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Aquatic Life Water Quality Criterion for Mercury in Idaho

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ACRONYMS

ACHe	acetylcholinesterase
ACR	Acute-to-Chronic Ratio
ALAd	aminolevulinic acid dehydratase
ALC	Aquatic Life Criterion
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ANOVA	Analysis of Variance
ASTM	American Society for Testing and Materials
AWQC	Ambient Water Quality Criteria
BAF	bioaccumulation factor
CAS	Chemical Abstracts Service
CDE	concentration day equivalents
CF	conversion factor
CdF	condition factor
C-R	concentration-response
CC	Chronic Criterion
CCC	Criterion Continuous Concentration
ChE	cholinesterase
CMC	Criterion Maximum Concentration
CWA	Clean Water Act
DER	Data Evaluation Record
d.f.	degrees of freedom
DOC	dissolved organic carbon
dpf	days post fertilization
dph	days post hatch
dw	dry weight
E2	17β-estradiol
ECOTOX	ECOTOXicology database
EC_x	Effect concentration at x percent level
EPA	U.S. Environmental Protection Agency
E Pro	early prometamorphosis
ESA	Endangered Species Act
EU	experimental unit
FACR	Final Acute-to-Chronic Ratio
FAV	Final Acute Value
FCV	Final Chronic Value
FE	forelimb emergence
FETAX	frog embryo teratogenesis assay–Xenopus
GLI	Great Lakes Initiative
GLU	glucose
GMAV	genus mean acute value
GMCV	genus mean chronic value
GS	Gosner Stage

GSD	genus sensitivity distribution
GSI	gonadal somatic index
Hg^0	elemental mercury
HgII	inorganic mercury
HL	hind-limb
hpf	hours post fertilization
HIS	hepatic somatic index
IC_x	Inhibitory concentration at x percent level
K_d	partitioning coefficients
K_{OC}	organic carbon water partitioning coefficient
K_{OW}	n-octanol-water partition coefficient
KT	11-Ketotestosterone
LC_x	lethal concentration at x percent level
LDH	lactate dehydrogenase
LOECs	Lowest Observed Effect Concentrations
LOQ	limit of quantification
L Pre	late premetamorphosis
LSI	liver somatic index
MATC	Maximum Acceptable Toxicant Concentration
MC	metamorphic climax
MDRs	minimum data requirements
MeHg	methylmercury
MeHg-Cys	methylmercury cysteinate
METAALICUS	Mercury Experiment to Assess Atmospheric Loading in Canada and the United States
mRNA	messenger ribonucleic acid
MT	metallothionein
NF	Nieuwkoop and Faber
NOAA	National Oceanic and Atmospheric Administration
NOECs	No Observed Effect Concentrations
OECD	Organization for Economic Cooperation and Development
OCSPP	Office of Chemical Safety and Pollution Prevention
ORD	Office of Research and Development
OW	Office of Water
PCR	polymerase chain reaction
pKa	acid dissociation constant
Post-M	post-metamorphosis
ppt	parts per thousand
Pre	premetamorphic
Pro	prometamorphosis
RAPD-PCR	Random Amplified Polymorphic DNA-polymerase chain reaction
RNA	ribonucleic acid
SAB	Science Advisory Board
SeMet	seleno-l-methionine
SGR	specific growth rate
SMACR	species mean acute-to-chronic ratio

SMAV	species mean acute value
SMCV	species mean chronic value
SOP	standard operating procedure
SRB	sulfate reducing bacteria
SSD	species sensitivity distribution
SVL	snout-vent length
T	testosterone
THg	total mercury
TL	trophic level
TMDLs	Total Maximum Daily Loads
TN	total nitrogen
TOC	total organic carbon
TR	tail resorption
TSCA	Toxic Substances Control Act
U.S.	United States
USGS	United States Geological Survey
WB:M CF	whole-body to-muscle conversion factor
WHO	World Health Organization
WQS	water quality standards
Wr	relative weight
ww	wet weight

EXECUTIVE SUMMARY

This document sets forth the basis for and derivation of the chronic water quality criterion for the protection of aquatic life in the State of Idaho from the harmful effects of mercury. Mercury is a naturally occurring element that can be toxic at environmentally relevant concentrations. Anthropogenic activities releasing mercury to the environment include historic mining, fossil fuel combustion, smelting and production of metals, cement production, oil refining, and mercury releases from the chlor-alkali industry. This assessment provides a critical review of all available data quantifying the toxicity of mercury to aquatic life and provides the basis for water quality criteria that will assure the protection of populations of fish, aquatic invertebrates, and aquatic life stages of amphibians in Idaho.

Although mercury may cause acute toxicity, most harmful effects on aquatic life are of a chronic nature and are primarily due to the bioaccumulation of the organic form of mercury (i.e., methylmercury). Aquatic organisms are exposed to mercury primarily through their diet, with direct exposures through water making only a minor contribution to organisms' overall exposure (U.S. EPA 1997a, b; Wentz et al. 2014). Consequently, in this action to develop proposed aquatic life criteria for the State of Idaho, the United States Environmental Protection Agency (U.S. EPA) has developed a criterion reflective of chronic dietary exposures of mercury, consistent with current guidance (Stephan et al. 1985). Studies considered for possible inclusion for criteria derivation utilized dietary exposures consisting of mercury (predominantly as methylmercury), consistent with available data. The proposed mercury criterion is expressed as total mercury (including inorganic and organic forms (i.e., methylmercury)) in biological tissue and in the water column since exposures in natural aquatic systems result from both inorganic and organic forms of mercury (e.g., methylmercury). Effects observed in most aquatic organisms

(e.g., fish) are expected primarily to be due to the toxicological effects of methylmercury. However, more recent studies in fish (Lescord et al. 2018), amphibians (Unrine and Jagoe 2004), and macroinvertebrates (Martins et al. 2021; Clarke et al. 2022) have demonstrated that the ratio of methylmercury to total mercury in the tissues of aquatic organisms varies, according to factors such as species identity and life stage-specific trophic ecology. Furthermore, although inorganic forms of mercury are excreted more easily, bioaccumulation in the tissues of digestive tract and excretory organs may play a role in toxicity of certain life stages and species if concentrations are sufficiently elevated (Unrine and Jagoe 2004; Clarke et al. 2022). Therefore, expressing the criterion as total mercury incorporates the range of mercury compound exposures in the environment, and their variable effects on aquatic organisms (Clarke et al. 2022). Further, most mercury monitoring results for fish tissue are reported as total mercury concentrations. For example, Bloom (1992) reported that the average concentration of methylmercury was greater than 95% of the total mercury concentration detected in fish tissue; this finding has been used as the basis to support the use of total mercury as a surrogate for methylmercury in fish muscle in modern studies used for human health risk assessment. However, more recently, this percent methylmercury has been shown to vary depending on fish species, size and age (Lescord et al. 2018). Thus, expressing the tissue- and water-based criterion as total mercury reflects the various forms of mercury, including both inorganic and organic forms, that aquatic organisms are exposed to and affected by in the environment. It also reflects the most common way mercury is reported in studies of mercury in tissues of fish and other aquatic organisms.

The proposed chronic criterion for mercury in Idaho is a tiered criterion composed of three parts, or elements. The tissue criterion elements take precedence over the water column criterion element due to the fact that tissue concentrations provide a more robust and direct

indication of potential mercury effects because the tissue criterion elements were derived using tissue data following dietary, not water column, exposures of aquatic organisms to mercury. The proposed criterion, applicable to all waters in Idaho, include: (1) a fish whole-body tissue criterion element, (2) a fish muscle tissue criterion element, and (3) a water column criterion element. The proposed criterion are intended to protect aquatic life from the chronic effects of exposure to all forms of mercury (i.e., total mercury). The outcome of assessing both reproductive and non-reproductive studies of aquatic vertebrates and invertebrates under both laboratory and field conditions ultimately led EPA to the conclusion that both reproductive and non-reproductive effects to aquatic vertebrates are likely of greater ecological concern than effects to invertebrates. EPA used acceptable toxicity data from a variety of aquatic organisms reflecting a range of mercury sensitivities to derive the proposed criterion element(s) of 225 ng Total Mercury/g wet weight (ng THg/g ww) for muscle tissue and 162 ng THg/g ww whole-body tissue. EPA used the tissue criterion elements based on all aquatic taxa in conjunction with Idaho-specific monitoring data for mercury in fish tissue and water to derive a bioaccumulation factor (BAF)-based water column criterion element for Idaho waters of 2.1 ng/L total mercury in whole water (not dissolved or filtered), described in **Section 2.9**, and **Section 3.6**. Therefore, similar to selenium (U.S. EPA 2016a), this proposal for mercury consists of one criterion with multiple elements. **Table ES-1** summarizes the mercury criterion for fish tissue and the water column for the state of Idaho.

Table ES-1. Proposed Chronic Mercury Ambient Water Quality Criterion for the Protection of Aquatic Life in Idaho.

Media Type	Fish Muscle Tissue [1, 2, 3] Total Mercury (ng THg/g wet weight)	Fish Whole Body Tissue [1, 2] Total Mercury (ng THg/g wet weight)	Water Column [1,4] Total Mercury (ng/L) in whole water
Magnitude	225	162	2.1
Duration	Instantaneous measurement [5]		30 day average
Frequency	The average tissue concentration must not be exceeded		Not more than once in three years on average

[1] The proposed criterion elements are hierarchical, with both tissue elements superseding the water column element. The fish muscle tissue and fish whole body tissue criterion elements are independently applicable.

[2] Tissue sample measurements must be based on measurement(s) of the total mercury concentration (in a composited tissue sample from each fish species or a central tendency estimate of individual tissue samples from each fish species) collected from a given site or waterbody in a discrete sampling period. These criterion elements support Idaho's aquatic life uses. Only samples of adult life stage trophic level (TL) 4 fish can be directly compared to the muscle or whole-body criterion elements.

[3] If adult life stage TL2 or TL3 fish are sampled, a Bioaccumulation Trophic Adjustment Factor (BTAF) must be applied to the muscle concentrations of those fish. If whole-body tissue from TL2 or TL3 fish is sampled, the fish whole body – muscle conversion factor of 0.72 must be applied to generate a translated muscle value before a BTAF is applied to the sample concentration. A TL2 sampled fish concentration must be multiplied by the TL2 BTAF of 5.6 and the resultant value compared to the muscle tissue criterion element. A TL3 sampled fish concentration must be multiplied by the TL3 BTAF of 3.5 and the resultant value compared to the muscle tissue criterion element. If multiple adults of different TLs are sampled, the TL4 fish result would supersede TL3 BTAF-applied or TL2 BTAF-applied value outcomes. If TL3 and TL2 fish are sampled, the TL3 BTAF-applied values supersede the TL2 BTAF-applied values.

[4] Water column values are based on total mercury in unfiltered or "whole water" samples. Total mercury includes all inorganic and organic species of mercury in the water column. Water samples collected during baseflow conditions would be most representative of the data used to derive this criterion element. This criterion element supports Idaho's aquatic life uses.

[5] Fish tissue data provide integrative measurements that reflect accumulation of mercury over time and space in aquatic organisms from a given site or waterbody in a discrete sampling period.

1 INTRODUCTION & BACKGROUND

EPA is proposing a water quality criterion to protect aquatic life in Idaho from the harmful effects of mercury. EPA developed this criterion following the general approach outlined in the Agency's "*Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses*" (Stephan et al. 1985). The proposed criterion presented herein are the Agency's best estimate of maximum concentrations of mercury, with associated frequency and duration, below which aquatic life in Idaho would be protected from unacceptable chronic effects.

Although mercury may cause acute toxicity, the most harmful effects on aquatic life are of a chronic nature and are due to mercury bioaccumulation. Aquatic organisms are exposed to mercury primarily through their diet, with direct exposures through water making only a minor contribution to organisms' overall exposure (U.S. EPA 1997b; Wentz et al. 2014). Consequently, the United States Environmental Protection Agency (U.S. EPA) has developed a criterion reflective of chronic dietary exposures of mercury, consistent with current guidance (Stephan et al. 1985). Studies considered for possible inclusion for criterion derivation utilized dietary exposures consisting of mercury in food (predominantly as methylmercury).

EPA is proposing this mercury criterion for Idaho expressed as total mercury (THg), including both inorganic and organic forms. EPA proposes this approach because exposures to organisms in the natural environment are to several forms of mercury, including inorganic and organic forms (i.e., methylmercury (MeHg)) while effects observed in aquatic toxicity tests are expected to be primarily due to the toxicological effects of methylmercury in tissues. Inorganic forms of Hg can be deposited and retained in aquatic and terrestrial environments and may be taken up by organisms at the base of food chains (Morel et al. 1998) or converted to

methylmercury through microbial action. Methylmercury can then be taken up at the base of the food web and bioaccumulate in higher trophic level organisms (U.S. EPA 1997c).

Most mercury monitoring results available for fish are reported as total mercury concentrations based on assumptions that total mercury is an adequate proxy for methylmercury in fish muscle (Bloom 1992). However, although the mercury in muscle tissue of higher trophic level (TL 3 & 4) fish is primarily methylmercury, more recent studies demonstrate that the forms of mercury present and ratio of inorganic to organic mercury in lower trophic level fish (Lescord et al. 2018), certain aquatic life stages of amphibians (Unrine and Jagoe 2004), and aquatic invertebrates (Martins et al. 2021; Clarke et al. 2022) is dependent on both life stage and their respective trophic ecology. Thus, expressing the criterion as total mercury both reflects the various forms of mercury aquatic organisms are exposed to and affected by in the environment, their variable effects on aquatic organisms, as well as the most common way mercury is reported in studies of mercury in tissues of aquatic organisms.

2 PROBLEM FORMULATION

A problem formulation provides a strategic framework for water quality criteria development under the Clean Water Act (CWA) by focusing on the most relevant chemical properties and endpoints. (U.S. EPA 1998a).

2.1 Overview of Mercury Sources and Releases

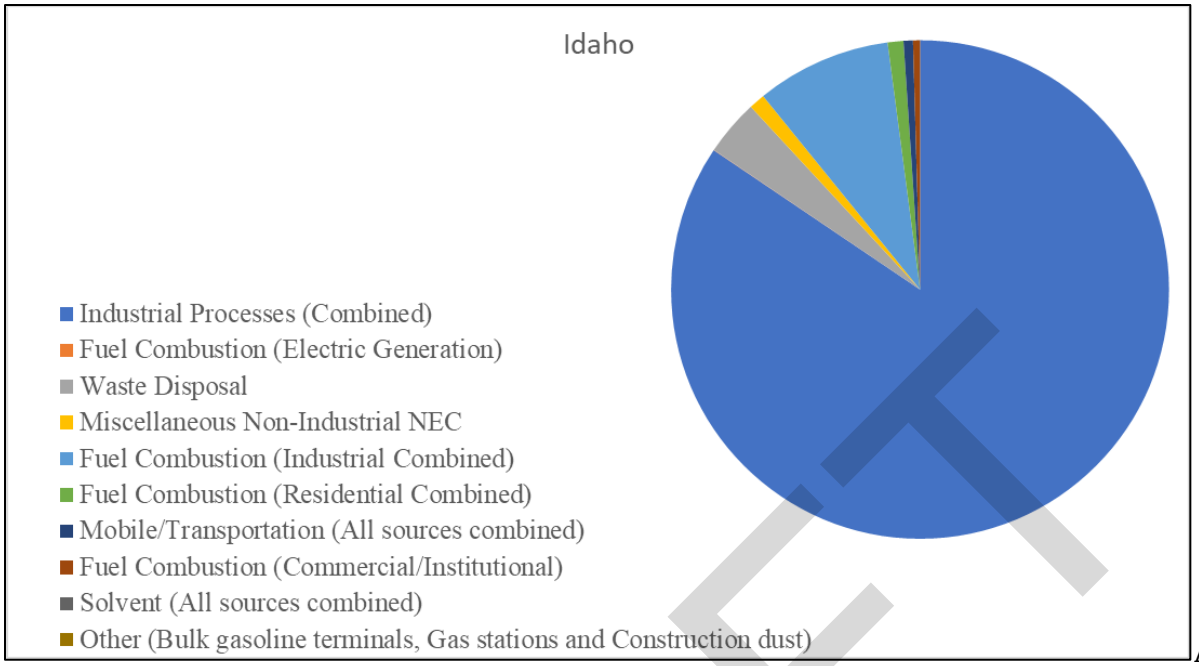
Mercury is a metal that occurs naturally in mineral deposits (e.g., cinnabar) and as an impurity in coal and geologic deposits of non-ferrous metals. Natural sources of mercury released from these deposits include the weathering of mercury-containing rocks, volcanoes (eruptive and non-eruptive activities), and geothermal activity (Nriagu and Becker 2003; Pyle and Mather 2003; Schuster et al. 2002; Varekamp and Buseck 1981). Natural sources collectively comprise approximately 10% of global atmospheric mercury emissions (Amos et al. 2013; U.N. Environment Programme 2013).

Naturally occurring mercury sources in Idaho include cinnabar deposits in central Idaho, silver deposits near Weiser, in southwest Idaho adjacent to the Oregon border (Gustafson 1987), and gold deposits (Berger and Bonham 1990) throughout central and northern Idaho (IDEQ 2005). In addition to these geologic deposits, there are numerous hot springs throughout the state that are associated with elevated mercury concentrations. USGS (1985) analyzed 142 hot springs in the Idaho Batholith (Boise, Payette, Clearwater, and Salmon Rivers) with mercury levels ranging from < 0.01 – 1.4 µg/L. Volcanic sources in Idaho are limited to the central Snake River Plain area, however Yellowstone National Park (Wyoming) represents a geothermally-related source of mercury emissions (releasing between 0.20 and 0.24 µg/m^2/hr), some of which is likely deposited in eastern Idaho following atmospheric transport (IDEQ 2005). Mercury emissions

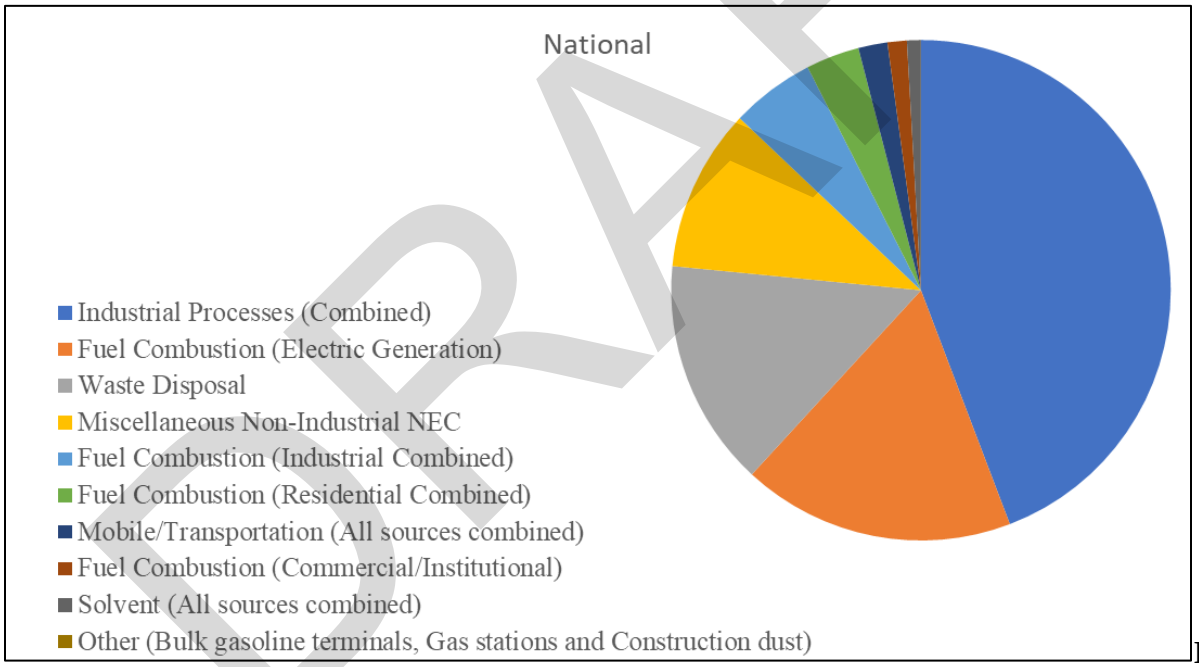
from volcanic formations in Nevada and California may also be contributing to elevated mercury concentrations across portions of southern Idaho (Engle et al. 2006).

Anthropogenic activities result in the release and transport of mercury to the aquatic environment primarily through atmospheric deposition of air emissions, discharges to water, and leaching from mercury-bearing strata exposed as a result of mining or other activities. Anthropogenic activities releasing mercury to the environment include historic mining (i.e., cinnabar deposits), fossil fuel combustion, smelting and production of metals, cement production, oil refining, mercury from the chlor-alkali industry, and cremation (from dental amalgam). Gold production (artisanal scale gold mining comprising 37% of annual global emissions) and fossil fuel combustion (comprising 25% of annual global emissions) are the top two sources of mercury release on a global scale (U.N. Environment Programme 2013). Industrial processes (e.g., chemical manufacture, ferrous and non-ferrous metals processing) are the predominant current source of emissions both in Idaho and nationally, comprising 84.4% of Idaho's total annual mercury emissions (U.S. EPA 2021a - National Emissions Inventory, 2017) (**Figure 2-1**).

Large historic industrial and widespread artisanal placer gold mining operations (Varley et al. 1919) have resulted in the release of mercury, both from the weathering of geological mercury-containing deposits and the leaching of mercury from gold mine waste materials (Fleck et al. 2016; Eckley et al. 2011a,b; Hsu-Kim et al. 2018). Several studies also in Idaho reported that sediments in streams downstream of historic mercury mining (Cinnabar Mine, Sugar Creek; Eckley et al. 2021), gold (Orofino Creek), and silver mines (Jordan Creek and Coeur d'Alene River) had elevated mercury concentrations compared to non-mining areas (Eckley et al. 2020).



A



B

Figure 2-1. Comparison of Major Mercury Emission Sources in Idaho (Panel A) and Nationally (Panel B).
NEC: not elsewhere classified; This simply means that those emissions processes were not appropriate to include in another Emission Inventory System sector and their emissions were too small individually to include as its own Emission Inventory System sector.
(Source: U.S. EPA (2021a), National Emissions Inventory, 2017)

2.2 Overview of Environmental Fate, Transformation, and Accumulation of Mercury in Freshwater Aquatic Systems

2.2.1 Environmental Fate of Mercury in the Freshwater Aquatic Environment

Mercury speciation influences the cycling, and thus fate, of mercury in aquatic ecosystems (**Figure 2-2**). Mercury cycling is dictated by physical, chemical, and biological reactions and thus may be affected by pH, temperature, reduction-oxidation (redox) potential, and the availability of nutrients, humic acids, and complexing agents (i.e., hydroxides, chlorides, and sulfides) (Driscoll et al. 2013; Morel et al. 1998; Ullrich et al. 2001). Mercury has a high affinity for sorbing to sediments as well as dissolved and particulate matter suspended within the water column. Sediments may serve as both a source and sink for mercury, facilitating sequestration and reduction through burial in the aquatic ecosystem (Ullrich et al. 2001; Branfireun et al. 2020). The main dissolved mercury species in the aquatic environment are inorganic mercury bound to organic matter (e.g., DOC) or other sulfur containing compounds and methylmercury (Morel et al. 1998; Ullrich et al. 2001).

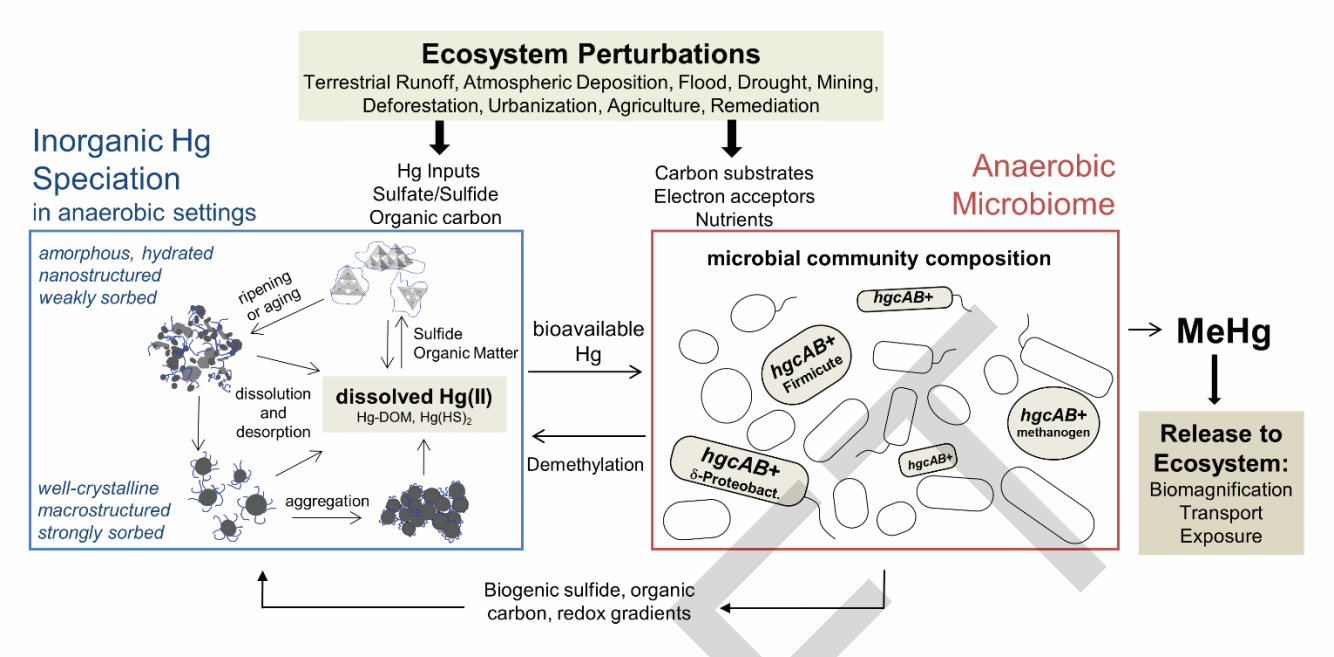


Figure 2-2. Diagram Depicting the Mercury Cycle within the Aquatic Ecosystem. (Source: Hsu-Kim et al. 2018, reprinted with permission).

2.2.1.1 Methylation of Mercury in Freshwater Aquatic Ecosystems

Mercury methylation occurs within anoxic environments (e.g., hypolimnion and sediments) by a diverse group of anerobic bacteria containing the hgcAB gene, which includes sulfate reducing bacteria (SRB), iron reducing bacteria, and methanogens (Compeau and Bartha 1985; Fitzgerald et al. 1991; Fleming et al. 2006; Gilmour et al. 1992; Kerin et al. 2006; Morel et al. 1998). Higher mercury methylation rates tend to occur in areas with higher anerobic microbial activity and when inorganic mercury is in a form that is bioavailable to the microbial community. Variables that can increase the activity of methylating anerobic bacteria can include an abundant source of labile organic material as well as terminal electron accepting compounds such as sulfate or ferric iron (among others), which often occur in wetland environments (Morel et al. 1998; Ullrich et al. 2001). Wetlands thus play a key role in the methylation of mercury due to the abundance of organic matter, nutrients and anoxic conditions in the water and sediment that

support the microbial communities involved with the methylation of inorganic mercury (Wentz et al. 2014; see **Figure 2-3**).

The breakdown of methylmercury through decomposition (demethylation) is important for mercury cycling within sediments and the water column. The breakdown of methylmercury occurs via abiotic processes involving chemical and photo-chemical reactions, as well as microbial processes via oxidative and reductive pathways (Barkay and Gu 2021; Benoit et al. 2003; Ullrich et al. 2001). The degradation of methylmercury yields methane and inorganic mercury species (Hg^{2+} or Hg^0), which can continue to cycle in the environment (Benoit et al. 2003; Morel et al. 1998; Ullrich et al. 2001).

2.2.1.2 Bioaccumulation of Mercury in the Freshwater Aquatic Environment

Mercury bioaccumulation in aquatic organisms is a function of mercury inputs to a system due to natural and anthropogenic perturbations (Hsu-Kim et al. 2018), speciation, transformation, and accumulation processes that involve trophic ecology (Greenfield et al. 2001; Ullrich et al. 2001; Selin 2009; Liu et al. 2012; Lucotte et al. 2012; Driscoll et al. 2013; Jardine et al. 2013; Hsu-Kim et al. 2013). Not all species of mercury are accumulated by aquatic organisms. Elemental mercury (Hg^0) and inorganic mercury complexes are not reactive and therefore are not accumulated by organisms, while the reactive forms of mercury (i.e., Hg(II) and methylmercury) are retained by organisms at the base of the food chain (Morel et al. 1998). Aquatic organisms can bioaccumulate inorganic (e.g., mercury) and organic (i.e., methylmercury) forms through passive diffusion across respiratory and other cellular membranes via direct water column exposures (e.g., gills, skin), and across intestinal, renal and other internal organ cellular membranes via dietary uptake (e.g., ingestion of contaminated food; U.S. EPA 1997a). Both organic and inorganic mercury (e.g., HgII) are bioconcentrated by primary

producers (e.g., algae, periphyton and macrophytes) through passive uptake (Mason et al. 1996; Moye et al. 2002) and other mechanisms (Dranguet et al. 2014).

Methylmercury is efficiently assimilated into the cytoplasm of primary producers (e.g., algae and cyanobacteria) and absorbed into tissues of higher trophic level organisms (e.g., invertebrates), becoming sequestered in proteins associated with skeletal muscle (U.S. EPA 1997c). Bioaccumulation factors (BAFs) ranging from 10^5 to 10^6 (U.S. EPA 1997c; Watras et al. 1998) (**Figure 2-3**) are typical in waterbodies with complex (i.e., multiple trophic positions) food webs, with predatory organisms (i.e., piscivorous) accumulating the highest mercury concentrations based on their trophic ecology and feeding strategies (Evers et al. 2005; Jackson et al. 2011; Rimmer et al. 2010; Tom et al. 2010; Johnson et al. 2015). Methylmercury can biomagnify (i.e., increase in concentration at successively higher trophic levels) within aquatic food webs, where inorganic mercury does not. As a result, most mercury in higher trophic level organisms is present as methylmercury.

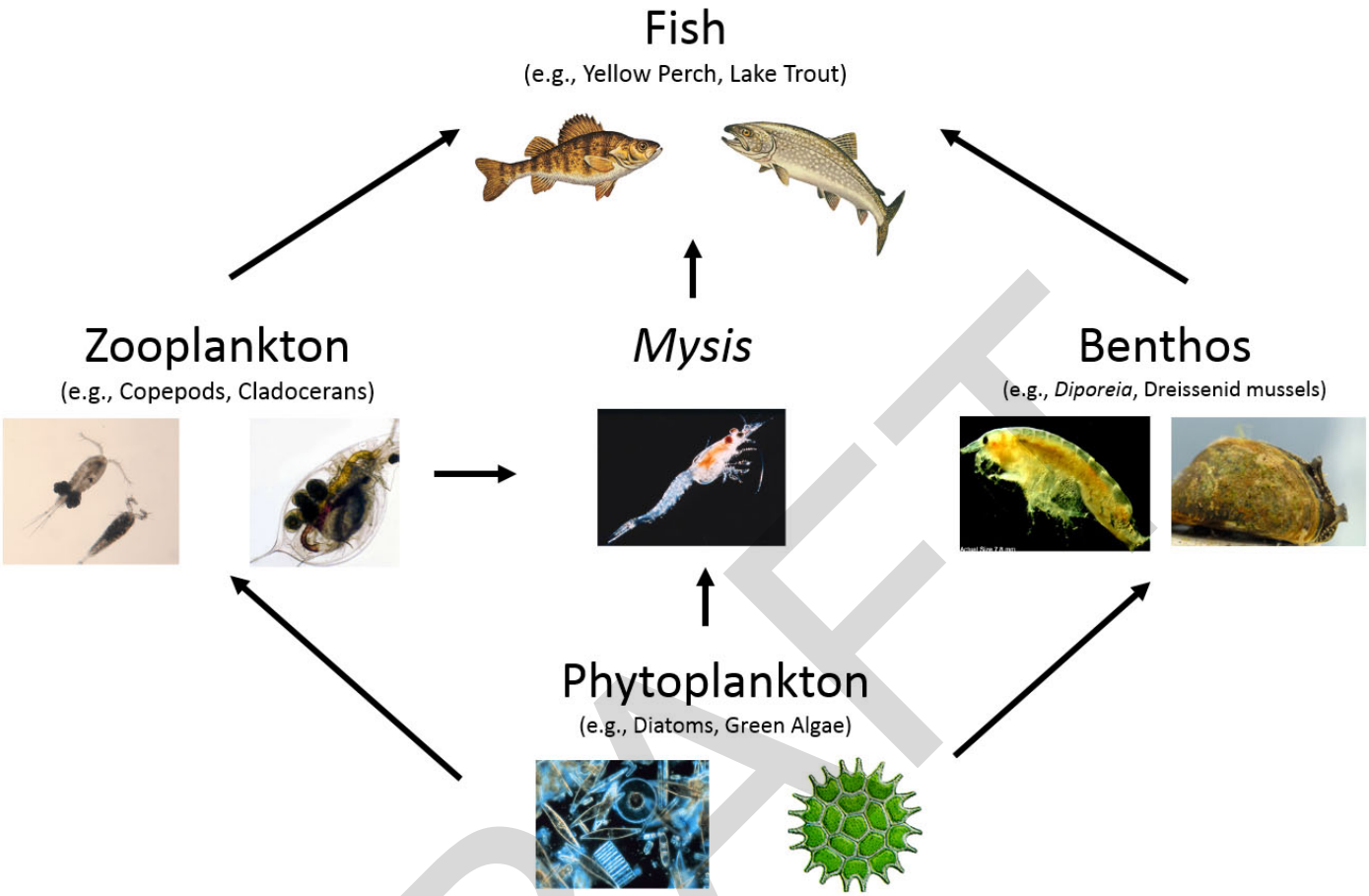


Figure 2-3. Diagram Demonstrating the Movement of Mercury in a Simplified (Great Lakes) Food Web.
The accumulation of methylmercury starts with bioconcentration by primary producers (i.e., phytoplankton). From there, methylmercury is accumulated and ultimately biomagnified through the rest of the trophic levels. Source: NOAA GLERL (*https://www.epa.gov/great-lakes-monitoring/great-lakes-biology-monitoring-program*).

2.2.1.3 Factors Influencing Methylmercury Bioaccumulation in Aquatic Ecosystems

Concentrations of methylmercury in organisms can vary widely between water bodies, even in regions having similar inorganic mercury inputs (Evers et al. 2005; Ward et al. 2010a). These differences in biotic methylmercury concentrations have been attributed to differences in a system's net methylation potential and differences in food web characteristics, such as species composition and abundance, trophic transfer efficiency, and foraging behavior (Burgess and

Meyer 2008; Clayden et al. 2013; Evers and Clair 2005; Mason et al. 1996; Scudder et al. 2009; Sorensen et al. 1990; Ward et al. 2010b; Watras et al. 1998).

Both organic and inorganic mercury are bioconcentrated by primary producers (e.g., algae, periphyton) through both passive uptake (Mason et al. 1996; Moye et al. 2002) and active uptake (Pickhardt and Fisher 2007) with subsequent accumulation via consumption through the food web. Additionally, although both inorganic and organic mercury accumulate in aquatic organism tissues, the uptake and toxicity manifested from these exposures may be very different. For example, Bradley et al. (2017) reviewed 25 studies on fish where assimilation efficiencies were measured, observing that assimilation of methylmercury ranged from 10% to 100% as compared to 2% to 51% for inorganic forms of mercury (e.g., Hg(II)). Once assimilated from the digestive system (primarily), both forms of mercury are distributed by metabolic processes and concentrate in various tissues based on biochemical affinities.

Methylmercury is more efficiently transferred and more slowly eliminated than inorganic mercury and biomagnifies with each trophic position (Mason et al. 1996; Tom et al. 2010). Further, methylmercury bioaccumulation in the consumer is influenced by the mercury burden of prey organisms, dietary ontogeny, growth efficiency, trophic position, foraging habits, and size-age relationships (Evers et al. 2005; Graeb et al. 2005; Galarowicz et al. 2006; Burgess and Meyer 2008; Clayden et al. 2013; Ward et al. 2010b; Watras et al. 1998), resulting in higher trophic level organisms frequently bearing the greatest body burdens, and likely associated risk, depending on their sensitivity to mercury.

2.3 Toxicity and Mode of Action of Mercury to Aquatic Life

For the purpose of this document, discussion of the toxicity and mode of action of mercury to aquatic life is primarily limited to methylmercury, consistent with available data. The toxicity of methylmercury is a function of its chemical structure and propensity to biomagnify in

higher trophic level organisms, putting long-lived organisms and predators at the highest risk for exposure and toxicity. In fish and amphibians, methylmercury accumulates in the blood, muscle, kidney, brain, and liver due to its high affinity for the sulfur-containing amino acid cysteine, forming methylmercury cysteinate (MeHg-Cys) complexes within fish tissues that hinder biological functions (Bridges and Zalups 2010; Lemes and Wang 2009).

With respect to the mode of action of mercury, several studies (Boudou and Ribeyre 1985; Rouleau et al. 1999; Berntssen et al. 2003) have reported both organic and inorganic mercury accumulation in fish brains. In vertebrates, methylmercury easily crosses the blood brain barrier due to its lipophilicity (Savari et al. 2020) and structural similarity between methylmercury cysteinate complexes and the amino acid, methionine (Zimmerman et al. 2013). The toxicological effects of methylmercury are largely related to its ability to form reactive oxygen species (Aschner et al. 2007; Roos et al. 2009), pro-oxidative effects resulting in depletion of GFH and antioxidant enzymes (Stringari et al. 2008; Roos et al. 2009; Mieiro et al. 2011), disturbing oxidative balance, disrupting homeostasis, and potentially altering signaling mechanisms in the nervous system (Cambier et al. 2012; Fretham et al. 2012). Berntssen et al. (2003) observed lipid peroxidation, vacuolation, and necrotic cell bodies in juvenile salmon brain following a 4-month dietary exposure to relatively low methylmercury exposure levels.

As a consequence of oxidative stress and neurological toxicity, downstream apical effects (e.g., reproduction and survival) can occur at the whole animal level. The effects of dietary methylmercury on fish reproduction (including endocrine modulating activity) have been reviewed (Crump and Trudeau 2009; Tan et al. 2009), with conclusions indicating that longer term dietary exposures at environmentally-relevant concentrations during initial sexual maturation from juvenile stages (Friedmann et al. 1996; Hammerschmidt et al. 2002) may result

in impacts to the reproductive systems of male and female fish, resulting in impaired reproduction. In addition to reproductive impairments in mature F_0 generation fish, studies in marine fish models (Alvarez et al. 2006; Matta et al. 2001) provide evidence that transgenerational effects can be manifested through maternal transfer of methylmercury in F_1 generations.

Invertebrates are typically more tolerant to both inorganic and organic mercury exposures than vertebrates (Boening 2000). Larval stages are usually the most sensitive, with mortality being the most common effect evaluated for most taxa (World Health Organization 1989). However, the mode of toxic action described above for vertebrates may be relevant to invertebrates as well, given the general nature of oxidative stress, and the presence of nervous tissue in many invertebrates. It is not well-understood why invertebrates tend to be less sensitive to mercury, once exposed.

2.4 Conceptual Model

The conceptual model depicted in **Figure 2-4** provides a visual representation depicting the prior discussions of how aquatic life could be exposed to and adversely affected by mercury in surface waters in the state of Idaho.

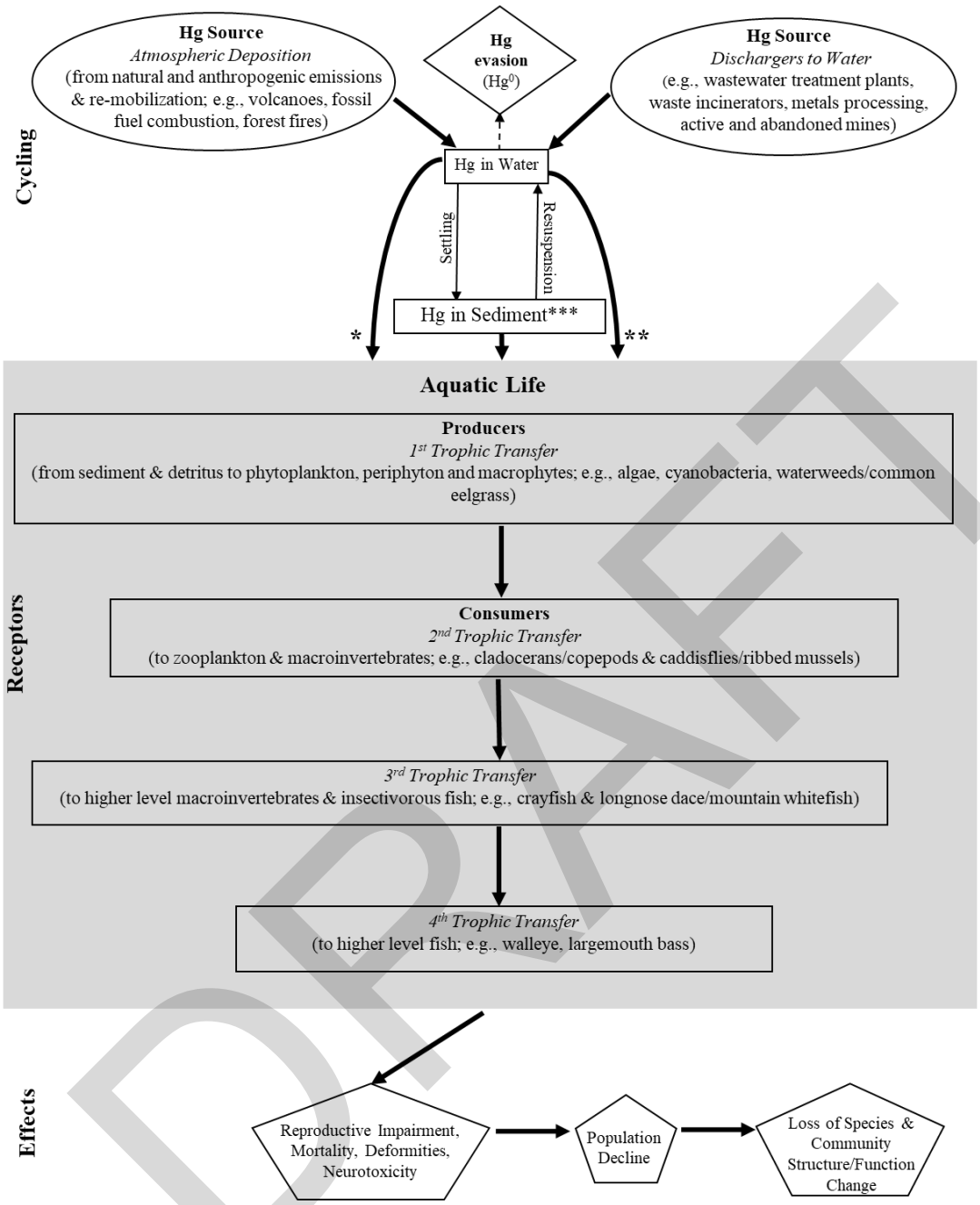


Figure 2-4. General and Broad Conceptual Model Diagram of Sources, Portioning, Bioaccumulation and Effects of Mercury in the Aquatic Environment.

