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Critical Review

Species Sensitivity Distribution Evaluation for Selenium in Fish Eggs: Considerations for Development of a Canadian Tissue-Based Guideline

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ABSTRACT

A freshwater Se guideline was developed for consideration based on concentrations in fish eggs or ovaries, with a focus on Canadian species, following the Canadian Council of Ministers of the Environment protocol for developing guideline values. When sufficient toxicity data are available, the protocol recommends deriving guidelines as the 5th percentile of the species sensitivity distribution (SSD). When toxicity data are limited, the protocol recommends a lowest value approach, where the lowest toxicity threshold is divided by a safety factor (e.g., 10). On the basis of a comprehensive review of the current literature and an assessment of the data therein, there are sufficient egg and ovary Se data available for freshwater fish to develop an SSD. For most fish species, Se EC10 values (10% effect concentrations) could be derived, but for some species, only no-observed-effect concentrations and/or lowest-observed-effect concentrations could be identified. The 5th percentile egg and ovary Se concentrations from the SSD were consistently 20 μg/g dry weight (dw) for the best-fitting distributions. In contrast, the lowest value approach using a safety factor of 10 would result in a Se egg and ovary guideline of 2 μg/g dw, which is unrealistically conservative, as this falls within the range of egg and ovary Se concentrations in laboratory control fish and fish collected from reference sites. An egg and ovary Se guideline of 20 μg/g dw should be considered a conservative, broadly applicable guideline, as no species mean toxicity thresholds lower than this value have been identified to date. When concentrations exceed this guideline, site-specific studies with local fish species, conducted using a risk-based approach, may result in higher egg and ovary Se toxicity thresholds. Integr Environ Assess Manag 2012;8:6–12. © 2011 SETAC

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INTRODUCTION

This paper provides an evaluation of species sensitivity distributions (SSDs) for selenium (Se) in fish eggs and ovaries, and considerations for developing a guideline based on Se concentration in fish eggs and ovaries, with a focus on Canadian species. The protocol for deriving national water quality guidelines for the protection of aquatic life in Canada is described by the Canadian Council of Ministers of the Environment (CCME 2007). Although the protocol does not specifically address bioaccumulation and tissue residues, instead addressing concentrations in water, the general methodology can be applied to these endpoints. The protocol specifies minimum data set requirements as well as the need for representation of fish and invertebrates (i.e., Type B2 requirements) and aquatic plants (i.e., Type B1 and Type A1 requirements). However, the scientific literature supports the notion that fish are the most sensitive group of aquatic organisms to Se (Janz et al. 2010); moreover, Se egg and ovary concentrations are the most appropriate tissues for evaluating whether Se concentrations have the potential to result in toxicity to larval fish as a result of maternal transfer (Lemly 1996; DeForest et al. 1999; Janz et al. 2010). These latter points comprise the justification for developing a Se guideline that is based on fish egg and ovary concentrations. Other jurisdictions are also using a tissue-based approach for developing Se criteria or guidelines. The US Environmental Protection Agency (USEPA), for example, developed a draft whole-body, fish-based criterion (USEPA 2004) and is currently revising the draft whole-body Se criterion to the Se concentration in fish eggs (CG Delos, personal communication).

This CCME protocol recommends the use of the SSD approach when adequate data are available. The protocol recognizes that consistently defined effects concentrations cannot always be defined between studies and recommends the following hierarchy: EC10 → EC11–25 → MATC → NOEC → LOEC → EC26–49 → nonlethal EC50. The ECx is the effect concentration for x% of the species, the NOEC is the no-observed-effect concentration, the LOEC is the lowest-observed-effect concentration, and the MATC (maximum allowable toxicant concentration) is the geometric mean of the NOEC and LOEC. For deriving a long-term exposure guideline, the protocol recommends the 5th percentile of the SSD. In addition to the SSD-based approach, the protocol includes a lowest value approach, which is a generic method to be used when toxicity data are limited. In this approach, the long-term exposure guideline is extrapolated from low-effects threshold data and by applying a safety factor (e.g., 10) to the lowest toxicity threshold identified.

