivity: 250 ± 0.6µS/cm
Slooff and Baerselman 1980	N.A.	N.A.	Y	Y	N.A.	**4**	19-21	N.A.	N.A.	stock solution	N.A.
Sobotka and Rahwan 1995	Y	N.A.	N.A.	N.A.	N.A.	**4**	20-26	110	7.7	stock solution	N.A.

Reference	QA/QC						Modifying Factors				
	Control	**Replication**	**Study design suitable**	**Data analysis**	**Treatment statistically significant relative to control**	**Rank**	**Temp. (°C)**	**Hardness (ppm $CaCO_3$)**	**pH**	**Sample type**	**Other**
Sparling *et al.* 2006	Y	Y	Y	Y	Y	**1**	21.6±1.7	7.3±4.59 mgCa/L	6.92±0.57	spiked	Water DO: 6.08 ± 1.22 mg/L; Conductivity: 168 ± 19 µS/cm; Ammonia: 0.39 ± 0.49 mg/L; Sediment: 8.25% Organic Carbon; 22.4% Sand; 38.4% Silt; 39.1% Clay
Sunderman *et al.* 1992	Y	Y	Y	Y	Y	**2**	23-24	N.A.	N.A.	metal added to FETAX[1] solution	N.A.
Woodall *et al.* 1988	Y	Y	Y	N.A.	N.A.	**2**	20-23	296	7	stock solution	N.A.

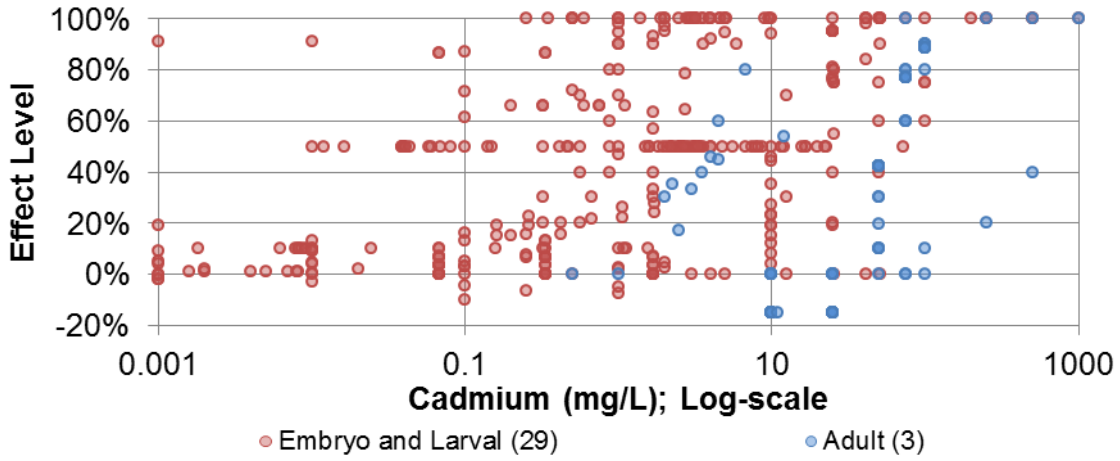
Y = Available/ Adequate; **N.A.** = Not Avaliable/Not Adequate; **DO** = Dissolved oxygen.

[1] FETAX: Frog Embryo Teratogenesis Assay-Xenopus

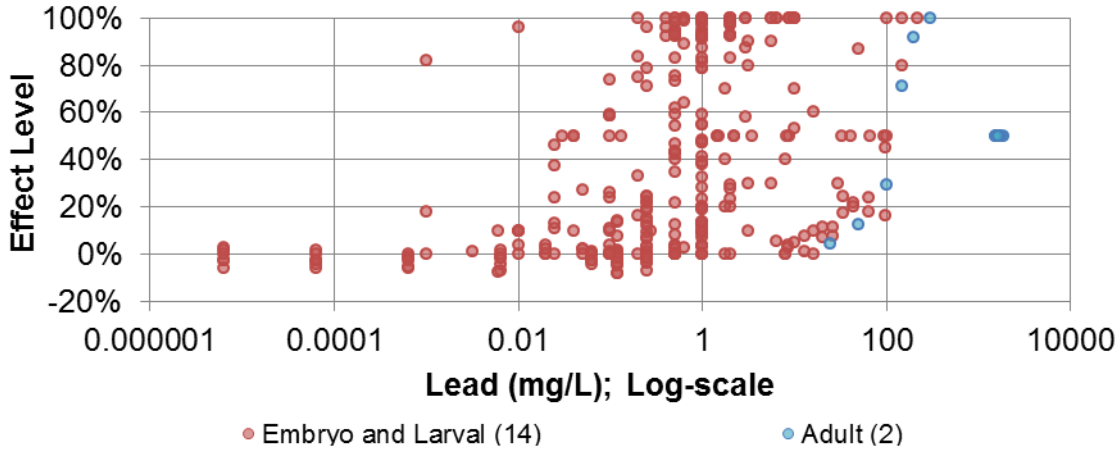
Appendix E. Life Stage

The literature-based toxicity data included in the multi-study concentration-response profiles cover a range of life stages. Most amphibian toxicity studies use the embryo or larval stage because they are easier and cheaper to maintain in a laboratory setting. As well, many of the critical developmental milestones occur during the embryonic or larval life stages prior to metamorphosis. Figure E1 presents the multi-study concentration-response profiles based on the life stages of the test species. In general, embryos and larvae (red dots) are much more susceptible to contaminants compared to adults (blue dots).

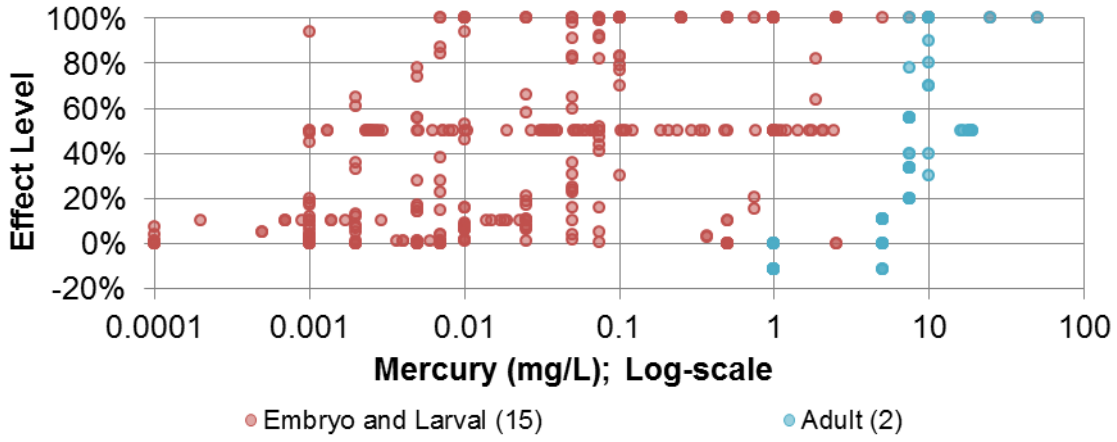
(a)



(b)



(c)



(d)

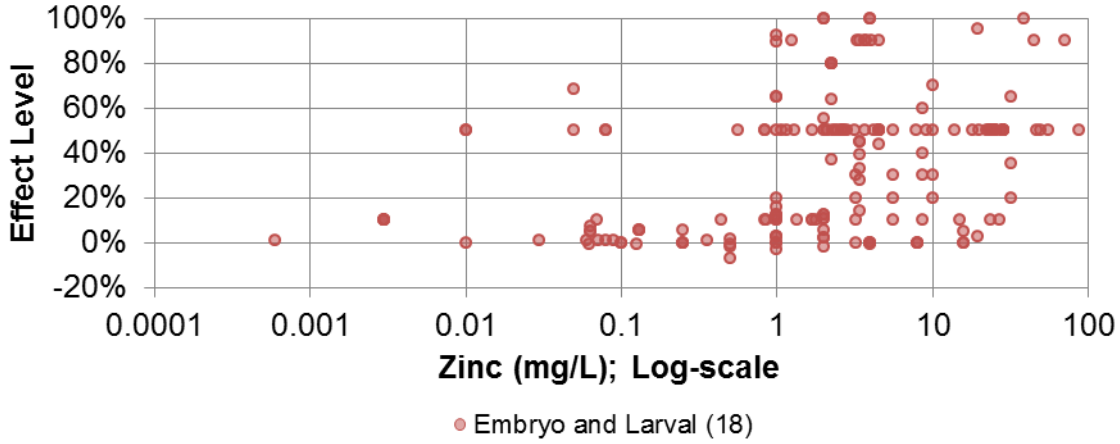


Figure E1. Multi-study concentration-response profile (water) by life stage, with the number of studies shown in parentheses.