Mercury sources represented in ovals, mercury evasion from aquatic ecosystem represented by diamond and dashed arrow, compartments within the aquatic ecosystem represented by rectangles, and effects (on all trophic levels represented by shaded box) represented as pentagons. Examples of organisms in each trophic transfer provided as freshwater/marine. Weighted arrows indicate relative proportion of mercury from each source, but it is recognized that relative proportion can be site-specific depending on the presence of local sources. Movement of mercury from water indicated by two separate pathways: bioconcentration by producers (*) and direct exposure to all trophic levels. (**) Relative proportion of mercury transferred between each trophic level is dependent on life history characteristics of each organism. (Weighted arrows indicate relative proportion of mercury from each source). Bacterial methylation of mercury (***) occurs primarily at the sediment water interface in anoxic sediments. In considering the effects of mercury on aquatic species and the development of a criterion, it is important to consider both the toxicity of mercury to species and the bioaccumulation of mercury by such species.

2.5 Assessment Endpoints

Assessment endpoints are defined as "explicit expressions of the actual environmental value that is to be protected" and are defined by an ecological entity (species, community, or other entity) and its attribute or characteristics (U.S. EPA 1998a). The protection of aquatic life and health of the aquatic community may be considered an assessment endpoint as indicated by survival, growth, and reproduction of the taxa present in the aquatic community. As defined under the CWA, these management goals are stated as designated uses for waters of the U.S. EPA's proposed aquatic life criterion described herein are expected to be protective of freshwater aquatic life in the state of Idaho. The assessment endpoint for this mercury criterion is thus the protection of freshwater aquatic life in Idaho. Although this action is specific to the state of Idaho, the aquatic taxa represented in the genus sensitivity distribution used to derive the tissue and BAF-based water criteria element concentrations for mercury are widely distributed in the U.S. and thus serve as surrogates for other untested species resident to the U.S.

2.6 Measures of Effect

In most cases, an assessment endpoint cannot be directly measured, so a measure of effect or measures of effect are selected that can be related, either qualitatively or quantitatively, to the assessment endpoint. For example, a decline in a sport fish population (an assessment endpoint) may be evaluated using laboratory studies that evaluate a toxicant's adverse effect on the mortality or reproduction of surrogate species, such as the fathead minnow (a measurement endpoint) (U.S. EPA 1998a). Measures of effect (**Table 2-1**) are used to characterize or quantify changes in the attributes of an assessment endpoint or changes in a surrogate entity or attribute, in this case a response to chemical exposure (U.S. EPA 1998a). Toxicity data are used as measures of direct and indirect effects on representative biological receptors. Studies have

demonstrated that vertebrates (amphibians and fish) are the most sensitive aquatic taxa to the chronic toxicological effects of mercury.

Table 2-1. Summary of Assessment Endpoints and Measures of Effect Used in Criterion Derivation for Mercury in the State of Idaho.

Assessment Endpoints for the Aquatic Community	Measures of Effect
Survival, growth, and reproduction of freshwater fish, other freshwater vertebrates, and invertebrates	EC_{10} NOEC, LOEC, MATC

MATC = Maximum acceptable toxicant concentration (geometric mean of NOEC and LOEC)
NOEC = No observed effect concentration
LOEC = Lowest observed effect concentration
EC_{10} = Effect concentration to10% of the test population

To ensure the protection of the entire aquatic community, EPA compiles toxicity test data from a minimum of eight diverse taxonomic groups based on Minimum Data Requirements (MDRs) set forth in the 1985 Guidelines (Stephan et al. 1985). The taxonomic requirements ensure that criteria are broadly protective of the range of taxa within typical aquatic ecosystems present in North America, including the state of Idaho.

> Chronic freshwater criteria using an empirical genus sensitivity distribution approach require data from the following taxonomic groups:
>
> a. Fish in the family Salmonidae in the class Osteichthyes
> b. a second family of fish in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish)
> c. a third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian)
> d. a planktonic crustacean (e.g., cladoceran, copepod)
> e. a benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish)
> f. an insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge)
> g. a family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca)
> h. a family in any order of insect or any phylum not already represented

Because methylmercury is bioaccumulative and significantly more toxic through chronic dietary exposure, EPA is not proposing a separate acute criterion element from the results of toxicity tests with water only exposure. EPA collected available chronic dietary toxicity test data meeting the minimum data requirements across the eight diverse taxonomic groups identified above per the 1985 Guidelines recommendations.

The 1985 Guidelines also specified that at least one quantitative test is needed for a freshwater alga or vascular plant to determine whether plants are more or less sensitive than animals. If plants are among the most sensitive aquatic organisms, toxicity test data from a plant in another phylum should also be available. EPA reviewed available data for aquatic plants and algae to determine if they were more sensitive to mercury than aquatic animals and found that they were not (see **Appendix A**, **Appendix B**, and **Appendix C**).

2.6.1 Measurement of Mercury Exposure Concentrations in Toxicity Tests

Data on the following mercury species were used for development of the proposed mercury criterion:

- Mercuric ion (Hg II) CAS # 7487-94-7 (DTXSID5020811)
- Methylmercury (MeHg) CAS # 22967-92-6 (DTXSID2031615)
- Total mercury (THg) CAS # 7439-97-6 (DTXSID1024172)

Because of the bioaccumulative nature and the significantly greater potential for toxic effect from dietary versus aqueous exposures, EPA based the proposed criterion on dietary exposures to both inorganic and organic mercury with tissue burdens measured as methylmercury or total mercury. Chronic dietary toxicity studies that only used mercury (predominantly as methylmercury) to expose freshwater aquatic animals were considered for possible inclusion in the criterion derivation (except amphibian tadpoles as noted below). Most of the dietary exposure studies reviewed and used for criterion derivation consisted of

methylmercury chloride (CH_3HgCl) that was spiked into dietary items. Chronic dietary toxicity studies that only used mercury (predominantly as methylmercury) to expose freshwater aquatic animals were considered for possible inclusion in the criterion derivation. Several dietary toxicity studies with amphibian tadpoles (Unrine and Jagoe, 2004; Bergeron et al. 2011a; Todd et al. 2011, 2012; Wada et al. 2011); however, were designed to mimic dietary mercury exposures from field observations reported at sites solely contaminated by atmospheric deposition (Cope and Rada 1992; Cleckner et al. 1998; Hill et al. 1996; Lewis et al. 2001). These particular studies, therefore, employed a combination of inorganic mercury and methylmercury combined in proportions intended to mimic mercury concentrations and speciation in aufwuchs (periphyton community and associated abiotic and biotic constituents present in aquatic systems; see further explanation provided in Unrine and Jagoe 2004).

To reiterate, the toxicological effects observed in most aquatic species used in the development of the mercury criterion for the state of Idaho are primarily due to chronic dietary exposure to methylmercury, however, the toxicity observed in some aquatic taxa (i.e., amphibian tadpoles and metamorphs) was likely due to the combined toxic effects of inorganic and organic forms of mercury (Unrine and Jagoe 2004). In the several amphibian studies evaluated by EPA for this document, both total mercury and methylmercury were measured so that the ratio of total mercury to methylmercury could be calculated. In contrast, the majority of dietary methylmercury toxicity studies involving fish analyzed the mercury concentrations in diets and tissues as total mercury rather than methylmercury because the test fish were fed mercury-contaminated food dosed with methylmercury sourced from analytical grade reagents. There was also low background mercury quantified in food prior to diet formulation in all of these studies.

Thus, measurements of total mercury in most dietary fish studies used for criterion derivation were a proxy for methylmercury.

EPA is proposing a criterion for the state of Idaho expressed as total mercury (THg). EPA has determined that it is justified to propose a criterion expressed as total mercury for Idaho because the analysis of total mercury incorporates the measurement of methylmercury, costs less, and uses less complex analytical methods which simplifies implementation activities. Additionally, measurement of total mercury in fish tissue (predominantly muscle) has served and will likely continue to serve as the basis for quantifying mercury concentrations in fish tissue monitoring programs implemented by EPA (Wathen et al. 2015) and many states, including Idaho (Essig 2010; Mebane and MacCoy 2016).

2.7 Mercury Toxicity Test Characteristics

When developing this proposed mercury ambient water quality criterion for the protection of freshwater aquatic life in Idaho, EPA applied the principles of systematic review (Rooney et al. 2014; NAS 2021; U.S. EPA 2021b) to ensure the data and information used to develop the criterion were collected and reviewed in an unbiased, reproducible, and transparent manner. The systematic review process consists of several steps.

The identification of acceptable data for possible inclusion in the toxicity dataset was guided by the problem formulation for mercury and data were collected from EPA's public Ecotoxicology Database (ECOTOX; *https://www.epa.gov/chemical-research/ecotoxicology-database*). ECOTOX is a curated, publicly available knowledgebase, providing test data and information on adverse effects of single chemical stressors to ecologically-relevant aquatic (and terrestrial) species based on peer reviewed science collected through comprehensive searches of the open literature. EPA conducted a systematic review of the literature on mercury toxicity to aquatic life via ECOTOX queries (2015, 2021) and a search of the open literature, e.g., via

Google Scholar (through August 2021) using a structured methodology to ensure the completeness of the dataset.

EPA comprehensively evaluated open literature studies that were collected through ECOTOX and the open literature. EPA then reviewed the studies for data relevance and quality using data quality review guidance in the 1985 Guidelines, EPA's Office of Chemical Safety and Pollution Prevention (OCSPP)'s Ecological Effects Test Guidelines (U.S. EPA 2016b), Guidance for Identifying, Selecting and Evaluating Open Literature Studies (U.S. EPA 2021b), and the EPA Office of Water's internal data quality Standard Operating Procedure to determine which studies were acceptable for the criterion derivation process. This process is consistent with OCSPP's data quality review approach (U.S. EPA 2018). This review process results in one of three determinations for study quality and utility: quantitative, qualitative, or unused. Quantitative studies are included in the effects assessment and used directly in the numeric derivation of the criterion; these are described in detail in **Appendix A**. Qualitative studies are typically discussed in the effects characterization as supporting information for quantitative studies; these are described in detail in **Appendix B**. Unused studies are summarized in **Appendix C** and/or bibliography depending on the study characteristics.

2.7.1 Taxonomic and Other Test Considerations

Based on EPA's interest in using all available high-quality data, EPA considered toxicity studies for possible inclusion regardless of the test species' residential status in North America, as is common practice with other published aquatic life criteria. Non-North American resident species used as laboratory test organisms serve as taxonomic surrogates for untested resident species. Nevertheless, because in this derivation the four most sensitive genera are resident species or closely related taxa (at the genus level) both in North America and in the State of Idaho (see **Section 3.5**), the influence of including non-resident species for the calculation of the

magnitude of the freshwater criterion for mercury is negligible. The influence of non-resident species in other portions of the genus sensitivity distribution has little effect on the criterion values.

Chronic values were based on endpoints and durations of exposure that were appropriate to the species. The chronic studies used in the derivation of the mercury criterion followed taxa-specific exposure duration requirements from various test guidelines (i.e., U.S. EPA's 1985 Guidelines (Stephan et al. 1985), and EPA's OCSPP's Ecological Effects Test Guidelines (U.S. EPA 2016b) when available. Thus, most studies consisted of partial life-cycle tests of sufficient length to ascertain whether dietary exposure to mercury had a deleterious effect on the endpoint of interest. Furthermore, for studies involving amphibian taxa, only dietary exposure studies using fully aquatic life stages (larvae, tadpoles, and metamorphs) of these species were considered since maternal transfer studies using aquatic-dependent or terrestrial adult life stages incorporate dietary exposures from non-aquatic food sources. Studies not included in the genus sensitivity distribution used for numeric criterion derivation, including some studies with dietary mercury exposures, were considered qualitatively as supporting information if they were deemed to be of sufficient quality. These studies are described in the Effects Characterization (**Section 4** and **Appendix B**).

2.8 Mercury Bioaccumulation Considerations

In considering bioaccumulation of mercury and deriving the criterion, EPA used the Bioaccumulation Factor (BAF) model (Burkhard et al. 1997) that numerically represents the relationship between the chemical concentrations in multiple environmental compartments. For the proposed criterion the BAFs are based on empirical data from site-specific measurements from the State of Idaho (Equation 1), with the exception of amphibians, where due to a lack of Idaho-specific amphibian BAF data, data from other states (ME and VT) were used.

$$Bioaccumulation\ Factor\ \left(\frac{L}{kg}\right) = \frac{Tissue\ [\frac{\mu g}{g}\ THg-ww]}{Water\ [\frac{\mu g}{L}]} \qquad \text{(Equation 1)}$$

BAFs were calculated for fish, amphibian, and invertebrate species, and considered and applied in development of both the tissue criterion elements and the water criterion element.

2.9 Approach to Calculating the Criterion Element Values

Protective mercury water column and tissue criterion elements should integrate consideration of both relative sensitivity to mercury and relative mercury bioaccumulation potential across the taxa considered. For example, some species may be very sensitive to mercury, showing effects at low body burdens, but may be less likely to be exposed to and accumulate significant concentrations of mercury from the environment. This may occur because the feeding strategy of the sensitive life stage of a particular species may involve herbivory or omnivory dominated by consumption of lower trophic level organisms, resulting in lower dietary mercury exposures, and the organism may not accumulate enough mercury from the environment to cause adverse effects, except under high environmental mercury conditions. Conversely, a species could be less inherently sensitive to mercury but may have a diet composed of higher trophic level organisms, resulting in exposure to potentially higher dietary mercury levels. Thus, the protective mercury water column and tissue criterion elements account for both organismal sensitivity (i.e., inherent toxicity) and exposure potential (i.e., bioaccumulation).

The following sections detail the process used for the evaluation of toxicity data and development of proposed chronic criterion element values, addressing the aspects of the relative sensitivity of aquatic organisms to mercury in criterion derivation.

2.9.1 Chronic Measures of Effect

The selected measure of effect for chronic dietary exposures to mercury is the effect concentration estimated to produce a chronic toxic effect on survival, growth, or reproduction in 10 percent of the test organisms (EC_{10}; **Table 2-1**), or an estimate of the NOEC, depending on the study design and nature of the available concentration-response data for each study. EPA selected an EC_{10} to estimate a low level of effect that would be different from controls but not cause severe effects at the population level for a bioaccumulative contaminant. The use of the EC_{10} is consistent with EPA criterion development for other bioaccumulative pollutants (e.g., Selenium Freshwater Aquatic Life Criterion (U.S. EPA 2016a) and with approaches used internationally. It is the recommended effect level in the harmonized guidelines from Organization for Economic Cooperation and Development (OECD) and the generally preferred effect level for other countries such as Canada, Australia, and New Zealand (CCME 2007; OECD 2001; Warne MSt.J. 2018).

As in other EPA aquatic life criteria documents, toxicity tests with a sufficient number of treatment levels to characterize a concentration-response (C-R) relationship, thus enabling estimation of chronic effects using approaches that yield an EC_x (x = % effect; e.g., regression analyses), are generally preferred over hypothesis-based study designs. However, the mercury toxicity dataset generated for mercury criterion derivation for Idaho includes studies with experimental designs that did not provide sufficient test concentrations to calculate an EC_{10} (see **Section 3.3**). Therefore, EPA used summary statistics including reported NOECs (No Observed Effect Concentrations) and LOECs (Lowest Observed Effect Concentrations) based on data in the source documents. Maximum Acceptable Toxicant Concentrations (MATCs) were also calculated and a LOEC:NOEC Adjustment Factor (U.S. EPA 1995b; MSRC, U.S. EPA 1997d) was used to help inform the derivation of chronic values. A NOEC is the highest test

concentration at which none of the observed effects were statistically different from the control. A LOEC is the lowest test concentration at which the observed effects are statistically different from the control. The MATC is the geometric mean of the NOEC and the LOEC. An uncertainty factor is employed to indicate uncertainty around the toxic threshold (i.e., LOEC to NOEC). An uncertainty factor of 3 was applied when chronic values were derived from a LOEC. This uncertainty factor accounts for the lack of an identifiable NOEC. This factor was based on a separate analysis (U.S. EPA 1995b) and is consistent with the uncertainty factor used for estimating a NOEC for dietary mercury toxicity studies in aquatic-dependent wildlife (U.S. EPA 1997d).

Although the NOEC has been criticized (Jager 2006) as a surrogate measure of a low EC_x (e.g., EC_{10}), recent evaluations (Beasley et al. 2015; Iwasaki et al. 2015) of statistical comparisons of EC_x (i.e., EC_{10} and EC_{20}) with NOECs for *Daphnia* spp. toxicity studies and other datasets revealed that an EC_{10} was a more suitable analog overall than EC_{20} for a NOEC when test conditions (e.g., pH, hardness). Further, Tanaka et al. (2018) determined, using Monte Carlo simulations, that performance of the NOEC was comparable to or slightly better in predicting concentration response than the EC_x (EC_5 and EC_{10}) when uncertainties in the data were small, and was applicable as the EC_x when concentrations were based on thresholds expected to ensure environmental safety.

2.9.1.1 Approach Used for Studies for which EC_{10} Could Not be Calculated

Because the available data for acceptable chronic tests generally did not allow calculation of an EC_{10}, EPA used estimation techniques to determine the chronic value that is an estimate of the EC_{10} (or an estimate of the NOEC) for each test. The estimate used for this analysis depended on the available effect endpoints (NOEC, LOEC, or MATC) and level of effect relative to the control (U.S. EPA 2013), and was determined according to the following:

a. When significant effects were observed at all treatment concentrations, such that no treatment concentration was classified as a NOEC, then the chronic value was assigned as "less than" (<) the lowest tested concentration (LOEC).

b. When the NOEC and LOEC were among the treatment concentrations, and the NOEC and LOEC were closely spaced (i.e., when the difference was less than ~ 10X), then a MATC was calculated as the geometric mean of the NOEC and LOEC.

c. When no significant effects were observed at any concentration, such that no treatment concentration was defined as a LOEC, then the chronic value was assigned as "greater than" (>) the highest tested concentration. If the greater than (>) chronic value also served as the Species Mean Chronic Value (SMCV), the value was designated as a >NOEC.

d. When all exposure concentrations of a study yielded either too little or too much effect to provide a point estimate of a chronic value (EC_{10}), the level of effect observed at the LOEC was compared with the control treatment and was used to help determine a chronic value equivalent to a NOEC (or EC_{10}) value. If the LOEC resulted in a level of effect greater than 25% when compared with control, than a LOEC:NOEC adjustment factor of 3 (U.S. EPA 1997d) was applied to the LOEC to obtain an estimate for the NOEC.

2.9.1.2 Evaluation Approach for Non-definitive Toxicity Values (greater or less than values)

A decision rule was applied to the mercury toxicity data when an author-reported NOEC or LOEC was used (U.S. EPA 2013). The rule was based on whether these chronic values with a "greater than" (>) or "less than" (<) sign added relevant information to the SMCV. The decision rule was based on the finding that "greater than" values for concentrations of low magnitude relative to the sensitivity range of the 4 most sensitive genera in the SSD, and "less than" values for concentrations of high magnitude relative to the 4 most sensitive genera in the SSD do not generally add significant information to the toxicity analysis. The decision rule was applied as follows: "greater than" (>) low chronic values and "less than" (<) high chronic values were not used in the calculation of the chronic criterion; but "less than" (<) low chronic values and

"greater than" (>) high chronic values were included in the chronic criterion. The latter, indeterminate (> NOEC) values provide a means of comparing the sensitivity of the species to more sensitive species with lower chronic values in studies with similar study designs. Hypothetical examples of this approach are provided below relative to the mercury data.

- A chronic value (NOEC or LOEC) reported as > 0.3 µg/g ww would not be found to provide additional useful information because the "unbounded" value would indicate that no significant effects were observed at the study's highest tested concentration of 0.3 µg/g ww (NOEC) and this data would provide no information to support derivation of an SMCV or Genus Mean Chronic Value (GMCV) since the (>) value is within the range of the lowest 4 genera.
- A chronic value reported as < 50 µg/g ww would not provide useful information for criterion derivation because the value would indicate effects are possible at concentrations below that concentration, providing no information to support derivation of an SMCV or GMCV relevant to criterion derivation since the "less than" value is two orders of magnitude above the range of the lowest 4 genera.
- However, a chronic value (LOEC) reported as < 0.75 µg/g ww would indicate that significant effects were observed even at the study's lowest tested concentration of 0.75 µg/g ww. Although this value is uncertain, it would provide information relevant to derivation of an SMCV. Therefore, to be consistent with principles set forth in the U.S. EPA (1997a-d), a LOEC:NOEC uncertainty factor (UF) of 3 would be applied to the (<) value to estimate the NOEC.
- Similarly, a chronic value reported as greater than the highest concentration tested (e.g., >2.0 µg/g ww) would indicate that no significant effects were observed in the study. This provides relevant information for the derivation of an SMCV since it provides a means of comparing the relative sensitivity of the tested species to more sensitive species with lower SMCVs.
- MATCs were paired with an evaluation of the effect of the LOEC relative to the control to be included in the SMCV calculation. When the LOEC was associated with a low effect compared to the control, EPA evaluated the MATC by comparing it to the study NOEC and control treatments to serve as a reasonable estimate for the EC_{10}. For

example, the differences in percent effect between the LOEC and control in two amphibian studies (Unrine and Jagoe 2004; Bergeron et al. 2011a) were < 20%; therefore, EPA selected the LOEC rather than the MATC as an estimate of the EC_{10} rather than the study NOEC.

2.9.2 Analysis Plan for the Derivation of a Chronic Tissue-Based Criterion Elements for Mercury

The following sections detail how EPA used toxicity effect estimates based on dietary exposures associated with the most sensitive aquatic life taxa to derive the whole-body and muscle tissue criterion elements in combination with consideration of bioaccumulation.

2.9.2.1 Analysis Plan for Derivation of the Chronic Tissue-Based Criteria Elements Magnitude

EPA screened chronic toxicity studies (both laboratory and field studies) to ensure they contained the relevant chronic exposure routes for aquatic organisms, measurement of chronic effects, and measurement of total mercury in tissue(s). EPA used only studies where test organisms were exposed to mercury in their diet, because such studies most closely replicate real-world chronic exposures (diet and/or diet plus water). This approach is consistent with the 2016 Selenium Aquatic Life Freshwater Criterion where diet was also the most significant source of pollutant exposure. (U.S. EPA 2016a). EPA identified a total of over 50 studies with exposure of aquatic life (amphibians, fish, or invertebrates) to mercury. EPA did not use studies quantitatively if either the experimental feeding regime was unclear or if concerns existed about the nature of the experimental results (e.g., control performance). In addition, studies with low (>) values were not used quantitatively (**Appendix B**), as explained above in **Section 2.9.1**.

EPA considered a total of 22 chronic aquatic life studies, resulting in quantitative data for 19 species and 18 genera (**Table 3-4**). The quantitative studies provided seven of the eight MDRs. In addition, there were 3 qualitative studies for fish and 3 studies with invertebrates that EPA reviewed and determined could not be used for criterion derivation due to issues related to

study design or test conditions (**Section 4.2, Section 4.3,** and **Appendix B**). EPA used the invertebrate studies to provide supporting information for the eighth MDR (additional insect order or other phyla). Based on the available data, EPA concluded that the 1985 Guidelines requirements for 8 MDRs were satisfied with inclusion of the invertebrate qualitative data, thus the database is sufficient to derive the chronic tissue criterion elements. EPA used the results from fish studies considered qualitatively to provide supporting information for endpoints and important species not considered quantitatively due to study design or uncertainty in dietary exposure due to effects observed in field collected individuals.

2.9.2.2 Analysis Plan for the Derivation of Whole Body and Muscle Tissue Criteria Elements

Mercury effect concentrations from acceptable chronic dietary toxicity tests for freshwater aquatic animals were reported as either muscle or whole-body concentrations, and therefore had to be translated, as appropriate, for derivation of the Final Chronic Value (FCV) expressed as total mercury in whole body or muscle tissue. For the whole-body and muscle criterion element concentrations, EPA either used chronic values directly as measured in the study or converted them to estimated equivalent whole-body or muscle chronic values. The majority of studies were based on muscle tissue concentrations in fish, and so those concentrations were converted to whole body concentrations in order to derive the whole-body tissue criterion element. To derive the muscle criterion element, EPA derived a whole-body to muscle conversion factor (WB:M CF) in **Appendix D**. EPA identified six studies (Bevelhimer et al. 1997; Boalt et al. 2012; Eagles-Smith et al. 2016; Goldstein et al. 1996; May and Brumbaugh 2007; Peterson et al. 2005) that evaluated the relationship between mercury in whole body and muscle in fish and reviewed them to develop a WB:M CF. These studies provided data for 13 species of freshwater fish (Family Centrarchidae, Cyprinidae, Catostomidae, Ictaluridae, and

Percidae) and two species of saltwater fish (Family Clupeidae and Percidae), and are discussed in the Effects Analysis below (**Section 3.2.1**)

EPA determined that the most scientifically appropriate approach to deriving the final chronic value (FCV) for the tissue criterion elements was to proceed in a manner broadly consistent with the 1985 Guidelines approach (Stephan et al. 1985), but with some adjustments to reflect the fact that mercury biomagnifies in aquatic ecosystems, with tissue concentrations greatly increasing in organisms at higher trophic levels. EPA gathered data on tissue-based measurements of aquatic organisms' sensitivity to mercury exposure via diet. EPA also gathered data on field-based measurements of mercury concentrations water and tissue in aquatic organisms in Idaho and used that data to calculate bioaccumulation factors (BAFs) for mercury in Idaho. These two data sets showed that aquatic species varied widely not only in their sensitivity to mercury, but also in their potential to bioaccumulate mercury from Idaho's aquatic environments, as expected based on scientific literature. The large difference in bioaccumulation potential across aquatic organisms in the data set suggested that it may not be appropriate to calculate the tissue criterion elements based solely on the species sensitivity to dietary exposures, when the sensitive species, amphibians, do not bioaccumulate mercury to as great an extent as fish and large invertebrates. Although EPA had used the 1985 Guidelines criteria derivation approach directly with tissue sensitivity values in its recent derivation of tissue-based aquatic life criteria element for selenium, the minimal variation among organisms in selenium bioaccumulation potential and trophic transfer factors in that data set made the direct application of the 1985 Guidelines approach to tissue data appropriate. For mercury, in the face of much greater variation in bioaccumulation potential among organisms, EPA determined that it was

important to synthesize both mercury sensitivity and mercury bioaccumulation data for aquatic species in deriving the tissue and water criterion elements.

In the data set compiled for Idaho mercury, EPA noted that the species most sensitive to mercury were also those that had (by far) the lowest bioaccumulation potential and, because these most sensitive taxa are the larval stages of amphibians, they are unlikely to be sampled for implementation, given the assumption that Idaho will most likely sample fish tissue for implementation based on their sampling programs for human health protection. EPA recognizes that the state may in the future also evaluate other methods such as dragonfly larvae or crayfish sampling to determine if they are useful quantitatively estimating risks to high TL fish as well, as amphibians. If EPA were to use the 1985 Guidelines criteria derivation approach directly using only tissue sensitivity values and the chronic tissue criterion elements were therefore driven by these amphibian GMCVs, such chronic criteria elements would a) not reflect the best available science regarding mercury bioaccumulation, considering the knowledge that fish are expected to be more likely to accumulate mercury to a body burden associated with toxic effect than amphibians, with their much lower bioaccumulation potential, and b) likely be inaccurate regarding potential effects in fish considering expected implementation via fish tissue sampling. EPA therefore used the following process to modify the chronic tissue criterion elements derivation approach so that tissue criterion elements were both protective of all aquatic species in the data set, including amphibians, *and* appropriate for implementation using fish tissue.

EPA used the 1985 Guidelines criteria derivation approach directly with tissue sensitivity values in its derivation of tissue-based aquatic life elements for selenium and, unlike mercury, did not need to integrate bioaccumulation differences across species in developing the tissue criteria elements. This is because there is minimal variation among organisms in

bioaccumulation potential and trophic transfer factors for selenium, making the direct application of the 1985 Guidelines approach to tissue data appropriate for selenium tissue criterion element derivation. The largest driver of selenium bioaccumulation is the integration of selenium into plankton, detritus, and sediment at the lowest end of the food web, which is quantified by an enrichment factor (EF). Selenium bioaccumulation through trophic levels has less impact on selenium accumulation in tissues overall. This is the opposite of the food web and bioaccumulation dynamics of mercury in aquatic ecosystems where biomagnification, or increasing bioaccumulation as one moves up trophic levels, is a driving factor in tissue accumulation of mercury in aquatic organisms.

Mercury chronic tissue criterion elements calculated following the 1985 Guidelines approach, modified to reflect mercury bioaccumulation dynamics, yielded an estimate of the muscle or whole-body tissue concentration protective of 95% of fish and invertebrates genera, based on available data. Following the rationale described above, EPA completed the chronic tissue criterion element calculations using sensitivity data (EC10s or NOECs) from fish and invertebrates in the numeric derivations, excluding amphibian tissue data from the tissue criteria calculation. Amphibian data are considered quantitatively in derivation of the water column criterion element, and amphibian protection from tissue criterion elements is addressed through an analysis comparing both sensitivity and mercury bioaccumulation potential to fish and invertebrates used in the tissue criteria derivation. (see **Section 4.1**).

Briefly, the tissue criterion elements are derived by first ranking the Genus Mean Chronic Values (GMCV) 1 to N, where N = 16, the number of genera in the sensitivity distribution. Then the cumulative probability, P, is calculated for each ranked GMCV as R/(N+1), where R represents assigned rank and N is the total number of GMCVs. Then, the four GMCVs having

cumulative probabilities closest to 0.05 (note: if N < than 59 GMCVs, these will always be the four lowest GMCVs) and their associated probabilities (Ps) are used to calculate the criterion using the following equations:

$$S^2 = \frac{\sum((lnGMCV)^2) - \left(\frac{(\sum(lnGMCV))^2}{4}\right)}{\sum(P) - \left(\frac{(\sum(\sqrt{P}))^2}{4}\right)}$$

$$\mathrm{L} = \left(\sum(\mathrm{lnGMCV}) - \mathrm{S}\left(\sum(\sqrt{P})\right)\right)/4$$

$$A = S\sqrt{0.05} + L$$

$$\mathrm{FCV} = \mathrm{e}^{\mathrm{A}}$$

Where: S = slope
L = X-axis intercept
A = lnFCV
P = cumulative probability

The proposed tissue criterion elements are expressed as whole-body wet weight or muscle wet weight total mercury concentrations. EPA selected this expression because it fully represents mercury present in the tissue, is a common measurement across all studies, and has wide use by EPA and states, including Idaho, in fish tissue monitoring programs.

2.9.2.3 Analysis Plan for Derivation of Duration of the Tissue Criterion Elements

EPA reviewed information on the duration of fish exposure experiments and the stability of mercury in fish tissue over time, to determine what would be an appropriate chronic tissue criterion element duration.

Test durations resulting in effects observed for chronically sensitive species exposed via diet to mercury (methylmercury) range from 30 – 249 days. Mercury concentrations in fish tissue are generally expected to change only gradually over time in response to environmental fluctuations in global, national, and regional mercury emissions (Sundseth et al. 2017; Angot et

al. 2018) and more localized sources, e.g., mining (Eckley et al. 2015, 2021), wildfires (Webster et al. 2016; Sever 2021), and deforestation (Eckley et al. 2018). Methylmercury has a half-life in adult fish of approximately 2 years (Stopford and Goldwater 1975; Tollefson and Cordle 1986), which is approximately two to five times longer than the half-life of inorganic mercury. However, growth rate can significantly influence mercury accumulation with faster growing juvenile life stages having lower mercury concentrations due to somatic growth dilution (Karimi et al. 2007; Simoneau et al. 2005). Ontogenic shifts in diet (particularly for piscivores), is also an important consideration (Galarowicz et al. 2006), and these characteristics are important to consider when evaluating mercury concentrations in fish tissue.

Typically, once a fish reaches the adult life stage, mercury concentrations in tissue are relatively stable. Hutcheson et al. (2014) found that mercury in largemouth bass and yellow perch from 23 Massachusetts lakes decreased an average of 13% and 19% respectively between 1999-2011, a period of twelve years. Also, Mathieu and McCall (2016) observed no change in mercury concentrations in fish tissue (largemouth and smallmouth bass) in four of five Washington lakes collected between 2005-2014, a nine-year period. However, the fish tissue mercury levels in one of the study lakes increased 44 percent between 2009 and 2014. Also, although Lake Whatcom (WA) exhibited a 60% reduction in mercury fish tissue concentrations between 2000 and 2014 based on comparisons to historical data (Mueller and Serdar 2002), the maximum concentrations in this waterbody were observed in an eight-year-old smallmouth bass, suggesting that observed reductions in mercury concentrations in fish may have been due to replacement by younger, less contaminated fish in the lake, rather than decreases in individual fish tissue body burdens over time, similar to findings from Blanchfield et al. (2022).

Finally, Grieb et al. (2020) reviewed 46 peer-reviewed studies in freshwater fish species yielding 119 "annual percent change" (APC) values demonstrating that for waters with negative trends, the average APC was equivalent to a 34% reduction in fish tissue mercury concentration in 10 years, whereas the average APC value for increasing trends corresponded to a 25% increase in fish tissue mercury concentrations in 10 years. Taken together, these studies indicate that mercury concentrations in fish in the environment are likely to be relatively stable at a given site over time (annual change of 2-3%). Therefore, fish tissue collected from that site can be assumed to integrate and represent the mercury bioaccumulation dynamics at that site over several years.

2.9.2.4 Analysis Plan for Derivation of Tissue Criterion Elements Return Frequency

Ecological recovery times following typical chemical disturbances are situation-specific and largely dependent on: (1) biological variables such as the presence of nearby source populations or generational time of affected taxa; (2) physical variables such as residence time and flow rate, and; (3) chemical variables such as chemical persistence and potential for residual effects. For mercury, where its presence is ubiquitous and sequestration rates are slow (e.g., sediment burial), variables affecting water chemistry (e.g., acidification) and microbial activity associated with methylation can have a significant impact on ecological recovery. In the *Mercury Experiment to Assess Atmospheric Loading in Canada and the United States* (METAALICUS) study, a whole lake and watershed mercury addition study conducted from 2001 – 2003 (Harris et al. 2007) and a set of follow-up experiments from 2002-present (Blanchfield et al. 2022) provided key studies on the subject. One of the key experiments included a 15-year whole-ecosystem monitoring follow-up study to determine the reductions in fish tissue methylmercury concentrations based on cessation in mercury additions to the experimental lake. Harris et al. (2007) determined that direct additions of inorganic mercury to the water resulted in increases in

tissue total mercury concentration in northern pike. Over the longer term, annual recruitment of young fish with low methylmercury and the loss of older more contaminated fish (based on stable population structure) enabled rapid recovery of the population from mercury contamination (Blanchfield et al. 2022), but total mercury concentration in more contaminated individuals did not significantly decrease.

Blanchfield et al. (2022) also observed a small contribution of terrestrially applied isotopic mercury to fish methylmercury concluding that (lentic) waters with larger watersheds will likely respond slower to reductions in atmospheric deposition. Such data are relevant to understanding how long it would take after implementation of pollution abatement for a system exceeding the criterion to reach a condition where it seldom exceeded the criterion; this would depend on the magnitude and duration of the exceedance and other factors, such as ongoing inputs of mercury from other sources such as atmospheric deposition, and the specific biogeochemistry in a waterbody. Two recent studies examining long term trends (e.g., 40 years) in lake trout and walleye in the Lake Ontario and Lake Erie (Bhavsar et al. 2010), and lake trout, northern pike, and walleye in Ontario, CA lakes (Gandhi et al. 2014), indicate that reductions in North American mercury emissions for the 1970s & 1980s yielded reductions in tissue mercury concentrations in these species early on (1990s); however, more recent tissue data from 2000-2007 (Bhavsar et al. 2010) and 1995 – 2012 (Gandhi et al. 2014) exhibit either a flattening trendline (e.g., walleye in Lake Ontario), or increasing concentrations (Lake Erie walleye). So despite the progress in North American mercury reduction, other factors such as global mercury emission sources, climate change (Schartup et al. 2019) and local watershed characteristics (i.e., biogeochemistry, land use changes, terrestrial and aquatic sediment mercury sinks, aquatic food web alterations; Eagles-Smith et al. 2018) are exerting more influence on the response time and

magnitude of mercury concentrations in fish tissue documented more recently than historical progress in mercury emission reductions in North America.

Given the empirical evidence for long recovery times related to reductions in atmospheric deposition of mercury (Blanchfield et al. 2022), the large variation in possible biological and physical variables influencing ecological recovery and continuing atmospheric mercury emissions on global and regional scales, EPA focused on the known chemical attributes of mercury in aquatic systems to inform the frequency of exceedance for the chronic tissue-based criterion elements.

2.9.3 Analysis Plan for Derivation of Chronic Water-Column Criterion Element

The water column criterion element for mercury inherently considers both sensitivity to mercury (EC10s) as well as the bioaccumulation potential of aquatic species with differing trophic ecologies. The relationship between the ambient concentration of mercury in water and the concentration of mercury in the tissue of fish or other aquatic life is primarily through the trophic transfer of mercury, which is greatly affected by site-specific conditions and species-specific trophic ecology. To translate the proposed muscle tissue criterion to an associated water column criterion, EPA used the bioaccumulation factor (BAF) approach (Burkhard et al. 1997; Burkhard 2021; Scudder-Eikenberry et al. 2015; U.S. EPA 2021c). A BAF is the ratio of the concentration of a chemical in the tissue of an aquatic organism to the concentration of the chemical dissolved in ambient water at the site of sampling (U.S. EPA 2001).

The BAF is expressed mathematically as:

$$BAF = \frac{C_{tissue}}{C_{water}}$$

Where: BAF = bioaccumulation factor derived from site-specific field-collected samples of tissue and water (L/kg);
C_{tissue} = concentration of chemical in tissue (µg Hg/g ww); and
C_{water} = ambient concentration of chemical in water (ng/L).

A BAF is a quantitative estimate that represents the ratio of the chemical concentrations in two environmental compartments (water and tissue [e.g., fish muscle] in this analysis). The BAF can then be used to translate a tissue concentration to a water concentration, which is expressed mathematically as:

$$C_{target} = \frac{C_{tissue}}{BAF}$$

Where: C_{target} = water concentration (ng/L);
C_{tissue} = tissue concentration (µg Hg/g ww); and
BAF = bioaccumulation factor derived from site-specific field-collected samples of tissue and water (L/kg).

EPA derived a chronic water column criterion by translating tissue SMCVs in the GSD to water column SMCVs using available BAF data to calculate BAFs for amphibian (frog tadpoles), crayfish, and fish. Although no frog BAF data were available from Idaho, EPA conducted a literature search and calculated a BAF for the wood frog (resident in Northern Idaho; https://idfg.idaho.gov/species/taxa/15529) from available paired tadpole tissue and water data collected in Maine and Vermont from Loftin et al. (2012) and Faccio et al. (2019). The crayfish BAF was calculated using crayfish (tail muscle) data collected from the Boise River in Idaho during the summer of 2021 (University of Idaho Crayfish Mercury Project; https://crayfish.nkn.uidaho.edu/wp-content/uploads/2022/02/Crayfish-Infographic-_FINAL.pdf) and paired with water collected from the Boise River by USGS in the fall of 2020. Fish BAFs for species in Idaho were calculated using paired total mercury measurements in fish tissue and water data collected from lentic and lotic sites in Idaho (Baldwin et al. 2020; Bauch et al. 2009; Eagles-Smith et al. 2016; Essig 2010; IDEQ 2007a, 2007b, 2007c, 2009; MacCoy and Mebane 2018; Poulin et al. 2020; Rutherford et al. 2020; USGS 2022; Willacker et al. 2023) compiled by EPA. Before calculating fish BAFs, data from several sites with high total mercury

concentrations (due to anthropogenic mercury contamination) were removed from the database to better reflect the range of BAFs derived from data for the majority of Idaho sites that were not influenced by anthropogenic contamination from legacy mining. The collection of Idaho fish species BAFs were also used to calculate BAFs representing low, medium, and high trophic magnitude categories, which were applied to fish species in the tissue toxicity database where a taxa-specific BAF could not be derived.

EPA considered paired tissue and water data acceptable for use in BAF derivation if the study identified the unit of measure, the media from which the measurement was made, the species and tissue type (for tissue samples), the location from where the sample was taken, and the date the sample was collected. Fish tissue and water data had to be collected at the same site within one year of each other in order to be used quantitatively to derive a BAF, consistent with the decision rule established for derivation of the enrichment factor (EF) during development of the selenium criterion (U.S. EPA 2016a). Site names were as defined by the respective study authors, and generally reflected a specific sampling location, although one of the lentic samples represented the average of several locations collected in a single sampling period, as those were the only data presented for that location. Combined measurements (e.g., averages of single measurements for water or composite measurements for fish from several locations in the same aquatic system) were also included if exposure conditions were considered similar. EPA only used data from studies where total mercury concentrations for tissue and water samples were within the bounds of concentrations found using analytical methods with suitable detection limits (e.g., EPA Method, 1631B, 1631E; U.S. EPA 2002). The spatial precision of field data sample collection locations was generally at the site level. The temporal precision of sample collection

times was usually at the level of the day they were collected, although some studies only provided enough information to determine the week, month, or year.

The derivation of the chronic water criterion element consisted of the following steps:

1. Fish, frog, and crayfish BAFs were calculated from field-collected tissue and water data (**Appendix E**) that were spatially (within same waterbody) and temporally (within one year) paired. Individual species level BAFs were calculated at the site level by dividing the average (composite or mathematical average) fish tissue mercury concentration by the paired site water mercury concentration. Then these individual species level BAFs for each site were combined across sites and years using medians to calculate a general species-level BAFs. All BAFs were expressed as muscle wet weight.
2. Fish species were binned into three trophic magnitude categories (low, medium, and high) largely corresponding to trophic levels designated in Essig (2010) based on Zaroban et al. (1999). In some instances, additional information regarding trophic ecology and other attributes of Pacific Northwest fish species resident in Idaho were also incorporated into the trophic level categorization determination (Zaroban et al. 1999, Fishbase.org).
3. After binning species into the three trophic magnitude categories, EPA calculated taxa-specific median BAFs when both chronic muscle tissue values and water measurements were available for fish at the species or genus level. EPA then calculated the 80^{th} centile BAF within each trophic magnitude category.
4. Chronic values for water were translated based on application of the fish BAF, frog BAF, or crayfish BAFs to each muscle tissue-based chronic value (as applicable based on

taxonomy or trophic ecology) to calculate translated water column chronic values. The BAF used for a water column translation was selected based on the following approach:

a. If available for a fish species, a BAF based on taxonomic relatedness at the species- or genus-level was applied to fish species chronic values as represented in the muscle tissue-based sensitivity distribution and used to translate water column values for those fish species.
b. If a taxa-specific BAF was not available for a fish species, the 80th centile BAF of the most applicable trophic magnitude category was applied to the fish species based on similar trophic ecology and used to translate the muscle tissue value into a water column chronic value.
c. The wood frog BAF was used as a representative for all species within the Order Anura and applied to the frog and toad chronic values to translate water column chronic values for those amphibian species.
d. The crayfish BAF (derived from Boise River crayfish tissue and water data) was applied to invertebrate chronic values to translate water column chronic values for invertebrates.

5. Once the muscle tissue chronic values were translated into water column chronic values, the FCV for Idaho waters was derived using the 1985 Guidelines approach (Stephan et al. 1985). Briefly, the water column based chronic values derived in Step 4 (a-d) above were ranked from most to least sensitive (1 – N). Then, consistent with the approach described above in **Section 2.9.2.2**, the cumulative probability, P, was calculated for each ranked translated water column chronic value as R/(N+1). Then, the four translated chronic water values having cumulative probabilities closest to 0.05 were used to calculate the

chronic water column criterion (FCV or continuous criteria concentration, CCC), providing a high level of protection, consistent with past practice for derivation of water quality criteria. The water column criterion element was expressed as nanograms per liter (ng/L) total mercury (THg, all mercury forms) in whole water (not filtered).

A more detailed description of the derivation methodology of the water column criterion element is provided in the Effects Analysis (**Section 3.6**). In determining the derivation methodology, EPA also considered scenarios using the 80th centile of taxa-specific BAFs, the effects of aggregating BAFs at a geographically broader waterbody level (vs. the site) for the Coeur d'Alene River, as well as examining the effect of calculating fish BAFs based on even more geographically broad Level III Ecoregion water total mercury concentrations (**Appendix E**; these scenarios were not used in the final chronic criterion).