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MATERIALS AND METHODS

Selenium toxicity values used to derive SSDs

Toxicity thresholds based on egg (or ovary) Se are currently available for 12 freshwater species (Table 1), although the distribution of 2 species, razorback sucker (*Xyrauchen texanus*) and Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*), does not include Canadian waters. The basic study design for the toxicity thresholds compiled included exposure of parent fish to dietary organic Se (either in a natural diet or a synthetic diet spiked with selenomethionine) and evaluation of deformities (e.g., craniofacial, skeletal, and finfold defects), edema, and mortality in larval offspring. The types of toxicity studies used to derive each threshold vary, and consequently it is not always possible to derive the toxicity threshold for each species by means of a consistent approach. For example, some studies were conducted in the laboratory, where the test organisms were exposed to a series of dietary organic Se concentrations, whereas in other studies, existing fish populations were naturally exposed to Se in the field at one or more exposure sites and a reference site. For the latter, Se had either previously been identified as the element responsible for observed toxicity at a site, or it was confirmed through experimental design, exposure and accumulation evaluation of other constituents, or both. Furthermore, the most sensitive endpoints in most studies with field-exposed fish were larval deformities (including craniofacial, skeletal, and finfold defects, and edema), which are diagnostic of Se exposure (Maier and Knight 1994; Lemly 1997) and is consistent with the mode of toxic action for Se (Janz et al. 2010).

Table 1 summarizes the toxicity thresholds extracted from each study and identifies the final threshold used for each species in this evaluation. When both egg and ovary Se concentrations were reported in a study, preference was given to egg Se-based concentrations because it is egg Se to which fish larvae are exposed during yolk sac absorption (Janz et al. 2010). Furthermore, if multiple endpoints were reported in a given study, the most sensitive endpoint is presented in Table 1. Following the hierarchy defined in CCME (2007), preference was given to EC10 values. For brook trout and white sucker, EC10 values could not be derived due to limitations with the available toxicity data; rather, EC10 values were approximated on the basis of the EC06 and EC13 values that could be derived. The brook trout EC06 was based on a 6% increase in craniofacial deformities in larvae from parent fish collected from a Se-exposed site relative to a reference site (and may also be considered a NOEC), whereas the EC13 for white sucker was the percentage of total deformities in larvae from parent fish collected from a Se-exposed site. Given the steepness of the concentration–response curve typically observed for Se (e.g., Doroshov et al. 1992; Coyle et al. 1993; CP&L 1997; Holm et al. 2005; McDonald et al. 2010), the EC06 and EC13 values are not expected to differ greatly from the EC10. For fathead minnow and razorback sucker, effects concentrations could only be defined on the basis of NOECs, LOECs, or the MATC. In the case of fathead minnow, a NOEC ($>10.92\ \mu g/g$ dry weight [dw]) and LOEC ($<23.6\ \mu g/g$ dw) were available from separate studies. The NOEC of $>10.92\ \mu g/g$ dw was not used as the fathead minnow threshold because this ovary Se concentration had less than a 2% effect on reproductive endpoints including a positive effect, relative to controls, for some endpoints. The LOEC of $<23.6\ \mu g/g$ dw was associated with 24.6% larval edema, which is within the range of acceptable effect concentrations in the second level of the effects concentration hierarchy (i.e., EC11–25). Accordingly, an effects threshold of $<23.6\ \mu g/g$ dw was used for the fathead minnow. Because the ranges of razorback sucker and Yellowstone cutthroat trout do not include Canada, they were not included in the primary SSD evaluation. However, because they may be considered surrogates for other untested Canadian sucker and salmonid species, they were included in a sensitivity analysis in order to evaluate whether they would influence the 5th percentile of the SSD.

It has been suggested that coldwater species, including trout, white sucker, and northern pike, are less sensitive to dietary Se than warmwater fish species (Chapman 2007); however, egg- or ovary-based Se thresholds for these coldwater species appear to bracket thresholds for what are sometimes referred to as warmwater species, including bluegill, largemouth bass, and fathead minnow. It should also be noted that all 3 of these species are native to parts of Canada (www.fishbase.org). Moreover, reducing the number of species in the SSD unnecessarily results in greater extrapolation to the 5th percentile of the distribution (i.e., more conservative estimates are required because the sample size is smaller). Accordingly, Se toxicity thresholds for both coldwater and warmwater fish species were included in the SSD.