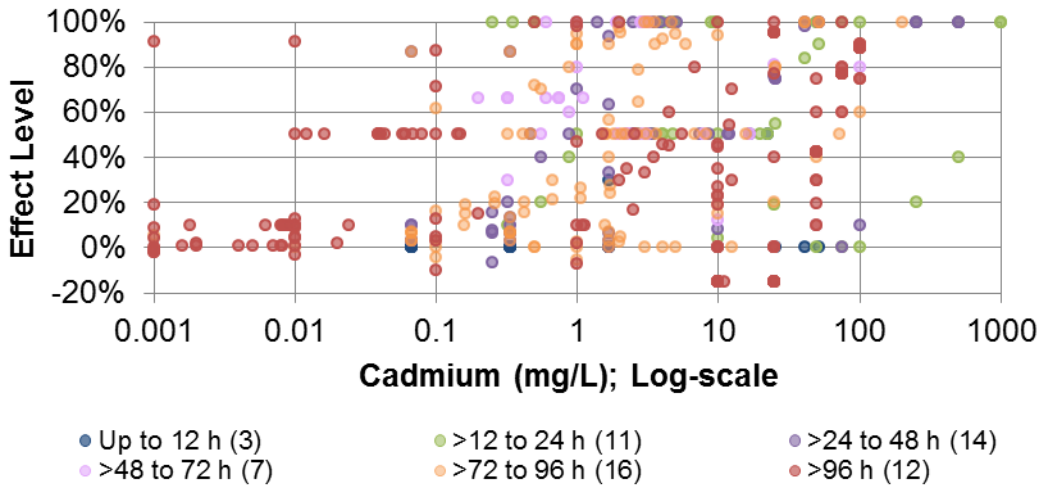
Appendix F. Exposure Time

The toxicological data included in the concentration-response profiles cover a range of exposure times. Section F1 examines whether exposure time has an impact on the effect level when other parameters (e.g., species, life stage) also vary. Section F2 presents results from individual studies that have assessed the change in effect levels due to increased exposure time, when other parameters (e.g., species, life stage) remain the same.

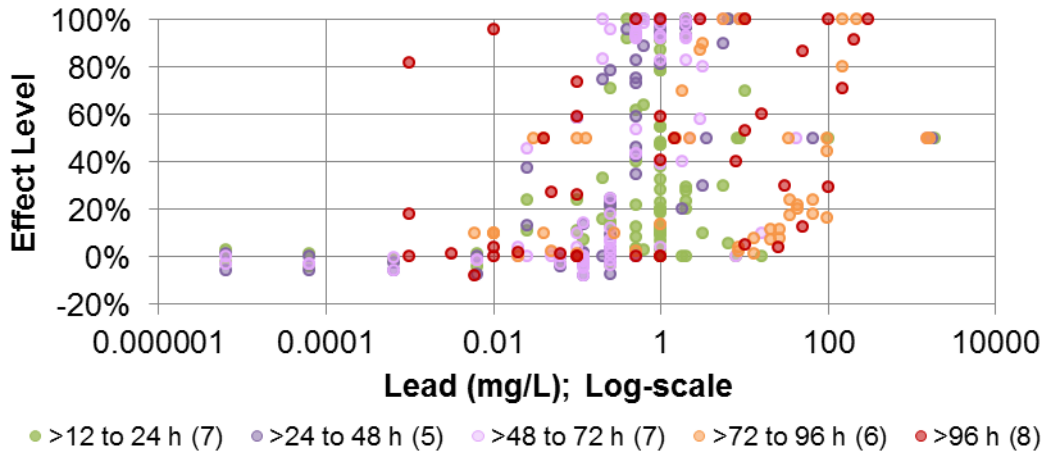
F1. Concentration-Response Profiles Plotted by Exposure Time

Figure F1 shows the concentration-response profiles with data displayed according to exposure time. In general, longer exposure times lead to higher effect levels, but the trend is confounded by other parameters such as modifying factors, life stage and species sensitivity.

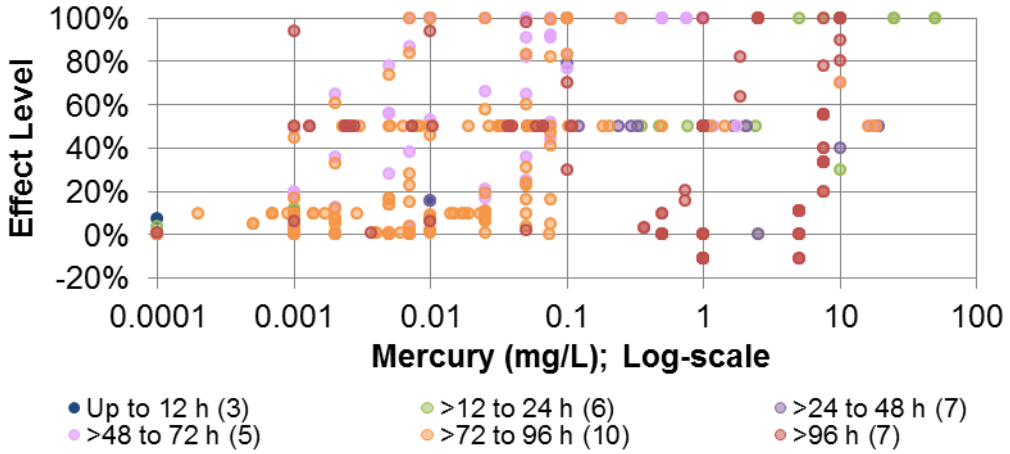
(a)


100%
80%
60%
40%
20%
0%
-20%
Effect Level
0.001
0.01
0.1
1
10
100
1000
Cadmium (mg/L); Log-scale
Up to 12 h (3)
>12 to 24 h (11)
>24 to 48 h (14)
>48 to 72 h (7)
>72 to 96 h (16)
>96 h (12)

(b)


100%
80%
60%
40%
20%
0%
-20%
Effect Level
0.000001
0.0001
0.01
1
100
10000
Lead (mg/L); Log-scale
>12 to 24 h (7)
>24 to 48 h (5)
>48 to 72 h (7)
>72 to 96 h (6)
>96 h (8)

(c)



(d)

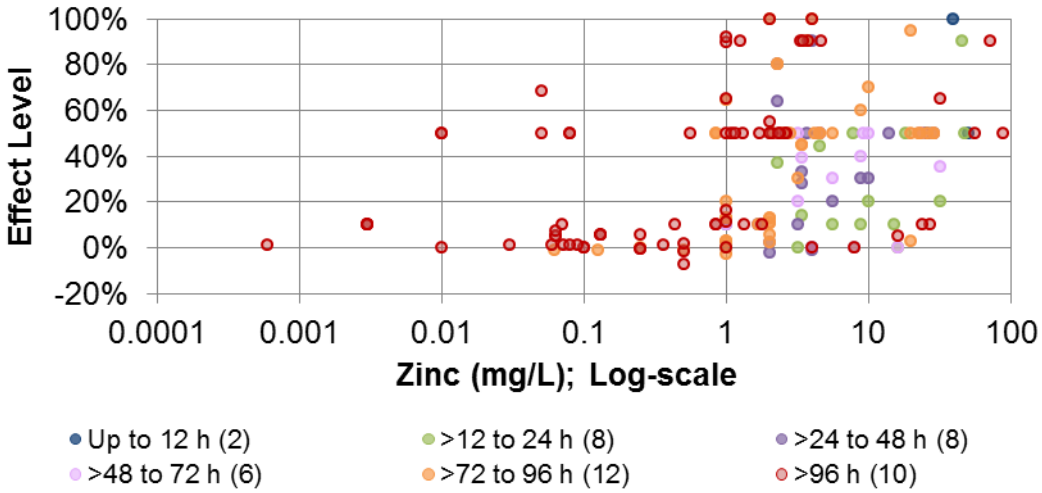


Figure F1. Multi-study concentration-response profiles (water), by exposure time, with the number of studies shown in parentheses.

F2. Individual Studies on Exposure Time

Several published studies investigated how prolonged exposure to contaminants in water influences the type or severity of effects experienced by amphibian receptors. These studies show that exposure time affects the magnitude of the effect.