2.9.3.1 Analysis Plan for Derivation of the Water Column Criterion Duration

In developing the duration aspect of the water column criterion, EPA considered mercury methylation processes affecting trophic transfer and observed durations of bioaccumulation and depuration processes in aquatic organisms (Bradley et al. 2017; Moye et al. 2002, Pickhardt et al. 2002, 2006; Stewart et al. 2008). Mercury bioaccumulation takes place over a longer period of time than often observed for acute and chronic effects on aquatic life based on exposure to aqueous concentrations of typical, non-bioaccumulative contaminants. Mercury cycling in aquatic ecosystems is controlled by various biotic and abiotic reactions interacting on a site-specific basis, which ultimately controls the rate of inorganic to methyl mercury conversion and biological uptake of mercury from the water to biota (Harris et al. 2007).

The determination of appropriate averaging periods for water concentrations of bioaccumulative pollutants, such as selenium and mercury, is explained in Appendix J of U.S.

EPA (2016a). Developing the averaging period, or duration, for the water column criterion included consideration of the characteristic time in the process of reaching a new steady-state plateau contaminant concentration in fish tissue after a change in water concentration yields either net accumulation or depuration. The characteristic time is related to the concept of a biological half-life and is defined as the reciprocal of the depuration rate coefficient (1/k) in a single compartment toxicokinetic model.

2.9.3.2 Analysis Plan for Derivation of the Water Column Criterion Return Frequency

The frequency aspect of water quality criteria is the number of times a chemical concentration (here, total mercury concentration in water) exceeding the criteria can occur over time without negatively affecting the aquatic community. The standard, current frequency recommendation (Stephan et al. 1985, U.S. EPA 1991) for water column criteria is once-in-3 years on average, based on the ability of an aquatic ecosystem to recover from a toxic stress. This frequency was applied for the water column criterion element for the bioaccumulative chemical selenium in the U.S. EPA (2016a) criterion document, and the Perfluorooctanoic acid (PFOA) and Perfluorooctane sulfonic acid (PFOS) Aquatic Life Criteria (U.S. EPA 2022a,b)

3 EFFECTS ANALYSIS FOR FRESHWATER AQUATIC ORGANISMS

The toxic effects of mercury in aquatic ecosystems are a function of its chemical structure and propensity to biomagnify, particularly in higher trophic level organisms, with longer-lived organisms and predators at the top of the food web at the highest risk for exposure and toxicity. BAFs are important to consider in development of the mercury criterion, in addition to direct dietary toxicity of mercury. BAFs are an empirical estimate of the relationship between mercury in water and biota reflecting the capability of mercury to bioaccumulate and cause observed dietary toxicity effects. EPA thus considered both bioaccumulation potential and toxicity in the derivation of tissue and water column criterion elements reflecting this knowledge.

3.1 Analysis of Bioaccumulation Data for Mercury in Idaho

EPA collected data for aquatic organism mercury body burdens and associated water column concentrations of mercury, and calculated BAFs for various aquatic organisms and taxa groupings. For all BAF calculations, tissue and water were considered spatially and temporally paired if they were collected at the same site within one year, consistent with the approach followed to calculate enrichment factors for selenium (U.S. EPA 2016a). These pairing conditions are also consistent with those for "high quality" BAFs defined in Burkhard (2021).

3.1.1 BAF Calculations

Fish species BAFs were calculated from a database of Idaho fish tissue and water samples compiled by EPA. This database included fish tissue and water measurements from a variety of sources (**Section 2.9.3**), as well as additional unpublished data (**Appendix E**). Fish BAFs were calculated by dividing the fish tissue concentration by a spatially and temporally paired water concentration (**Section 2.9.3**).

A wood frog (*Lithobates sylvaticus*) BAF was calculated from paired water and tissue data from two field studies (Loftin et al. 2012; Faccio et al. 2019 - **Appendix E**). These data

were collected in vernal pools located in Acadia National Park, ME and forests in east-central Vermont. A crayfish BAF was calculated using tissue and water data collected in the Boise River, ID by the Crayfish Mercury Project (University of Idaho) and USGS. (**Appendix E**).

All BAFs were expressed as L/kg-ww based on muscle tissue. Most of the sampled fish tissues were muscle. The frog tissues (Loftin et al. 2012 and Faccio et al. (2019), and the remainder of fish from Idaho were whole-body, which were converted to muscle concentrations using a whole-body to muscle conversion factor of 0.72 for fish and 0.97 for frogs (**Appendix D**). All of the crayfish tissue samples were muscle.

Four hundred and seventy-four BAFs were initially calculated from the Idaho fish tissue and water database for all possible tissue and water pairings. This initial dataset was then censored to remove seven sites (84 BAFs) across five mercury-contaminated watersheds (Cinnabar Creek, Jordan Creek, Orofino Creek, Portneuf River, and Sugar Creek) with high water total mercury concentrations (4.25-92.7 ng/L) due to legacy mining activities. Mercury BAFs are inversely correlated with mercury water concentrations; this censoring was intended to produce a set of BAFs that are more representative of bioaccumulation levels at concentrations that are reflected broadly across the state, based on atmospheric deposition. This resulted in 390 individual fish BAFs from 43 sites, predominantly lotic sites, as summarized in **Table 3-1** and **Table 3-2**. This dataset is the starting point for all options considered by EPA and presented below and in **Appendix E**. The detailed methods for calculating fish, invertebrate and frog BAFs are provided below.

Table 3-1. Summary of Idaho Fish BAF Database

Characteristic	Value (full BAF dataset)	Value (censored BAF dataset)
Number of BAFs	474	390
Number of Waterbodies	31 (27 lotic, 4 lentic)	28 (24 lotic, 4 lentic)
Number of Sites	50 (45 lotic, 5 lentic)	43 (38 lotic, 5 lentic)
Number of Fish Species	32[a]	30[a]
Water THg (ng/L)	Min.-Max.: 0.17-92.68 Median: 1.00 20^{th}-80^{th} centile: 0.49-2.35	Min.-Max.: 0.17-6.21 Median: 0.77 20^{th}-80^{th} centile: 0.41-1.20
Fish Tissue THg (mg/kg-ww)	Min.-Max.: <0.20-1.25 Median: 0.17 20^{th}-80^{th} centile: 0.08-0.28	Min.-Max.: <0.02 – 1.25 Median: 0.14 20^{th}-80^{th} centile: 0.07-0.25
BAF (L/kg-ww)	Min.-Max.: 2,104-4,142,857 Median: 127,826 20^{th}-80^{th} centile: 45,474-341,463	Min.-Max.:16,991-4,142,857 Median: 161,524 20^{th}-80^{th} centile: 89,796 – 397,316
# Fish/Tissue Sample	Single: 368 Composite: 77 (Range 2-207; 64 ≤20) Not Reported: 29	Single: 303 Composite: 76 (Range 2-207; 64 ≤20) Not Reported: 11
Fish Length (mm)	Min.-Max.: 44-720 Median: 322 20^{th}-80^{th} centile: 207-540 Not Reported: 68	Min.-Max.:44-720 Median: 335 20^{th}-80^{th} centile: 260-570 Not Reported: 38
Fish Weight (g)	Min.-Max.: 2-4,902 Median: 340 20^{th}-80^{th} centile: 47-1,727 Not Reported: 88	Min.-Max.:2-4,902 Median: 486 20^{th}-80^{th} centile: 159-2,051 Not Reported: 56

[a] Brook trout and Northern pikeminnow were subdivided into large and small categories based on length, and bull trout were also redesignated as a small category based on length. The BAFs associated with the small size category for all three species were designated as medium trophic magnitude, vs. the high trophic magnitude designations for these species representing (large) adult life stages exhibiting a piscivorous trophic ecology.

3.1.1.1 Calculations of BAFs for Fish Species

The fish BAF dataset was reduced to a set of species level fish BAFs through a series of steps, as follows:

1) Calculation of Fish Species by Site BAFs:

   a) BAFs were calculated for every unique fish species by site combination.

   b) There was a mixture of fish tissue sample data composed of both physical composites and individual samples. To create parity across the fish samples, if more than one individual

fish tissue sample for the same species during the same year at the same site was available, then the arithmetic mean total mercury tissue concentration was calculated. The species calculated composites and physical composites at each site were then used in calculating BAFs.

c) A Species by Site BAF was calculated as the combination of the average total mercury tissue concentration (arithmetic mean or physical composite) divided by the paired site water total mercury concentration, when both samples were collected within the same calendar year. These calculations resulted in 119 BAFs representing every unique fish species-by-site-by-year combination from the initial set of 390 BAFs (**Table E-1**).

d) When a BAF for a fish species at a particular location was available for more than one year, yielding multiple temporally separated BAFs for the same fish species at a given location, then the median of those multiple year BAFs was calculated to represent the species-location combination.

   i) These calculations yielded a total of 101 BAFs representing every unique "fish species by site" combination calculated from the set of 119 fish species-site-year BAFs in step 1c.

2) Calculation of Fish Species BAFs across sites

   a) If more than one site had data sufficient to calculate a BAF for a given species, a median fish species BAF was calculated using the "fish species by site" BAFs from across all sites.

      i) A total of 30 median BAFs for each fish species were calculated from the set of 101 fish species-by site BAFs from step 2a. The use of medians to reduce the dataset from 101 species-site BAFs to 30 species BAFs across sites was employed to reduce the

effect of outliers and was consistent with the approach followed when deriving the enrichment factors (EFs) for the national selenium aquatic life criterion (U.S. EPA 2016a).

Table 3-2. Fish Species BAFs Used in the Tissue to Water Translation Procedure.

Fish Common Name	Trophic Magnitude Category	Fish Length Range[a] (Min.-Max.) (mm)	THg BAF[b] (L/kg)	Muscle THg Median[b] (µg/g ww)	Muscle THg Range[a] (Min.-Max.) (µg/g ww)
Banded killifish	Medium	51.81-56.8 (n=2)	34,055 (n=2)	0.070 (n=2)	0.066-0.075 (n=2)
Black crappie	Medium	250-250 (n=1)	45,089 (n=1)	0.280 (n=1)	0.28-0.28 (n=1)
Bluegill	Medium	82.36-117.0 (n=3)	77,925 (n=2)	0.160 (n=2)	0.147-0.181 (n=3)
Bridgelip sucker	Low	44-550 (n=4)	144,915 (n=3)	0.086 (n=3)	0.04-0.234 (n=4)
Small Brook trout	Medium	250-250 (n=1)	66,667 (n=1)	0.064 (n=1)	0.064-0.064 (n=1)
Large Brook trout	High	400-430 (n=2)	586,705 (n=2)	0.164 (n=2)	0.153-0.174 (n=2)
Brown trout	High	360-450 (n=2)	302,721 (n=2)	0.174 (n=2)	0.052-0.253 (n=3)
Bull trout	Medium	143-218 (n=27)	108,418 (n=2)	0.065 (n=2)	0.023-0.2 (n=27)
Channel catfish	Medium	309.5-720 (n=88)	205,123 (n=6)	0.247 (n=6)	0.06-0.738 (n=88)
Common carp	Medium	570-610 (n=2)	175,835 (n=2)	0.195 (n=2)	0.138-0.252 (n=2)
Crappie sp.	Medium	182.7-244.4 (n=2)	100,894 (n=2)	0.209 (n=2)	0.203-0.214 (n=2)
Cutthroat trout	Medium	230-530 (n=12)	165,114 (n=8)	0.061 (n=8)	0.037-0.87 (n=12)
Cutthroat trout x Rainbow trout hybrid	Medium	460-460 (n=1)	333,333 (n=1)	0.240 (n=1)	0.24-0.24 (n=1)
Flathead catfish	Medium	537-537 (n=1)	256,008 (n=1)	0.477 (n=1)	0.477-0.477 (n=1)
Kokanee salmon	Medium	320-320 (n=1)	491,304 (n=1)	0.113 (n=1)	0.113-0.113 (n=1)

Fish Common Name	Trophic Magnitude Category	Fish Length Range[a] (Min.-Max.) (mm)	THg BAF[b] (L/kg)	Muscle THg Median[b] (µg/g ww)	Muscle THg Range[a] (Min.-Max.) (µg/g ww)
Largemouth bass	High	500-500 (n=1)	92,110 (n=1)	0.572 (n=1)	0.572-0.572 (n=1)
Largescale sucker	Medium	257.4-550 (n=8)	191,430 (n=8)	0.194 (n=8)	0.083-0.489 (n=8)
Mountain whitefish	Medium	135-460 (n=80)	179,367 (n=17)	0.097 (n=17)	0.04-0.63 (n=90)
Small Northern pikeminnow	Medium	83.3-228 (n=2)	69,347 (n=2)	0.136 (n=2)	0.067-0.205 (n=2)
Large Northern pikeminnow	High	330-330 (n=1)	687,755 (n=1)	0.674 (n=1)	0.674-0.674 (n=1)
Pumpkinseed	Medium	104.2-137.5 (n=2)	63,802 (n=2)	0.128 (n=2)	0.089-0.167 (n=2)
Rainbow trout	Medium	250-510 (n=24)	161,685 (n=7)	0.132 (n=7)	0.02-0.48 (n=24)
Salmonidae sp.	Medium	ND[c]	84,810 (n=1)	0.134 (n=1)	0.134-0.134 (n=1)
Sculpin	Medium	ND[c]	91,874 (n=2)	0.056 (n=2)	0.02-0.086 (n=26)
Smallmouth bass	High	156.6-452 (n=75)	258,163 (n=15)	0.253 (n=15)	0.04-1.02 (n=75)
Sucker sp.	Low	208.8-208.8 (n=1)	35,385 (n=1)	0.066 (n=1)	0.066-0.066 (n=1)
Utah sucker	Low	380-440 (n=2)	73,651 (n=2)	0.112 (n=2)	0.032-0.192 (n=2)
Walleye	High	442-457 (n=2)	453,578 (n=1)	1.002 (n=1)	0.753-1.25 (n=2)
Warmouth	Medium	98-98 (n=1)	54,616 (n=1)	0.128 (n=1)	0.128-0.128 (n=1)
Yellow perch	Medium	207.2-264 (n=4)	131,289 (n=4)	0.225 (n=4)	0.108-0.587 (n=4)

[a] n based on number of reported length or muscle total mercury (THg) measurements for each species from the set of 390 fish BAFs.

[b] n based on number of reported BAF or muscle total mercury (THg) measurements for each species from the set of 101 unique "site x species" fish BAFs.

[c] ND – no data.

3.1.1.1.1 Characterization of Idaho Fish Tissue Data for Derivation of Fish Trophic Magnitude BAFs

After calculating species-level BAFs, Idaho fish species were assigned to low, medium, and high trophic magnitude categories, which largely correspond to trophic levels 2, 3, and 4. Trophic magnitude category assignments designated in Essig (2010) based on Zaroban et al. (1999) were used, with the following exceptions:

Kokanee salmon was assigned to a trophic level of 2 by Essig (2010), consistent with a diet of aquatic plants and algae. However, Kokanee salmon are primarily planktivorous, with diets consisting largely of freshwater zooplankton, but which can also include some aquatic insects, as well as aquatic plants. Because they mainly consume zooplankton, the Kokanee salmon was assigned to the medium trophic magnitude category for purposes of BAF calculation. Sculpin were present in the BAF dataset but were not reported in Essig (2010). This benthic species is classified here as a medium trophic magnitude category species based on their omnivorous diet (Zaroban et al. 1999; Natureserve.org - Accessed 2023). Bull trout collected in Idaho by Essig (2010) were also classified as a medium trophic magnitude category species based on size, as Guy et al. (2011) reported that Bull trout became primarily piscivorous at lengths greater than 506 mm, more than double the upper end of the range of bull trout in the BAF dataset (**Table 3-2**). Generally smaller bull trout consume primarily invertebrates (aquatic insects, crayfish); and only bull trout > 300 mm were observed to consume fish in an assessment in Utah waters (Budy et al. 2004). Brook trout and northern pikeminnow were also subdivided into medium and high trophic magnitude categories based on fish length and relationship to dietary changes over the lifespan of the organism. Brook trout less than 250 mm were classified as medium trophic magnitude, and those greater than 250 mm were classified as high trophic magnitude, based on the observation that diets of individuals greater than 250 mm included more

large prey fish (Brown and Rasmussen 2009). Northern pikeminnow less than 300 mm were classified as medium trophic magnitude, and those greater than 300 mm as high trophic magnitude, based on the observation that northern pikeminnow in the smaller size range consumed a diet consisting primarily of invertebrates (Northwest Power and Conservation Council 2004), whereas the diet of larger pikeminnow consists mainly of fish and crayfish increasing in importance as fish size increases (Poe et al. 1991).

Finally, the sucker taxa (Bridgelip sucker, Utah sucker, and unidentified sucker species) were assigned to the low trophic magnitude category as they were the closest surrogate for the low trophic magnitude category, although they were classified as trophic level 3 by Essig (2010). With the exception of Kokanee salmon, Essig (2010) classified all species in the Idaho fish tissue dataset as trophic level 3 or 4, whereas four fish species in the muscle tissue criterion dataset were classified as low trophic magnitude species where no taxa-specific BAF was available. Because of this, a sucker-based BAF was considered the most representative surrogate low trophic magnitude BAF for these species. Sucker species are omnivorous, although the relative importance of plants or animals in their diets can vary greatly depending on fish size, prey availability, and other site-specific factors. In addition, both the Utah and Bridgelip sucker trophic levels were designated as 2.8 by Fishbase.org, indicating some herbivory in their diets. Finally, the sucker-based low trophic magnitude category BAF of 144,915 L/kg was similar to the crayfish BAF of 128,414 L/kg, which also has an omnivorous diet. Although there is some uncertainty in the low trophic magnitude fish BAF, it is the best available BAF for this category given the available data and is likely a conservative value given the likelihood of omnivory in the field-collected sucker species.

After assigning trophic magnitude categories to all fish species, representative BAFs for each trophic magnitude category were calculated by selecting the 80th centile of the (median) species-specific BAFs within each trophic magnitude category. Selection of the 80th centile is consistent with past approaches for selecting protective water column values (e.g., U.S. EPA 2016a) as it provides a high probability of protection for most aquatic species. Because there were only three fish species within the low trophic magnitude category, the highest BAF within that category was selected.

3.1.1.1.2 Development of Fish Taxon-Specific BAFs

For Idaho fish species with BAFs that are represented in the muscle tissue sensitivity distribution the species-specific BAF for the species or its taxonomic surrogate was calculated (**Table 3-3**). Four species in the muscle tissue criterion dataset (Atlantic salmon, *Salmo salar*; channel catfish, *Ictalurus punctatus*; rainbow trout, *Oncorhynchus mykiss*; and walleye, *Stizostedion vitreus*) also had tissue data corresponding to three species and one genus level surrogate (brown trout; *Salmo trutta*) in the BAF database. Similar to the trophic magnitude category BAFs, taxon specific BAFs were derived in the same way, using the median species by site BAF from each species, except walleye. The lack of paired tissue and water samples for walleye from different sites required treating the two BAFs calculated from the same site during different years separately, and so the median was based on two temporally separate samples from the same site.

Table 3-3. Taxon-Specific Fish BAFs Used in the Tissue to Water Translation Procedure.

Trophic Magnitude Category	Common Name (Scientific Name)	Median THg (mg/kg ww)	BAF (L/kg muscle-ww)
Low		NA	144,915 (80th centile)
Medium		NA	199,646 (80th centile)
High		NA	647,335 (80th centile)
	Wood Frog *L. sylvaticus*	NA	8,222 (median)
	Crayfish (sp.)	NA	128,414 (geomean)
	Walleye (*Stizostedion vitreus*)	1.00	453,578 (median)
	Channel catfish (*Ictalurus punctatus*)	0.247	205,123 (median)
	Rainbow Trout (*Oncorhynchus mykiss*)	0.132	161,685 (median)
	Brown trout (*Salmo trutta*)	0.174	302,721 (median)

3.1.1.2 Development of Amphibian (Wood Frog) BAF

Because of the different bioaccumulation dynamics of amphibians, a separate BAF was calculated from wood frogs (Loftin et al. 2012; Faccio 2019) to represent all frog species. Paired total mercury tissue concentrations and water data were available from two studies for the wood frog (*L. sylvaticus*) collected from seasonal woodland pools in Acadia National Park, ME, and from vernal pools in Vermont. Loftin et al. (2012) reported individual late larval whole-body total mercury ww measurements and paired water concentrations at three sites, whereas Faccio et al. (2019) reported individual whole-body methylmercury dw measurements and paired methylmercury and total mercury water concentrations at six sites for four life stages (embryo, early larvae, late larvae, adult). EPA first converted the dry weight concentration data reported in Faccio et al. (2019) to equivalent wet weight total mercury concentrations using the average percent moisture (86.23%) from measurements of post metamorphic amphibian life stages. EPA then converted the whole-body concentrations to estimated muscle concentrations using a

conversion factor of 0.97. The final amphibian BAF of 8,222 L/kg was calculated based on study level BAFs using three site medians from Loftin et al. (2012) and the median of six site BAFs based on tissue concentrations of the late larval stage frogs measured in both studies. Although the available BAF data for amphibian (frog) tadpoles were collected from different states, the setting of the collection (influenced by atmospheric deposition only), and total mercury concentrations in water and total and methylmercury concentrations in tadpole tissue were comparable to water and tissue concentrations in toxicity tests (Unrine and Jagoe 2004) as well as tissue concentrations observed at field sites in other states (Carolina Bays wetlands; Unrine et al 2004) and likely similar to settings in Idaho waters. Details of the wood frog BAF calculation procedure are described in **Appendix E.2**.

3.1.1.3 Development of Invertebrate (Crayfish) BAF

A crayfish BAF was calculated and used to represent all invertebrate species. Native signal crayfish (*Pacifastacus leniusculus*) and nonnative red swamp crayfish (*Procambarus clarkii*) were collected from the Boise River ID in 2021 by the Idaho Crayfish Project (https://crayfish.nkn.uidaho.edu/wp-content/uploads/2022/02/Crayfish-Infographic-_FINAL.pdf) and paired with water samples obtained from the USGS National Water Information System (NWIS) in 2022 (USGS 2022). The final crayfish BAF of 128,414 L/kg was used as a surrogate BAF for all invertebrate taxa and was calculated as the average tail muscle tissue from crayfish collected from the Boise River, ID paired with the geometric mean total mercury concentration from three water samples collected in 2020-2021 (within 1 year) by USGS. Although three of the four invertebrate species in the toxicity dataset were not crayfish species, the crayfish BAF was considered the most representative surrogate BAF for invertebrate taxa, because three of the four species were from phylum Arthropoda. In addition, the trophic ecology of all invertebrate species in the sensitivity distribution was most closely related with the low trophic magnitude

category, and the crayfish BAF was relatively similar to the sucker species BAF of 144,915 L/kg that was used as a surrogate BAF for low trophic magnitude fish species. Although there is some uncertainty in the application of the crayfish BAF to non-crayfish invertebrate species, it is the only available invertebrate BAF, and is most likely protective given the likelihood of omnivory in field-collected crayfish. All BAFs used in the tissue to water translation are shown in **Table 3-3**.

3.1.2 Characterization of Idaho Water Data Used for Derivation of BAFs

EPA derived bioaccumulation factors (BAFs) for Idaho waters using available paired water and tissue data (Essig 2010; USGS 2022 (National Water Information System (NWIS) - accessed 2022; Willacker et al. 2023). Most of the water data consisted of whole (unfiltered) water samples collected with fish tissue during periods of seasonally low discharge (July-October). First EPA evaluated the dataset and removed whole water samples that contained high concentrations of recalcitrant particle-bound Hg from an industrial source, primarily legacy gold mining. These were identified using water quality investigations generated by Idaho to support Total Maximum Daily Loads for mercury and resulted in exclusion of data for five waterbodies. In these waterbodies it is very common to observe high whole water mercury concentrations driven by particulate bound mercury (e.g., Jordan Creek – mean THg = 20.9 ng/L (13.3 – 92.7 ng/L; IDEQ 2009). In these instances, the particulate fraction may have low bioavailability because it represents the entrainment of tailings particles where Hg may be in a recalcitrant particle-bound form (Eckley et al. 2021). Therefore, after removing samples from these waterbodies, the remaining whole water samples used for BAF derivation did not contain high concentrations of recalcitrant particle-bound Hg from an industrial source.

Most of the BAFs were derived using available paired tissue and water collected during lower flow regimes (July – October) when transport of particle-bound mercury would be

expected to be lower due to stream discharge rate. Several gauged river sites were available for EPA to investigate the relationship between the river flow and paired mercury samples collected over time at these sites. The data from three lotic sites (Payette, Boise, and Salmon Rivers – **Figure 3-1**, **Figure 3-2**, **Figure 3-3**) illustrate the relationship between seasonality of river flow and total mercury concentrations collected (open diamond) from representative sites in three Idaho rivers, with the highlighted samples (filled diamond) representing the paired tissue and water collections used for BAF derivation. These figures demonstrate that the Hg data used in the BAF calculations were representative of lower discharge conditions when the Hg concentrations were on the lower-end and more likely to represent dissolved-phase concentrations.

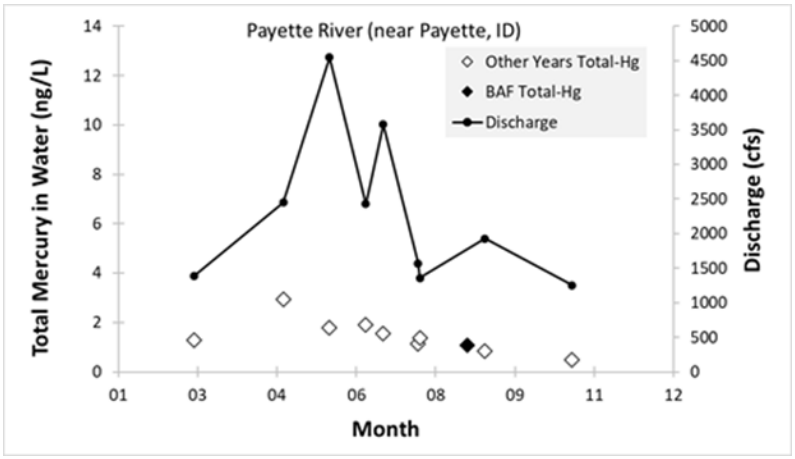


Figure 3-1. Relationship of Total Mercury and monthly average discharge in the Payette River, Idaho.

Diamonds represent water sampling [THg] by month of collection for various years where mercury in water was assessed. The closed diamond is the water concentration collected in August 2008 that was paired with concurrently collected fish tissue to derive BAFs for fish species collected from the Payette River.

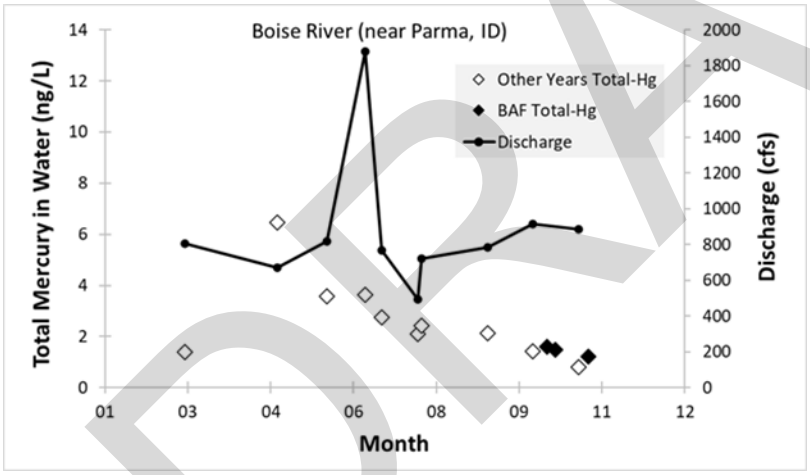


Figure 3-2. Relationship of Total Mercury in water to Seasonal flow of Boise River, Idaho.

Diamonds represent water sampling [THg] by month of collection for various years where mercury in water was assessed. The closed diamonds are the water concentrations collected in October 2013, 2015, and 2017-18 that were paired with concurrently collected fish tissue to derive BAFs for fish species collected from various sites on the Boise River.

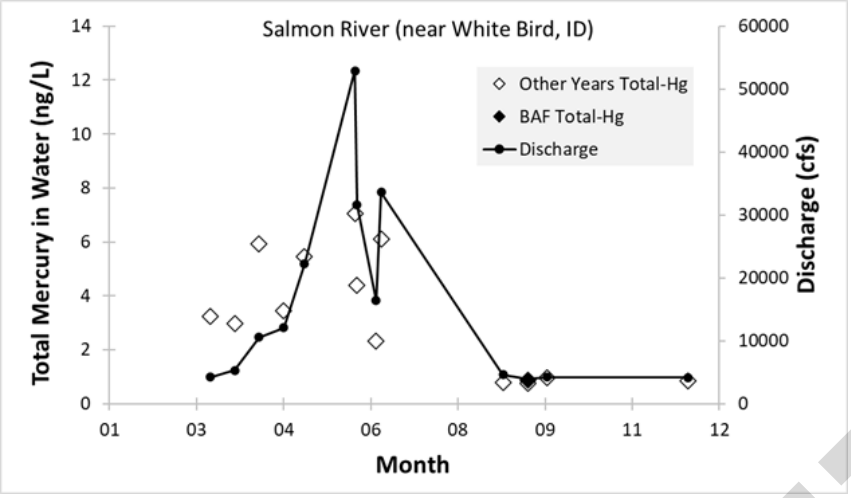


Figure 3-3. Relationship of Total Mercury in water to Seasonal flow of Salmon River, Idaho.

Diamonds represent water sampling [THg] by month of collection for various years where mercury in water was assessed. The closed diamonds are the water concentrations collected in August 2006, and September 2008 that were paired with concurrently collected fish tissue to derive BAFs for fish species collected from various sites on the Salmon River.

EPA estimated the percent dissolved mercury at lotic and lentic sites during baseflow conditions using data from 24 sampling sites within Idaho where both dissolved and whole water mercury data obtained from the National Water Information System (NWIS); https://waterdata.usgs.gov/nwis downloaded 5/24/2022). Of these locations, 10 are lotic and 14 are lentic (all of which are from reservoirs). All of the locations are predominantly impacted by mercury from atmospheric and watershed sources, and none are directly downstream of contaminated sites. EPA focused its evaluation on samples collected during baseflow conditions (July to mid-October), because the majority of water and fish that were used in the BAF calculations were collected during that period. For the lentic sites, the mean percent Hg in the dissolved phase was 61±15%, (n=438) and for the lotic sites the mean percent Hg in the dissolved phase was 56±16% (n=43). Although the remaining mercury is in the particulate phase, the whole water samples will not contain high concentrations of recalcitrant particle-bound Hg from an industrial source, since these sites were omitted from consideration. Instead,

the particulate bound fraction of mercury in these samples is more likely to be mercury associated with fine particulates (including detritus and planktonic matter) where the mercury is less tightly bound and may become part of the dissolved phase, especially following deposition to the sediment.

Most of the BAFs used were derived using available paired tissue and whole water samples collected during lower flow regimes (July – October), when turbidity from spring snowmelt has subsided in Idaho. During this period, the transport of particulate mercury would be expected to be lower, and greater than half (50-55%) of the total mercury in surface water was present in the dissolved phase (NWIS, accessed 10/5/2021). EPA removed sampling data associated with waters impacted by anthropogenic sources (e.g., legacy mining), so the water data used for BAFs will not reflect THg influenced by samples containing high concentrations of recalcitrant particle-bound Hg from an industrial source. Instead, the particulate bound fraction of Hg in these samples may be Hg associated with particulate organic matter where the Hg is less tightly bound and may become part of the dissolved phase, especially following deposition to the sediment (Eckley 2023, personal communication). Therefore, EPA has concluded that whole water (unfiltered) samples provide an appropriate representation of the maximum amount of Hg that could become methylated and bioaccumulate in biota.

3.2 Summary of Mercury Toxicity Studies Used to Derive the Aquatic Life Criterion

EPA reviewed all available and relevant chronic toxicity studies relating to the toxicological effects of mercury for data quality and evaluated them for incorporation into the derivation of the criterion for Idaho. Quantitative data for chronic dietary toxicity of mercury to aquatic life were available for 19 freshwater species, representing 18 genera and 12 families in 11 orders (**Table 3-4**). Study summaries of the six most sensitive genera are presented below (**Section 3.3**). **Section 3.5** presents the ranked GMCVs used in the derivation of the FCV based

on either whole body (**Table 3-6**) or muscle (**Table 3-7**). **Section 3.5** also examines the relative mercury bioaccumulation of amphibians to invertebrates and fish (**Table 3-8**), presents the calculation of the tissue criteria elements for fish and invertebrates only (**Table 3-10** and **Table 3-11**), and provides graphical presentations of genus sensitivity distributions (**Figure 3-4** and **Figure 3-5**). Discussion of additional acceptable studies that provided quantitative information for less sensitive genera that were included in the derivation of the chronic criteria elements are presented in detail in **Appendix A**.

Table 3-4. Summary Table of Minimum Data Requirements per the 1985 Guidelines Reflecting the Taxonomic Classifications for Acceptable Quantitative Studies in the Freshwater Toxicity Dataset for Mercury.

MDR	Freshwater			
	SMCV	GMCV	Family	Order
Family Salmonidae in the class Osteichthyes	2	2	1	1
Second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species	11	10	5	5
Third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)	2	2	2	1
Planktonic Crustacean	1	1	1	1
Benthic Crustacean	1	1	1	1
Insect	1	1	1	1
Family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, or Mollusca)	1	1	1	1
Family in any order of insect or any phylum not already represented[a]	0	0	0	0
Total	**19**	**18**	**12**	**11**

[a] One MDR, (Requirement H - Family in any order of insect or any phylum not already represented), was not fulfilled with acceptable quantitative chronic data. However, EPA considered qualitative data for annelids and rotifers as discussed below (Section 4.3). Taken together with the invertebrate MDRs that were met, EPA concluded that not having met MDR H would not substantively affect the resulting FCV to develop a chronic freshwater criterion.

3.2.1 Derivation of Whole Body and Muscle Tissue Values

Mercury effect concentrations from acceptable chronic dietary toxicity tests for freshwater aquatic animals were reported as either muscle or whole-body concentrations, and therefore had to be translated, as appropriate, for derivation of the Final Chronic Value (FCV)

expressed as total mercury in whole body or muscle tissue. For the whole-body and muscle criterion element concentrations, EPA either used chronic values directly as measured in the study or converted them to estimated equivalent whole-body or muscle chronic values. The majority of studies were based on muscle tissue concentrations in fish, and so those concentrations were converted to whole body concentrations in order to derive the whole-body tissue criterion element. To derive the muscle criterion element, EPA derived a whole-body to muscle conversion factor (WB:M CF). EPA identified six studies (Bevelhimer et al. 1997; Boalt et al. 2012; Eagles-Smith et al. 2016; Goldstein et al. 1996; May and Brumbaugh 2007; Peterson et al. 2005) that evaluated the relationship between mercury in whole body and muscle in fish and reviewed them to develop a WB:M CF. These studies provided data for thirteen species of freshwater fish (Family Centrarchidae, Cyprinidae, Catastomidae, Ictaluridae, and Percidae) and two species of saltwater fish (Family Clupeidae and Percidae). Conversion factors ranged from 0.57 (common carp, *Cyprinus carpio*; Goldstein et al. 1996) to 0.86 (herring, *Clupea harengus*; Boalt, 2012). Six of the studies contained either equations to calculate mean and median WB:M CFs, or WB:M CFs that can be used directly for EPA purposes allowing EPA to derive a WB:M CF of 0.72 based on the median of the available data for use in tissue criterion element derivation. This factor is consistent with WB:M CF for mercury reported elsewhere (e.g., 0.74; Eagles-Smith et al. 2016).

EPA also conducted a literature search for information regarding paired whole body and muscle total mercury concentrations in amphibians, with emphasis on fully aquatic life stages (late-stage tadpoles and early metamorphs) of frog or toads. No such information was found specific to these life stages via preliminary search; however, Hothem et al. (2009) provided results of paired muscle (hind leg) and total body mercury in bullfrog (*Lithobates catesbeianus*)

tissues from the Cache Creek watershed, Northern California. The author found that the majority of mercury (82-84%) was present in the whole body (carcass), with the remainder in leg muscle (11%) and liver (~6%). EPA used the available tissue data from a mix of 10 juvenile and adult bullfrogs to calculate a mean WB:M CF of 0.97.

It is currently unknown whether this conversion factor is representative of larval (aquatic life stages) of the Order Anura and other amphibians. However, given the relatively high ratio of inorganic to methylmercury in the food (*aufwuchs*) and resultant tissue concentration ratios observed in tadpoles (Unrine and Jagoe 2004; Faccio et al. 2019), and the influence of the form of mercury on the differential uptake and assimilation between inorganic mercury and methylmercury observed in fish tissues (Kidd and Batchelar 2012; Bradley et al. 2016), it is biologically plausible for the whole body to muscle ratio for total mercury to be higher in larval amphibians than in fish. Since methylmercury is typically higher in muscle (due to the presence of high levels of sulfhydryl groups in muscle) this mechanism may help explain the ratio of mercury observed in whole body and muscle tissues during (and just after) metamorphosis in anuran amphibians.

For aquatic invertebrate studies (aquatic insect, cladoceran, mollusk, crayfish) EPA determined it was unnecessary to use a conversion factor for expression as an equivalent concentration in muscle since the most sensitive invertebrate was a crayfish (see **Table 3-5**) and the crayfish toxicity value was measured in tail (abdominal) muscle. For the invertebrate whole body to muscle conversion factor, EPA used the inverse of the fish WB:M conversion factor of 0.72 (1/0.72, or 1.39) since a conversion factor for crayfish was not available. The remainder of the invertebrate taxa were relatively insensitive to mercury and the chronic values from these

studies had only a minor effect on the magnitude of the mercury tissue-based criterion through their inclusion in the criterion calculation sample size parameter ("N").

In addition to whole-body and muscle conversion factors, it was necessary to also convert tissue mercury concentrations reported as dry weight to wet weight. For toxicity tests using fish, wet weight was calculated from dry weight tissue concentrations or dietary concentrations using percent moisture estimates reported in the source document. In the absence of a reported value for the tested species, EPA used a species-specific or taxonomic surrogate-specific percent moisture estimate reported in compilations from the open literature (Appendix E; GEI 2014; National Contaminant Biomonitoring Program (NCBP) USGS (2016)). For toxicity tests using amphibian (Anuran) tadpoles and metamorphs, wet weight was calculated from dry weight tissue concentrations for species with data from the American toad; *Bufo americanus* (Bergeron et al. 2011a; Bergeron et al. 2011b; Todd et al. 2011), European Common Frog, *Lithobates temporaria* (Fletcher and Myant 1959) and the wood frog, *Lithobates sylvatica* (Wada et al. 2011) using the average percent moisture (86.23%) from measurements of pre-metamorphic life stages of the three species above as reported in the source documents. Background information and data for these estimates are provided in **Appendix D**.

3.3 Acceptable Studies of Dietary Effects of Mercury for the Six Most Sensitive Genera

Below is a brief synopsis of the experimental design, test duration, relevant test endpoints, and other critical information regarding the four sensitive genera most influential to the calculation of chronic tissue criterion elements. The studies in this section involve effects of dietary mercury on survival, growth, and reproduction of aquatic life. Effect concentrations are reported both as whole body and muscle tissue equivalents, based on application of the appropriate tissue conversion factors. Data for all taxa used for criterion element derivation are

summarized in **Table 3-5**. Details of these studies and other chronic studies considered for criterion element derivation are contained in **Appendix A**.

3.3.1 Most Sensitive Genera: Lithobates (Rana) sphenocephala (Southern leopard frog) Family Ranidae (true frogs)

Unrine and Jagoe (2004) exposed southern leopard frog (*Lithobates* (*Rana) sphenocephala*) larvae to experimental diets formulated using *aufwuchs* (surface growth/periphyton and associated biotic and abiotic components) from control and mercury-enriched mesocosms (See **Appendix A.2.1** for detailed description of diet and study design) to examine the chronic effects of dietary mercury exposure on larval and metamorph stages of this species. Tadpoles (Gosner Stage (GS) 25) were assigned to a control or one of three mercury-contaminated dietary treatment groups. The measured total mercury and methylmercury concentrations in the dietary treatments were 0.054 µg total mercury/g dw (0.012 µg methylmercury/g dw or 22 % as methylmercury) in the control; 0.423 µg total mercury/g dw (0.014 µg methylmercury/g dw, or 3.4 % as methylmercury) in the low treatment, 1.409 µg total mercury/g dw (0.27 µg/g dw, or 1.9%, methylmercury) in the medium treatment, and 3.298 µg total mercury/g dw (0.47 µg/g dw methylmercury, or 1.5% as methylmercury) in the high treatment. The tadpoles were observed every one to two days for survival, food consumption, and developmental abnormalities for a total of 254 days. Complete tail resorption was defined as completion of metamorphosis, at which point the study was terminated. Dietary mercury exposure (duration of 194 days) resulted in total and methylmercury whole body tissue concentrations of 0.049 µg THg/g dw (0.021 µg MeHg/g dw) in the control treatment, 0.095 µg THg/g dw (0.018 µg MeHg/g dw) in the low treatment, 0.23876 µg THg/g dw (0.020 µg MeHg/g dw) in the medium treatment, and 0.412 µg THg/g dw (0.028 µg MeHg/g dw) in the high treatment.

The authors determined survival, metamorphic success, and malformation rate to be dependent on mercury treatment but did not report NOECs or LOECs. Survival was 88.2%, 100%, 72.2% and 72.2% in control, low, medium and high doses, respectively, and log-likelihood ratio tests (*G* tests) of independence were used to examine the effect of treatments on survival ($p = 0.406$), and exact *p* values for *G* statistics were estimated by Monte Carlo simulation. Metamorphic success rates, the difference in time to reach benchmark developmental stages (hindlimb formation, forelimb formation, and tail resorption) were evaluated using survival time analysis using developmental benchmarks instead of mortality and log-likelihood ratio tests (G Tests) to assess effect of dietary treatments. Metamorphic success rates were 82.4, 100, 66.7, and 72.2% for control, low, medium, and high mercury diets, respectively ($G = 10.4703$, $p = 0.0293$). Malformation rates, evaluated using a log-logistic concentration response model ($r^2 = 0.9945$, $p = 0.0475$) were 5.9% (1/17), 5.6% (1/18), 11.1% (2/18), and 27.8% (5/18) in control, low, medium, and high treatments, respectively,

Although observed effects (malformation rate, metamorphic success, and mortality) were higher in the two highest dietary mercury concentrations tested compared to controls, effects observed in the low dietary mercury treatment were less than controls. This may be due to a threshold effect resulting from the slightly higher tissue methylmercury concentration in the control treatment (0.021 µg MeHg/g dw) as compared to the low treatment (0.018 µg MeHg/g dw) and is not unexpected if methylmercury has effects at low concentrations.

EPA evaluated these effects data to estimate the NOEC (low mercury treatment) and the LOEC (medium mercury treatment). Based on the whole-body accumulation data reported by Unrine and Jagoe (2004), the corresponding whole-body total mercury NOEC and LOEC for combined effects (survival, malformation rate, and metamorphic success) were 0.095 and 0.2376

µg THg/g dw respectively. This corresponds to a 16% difference in survival and a 15.7% difference in metamorphic success between the control and the LOEC. The LOEC also corresponds to an 11.1% malformation rate, and this endpoint provides the best evidence of dose-response from dietary exposure. This malformation rate was consistent with a 10% effect level, and the difference between the control and LOEC for survival and metamorphic success are relatively small (< 20%). Therefore, EPA selected the LOEC of the study (0.2376 µg THg/g dw) as the surrogate for the EC_{10}. EPA then used the average post-metamorphic stage percent moisture of 86.23% based on data for species in Bufonidae and Lithobatidae (Ranidae) as described in **Section 2.9.2.2** for the dry weight to wet weight conversion. The LOEC for survival and metamorphic success in southern leopard frog based on whole body total mercury is 0.03272 µg THg/g ww (0.2376 µg/g dw ÷ 7.26), the value EPA selected for criterion element derivation from the study. [7.26 is the dw to ww conversion factor for amphibians.] This whole-body total mercury value is equivalent to 0.03373 µg THg/g ww total mercury in muscle after applying the WB:M conversion factor of 0.97.