SSD development

Selenium SSDs were developed on the basis of the species mean toxicity thresholds summarized in Table 1. In addition, sensitivity analyses were conducted to evaluate the influence of removing certain species from the SSD. For example, SSDs were developed with brook trout and white sucker removed from the data set because there was higher uncertainty associated with these toxicity thresholds. In addition, a coldwater species SSD was developed by considering only toxicity data for trout, northern pike, and white sucker. As noted above, a sensitivity analysis was also conducted with and without razorback sucker and Yellowstone cutthroat trout included in the SSD. The best-fitting distributions to the log-transformed Se toxicity threshold data were identified by Decision Tools software (Palisade Corporation 2008). This software uses the following 3 goodness-of-fit statistics to describe each distribution's fit to the raw toxicity data: 1) chi-square; 2) Komolgorov-Smirnov; and 3) Anderson-Darling. The 3 best-fitting distributions were selected for each SSD in order to evaluate whether the SSD and its associated 5th percentile were sensitive to the statistical distribution type selected.

RESULTS AND DISCUSSION

We first briefly summarize the results of the lowest value approach; the remainder of this section focuses on the results of the SSD evaluations.

Lowest value approach

By means of the CCME's lowest value approach, the lowest toxicity value identified is divided by a safety factor (CCME 2007). In this evaluation, the lowest species mean toxicity value identified was a NOEC of $20\ \mu g/g$ dw. Division

Table 1. Summary of studies evaluating selenium toxicity to embryos and larvae resulting from maternal transfer.[a]

Species	Reference	Adult exposure	Endpoint	Tissue	Endpoint statistic[b]	Se (μg/g dw)	Species mean Se threshold (μg/g dw)
Bluegill	Bryson et al. (1984)	Field	Larval mortality	Ovary	LOEC	<49	21.5
	Bryson et al. (1985a)	Field	Hatchability, swim-up	Ovary	NOEC	>9.1	—
		Field	Hatchability, swim-up	Ovary	LOEC	<30	—
	Bryson et al. (1985b)	Field	Hatchability, swim-up	Ovary	NOEC	>14.8	—
		Field	Hatchability, swim-up	Ovary	NOEC	>9.2	—
	Gillespie and Baumann (1986)	Field	Larval edema	Ovary	LOEC	<38.6[d]	v
	Doroshov et al. (1992)	Lab	Larval edema	Egg	EC10	*21*[c]	—
	Coyle et al. (1993)	Lab	Larval mortality	Egg	EC10	*22*[c]	—
	Hermanutz et al. (1996)	Mesocosm	Larval edema	Ovary	EC10	30[c]	
Brook trout	Holm (2002); Holm et al. (2003, 2005)	Field	Larval deformities	Egg	NOEC	*20*[e]	20
Brown trout	Formation Environmental (2011a)	Field	Alevin mortality	Egg	EC10	*20.8*	20.8
			Larval deformities	Egg	EC10	22.0	—
Westslope cutthroat trout	Kennedy et al. (2000)	Field	Larval deformities, mortality	Egg	NOEC	>21	21
	Rudolph et al. (2008)	Field	Alevin mortality	Egg	EC10	*17*[c]	—
	Nautilus Environmental (2011)	Field	Alevin mortality	Egg	EC10	*24.8*	—
Yellowstone cutthroat trout	Hardy et al. (2010)	Lab	Larval deformities, mortality	Egg	NOEC	>16.04	—
	Formation Environmental (2011b)	Field	Alevin mortality	Egg	MATC	*25*	25
Dolly Varden	McDonald et al. (2010)	Field	Larval deformities	Egg	EC10	*54*	54
Fathead minnow	Ogle and Knight (1989)	Lab	Reproduction	Ovary	NOEC	>10.92	<23.6
	Schultz and Hermanutz (1990)	Mesocosm	Larval edema, lordosis	Ovary	LOEC	*<23.6*[d]	—
Largemouth bass	CP&L (1997)	Lab	Larval mortality	Ovary	EC10	*22*	22
Northern pike	Muscatello et al. (2006)	Field	Larval deformities	Egg	EC10	*20.4*	20.4
Rainbow trout	Holm (2002); Holm et al. (2003, 2005)	Field	Larval deformities	Egg	EC10	*23*[c e]	23
Razorback sucker	Hamilton et al. (2005a, 2005b)	Field	Larval deformities	Egg	MATC	*41.9*	41.9
White sucker	de Rosemond et al. (2005)	Field	Larval deformities	Egg	EC13	*26*	26