Exposure Time - Greater than 96 h

Sobotka and Rahwan (1995) measured malformations in *Xenopus laevis (*African clawed frog) at different times post-fertilization. For short-term exposure, measurements were taken after exposing *X. laevis* to contaminants from day 1 to 4 post-fertilization, from day 2 to 4 post-fertilization and from day 3 to 5 post-fertilization. The effect levels for short-term exposure vary due to the difference in exposure times. Long-term exposure involved exposing individuals to contaminants from day 1 to > 21 days post-fertilization. Comparing short- and long-term exposures, Figure F2 shows that effect concentrations for long-term exposures were at least one order of magnitude lower than the effect concentrations for short-term exposures.

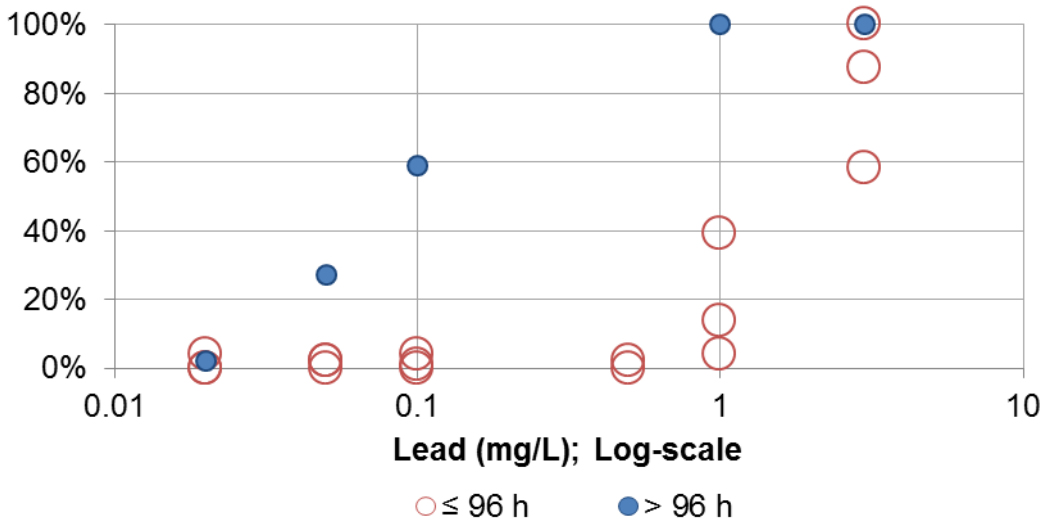
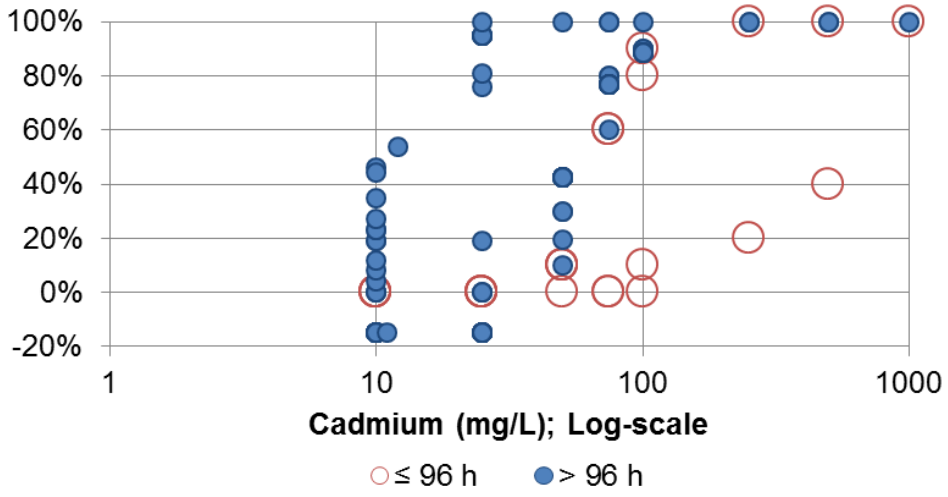


Figure F2. Adverse effects on *Xenopus laevis* from exposures to lead in water of less than or greater than 96 h (malformation). Data from Sobotka and Rahwan (1995).

Birge and Just (1973) measured cadmium and mercury induced mortality in *Lithobates pipiens* (Leopard Frog) exposed to cadmium and mercury for 1 to 24 days (increments of 1 day). As shown in Figure F3 for cadmium, longer exposure times (>96 h) resulted in effects at lower concentrations compared with shorter exposure times (≤96 h). For mercury, there is no apparent relationship between exposure times and effect concentrations.

(a)



(b)

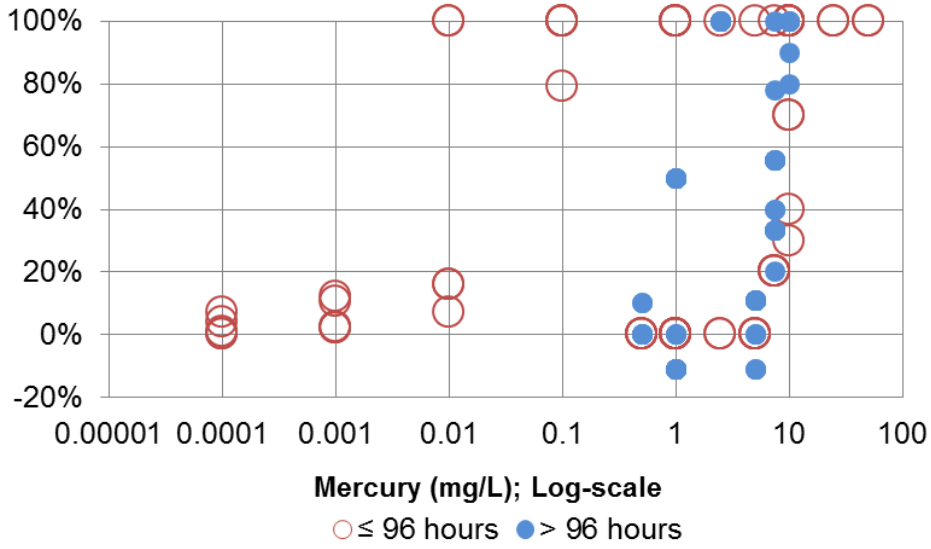


Figure F3. Adverse effects on *Lithobates pipiens* exposed to cadmium or mercury in water for 1 to 24 days (mortality). Data from Birge and Just (1973).

Brodeur *et al.* (2009) measured zinc-induced mortality in Argentine Common Toads (*Rhinella arenarum*). For toads exposed to zinc for longer than 96 h, effects appeared at lower concentrations compared with effects reported at ≤ 96 h (Figure F4).