3.3.2 2nd Most Sensitive Genera: Anaxyrus (Bufo) americanus (American toad) Family Bufonidae

Bergeron et al. (2011a) examined the effects of maternally- and trophically-derived (dietary) mercury on larval development of American toads (*Anaxyrus americanus*). Eggs were collected from breeding-pair females found in pools along historic mercury-contaminated and reference stretches of the South River, Virginia, however, only dietary effects of mercury on tadpoles spawned from reference site females were considered here for criterion derivation; the maternally-derived mercury effects are not included in this criterion analysis. Larvae (approximately 4 days post-hatch) were fed a control (0.010 µg THg/g dw), low (2.50 µg THg/g dw), or high (10.1 µg THg/g dw) mercury contaminated diet, similar to the formulation by

Unrine and Jagoe (2004) for 26-28 days. The percent of methylmercury was quantified in each diet also measuring 56.7% (0.0057 µg MeHg/g dw) in control diet, 3.19% (0.0798 µg MeHg/g dw) in low diet, and 1.05% (1.061 µg MeHg/g dw) in the high diet.

Larval survival was high in all treatments until the onset of metamorphic climax (80, 92, and 96% for larvae from reference mothers fed control, low, and high mercury diets, respectively), but decreased during metamorphic climax to 60, 44, and 48%, respectively, for metamorphs fed those same diets. Therefore, only the results collected for survival, development, and swimming performance before metamorphic climax were further considered for mercury criterion derivation. There was no effect of dietary mercury exposure on survival or average swimming speed of larvae. However, dietary exposure to mercury had a significant effect on mass at GS 42 (Component ANOVA, $p = 0.004$), but not on the duration of larval period (Component ANOVA, $p = 0.79$). Post hoc Tukey's tests showed that mass at GS 42 differed significantly between larvae fed the control diet and high mercury diet ($p = 0.004$). On average, animals fed the high mercury diet were 16% smaller than those fed control diet. The mean whole-body total mercury concentrations at the dietary NOEC and LOEC for mass at GS 42 were roughly 0.800 and 1.800 µg THg/g dw, respectively, resulting in an MATC of 1.2 µg THg/g dw. EPA selected the MATC as a surrogate for the EC_{10} rather than the NOEC because the percent effect between the LOEC and the control was small (16%). EPA used the average post-metamorphic stage percent moisture of 86.23% based on data for species in Bufonidae and Lithobatidae (Ranidae) as described in **Section 2.9.2.2** to convert the dry weight effects concentration to an equivalent wet weight concentration. The MATC for decreased mass at GS 42 in American Toad based on whole body total mercury is 0.1653 µg/g ww (1.2 µg THg/g dw ÷ 7.26), the value EPA selected for criteria derivation from the study. This whole-body total

mercury value is equivalent to 0.1704 µg THg/g ww in muscle after applying the WB:M conversion factor of 0.97.

3.3.3 3rd Most Sensitive Genera: Pimephales promelas (Fathead Minnow) Family Cyprinidae

In the first of a series of related laboratory experiments, Hammerschmidt et al. (2002) examined the effects of either dietary or maternally-transferred methylmercury on fathead minnows (*Pimephales promelas*) in a full life-cycle test. The study included four sequential phases corresponding to life stages of the fathead minnow (F_0 juvenile, F_0 sexual maturation and spawning, F_1 embryogenesis, and F_1 larval growth). For Phase 1, juvenile (~3 month old) fathead minnows were fed one of four diets (Soft-moist fish food, Nelson and Sons, Inc.) contaminated with methylmercuric chloride until sexual maturity (assessed as sexual dimorphism; ~240 days). Mean dietary concentrations were 0.060 µg/g dw (control), 0.88 µg/g dw (low), 4.11 µg/g dw (medium), and 8.46 µg/g dw (high) exposure, respectively, analyzed as total mercury. Sexually mature males and females from each dietary exposure were paired randomly for reproduction studies in Phase 2. Dietary exposures were manipulated during the 136-day period of the reproductive phase (see details in **Appendix A.2.3**) to evaluate the effects of dietary methylmercury during gametogenesis, as well as relative effects of either male or female exposure during gametogenesis and spawning. Spawning behaviors and reproductive success were observed. Finally, the 7-d survival and growth of fathead minnow progeny were determined in Phase 4.

Several aspects of the reproductive process were negatively impacted, particularly in fish exposed in Phase 1 (from juvenile stage to sexual maturity) and as mating pairs exposed in Phase 2 to methylmercury in the diet. EPA re-evaluated study data and found that reproductive effort (defined by number of eggs laid/day and the total number of eggs laid) of fathead minnow was

significantly [negatively] affected (total eggs laid, p = 0.03163, n = 13; and number eggs laid/day, p = 0.01765, n = 13; Wilcoxon rank sum test). Also, dietary exposure reduced overall spawning success of mating pairs of exposed fathead minnows. Spawning success is defined as the percentage of pairs within a dietary treatment that spawned a clutch of (5 or more) eggs within 21 days after placement in breeding aquaria. Spawning success of mating pairs fed the control diet during Phase 1 and 2 was 81%, whereas pairs fed the low and medium mercury-contaminated diets was 50%, and spawning success was 36% for the high methylmercury diet. This result represents a reduction in spawning success relative to control levels of 31% and 45% in low/medium and high methylmercury diets, respectively. Also, for those mating pairs that spawned successfully, the average time to spawn a clutch of 5 or more eggs was 4 days, 7.8 days, 7.6 days, and 14 days for control, low, medium, and high dietary exposures, respectively.

The mean whole-body total mercury concentrations attained by male and female fish exposed to the same diet during Phases 1 and 2 were 0.32 and 0.48 (THg µg/g dw, control diet), 2.83 and 3.40 (THg µg/g dw, low methylmercury diet), 11.7 and 14.0 (THg, µg/g dw, medium methylmercury diet), and 18.4 and 22.2 (THg µg/g dw, high methylmercury diet), respectively. The arithmetic means of the average male and female whole-body total mercury concentrations (0.40, 3.102, 12.85, and 20.3 (THg, µg/g dw) were used to represent effect concentrations. Dietary methylmercury was observed to reduce reproductive capacity based on daily and total number of eggs laid by spawning female fathead minnows in the study, resulting in a 31% reduction of reproductive capacity from control levels observed in the low methylmercury diet fed in Phases 1 and 2. The authors also observed reduced gonadal development ($r^2 = 0.15$, p = 0.005, n = 52) due to mercury exposure; and EPA notes that this result could contribute to effects on reproductive capacity. For the LOEC, the whole-body mean total mercury concentration of

male and female fish fed the low methylmercury diet in Phases 1 and 2 is 3.102 µg THg/g dw, or 0.7246 µg THg/g ww (3.102 µg THg/g ww ÷ 4.28) based on 76.64% moisture content in fathead minnow (U.S. EPA 2021c). EPA applied a LOEC:NOEC uncertainty factor of 3 (U.S. EPA 1997d) to the LOEC (0.7246 µg/g ww), yielding an estimate for the NOEC of 0.2415 µg THg/g ww, based on whole body, or 0.3355 µg THg/g ww based on muscle after application of a WB:M conversion factor of 0.72. EPA recommended these values for use in deriving the mercury criterion elements from this study.

Drevnick and Sandheinrich (2003) conducted a study similar in design as Phase 1 of Hammerschmidt et al. (2002). Juvenile fathead minnow (ninety days post-hatch) were fed a control or methylmercury-contaminated diet (0.058, 0.87, and 3.93 µg/g dw measured as total mercury) until sexual maturity (approximately 250 days). After fathead minnows became sexually dimorphic (~ 300-320 days post-hatch), five breeding pairs were selected and randomly assigned, within treatment, pre-sexual maturation for reproductive trials and subsequent blood and tissue sample collection.

Methylmercury suppressed testosterone levels in males (ANOVA, $F_{2,12} = 4.941$, $P = 0.03$), as well as estrogen levels in females (ANOVA, $F_{2,12} = 9.135$, $P < 0.01$). Dietary methylmercury also adversely affected the reproductive success (proportion of pairs spawning within 21 days) of fathead minnows in a dose-dependent manner ($X^2_{df=2} = 10.439$, $P < 0.01$). Spawning success was 32% in controls, 12% in the low treatment, and 0% in the highest treatment. The mean total mercury carcass (whole body less plasma and gonads) concentrations (µg THg/g ww) for males and females were 0.071 and 0.079 in controls, 0.864 and 0.917 in the low treatment, and 3.557 and 3.842 in the highest treatment, respectively. The arithmetic mean of the average male and average female carcass total mercury concentrations was used to

represent effect concentrations (0.0750, 0.8901, and 3.70 µg THg/g ww in control, low and high treatments, respectively). Since there was no study concentration between the control and the lowest concentration eliciting a toxic effect (NOEC), EPA estimated the NOEC for this study by applying an uncertainty factor of 3 (U.S. EPA 1997d), to the LOEC carcass concentration of 0.8901 µg THg/g ww (the low exposure) resulting in an estimated NOEC of 0.2967 µg THg/g ww, based on whole body concentrations, or 0.4121 µg THg/g ww based on muscle tissue after application of a WB:M conversion factor of 0.72. EPA selected these values for use in criterion derivation.

Sandheinrich and Miller (2006) used a similar study design as Hammerschmidt et.al. (2002) and Drevnick and Sandheinrich (2003) to examine the effects of dietary methylmercury on the production of testosterone in and the reproductive behavior of male fathead minnows, expanding on the previous experiments designed to elucidate reproductive effects in fathead minnow from dietary exposure to methylmercury at ecologically-relevant concentrations. Using the exposure scenario previously described for Drevnick and Sandheinrich (2003), 200 juveniles were exposed to dietary total mercury concentrations of 0.058 µg THg/g dw (control), 0.87 µg THg/g dw (low), and 3.93 µg THg/g dw (medium treatment). After fathead minnows became sexually mature, breeding pairs (one male and one female fish) were selected from each dietary exposure and assigned randomly to a breeding aquarium and this procedure was repeated until sufficient pairs were assigned to evaluate effects on reproductive endpoints planned for this study.

As previously found by Hammerschmidt et al. (2002) and Drevnick and Sandheinrich (2003), dietary exposure to methylmercury at ecologically-relevant concentrations did not impact growth or survival of fathead minnows. However, dietary methylmercury did alter the

reproductive behavior of male fathead minnows. Exposure suppressed mating behavior ($F_{2,12}$ = 3.263, p = 0.07) resulting in the reduction of reproductive success of pairs of fish exposed at both mercury-contaminated levels (chi-square statistic = 17.5, degrees of freedom = 5, p <0.05). Control fish had a spawning success of 40%, but low- and medium-treatment level fish both had spawning success of 20%. Mean male total mercury carcass (whole body less plasma, measured as total mercury) concentrations were 0.068 µg THg/g ww in controls, 0.7140 µg THg/g ww in the low exposure, and 4.225 µg THg/g ww in the high exposure treatment. Since there was no study concentration between the control and the lowest concentration eliciting a toxic effect (NOEC), EPA estimated the NOEC for this study by applying an uncertainty factor of 3 (U.S. EPA 1997d), to the LOEC carcass concentration of 0.7140 µg THg/g ww, resulting in an estimated NOEC of 0.2380 µg THg/g ww. EPA selected this value for criterion derivation. EPA calculated the geometric mean of the three studies (0.2574 µg THg/g ww) and used that value as the SMCV for fathead minnow. After applying the WB:M conversion factor of 0.72, this whole-body total mercury value is equivalent to 0.3575 µg THg/g ww in muscle.

3.3.4 4th Most Sensitive Species: Procambarus clarkii (Red Swamp Crayfish) Family Cambaridae

Brant (2004) evaluated the relationship of sex and age on uptake, elimination, and potential adverse effects of dietary methylmercury on three different age classes (3-week [4th molt], five-week [6th molt], and 8-week old [8th molt]) of juvenile red swamp crayfish (*Procambarus clarkii*). Mean total mercury concentration in brood females was 32.16 ng/g dw, indicating a low potential for maternal transfer. Juvenile crayfish were fed one of two mercury-contaminated diets for 142 days: a low mercury diet (farm-raised channel catfish; *Ictalurus punctatus*) containing a mean concentration of 0.009 µg THg/g ww (80% methylmercury), which was used as the presumed control, and high mercury diet (wild-caught largemouth bass;

Micropterus salmoides) containing a mean concentration of 0.278 µg THg/g ww (98% methylmercury). Age classes composed of both males and females were randomly assigned a diet containing either the low or high mercury concentration until each treatment had 36 crayfish.

Survival and growth and molting were observed throughout the exposure, and behavior (time to find and enter shelter, and forced escape response from shelter area) was also evaluated. Chronic exposure to the high mercury diet resulted in higher mortality than in the low (presumed control) mercury diet treatment ($p=0.025$, $\chi^2=5.25$). Nine of 36 crayfish (25%) died in the high mercury dietary treatment, whereas only 2 (5.5%) died in the low mercury treatment: 75% vs 94% survival, respectively. Crayfish weight did not differ between diet treatments in any age group, but crayfish fed the high mercury diet took approximately twice the time to find refuge as those fed the low mercury diet in behavioral trials. Total mercury was measured in tail (abdominal) muscle and reported in deceased crayfish; mean total mercury of the nine crayfish killed by high dietary mercury exposure was 7.757 µg THg/g dw (< LOEC) versus 0.3033 µg THg/g dw in the low mercury diet. Brant (2004) did not provide an estimate for percent moisture, however EPA used the equation, "Wet weight =5.28607*dry weight$^{0.937422}$" (Anastacio et al. 1999) yielding an average percent moisture of 80.77% (n = 9). The swamp crayfish LOEC of 1.492 µg THg/g ww for reduced survival, the value EPA selected for criterion derivation from the study, was converted from a dry weight estimate of 7.757 µg THg/g dw (7.757 µg THg/g dw ÷ 5.20). EPA divided the LOEC of 1.492 µg THg/g ww by an uncertainty factor of 3 (U.S. EPA 1997d) to estimate a NOEC for the study of 0.4973 µg THg/g ww in abdominal muscle tissue. This muscle total mercury value is equivalent to 0.3581 µg/g ww total mercury in whole body after applying the inverse of the average fish WB:M conversion factor of 0.72 since a conversion factor for crayfish was not available.

3.3.5 5th Most Sensitive Species: Sander vitreus (Walleye) Family Percidae

Friedmann et al. (1996) randomly assigned and acclimated hatchery-raised juvenile (6-month-old) walleye in four 180 L aquaria (22 animals per tank) over a period of two and a half months. Fish were maintained on a natural diet (farm-raised catfish fillets), prior to the study, and this same diet was used in the exposures. Fish length (total) and weight were recorded after acclimation, then exposed to methylmercury for six months via a natural diet. Catfish fillets were injected with methylmercury (Sigma Chemical Company, St. Louis, MO) dissolved in distilled water resulting in a low mercury diet (0.1 µg Hg/g food) and a high mercury diet (1.0 µg Hg/g food). Analyses confirmed dietary concentrations to which walleye were exposed as control (< 0.04 µg THg/g ww]), low dose (0.137 µg THg/g ww), and high dose diet (0.987 µg THg/g ww). Test organisms were fed 1gram pieces of methylmercury injected fish three times per week, increased to 1.5 grams at three and half months into the 6-month exposure period. Diets were supplemented with uncontaminated and MeHg-injected fathead minnow (1.3-1.5 grams) approximating the MeHg doses in the catfish fillets at 6 weeks after exposure initiation. At the end of the six-month exposure, mercury body burdens in walleye were determined, as well as dietary methylmercury effects on growth, gonadosomatic index (GSI), and cortisol levels. Walleye body burdens were 0.06 µg THg/g ww (control fish), 0.25 µg THg/g ww (low dose diet) and 2.37 µg THg/g ww (high dose diet).

Mortality in low dose (45%) and high dose (32%) were evaluated against control mortality rates (28%) using Kaplan-Meier survival statistics, and differences were not significant. EPA therefore used this study, even though control mortality was slightly elevated. Elevated control mortality illustrates the difficulty in maintaining larger wild fish species for long exposure durations. Methylmercury exposure did have a significant negative effect on both fish length (r=0.82; P-C 0.004) and weight (approximately 25-30% reduction; r= 0.74; P < 0.02).

Also, gross measurement and histological assessment of the gonads revealed effects of dietary methylmercury exposure on reproductive potential in walleye. Pooled analyses of control versus exposed fish also showed a significant decrease in GSI for male fish, and histological examination revealed testicular atrophy in both mercury-exposed groups, with severity being dependent on dietary dose. Based on the approximately 25-30% reduction in weight gain at the high mercury exposure, the tissue total mercury NOEC and LOEC for walleye were determined to be 0.25 and 2.37 µg/g ww, respectively, yielding an MATC of 0.7697 µg THg/g ww as a whole-body concentration, and 1.069 µg THg/g ww as a muscle concentration equivalent based on application of a WB:M conversion factor of 0.72. These values were utilized by EPA in criterion derivation.

3.3.6 6th Most Sensitive Species: Huso huso (Beluga Sturgeon) Family Acipenseridae

Gharaei et al. (2008) examined the effects of dietary methylmercury exposure on bioenergetics of beluga sturgeon focusing on mortality, food consumption, and specific growth rate (SGR) based on a 70-day dietary exposure. A fish meal (62.8 % herring powder) based diet containing sufficient nutrients to meet the sturgeons' dietary needs was prepared. The prepared diet was stabilized with gelatin to reduce dissolution of the pellets in water, minimizing methylmercury release. Then methylmercuric chloride dissolved in ethanol was combined with the fish meal preparation to achieve dietary concentrations of 0.04 mg/kg (control); 0.76 mg/kg (low mercury); 7.88 mg/kg (medium mercury) and 16.22 mg/kg (high mercury). The low mercury dose is similar to total mercury observed in sturgeon prey items collected from the Caspian Sea (Agusa 2004). Total mercury content in the diet was confirmed from three random samples per treatment.

One hundred juvenile beluga sturgeon were transferred from the reproduction facility to the laboratory to acclimate to the feeding regimen and test conditions for three weeks. Animals

were fed an experimental diet three times per day based on fish biomass. After acclimation, 20 fish were distributed to twenty 500 L tanks each. Each treatment was replicated five times with 100 fish total per treatment. Mean muscle concentrations at day 70 were <0.05, 3, and 9 µg THg/g ww for the control, 0.76 and 7.88 µg/g total mercury dw diets, respectively. While only 2-4% percent mortality was observed in the control, low, and mid-level treatment diets, 100% mortality was observed in the highest test diet (16.22 mg/kg) with death occurring between 40 and 42 days. The most sensitive apical endpoint from this study was SGR measured from day 36 to day 70 with the two lowest mercury supplemented test diets having SGR significantly less than the control. The control SGR in this time period averaged 2.3 g, whereas SGR for the low and medium treatments were 2.06 g and 1.31 g, a 10.4% and 41% difference from the SGR of the control, respectively. Since the percent effect of the low dietary mercury treatment approximated an EC_{10} level of effect, EPA selected the muscle tissue concentration of 3 µg THg/g ww (or 2.16 µg THg/g ww as an estimated whole-body concentration based on application of a WB:M conversion factor of 0.72) as the values to represent the sensitivity of this species to dietary mercury exposure in the chronic dataset.

Note: Beluga sturgeon is ranked 9th in the whole-body sensitivity table below because tigerfish (*Hoplias malabaricus)*, Channel catfish (*Ictalurus punctatus*), and goldfish (Carassius auratus), are listed in the table for reference, despite their non-definitive qualitative values which were reported as lower than the definitive beluga sturgeon value.

3.4 Summary of Acceptable Studies of Dietary Mercury Exposure to Vertebrates

Table 3-5 summarizes the dietary information and effect concentrations obtained from all acceptable toxicity studies with fish and fully aquatic life stages of amphibians (tadpoles/metamorphs). Detailed summaries of the remainder of the toxicity studies (beyond the six most sensitive genera described above) can be found in **Appendix A**.

Table 3-5. Acceptable Dietary Mercury Exposure Studies.

Basis for chronic values are highlighted in bold[1]. Ranked by sensitivity based on whole body values.

Rank	Species	Dietary Description	Dietary Effect Concentrations (µg THg/g)	Tissue Effect Concentrations (µg THg/g)	Conversion Factors Applied	Chronic Value THg (µg/g ww)[2]	Endpoint and Reported Level of Effect	SMCV THg (µg/g ww)[2]	GMCV THg (µg/g ww)[2]	Reference
1	Southern leopard frog (*Lithobates sphenocephala*)	Aufwuchs from control and mercury-enriched mesocosms, enriched rabbit food embedded in agar medium	NOEC: 0.423 MATC: 772 LOEC: 1.409 (dry weight)	NOEC: 0.095 MATC:0.1502 **LOEC: 0.2376** (dry weight; whole body)	DW:WW (86.23 % Moisture; 7.26) WB:M CF = 0.97	0.03272 (whole body) 0.03373 (muscle)	Malformation Rate[3] LOEC is an 11.1 % malformation rate	0.03272 (whole body) 0.03373 (muscle)	0.03272 (whole body) 0.03373 (muscle)	Unrine and Jagoe 2004
2	American toad (*Anaxyrus americanus*)	Dry feed mix spiked with or without [inorganic mercury; (HgII) and methylmercury chloride; Alfa Aesar] in agar gelatin mixture similar to Unrine and Jagoe (2004).	NOEC: 2.5 MATC:5.025 LOEC: 10.1 (dry weight)	NOEC: 0.8 **MATC: 1.2** LOEC: 1.8 (dry weight; whole body)	DW:WW (86.23 % Moisture; 7.26) WB:M CF = 0.97	0.1653 (whole body) 0.1704 (muscle)	Growth LOEC is 16% reduction in mass @ Gosner Stage 42	0.1653 (whole body) 0.1704 (muscle)	0.1653 (whole body) 0.1704 (muscle)	Bergeron et al. 2011a
3	Fathead minnow (*Pimephales promelas)*	Soft-moist fish food (Nelson and Sons, Inc.) mixed with methylmercury chloride	NOEC:< 0.88 MATC:< 0.88 LOEC: 0.88 (dry weight)	NOEC: < 3.102 MATC: < 3.102 **LOEC: 3.102** (dry weight; whole body)	Chronic Value = LOEC/3 (GLI) DW:WW (76.64 % Moisture; 4.28) WB:M CF = 0.72	0.2415 (whole body) 0.3355 (muscle)	Reproduction: Reduction in reproductive capacity (daily & total number of eggs/female) vs controls LOEC is 31%	0.2574 (whole body) 0.3575 (muscle)	0.2574 (whole body) 0.3575 (muscle)	Hammerschmidt et al. 2002
		Same as Hammerschmidt et.al. 2002	NOEC:< 0.87 MATC:< 0.87 LOEC: 0.87 (dry weight)	NOEC: < 0.8901 MATC: < 0.8901 **LOEC: 0.8901** (wet weight; whole body)	Chronic Value = LOEC/3 (GLI) WB:M CF = 0.72	0.2967 (whole body) 0.4121 (muscle)	Reproduction: Reduction in spawning success vs controls LOEC is 62.5% reduction			Drevnick and Sandheinrich 2003

Rank	Species	Dietary Description	Dietary Effect Concentrations (µg THg/g)	Tissue Effect Concentrations (µg THg/g)	Conversion Factors Applied	Chronic Value THg (µg/g ww)[2]	Endpoint and Reported Level of Effect	SMCV THg (µg/g ww)[2]	GMCV THg (µg/g ww)[2]	Reference
		Same as Hammerschmidt et.al. 2002	NOEC:< 0.87 MATC:< 0.87 LOEC: 0.87 (dry weight)	NOEC: < 0.714 MATC: < 0.714 **LOEC: 0.714** (wet weight; whole body)	Chronic Value = LOEC/3 (GLI) WB:M CF = 0.72	0.2380 (whole body) 0.3306 (muscle)	Reproduction: Reduction in spawning success vs controls LOEC is 50 % reduction			Sandheinrich and Miller 2006
4	Red Swamp Crayfish (*Procambarus clarkii*)	Farm-raised catfish (low mercury diet) or wild caught largemouth bass (high mercury diet)	NOEC:< 0.278 MATC:< 0.278 LOEC: 0.278 (wet weight)	NOEC: MATC: **LOEC: 7.757** (dry weight; muscle)	Chronic Value = LOEC/3 (GLI) DW:WW (80.77 % Moisture; 5.2) WB:M CF = 0.72	0.3581 (whole body) 0.4973 (muscle)	Survival LOEC is 25 % mortality vs controls	0.3581 (whole body) 0.4973 (muscle)	0.3581 (whole body) 0.4973 (muscle)	Brant 2004
5	Walleye (*Sander vitreus*)	Farm-raised catfish fillets injected with methylmercury, supplemented at 6 weeks with fathead minnows injected with methylmercury	NOEC: 0.137 MATC: 0.3677 LOEC: 0.987 (wet weight)	NOEC: 0.25 **MATC: 0.7697** LOEC: 2.37 (wet weight; whole body)	WB:M CF = 0.72	0.7697 (whole body) 1.069 (muscle)	Growth: Reduction in weight gain LOEC is 30% reduction	0.7697 (whole body) 1.069 (muscle)	0.7697 (whole body) 1.069 (muscle)	Friedmann et al. 1996
6	Tigerfish (*Hoplias malabaricus*)	*Astyanax* spp (Tetra fish) IP injected with methylmercury chloride	NOEC: NA MATC: NA LOEC: NA	NOEC: 1.45 **MATC: > 1.45** LOEC: > 1.45 (wet weight; muscle)	WB:M CF = 0.72	>1.04 (whole body) >1.45 (muscle)	Survival No Effect observed	>1.04 (whole body) >1.45 (muscle)	>1.04 (whole body) >1.45 (muscle)	Olivera-Riberio et al. 2006; Costa et al. 2007; Mela et al. 2007
7	Channel catfish (*Ictalurus punctatus*)	Fed Japanese medaka injected with methylmercury	NOEC: NA MATC: NA LOEC: NA	NOEC: 1.6 **MATC: > 1.6** LOEC: > 1.6 (wet weight; muscle)	WB:M CF = 0.72	>1.15 (whole body) >1.6 (muscle)	Growth: Condition Factor No Effect observed	>1.15 (whole body) >1.6 (muscle)	>1.15 (whole body) >1.6 (muscle)	Schlenk et al. 1997
8	Goldfish (*Carassius auratus*)	Floating trout pellets combined with methylmercury chloride	NOEC: 7.78 MATC:> 7.78 LOEC:> 7.78 (wet weight)	NOEC: 2.037 **MATC: > 2.037** LOEC: > 2.037 (wet weight; muscle)	WB:M CF = 0.72	>1.47 (whole body) >2.037 (muscle)	Survival and Growth No Effect observed	>1.47 (whole body) >2.037 (muscle)	>1.47 (whole body) >2.037 (muscle)	Crump et al. 2008

Rank	Species	Dietary Description	Dietary Effect Concentrations (µg THg/g)	Tissue Effect Concentrations (µg THg/g)	Conversion Factors Applied	Chronic Value THg (µg/g ww)[2]	Endpoint and Reported Level of Effect	SMCV THg (µg/g ww)[2]	GMCV THg (µg/g ww)[2]	Reference
9	Beluga Sturgeon (*Huso huso*)	Fishmeal-based experimental diet amended with methylmercury	NOEC: 0.76 LOEC: 0.76 (dry weight)	NOEC < 3.0 LOEC: 3.0 (wet weight; muscle)	WB:M CF = 0.72	2.16 (whole body) 3.0 (muscle)	Growth 10.4% reduction in specific growth rate	2.16 (whole body) 3.0 (muscle)	2.16 (whole body) 3.0 (muscle)	Gharaei et al. 2008
10	Atlantic Salmon (*Salmo salar*)	Dry food formulation mixed with methylmercury chloride	NOEC:< 8.48 MATC:< 8.48 LOEC: 8.48 (dry weight)	NOEC: 3.07 **MATC: > 3.07** LOEC: > 3.07 (wet weight; muscle)	WB:M CF = 0.72	>2.210 (whole body) >3.07 (muscle)	Survival and Growth No Effect observed	>2.210 (whole body) >3.07 (muscle)	>2.210 (whole body) >3.07 (muscle)	Berntssen et al. 2003, 2004
11	Rainbow trout (*Oncorhynchus mykiss*)	Commercial trout food mixed with aqueous solution of methylmercury chloride (2% ration)	NOEC:< 23.9 LOEC: 23.9 (dry weight)	NOEC: < 9 **LOEC: 9** (wet weight; whole body)	Chronic Value = LOEC/3 (GLI) WB:M CF = 0.72	3.0 (whole body) 4.17 (muscle)	Growth reduction LOEC is 30.1% reduction	3.162 (whole body) 4.392 (muscle)	3.162 (whole body) 4.392 (muscle)	Rodgers and Beamish 1982
		Commercial trout food mixed with aqueous solution of methylmercury chloride (ad libitum)	NOEC:< 23.9 LOEC: 23.9 (dry weight)	NOEC: < 10 **LOEC: 10** (wet weight; whole body)	Chronic Value = LOEC/3 (GLI) WB:M CF = 0.72	3.33 (whole body) 4.63 (muscle)	Growth LOEC is 17.7% reduction in growth			Rodgers and Beamish 1982
12	Zebrafish (*Danio rerio*)	Methylmercury chloride dissolved in the diet lipid fraction of a formulated basal diet.	NOEC:< 11.98 LOEC: 11.98 (dey weight)	NOEC: < 33.31 **LOEC: 33.31** (dry weight; whole body)	Chronic Value = LOEC/3 (GLI) DW:WW (75 % Moisture; 3.48) WB:M CF = 0.72	3.187 (whole body) 4.426 (muscle)	Survival and Growth reduction LOEC is 25% reduction in survival and 36% reduction in weight	3.187 (whole body) 4.426 (muscle)	3.187 (whole body) 4.426 (muscle)	Penglase et al. 2014a,b
13	Burrowing mayfly (*Hexagenia* sp.)	Natural sediments with dried, finely ground leaves of submersed aquatic plants (curly pondweed and wild celery).	NOEC: NA MATC: NA LOEC: NA	NOEC: 10.819 **LOEC:> 10.819** (dry weight; whole body)	DW:WW (67.5 % Moisture:3.08)	>3.516 (whole body)[4]	Survival and Growth No Effect .observed	>3.516 (whole body)[4]	>3.516 (whole body)[4]	Naimo et al. 2000

Rank	Species	Dietary Description	Dietary Effect Concentrations (µg THg/g)	Tissue Effect Concentrations (µg THg/g)	Conversion Factors Applied	Chronic Value THg (µg/g ww)[2]	Endpoint and Reported Level of Effect	SMCV THg (µg/g ww)[2]	GMCV THg (µg/g ww)[2]	Reference
14	Sacramento blackfish (*Orthodon microlepidotus*)	Trout chow crumble ground mixed with methylmercury chloride dissolved in 100% ethanol	NOEC: 0.52 MATC:3.398 LOEC: 22.2 (dry weight)	NOEC: 2.3 **MATC: 7.583** LOEC: 25 (wet weight; muscle)	WB:M CF = 0.72	5.460 (WB) 7.583 (muscle)	Growth reduction LOEC is 10.2% reduction in weight vs control	5.460 (whole body) 7.583 (muscle)	5.460 (whole body) 7.583 (muscle)	Houck and Cech 2004
15	Asiatic clam (*Corbicula fluminea*)	Natural food from indoor experimental unit where Hg contamination levels in sediment were achieved by methylmercury chloride and inorganic mercury chloride addition.	NOEC: NA MATC: NA LOEC: NA	NOEC: 6.0 LOEC: > 6.0 (wet weight; whole body)	NA	>6.0 (whole body)[5]	Survival and Growth No Effect Observed	>6.0 (whole body)[4]	>6.0 (whole body)[4]	Inza et al. 1997
16	Sacramento splittail (*Pogonichthys macrolepidotus*)	Methylmercury chloride (pre-dissolved in 100% ethanol) added to a dry basal diet	NOEC:< 11.7 MATC:< 11.7 LOEC: 11.7 (dry weight)	NOEC: 6.0 LOEC: > 6.0 (wet weight; whole body)	WB:M CF = 0.72	>6.0 (WB) >8.33 (muscle)	Survival and Growth No Effect observed	>6.0 (whole body) >8.33 (muscle)	>6.0 (whole body) >8.33 (muscle)	Deng et al. 2008
17	Cladoceran (*Daphnia magna*)	Green alga in the exponential phase spiked with radiolabeled methylmercury[203] at 148 kilobecquerel per liter	NOEC: NA MATC: NA LOEC: NA	NOEC:< 33.3 **LOEC: 33.3** (wet weight; whole body)	Chronic Value = LOEC/3 (GLI)	11.1 (whole body)[4]	Reproduction reduction LOEC is 79% average reduction in neonates per day	11.1 (whole body)[4]	11.1 (whole body)[4]	Tsui and Wang 2004
18	Green Sturgeon (*Acipenser mediorostris*)	Methylmercury chloride dissolved in 100% ethanol was added to a purified diet	NOEC: 25 MATC: 35.36 LOEC: 50 (dry weight)	NOEC: 50.8 **MATC: 76.50** LOEC: 115.2 (dry weight; muscle)	DW:WW (76.5 % Moisture; 4.26) WB:M CF = 0.72	12.94 (whole body) 17.98 (muscle)	Survival LOEC is 89% reduction in survival vs controls	12.94 (whole body) 17.98 (muscle)	18.45 (whole body) 25.64 (muscle)	Lee et al. 2011

Rank	Species	Dietary Description	Dietary Effect Concentrations (µg THg/g)	Tissue Effect Concentrations (µg THg/g)	Conversion Factors Applied	Chronic Value THg (µg/g ww)[2]	Endpoint and Reported Level of Effect	SMCV THg (µg/g ww)[2]	GMCV THg (µg/g ww)[2]	Reference
	White Sturgeon (*Acipenser transmontanus*)	Methylmercury chloride dissolved in 100% ethanol was added to a purified diet	NOEC: 50 MATC: 70.71 LOEC: 100 (dry weight)	NOEC: 104.4 **MATC: 155.6** LOEC: 231.8 (dry weight; muscle)	DW:WW (76.5 % Moisture; 4.26) WB:M CF = 0.72	26.32 (whole body) 36.56 (muscle)	Survival LOEC is 38.5% reduction in survival vs control	26.32 (whole body) 36.56 (muscle)		

[1] See Appendix A for additional details regarding quantitative studies.

[2] Converted to a wet weight basis and to a whole body or muscle tissue basis using the conversion factors from Appendix D. Some values include application of an uncertainty factor - see detailed study summaries in Appendix A.

[3] Combined effects: Malformation rate, Metamorphic success, and Survival

[4] No whole body-muscle conversation factor developed or applicable for this species.

NA=Not applicable

3.5 Derivation of the Mercury Aquatic Life Criterion

3.5.1 Derivation of the Chronic Tissue Values for Mercury for Whole Body Tissue

The mercury chronic data set based on dietary exposures to methylmercury (and inorganic mercury for amphibians) contained data for seven of the eight MDRs. Quantitative data were not available for the 8th MDR (Group H, a family in any order of insect or any phylum not already represented). Following the approach of U.S. EPA (2008), which was reviewed by the EPA Science Advisory Board (available online), if information is available to demonstrate that an MDR is not sensitive, then a surrogate value can be used in place of actual toxicity data to represent the missing MDR. Evaluating the available quantitative invertebrate data provided insight on the sensitivity of aquatic invertebrates relative to vertebrates based on dietary exposures to methylmercury. The qualitative studies (Vidal and Horne 2003a; Vidal and Horne 2003b) provided supporting information for two additional phyla - annelids and rotifers, both representatives for the remaining MDR Group H. Therefore, EPA concluded that there are sufficient data to derive chronic tissue criterion elements using the Guidelines approach from empirical tissue data from chronic dietary toxicity studies. The tissue FCV for muscle and whole body were calculated directly using the GMCVs representing low effect levels for 16 freshwater genera (**Table 3-6** and **Table 3-7**).

Table 3-6. Ranked Freshwater Genus Mean Chronic Values based on Total Mercury Concentrations in Whole Body of Aquatic Organisms.

Rank[a]	GMCV (µg THg/g ww)	GMCV (ng THg/g ww)	MDR Group[c]	Genus	Species	SMCV[b] (ng THg/g ww)
*	0.03272	32.72	C	*Lithobates*	Southern Leopard frog (*Lithobates sphenocephala*)	32.72
*	0.1653	165.3	C	*Anaxyrus*	American toad (*Anaxyrus americanus*)	165.3
1	0.2574	257.4	B	*Pimephales*	Fathead minnow (*Pimephales promelas*)	257.4
2	0.3581	358.1	E	*Procambarus*	Red Swamp Crayfish (*Procambarus clarkii*)	358.1[a]
3	0.7697	769.7	B	*Sander*	Walleye (*Sander vitreus*)	769.7
4	> 1.04	>1,040	B	*Hoplias*	Tigerfish *(Hoplias malabaricus)*	>1,040[a]
5	> 1.15	>1,150	B	*Ictalurus*	Channel catfish (*Ictalurus punctatus*)	>1,150[a]
6	> 1.47	>1,470	B	*Carassius*	Goldfish (*Carassius auratus*)	>1,470[a]
7	2.16	2,160	B	*Huso*	Beluga sturgeon (*Huso huso*)	2,160[a]
8	> 2.210	>2,210	A	*Salmo*	Atlantic salmon (*Salmo salar*)	>2,210[a]
9	3.162	3,162	A	*Oncorhynchus*	Rainbow trout *Oncorhynchus mykiss*)	3,162
10	3.187	3,187	B	*Danio*	Zebrafish (*Danio rerio*)	3,187
11	>3.516	>3,516	F	*Hexagenia*	Burrowing mayfly (*Hexagenia spp.*)	>3,516
12	5.460	5,460	B	*Orthodon*	Sacramento blackfish (*Orthodon microlepidotus*)	5,460[a]
13	> 6.0	> 6,000	G	*Corbicula*	Asiatic clam (*Corbicula fluminea*)	> 6,000
14	> 6.0	> 6,000	B	*Pogonichthys*	Sacramento splittail (*Pogonichthys macrolepidotus*)	> 6,000
15	11.1	11,100	D	*Daphnia*	Cladoceran (*Daphnia magna*)	11,100

Rank[a]	GMCV (µg THg/g ww)	GMCV (ng THg/g ww)	MDR Group[c]	Genus	Species	SMCV[b] (ng THg/g ww)
16	18.45	18,450	B	*Acipenser*	Green Sturgeon (*Acipenser medirostris*)	12,940[a]
			B		White Sturgeon (*Acipenser transmontanus*)	26,320[a]

[a] Converted from muscle concentration to whole body concentration based on conversion factor of 0.72 (WB:M ratio).
[b] SMCV = Species Mean Chronic Value
[c] MDR Group – refers to the 8 family level minimum data requirements for derivation of water quality criteria using the 1985 Guidelines (Stephan et al. 1985)
* EPA excluded the amphibian tissue data from the criteria calculation considering mercury bioaccumulation dynamics, as noted above, so that tissue criterion elements are protective of all aquatic species, including amphibians, *and* appropriately protective (see text for details.).

To derive the chronic whole-body values, EPA applied the WB:M conversion factor of 0.72 to the 4th most sensitive genera (*Procambarus*), as well as the remainder of the fish genera in the sensitivity distribution since these effect concentrations were expressed as total mercury in muscle tissue in those studies. The remainder of the chronic values were based on effect concentrations for amphibians, fish, and invertebrates expressed as total mercury in whole body tissue, therefore no conversion factor was necessary.

3.5.2 Derivation of the Chronic Tissue Values for Mercury in Muscle Tissues

To derive the chronic muscle tissue values, EPA applied the whole-body – muscle conversion factor of 0.97 to amphibian whole body chronic values for the genera *Lithobates* and *Anaxyrus*, to derive muscle tissue values. The remainder of the toxicity studies were comprised of fish taxa (and one invertebrate taxa, the red swamp crayfish) with endpoints expressed as total mercury in muscle (or fillet) or whole body (varying by species and study), necessitating the application of a conversion factor. The whole-body – muscle conversion factor of 0.72 was applied to the whole-body chronic value of the most sensitive fish genera (*Pimephales*) and the red swamp crayfish to calculate an equivalent whole body total mercury chronic value. EPA did

not apply the conversion factor to the remainder of the invertebrate genera (representing aquatic insects, cladocerans, and mollusks) in the sensitivity distribution due to the uncertainty of applying a conversion factor based on fish. However, these taxa were insensitive relative to the taxa present in the lower tail of the distribution, and therefore did not materially affect the derivation of the chronic criterion elements.

Table 3-7. Ranked Freshwater Genus Mean Chronic Values based on Total Mercury Concentrations in Muscle Tissues of Aquatic Organisms.

Rank[a]	GMCV (µg THg/g ww)	GMCV (ng THg/g ww)	MDR Group[c]	Genus	Species	SMCV[b] (ng THg/g ww)
*	0.03373	33.73	C	*Lithobates*	Southern Leopard Frog (*Rana sphenocephala*)	33.7[e]
*	0.1704	170.4	C	*Bufo/Anaxyrus*	American toad (*Anaxyrus americanus*)	170.4[e]
1	0.3575	357.5	B	*Pimephales*	Fathead minnow (*Pimephales promelas*)	357.5[d]
2	0.4973	497.3	D	*Procambarus*	Red Swamp Crayfish (*Procambarus clarkii*)	497.3
3	1.069	1,069	B	*Sander*	Walleye (*Sander vitreus*)	1,069[d]
4	> 1.45	>1,450	B	*Hoplias*	Tigerfish *(Hoplias malabaricus)*	>1,450
5	> 1.6	>1,600	B	*Ictalurus*	Channel catfish (*Ictalurus punctatus*)	>1,600
6	> 2.037	>2,037	B	*Carassius*	Goldfish (*Carassius auratus*)	>2,037
7	3.0	3,000	B	*Huso*	Beluga sturgeon (*Huso huso*)	3,000
8	> 3.07	>3,070	A	*Salmo*	Atlantic salmon (*Salmo salar*)	>3,070
9	> 3.516	>3,516	F	*Hexagenia*	Burrowing mayfly (*Hexagenia spp*.)	>3,516[f]
10	4.392	4,392	A	*Oncorhynchus*	Rainbow trout *Oncorhynchus mykiss*)	> 4,392[d]
11	4.426	4,426	B	*Danio*	Zebrafish (*Danio rerio*)	4,426[d]
12	> 6.0	> 6,000	G	*Corbicula*	Asiatic clam (*Corbicula fluminea*)	> 6,000[f]

Rank[a]	GMCV (µg THg/g ww)	GMCV (ng THg/g ww)	MDR Group[c]	Genus	Species	SMCV[b] (ng THg/g ww)
13	7.583	7,583	B	*Orthodon*	Sacramento blackfish (*Orthodon microlepidotus*)	7,583
14	> 8.33	>8,330	B	*Pogonichthys*	Sacramento splittail (*Pogonichthys macrolepidotus*)	>8,330[d]
15	11.1	11,100	D	*Daphnia*	Cladoceran (*Daphnia magna*)	11,100[f]
16	25.64	25,640	B	*Acipenser*	Green Sturgeon (*Acipenser medirostris*)	17,980
			B		White Sturgeon (*Acipenser transmontanus*)	36,560

[a] Ranked from the most to least sensitive based on Genus Mean Chronic Value.
[b] From Appendix A.
[c] MDR Groups identified by list provided in Section 2.6 above.
[d] Converted from whole body concentration to muscle concentration based on a conversion factor of 0.72 (whole body:muscle ratio).
[e] Converted from whole body concentration to muscle concentration based on a conversion factor of 0.97 (whole body:muscle ratio).
[f] Whole body value; no WB:M conversion factor available or applicable.
*EPA excluded the amphibian tissue data from the criteria calculation considering mercury bioaccumulation dynamics, as noted above, so that tissue criterion elements are protective of all aquatic species, including amphibians, *and* appropriately protective (see text for details.).