[a] Italic values were used to derive the species mean Se thresholds in the far right column. EC10 = 10% effective concentration; EC13 = 13% effective concentration; NOEC = no-observed-effect concentration; LOEC = lowest-observed-effect concentration; MATC = maximum allowable toxicant concentration (geometric mean of NOEC and LOEC).
[b] The endpoint statistics were reported by the original study authors unless otherwise noted.
[c] We calculated the EC10 values from the concentration–response data reported in the original studies. EC10 values were derived as follows: 1) Doroshov et al. (1992): probit model fit to concentration-response data for larval edema; 2) Coyle et al. (1993): probit model fit to concentration-response data for larval mortality at 5 days after hatching; 3) Hermanutz et al. (1996): probit model fit to concentration–response data for larval edema; 4) Rudolph et al. (2008): linear model fit to concentration–response shown in figure 1 in Rudolph et al. (2008); 5) Holm (2002); Holm et al. (2003, 2005): derived from figure 2a in Holm et al. (2005).
[d] Dry weight ovary Se concentrations were estimated assuming 85% moisture, based on data for bluegill ovaries (Gillespie and Baumann 1986).
[e] Dry weight ovary Se concentrations were estimated assuming 61% moisture, based on data for rainbow trout eggs (Holm et al. 2005).

of this value by a safety factor of 10 would result in an egg and ovary Se guideline of 2 µg/g dw. CCME (2007) notes that this approach should only be applied where limited toxicological data are available. For Se, there are sufficient data available to support a threshold for toxicity—specifically, that it begins to occur near 20 µg/g dw, precluding the need for application of the lowest value approach. Furthermore, and more importantly, the safety factor of 10 is not readily applicable to a naturally occurring essential element such as Se, because it results in an egg and ovary Se concentration of 2 µg/g dw that is within the range of background concentrations from fish unexposed to elevated Se, and well below the egg and ovary Se concentrations that have been measured in fish collected from reference (i.e., clean) sites in Canada. As illustrated in Figure 1, mean egg and ovary Se concentrations in fish collected from reference sites in the coal mining regions of Alberta and British Columbia and uranium mining regions of Saskatchewan are all well above 2 µg/g dw (McDonald and Strosher 1998; Casey and Siwik 2000; Kennedy et al. 2000; Holm 2002; Holm et al. 2003, 2005; Golder Associates 2005; Mackay 2006; Mainstream Aquatics 2006; Minnow Environmental and Paine, Ledge and Associates 2006; Muscatello et al. 2006; Rudolph et al. 2008; McDonald 2009; Muscatello and Janz 2009; McDonald et al. 2010). In addition, the mean egg and ovary Se concentrations in the control fish from laboratory and mesocosm studies typically exceed a value of 2 µg/g dw (Figure 1).

Selenium SSDs and predicted guideline values

On the basis of the toxicity thresholds summarized in Table 1, the inverse Gaussian, log-logistic, and Pearson V distributions provided the overall best fits on the basis of the 3 goodness-of-fit tests. The SSDs based on all 3 of these distributions resulted in a 5th percentile of 20 µg/g dw (Figure 2).