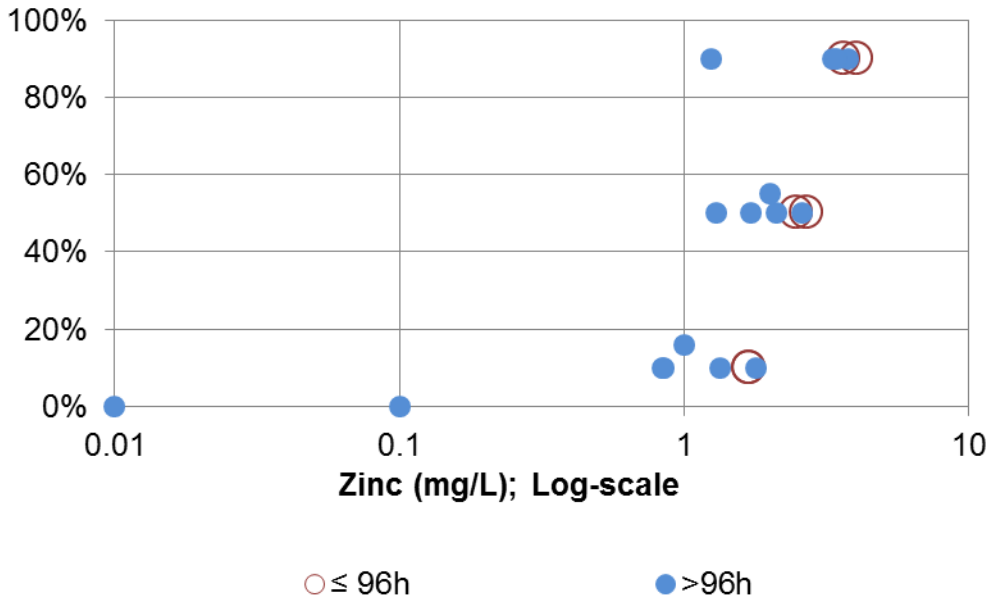
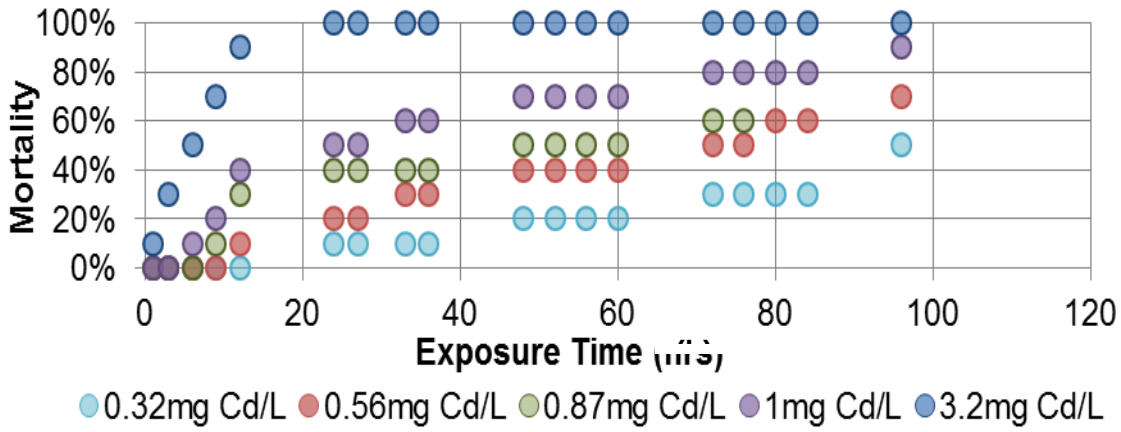


Figure F4. Mortality of *Rhinella arenarum* exposed to zinc in water for less than or greater than 96 h (mortality). Data from Brodeur *et al.* (2009).

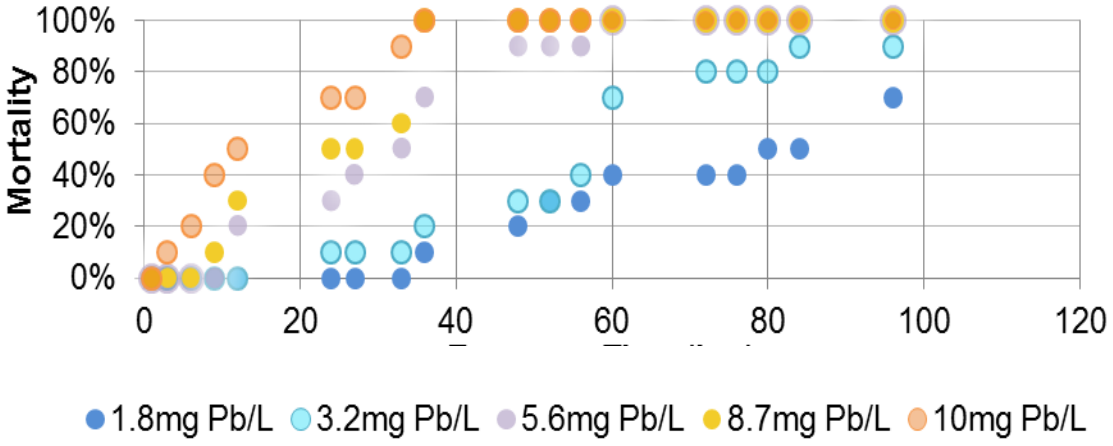
Exposure Time - Less than 96 h

Shuhaimi-Othman *et al.* (2012) reported metal-induced mortality (for cadmium, lead, and zinc) in *Duttaphrynus melanostictus* (Asian Common Toad) exposed across a range of exposure periods (1 to 96 h, increments of 3 h). Figure F5 shows mortality as a function of time. Effect levels increased with an increase in exposure time for all concentrations.

(a)



(b)



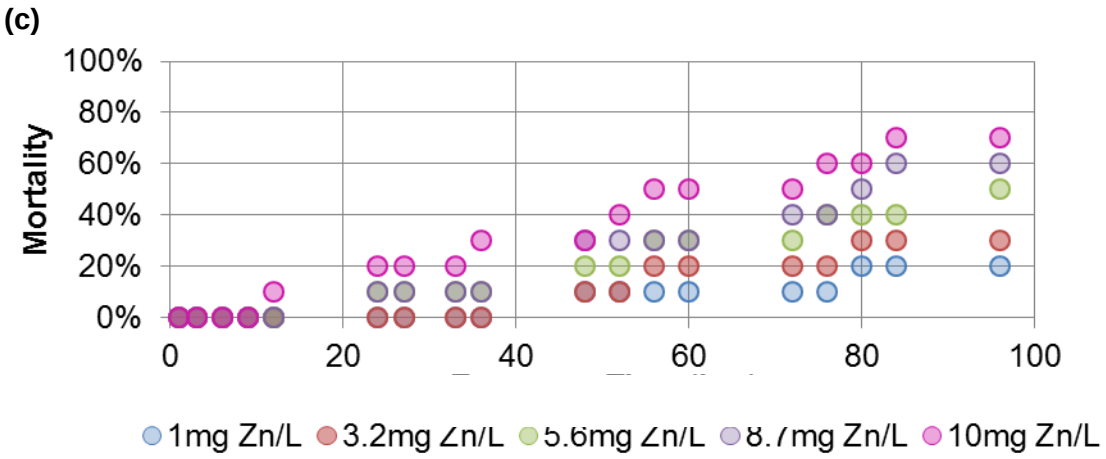


Figure F5. Mortality of *Duttaphrynus melanostictus* exposed for up to 96 h to cadmium (a), lead (b), and zinc (c) in water. Data from Shuhaimi-Othman *et al.* (2012).

Khangarot *et al.* (1985) reported LC_{50} values as a function of time for the Green Pond Frog (*Euphlyctis hexadactylus*). The biggest change in LC_{50} occurred between 24 h and 48 h (Figure F6**Error! Reference source not found.**).

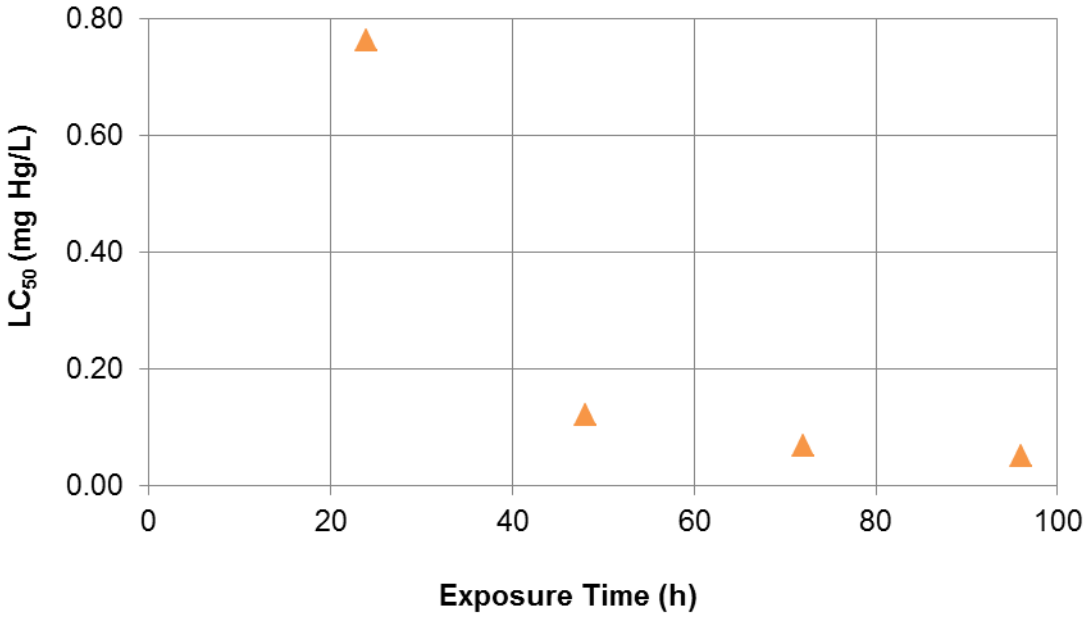


Figure F6. Mortality of *Euphlyctis hexadactylus* exposed to mecury in water, represented by LC_{50} values measured at different exposure durations. Data from Khangarot *et al.* (1985).