3.5.2.1 Deriving Tissue-based Chronic Criterion

As noted above in **Section 2.9** (Approach to Calculating the Criterion Values), protective mercury tissue criterion should integrate consideration of both relative sensitivity to mercury and relative mercury bioaccumulation potential across the taxa considered. EPA has thus developed a proposed tissue-based chronic criterion for mercury that reflect both sensitivity of aquatic species and bioaccumulation potential across aquatic taxa, based on the latest scientific information. As discussed below, this draft tissue-based criterion, derived using both fish and invertebrate data, is protective of the vast majority of aquatic organisms, including amphibians.

Available dietary toxicity data indicate that the aquatic life stages of amphibians (e.g., tadpoles) are more sensitive than fish or invertebrates to total mercury, as indicated by their

rankings as the two most sensitive taxa for both whole-body and muscle EC_{10} concentrations (**Table 4-1** and **Table 4-2**). The EC_{10} value of the most sensitive amphibian, southern leopard frog tadpole (*R. sphenocephala*) is 7 to 11 times lower than that the EC_{10} for the most sensitive fish, the fathead minnow (*P. promelas*), for whole body and muscle tissue, respectively.

Despite their relative sensitivity to direct exposure to mercury, amphibians do not bioaccumulate mercury as readily as fish and large invertebrates such as crayfish, due to trophic ecology and feeding dynamics. The diet of larval/aquatic stages of amphibians is largely composed of degrading organic matter (aufwuchs) and plants, and both food sources typically have lower mercury concentrations than higher trophic-level aquatic organisms. In contrast, fish and invertebrates such as crayfish, although less sensitive to mercury than amphibians, consume prey at higher trophic levels in which mercury has generally bioaccumulated to relatively higher levels, following bioaccumulation and biomagnification, increasing concentrations of mercury at higher trophic levels, within the food web.

Therefore, EPA analyzed mercury bioaccumulation differences across tested aquatic taxa to determine the potential impact of the proposed tissue-based chronic criterion elements on amphibians (**Table 3-8**). The analysis provides estimated tissue mercury concentrations for amphibians based on the relative relationship of the median BAFs for fish (either species specific or based on trophic ecology) and amphibians, providing an estimate at the muscle tissue criterion element concentration. Fish and crayfish BAFs are significantly greater than those for amphibians. The ratio of the median fish BAF for all fish species collected in Idaho (138,101) to the amphibian BAF (8,222) is 16.79, meaning that, overall, fish are expected to accumulate mercury to approximately 17 times higher concentrations than amphibians (**Table 3-8**). The relationship of the median for low, medium and high trophic magnitude fish species to the

amphibian BAF are 8.96, 13.18, and 45.99 respectively, and the BAF of the 20th centile fish in the dataset is 8.2 times greater than that of amphibians, yielding an estimated amphibian tissue total mercury concentration of 27.5 ng/g ww, below the southern leopard frog EC_{10} of 33.7 ng/g ww, if the criterion were met in a 20th centile fish sample The southern leopard frog EC_{10} is also approximately the 2.5th centile of the mercury concentrations in muscle tissue for all fish species in the Idaho BAF database by site and year (n = 119), indicating that most fish will have mercury concentrations substantially higher than tissue concentrations of an amphibian tadpole if sampled from the same waterbody. Finally, the BAFs for several common and important fish species considered for protection in the environment, and that are resident in Idaho, are 15 times to 55 times greater than amphibian BAFs: rainbow trout (20 times greater), channel catfish (25 times greater), brown trout (37 times greater), and walleye (55 times greater). The ratio of the BAF of the relatively sensitive invertebrate red swamp crayfish (BAF of 128,414), which has a low to medium trophic level position, to the amphibian BAF (8,222) is 15.6. These data indicate that fish and crayfish will bioaccumulate mercury in their tissues to a substantially greater extent than amphibians, hence the fish tissue criterion elements are expected to be protective of amphibians, over 80% of the time where they co-occur.

Table 3-8. Relative Magnitude of BAFs for Invertebrates (crayfish) and Fish Relative to Amphibians.

Taxa	Median BAF	Amphibian BAF	Fold Difference of Various Taxa BAFs to Amphibian BAF
20th Centile Fish (based on species specific medians for all fish species collected in Idaho)	67,203	8,222	8.17
Crayfish (Low – Medium Trophic Magnitude dependent on species and life stage)	128,414	8,222	15.6
Medium Trophic Magnitude Fish	108,418	8,222	13.18
High Trophic Magnitude Fish	378,150	8,222	45.99
All fish pooled	138,101	8,222	16.79
Walleye (driver of water column criterion) (*Sander vitreus*)	453,578	8,222	55.2
Channel catfish (*Ictalurus punctatus*)	205,123	8,222	24.9
Rainbow Trout (*Oncorhynchus mykiss*)	161,685	8,222	19.7
Brown trout (*Salmo trutta*)	302,721	8,222	36.8

To further explore the protection of amphibians from mercury exposure, EPA compared relative mercury bioaccumulation and sensitivity to dietary mercury across several fish species to the most sensitive amphibian species. The ratio of the fathead minnow (Low Trophic Magnitude Fish) BAF to the amphibian BAF is 8.9, while the most sensitive amphibian, southern leopard frog tadpole (*R. sphenocephala*), is seven to 11 times more sensitive to mercury (based on EC_{10}) than the fathead minnow (*P. promelas*), for whole-body and muscle tissue, respectively (**Table 3-6** and **Table 3-7**). Thus, more generally, the proposed tissue-based chronic criterion elements based primarily on fish data are expected to be protective of amphibians because the generally less sensitive fish bioaccumulate mercury to a much greater extent than amphibians, and this will be reflected in tissue samples collected from the environment. Further, as a practical matter,

since Idaho's monitoring programs focus primarily on the measurement of mercury concentrations in fish muscle filet tissue or whole-body, not amphibians, tissue criterion elements based on the mercury concentrations in fish may be more useful for Idaho's water quality programs for evaluating any total mercury tissue criterion element exceedances (IDEQ 2005).

Although the state of Idaho's mercury monitoring program prioritizes collection of fillet (muscle) tissue from TL4 fish (IDEQ 2005), the state (Essig 2010) or other entities (e.g., MacCoy and Mebane 2018) may collect fish species (e.g., bridgelip sucker, mountain whitefish) or juvenile fish representing lower trophic ecologies (i.e. Trophic Level 2 and Trophic Level 3). Comparing the mercury concentration from a whole body or muscle tissue sample of a trophic level 2 or trophic level 3 fish species does not demonstrate protection of the higher trophic level 4 fish if the mercury concentration is below the applicable tissue criterion threshold, because of the lesser bioaccumulation of mercury in lower trophic level fish. Therefore, EPA developed an adjustment factor approach to estimate TL4 fish tissue concentrations when actual tissue samples from TL4 fish species are not available for a waterbody. The Bioaccumulation Trophic Adjustment Factor (BTAF) should be applied to fish tissue monitoring data for lower trophic level species that may not bioaccumulate as much mercury, but may be the only fish sampled in a waterbody to ensure protection of the aquatic ecosystem in the waterbody and downstream of it. Application of the BTAFs to available data ensures protection of high trophic level species when mercury tissue data is not available in a given waterbody.

The BTAFs for Idaho are based on the relationship between the median BAFs of trophic magnitude categories for Idaho fish species sampled between 2008 and 2017 at lotic and lentic waterbodies. BTAFs are calculated as the ratio of the median BAF for the high trophic

magnitude to the median BAF of the lower trophic magnitude categories (**Table 3-9**). Due to the paucity of low trophic level fish species (TL 2.0 – 2.5; e.g., fathead minnow) in the Idaho database, EPA used the median BAF for the 20th centile fish species to ensure protection of high trophic level species if data for fathead minnow (or some other low trophic level 2 fish) was all that was available for a certain waterbody. The use of the 20th centile is to provide appropriate protection for all species in Idaho and is consistent with approaches used for BAFs for PFAS (U.S. EPA 2022a,b) and the threshold used for calculation of the lentic and lotic water column criterion elements from the distribution for site-based water quality thresholds calculated for selenium (U.S. EPA 2016a).

Table 3-9. Bioaccumulation trophic adjustment factor (BTAF) for Protection of High Trophic Level Fish in Idaho

Trophic Magnitude Category	Median Trophic Magnitude BAFs (L/kg muscle ww)	BTAF
20th centile of median species BAFs for Idaho fish	67,203	
Low Trophic Magnitude Median BAF for Idaho fish	73,651	
Medium Trophic Magnitude Median BAF for Idaho fish	108,418	3.5[a]
High Trophic Magnitude Median BAF for Idaho fish	378,150	5.6[b]

[a] TL3 BTAF. If TL3 fish are sampled, ensures protection of TL4 fish. This value is the ratio of the high TL level median to the medium TL BAF median.

[b] TL2 BTAF. If TL2 fish are sampled, ensures protection of TL3 and TL4 fish. This value is the ratio of the high TL level median fish BAF to the 20th centile of all median fish BAFs.

In practice, if fish fillet tissue sample data collected according to Idaho's guidance (IDEQ 2005) is available from TL 4 species (e.g., smallmouth bass), then the BTAF is not used and the total mercury tissue concentration is compared directly to the whole-body criterion element (162 ng/g ww) or the muscle criterion element (225 ng/g ww), depending on the tissue sampled. This

is because their tissue concentration adequately reflects the trophic ecology that is impacted by mercury if mercury bioaccumulation is of concern in a particular waterbody, and a TL4 fish sample concentration lower than the criterion concentration would be protective of all aquatic species including aquatic phases of amphibians. However lower trophic level fish (TL 2 and 3) have lower rates of bioaccumulation from dietary exposure of mercury and tissue concentrations typically associated with fish in these trophic levels do not provide evidence that higher trophic level fish will be protected. If muscle tissue from a TL2 or TL3 fish species is the only data available, then the appropriate BTAF is applied to yield a representative estimated tissue concentration for a TL4 fish in that particular waterbody. Since the BTAFs are based on BAFs calculated from fish muscle tissue concentrations, if whole body tissue from a TL2 or TL3 fish species is the only data available, the whole-body tissue concentration is first converted to an equivalent muscle concentration using the WB:M conversion factor (0.72), then the appropriate BTAF is applied to yield an estimated representative tissue concentration for a TL4 fish in that particular waterbody. The BTAF must be applied to any tissue sample that is not from an adult life stage trophic level 4 fish to assess the estimated TL4 muscle tissue concentration against the mercury muscle tissue criterion element.

Typically, for Idaho and most state fish tissue monitoring programs, muscle (fillet) samples are available predominantly for trophic level 3 and 4 fish species of sufficient length to assess the mercury concentrations in edible (muscle) tissue from sport fish to protect human health. Although some monitoring programs may target lower trophic levels (TL2) or more juvenile lifestages for ecological risk assessments associated with aquatic dependent wildlife, fish tissue data available for Idaho from 2008-2017 show that only 9 of 390 (2.3%) tissue samples were whole-body tissue samples and 7 of 390 (1.7%) tissue samples were from fish

species below TL 3.0 as defined by Fishbase.org (accessed 2022), indicating that most of the fish tissue data available to EPA for evaluating whether samples indicate the criterion is not exceeded would be based on muscle tissue sample data, and the use of the TL4:TL2 BTAF and WB:M conversions of whole-body sample concentrations would be uncommon.

In addition to fish tissue sampling, more recently, monitoring programs focusing on aquatic invertebrates such as crayfish (Idaho University Crayfish Mercury Project; https://crayfish.nkn.uidaho.edu/wp-content/uploads/2022/02/Crayfish-Infographic-_FINAL.pdf) in the Columbia River Basin and dragonfly nymphs in the National Parks (USGS Dragonfly Mercury Project; https://geonarrative.usgs.gov/dmp) have been initiated and are gaining popularity, particularly as participatory (citizen) science projects. These aquatic taxa may serve as indicator species and provide value in prioritizing locations for fish tissue monitoring in waters where tissue samples have not been collected. However, Hg concentrations from these taxa should not be used for direct comparison to the tissue criterion. unless data have been collected and analyzed characterizing the crayfish or dragonfly nymph's relative bioaccumulation of mercury with respect to fish and amphibians collected from the same waterbody and time period, to ensure protection of TL 4 fish species. Discussion of the protectiveness of the muscle and whole-body tissue criterion elements for amphibians is provided in the Effects Characterization, **Section 4** of this criterion document.

3.5.2.1.1 Calculation of Total Mercury Whole Body Tissue Criterion Element using Fish and Invertebrate Data

The fish tissue whole-body criterion element calculated using the fish and invertebrate sensitivity distribution data following the statistical procedure described in the 1985 Guidelines (Stephan et al. 1985) is 162 ng THg/g ww (**Table 3-10**). This value was calculated excluding

amphibian tissue values due to their lower bioaccumulation potential (see the Effects Characterization, **Section 4** for further discussion of amphibians).

Table 3-10. Whole Body tissue criterion element for taxa with higher bioaccumulation potential (e.g., fish and invertebrates included, amphibians excluded) in ng THg/g ww.

Genus	*N*	Rank	GMCV	ln(GMCV)	ln(GMCV)^2	P=R/(N+1)	sqrt(P)
Pimephales	16	1	257.40	5.55	30.81	0.059	0.243
Procambarus		2	358.10	5.88	34.58	0.118	0.343
Sander		3	769.70	6.65	44.17	0.176	0.420
Huso		4	2160.00	7.68	58.95	0.235	0.485
		Sum:		**25.76**	**168.51**	**0.59**	**1.49**
				S^2 =	81.91		
				L =	3.066		
				A =	5.090		
				FCV =	**162.3**	**ng THg/g ww**	

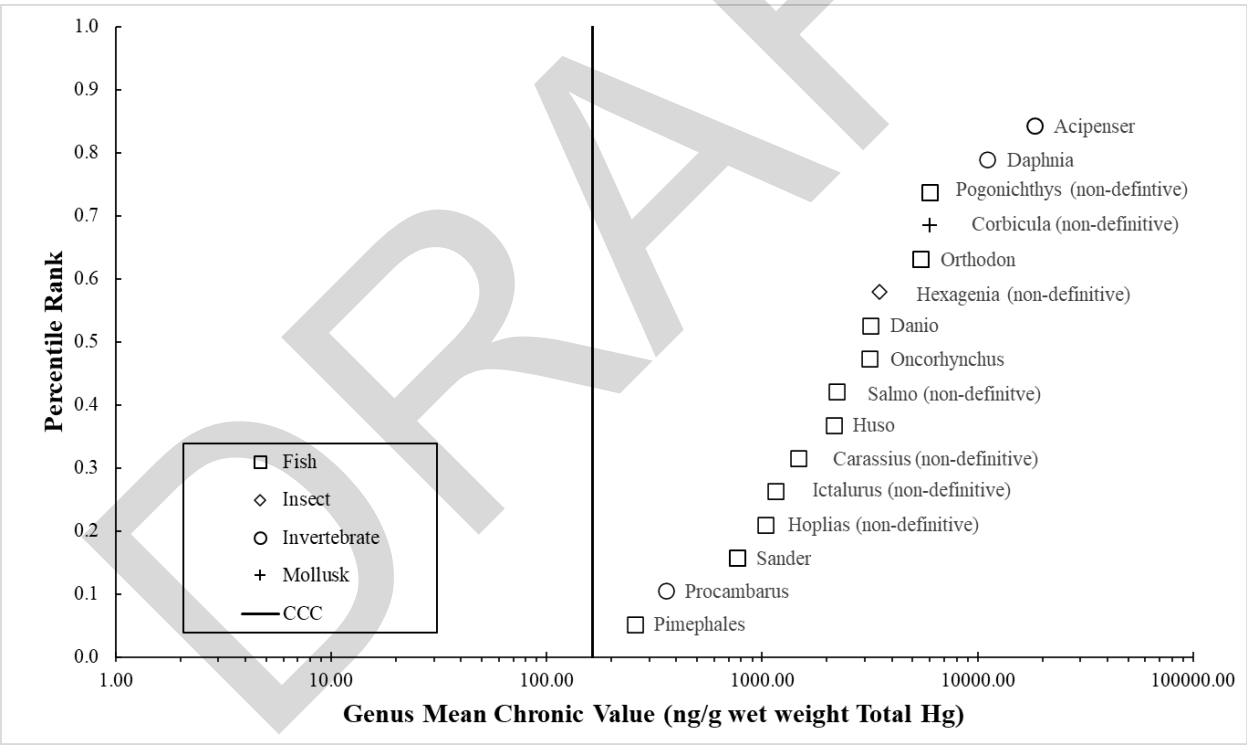


Figure 3-4. Distribution of Measured Dietary Mercury Effect GMCVs (fish and invertebrates) expressed as Whole Body (THg, ng/g ww).

3.5.2.1.2 Calculation of Total Mercury Muscle Tissue Criterion Element using Fish and Invertebrate Data

The freshwater chronic muscle tissue criterion element calculated using fish and invertebrate sensitivity distribution data generally following the procedures described in the 1985 Guidelines (Stephan et al. 1985) is 225 ng THg/g ww (**Table 3-11**). This value was calculated excluding amphibians due to their lower bioaccumulation potential (see Effects Characterization, **Section 4** for further discussion of amphibians).

Table 3-11. Muscle Tissue Criterion Element for taxa with higher bioaccumulation potential (e.g., fish and invertebrates) in ng THg/g ww.

Genus	***N***	**Rank**	**GMCV**	**ln(GMCV)**	**ln(GMCV)^2**	**P=R/(N+1)**	**sqrt(P)**
Pimephales	16	1	357.50	5.88	34.56	0.059	0.243
Procambarus		2	497.30	6.21	38.55	0.118	0.343
Sander		3	1069.00	6.97	48.64	0.176	0.420
Huso		4	3000.00	8.01	64.10	0.235	0.485
		Sum:		**27.07**	**185.86**	**0.59**	**1.49**
				S^2 =	81.92		
				L =	3.394		
				A =	5.418		
				FCV =	**225.45553**		
				CCC =	**225.456**	**ng THg/g ww**	

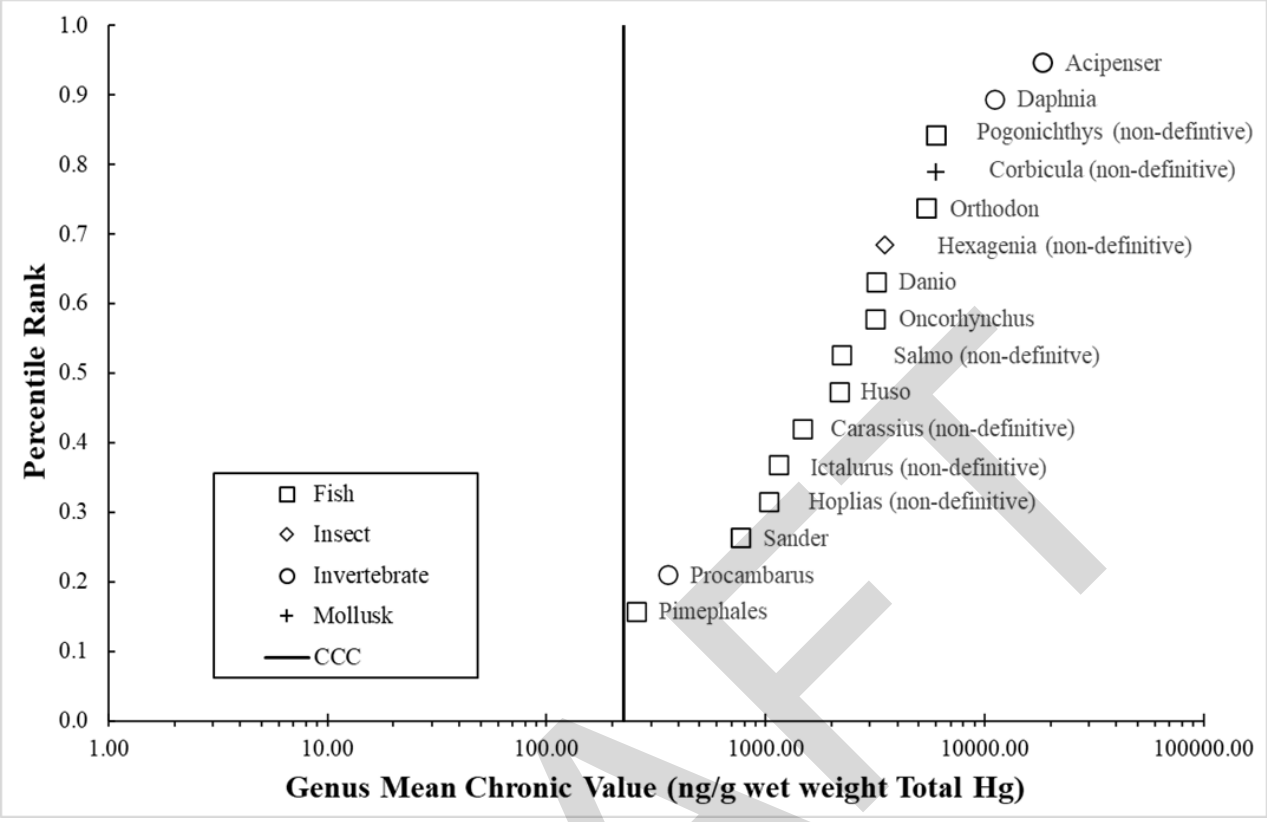


Figure 3-5. Distribution of Measured Dietary Mercury Effect GMCVs expressed as Muscle (THg, ng/g ww).

The proposed whole-body criterion element is 162 ng THg/g ww, and the proposed muscle tissue criterion element is 225 ng THg/g ww.

The freshwater tissue criterion element values for muscle and whole-body tissues are expected to be protective of approximately 95% of freshwater genera, including amphibians due to their lower bioaccumulation, that are exposed to total mercury through dietary exposure under long term conditions. The difference in the whole-body and muscle-based fish tissue criterion element values is due to the application of the WB:M CF for the aquatic life taxa present in the respective sensitivity distributions. The effect concentration for each aquatic taxa (e.g., fathead minnow, *Pimephales promelas*) in the distribution was analyzed in the respective studies used by EPA. Because it is useful for implementation to have tissue criterion elements expressed as both

a whole-body concentration and a muscle concentration, EPA applied taxa-specific conversion factors to the available study data as applicable to yield sensitivity distributions expressed as muscle and whole-body tissue concentrations. The whole-body criterion element value of 162 ng THg/g ww is similar to previous estimates of ecologically relevant thresholds, such as an approximately 200 ng THg/g ww (in whole body), proposed as a protective value for juvenile and adult fish (Beckvar et al. 2005). Effects on reproduction in fish were indicated below 500 ng THg/g ww, consistent with the EPA's criterion recommendations that are protective for mercury-induced reproductive effects in fish (Depew et al. 2012).

3.5.2.2 Deriving A Protective Duration for the Tissue-based Chronic Criterion Elements

Test durations resulting in effects observed for chronically sensitive species exposed via diet to mercury (methylmercury) range from 30 – 249 days. One study (used qualitatively in the assessment) examined the latency of methylmercury exposed embryos on feeding behavior of 2-year-old grayling (Fjeld et al. 1988) suggesting that the effects observed from dietary exposure to methylmercury typically occur over long periods of exposure and may result in latent effects occurring long after the exposure. Furthermore, mercury has a prolonged half-life in fish tissue and these tissue concentrations are further stabilized in natural systems since concentrations in aquatic media compartments (water, sediment, particulate, producer, and animal tissues) change only gradually over time due to environmental fluctuations. The chronic tissue-based criterion elements averaging period, or duration, is specified as instantaneous, because tissue data provide point, or instantaneous, measurements that reflect integrative accumulation of mercury over time and space in population(s) at a given site.

3.5.2.3 Deriving a Protective Frequency for the Mercury Criterion: Chronic Tissue-Based Criterion Elements

EPA is proposing that the mercury tissue criterion elements' magnitudes, or concentrations, have a frequency of "not to be exceeded" based on average mercury concentrations in fish tissue samples, reflecting the expected slow recovery of populations after tissue bioaccumulation of mercury and expected ongoing atmospheric and local watershed sources.

A recent fish tissue mercury status and trends report by New Hampshire Department of Environmental Services (NHDES 2018) (https://www.des.nh.gov/sites/g/files/ehbemt341/files/documents/2020-01/r-wd-17-22.pdf) included a statewide analysis (Table 6 in the report) of mercury in largemouth bass collected from NH waterbodies between 1994 and 2015. This comprehensive study (a minimum of 5 fish were collected from 1 to 7 waterbodies per year for 20 of 22 years) reported average total mercury in years where fish tissue was collected. In 1994, the average total mercury was 0.45 mg/kg, and the average total mercury was 0.35 mg/kg in 2015, a 22% decrease over 21 years, about 1% a year.

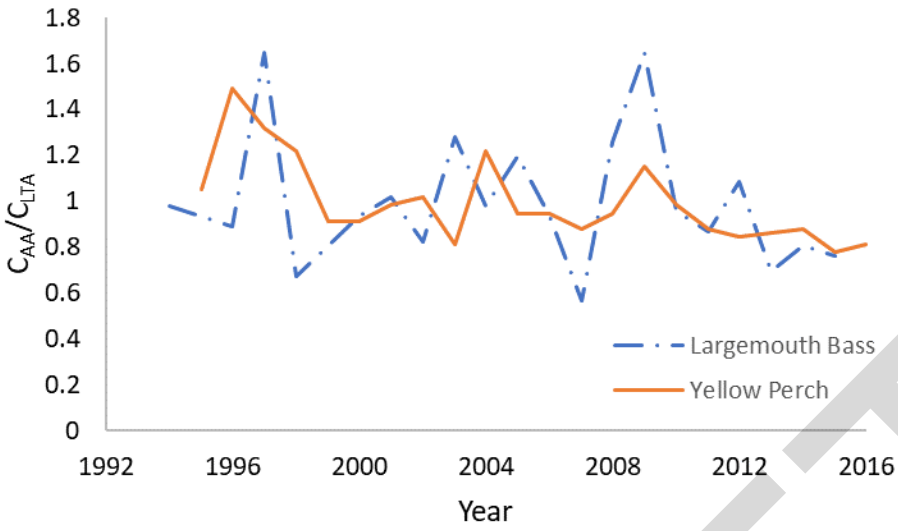


Figure 3-6. Year-to-year variations in mercury concentrations in New Hampshire largemouth bass and yellow perch collected across a limited number of similar water bodies (NHDES 2018).
To focus on variability, annual average concentrations, C_{AA}, have been normalized to their long-term average, C_{LTA}.

To understand how EPA decided to propose a return frequency of "not to be exceeded," note some features of both of these time series (**Figure 3-6**). Although there is a slight overall downward trend in the tissue data stemming from reduced mercury deposition over approximately 22 years of regular monitoring, the trend reflects some variability. These time series illustrate variability in situations where the pollution control effort is stable over time.

To compare the protectiveness of different target return intervals, **Figure 3-7** shows the probability distribution of concentrations that would occur given various exceedance return intervals ranging from 2 years to 50 years. Two levels of year-to-year variability are considered. The left graph in **Figure 3-7** assumes a coefficient of variation (CV) = 0.3, a comparatively high value slightly greater than that of the largemouth bass reported in NHDES (2018) (**Figure 3-6**). The right graph assumes CV=0.15, slightly lower than that of the yellow perch (**Figure 3-6**).

The findings of EPA's analysis of NHDES (2014) lake datasets are consistent with modeling performed by Vijayaraghavan et al. (2014). Vijayaraghavan et al. (2014) evaluated long-term response in mercury concentrations in fish to reductions in local and national emissions, as well as modeled increases or decreases in global (non-US) emissions, using samples collected from a Northeastern US lake that was impacted by atmospheric deposition. Modeling results indicated that reductions in fish tissue mercury concentrations could begin in the first 3-8 years, with reductions in tissue proportionally linked to reductions in emissions over 50 years, with increases in non-U.S. emissions potentially offsetting reductions in the U.S.

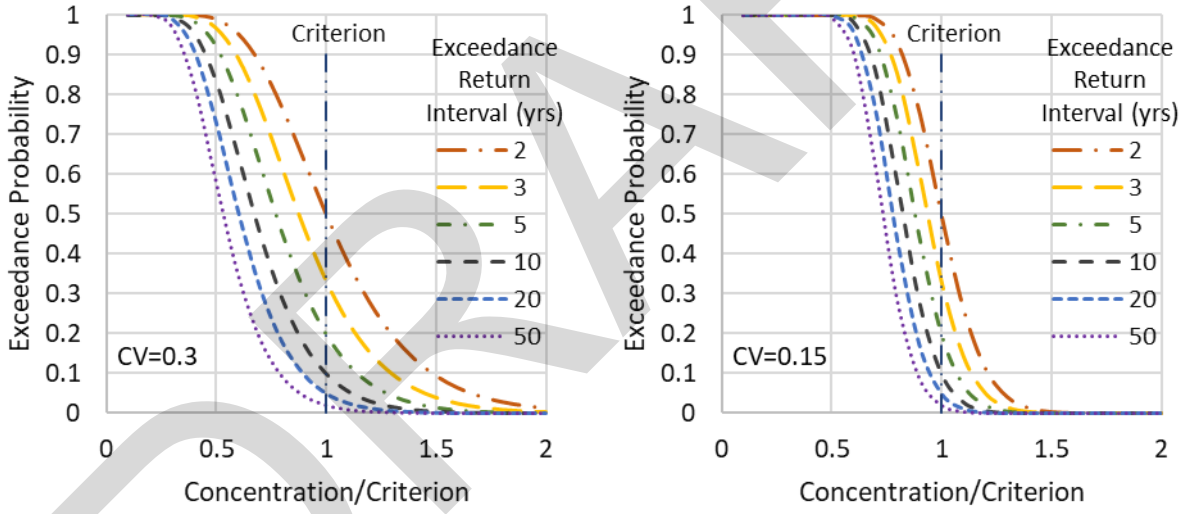


Figure 3-7. Tissue concentration distributions that would occur for different target exceedance return intervals, ranging from 2 years to 50 years, assuming the year-to-year concentrations have a Coefficient of Variation (CV) with a comparatively high value of 0.3 (on the left) or a comparatively low value of 0.15 (on the right).
Lognormal distributions are assumed, with concentrations normalized to the criterion (that is, the criterion has a normalized value of 1.0). Each curve intersects the criterion line of 1.0 at an exceedance probability equal to the reciprocal of the return interval. Hence, the fraction of the 10-year return interval curve exceeding the criterion is 0.1. Based on data in Vijayaraghavan et al. (2014).

In addition to the evidence in the literature presented by EPA supporting a return frequency of "Not to be exceeded" for the mercury tissue criterion elements, this return

frequency is consistent with the selenium aquatic life criterion (U.S. EPA 2016a) and the human health methylmercury tissue criterion (U.S. EPA 2001). The return frequency selected for these criterion elements recognize the relative stability of these pollutants in fish tissue following bioaccumulation processes in aquatic systems and also the ongoing hazard that elevated concentrations of these pollutants pose for sensitive aquatic taxa and human receptors for selenium and mercury, respectively. Since the goal of water quality criteria derived for aquatic life is the protection of aquatic organisms and their uses at the level of population, EPA recommends that concentrations of mercury in fish tissue can be based on a central tendency estimate (e.g., average concentration) for a sample (either composited tissue or individuals) in a given species for a site or water body.

EPA has provided a scientific rationale for a tissue-criterion element return frequency of "not to be exceeded" based on results from trends in tissues (Bhavsar et al. 2010; Gandhi et al. 2014; and NHDES 2018), modeling (Vijayaraghavan et al. 2014), and field studies associated with the METAALICUUS Project (Blanchfield et al. 2022). In addition to these lines of evidence, recently, Grieb et al. (2020) assessed trends in gamefish tissue mercury and concentrations in North American lakes over the period 1972–2016 based on eight studies (Azim et al. 2011; Blukacz-Richards et al. 2017; Gandhi et al. 2014; Monson 2009; Monson et al. 2011; Paller and Lintrell 2007; Sadradinni et al. 2011; Weis 2004). An early period of generally decreasing trends were noted during the period 1970–1995, followed by increasing trends observed between 1995–2012. Although analyses of fish tissue data from 46 peer-reviewed studies indicated an average 34% reduction over 25 years (1970-1995) correlating with reductions in North American mercury emissions, the same study observed fish tissue mercury

concentrations collected more recently during 1995-2012 increased 25% in 10 years (Grieb et al. 2020).

In addition to the fish tissue studies discussed above, research evaluating aquatic (Ullrich et al. 2001) and terrestrial (Gworek et al. 2020; Sever 2021) mercury sinks in the environment indicate that decades of atmospheric deposition of mercury retained in sediments and/or soil can be remobilized due to hydrologic events (e.g., floods, reservoir management) and landscape disturbances (e.g., wildfire, Sever 2021). This can result in residual effects over time that further delay ecosystem recovery.

The bioaccumulative nature and persistence of mercury in aquatic systems and its biota (Trudel and Rasmussen 1997, 2006; Peng et al. 2016), in combination with the estimates of recovery times of mercury-contaminated waters (Vijayaraghavan et al. 2014) impacted by atmospheric deposition and other anthropogenic inputs, suggest that the return frequency for the tissue-based criterion elements for mercury should be "not to be exceeded," similar to other bioaccumulative contaminants criteria, such as the selenium aquatic life criterion (U.S. EPA 2016a). EPA therefore proposes that the tissue-based criterion elements are protective if they are not exceeded based on a central tendency estimate of total mercury concentrations in the tissues of sensitive populations of aquatic life at a site or waterbody. This return frequency addresses uncertainties regarding how mercury concentrations built up in tissues and source reservoirs in the freshwater system may or may not diminish over time, yielding limited opportunities for aquatic life to recover following elevated mercury bioaccumulation in tissues.

3.5.2.4 Summary of Total Mercury Tissue Criterion Elements

The chronic freshwater criterion for the protection of aquatic life (including amphibians) consists of a muscle tissue criterion element magnitude of 225 ng THg/g wet weight (ww) and a whole-body tissue criterion element magnitude of 162 ng THg/g wet weight (ww). The chronic

tissue-based criterion elements averaging periods, or durations, are specified as instantaneous, because tissue data provide point, or instantaneous, measurements that reflect integrative accumulation of mercury over time and space in population(s) at a given site. The chronic frequencies for the mercury tissue criterion elements are "not to be exceeded" based on measurement(s) of the total mercury concentration in a composited tissue sample or a central tendency estimate of tissue concentrations collected from a given site or waterbody in a discrete sampling period.

3.6 Chronic Water Column-Based Mercury Criterion Element

3.6.1 Translation of the Chronic Tissue Criterion Element to Water Column Criterion Element

EPA derived the chronic water column total mercury criterion element for Idaho waters by translating the total mercury muscle tissue criterion element to an equivalent water concentration using bioaccumulation factors (BAFs). EPA applied the Bioaccumulation Factor (BAF) model (Burkhard et al. 1997), which numerically represents the relationship between the chemical concentrations in multiple environmental compartments based on empirical data from site-specific measurements, to data collected in the State of Idaho (Equation 1).

$$Bioaccumulation\ Factor\ \left(\frac{L}{kg}\right) = \frac{Tissue\ [\frac{ng}{g}\ THg{-}ww]}{Water\ [\frac{\mu n}{L}]} \qquad \text{(Equation 1)}$$

BAFs were calculated for fish, amphibian, and invertebrate species, as described in **Section 3.1.1.1**, and then each SMCVs in the muscle tissue criterion dataset was multiplied by the most representative BAF to calculate a distribution of SMCVs expressed as water column concentrations. Translated SMCVs were grouped into GMCVs and a translated water FCV and

CCC was calculated as was done for the tissue-based criterion elements. An overview of this translation approach is shown in **Figure 3-8**, and the details of the approach are described below.

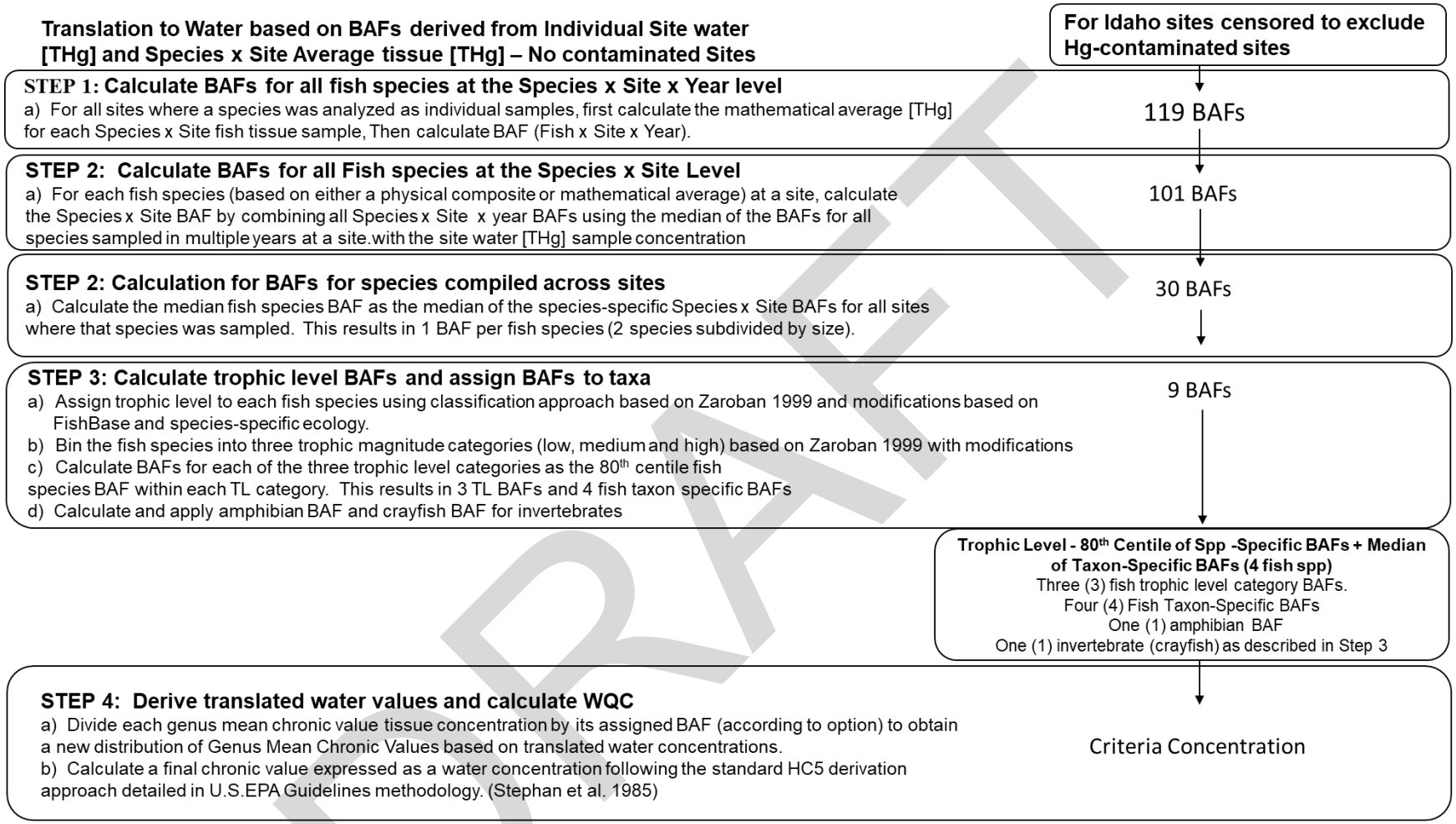


Figure 3-8. Overview of Tissue Criterion Element Translation Process to Generate a Protective Water Column Total Mercury Criterion for Idaho.

Table 3-12. BAFs Used in the Tissue to Water Translation Procedure.

Trophic Magnitude Category	Common Name (Scientific Name)	Median THg (mg/kg ww)	BAF (L/kg muscle-ww)
Low		NA	144,915 (80th centile)
Medium		NA	199,646 (80th centile)
High		NA	647,335(80th centile)
	L. sylvaticus	NA	8,222 (median)
	Crayfish (sp.)	NA	128,414 (geomean)
	Walleye (*Stizostedion vitreus*)	1.00	453,578 (median)
	Channel catfish (*Ictalurus punctatus*)	0.247	205,123 (median)
	Rainbow Trout (*Oncorhynchus mykiss*)	0.132	161,685 (median)
	Brown trout (*Salmo trutta*)	0.174	302,721 (median)

3.6.2 Development of Water Column Criterion Element

Water column values were developed by dividing each muscle tissue value (SMCV) by its associated BAF (**Table 3-12**). Fish species in the criterion dataset were translated using either a median species- or genus-specific BAF, if available, or the most representative trophic magnitude category BAF, if no species- or genus-specific BAF was available. Fish species BAFs were the median BAF across all sites where a BAF for that species was available. Taxon-specific BAFs were not applied beyond the genus level because mercury bioaccumulation increases at higher trophic levels, and BAFs across species at the family level and above were expected to be less representative than BAFs within a trophic category. Fish species in the toxicity dataset were assigned to trophic magnitude categories based on trophic level designations as described above. Trophic magnitude category fish BAFs were calculated as the 80th centile fish species BAFs within that category. Frog species were translated using the wood frog BAF. Invertebrate species were translated using the crayfish BAF.

The water criterion element was calculated by first dividing the chronic value of each species (SMCV) in the muscle species sensitivity distribution (**Section 3.5.2**) by the most

appropriate BAF described above, using Equation 2, to generate a new species sensitivity distribution of translated water column values.

$$Species\ Chronic\ Water\ Column\ Value\ \left(\frac{ng}{L}\right) = \frac{SMCV\ [\frac{ng}{g}]}{BAF\ [\frac{L}{kg}]}\ (1000g/kg) \qquad \text{(Equation 2)}$$

Translated SMCVs represent the water concentrations at which adverse effects for the taxa in the muscle criterion element dataset would be expected to occur, based on the BAFs for those taxa. Translated SMCVs were grouped into GMCVs, where data for multiple species in a genus were available, and a translated water final chronic value (FCV) and chronic criterion (CCC) were calculated following the 1985 Guidelines calculation method. **Table 3-13** shows the distribution of tissue based SMCVs, BAFs, and translated water SMCVs and GMCVs for the species in the criterion dataset. **Table 3-14** shows the translated water FCV calculation, and **Figure 3-9** shows the distribution of translated water GMCVs ranked by sensitivity centile. The translated water FCV is 2.118 ng/L, and the translated water chronic criterion (CCC) is 2.1 ng/L.

As noted above, adverse effects to mercury were observed at lower tissue concentrations in frogs than in fish (see **Section 3.5.2.1**), but mercury bioaccumulation is higher in fish than in frogs (**Table 3-8** and **Appendix E**). The frog genera *Lithobates* and *Anaxyrus*, represented by the southern leopard frog and American toad, respectively, are the two most sensitive genera with respect to muscle and whole-body tissue concentrations and associated adverse effects, but because of their lower bioaccumulation factors, relative to other aquatic taxa, they are the fourth and ninth most sensitive genera with respect to translated water concentrations. The lowest translated water GMCV is for the *Sander* (walleye), a high trophic magnitude fish species (piscivore) with a relatively large BAF, and moderate sensitivity (Rank 5) to mercury based on

its tissue chronic value. *Pimephales* (fathead minnow) and *Procambarus* (red swamp crayfish) are the second and third most sensitive species in the translated genus sensitivity distribution (GSD), similar to their rankings as the third and fourth most sensitive genera in the muscle tissue mercury GSD (**Table 3-7**, **Figure 3-5**).