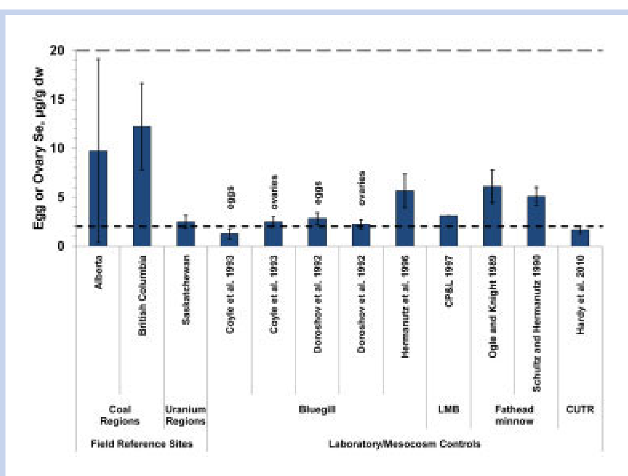

Figure 1. Comparison of Se values from the lowest value approach (2 µg/g dw) and species sensitivity distribution (SSD) approach (20 µg/g dw) to Se concentrations measured in reference site fish and control fish from laboratory and mesocosm studies. Reference site Se data from Casey and Siwik (2000); Golder Associates (2005); Holm 2002; Holm et al. (2003, 2005); Kennedy et al. (2000); Mackay (2006); Mainstream Aquatics (2006); McDonald (2009); McDonald and Strosher (1998); Minnow Environmental and Paine, Ledge and Associates (2006); Muscatello and Janz (2009); Muscatello et al. (2006); Rudolph et al. (2008); McDonald et al. (2010). LMB = largemouth bass; CUTR = cutthroat trout.

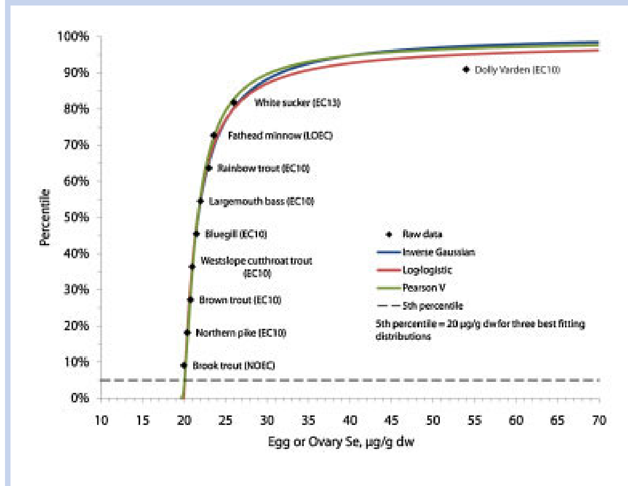

Figure 2. Species sensitivity distributions (SSDs) based on all egg and ovary Se toxicity thresholds for fish species that occur in Canada. See Table 1 for sources of toxicity thresholds.

Sensitivity analyses

Coldwater versus warmwater species. As discussed above, it has been suggested that coldwater fish such as trout, northern pike, and suckers are less sensitive to Se than fish that may be considered warmwater species, such as bluegill, largemouth bass, and fathead minnow, although the latter warmwater species can also inhabit relatively cold waters, including some Canadian waters. In one of the sensitivity analyses, bluegill, largemouth bass, and fathead minnows were excluded from the SSD evaluation. On the basis of the 3 goodness-of-fit tests, the best-fitting distributions were the inverse Gaussian, exponential, and Pareto. All 3 distribution types are illustrated in Figure 3. The 5th percentiles of the SSDs are again 20 µg/g dw on the basis of all 3 distribution types. Thus, removal of the warmwater species had no influence on the 5th percentiles. For comparative purposes, removal of salmonids from the data set likewise resulted in SSDs with

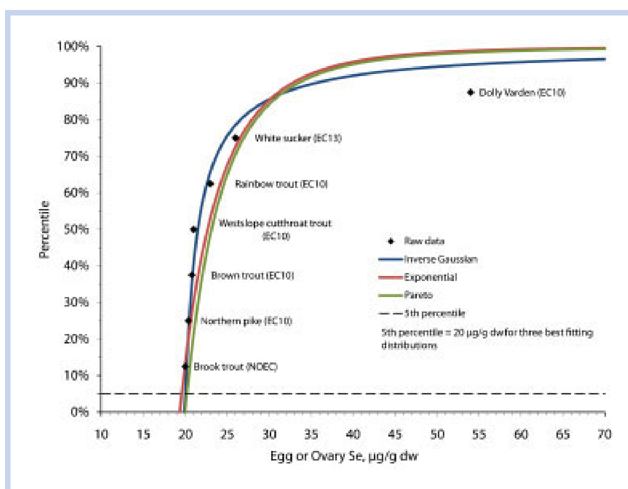

Figure 3. Species sensitivity distributions (SSDs) based on egg and ovary Se toxicity thresholds for coldwater fish species that occur in Canada (bluegill, largemouth bass, and fathead minnow excluded). See Table 1 for sources of toxicity thresholds.

a 5th percentile of 20 μg/g dw for the best-fitting distributions (not shown).