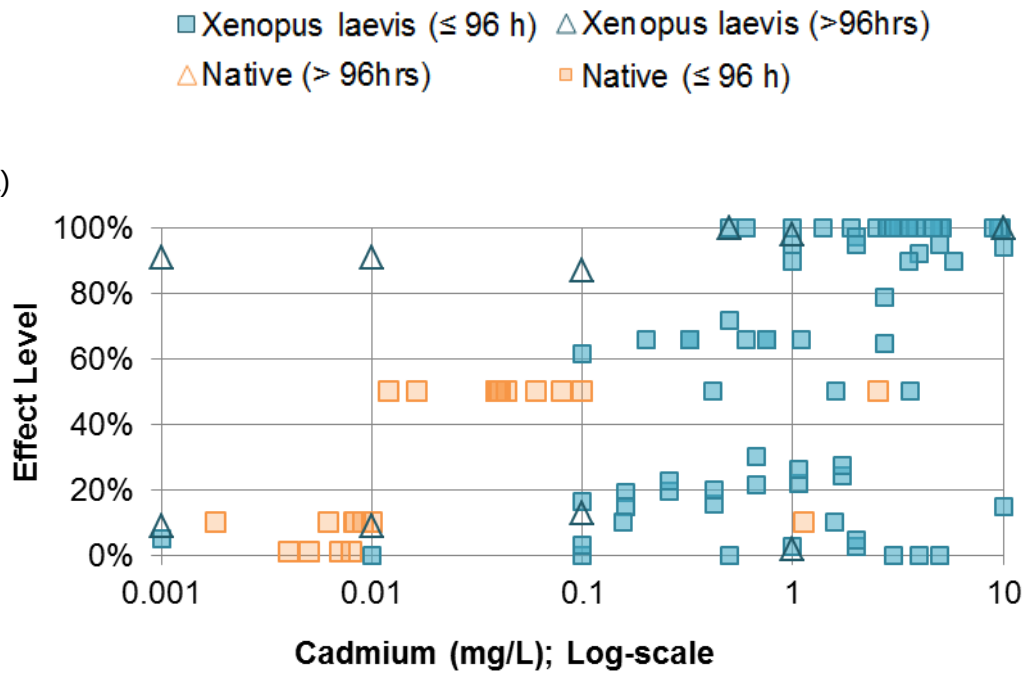
Appendix G. Species Sensitivity

The toxicological data included in the concentration-response profiles cover a range of amphibian taxa. Risk assessment practitioners have the option of selecting species that are relevant to the site if the required data are available. Section G1 includes concentration-response profiles that compare effects on species native to Canada to effects on *Xenopus laevis,* the most common laboratory test species. Individual studies that have investigated the difference in sensitivity among several species are presented in Section G2.

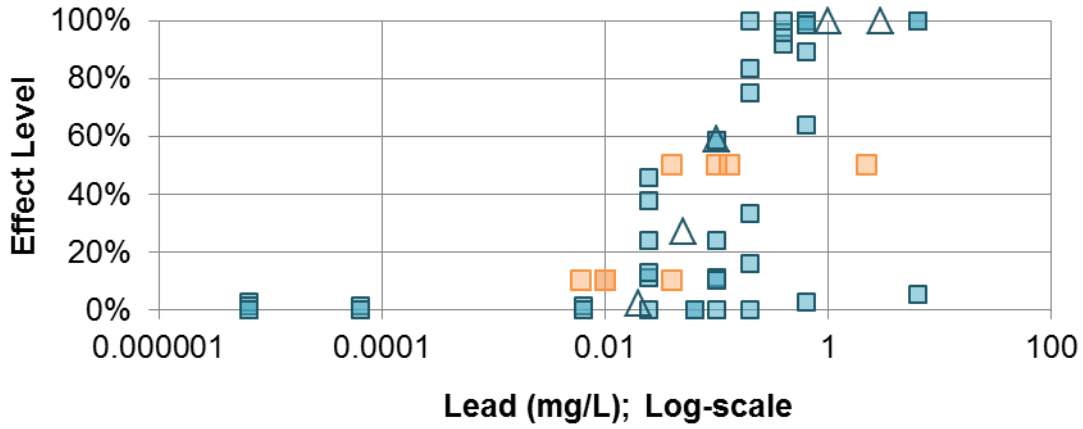
G1. Concentration-Response Profiles Plotted by Species

Xenopus laevis is commonly used for laboratory testing. The multi-study concentration-response profiles below have been filtered for *X. laevis* and species that are native to Canada (Figure G1**Error! Reference source not found.**). *X. laevis* appears to be less susceptible to contaminants compared to native amphibian species; however, a limited number of native species have been studied.

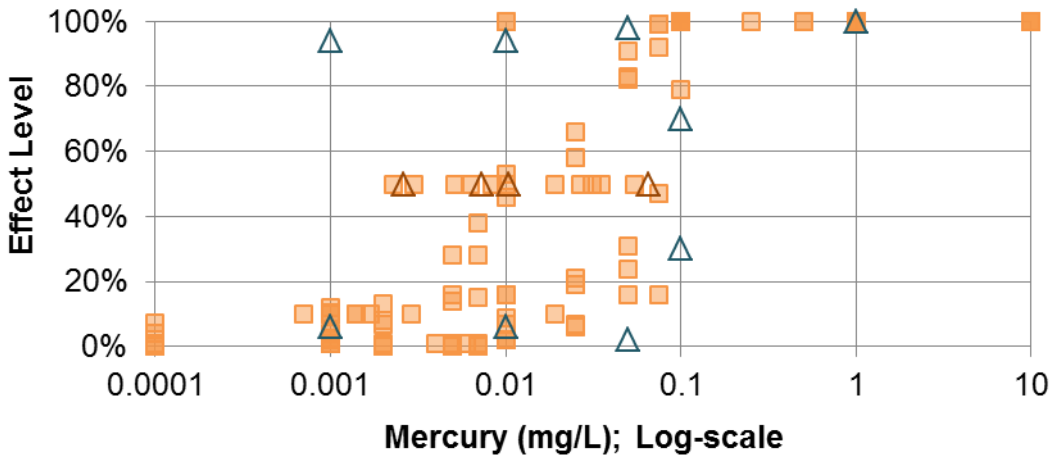
(a)


Xenopus laevis (≤ 96 h)
Xenopus laevis (>96hrs)
Native (> 96hrs)
Native (≤ 96 h)
100%
80%
60%
40%
20%
0%
Effect Level
0.001
0.01
0.1
1
10
Cadmium (mg/L); Log-scale

(b)


100%
80%
60%
40%
20%
0%
Effect Level
0.000001
0.0001
0.01
1
100
Lead (mg/L); Log-scale

(c)



(d)