The tigerfish water column GMCV of >2.240 ng/L, translated from a muscle tissue GMCV of >1.45 µg/g ww, would have been the lowest translated water GMCV. However, it was removed from the translated water sensitivity distribution because of the uncertainty that would have arisen from including a non-definitive (greater-than) value as the most sensitive GMCV, most notably because this experiment resulted in no measurable effect on these fish. This approach is consistent with the practice described in **Section 2.9.1.2** and established in past criteria, including the 2013 Ammonia Aquatic Life Ambient Water Quality Criteria (U.S. EPA 2013) to exclude studies with greater than values quantitatively, because they do not provide useful information in criteria calculations other than indicate that the species is not sensitive to the test substance. Although the translated value was not used quantitatively in derivation of the water column criterion element, the indeterminate tigerfish value was retained qualitatively as part of the N in the criterion element calculation, thus the water value was still 2.1 ng/L calculated from an N = 18 genera.

The calculation of a water column criterion element based on the translation of tissue values using the most appropriate BAF for that species better reflects species-level exposure conditions and links the potential for effects with its trophic ecology. The translated water column sensitivity distribution results in fish species being identified as the most sensitive group, particularly those species whose trophic ecology results in higher tissue total mercury

concentrations (more bioaccumulative) and are relatively more sensitive based on the study endpoints assessed.

Table 3-13. Ranked Freshwater Genus Mean Chronic Values based on Muscle Concentrations Translated to Water Concentrations using Bioaccumulation Factors.

Median species- and genus-specific fish BAFs used, when available.

Rank[a]	MDR Group[b]	Genus	Species	Muscle SMCV[c] (ng THg/g ww)	BAF (L/kg ww)	Water SMCV (ng THg/ L)	Water GMCV (ng THg/ L)	BAF Source[d]
1	B	*Hoplias*	Tigerfish (Hoplias malabaricus)	>1,450 (No Effect observed)	647,335	2.240	2.240 (Not used quantitatively because tissue value is a non-definitive value)	High Trophic Magnitude
2	B	*Sander*	Walleye (*Sander vitreus*)	1,069	453,578	2.357	2.357	*S. vitreus*
3	B	*Pimephales*	Fathead minnow (*Pimephales promelas*)	357.5	144,915	2.467	2.467	Low trophic magnitude
4	E	*Procambarus*	Red swamp crayfish (*Procambarus clarkii)*	497.3	128,414	3.873	3.873	Crayfish
5	C	*Lithobates*	Southern leopard frog (*Lithobates sphenocephala*)	33.73	8,222	4.103	4.103	Anura
6	B	*Huso*	Beluga sturgeon (*Huso huso*)	3,000	647,335	4.634	4.634	High trophic magnitude
7	B	*Ictalurus*	Channel catfish (*Ictalurus punctatus*)	>1,600	205,123	7.800	7.800	*I. punctatus*
8	A	*Salmo*	Atlantic Salmon (*Salmo salar*)	>3,070	302,721	10.14	10.14	Salmo
9	B	*Carassius*	Goldfish (*Carassius auratus*)	>2,037	144,915	14.06	14.06	Low trophic magnitude
10	C	*Anaxyrus*	American toad (*Anaxyrus americanus*)	170.4	8,222	20.73	20.73	Anura
11	B	*Danio*	Zebrafish (*Danio rerio*)	4,426	199,646	22.17	22.17	Medium trophic magnitude

Rank[a]	MDR Group[b]	Genus	Species	Muscle SMCV[c] (ng THg/g ww)	BAF (L/kg ww)	Water SMCV (ng THg/ L)	Water GMCV (ng THg/ L)	BAF Source[d]
12	A	*Oncorhynchus*	Rainbow trout (*Oncorhynchus mykiss*)	4,392	161,685	27.16	27.16	*O. mykiss*
13	F	*Hexagenia*	Mayfly (*Hexagenia sp.*)	>3,516	128,414	27.38	27.38	Crayfish
14	G	*Corbicula*	Asiatic clam (*Corbicula fluminea*)	>6,000	128,414	46.72	46.72	Crayfish
15	B	*Orthodon*	Sacramento blackfish (*Orthodon microlepidotus*)	7,583	144,915	52.33	52.33	Low trophic magnitude
16	B	*Pogonichthys*	Sacramento splittail (*Pogonichthys macrolepidotus*)	>8,330	144,915	57.48	57.48	Low trophic magnitude
17	B	*Acipenser*	Green sturgeon (*Acipenser medirostris*)	17,980	647,335	27.78	71.32	High trophic magnitude
			White sturgeon (*Acipenser transmontanus*)	36,560	199,646	183.1		Medium trophic magnitude
18	D	*Daphnia*	Cladoceran (*Daphnia magna)*	11,100	128,414	86.44	86.44	Crayfish

[a] Ranked from the most to least sensitive based on GMCV
[b] MDR Groups identified by list provided in **Section 2.6** above.
[c] Muscle-based SMCVs from **Table 3-7** above.
[d] BAFs from **Table 3-12** above.
.

Table 3-14. Freshwater Final Translated Water Column Chronic Value (Criterion Continuous Concentration).

Median species- and genus-specific fish BAFs, when available. N=18

Genus	*N*	Rank	GMCV	ln(GMCV)	$ln(GMCV)^2$	P=R/(N+1)	sqrt(P)
Sander	18	1	2.357	0.86	0.74	0.053	0.229
Pimephales		2	2.467	0.90	0.82	0.105	0.324
Procambarus		3	3.873	1.35	1.83	0.158	0.397
Lithobates		4	4.103	1.41	1.99	0.211	0.459
		Sum:		**4.53**	**5.38**	**0.53**	**1.41**
				S^2 = 8.73			
				L = 0.090			
				A = 0.751			
				FCV = 2.118		**ng/L**	
				CCC = 2.10		**ng/L**	

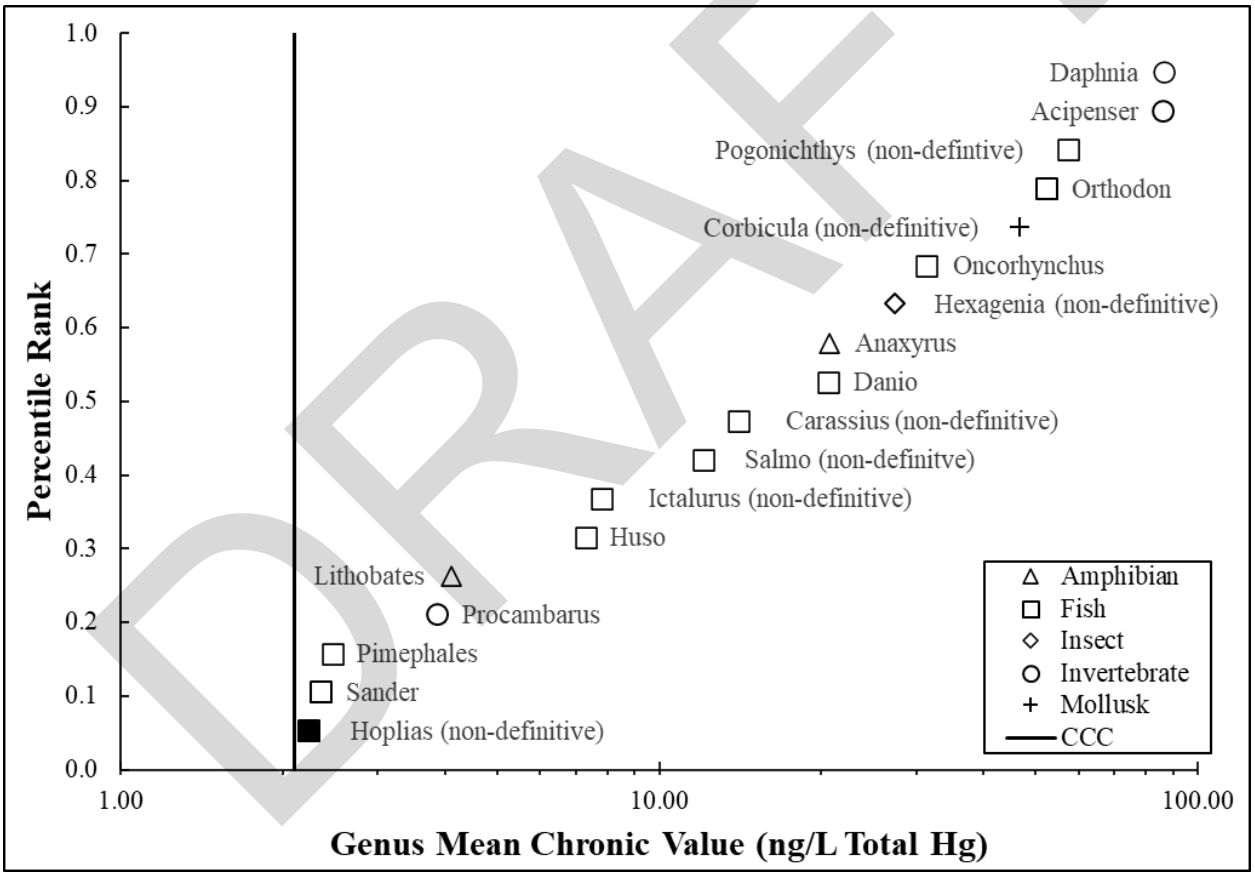


Figure 3-9. Distribution of Mercury Water Column GMCVs (THg, ng/L) Translated from Measured Dietary Mercury Effect GMCVs Expressed as Muscle (THg, µg/g ww).

Median species- and genus-specific fish BAFs, when available.

Note: The water based GMCV for *Hoplias* is based on a greater than NOEC (there was no measured effect in the test), therefore the criterion element is based on the four lowest translated water GMCVs with observed effects (**Table 3-14**).

3.6.2.1 Deriving Protective Duration for Water Column Mercury Criterion Element

The water criterion element averaging period, or duration, is specified as a 30-day average concentration of total mercury. This characteristic duration is based on mercury methylation processes affecting trophic transfer and observed durations of bioaccumulation and depuration processes in aquatic organisms (Bradley et al. 2017; Moye et al. 2002, Pickhardt et al. 2002, 2006; Stewart et al. 2008; Viera et al. 2021). The bioaccumulation process for mercury takes place over a longer period of time than often observed for acute and chronic effects on aquatic life based on exposure to aqueous concentrations of contaminants. Mercury cycling in aquatic ecosystems is controlled by various biotic and abiotic reactions interacting on a site-specific basis, which ultimately controls the rate of inorganic to methyl mercury conversion and biological uptake of mercury from the water to biota (Harris et al. 2007).

The proposed averaging period or duration for the mercury water column criterion is the same as recommended for the water column criterion or criterion elements for selenium, PFOA, and PFOS (U.S. EPA 2016a, 2022a, b, respectively). For setting averaging periods for aquatic life criteria, U.S. EPA (1995b) used the concept that the criterion averaging period should be less than or equal to the "characteristic time" describing the toxic speed of action. The determination of appropriate averaging periods for water concentrations of bioaccumulative pollutants, such as selenium and mercury, is explained in Appendix J of U.S. EPA (2016a). The averaging period was set by considering the characteristic time in the process of reaching a new steady-state plateau contaminant concentration in fish tissue after a change in water concentration yields either net accumulation or depuration. The characteristic time is related to the concept of a biological half-life and is defined as the reciprocal of the depuration rate coefficient (1/k) in a single compartment toxicokinetic model.

Previously, for selenium in U.S. EPA (2016a), a characteristic time of approximately 60-days was calculated assuming that a fish with 50-day characteristic time (reflecting observed depuration rates of small fish) was feeding on invertebrates with an environmentally conservative 5-day characteristic time, in turn feeding on algae with a 5-day characteristic time. In such a sequential exposure system, the characteristic times are approximately additive, thereby yielding 60 days. Similarly, for methylmercury, a laboratory study with mosquitofish by Pickhardt et al. (2006) found a 61 – 63-day characteristic time for bioaccumulation processes. In contrast, in lakes, the mercury accumulation curve of Harris et al. (2007) suggests that averaging durations of a year or more could be appropriate in such systems with longer hydraulic retention times. However, Riva-Murray et al. (2013) found that elevated methylmercury typically occurred during a one- to three-month period (growing season) during the year in 11 systems located in southern (South Carolina and Florida) and northern (New York, Oregon, and Wisconsin) states. The study also concluded that higher methylmercury did not occur during winter months and particularly at sites in northern states and recommended monitoring for BAF development that focuses on the growing season based on the geographic site of interest. Idaho is located at a similar latitude as the northern states in the Riva-Murray et al. (2013) study, and growing season conditions when elevated methylmercury occurs is likely similar. Taken together, the available science suggests that the use of a 30-day averaging period, as was used for the 2016 selenium freshwater water criterion, would yield an appropriate water column criterion for all systems in Idaho, including lotic systems with rapid turnover of mercury.

3.6.2.2 Deriving a Protective Frequency for Mercury Criterion: Chronic Water Column Criterion Element

The frequency aspect of water quality criteria is the number of times a chemical concentration (here, total mercury concentration in water) exceeding the criteria can occur over

time without negatively affecting the aquatic community. The standard, current frequency recommendation (Stephan et al. 1985; U.S. EPA 1991) is once-in three years on average, based on the ability of an aquatic ecosystem to recover from a toxic stress. This once-in-three years frequency was applied in other bioaccumulative chemical criteria documents, (U.S. EPA 2016a, 2023 a, b), and is also applied here as the frequency for the chronic mercury water column criterion element.

3.6.2.3 Summary of Total Mercury Water Column Criterion Element

The chronic freshwater water column criterion element for the protection of aquatic life (including amphibians) consists of a magnitude of 2.1 ng THg/L. The chronic water column criterion element averaging period, or duration, is specified as 30 days, and the chronic frequency is not to be exceeded more than once in three years.

3.7 Summary of the Total Mercury Aquatic Life Criterion for Idaho Freshwaters

The proposed mercury aquatic life criterion was developed to protect freshwater aquatic life against adverse effects, such as mortality, altered growth, and reproductive impairments, associated with chronic dietary exposure to mercury in Idaho freshwaters. The proposed criterion for Idaho waters includes two tissue criterion elements and one water column-based criterion element for freshwaters. The chronic tissue criterion elements for the protection of aquatic life consist of a muscle tissue criterion element of 225 ng THg/g wet weight (ww) and whole-body tissue criterion element of 162 ng THg/g wet weight (ww). The chronic water column criterion element of 2.1 ng THg/L was translated from the muscle tissue SMCVs using BAFs representative of all taxa in the sensitivity distribution (**Table 3-15**). The proposed chronic criterion for mercury in Idaho is a tiered criterion composed of three elements, two tissue criterion elements and one water column criterion element. The tiering of the criterion indicates that the tissue criterion elements have primacy over the water column criterion element when

both media are measured. The fish tissue criterion elements have primacy over the water column criterion element due to the fact that fish tissue concentrations provide a more robust and direct indication of potential mercury effects, because the criterion was derived using tissue data following dietary, not water column, exposures of aquatic organisms to mercury. Thus, the proposed criterion, applicable to all waters in Idaho, include: (1) a fish whole-body tissue criterion element, (2) a fish muscle tissue criterion element, and (3) a water column criterion element. The proposed criterion is intended to protect aquatic life (i.e., fish, amphibians, and aquatic invertebrates) from the chronic effects of exposure to all forms of mercury (i.e., total mercury) in Idaho.

Table 3-15. Proposed Chronic Mercury Ambient Water Quality Criterion for the Protection of Aquatic Life in Idaho Freshwaters

Media Type	Fish Muscle Tissue [1, 2, 3] Total Mercury (ng THg/g wet weight)	Fish Whole Body Tissue [1, 2] Total Mercury (ng THg/g wet weight)	Water Column [1,4] Total Mercury (ng/L) in whole water
Magnitude	225	162	2.1
Duration	Instantaneous measurement [5]		30-day average
Frequency	The average tissue concentration must not be exceeded		Not more than once in three years on average

[1] The proposed criterion elements are hierarchical, with both tissue elements superseding the water column element. The fish muscle tissue and fish whole body tissue criterion elements are independently applicable.

[2] Tissue sample measurements must be based on measurement(s) of the total mercury concentration (in a composited tissue sample from each fish species or a central tendency estimate of individual tissue samples from each fish species) collected from a given site or waterbody in a discrete sampling period. These criterion elements support Idaho's aquatic life uses. Only samples of adult life stage trophic level (TL) 4 fish can be directly compared to the muscle or whole-body criterion elements.

[3] If adult life stage TL2 or TL3 fish are sampled, a Bioaccumulation Trophic Adjustment Factor (BTAF) must be applied to the muscle concentrations of those fish. If whole-body tissue from TL2 or TL3 fish is sampled, the fish whole body – muscle conversion factor of 0.72 must be applied to generate a translated muscle value before a BTAF is applied to the sample concentration. A TL2 sampled fish concentration must be multiplied by the TL2 BTAF of 5.6 and the resultant value compared to the muscle tissue criterion element. A TL3 sampled fish concentration must be multiplied by the TL3 BTAF of 3.5 and the resultant value compared to the muscle tissue criterion element. If multiple adults of different TLs are sampled, the TL4 fish result would supersede TL3 BTAF-applied or TL2 BTAF-applied value outcomes. If TL3 and TL2 fish are sampled, the TL3 BTAF-applied values supersede the TL2 BTAF-applied values.

[4] Water column values are based on total mercury in unfiltered or "whole water" samples. Total mercury includes all inorganic and organic species of mercury in the water column. Water samples collected during baseflow conditions would be most representative of the data used to derive this criterion element. This criterion element supports Idaho's aquatic life uses.

[5] Fish tissue data provide integrative measurements that reflect accumulation of mercury over time and space in aquatic organisms from a given site or waterbody in a discrete sampling period.

4 EFFECTS CHARACTERIZATION FOR AQUATIC LIFE

The Effects Characterization summarizes the remainder of the available toxicity data used to derive the criterion as well as studies providing supporting information that contributed to the weight-of-the evidence for the criterion derivation process. For the proposed Idaho aquatic life criterion for mercury, this section includes: 1) a discussion of the protectiveness of the fish tissue and water column criterion elements for sensitive amphibian taxa (**Section 4.1**); 2) a summary of the remaining acceptable (quantitative) toxicity studies beyond the four most sensitive taxa in fish and invertebrates with apical endpoints (e.g., effects on survival, growth, or reproduction) that were used directly to derive the criterion (**Section 4.2**); 3) discussion on use of qualitative invertebrate data to waive MDR H (invertebrate family in any order of insect or any phylum not already represented) (**Section 4.3**); 4) a summary of the toxicity studies with apical as well as non-apical endpoints (e.g., effects on behavior, neurotoxicity or biochemical/histological endpoints) that were not used directly to derive the criterion, but were used qualitatively to support the mercury criterion discussions comparing these endpoints to toxicity endpoints for key quantitative studies (**Section 4.4**); and 5) a characterization of uncertainty and variability with respect to criterion derivation (**Section 4.5**). The additional analyses presented here are solely intended to support the proposed tissue-based and water column criterion elements through a weight-of-evidence approach and characterize variability and uncertainties with respect to criterion derivation.

4.1 Protectiveness of the Fish Tissue and Water Column Criterion Elements for Sensitive Amphibian Taxa

As discussed above in the Effects Analysis, in **Section 3.5.2.1**, in considering development of the mercury tissue criterion elements that consider both relative sensitivity and relative bioaccumulation of mercury across taxa, EPA concluded that the quantitative analysis to

develop the total mercury tissue criterion elements should include only fish and invertebrate species. EPA determined that this approach was appropriate because while amphibians are sensitive to mercury, they do not bioaccumulate mercury in their tissues to the same extent as fish or larger invertebrates, such as crayfish. Thus, EPA's proposed criterion elements based on fish and invertebrate data are expected to be protective of sensitive amphibian species in Idaho (e.g., aquatic stages of the northern leopard frog (*Lithobates pipiens*) and the American toad (*Anaxyrus americanus*)) and related species.

In addition to calculating the relative bioaccumulation and sensitivity of aquatic taxa provided in the Effects Analysis section above, EPA conducted an analysis to determine an example criterion element if amphibians were included in the tissue sensitivity distribution calculations.

Table 4-1. Freshwater Chronic Value: Whole Body Tissue for Aquatic Life, if Amphibians were included (ng/g ww).

Genus	***N***	**Rank**	**GMCV**	**ln(GMCV)**	**ln(GMCV)^2**	**P=R/(N+1)**	**sqrt(P)**
Lithobates	18	1	32.72	3.49	12.17	0.053	0.229
Anaxyrus		2	165.30	5.11	26.09	0.105	0.324
Pimephales		3	257.40	5.55	30.81	0.158	0.397
Procambarus		4	358.10	5.88	34.58	0.211	0.459
		Sum:		**20.03**	**103.65**	**0.53**	**1.41**
				S^2 =	115.42		
				L =	1.220		
				A =	3.622		
				FCV =	**37.410**	**ng THg/g ww**	
				CCC =	**37**	**ng THg/g ww**	

When the sensitive amphibian species are included in the tissue calculation, the freshwater chronic whole-body tissue value for total mercury would be 37 ng THg/g ww (**Table 4-1**), calculated using the procedures described in the 1985 Guidelines (Stephan et al. 1985) and

is the 5th percentile of the chronic sensitivity distribution. Amphibians represent the two most sensitive taxa, as represented by the GMCVs for the southern leopard frog and American toad (**Figure 4-1**).

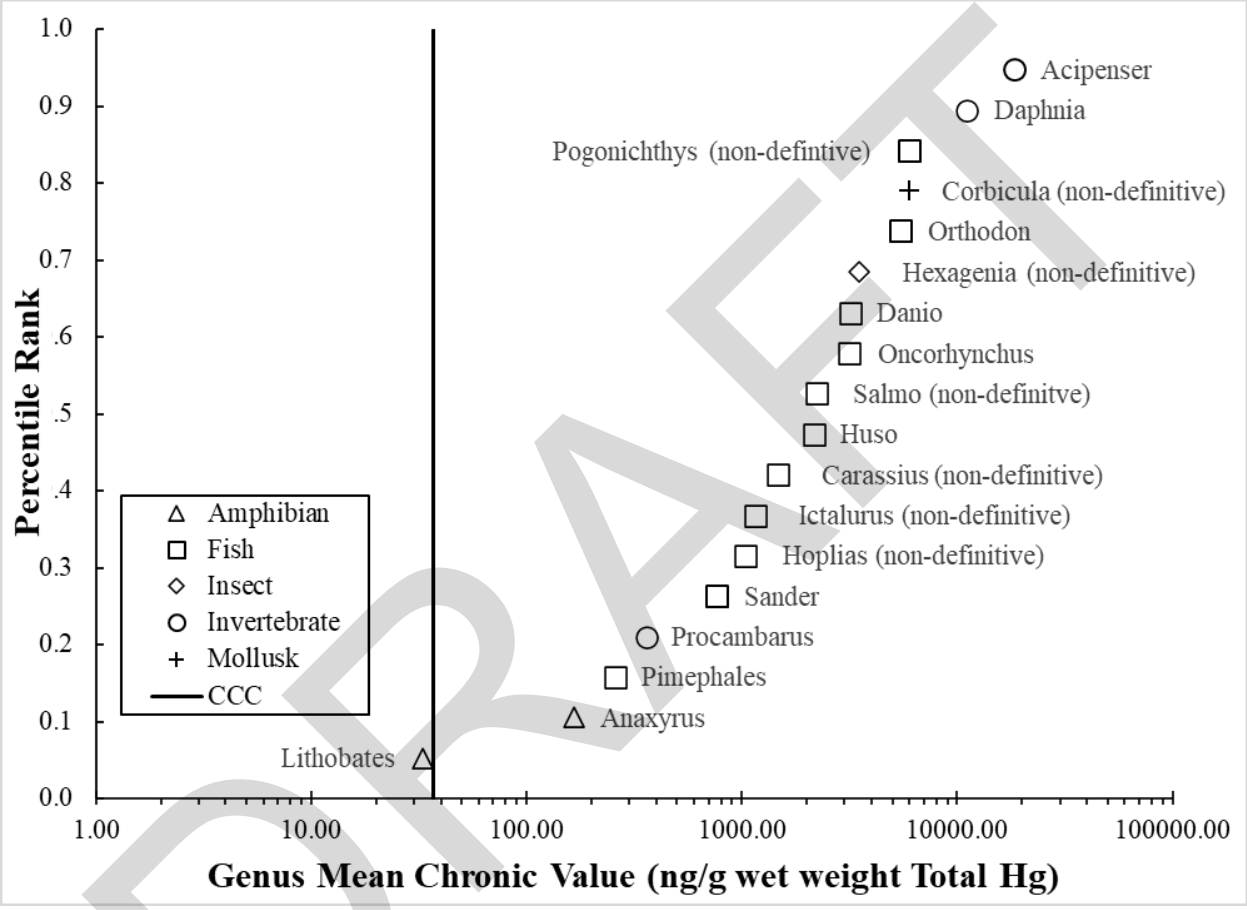


Figure 4-1. Distribution of Measured Dietary Mercury Effect GMCVs expressed as Whole-Body (THg, ng/g ww), including Amphibians.

If the sensitive amphibian species were included in the muscle tissue value calculation, the freshwater chronic muscle tissue value for total mercury would be 37 ng THg/g ww (**Table 4-2**), the same as the parallel whole-body amphibian-inclusive value, calculated using the procedures described in the 1985 Guidelines (Stephan et al. 1985), the 5th percentile of the

chronic sensitivity distribution. Amphibians represent the two most sensitive taxa, based on the GMCVs for the southern leopard frog and American toad (**Figure 4-2**).

Table 4-2. Freshwater Chronic Value: Muscle Tissue, if Amphibians were included.

Genus	*N*	Rank	GMCV	ln(GMCV)	$ln(GMCV)^2$	P=R/(N+1)	sqrt(P)
Lithobates	18	1	33.73	3.52	12.38	0.053	0.229
Anaxyrus		2	170.40	5.14	26.40	0.105	0.324
Pimephales		3	357.30	5.88	34.56	0.158	0.397
Procambrus		4	497.30	6.21	38.55	0.211	0.459
		Sum:		**20.74**	**111.89**	**0.53**	**1.41**
				S^2 =	147.32		
				L =	0.908		
				A =	3.622		
				FCV =	**37.39379**	**ng THg/g ww**	
				CCC =	**37.0**	**ng THg/g ww**	

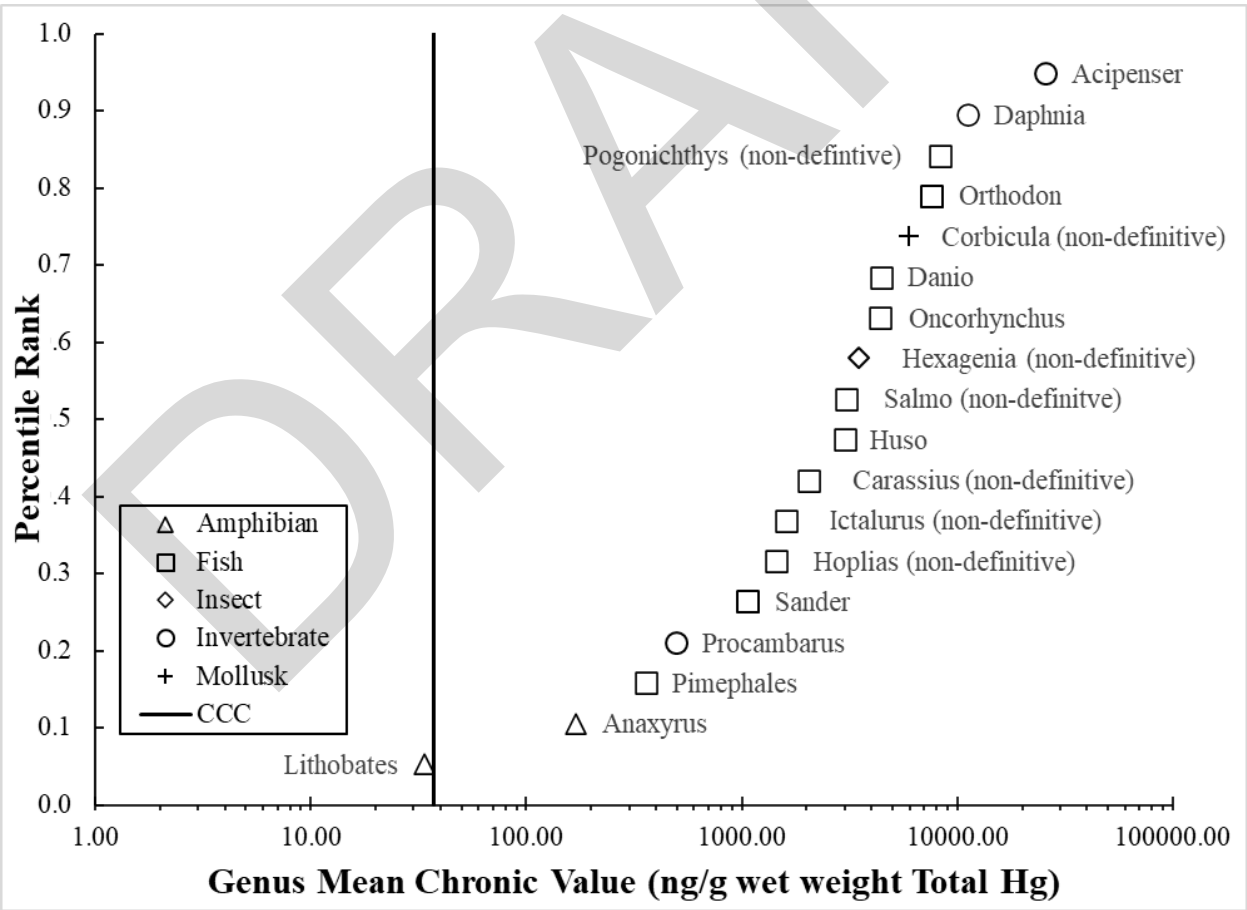


Figure 4-2. Distribution of Measured Dietary Mercury Effect GMCVs for Aquatic Life (including Amphibians) expressed as Muscle (ng THg/g ww).

One can further consider the protectiveness of the proposed chronic tissue criterion for fish by examining the expected protection of amphibians at the muscle criterion element concentration (225 ng THg/g ww). To demonstrate the protectiveness of the mercury criterion for amphibians, EPA compared the BAF for the walleye (*Sander vitreus*) – the fish species in the dataset with the greatest bioaccumulation potential and the most sensitive species (i.e., with the lowest acceptable water column concentration) – to the BAF for sensitive amphibians. Considering the relative mercury bioaccumulation potential and sensitivity, by examining the outcomes in the most bioaccumulative fish and the most sensitive amphibian in the criterion dataset, the ratio of the walleye BAF to the amphibian BAF is 55 (**Table 3-12 -** the walleye BAF of 453,578 divided by the amphibian BAF of 8,222), meaning the walleye would be expected to bioaccumulate mercury to a level 55 times greater than the level accumulated in amphibians. The most sensitive amphibian, southern leopard frog tadpole (*R. sphenocephala*), EC_{10} expressed as muscle (33.73 ng THg/g ww) is 10.6 times lower than the EC_{10} (357.3 ng THg/g ww) of the most sensitive fish, the fathead minnow, *Pimephales promelas* and 6.7 times lower than the muscle criterion element value of 225 ng THg/g ww. For a given water body, if walleye was sampled for whole-body mercury concentrations and was at the muscle tissue criterion of 225 ng THg/g ww, then the amphibian concentrations expressed as a muscle tissue concentration would be expected to be 55 times below the criterion element (225/55) or approximately 4 ng THg/g ww. This mercury concentration bioaccumulated in the southern leopard frog would be substantially below (> 8 times below) its EC_{10} (33.73 ng THg/g ww), indicating that even the most sensitive amphibian would be well-protected at the proposed fish/invertebrate whole-body tissue criterion element. This analysis can be repeated using the 20^{th} centile BAF from the distribution of all species-specific fish BAFs for Idaho when compared with the amphibian BAF.

This conservative analysis would yield a ratio of 8.17, that when applied to the muscle tissue criterion in a manner similar to the walleye above would yield an estimated tissue concentration for amphibians of 27.5 ng THg/g ww, still below the most sensitive amphibian EC10 of 33.7 ng THg/g ww. EPA repeated this analysis for all of the BAFs used for translation of tissue concentrations to water to illustrate the protectiveness of the tissue criteria for sensitive amphibians when sensitivity and bioaccumulation potential are considered together (**Table 4-3**).

Table 4-3. Relative Bioaccumulation of Mercury Across Taxa and Expected Amphibian Tissue Concentrations at Fish Muscle Tissue-based Criterion Element

Taxa	Median BAF (L/Kg)	Amphibian BAF (L/Kg)	Ratio of Various Taxa BAFs to Amphibian BAF	Draft Fish Muscle Tissue Criterion (ng THg/g ww)	Most sensitive Amphibian muscle EC10 (ng THg/g ww)	Estimated most sensitive amphibian tissue concentrations at muscle fish tissue criterion element value (i.e., 225 ng THg/BAF ratio)
20th centile Idaho Fish Species	67,203	8,222	8.17	225	33.7	27.5
Low Trophic Magnitude Fish	73,651	8,222	8.96	225	33.7	25.1
Medium Trophic Magnitude Fish	108,418	8,222	13.18	225	33.7	17.1
Median Idaho Fish Species (All Species Pooled)	138,102	8,222	16.79	225	33.7	13.4
High Trophic Magnitude Fish	378,150	8,222	45.99	225	33.7	4.9
Species Specific BAFs for Idaho Aquatic Taxa						
Crayfish (Low – Medium Trophic Magnitude	128,414	8,222	15.6	225	33.7	14.4
Rainbow Trout (*Oncorhynchus mykiss*)	161,685	8,222	19.7	225	33.7	11.4
Channel catfish (*Ictalurus punctatus*)	205,123	8,222	24.9	225	33.7	9.0
Brown trout (*Salmo trutta*)	302,721	8,222	36.8	225	33.7	6.1
Walleye (driver of water column criterion element) (*Sander vitreus*)	453,578	8,222	55.2	225	33.7	4.1

Based on the analyses in **Table 4-3**, even the most sensitive amphibian, Southern leopard frog, is expected to be protected at the fish tissue criterion elements, based on muscle tissue analyses, for nearly all fish species in the dataset. If tissue concentrations measured in the fish with a BAF similar to the 20th centile fish (the lowest BAF) met the muscle tissue criterion element, the expectation would be that the most sensitive amphibians would be protected with the most sensitive amphibian body burden being below their EC_{10} level.

At sites where uncertainty may occur regarding the protectiveness of the fish tissue criterion elements for amphibians, late-stage (pre-metamorph) amphibian tadpole tissue could be collected and evaluated, using the amphibian-inclusive tissue value of 37 ng THg/g ww for whole-body or muscle tissue, as derived above, to further verify that the fish criterion elements are protective of amphibians.

4.2 Studies Acceptable for quantitative use for Taxa that were not among the Four Most Sensitive Genera

The following is a brief summary providing an overview of toxicity tests on fish and aquatic invertebrate taxa that were not among the four most sensitive genera but were included in the number of GMCVs in the dataset (see **Section 3.5**), and how these studies compare to the chronic aquatic life criterion derived for mercury. Data are summarized as whole body in **Table 3-6** and as muscle (based on a whole-body – muscle conversion factor) in **Table 3-7**. Details of these studies for fourteen additional genera used directly in the derivation of the mercury criterion are contained in **Appendix A**.

4.2.1 Characterization of Acceptable Fish Studies on the Tissue-based Final Chronic Value Not among the Four Most Sensitive Genera

Acceptable chronic values were available for eleven fish genera in six families reflecting ecological niches and interactions (primary consumer to piscivore) at all trophic levels in freshwater systems. Chronic values reported (or converted) to muscle tissue equivalents ranged

from 1.069 µg THg/g ww (MATC for reduced growth observed in walleye, Friedmann et al. 1996) to 25.64 µg THg/g ww (geometric mean of the MATCs based on survival and growth in green and white sturgeon, Lee et al. 2011).

Friedmann et al. (1996) exposed hatchery-raised juvenile (6-month old) walleye (*Sander vitreus*) to a diet of methylmercury-injected catfish fillets and fathead minnows for six months yielding a muscle tissue-based NOEC, LOEC and MATC of 0.347, 2.392, and 1.069 µg THg/g ww (whole body-based equivalent NOEC, LOEC and MATC of 0.25, 2.37 and 0.7697 µg THg/g ww respectively) based on reduced growth. This chronic value (MATC of 1.069 µg THg/g ww) is five times higher than the muscle criterion element value of 225 ng THg/g ww, and approximately three times higher than the most sensitive fish genera muscle value of 0.3575 µg THg/g ww for *Pimephales* (fathead minnow). The chronic value for walleye also provides a surrogate value for fish species in the family Percidae.

The effect of mercury on condition factor (CdF) and expression of hepatic metallothionein (MT) in juvenile channel catfish (*Ictalurus punctatus*) was investigated by Schlenk et al. (1997). Catfish (12-15 cm) were fed a diet of methylmercury-injected Japanese medaka (*Oryzias latipes*) and commercial catfish food for 30 days. There was no effect on condition factor, liver somatic index (LSI) and hepatic metallothionein (MT) expression between the control and mercury fed fish. The indeterminate muscle tissue-based NOEC > 1.6 µg THg/g ww (whole body equivalent NOEC of > 1.15 µg THg/g ww) based on growth is seven times higher than the muscle criterion element of 225 ng THg/g ww and represents the relative sensitivity of this species to dietary mercury exposure in the chronic criterion dataset.

The effects of mercury on four additional cyprinid genera including goldfish (*Carassius auratus*; Crump et al. 2008), zebrafish (*Danio rerio:* Amlund et al. 2015; Penglase et al. 2014a,b;

Lerebours et al. 2013; Cambier et al. 2009, 2010; Oliviera-Riberio et al. 2008; Gonzalez et al. 2005), Sacramento blackfish (*Orthodon microlepidotus:* Houck and Cech 2004) and Sacramento splittail (*Pogonichthys macrolepidotus*: Deng et al. 2008) were investigated using a variety of study designs featuring different dietary regimens and a range of exposure concentrations using commercially prepared diets formulated with methylmercury. Survival and growth as well as biochemical, genomic, and histological endpoints were measured following exposure durations ranging from 28 – 247 days. Studies yielded a combination of indeterminate and defined whole body chronic values ranging from > 1.47 µg THg/g ww to > 6.0 µg THg/g ww. The lowest translated muscle tissue value, representing the most sensitive of these four species, was an indeterminate NOEC > 2.037 µg THg/g ww for survival and growth in goldfish is nine times higher than the muscle criterion element value of 225 ng THg/g ww, and approximately six times higher than the lowest muscle tissue-based chronic value for a species in the Family Cyprinidae (*Pimephales promelas*; 0.3575 µg THg/g ww). The lowest translated muscle tissue value for goldfish confirms the relative insensitivity of this fish family to dietary mercury exposure in the chronic criterion dataset compared to the tissue criterion elements based on the endpoints evaluated, and the other species (zebrafish, Sacramento blackfish, Sacramento splittail) were all less sensitive to mercury in these experiments than the goldfish.

The effects of dietary methylmercury exposure were also evaluated in sturgeon. Gharaei et al. (2008, 2011) investigated the dietary exposures on the non-resident beluga sturgeon (*Huso huso*) whereas Lee et al. (2011) investigated two North American species the green sturgeon (*Acipenser mediorostris*) and the white sturgeon (*Acipenser transmontanus*). Dietary exposures ranging from 60-70 days in juvenile sturgeon resulted in reduced growth observed in all 3 species yielding muscle tissue-based chronic values ranging from a LOEC of 3.0 µg THg/g ww

for beluga sturgeon to a GMCV of 25.64 µg THg/g ww based on the MATC for reduced growth of green sturgeon (17.98 µg THg/g ww) and the MATC for reduced growth in white sturgeon (36.56 µg THg/g ww). The lowest value for the family Acipenseridae (3.0 µg THg/g ww for beluga sturgeon) is 13 times higher than the muscle criterion element of 225 ng THg/g ww, providing an estimate of the relative sensitivity of this fish family for the endpoints evaluated.

Several investigators also investigated the effects of dietary methylmercury exposure on salmonids. Berntssen et al. (2003, 2004) tested Atlantic salmon (*Salmo salar*) parr (14.7±3.8 g) whereas fingerling rainbow trout (*Oncorhynchus mykiss*) were tested by Rodgers and Beamish (1982), Phillips and Buhler (1978), and Wobeser (1975) in exposures to fish meal diets ranging from 24-105 days. Studies evaluated survival and growth of early juvenile life stages as well as brain lipid peroxidation and the neurotoxic effects of mercury on behavior. No effects of dietary methylmercury on survival or growth (final weight) were observed in Atlantic salmon resulting in an indeterminate NOEC of > 3.07 µg/g THg ww in muscle tissue. For rainbow trout, EPA selected the geometric mean of the two estimated NOECs (value of 4.392 µg THg/g ww) in fish muscle tissue from Rogers and Beamish (1982) because the fingerlings for this study were approximately 4 times smaller (5.6 g vs 20.9 g), thus younger and potentially more sensitive than fingerlings used in the Wobeser study and the exposure was approximately 3.5 times longer (84 days vs 24 days) than the Phillips and Buhler (1978) study. The lowest value for salmonids, the indeterminate NOEC for Atlantic salmon of >3.07 µg/g THg ww is 14 times higher than the muscle tissue criterion element of 225 ng/g THg ww and provides an estimate of relative sensitivity for the family Salmonidae for the endpoints evaluated.

Finally, Olivera-Riberio et al. (2006), Costa et al. (2007) and Mela et al. (2007) examined the effects of dietary exposure on survival and multiple biochemical endpoints in the non-

resident piscivore, tigerfish (*Hoplias malabaricus*). Methylmercury-injected prey (*Astyanax* spp.; "Tetra fish") were fed to tigerfish over 70 days, however no mortality was observed, yielding an indeterminate NOEC value of >1.45 µg THg/g ww measured in muscle tissue, approximately six times higher than the muscle tissue criterion element of 225 ng THg/g ww. The effects of dietary methylmercury on biochemical endpoints are discussed further in **Appendix A**, however these effects were not within the low range of apical effects in sensitive species that influence criterion derivation. Although this species is non-resident and does not have a close taxonomic relationship with North American species, EPA included this species as a trophic surrogate for high level piscivores such as northern pike and muskellunge in the Order Esociformes that are not represented in the toxicity dataset.

The chronic values reported for insensitive fish discussed above range from 4.392 µg THg/g ww to 25.64 µg THg/g ww in muscle tissue, and 3.162 to 18.46 on whole body concentration basis. Five of the eleven dietary exposures in the fish studies included by EPA resulted in indeterminate (>) chronic values due to a lack of observed effects at methylmercury doses and exposure durations tested. Mortality was uncommon, and typically occurred at higher mercury doses for exposure durations exceeding 49 days. Taken together, these studies provide important insight on the sensitivity of these fish taxa considering the experimental doses, exposure durations, and effects evaluated relative to the most sensitive endpoint for fish taxa in the sensitivity distribution, reproductive effects in the fathead minnow (*Pimephales promelas*), as well as demonstrating the protectiveness of the muscle and whole body tissue criterion element values relative to the sensitivity of tested species representing the families Acipenseridae, Alestidae, Cyprinidae, Ictaluridae, and Salmonidae.

4.2.2 Characterization of Quantitatively Acceptable Invertebrate Studies not among the Four Most Sensitive Genera

In addition to the chronic value for *Procambarus*, acceptable chronic values were available for three invertebrate taxa representing aquatic insects (burrowing mayfly, *Hexagenia* spp), mollusks (Asiatic clam, *Corbicula fluminea*), and cladocerans (Water flea, *Daphnia magna*) reflecting diverse ecological niches and trophic interactions.