Uncertainties in toxicity thresholds. Much of the toxicity data used to derive the egg-based SSDs are based on studies in which wild fish were exposed to Se in the field. The variability in sampling designs and the nature of the data made it difficult to calculate EC10 values in a consistent manner. For example, the brook trout (Holm et al. 2005) and white sucker (de Rosemond et al. 2005) toxicity values are based on approximated EC06 and EC13 values, respectively; the brook trout threshold may also be considered a NOEC because no significant effects ($p > 0.05$) were observed relative to the reference site. These endpoints were included in the SSD of EC10 values because they provide useful information pertaining to the sensitivity of these fish species (based on egg Se concentrations), and because they are not expected to differ substantially from EC10 values because of the steepness of typical concentration–response curves for Se (e.g., Doroshov et al. 1992; Coyle et al. 1993; CP&L 1997; Holm et al. 2005; McDonald et al. 2010). For white sucker, data were only available from a Se exposure site, where the mean egg Se concentration was 25.6 μg/g dw and the mean percentage of larval deformities was 12.6%. The mean egg Se concentrations from individual fish in this study (de Rosemond et al. 2005) were variable, and data for a reference site were unavailable; accordingly, the sensitivity of white sucker, based on egg Se concentrations, has a relatively high uncertainty. Nevertheless, the egg Se concentration of 25.6 μg/g dw was considered a reasonable estimate of the threshold for the purposes of SSD development in this evaluation. Moreover, we determined that excluding the white sucker threshold from the data set has no effect on the 5th percentile, which remains at 20 μg/g dw. We also evaluated the influence of the brook trout threshold (Holm et al. 2005) being removed from the data set, as the threshold was associated with an effect less than 10%. Again, the 5th percentile is unchanged at 20 μg/g dw. Finally, removing both brook trout and white sucker from the SSD still results in no change to the 5th percentile (i.e., 20 μg/g dw) on the basis of the 3 best-fitting distributions (Figure 4). Overall, therefore, removing either the brook trout or white sucker thresholds or both from the data set does not strongly influence the steepness of the SSD, and accordingly, the 5th percentile is unchanged at 20 μg/g dw.

Inclusion of Yellowstone cutthroat trout and razorback sucker. Inclusion of Yellowstone cutthroat trout and razorback sucker in the SSD increased the total number of species from 10 to 12; the EC10 of 25 μg/g dw for Yellowstone cutthroat trout and MATC of 41.9 μg/g dw were the 9th and 11th highest. Inclusion of Yellowstone cutthroat trout and razorback sucker did not have a significant influence on the SSD, with the 5th percentile remaining at 20 μg/g dw (Figure 5).

Practical difficulties in implementing an egg-based Se criterion. It is recognized that in some water bodies, it may not be possible to collect fish eggs or mature ovaries for Se analysis. In northern Canadian waters, for example, ice cover may impair the ability to collect the necessary samples. One option is to develop a relationship between Se concentrations in eggs or ovaries relative to Se concentrations in whole body or muscle tissue. Such relationships were provided in USEPA (2004) to maximize the amount of fish tissue-based Se toxicity data for criteria development. For example, on the basis of the relationship in USEPA (2004), the whole body Se concentration predicted to be associated with an egg Se guideline of 20 μg/g dw would be 9.3 μg/g dw. However, as noted in Holm et al. (2005) and evaluated in detail in deBruyn et al. (2008), relationships in Se concentrations between fish tissues can be species and site specific. Accordingly, in the absence of site-specific data, there is higher uncertainty in implementing a whole body–based Se guideline. For example, mean ratios between egg and ovary Se and whole body Se ranges include approximately 2.4 and 2.0 for bluegill (Coyle et al. 1993; Hermanutz et al. 1996), 2.1 for fathead minnow (Ogle and Knight 1989), and 1.3 for cutthroat trout (Hardy et al. 2010). The estimated whole body Se concentration associated with an egg Se guideline of 20 μg/g dw would be 8.3 μg/g dw assuming an egg to whole body Se ratio of 2.4 and 15.4 μg/g dw assuming an egg to whole body Se ratio of 1.3. Accordingly, this ratio can have important implications if implemented to monitor or regulate Se concentrations in a