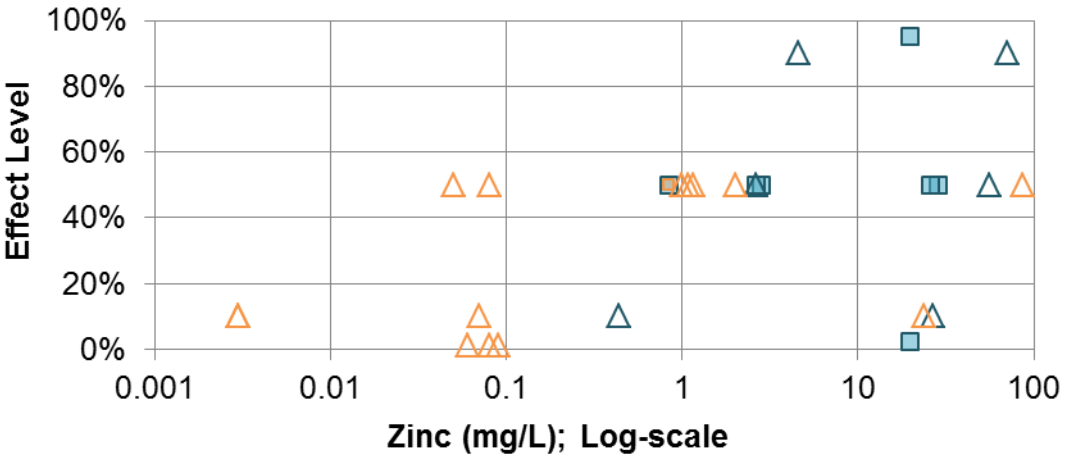


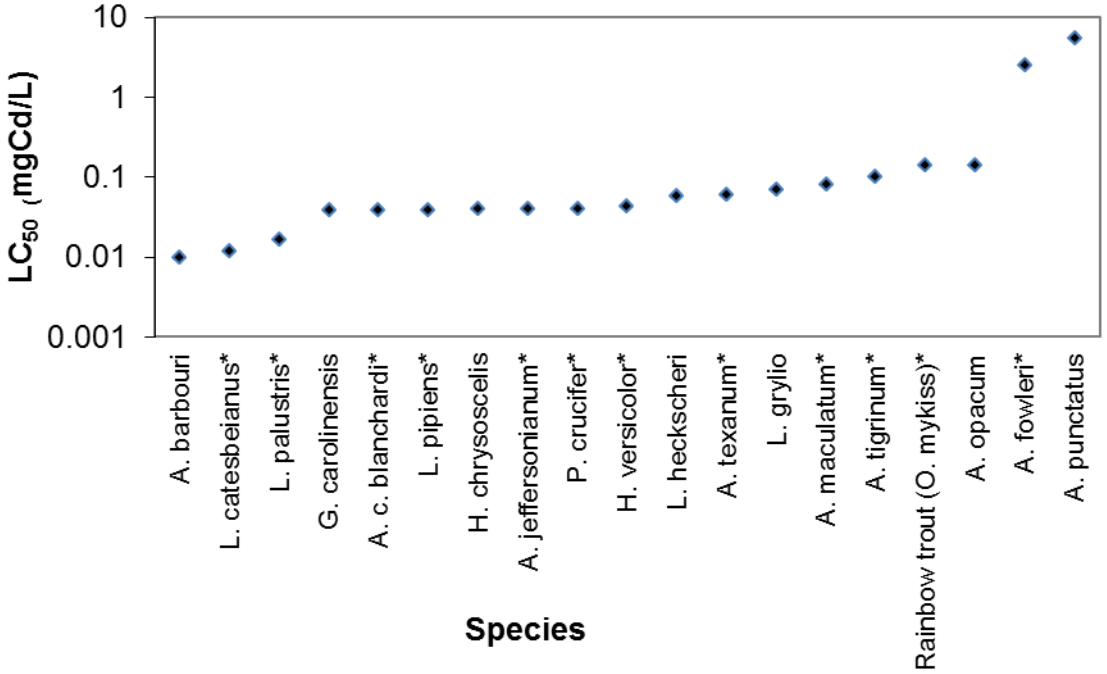
Figure G1. Multi-study concentration-response profiles (water) for embryos, by species and exposure time.[1]

[1] Darker shaded symbols indicate several overlaying data points.

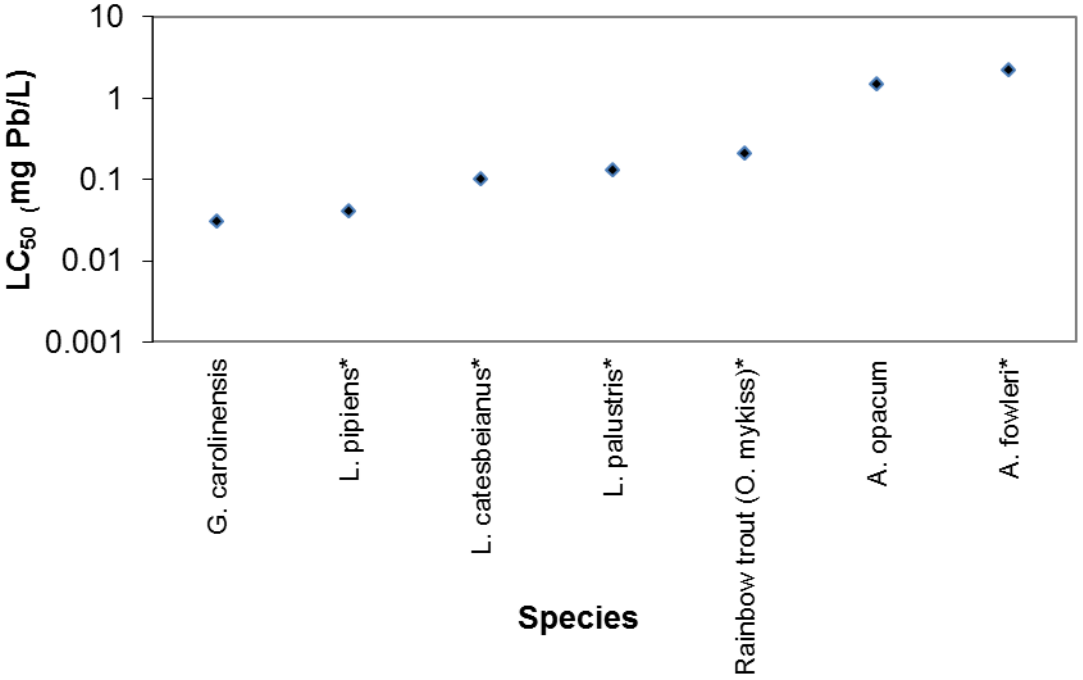
G2. Birge *et al.* (2000) Species Sensitivity Analysis

Birge *et al.* (2000) conducted the 96 h FETAX assay on various amphibian species. The goal of the study was to investigate the sensitivity of different amphibian species and determine how amphibians compare to juvenile rainbow trout. The authors evaluated amphibian sensitivity to 34 metals, and reported LC_{10} and LC_{50} values for each species. LC_{50} data from Birge *et al.* (2000) are presented in Figure G2 below for the four metals used in the multi-study concentration-response profiles developed in this ERA module. Concentration-response data that directly compare different species for a given COC are not available from this study.

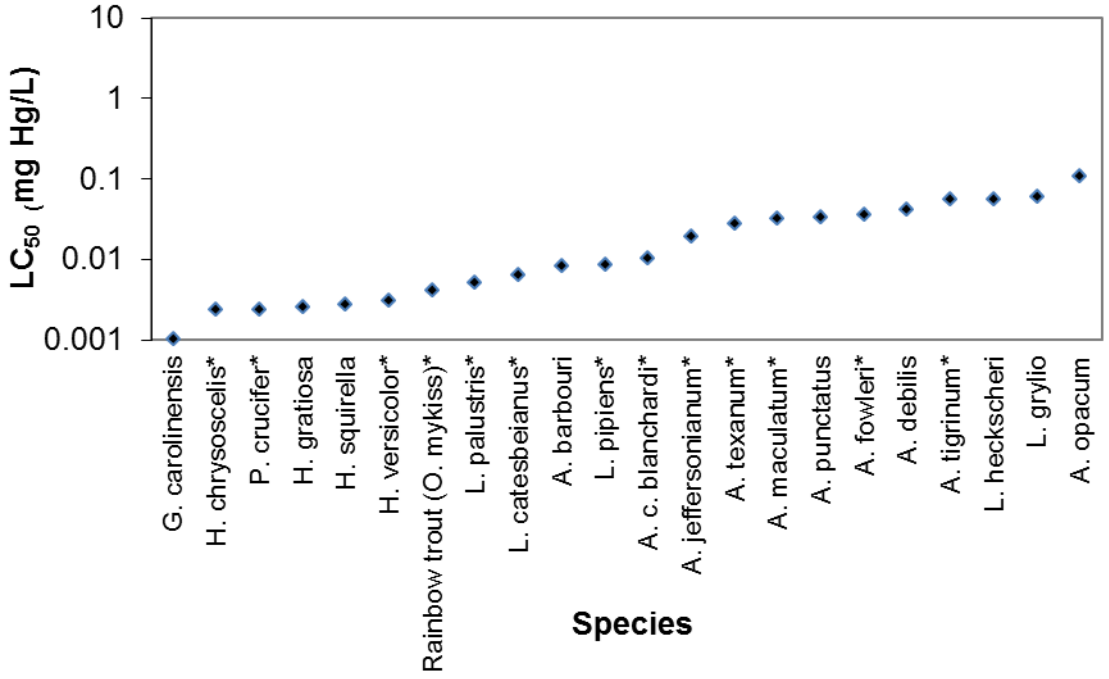
(a)