Naimo et al. (2000) exposed mayfly nymphs (almost entirely *Hexagenia bilineata*) in 21-day bioaccumulation tests to mercury-contaminated and reference sediments collected from Sudbury River, Massachusetts. The overall survival of *Hexagenia* mayflies ranged from 90 - 96% in all treatments and growth was not correlated with mercury concentrations in test sediment resulting in an indeterminate LOEC of > 3.516 µg THg/g ww based on whole body measurements. EPA selected this value to represent the relative sensitivity of this species to dietary mercury exposure and it is approximately ten times less sensitive than the chronic value (0.3581 µg THg/g ww) for the most sensitive invertebrate, the red swamp crayfish (*Procambarus clarkii*) and the value is 21.6 times higher than the whole-body criterion value of 162 ng THg/g ww.

Mollusks, represented by the Asiatic clam (*Corbicula fluminea*) were used by Inza et al. (1997) to evaluate water and sediment exposures to methylmercury. No mortality or effect on growth was observed following the 14-day experiments, resulting in an indeterminate LOEC of >6.000 µg THg/g ww based on whole body measurements. This value is 16.8 times higher than the crayfish whole-body chronic value of 0.3581 µg THg/g ww and 36.8 times higher than the whole-body criterion element of 162 ng THg/g ww representing the relative sensitivity of this genera in the chronic criterion dataset.

Finally, Tsui and Wang (2004) evaluated the effect of dietary methylmercury exposure on 3-day old *Daphnia magna* based on a 5-day exposure to methylmercury-radiolabeled *C. reinhardtii* [5.3 x 10^4 cells/mL]. Significant effects were observed in survival and egg production of the F_0 generation, as well as survival of the F_1, despite low maternal transfer efficiency resulting in an estimated NOEC of 11.1 µg THg/g ww tissue measured in whole body. This chronic value is 31 times higher than the crayfish chronic value and 68 times higher than the 162 ng THg/g ww whole-body criterion element representing the relative sensitivity of this genera to dietary mercury exposure in the chronic criterion dataset.

Taken together, these studies represent a range of sensitivity for three diverse invertebrate taxa and provide important insight on the sensitivity of aquatic invertebrates to dietary exposure of methylmercury relative to the most sensitive invertebrate taxa in the sensitivity distribution, the red swamp crayfish (*Procambarus clarkii*), as well as demonstrating the protectiveness of the whole-body tissue-based criterion element for aquatic invertebrate taxa.

4.3 Use of Qualitative Invertebrate Data to Address the Minimum Data Requirement H, (Invertebrate family in any order of insect or any phylum not already represented)

EPA has met the minimum data requirements for invertebrates including the pelagic crustacean (D), benthic crustacean (E), aquatic insect family (F), and mollusks (G), as described in **Section 3.2** above. Quantitatively acceptable data for data requirement H, described as "an invertebrate family in any order of insect or any phylum not already represented" were not available. This data requirement provides taxonomic flexibility in its fulfillment; therefore, EPA evaluated three additional studies with invertebrates to evaluate their potential influence on criterion derivation for mercury as well as considerations to serve as surrogates for MDR H.

4.3.1 Family Sparganophilidae and Naididae (Oligochaeta)

Two studies evaluated the effect of aqueous mercury exposure on the oligochaete worm *Sparganophilus pearsei* (Vidal and Horne 2003b) and *Tubifex tubifex* (Vidal and Horne 2003a). Oligochaetes may develop tolerance to mercury over generations or have evolved physiological mechanisms to tolerate sublethal stress (i.e., autotomy). Both studies acclimated worms to Hg-contaminated segments as well as low mercury reference sediments and the worms were subsequently exposed to aqueous mercury chloride. The 24-hour LC_{50} for *S. pearsei* reference organisms was 0.12 mg/L (95% CI; 0.10–0.16) whereas the 96-h LC_{50}s for *T. tubifex* worms acclimated to reference sediments ranged from 0.16-0.19 mg/L. Although the studies used Hg concentrations well above ecologically relevant ranges, the studies provide an estimate of mercury insensitivity relative to more sensitive taxa used in criterion derivation.

4.3.2 Family Euchlanidae (Rotifera): Euchlanis dilatata

Hernandez-Flores et al. (2020) evaluated the bioconcentration and toxicity of five metals in the freshwater rotifer *Euchlanis dilatata*, a widely distributed, benthic littoral rotifer. Rotifers were exposed for 24 hrs to HgIICl at 0.001, 0.003, 0.006, 0.009, and 0.013 µg/L (nominal) for lethality, 0.04, 0.06, 0.08, 0.1, and 0.12 µg/L (nominal) for ingestion rate, and 0.05, 1.0, 1.5, 2.0, and 2.5 µg/L (nominal) for reproduction. NOECs for mortality, ingestion rate, and reproduction were 0.081, 0.033, and 0.417 µg/L respectively. Although the effect concentrations observed here are difficult to directly compare to the relative sensitivity of taxa used in the criterion due to route of exposure and form of mercury, this taxon is expected to be protected by the proposed criterion since even the lowest concentration is more than an order of magnitude above the proposed water column mercury criterion element (0.0021 ug/L - 2.1 ng/L). Therefore, these studies allow EPA to conclude that these MDR H is fulfilled.

4.4 Qualitative Studies Assessing Sublethal Effects

EPA evaluated three qualitative studies assessing sublethal effects. One was a field study (Webb et al. 2006) determined to be qualitatively acceptable on sublethal effects that provided observations of potential reproductive effects of dietary mercury exposure in juvenile white sturgeon. Two additional laboratory studies (Fjeld et al. 1988; Webber and Haines 2003) evaluated the effects of dietary methylmercury on behavior related to predator-prey relationships relative to controls. EPA compared the relative sensitivity of effects observed in these qualitative studies to quantitative chronic values for sensitive species in the distribution used to derive the tissue criterion elements.

4.4.1 Family Acipenseridae, White Sturgeon (Acipenser transmontanus)

EPA evaluated a field study even though the qualitative study was not acceptable for use in criterion derivation, because of a lack of information on potential co-contaminants in the field. Webb et al. (2006) investigated the relationship between tissue mercury concentrations and various physiological parameters in juvenile male and female white sturgeon from four sites in the Columbia River Basin. Webb et al. observed reduced testosterone in male fish, and a reduction in the GSI of immature male sturgeon correlating to an average mercury concentration in muscle of 0.176 µg THg/g ww. Although this study does not establish a causal link of mercury exposure to reproductive effects in white sturgeon, EPA compared this value against the muscle tissue criterion element (225 ng THg/g ww). The muscle tissue criterion element is approximately 1.3 times higher than the concentration reported to affect juvenile sturgeon reproductive capacity as measured in juvenile male sturgeon in this study, however, only mercury was measured in this field study, and other contaminants (e.g., endocrine disruptors or other contaminants) could have played a role in the effects observed in sturgeon in the Columbia River Basin.

4.4.2 Family Cyprinidae: Golden shiner (Notemigonus crysoleucas)

Webber and Haines (2003) exposed golden shiner (*Notemigonus crysoleucas*) to methylmercury via diet for 90 days to assess the effects of methylmercury on predator avoidance using a model of a belted kingfisher, *Ceryle alcyon*. Measured dietary concentrations were 0.012, 0.0455, and 0.0959 µg THg/g dw for the control, low-Hg diet and a high-Hg diet, respectively. There were no effects on growth or survival during the 90-day dietary exposure, however dietary methylmercury exposure impacted predator avoidance. There was a significant difference among treatments in shoal area (Kruskal-Wallis one-way ANOVA, $p = 0.0463$) mean maximum shoal heights (ANOVA, $p = 0.0417$), and marginally significant mean time to settle in the high-Hg treatment (Kruskal-Wallis one-way ANOVA, $p < 0.0702$). The whole-body Hg concentrations attained by the fish in the study were 0.041, 0.230 and 0.536 µg/g ww, for the control, low-Hg and high-Hg diets, respectively. Analyses using these whole-body tissue concentrations yielded a NOEC, LOEC, and MATC of 0.230, 0.536, and 0.351 µg THg/g ww, (0.319, 0.744, and 0.4875 µg THg/g was muscle concentrations after application of the WB:M conversion factor of 0.72). EPA considered the MATC of 0.4875 µg THg/g ww based on muscle as the chronic value because of the 11% difference from control in shoal area after settling post predator exposure. This MATC is more than twice as high as the muscle tissue criterion element (225 ng THg/g ww), indicating the proposed criterion would be protective of this species.

4.4.3 Family Salmonidae: Grayling (Thymallus thymallus)

Fjeld et al. (1988) exposed grayling embryos to aqueous exposures of methylmercury (nominal concentrations of 0.16, 0.8, 4.0 and 20 µg/L) yielding tissue concentrations of 0.01, 0.09, 0.27, 0.63, and 3.8 µg THg/g ww in larvae (13 mm) categorized as Groups A-E, respectively. EPA categorized this study as qualitative since the study design used a short duration, high concentration aqueous exposure to methylmercury to simulate a chronic dietary

exposure. Fish were held for 2 years post-exposure under background exposure conditions to examine latent sublethal effects on fish foraging behavior from embryonic exposure to methylmercury. First, the feeding efficiency of exposed fish was assessed in single fish feeding trials, where mean number of prey caught decreased from control (Group A) by~13-15% in pooled means for low (Group C) and medium (Group D) embryonic exposures compared to a 23.9% difference from control in the highest exposure group (Group E) (ANOVA $F = 9.62$, df. = 4,47, $P < 0.001$). This yielded a NOEC, LOEC, and MATC of 0.0900, 0.2700 and 0.1559 µg THg/g ww, as whole-body tissue (0.125, 0.375 and 0.2165 µg THg/g ww as muscle tissue, after application of the WB:M conversion factor of 0.72). EPA considered the MATC expressed as a muscle concentration (0.2165 µg THg/g ww). This value again is similar to the muscle tissue criterion (225 ng THg/g ww), however the aqueous exposure (vs. dietary exposure) makes direct comparison of tissue concentrations uncertain.

4.5 Characterization of Uncertainty and Variability with Respect to Criterion Element Derivation

4.5.1 Conversion Factors

As explained in **Section 2**, EPA derived tissue-based criterion elements for the protection of aquatic life in the State of Idaho due to the importance of the dietary route for mercury exposure in aquatic life. A tissue-based criterion element for the receptor organisms was determined to be a better approach than a dietary-based criterion due the wide variability in diet types used for mercury exposures found in scientific publications, and because of some uncertainty with the composition and form of mercury in diets. In **Appendix D**, EPA further explains that for the purpose of implementation in the state of Idaho, it was also important to be able express the tissue criterion element as a wet weight (ww) concentration, as either whole body or muscle concentration equivalents. The latter are due to the recognition by EPA that it is

important to be able to compare EPA's proposed tissue-based values (whole body and muscle) to monitoring data for aquatic life collected as muscle (fillet or muscle plug) in fish or as whole-body tissue concentrations for fish or other aquatic life. Since the aquatic life criterion element will be reported as wet weight fish (muscle), it was necessary to also convert tissue concentrations reported in dry weight in toxicity tests to wet weight. Therefore, EPA collected available data for derivation of dry weight (ww) to wet weight (dw) and whole body to muscle conversion factors, as described in detail in **Appendix D**. EPA characterized the variability associated with the application of the weight measurement conversion factors applied in the derivation of mercury tissue criterion element for State of Idaho to the four most sensitive taxa.

4.5.2 Dry Weight to Wet Weight Conversion Factors

4.5.2.1 Southern leopard frog (Lithobates sphenocephalus) dry weight to wet weight conversion:

The LOEC of 0.2376 µg/g THg dw from Unrine et al. (2004) was divided by a factor of 7.26 to derive an SMCV for the species of 0.03272 µg/g THg ww which is used as the GMCV for *Lithobates*. The factor represents the grand average percent moisture value of 86.23% from three other anuran amphibian species: *Bufo arenarum* (88.93), *Rana temporaria* (86.25), and *Lithobates sylvaticus* (83.5). The 10^{th} and 90^{th} percentile average moisture content for these taxa (reported in Table D-5) ranges from 80.7 to 91.4, resulting in conversion factors ranging from 5.18 to 11.64 and corresponding alternate SMCVs that are within a factor of 1.6 of 0.03272 µg/g THg ww.

4.5.2.2 American toad (Anaxyrus americanus) dry weight to wet weight conversion:

The NOEC and LOEC of 0.800 and 1.800 µg THg/g dw, respectively from Bergeron et al. (2011a) were divided by 7.26 as described above and are equal to 0.1102 and 0.2479 µg/g THg ww, respectively. The geometric mean of these two values (0.1653 µg THg/g ww) represents the MATC and SMCV for the species. Using the same 10^{th} and 90^{th} percentile average

moisture for these taxa as above (i.e., 80.7 to 91.4, resulting in conversion factors 5.18 and 11.64, respectively), the corresponding alternate SMCVs thar are within the same factor (1.6) of 0.1653 µg THg/g ww.

4.5.2.3 Fathead minnow (Pimephales promelas) dry weight to wet weight conversion:

Only one of the three studies used to calculate the SMCV for the fathead minnow reported mercury tissue concentrations as dry weight: Hammerschmidt et al. (2002). An average percent moisture value (76.64%) for the species from close to 300 whole body samples (see in Table D-5) was used to convert the chronic value from the test to wet weight. The LOEC of 3.102 µg THg/g dw whole body tissue from the study was divided by a factor of 4.28 based on the average species-specific percent moisture value for *P. promelas* of 76.64 and is equal to 0.7246 µg THg/g ww. The 10^{th} and 90^{th} percentile average moisture content for several other fish in the family Cyprinidae (reported in Table D-5), ranges from 71.05 to 76.90, resulting in conversion factors ranging from 3.45 to 4.33 and corresponding alternate LOEC values are within a factor of 1.2 of 0.7246 µg THg/g ww.

4.5.2.4 Red swamp crayfish (Procambarus clarkia) dry weight to wet weight conversion:

The relationship between wet weight and dry weight of the red swamp crayfish was previously described in and Anastacio et al. (1999). Based on this relationship, percent muscle moisture decreases as crayfish grow. To translate the chronic tissue mercury value for red swamp crayfish from Brant (2004), the wet weight of crayfish that died during the test was estimated from figures in the publication. These weights were then translated to dry weight using the equation presented by Anastacio: Wet Weight = 5.28607 x Dry Weight$^{0.937422}$. The percent moisture of the deceased crayfish from Brant (2004) ranged from 80.55 to 81.51, with an average value of 80.77 (Table D 3). There was very little variation in the percent moisture for the crayfish despite the range in sizes of deceased organisms (~3.75 – 8 g ww). The average

abdominal muscle tissue Hg concentration of the deceased crayfish (7.757 µg THg/g dw) was divided by a factor of 5.20 and is equal to 1.492 µg THg/g ww abdominal muscle tissue. Changing the average moisture content of 80.77 of *P. clarkia* by plus or minus 10% results in conversion factors ranging from 3.66 to 8.97 and corresponding alternate LOEC values that are within a factor of 1.7 of 1.492 µg THg/g ww.

4.5.3 Whole-body:muscle (WB:M) conversion factors (CF) Factors

EPA also characterized the variability in tissue concentrations associated with application of conversion factors based on tissue type (i.e., whole-body:muscle (WB:M) conversion factor (CF)) for the species and tests identified above. The necessary information was provided in only a few toxicity studies for mercury and was determined to be too limited in scope to be useful. Therefore, EPA performed an additional literature search for other studies that could be used for deriving a WB:M CF for mercury, as described in **Appendix D.3**.

4.5.3.1 Amphibian whole-body:muscle (WB:M) CF:

For the two amphibians (Genus *Lithobates* and *Anaxyrus*), EPA conducted a literature search for information regarding paired whole body and muscle total mercury concentrations in amphibians, with emphasis on aquatic life stages and or fully aquatic amphibians, but no such information was found specific to these life stages. Instead, EPA relied on data from a field study (Hothem et al. 2009), which provided results of paired muscle (hind leg) and total body mercury in bullfrog tissues from Bear Creek in the Cache Creek Watershed, Northern California. The mean WB:M CF for a mix of 10 juvenile and adult bullfrogs of mixed gender was 0.97. Assuming a conservative 20% variation in this factor, the converted muscle SMCV for *Lithobates* and *Anaxyrus* could vary by a maximum factor of only 1.25.

4.5.3.2 Fish whole-body:muscle (WB:M) CF:

The fish WB:M CFs for mercury EPA gathered from the literature ranged from a low CF of 0.57 to up to 0.86 (see in **Table D-6**). Application of the minimum and maximum WB:M CFs to the NOEC of 0.2415 µg/g THg ww whole body tissue for *P. promelas*, as converted from LOEC of 3.102 µg/g THg dw whole body reported in Hammerschmidt et al. (2002), results in alternate LOEC values that could vary by a maximum factor of only 1.26.

4.5.3.3 Crayfish whole-body:muscle (WB:M) conversion factor (CF):

No studies were identified that could be used to determine a WB:M CF for the crayfish. Given the lack of data, the abdominal muscle concentrations for the crayfish were converted to whole body concentrations based on the 0.72 WB:M CF recommended for fish. EPA acknowledges the application of the fish WB:M CF to crayfish is uncertain.

The 1985 Guidelines recommend that variability should be less that ten-fold across toxicity tests from a given genus level taxon used for criterion derivation. Findings from previous interlaboratory and intra-laboratory acute toxicity test comparisons have demonstrated that a two- to five-fold range in LC_{50}s from water-only tests using the same species and chemical combinations and test conditions is expected (Mayer et al. 2008). The variability in chronic tissue values derived from dietary mercury exposure and converted to common values for the derivation of mercury criterion for Idaho are not inconsistent with these expectations.

4.5.4 Comparison of Paired and Unpaired Fish Sizes and Mercury Tissue Concentrations

The fish BAF database consists of spatially and temporally paired total mercury concentrations in fish tissue and water from a variety of sources (see **Section 3.6**). Many additional mercury tissue measurements are available; however, they could not be used to calculate BAFs because there were no available paired water mercury samples. In order to examine whether the fish mercury BAF dataset was representative of waters throughout Idaho,

the fish total mercury concentrations in the BAF dataset were compared to a larger dataset of fish total mercury concentrations from Idaho waters compiled for the Western North America mercury synthesis (WNAMS - Eagles-Smith et al. 2016), to see if total mercury tissue concentrations were similar. Because of the relationship between fish size and mercury tissue concentration, comparison of WNAMS data to the Idaho mercury aquatic life criterion (ALC) dataset is limited to those fish species that had tissue sample data with both a fish length and total mercury fish tissue measurement (mg/kg dw). All mercury measurements were expressed as muscle tissue. The majority of samples were measured in muscle, and the remaining whole-body samples were converted to muscle using a whole body to muscle conversion factor of 0.72.

Median values and ranges for fish length and size were calculated for all fish species within the two databases where both length and tissue concentration measurements were available. Median lengths and mercury concentrations were calculated following the procedure used to calculate fish BAFs in Section 3.6. In the BAF dataset, there were a total of 352 individual fish samples with length and tissue measurements, 111 unique "site-species-year" measurements, 93 unique "site-year" measurements, and 30 species measurements. In the WNAMS dataset, there were a total of 1,259 individual fish samples with length and tissue measurements, 331 unique "site-species-year" measurements, 252 unique "site-year" measurements, and 41 species measurements. As with the fish BAF calculations, brook trout and northern pikeminnow were subdivided into large and small size classes and are referred to as separate species here for convenience (see **Section 3.6**). Median lengths, mercury concentrations, and associated ranges for the two datasets are shown in **Table 4-4**.

Twenty-three fish species were represented by both datasets, seven species were in the ALC dataset but not the WNAMS dataset, and 18 were in the WNAMS dataset but not the ALC

dataset. Of the 23 taxa where both fish length and tissue total mercury concentrations were available from both data sources, the median tissue total mercury for 19 taxa were within a factor 2 of each other, and 12 differed by less than a factor 1.5. With only a few exceptions (e.g., channel catfish, crappie sp.), higher tissue total mercury concentration were associated with greater length. Overall, these data suggest that the tissue total mercury concentrations used for the calculation of BAFs for the Hg criterion are representative of Idaho. Additionally, both data sources produce values that conform to the expectation of increased tissue total mercury concentration with greater fish size.

Table 4-4. Comparison of medians and ranges of fish lengths and total mercury concentrations (THg in mg/kg dw) between samples used to calculate BAFs (ALC dataset) and samples in the Western North America mercury synthesis (WNAMS) database.
Comparisons limited to those fish samples with both a length and THg measurement. Numbers within parentheses represent counts of unique "site-species" measurements (medians) and total measurements (ranges). Blank cells indicate no measurements available for that species from the respective data source.

	Length (mm)		THg (mg/kg dw)	
Species	**Used in ALC**	**WNAMS**	**Used in ALC**	**WNAMS**
Banded killifish	54 (2) 52-57 (2)		0.07 (2) 0.066-0.075 (2)	
Black crappie	250 (1) 250-250 (1)	250 (9) 153-270 (29)	0.28 (1) 0.28-0.28 (1)	0.112 (9) 0.043-0.28 (29)
Bluegill	96 (2) 82-117 (3)		0.16 (2) 0.147-0.181 (3)	
Bonneville whitefish		374 (1) 270-491 (20)		0.043 (1) 0.021-0.087 (20)
Bridgelip sucker	235 (3) 44-550 (4)	495 (2) 440-550 (3)	0.086 (3) 0.04-0.234 (4)	0.16 (2) 0.079-0.234 (3)
Small Brook trout	250 (1) 250-250 (1)	233 (6) 165-250 (28)	0.064 (1) 0.064-0.064 (1)	0.082 (6) 0.026-0.577 (28)
Large Brook trout	415 (2) 400-430 (2)	360 (4) 290-430 (5)	0.164 (2) 0.153-0.174 (2)	0.144 (4) 0.013-0.174 (5)
Brown bullhead		260 (1) 260-260 (1)		0.065 (1) 0.065-0.065 (1)
Brown trout	405 (2) 360-450 (2)	393 (7) 165-582 (45)	0.153 (2) 0.052-0.253 (2)	0.161 (7) 0.035-1.2 (45)
Bull trout	188 (2) 143-218 (27)	390 (3) 240-406 (3)	0.065 (2) 0.023-0.2 (27)	0.117 (3) 0.039-0.163 (3)
Bullhead sp.		224 (3) 162-283 (99)		0.046 (3) 0.02-0.21 (99)
Catfish sp.		714 (1) 714-714 (2)		0.401 (1) 0.349-0.453 (2)
Channel catfish	604 (6) 310-720 (88)	381 (7) 180-710 (14)	0.247 (6) 0.06-0.738 (88)	0.21 (7) 0.079-0.81 (14)
Chiselmouth		180 (3) 140-222 (9)		0.087 (3) 0.031-0.29 (9)
Coho salmon		305 (1) 305-305 (1)		0.12 (1) 0.12-0.12 (1)
Common carp	590 (2) 570-610 (2)	447 (7) 138-710 (41)	0.195 (2) 0.138-0.252 (2)	0.185 (7) 0.01-0.561 (41)
Crappie sp.	214 (2) 183-244 (2)	322 (2) 309-335 (2)	0.209 (2) 0.203-0.214 (2)	0.094 (2) 0.016-0.172 (2)
Cutthroat trout	330 (8) 230-530 (12)	329 (17) 100-657 (62)	0.061 (8) 0.037-0.87 (12)	0.067 (17) 0.014-0.87 (62)

Species	Length (mm)		THg (mg/kg dw)	
	Used in ALC	WNAMS	Used in ALC	WNAMS
Cutthroat trout x Rainbow trout	460 (1) 460-460 (1)		0.24 (1) 0.24-0.24 (1)	
Flathead catfish	537 (1) 537-537 (1)		0.477 (1) 0.477-0.477 (1)	
Kokanee salmon	320 (1) 320-320 (1)	312 (8) 200-485 (9)	0.113 (1) 0.113-0.113 (1)	0.126 (8) 0.048-0.25 (9)
Lahontan cutthroat trout		325 (2) 300-350 (2)		0.411 (2) 0.319-0.502 (2)
Lake trout		642 (4) 425-880 (13)		0.321 (4) 0.037-0.723 (13)
Largemouth bass	500 (1) 500-500 (1)	381 (18) 260-500 (25)	0.572 (1) 0.572-0.572 (1)	0.273 (18) 0.132-0.586 (25)
Largescale sucker	473 (8) 257-550 (8)	411 (14) 108-550 (68)	0.194 (8) 0.083-0.489 (8)	0.22 (14) 0.014-0.839 (68)
Longnose sucker		420 (1) 420-420 (1)		0.147 (1) 0.147-0.147 (1)
Minnow sp.		59 (2) 51-76 (4)		0.452 (2) 0.16-0.84 (4)
Mountain whitefish	320 (12) 135-460 (80)	319 (16) 170-379 (25)	0.084 (12) 0.04-0.3 (80)	0.052 (16) 0.033-0.247 (25)
Northern pike		546 (3) 375-828 (61)		0.115 (3) 0.02-0.48 (61)
Small Northern pikeminnow	156 (2) 83-228 (2)	203 (5) 136-284 (13)	0.136 (2) 0.067-0.205 (2)	0.24 (5) 0.028-1.2 (13)
Large Northern pikeminnow	330 (1) 330-330 (1)	367 (2) 320-394 (9)	0.674 (1) 0.674-0.674 (1)	0.631 (2) 0.2-1.7 (9)
Peamouth		201 (2) 165-241 (4)		0.352 (2) 0.042-0.62 (4)
Perch sp.		90-211 (84)		0.02-0.23 (84)
Pumpkinseed	121 (2) 104-138 (2)		0.128 (2) 0.089-0.167 (2)	
Rainbow trout	355 (7) 250-510 (24)	320 (35) 120-550 (116)	0.132 (7) 0.02-0.48 (24)	0.08 (35) 0.014-0.652 (116)
Redband trout		172 (11) 51-381 (128)		0.393 (11) 0.04-2.4 (128)
Redside shiner		98 (2) 51-148 (12)		0.429 (2) 0.148-1.03 (12)
Sculpin sp.		51 (2) 51-51 (6)		0.816 (6) 0.181-1.431 (6)
Smallmouth bass	290 (15) 157-452 (75)	295 (12) 149-473 (49)	0.253 (15) 0.04-1.02 (75)	0.232 (12) 0.056-1.229 (49)
Sucker sp.	209 (1) 209-209 (1)	348 (11) 89-575 (49)	0.066 (1) 0.066-0.066 (1)	0.233 (11) 0.035-2.26 (49)

Species	Length (mm)		THg (mg/kg dw)	
	Used in ALC	WNAMS	Used in ALC	WNAMS
Sunapee trout		190 (1) 190-190 (1)		0.02 (1) 0.02-0.02 (1)
Utah chub		287 (1) 263-311 (2)		0.576 (1) 0.535-0.618 (2)
Utah sucker	410 (2) 380-440 (2)	412 (6) 138-486 (8)	0.112 (2) 0.032-0.192 (2)	0.063 (6) 0.032-0.192 (8)
Walleye	450 (1) 442-457 (2)	370 (3) 310-710 (15)	1.002 (1) 0.753-1.25 (2)	0.564 (3) 0.167-1.38 (15)
Warmouth	98 (1) 98-98 (1)		0.128 (1) 0.128-0.128 (1)	
Whitefish sp.		305 (6) 131-481 (15)		0.132 (6) 0.042-0.25 (15)
Yellow perch	228 (4) 207-264 (4)	221 (8) 42-305 (176)	0.225 (4) 0.108-0.587 (4)	0.229 (8) 0.01-0.9 (176)

5 REFERENCES

Agusa T, T. Kunito, S. Tanabe, M. Pourkazemi, and D.G. Aubrey. 2004. Concentration of trace elements in muscle of sturgeons in the Caspian Sea. Mar. Pollut. Bull. 49: 789-800.

Alvarez, M.C., C.A. Murphy, K.A. Rose, I.D. McCarthy and L.A. Fuiman. 2006. Maternal body burdens of methylmercury impair survival skills of offspring in Atlantic croaker (*Micropogonias undulatus*). Aquat. Toxicol. 80(4): 329–337.

Amlund, H., A.K. Lundebye, D. Boyle and S. Ellingsen. 2015. Dietary selenomethionine influences the accumulation and depuration of dietary methylmercury in zebrafish (*Danio rerio*). Aquat. Toxicol. 158: 211-217.

Amos, H.M., D.J. Jacob, D.G. Streets and E.M. Sunderland. 2013. Legacy impacts of all-time anthropogenic emissions on the global mercury cycle. Global Biogeochem. Cycles. 27: 410-421.

Anastacio, P.M., S.N. Nielsen and J.C. Marques. 1999. CRISP (crayfish and rice integrated system of production): 2. Modelling crayfish *(Procambarus clarkii*) population dynamics. Ecol. Model. 123: 5-16.

Angot, H., N. Hoffman, A. Giang, C.P. Thackray, A.N. Hendricks, N.R. Urban and N.E. Selin. 2018. Global and Local Impacts of Delayed Mercury Mitigation Efforts. Environ. Sci. Technol. 52(22): 12968-12977.

Arcagni, M., R. Juncos, A. Rizzo, M. Pavlin, V. Fajon, M.A. Arribere, M. Horvat and S. Ribeiro-Guevara. 2018. Species- and habitat-specific bioaccumulation of total mercury and methylmercury in the food web of a deep oligotrophic lake. Sci. Total. Environ. 612: 1311–1319.

Aschner, M., T. Syversen, D.O. Souza, J.B.T. Rocha and M. Farina. 2007. Involvement of glutamate and reactive oxygen species in methylmercury neurotoxicity. Braz. J. Med. Biol. Res. 40: 285-291.

Azim, M.E., A. Kumarappah, S.P. Bhavsar, S.M. Backus, and G. Arhonditsis. 2011. Detection of the spatiotemporal trends of Mercury in Lake Erie fish communities: A Bayesian approach. Environ. Sci. Technol. 45: 2217-2226.

Baldwin, A.K., B.A. Poulin, J. Naymik, C. Hoovestol, G.M. Clark and D.P. Krabbenhoft. 2020. Seasonal Dynamics and Interannual Variability in Mercury Concentrations and Loads through a Three-Reservoir Complex. Environ. Sci. Tech. 54: 9305-9314.

Barkay T. and B. Gu. 2021. Demethylation-the other side of the mercury methylation coin: A critical review. ACS Environ. Au. 2: 77-97

Bauch, N.J., L.C. Chasar, B.C. Scudder, P.W. Moran, K.J. Hitt, M.E. Brigham, M.A. Lutz, and D.A. Wentz. 2009. Data on mercury in water, bed sediment, and fish from streams across the United States, 1998-2005. United States Geological Survey Data Series 307, 33p.

Beasley, A., S.E. Belanger, J.L. Brill and R.R. Otter. 2015. Evaluation and comparison of the relationship between NOEC and EC10 or EC20 values in chronic *Daphnia* toxicity testing. Environ. Toxicol. Chem. 34(10): 2378-2384.

Beckvar, N., T.M. Dillon, and L.B. Read. Approaches for linking whole-body fish tissue residues of mercury or DDT to biological effects thresholds. Envrion. Toxicol. Chem. 24(8): 2094-2105.

Belz, R.G. and H.P. Piepho. 2015. Statistical modeling of the hormetic dose zone and the toxic potency completes the quantitative description of hormetic dose responses. Environ. Toxicol. Chem. 34(5): 1169–1177.

Bender, M.C., C. Hu, C. Pelletier and R.J. Denver. 2018. To eat or not to eat: Ontogeny of hypothalamic feeding controls and a role for leptin in modulating life-history transition in amphibian tadpoles. Proc. R. Soc. B 285: 20172784.

Benoit, J.M., C.C. Gilmour, A. Heyes, R.P. Mason and C.L. Miller. 2003. Geochemical and biological controls over methylmercury production and degradation in aquatic ecosystems. Am. Chem. Soc. Symp. Ser. 835: 262-297.

Berger, B.R. and H.F. Bonham, Jr. 1990. Epithermal gold-silver deposits in the western United States: time-space products of evolving plutonic, volcanic and tectonic environments. J. Geochem. Explor. 36: 103-142.

Bergeron, C.M., W.A. Hopkins, B.D. Todd, M.J. Hepner and J.M. Unrine. 2011a. Interactive effects of maternal and dietary mercury exposure have latent and lethal consequences for amphibian larvae. Environ. Sci. Tech. 45(8): 3781-3787.

Bergeron, C.M., W.A. Hopkins, C.M. Bodinof, S.A. Budischak, H. Wada and J.M. Unrine. 2011b. Counterbalancing effects of maternal mercury exposure during different stages of early ontogeny in American toads. Sci. Total Environ. 409: 4746-4752.

Berntssen, M.H.G., A. Aatland and R.D. Handy. 2003. Chronic dietary mercury exposure causes oxidative stress, brain lesions, and altered behaviour in Atlantic salmon (*Salmo salar*) parr. Aquat. Toxicol. 65(1): 55–72.

Berntssen, M., K. Hylland, K. Julshamn, A. Lundebye and R. Waagbø. 2004. Maximum limits of organic and inorganic mercury in fish feed. Aquaculture Nutrition. 10(2): 83-97. doi:10.1046/j.1365-2095.

Bevelhimer, M.S., J.J. Beauchamp, B.E. Sample and G.R. Southworth. 1997. Estimation of whole-fish contaminant concentrations from fish fillet data. ES/ER/TM-202. Prepared by the Risk Assessment Program, Oak Ridge National Laboratory, Oak Ridge, TN. 23 pp.

Bhavsar, S.P., S.B. Gewurtz, D.J. McGoldrick, M.J. Keir, and S.M. Backus. 2010. Changes in mercury levels in Great Lakes fish between 1970s and 2007. Environ. Sci. Technol. 44: 3273-3279.

Biesinger, K.E., L.E. Anderson and J.G. Eaton. 1982. Chronic effects of inorganic and organic mercury on *Daphnia magna*: Toxicity, accumulation, and loss. Arch. Environ. Contam. Toxicol. 11: 769-774.

Blanchfield, P.J., J.W.M. Rudd, L.E. Hrenchuk, M. Amyot, C.L. Babiarz, K.G. Beaty, R.A.D. Bodaly, B.A. Branfireun, C.C. Gilmour, J.A. Graydon, B.D. Hall, R.C. Harris, A. Heyes, H. Hintelmann, J.P. Hurley, C.A. Kelly, D.P. Krabbenhoft, S.E. Lindberg, R.P. Mason, M.J. Paterson, C.L. Podemski, K.A. Sandilands, G.R. Southworth, V.L. St Louis, L.S. Tate and M.T. Tate. 2022. Experimental evidence for recovery of mercury-contaminated fish populations. Nature. 601: 74-78.

Bloom, NS. 1992. On the chemical form of mercury in edible fish and marine invertebrate tissue. Can. J. Fish. Aquat. Sci. 49: 1010–1017.

Blukacz-Richards, E.A., A. Visha, M.L. Graham, D.I. McGoldrick, S.R. de Solla, D.J. Moore, and G.B. Arhonditsis. 2017. Mercury levels in herring gulls and fish: 42 years of spatio-temporal trends in the Great Lakes. Chemosphere. 172: 476-487.

Boalt, E., H. Dahlgren and A. Miller. 2012. Cadmium, lead, and mercury concentrations in whole-fish, liver, and muscle of herring (*Clupea harengus*) and perch (*Perca fluviatilis*). Report nr 6:2012. Swedish Museum of Natural History, Department of Contaminant Research, Stockholm, Sweden. 11 pp.

Boening, D.W. 2000. Ecological effects, transport, and fate of mercury: A general review. Chemosphere. 40: 1335-1351.

Boudou, A., and F. Ribeyre. 1985. Experimental study of trophic contamination of *Salmo gairdneri* by two mercury compounds — HgCl2 and CH3HgCl — analysis at the organism and organ levels. Water Air Soil Pollut. 26: 137–148.

Bradford, D.F. 1984. Water and osmotic balance in overwintering tadpoles and frogs, *Rana muscosa*. Physiol. Zool. 57(4): 474-480.

Bradford, D.F., J.L. Kramer, S.L. Gerstenberger, N.G. Tallent-Halsell and M.S. Nash 2012. Mercury in tadpoles collected from remote alpine sites in the Southern Sierra Nevada Mountains, California, USA. Arch. Environ. Contam. Toxicol. 62: 135-140.

Bradley, M.A., B.D. Barst, and N. Basu. 2017. A Review of Mercury Bioavailability in Humans and Fish. Int. J. Environ. Res. Public Health. 14(2): 169-189.

Bradley, P.M., D.A. Burns, J.W. Harvey, C.A. Journey, M.E. Brigham, and K. Riva-Murray. 2016. Hydraulic and biochemical gradients limit wetland mercury supply to an Adirondack stream. SDJ Aq. Res. 1(1): 1-9.

Branfireun, B.A., C. Cosio, A.J. Poulain, G. Riise and A.G. Bravo. 2020. Mercury cycling in freshwater systems – An updated conceptual model. Sci. Total Environ. 745: 1-12.

Brant, H.A, 2004. Chronic dietary methylmercury exposure on three juvenile life stages of the crayfish *Procambarus clarkii.* University of Georgia, MS Thesis, under direction of C. Jagoe.

Bridges, C.C. and R.K. Zalups. 2010. Transport of inorganic mercury and methylmercury in target tissues and organs. J. Toxicol. Environ. Health, Part B. 13: 385-410.

Brown D.D. and L. Cai. 2007. Amphibian metamorphosis. Dev. Biol. 306(1): 20-33.

Brown, D.R. and J.B. Rasmussen. 2009. Shift in the trophic ecology of brook trout resulting from interactions with yellow perch: An interguild predator-prey interaction. Trans. Amer. Fish. Soc. 138: 1109-1122.

Brown, P.S., M.J. Murphy and S.C. Brown. 1989. Effects of prolactin on water balance and kidney function in bullfrog tadpoles. Gen. Comp. Endocrinol. 75(3): 389-396.

Brown, S.C., E.A. Horgan, L.M. Savage and P.S. Brown. 1986. Changes in body water and plasma constituents during bullfrog development: Effects of temperature and hormones. J. Exper. Zool. 237(1): 25-33.

Budy, P., R. Al-Chokhachy, and G.P. Thiede. 2004. Bull trout population assessment and life-history characteristics in association with habitat quality and land use: a template for recovery planning. Annual Progress Report for 2003. 55p.

Burger, J. and J. Snodgrass. 1998. Heavy metals in bullfrog (*Rana catesbeiana*) tadpoles: Effects of depuration before analysis. Environ. Toxicol. Chem. 17(11): 2203-2209.

Burgess, N.M. and M.W. Meyer. 2008. Methylmercury exposure associated with reduced productivity in common loons. Ecotoxicol. 17: 83-91.

Burke, J.N., C.M. Bergeron, B.D. Todd and W.A. Hopkins. 2010. Effects of mercury on behavior and performance of northern two-lined salamanders (*Eurycea bislineata*). Environ. Pollut. 158: 3546–3551.

Burkhard. L.P. 2021. Evaluation of published Bioconcentration Factor (BCF) and Bioaccumulation Factor (BAF) data for per- and polyfluoroalkyl substances across aquatic species. Environ. Toxicol. Chem. 40(6): 1530-1543.

Burkhard, L.P., B.R. Sheedy, D.J. McCauley and G.M. DeGraeve. 1997. Bioaccumulation factors for chlorinated benzenes, chlorinated butadienes and hexachloroethane. Environ. Toxicol. Chem. 16(8): 1677-1686.

Buss, N., L. Swierk and J. Hua. 2021. Amphibian breeding phenology influences offspring size and response to a common wetland contaminant. Front. Zool. 18:31.

Calabrese, E.J. 2008. Hormesis: Why it is important to toxicology and toxicologists. Environ. Toxicol. Chem. 27(7): 1451-1474.

Cambier, S., G. Bénard, N. Mesmer-Dudons, P. Gonzalez, R. Rossigno, D. Brèthes and J.P. Bourdineaud. 2009. At environmental doses, dietary methylmercury inhibits mitochondrial energy metabolism in skeletal muscles of the zebrafish (*Danio rerio*). Int. J. Biochem. Cell Biol. 41: 791–799.

Cambier, S., P. Gonzalez, G. Durrieu, R. Maury-Brachet, A. Boudou and J.P. Bourdineaud. 2010. Serial analysis of gene expression in the skeletal muscle fibres of zebrafish fed with a methylmercury contaminated diet. Environ. Sci. Technol. 44: 469–475.

Cambier, S., P. Gonzalez, N. Mesmer-Dudons, D. Brèthes, M. Fujimura, and J.P. Bourdineaud. 2012. Effects of dietary methylmercury on the zebrafish brain: histological, mitochondrial, and gene transcription analyses. Biometals. 25(1): 165-180.

CCME (Canadian Council of Ministers of the Environment). 2007. A protocol for the derivation of water quality guidelines for the protection of aquatic life 2007. In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment, 1999, Winnipeg.

Cedergreen, N., C. Ritz and J.C. Streibig. 2005. Improved empirical models describing hormesis. Environ. Toxicol. Chem. 24(12): 3166–3172.

Clarke, R.G., S.J. Klapstein, N.K. Hillier and N.J. O'Driscoll. 2022. Methylmercury in caddisflies and mayflies: Influences of water and sediment chemistry. Chemosphere. 286(3): 131785.

Clayden, M.G., K.A. Kidd, B. Wyn, J.L. Kirk, D.C. Muir and N.J. O'Driscoll. 2013. Mercury biomagnification through food webs is affected by physical and chemical characteristics of lakes. Environ. Sci. Technol. 47: 12047-12053.

Cleckner, L.B., P.J. Garrison, J.P. Hurley, M.L. Olson and D.P. Krabbenhoft. 1998. Trophic transfer of methyl mercury in the northern Florida Everglades. Biogeochem. 40: 347-361.

Compeau, G.C. and R. Bartha. 1985. Sulfate-reducing bacteria: principal methylators of mercury in anoxic estuarine sediment. Appl. Environ. Microbiol. 50(2):498-502.

Cope, W.G. and R.G. Rada. 1992. Accumulation of mercury by aufwuchs in Wisconsin seepage lakes: Implications for monitoring. Arch. Environ. Contam. Toxicol. 23: 172-178.

Costa, J.R.M.A., M. Mela, H.C. da Silva de Assis, E. Pelletier, M.A.F. Randi and C.A. Oliveira Ribeiro. 2007. Enzymatic inhibition and morphological changes in *Hoplias malabaricus* from dietary exposure to lead(II) or methylmercury. Ecotoxicol. Environ. Saf. 67: 82-88.

Crump, K. 2008. The effects of methylmercury on the reproductive axis of goldfish (*Carassius auratus*). M.S. Thesis, University of Ottawa, Canada. 117 pp.

Crump, K.L. and V.L. Trudeau. 2009. Mercury-induced reproductive impairment in fish. Environ. Toxicol. Chem. 28(5): 895-907.

Deng, D.F., F.C. Teh and S.J. Teh. 2008. Effect of dietary methylmercury and seleno-methionine on Sacramento splittail larvae. Sci. Total Environ. 407(1): 197-203.

Depew, D.C., N. Basu, N.M. Burgess, L.M. Campbell, E.W. Devlin, P.E. Drevnick, C.R. Hammerschmidt, C.A. Murphy, M.B. Sandheinrich, and J.G. Wiener. 2012. Toxicity of dietary methylmercury to fish: Derivation of ecologically meaningful threshold concentrations. Environ. Tox. Chem. 31(7): 1536-1547.

Dranguet P., R. Flück, N. Regier, C. Cosio, S. Le Faucheur, and V.I. Slaveykova. 2014. Towards Mechanistic Understanding of Mercury Availability and Toxicity to Aquatic Primary Producers. Chimia (Aarau). 68(11): 799-805.