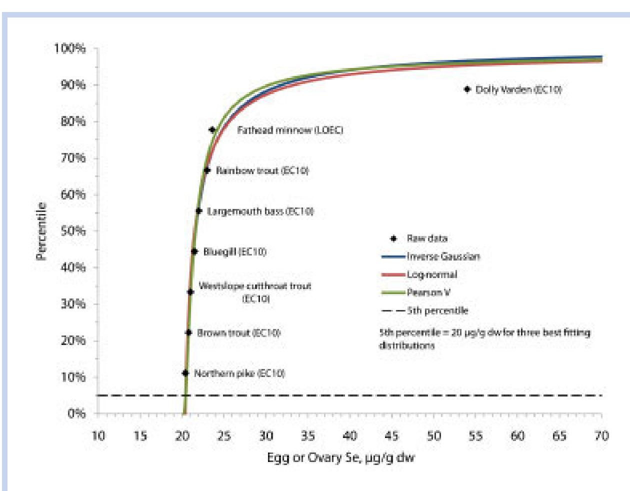

Figure 4. Species sensitivity distributions (SSDs) based on egg and ovary Se toxicity thresholds for fish species that occur in Canada, but with brook trout and white sucker excluded. See Table 1 for sources of toxicity thresholds.

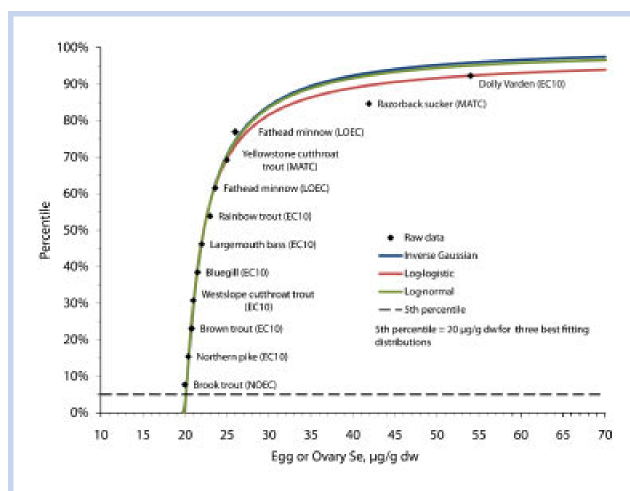

Figure 5. Species sensitivity distributions (SSDs) based on egg and ovary Se toxicity thresholds with Yellowstone cutthroat trout and razorback sucker included. See Table 1 for sources of toxicity thresholds.

water body receiving Se inputs. If possible, development of a species- and site-specific egg to whole body Se ratio, which would allow for subsequent Se monitoring in whole body tissue, may be desirable for certain conditions.

CONCLUSIONS

The 5th percentiles of egg and ovary Se SSDs based on all Canadian fish species and all fish species (including Yellowstone cutthroat trout and razorback sucker) were 20 μg/g dw for the best-fitting statistical distribution types. The 5th percentiles of SSDs with the more uncertain toxicity thresholds for brook trout and white sucker excluded, or with data for just coldwater species (excluding bluegill, largemouth bass, and fathead minnow), were likewise 20 μg/g dw. Accordingly, the uncertainties in the brook trout and white sucker toxicity thresholds have no influence on the 5th percentiles of the SSDs. The consistency in the 5th percentile of 20 μg/g dw between the various data sets evaluated is attributed to the narrow range in egg and ovary Se thresholds for most fish species, which ranged from between just 20 and 23.6 μg/g dw for 8 of the 10 Canadian species. Furthermore, the narrow range in egg and ovary Se toxicity thresholds between the most sensitive species results in a steep SSD that results in negligible extrapolation from the lowest toxicity threshold to the 5th percentile. An egg and ovary Se guideline of 20 μg/g dw, which was derived following the CCME (2007) protocol, should be considered a conservative, broadly applicable guideline to Canadian species, as no species mean toxicity thresholds lower than this value have been identified to date. When concentrations exceed this guideline, site-specific studies with local fish species, conducted by means of a risk-based approach, may result in higher egg and ovary Se toxicity thresholds (e.g., McDonald et al. 2010).

Disclaimer—The peer-review process for this article was managed by the Editorial Board without the involvement of G. Gilron. In addition, although this initiative follows Canadian Council of Ministers of the Environment (CCME) methodology, neither CCME nor Environment Canada were consulted or directly involved with the derivation of the guideline presented for consideration within this manuscript.

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