10
1
0.1
0.01
0.001
LC50 (mgCd/L)
A. barbouri
L. catesbeianus*
L. palustris*
G. carolinensis
A. c. blanchardi*
L. pipiens*
H. chrysoscelis
A. jeffersonianum*
P. crucifer*
H. versicolor*
L. heckscheri
A. texanum*
L. grylio
A. maculatum*
A. tigrinum*
Rainbow trout (O. mykiss)*
A. opacum
A. fowleri*
A. punctatus
Species

(b)


10
1
0.1
0.01
0.001
LC50 (mg Pb/L)
G. carolinensis
L. pipiens*
L. catesbeianus*
L. palustris*
Rainbow trout (O. mykiss)*
A. opacum
A. fowleri*
Species

(c)



(d)

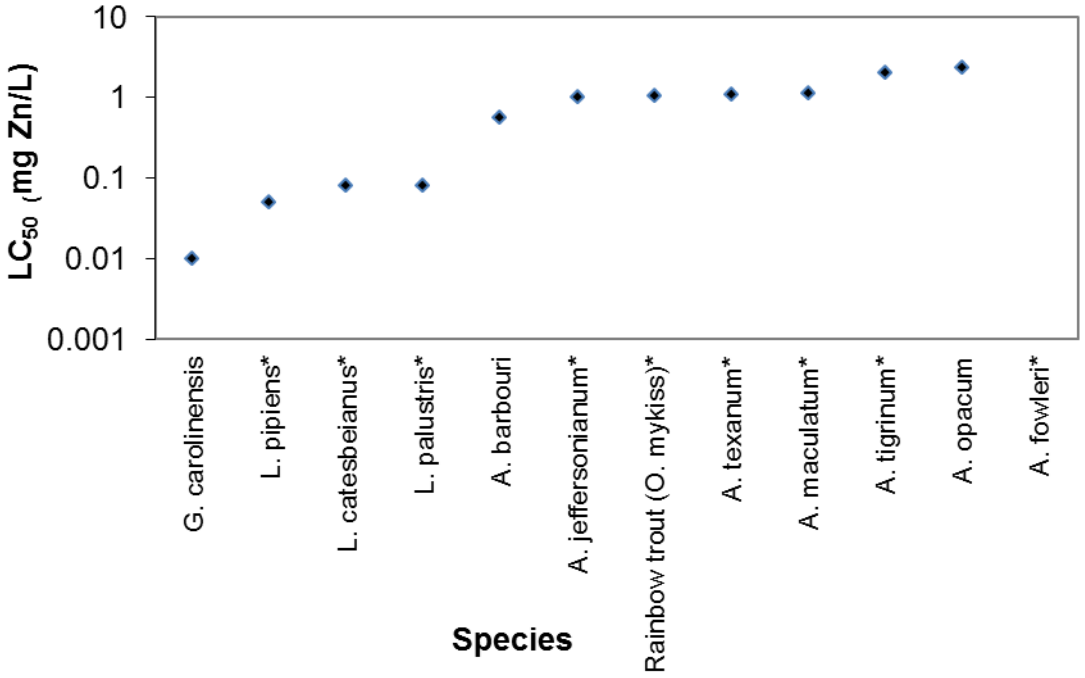
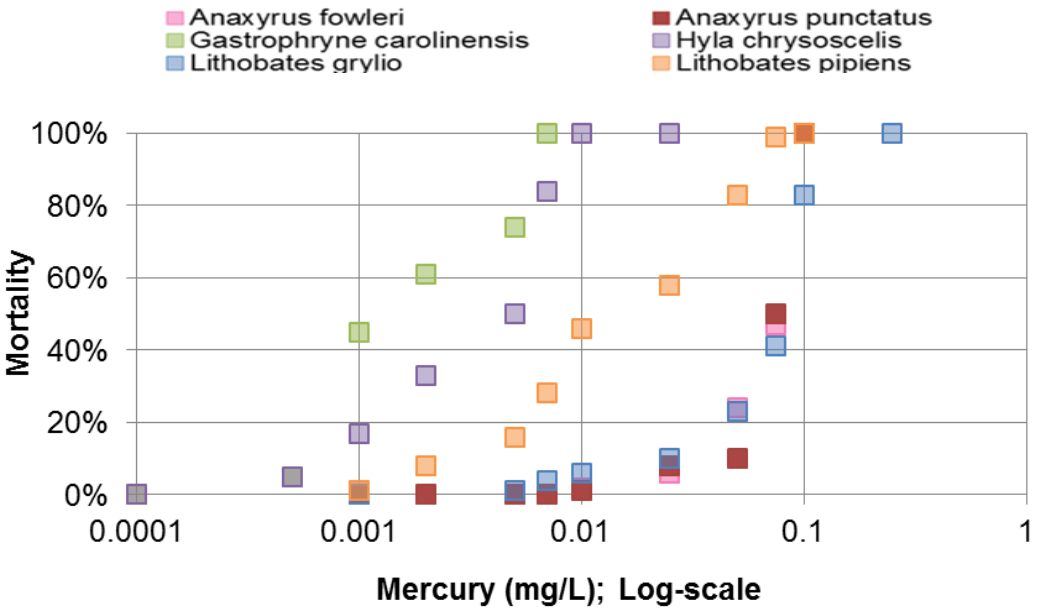


Figure G2. LC_{50} for several amphibian species and Rainbow Trout exposed to cadmium, lead, mercury, and zinc in water. Data from Birge *et al.* (2000).[1]

[1] The asterisk (*) indicates that the species is native to Canada.

Birge *et al.* (1983) reported mortality and malformations in amphibian larvae of six different species at several mercury concentrations. The shape of the concentration-response curves is similar for all six species; however, different species experienced effects at different contaminant concentrations. Figure G3 below summarizes the mortality and malformation responses for different amphibian species exposed to mercury.

a)



b)

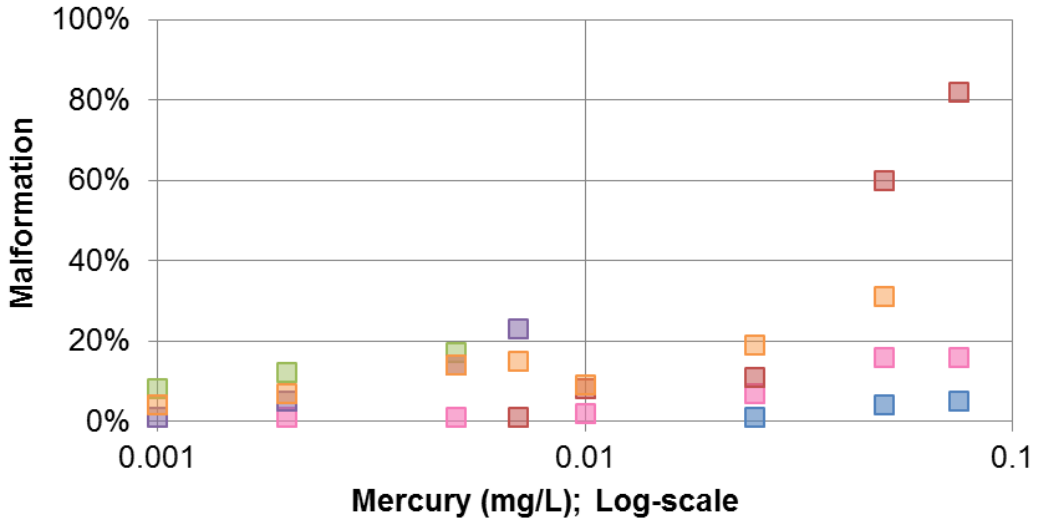


Figure G3. Concentration-response curves for survival (a), and malformation (b) endpoints, measured in six different amphibian species exposed to mercury in water. Data from Birge *et al.* (1983).

Appendix H. Common Names of Amphibian Species

Scientific Name	English Common Name	French Common Name	Reference
Acris blanchardi	Blanchard's Cricket Frog	Rainette grillon de Blanchard	GC 2019
Ambystoma barbouri	Streamside Salamander	Salamandre pourpre	IUCN 2018
Ambystoma gracile	Northwestern Salamander	Salamandre foncée	GC 2019
Ambystoma jeffersonianum	Jefferson Salamander	Salamandre de Jefferson	GC 2019
Ambystoma laterale	Blue-spotted Salamander	Salamandre à points bleus	AARQ 2019
Ambystoma macrodactylum	Long-toed salamander (Western subspecies)	Salamandre à longs doigts	GC 2019; IUCN 2018
Ambystoma macrodactylum columbianum	Long-toed Salamander (Eastern subspecies)	Salamandre à longs doigts (sous-espèce de l'Est)	GC 2019; IUCN 2018
Ambystoma macrodactylum krausei	Long-toed Salamander (Northern subspecies)	Salamandre à longs doigts (sous-espèce du Nord)	GC 2019; IUCN 2018
Ambystoma maculatum	Yellow-Spotted Salamander	Salamandre maculée	AARQ 2019
Ambystoma mavortium diaboli	Gray Tiger Salamander	Salamandre tigrée de Gray	IUCN 2018
Ambystoma mavortium melanostictum	Western Tiger Salamander (Blotched subspecies)	Salamandre tigrée de l'Ouest	GC 2019; IUCN 2018
Ambystoma mexicanum	Mexican Salamander	Salamandre du Mexique (Axolotl)	CITES 2019
Ambystoma opacum	Marbled Salamander	N/A	IUCN 2018
Ambystoma texanum	Small-mouthed Salamander	Salamandre à nez court	GC 2019
Ambystoma tigrinum	Eastern Tiger Salamander	Salamandre tigrée de l'Est	GC 2019
Anaxyrus americanus (formerly known as *Bufo americanus)*	Eastern American Toad	Crapaud d'Amérique	AARQ 2019
Anaxyrus boreas (formerly known as *Bufo boreas*)	Western Toad	Crapaud de l'Ouest	IUCN 2018

Scientific Name	English Common Name	French Common Name	Reference
Anaxyrus cognatus (formerly known as *Bufo cognatus*)	Great Plains Toad	Crapaud des steppes	GC 2019
Anaxyrus debilis (formerly known as *Bufo debilis*)	Green Toad	Crapaud vert (Author's translation)	IUCN 2018
Anaxyrus fowleri (formerly known as *Bufo fowleri*)	Fowler's Toad	Crapaud de Fowler	GC 2019
Anaxyrus hemiophrys	Canadian Toad	Crapaud du Canada	NW 2019
Anaxyrus punctatus (formerly known as *Bufo punctatus*)	Red-spotted Toad	N/A	Amphibiaweb 2019
Aneides vagrans	Wandering Salamander	Salamandre errante	Blouin-Demers 2012, GC 2019
Ascaphus montanus	Rocky Mountain Tailed Frog	Grenouille-à-queue des Rocheuses	GC 2019
Ascaphus truei	Coastal Tailed Frog	Grenouille-à-queue côtière	GC 2019
Desmognathus fuscus	Northern Dusky Salamander	Salamandre sombre du Nord	AARQ 2019, GC 2019
Desmognathus ochrophaeus	Allegheny Mountain Dusky Salamander	Salamandre sombre des montagnes	AARQ 2019, Blouin-Demers 2012, GC 2019
Dicamptodon tenebrosus	Coastal Giant Salamander	Grande Salamandre du Nord	GC 2019
Duttaphrynus melanostictus (formerly known as *Bufo melanostictus*)	Asian common toad	N/A	IUCN 2018
Ensatina eschscholtzii oregonensis	Oregon Ensatina	Salamandre variable de l'Oregon	Blouin-Demers 2012, GC 2019
Euphlyctis ehrenbergii (formerly known as *Rana cyanophlyctis*)	Arabian Skittering Frog	N/A	IUCN 2018

Scientific Name	English Common Name	French Common Name	Reference
Euphlyctis hexadactylus (formerly known as *Rana hexadactyla*)	Indian Bullfrog; Indian Five-fingered Frog Green Pond Frog; Indian Green Frog	N/A	IUCN 2018
Eurycea bislineata	Northern Two-lined Salamander	Salamandre à deux lignes	AARQ 2019
Gastrophryne carolinensis	Eastern Narrow-mouthed Toad	N/A	IUCN 2018
Gyrinophilus porphyriticus	Spring Salamander	Salamandre pourpre	AARQ 2019, Blouin-Demers 2012
Hemidactylium scutatum	Four-toed Salamander	Salamandre à quatre orteils	AARQ 2019, GC 2019
Hoplobatrachus tigerinus (formerly known as *Rana tigerina*)	Indian Bullfrog	Crapaud indien	IUCN 2018
Hyla chrysoscelis	Cope's Gray Treefrog	Rainette criarde	Blouin-Demers 2012
Hyla gratiosa (also known as *Dryophytes gratiosus*)	Barking Treefrog	Rainette jappeuse	Blouin-Demer, 2012
Hyla squirella	Squirrel Treefrog	Rainette écureuil	Blouin-Demer, 2012
Hyla versicolor	Gray Treefrog	Rainette versicolore	AARQ 2019, Blouin-Demers 2012
Lithobates catesbeiana (*Rana catesbeianus*)	American Bullfrog	Ouaouaron	AARQ 2019, Blouin-Demers 2012
Lithobates clamitans melanota (formerly known as *Rana clamitans*)	Green Frog	Grenouille verte	AARQ 2019, Blouin-Demers 2012
Lithobates grylio (formerly known as *Rana grylio*)	American Pig Frog	Creux-creux	Blouin-Demers 2012
Lithobates palustris (formerly known as *Rana palustris*)	Pickerel Frog	Grenouille des marais	AARQ 2019, Blouin-Demers 2012

Scientific Name	English Common Name	French Common Name	Reference
Lithobates pipiens (formerly known as *Rana pipiens*)	Northern Leopard Frog	Grenouille léopard du Nord	Blouin-Demers 2012
Lithobates septentrionalis	Mink Frog	Grenouille du Nord	AARQ 2019, Blouin-Demers 2012
Lithobates sphenocephalus (formerly known as *Rana sphenocephala*)	Southern Leopard Frog	Grenouille léopard de Floride	Blouin-Demers 2012
Lithobates sylvaticus	Wood Frog	Grenouille des bois	AARQ 2019, Blouin-Demers 2012
Microhyla ornata	Ant Frog	N/A	IUCN 2018
Necturus maculosus maculosus	Common Mudpuppies	Necture tacheté	AARQ 2019, Blouin-Demers 2012
Notophthalmus viridescens	Eastern Newt	Triton vert	AARQ 2019, Blouin-Demers 2012
Pelophylax ridibundus (formerly known as *Rana ridibunda*)	Eurasian Marsh Frog	N/A	IUCN 2018
Plethodon cinereus	Eastern Red-backed Salamander	Salamandre cendrée	AARQ 2019
Plethodon idahoensis	Coeur d'Alene Salamander	Salamandre de Cœur d’Alène	Blouin-Demers 2012, GC 2019
Plethodon vehiculum	Western Red-backed Salamander	Salamandre à dos rayé	Blouin-Demers 2012, GC 2019
Pseudacris crucifer (formerly known as *Hyla crucifer*)	Spring Peeper	Rainette crucifère	AARQ 2019, Blouin-Demers 2012
Pseudacris maculata	Boreal Chorus Frog	Rainette faux-grillon boréal	AARQ 2019, Blouin-Demers 2012
Pseudacris regilla	Pacific Treefrog	Rainette du Pacifique	Blouin-Demers 2012, IUCN 2018
Pseudacris triseriata	Western Chorus Frog	Rainette faux-grillon de l’Ouest	AARQ 2019, Blouin-Demers 2012
Rana aurora	Northern Red-legged Frog	Grenouille à pattes rouges du Nord	Blouin-Demers, 2012

Scientific Name	English Common Name	French Common Name	Reference
Rana heckscheri (*Lithobates heckscheri*)	River Frog	Grenouille des rivières	Blouin-Demers 2012
Rana luteiventris	Columbia Spotted Frog	Grenouille maculée de Columbia	Blouin-Demers 2012
Rana pretiosa	Oregon Spotted Frog	Grenouille maculé de l'Orégon	AARQ 2019, Blouin-Demers 2012
Rhinella arenarum (formerly known as *Bufo arenarum*)	Argentine Common Toad	N/A	IUCN 2018
Spea bombifrons	Plains Spadefoot	Crapaud pied-bêche des Plaines	Blouin-Demers 2012
Spea intermontana	Great Basin Spadefoot	Crapaud pied-bêche du Grand Bassin	IUCN 2018, Blouin-Demers 2012
Sphaerotheca breviceps (formerly known as *Rana breviceps*)	Southern Burrowing Frog	N/A	IUCN 2018
Taricha granulosa	Rough-skinned Newt	Triton rugueux	Blouin-Demers 2012
Taricha granulosa	Rough-skinned Newt	N/A	IUCN 2018
Xenopus laevis	African Clawed Frog	N/A	IUCN 2018

N/A = not available

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