Drevnick, P.E. and M.B. Sandheinrich. 2003. Effects of dietary methylmercury on reproductive endocrinology of fathead minnows. Environ. Sci. Technol. 3-7(19): 4390-4396.

Drevnick, P.E., M.B. Sandheinrich and J.T. Oris. 2006. Increased ovarian follicular apoptosis in fathead minnows (*Pimephales promelas*) exposed to dietary methylmercury. Aquat. Toxicol. 79: 49-54.

Driscoll, C.T., R.P. Mason, H.M. Chan, D.J. Jacob, and N. Pirrone. 2013. Mercury as a global pollutant: sources, pathways, and effects. Environ Sci Technol. 47(10): 4967-4983.

Eagles-Smith, C.A., J.T. Ackerman, J.J. Willacker, M.T. Tate, M.A. Lutz, J.A. Fleck, A.R. Stewart, J.G. Wiener, D.C. Evers, J.M. Lepak, J.A. Davis and C. F. Pritz. 2016. Spatial and temporal patterns of mercury concentrations in freshwater fish across the Western United States and Canada. Sci. Total Environ. 568: 1171-1184.

Eagles-Smith, C.A., E.K. Silbergeld, N. Basu, P. Bustamante, F. Diaz-Barriga, W.A. Hopkins, and J.F. Nyland. 2018. Modulators of mercury risk to wildlife and humans in the context of rapid global change. Ambio. 47: 170-197.

Eckley, C.S., M. Gustin, F. Marsik and M.B. Miller. 2011a. Measurement of surface mercury fluxes at active industrial gold mines in Nevada (USA). Sci. Total Environ. 409: 514-522.

Eckley, C.S., M. Gustin, M.B. Miller and F. Marsik. 2011b. Scaling non-point-source mercury emissions from two active industrial gold mines: Influential variables and annual emission estimates. Environ. Sci. Technol. 45: 392-399.

Eckley, C.S., T.P. Luxton, J.L. McKernan, J. Goetz, and J. Goulet. 2015. Influence of reservoir water level fluctuations on sediment methylmercury concentrations downstream of the historical Black Butte mercury mine, OR. App. Geochem. 61: 284–293.

Eckley, C.S, C. Eagles-Smith, M.T. Tate, B. Kowalski, R. Danehy, S.L. Johnson, and D.P. Krabbenhoft. 2018. Stream mercury export in response to contemporary timber harvesting methods (pacific coastal mountains, Oregon, USA). Environ. Sci. Technol. 52: 1971-1980.

Eckley, C.S., C.C. Gilmour, S. Janssen, T.P. Luxton, P.M. Randall, L. Whalin and C. Austin. 2020. The assessment and remediation of mercury contaminated sites: A review of current approaches. Sci. Total Environ. 707: 136031.

Eckley, C.S., T.P. Luxton, B. Stanfield, A. Baldwin, J. Holloway, J. McKernan and M.G. Johnson. 2021. Effect of organic matter concentration and characteristics on mercury mobilization and methylmercury production at an abandoned mine site. Environ. Pollut. 271: 116369.

Ecology and Environmental, Inc. 2018. Cinnabar Mine site. 2018 Removal site evaluation summary report. Yellow Pine, Idaho. Prepared for USEPA. Contract Number EP-S7-13-07. Seattle, WA.

Eddy, L.J. 1979. Prolactin action on extracellular fluid volume in tails of stage XII bullfrog tadpoles. Gen. Comp. Endocrinol. 37(3): 369-373.

Egea-Serrano, A., R.A. Relyea, M. Tejedo, and M. Torralva. 2012. Understanding of the impact of chemicals on amphibians: A meta-analytic review. Ecol. Evol. 2(7): 1382–1397.

Eikenberry, B.C.S., K. Riva-Murray, C.D. Knightes, C.A. Journey, L.C. Chasar, M.E. Brigham, and P.M. Bradley. 2015. Optimizing fish sampling for fish–mercury bioaccumulation factors. Chemosphere 135: 467-473.

Elissen, H.J.H., W.J. Mulder, T.L.G. Hendrickx, H.W. Elbersen, B. Beelen, H. Temmink and C.J.N. Buisman. 2010. Aquatic worms grown on biosolids: Biomass composition and potential applications. Biores. Technol. 101: 804-811.

Engle, M.A., M. S. Gustin, F. Goff, D. A. Counce, C. J. Janik, D. Bergfeld, and J. J. Rytuba, 2006. Atmospheric mercury emissions from substrates and fumaroles associated with three hydrothermal systems in the western United States. J. of Geophys. Res.: Atmospheres. 111(17): 1-16.

Essig, D.A. 2010. Arsenic, mercury, and selenium in fish tissue and water from Idaho's major rivers: A statewide assessment: Boise, Idaho, Idaho Department of Environmental Quality, 64 pp. plus appendixes. Available online at: http://www.deq.idaho.gov/media/639752-arsenic_mercury_fish_tissue_report_0310.pdf.

Etkin, W. 1932. Growth and resorption phenomena in anuran metamorphosis. Physiol. Zool. 5: 275-300.

Evers, D.C. and T.A. Clair. 2005. Mercury in northeastern North America: A synthesis of existing databases. Ecotoxicol. 14: 7-14.

Evers, D.C., N.M. Burgess, L. Champoux, B. Hoskins, A. Major, W.M. Goodale, R.J. Taylor, R. Poppenga and T. Daigle. 2005. Patterns and interpretation of mercury exposure in freshwater avian communities in northeastern North America. Ecotoxicol. 14: 193-221.

Faccio, S.D., K.L. Buckman, J.D. Lloyd, A.N. Curtis and V.F. Taylor. 2019. Bioaccumulation of methylmercury in wood frogs and spotted salamanders in Vermont vernal pools. Ecotoxicol. (7): 717-731

Farrell, M.P. and J.A. MacMahon. 1969. An eco-physiological study of water economy in eight species of tree frogs (Hylidae). Herpetolo. 25(4): 279-294.

Ferrari, L., R.J. Lombardo and A.S. Alibi. 1995. Quantitative evaluation of water balance in *Bufo arenarum* young tadpoles after acute exposure to D-mannitol solutions: a multivariate approach. Biol. Res. 28: 251-259.

Fitzgerald, W.F., R.P. Mason and G.M. Vandal. 1991. Atmospheric cycling and air-water exchange of mercury over mid-continental lacustrine regions. Water, Air, Soil Pollut. 56: 745-767.

Fjeld, E., T.O. Haugen, and L.A. Vøllestad. 1998. Permanent impairment in the feeding behavior of grayling *Thymallus thymallus* exposed to methylmercury during embryogenesis. Sci. Total Environ. 213: 247-254.

Fox, D.R. 2009. Is the ECx a legitimate surrogate for a NOEC? Integr. Environ. Assess. Manag. 5: 351– 353.

Fleck, J.A., M. Marvin-DiPasquale, C.A. Eagles-Smith, J.T. Ackerman, M.A. Lutz, M. Tate, C.N. Alpers, B.D. Hall, D.P. Krabbenhoft, and C.S. Eckley. 2016. Mercury and methylmercury in aquatic sediment across western North America. Sci Total Environ. 568: 727-738.

Fleming, E., E.E. Mack, P.G. Green and D.C. Nelson. 2006. Mercury methylation from unexpected sources: Molybdate-inhibited freshwater sediments and an iron-reducing bacterium. Appl. Environ. Micobiol. 72(1): 457-464.

Fletcher, K. and N.B. Myant. 1959. Oxygen consumption of tadpoles during metamorphosis. J. Physiol. 145: 353-368.

Fretham, S.J., S. Caito, E.J. Martinez-Finley and M. Aschner. 2012. Mechanisms and modifiers of methylmercury-induced neurotoxicity. Toxicol. Res. 1(1): 32-38.

Friedmann, A.S., M.C. Watzin, T. Brinck-Johnsen and J.C. Leiter. 1996. Low levels of dietary methylmercury inhibit growth and gonadal development in juvenile walleye (*Stizostedion vitreum*). Aquat. Toxicol. 35: 265-278.

Galarowicz, T.L. J.A. Adams, and D.H. Wahl. 2006. The influence of prey availability on ontogenetic diet shifts of a juvenile piscivore. Canadian Journal of Fisheries and Aquatic Sciences. 63(8): 1722-1733.

Gandhi, N., R.W. Tang, S.P. Bhavsar, and G.B. Arhonditsis. 2014. Fish mercury levels appear to be increasing lately: A report from 40 years of monitoring in the province of Ontario, Canada. Environ. Sci. Technol. 48(10): 5404–5414.

GEI Consultants. 2013. Proposal for resegmentation and site-specific selenium water quality criteria for Sand Creek segment 16a – Updated January 2013. Technical memorandum. Exhibit B. 4601 DTC Blvd. Suite 900. Denver, CO 80237. 63 pp.

GEI Consultants. 2014. Review of EPA 2014 draft Se criteria document EPA 822-P-14-001. GEI Consultants, Inc., Ecological Division. Denver, CO. June 13, 2014. Appendix A supplemental data and supporting unpublished spreadsheet.

Gharaei A, A. Esmaili-Sari, V. Jafari-shamoshaki and M. Ghaffari. 2008. Beluga (*Huso huso* Brandet 1869) bioenergetics under dietary methylmercury. Fish Phyiol. Biochem. 34: 473–482.

Gharaei A, M. Ghaffari, S. Keyvanshokooh and R. Akrami. 2011. Changes in metabolic enzymes, cortisol and glucose concentrations of beluga (*Huso huso*) exposed to dietary methylmercury. Fish Physiol. Biochem. 37: 485–493.

Gillis, J.E. 1979. Adaptive differences in the water economies of two species of leopard frogs from Eastern Colorado (Amphibia, Anura, Ranidae). J. Herpetol. 13(4): 445-450.

Gilmour. C.C., E.A. Henry, R. Mitchell. 1992. Sulfate stimulation of mercury methylation in freshwater sediments. Environ. Sci. Techn. 26(11): 2281-2287.

Goldstein, R.M., M.E. Brigham and J.C. Stauffer. 1996. Comparison of mercury concentrations in liver, muscle, whole bodies, and composites of fish from the Red River of the North. Can. J. Fish. Aquat. Sci. 53: 244-252.

Gonzalez P, Y. Dominique, J.C. Massabuau , A. Boudou and J.P. Boourdineaud. 2005. Comparative effects of dietary methylmercury on gene expression in liver, skeletal muscle, and brain of the zebrafish (*Danio rerio*). Environ. Sci. Technol. 39: 3972–3980.

Graeb, B.D.S., T. Galarowicz, D.H. Wahl, J.M. Dettmers, and M.J. Simpson. 2005. Foraging behavior, morphology, and life history variation determine the ontogeny of piscivory in two closely related predators Can. J. Fish. Aquat. Sci. 62: 2010–2020.

Gray, J.E. and M.E. Hines. 2009. Biogeochemical mercury methylation influenced by reservoir eutrophication, Salmon Falls Creek Reservoir, Idaho, USA. Chem. Geo. 258: 157-167.

Grieb, T.M., N.S. Fisher, R. Karimi and L. Levin. 2020. An assessment of temporal trends in mercury concentrations in fish. Ecotoxicol. 29: 1739–1749.

Greenfield, B.K., T.R. Hrabik, C.J. Harvey, and S.R. Carpenter, 2001. Predicting mercury levels in yellow perch: use of water chemistry, trophic ecology, and spatial traits. Can. J. Fish. Aquat. Sci. 58: 1419–1429.

Guy, C.S., T.E. MaMahon, C.J. Smith, B.S. Cox, W.A. Fredenberg, D.W. Garfield. 2011. Diet overlap of top-level predators in recent sympatry: Bull trout and nonnative lake trout. J. Fish Wild. Manage. 2(2): 183-189.

Gustafson, J. 1987. Mining districts of the state of Idaho, redrawn version: Idaho Geological Survey Map 6, scale 1:1,000,000.

Gworek, B., W. Dmuchowski, and A.H. Baczewska-Dąbrowska. 2020. Mercury in the terrestrial environment: a review. Environ. Sci. Eur. 32: 128-147.

Hall, B.D., R.A. Bodaly, R.J.P. Fudge, J.W.M. Rudd and D.M. Rosenberg. 1997. Food as the dominant pathway of methylmercury uptake by fish. Water Air Soil Pollut. 100(1-2): 13-24.

Hammerschmidt, C.A., M.B. Sandheinrich, J.C. Weiner and R.C. Rada. 2002. Effects of dietary methylmercury on reproduction of fathead minnows. Environ. Sci. Technol. 36: 877-883.

Hansen, J.A., J. Lipton, P.G. Welsh, D. Cacela and B. MacConnell. 2004. Reduced growth of rainbow trout (*Oncorhynchus mykiss*) fed a live invertebrate diet pre-exposed to metal-contaminated sediments. Environ. Toxicol. Chem. 23(8): 1902-1911.

Harris, H.H., I.J. Pickering, and G.N. George. 2003. The chemical form of mercury in fish. Science. 301(5637): 1203.

Harris, R.C., J.W.M. Rudd, M. Amyot, C.L. Babiarz, K.G. Beaty, P.J. Blanchfield, R.A. Bodaly, B.A. Branfireun, C.C. Gilmour, J.A. Graydon, A. Heyes, H. Hintelmann, J.P. Hurley, C.A. Kelly, D.P. Krabbenhoft, S.E. Lindberg, R.P. Mason, M.J. Paterson, C.L. Podemski, A. Robinson, K.A. Sandilands, G.R. Southworth, V.L. St. Louis and M.T. Tate. 2007. Whole-ecosystem study shows rapid fish-mercury response to changes in mercury deposition. Proc. Natl. Acad. Sci. USA. 104(42): 16586-16591.

Hernández-Flores, S., G.E. Santos-Medrano, I. Rubio-Franchini and R. Rico-Martínez. 2020. Evaluation of bioconcentration and toxicity of five metals in the freshwater rotifer *Euchlanis dilatata* Ehrenberg, 1832. Environ. Sci. Pollut. Res. 27: 14058–14069.

Hill, W.R., A.J. Stewart and G.E. Napolitano. 1996. Mercury speciation and bioaccumulation in lotic primary producers and primary consumers. Can. J. Fish Aquat. Sci. 53: 812-819.

Hocking, D.J. and J.K. Babbitt. 2014. Amphibian contributions to ecosystem services. Herpetolog. Conserv. Biol. 9(1): 1−17.

Hothem, R.L., D.R. Bergen, M.L. Bauer, J.J. Crayon and A.M. Meckstroth. 2007. Mercury and trace elements in crayfish from Northern California. Bull Environ. Contam. Toxicol. 79: 628–632.

Hothem, R.L., M.R. Jennings and J.J. Crayon. 2009. Mercury contamination in three species of anuran amphibians from the Cache Creek Watershed, California, USA. Environ. Monit. Assess.163: 433-448.

Houck A. and J. Cech. 2004. Effects of dietary methylmercury on juvenile Sacramento blackfish bioenergetics. Aquat. Toxicol. 69: 107–123.

Hsu-Kim, H., K.H. Kucharzyk, T. Zhang, and M.A. Deshusses. 2013. Mechanisms regulating mercury bioavailability for methylating microorganisms in the aquatic environment: A critical review. Environ. Sci. Technol. 47: 2441-2456.

Hsu-Kim, H., C.S. Eckley, D. Achá, X. Feng, C.C. Gilmour, S. Jonsson and C.P.J. Mitchell. 2018. Challenges and opportunities for managing aquatic mercury pollution in altered landscapes. Ambio 47: 141-169.

Hutcheson, M.S., C. Mark-Smith, J. Rose, C. Batdorf, O. Pancorbo, C. Rowan-West, J. Strube, and C. Francis. 2014. Temporal and Spatial Trends in Freshwater Fish Tissue Mercury Concentrations Associated with Mercury Emissions Reductions. Environ. Sci. Technol. 48(4): 2193-2202.

IDEQ (Idaho Department of Environmental Quality). 2005. Implementation Guidance for the Idaho Mercury Water Quality Criteria.

IDEQ (Idaho Department of Environmental Quality). 2007a. Orofino Creek Mercury Monitoring Project. February 2007. Lewiston Regional Office, Lewiston, ID. 7 pp.

IDEQ (Idaho Department of Environmental Quality). 2007b. Salmon Falls Creek subbasin assessment and total maximum daily loads.

IDEQ (Idaho Department of Environmental Quality). 2007c. Upper Portneuf River fish tissue and water column mercury sampling results. Boise, ID. 9 pp.

IDEQ (Idaho Department of Environmental Quality). 2009. Jordan Creek subbasin assessment and total maximum daily loads. State Office Surface Water Program. Boise, ID.

Inza, B., F. Ribeyre, R. Maury-Brachet and A. Boudou. 1997. Tissue distribution of inorganic mercury, methylmercury and cadmium in the Asiatic clam (*Corbicula fluminea*) in relation to the contamination levels of the water column and sediment. Chemosphere. 35(12): 2817-2836.

Iwasaki, Y., K. Kotani, S. Kashiwada and S. Masunaga. 2015. Does the choice of NOEC or EC10 affect the hazardous concentration for 5% of the species? Environ. Sci. Technol. 49(15): 9326-9330.

Jackson, A.K., D.C. Evers, S.B. Folsom, A.M. Condon, J. Diener, L.F. Goodrick, A.J. McGann, J. Schmerfed and D.A. Cristol. 2011. Mercury exposure in terrestrial birds far downstream of an historical point source. Environ. Pollut. 159: 3302-3308.

Jager, T., E.H.W. Heugens, and S.A.L.M. Kooijman. 2006. Making sense of ecotoxicological test results: Towards application of process-based models. Ecotoxicol. 15: 305– 314.

Jardine, T.D., K. A. Kidd, and N. O' Driscoll. 2013. Food web analysis reveals effects of pH on mercury bioaccumulation at multiple trophic levels in streams. Aquat. Tox. 132–133: 46-52.

Jensen, J.B. and C.D. Camp. 2003. Human exploitation of amphibians: direct and indirect impacts. Pgs. 199-213 in R. D. Semlitsch, editor. Amphibian Conservation. Smithsonian Institution, Washington.

Johnson. B.M., J.M. Lepak, and B.A. Wolff. 2015. Effects of prey assemblage on mercury bioaccumulation in a piscivorous sport fish. Sci. Tot. Environ. 506–507: 330-337.

Karimi, R., C.Y. Chen, P.C. Pickhardt, N.S. Fisher and C.L. Folt. 2007. Stoichiometric controls of mercury dilution by growth. Proc. Natl. Acad. Sci. USA. 104(18): 7477-7782.

Kerin, E.J., C.C. Gilmour, E. Roden, M.T. Suzuki, J.D. Coates and R.P. Mason. 2006. Mercury methylation by dissimilatory iron-reducing bacteria. Appl. Environ. Microbiol. 72(12): 7917-7921.

Kidd, K. and K. Batchelar. 2012. Homeostasis and toxicology of non-essential metals – Mercury. Fish. Phys. 31B: 237-295.

Klaper, R., C.B. Rees, P. Drevnick, D. Weber, M. Sandheinrich and M.J. Carvan. 2006. Gene expression changes related to endocrine function and decline in reproduction in fathead minnow (*Pimephales promelas*) after dietary methylmercury exposure. Environ. Health Perspect. 114(9): 1337-1343.

Klaper, R., B.J. Carter, C.A. Richter, P.E. Drevnick, M.B. Sandheinrich and D.E. Tillitt. 2008. Use of a 15k gene microarray to determine gene expression changes in response to acute and chronic methylmercury exposure in the fathead minnow *Pimephales promelas* Rafinesque. J. Fish Biol. 72(9): 2207-2280.

Kooijman, S.A.L.M. 2006. An alternative for NOEC exists, but the standard model has to be abandoned first. Oikos. 75: 310–316.

Kuwabara, J.S., Y. Arai, B.R. Topping, I.J. Pickering and G.N. George. 2007. Mercury speciation in piscivorous fish from mining-impacted reservoirs. Environ. Sci. Technol. 41: 2745-2749.

Larose, C., R. Canuel, M. Lucotte and R.T. Di Giulio. 2008. Toxicological effects of methylmercury on walleye (*Sander vitreus*) and perch (*Perca flavescens*) from lakes of the boreal forest. Comp. Biochem. Physiol. Part C. 147(2): 139-149.

Laskowski, R. 1995. Some good reasons to ban the use of NOEC, LOEC and related concepts in ecotoxicology. Oikos. 73: 140–144.

Latif, M.A., R.A. Bodaly, T.A. Johnston and R.J.P. Fudge. 2001. Effects of environmental and maternally derived methylmercury on the embryonic and larval stages of walleye (*Stizostedion vitreum*). Environ. Poll. 111: 139-148.

Lee, J-W., N.D. Riu, S. Lee, S.C. Bai, G. Moniello and S.S.O Hung. 2011. Effects of dietary methylmercury on growth performance and tissue burden in juvenile green (*Acipenser medirostris*) and white sturgeon (*A. transmontanus*). Aquat. Toxicol. 105: 227–234.

Lemes, M. and F. Wang. 2009. Methylmercury speciation in fish muscle by HPLC-ICP-MS following enzymatic hydrolysis. J. Anal. At. Spectrom. 24: 663–668.

Lerebours, A., S. Cambier, L. Hislop, C. Adam-Guillermin and J.P. Bourdineaud. 2013. Genotoxic effects of exposure to waterborne uranium, dietary methylmercury and hyperoxia in zebrafish assessed by the quantitative RAPD-PCR method. Mutat. Res. 755(1): 55-60.

Lescord, G.L., T.A. Johnston, B.A. Branfireun and J.M. Gunn. 2018. Percentage of methylmercury in the muscle tissue of freshwater fish varies with body size and age and among species. Environ. Toxicol. Chem. 37(10): 2682–2691.

Lewis, M.A., D.E. Weber, R.S. Stanley and J.C. Moore. 2001. Dredging impact on an urbanized Florida bayou: Effects on benthos and algal-periphyton. Environ. Pollut. 115: 161-171.

Lin, F., J. Lin., X. Liu, Y. Yuan, G. Liu and X. Ye. 2022. Effects of temperature on muscle growth and collagen deposition in zebrafish (*Danio rerio*). Aquacult. Reports. 22: 100952.

Liu, G., Y. Cai, N. O'Driscoll, X. Feng, and J. Guibin. 2012. Overview of Mercury in the Environment. Environ. Chem. Tox. Mercury. 568 pp.

Loftin, C.S., A.J.K. Calhoun, S. Nelson, A. Elskus and K. Simon. 2012. Does mercury bioaccumulate in wood frogs developing in seasonal woodland pools in Maine, USA? Northeastern Nat. 19: 579-600.

Lucotte, M. S. Paquet, and M. Moingt. 2016. Climate and Physiography Predict Mercury Concentrations in Game Fish Species in Quebec Lakes Better than Anthropogenic Disturbances Arch. Environ. Contam. Toxicol. 70: 710–723.

MacCoy, D.E. and C.A. Mebane. 2018. Mercury concentrations in water and mercury and selenium concentrations in fish from Brownlee Reservoir and selected sites in the Boise and Snake Rivers, Idaho and Oregon, 2013-17. Open-File Report, Reston, VA.

Malley, D.F., A.R. Stewart and B.D. Hall. 1996. Uptake of methyl mercury by the floater mussel, *Pyganodon grandis* (Bivalvia, Unionidae), caged in a flooded wetland. Environ. Toxicol. Chem. 15(6): 928-936.

Manar, R., H. Bessi and P. Vasseur. 2009. Reproductive effects and bioaccumulation of chlordane in *Daphnia magna*. Environ. Toxicol. Chem. 28(10): 2150-2159.

Martin, T., K. Tsui, and W-X. Wang. 2004. Maternal transfer efficiency and transgenerational toxicity of methylmercury in *Daphnia magna*. Environ. Toxicol. Chem. 23(6): 1504–1511.

Martins B.M., N.J. O'Driscoll, M.L. Mallory, and J. Canário.. 2021. A Review of Freshwater Invertebrates as Biomonitors of Methylmercury: the Importance of More Complete Physical and Chemical Reporting. Bull Environ Contam Toxicol. 107(5): 801-808.

Mason, R.P., J.R. Reinfelder and F.M.M. Morel. 1996. Uptake, toxicity and trophic transfer of mercury in coastal diatom. Environ. Sci. Technol. 30: 1835-1845.

Mason, R.P., J.M. Laporte and S. Andres 2000. Factors controlling the bioaccumulation of mercury, methylmercury, arsenic, selenium, and cadmium by freshwater invertebrates and fish. Arch. Environ. Contam. Toxicol. 38: 283–297.

Mathieu, C. and M. McCall. 2016. Measuring Mercury Trends in Freshwater Fish in Washington State; 2014 Sampling Results. Washington State Department of Ecology. Publication No. 16-03-033. https://apps.ecology.wa.gov/publications/documents/1603033.pdf.

Matida, Y., H. Kumada, S. Kimura, Y. Saiga, T. Nose, M. Yokote and H. Kawatsu. 1971. Toxicity of mercury compounds to aquatic organisms and accumulation of the compounds by the organisms. Bull. Freshwat. Fish Res. Lab. 21: 197–227.

Matta, M.B., J. Linse, C. Cairncross, L. Francendese and R.M. Kocan. 2001. Reproductive and transgenerational effects of methyl-mercury or Aroclor 1268 on *Fundulus heteroclitus*. Environ. Toxicol. Chem. 20: 327–335.

May, T.W. and W.G. Brumbaugh. 2007. Determination of total mercury in whole-body fish and fish muscle plugs collected from the South Fork of the Humboldt River, Nevada, September 2005: U.S. Geological Survey Open-File Report 2007–1059, 4 pp.

May, T.W., M.J. Walther, W.G. Brumbaugh and M. McKee. 2009. Concentrations of elements in whole-body fish, fish fillets, fish muscle plugs, and fish eggs from the 2008 Missouri Department of Conservation general contaminant monitoring program: U.S. Geological Survey Open-File Report 2009–1278, 11 pp.

Mayer, F.L., D.R. Buckler, F.J. Dwyer, M.R. Ellersieck, L.C. Sappington, J.M. Besser, and C.M. Bridges. 2008. Endangered aquatic vertebrates: Comparative and probabilistic-based toxicology. U.S. EPA Rept. No. EPA/600/R-08/045, Washington, DC.

Mcgee, B.N., D.L. Rutherford, M. Pribil. 2020. Hg Concentrations of Fish Tissue Samples in the Vicinity of Yellow Pine, Idaho. https://www.usgs.gov/data/hg-concentrations-fish-tissue-samples-vicinity-yellow-pine-idaho

MacCoy, D.E., and C.A. Mebane. 2018. Mercury concentrations in water and mercury and selenium concentrations in fish from Brownlee Reservoir and selected sites in the Boise and Snake Rivers, Idaho and Oregon, 2013-2017. U.S. Geological Society Open-File Report 2018-1122.

Mebane, C.A. and D.E. MacCoy. 2016. Monitoring plan for mercury in fish tissue and water from the Boise River, Snake River, and Brownlee Reservoir, Idaho and Oregon. USGS Open-File Report. pp. 2013–1068.

Mela, M., M.A.F. Randi, D.F. Ventura, C.E.V. Carvalho, E. Pelletier and C.A. Oliveira Ribeiro. 2007. Effects of dietary methylmercury on liver and kidney histology in the neotropical fish *Hoplias malabaricus*. Ecotoxicol. Environ. Saf. 68: 426–435.

Mieiro, C.L., M.E. Pereira, A.C. Duarte and M. Pacheco. 2011. Brain as a critical target of mercury in environmentally exposed fish (*Dicentrarchus labrax*) - Bioaccumulation and oxidative stress profiles. Aquat. Tox. 103(3-4): 233-240.

Mierzykowski, S.E. 2012. Contaminants in Atlantic sturgeon and shortnose sturgeon recovered from the Penobscot and Kennebec Rivers, Maine. USFWS. Spec. Proj. Rep. FY09-MEFO-3-EC. Maine Field Office. Orono, ME. 50 pp.

Monson, B.A. 2009. Trend reversal of mercury concentrations in piscivorous fish from Minnesota Lakes: 1982–2006. Environ. Sci. Technol. 43(6): 1750–1755.

Monson, B.A., D.F. Staples, S.P. Bhavsar, T.M. Holsen, C.S. Schrank, S.K. Moses, D.J. McGoldrick, S.M. Backus and K.A. Williams. 2011. Spatiotemporal trends of mercury in walleye and largemouth bass from the Laurentian Great Lakes region. Ecotoxicol. 20(7): 1555–1567.

Moore, D.R., R.S. Teed and G.M. Richardson. 2003. Derivation of an ambient water quality criterion for mercury: Taking account of site-specific conditions. Environ. Toxicol. Chem. 22(12): 3069-3080.

Morel, R.M., A.M.L. Kraepiel and M. Amyot. 1998. The chemical cycle and bioaccumulation of mercury. Annu. Rev. Syst. 29: 54-566.

Moye, H.A., C.J. Miles, E.J. Philips, B. Sargent and K.K. Merritt. 2002. Kinetics and uptake mechanisms for monomethylmercury between freshwater algae and water. Environ. Sci. Technol. 36: 3550-3555.

Mueller, K.W. and D.M. Serdar. 2002. Total Mercury Concentrations among Fish and Crayfish Inhabiting Different Trophic Levels in Lake Whatcom, Washington. Journal of Freshwater Ecology. 71(4): 621-633.

Murphy, C. 2014. Idaho's Wetland Program Plan: A plan for implementing the Idaho Wetland Conservation Strategy focused on Idaho Department of Fish and Game's wetland and riparian habitats. EPA Wetland Program Development Grant CD–00J49001-0. https://www.epa.gov/sites/default/files/2015-10/documents/idfg-wetland-program-plan-2015.pdf

Naimo, T.J., J.G. Wiener, W.G. Cope and N.S. Bloom. 2000. Bioavailability of sediment-associated mercury to *Hexagenia* mayflies in a contaminated floodplain river Can. J. Fish. Aquat. Sci. 57: 1092–1102.

NAS (National Academies of Sciences, Engineering, and Medicine). 2021. The use of systematic review in EPA's Toxic Substances Control Act risk evaluations. Washington, DC. The National Academies Press.

NHDES (New Hampshire Department of Environmental Services). 2018. Status and Trends of Mercury in Fish Tissue in New Hampshire Waterbodies, 1992–2016. Prepared by: Neils, D. and K. Nelson. Concord, NH. https://www.des.nh.gov/sites/g/files/ehbemt341/files/documents/2020-01/r-wd-17-22.pdf

Northwest Power and Conservation Council. 2004. Salmon and steelhead recovery and subbasin plan. Technical foundation volume III, other species. Prepared by: Lower Columbia Fish Recovery Board. May 28, 2004 draft.

Nriagu, J. and C. Becker. 2003. Volcanic emissions of mercury to the atmosphere: Global and regional inventories. Sci. Total Environ. 304: 3-12.

Nybroe, O., P. Rosenkilde and L. Ryttersgaard. 1985. Effects of hypophysectomy and substitution with growth hormone, prolactin, and thyroxine on growth and deposition in juvenile frogs, *Xenopus laevis*. Gen. Comp. Endocrinol. 51: 257-265.

OECD (Organisation for Economic Co-operation and Development). 2001. Guidance document on the use of the harmonised system for the classification of chemicals which are hazardous for the aquatic environment. OECD series on Testing and Assessment No. 27. ENV/JM/MONO(2001)8.

Oliveira Ribeiro, C.A., F. Filipak Neto, M. Mela, P.H. Silva, M.A.F. Randi, I.S. Rabitto, J.R.M. Alves Costa and E. Pelletier. 2006. Hematological findings in neotropical fish *Hoplias malabaricus* exposed to subchronic and dietary doses of methylmercury, inorganic lead, and tributyltin chloride. Environ. Res. 101(1): 74-80.

Oliviera-Ribeiro C.A.D., N. Mesmer-Dudons, P. Gonzalez, Y. Dominique, J.P. Bourdineaud, A. Boudou and J.C. Massabuau. 2008. Effects of dietary methylmercury on zebrafish skeletal muscle fibres. Environ. Toxicol. Pharmacol. 25: 304–309.

Paller, M.H. and J.W. Littrell. 2007. Long-term changes in mercury concentrations in fish from the middle Savannah River. Sci.Tot. Environ. 382: 375-382.

Peng, X., F. Liu, and W.X. Wang. 2016. Organ-specific accumulation, transportation, and elimination of methylmercury and inorganic mercury in a low Hg accumulating fish. Environ. Toxicol. Chem. 35(8): 2074–2083.

Penglase, S., K. Hamre and S. Ellingsen. 2014a. Selenium and mercury have a synergistic negative effect on fish reproduction. Aquat. Toxicol. 149: 16-24.

Penglase,S., K. Hamre, and S. Ellingsen. 2014b. Selenium Prevents Downregulation of Antioxidant Selenoprotein Genes by Methylmercury. Free Radic. Biol. Med.75:95-104.

Peterson, S.A., J. Van Sickle, R.M. Hughes, J.A. Schacher and S.F. Echols. 2005. A biopsy procedure for determining filet and predicting whole-fish mercury concentration. Arch. Environ. Contam. Toxicol. 48: 99-107.

Phillips, G.R. and D.R. Buhler. 1978. The relative contributions of methylmercury from food or water to rainbow trout (*Salmo gairdneri*) in a controlled laboratory environment. Trans. Am. Fish. Soc. 107: 853-861.

Pickhardt, P.C., C.L. Folt, C.Y. Chen, B. Klaue and J.D. Blum. 2002. Algal blooms reduce the uptake of toxic methylmercury in freshwater food webs. PNAS 99(7): 4419-4423.

Pickhardt, P.C. M. Stepanova and N.S. Fisher. 2006. Contrasting uptake routes and tissue distributions of inorganic and methylmercury in mosquitofish (*Gambusia affinis*) and redear sunfish (*Lepomis microlophus*). Environ. Toxicol. Chem. 25(8): 2132-2142.

Pickhardt, P.C. and N.S. Fisher. 2007. Accumulation of Inorganic and Methylmercury by Freshwater Phytoplankton in Two Contrasting Water Bodies. Environ. Sci. Technol. 41(1): 125–131.

Platt, J.E. and M.A. Christopher. 1977. Effects of prolactin on the water and sodium content of larval tissues from neotenic and metamorphosing *Ambystoma tigrinum*. Cert. Comp. Endocrinol. 31: 243-248.

Poe, T.P., H.C. Hansel, S. Vigg, D.E. Palmer, and L.A. Prendergast. 1991. Feeding of predaceous fishes on out-migrating juvenile salmonids in the John Day Reservoir, Columbia River. Trans. Am. Fish. Soc. 120: 405-420.

Poulin, B. A.; Breitmeyer, S. E.; Krabbenhoft, D. P.; Tate, M. T.; DeWild, J. F.; Ogorek, J. M.; Babiarz, C. L.; Janssen, S. E.; DiPasquale, M. C.; Agee, J. L.; Kakouros, E.; Kieu, L.; Arias, M.; Conaway, C.; Antweiler, R. C.; Baldwin, A. K.; Yoder, A.; Clark, G. M.; Aiken, G. R. 2020. Chemical Characterization of Water and Suspended Sediment of the Snake River and Hells Canyon Complex (Idaho, Oregon); U.S. Geological Survey Data Release. Link to most recent version of data release: Chemical characterization of water and suspended sediment of the Snake River and Hells Canyon Complex (Idaho, Oregon) (ver. 3.0, November 2023) | U.S. Geological Survey (usgs.gov)

Prati, M., R. Gornati, P. Boracchi, E. Biganzoli, S. Fortaner, R. Pietra, E. Sabbioni and G. Bernardini. 2002. A comparative study of the toxicity of mercury dichloride and methylmercury, assayed by the frog embryo teratogenesis assay–Xenopus (FETAX). Altern Lab Anim. 30: 23–32.

Pyle, D.M. and T.A. Mather. 2003. The importance of volcanic emissions for the global atmospheric mercury cycle. Atmospheric Environ. 37: 5115-5124.

Rak, A.E., S.N.A.M. Nasir, M.M. Nor, D.K. Han, S. Appalasamy, F. Abdullah and R.M. Ghazi. 2020. Proximate analysis and fatty acid of *Corbicula fluminea* (*C. fluminea*) tissue in Kelantan, Malaysia. Environ. Sci. Pollut. Res. 27: 24772-24785.

Richter, C.A., C.J. Martyniuk, M.L. Annis, W.G. Brumbaugh, L.C. Chasar, N.D. Denslow and D.E. Tillitt. 2014. Methylmercury-induced changes in gene transcription associated with neuroendocrine disruption in largemouth bass (*Micropterus salmoides*). General Comp. Endocrin. 203: 215–224.

Rimmer, C.C., E.K. Miller, K.P. McFarland, R.J. Taylor and S.D. Faccio. 2010. Mercury bioaccumulation and trophic transfer in the terrestrial food web of a montane forest. Ecotoxicol. 19: 697-709.

Riva-Murray, K., P. Bradley, B.C. Scudder Eikenberry, C.D. Knightes, C.A. Journey, M.E. Bringham, and D.T. Button. 2013. Optimizing Stream Water Mercury Sampling for Calculation of Fish Bioaccumulation Factors. Environ. Sci. Technol. 47: 5904−5912.

Rodgers, D.W. and F.W.H. Beamish. 1982. Dynamics of dietary methylmercury in rainbow trout, *Salmo gairdneri*. Aquat. Toxicol. 2: 271–290.

Rooney, A.A., A.L. Boyles, M.S. Wolfe, J.R. Bucher and K.A. Thayer. 2014. Systematic review and evidence integration for literature-based environmental health science assessments. Environ. Health Perspect. 122(7): 711-718.

Roos, D.H., R.L. Puntel, M.M. Santos, D.O.G. Souza, M. Farina, C.W. Nogueira, M. Aschner, M.E. Burger, N.B.V. Barbosa and J.B.T. Rocha. 2009. Guanosine and synthetic organoselenium compounds modulate methylmercury-induced oxidative stress in rat brain cortical slices: Involvement of oxidative stress and glutamatergic system. Toxicol. Vitro. 23(2): 302-307.

Rouleau, C., K. Borg-Neczak, J. Gottofrey, and H. Tjälve. 1999. Accumulation of waterborne mercury(II) in specific areas of fish brain. Environ. Sci. Technol. 33: 3384-3389.

Rutherford, DL, B.N. McGee and M. Pribil. 2020. Hg concentrations of fish tissue samples in the vicinity of Yellow Pine, Idaho: U.S. Geological Survey data release. Available online at: https://doi.org/10.5066/P9G2B6HF.

Sadraddini, S., M.E. Azim, Y. Shimoda, M. Mahmood, S.P. Bhavsar, S.M. Backus and G.B. Arhonditsis. 2011. Temporal PCB and mercury trends in Lake Erie fish communities: A dynamic linear modeling analysis. Ecotoxicol. Environ. Saf. 74(8): 2203–2214.

Saiki, M.K., B.A. Martin, L.D. Thompson and D. Welsh. 2001. Copper, cadmium, and zinc concentrations in juvenile chinook salmon and selected fish-forage organism (aquatic insects) in the Upper Sacramento River, California. Water Air Soil Pollut. 132: 127-139.

Sandheinrich, M.B. and K.M. Miller. 2006. Effects of Dietary Methylmercury on Reproductive Behavior of Fathead Minnows (*Pimephales promelas)*. Environ. Toxicol. Chem. 25(11): 3053-3057.

Santos, A.B, J.F. Bibiano Melo, P.R. Soares Lopes and M.B. Malgarim. 2001. Chemical composition and filet yield in traira (*Hoplias malabaricus*). Uruguaiana. 7/8(1): 140-150.

Sarazudin, M.S. 2019. Investigation of proximate composition in two different treatments of Asian clams (*Corbicula fluminea*) at selected district in Kelantan. Bachelor of Applied Science report, University Malaysia Kelantan.

Savari, S., A. Safahieh, B. Archangi, A. Savari and R. Abdi. 2020. The histopathological effect of methylmercury on the brain in orange spotted grouper (*Epinephelus coioides*) in Zangi Creek and laboratory. Iranian J. Fish. Sci. 19(1): 457-470.

Schartup, A.T., C.P. Thackray, A. Qureshi, C. Dassuncao, K. Gillespie, A. Hanke, and E.M. Sunderland. 2019. Climate change and overfishing increase neurotoxicant in marine predators. Nat. 572: 648–650.

Scherer, E., F.A.J Armstrong and S.H. Nowak. 1975. Effects of mercury contaminated diet upon walleyes, *Stizostedion vitreum* (Mitchill). Fisheries and Marine Service Research and Development Technical Report 597. Fisheries and Oceans Canada, Ottawa, ON.

Schlenk, D., M. Chelius, L. Wolford, S. Khan and K.M. Chan. 1997. Characterization of Hepatic Metallothionein Expression in Channel Catfish (*Ictalurus punctatus*) by Reverse-Transcriptase Polymerase Chain Reaction. Biomarkers. Lond. 2(3): 161-167.

Schuster, P. F., D.P., Krabbenhoft, D.L., Naftz, L.D. Cecil, M.L. Olson, J.F. Dewild, D.D. Susong, J.R. Green and M.L. Abbott. 2002. Atmospheric mercury deposition during the last 270 years: A glacial ice core record of natural and anthropogenic sources. Environ. Sci. Technol. 36: 2303-2310.

Scudder, B.C., LC. Chasar, D.A. Wentz, N.J. Bauch, M.E. Brigham, P.W. Moran and D.P. Krabbenhoft. 2009. Mercury in fish, bed sediment, and water from streams across the United States, 1998-2005. U.S. Geological Survey Scientific Investigations Report 2009-5109, 74 pp. http://pubs.usgs.gov/sir/2009/5109/

Scudder Eikenberry, B.C., K. Riva-Murray, C.D. Knightes, C.A. Journey, L.C. Chasar, M.E. Brigham and P.M. Bradley. 2015. Optimizing fish sampling for fish–mercury bioaccumulation factors. Chemosphere. 135: 467-473.

Selin, N. E. 2009. Global Biogeochemical Cycling of Mercury: A Review. Annual Review of Environment and Resources. Vol. 34:43-63.

Sever, M. 2021. Big wildfiers mobilize mercury. What are the risks to surface water? Proceedings of the National Academy of Sciences of the United States of America. 118(27): 1-4.

Sheffy, T.B. 1978. Mercury burdens in crayfish from the Wisconsin River. Environ. Pollut. 17: 219–225.

Simoneau, M., M. Lucotte, S. Garceau and D. Laliberté. 2005. Fish growth rates modulate mercury concentrations in walleye (*Sander vitreus*) from eastern Canadian lakes. Environ. Res. 98(1): 73-82.

Sorensen, J.A., G.E. Glass, K.W. Schmidt, J.K. Huber and G.R. Rapp. 1990. Airborne mercury deposition and watershed characteristics in relation to mercury concentrations in water, sediments, plankton, and fish of eighty northern Minnesota lakes. Environ. Sci. Technol. 24(11): 1716-1727.

Stefansson, E.S., A. Heyes and C.L. Rowe. 2013. Accumulation of dietary methylmercury and effects on growth and survival in two estuarine forage fish: *Cyprinodon variegatus* and *Menidia beryllina*. Environ. Toxicol. Chem. 32: 848–856.

Steingraeber, M.T., T.R. Schwartz, J.G. Wiener and J.A. Lebo. 1994. Polychlorinated biphenyl congeners in emergent mayflies from the Upper Mississippi River. Environ. Sci. Technol. 28(4): 707-714.

Stephan, C.E., D.I. Mount, D.J. Hansen, J.H, Gentile, G.A. Chapman and W.A. Brungs. 1985. Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses. PB85-227049. National Technical Information Service, Springfield, VA.

Stewart, A.R. M.K. Saiki, J.S. Kuwabara, C.N. Alpers, M. Marvin-DiPasquale and D.P. Krabbenhoft. 2008. Influence of plankton mercury dynamics and trophic pathways on mercury concentrations of top predator fish of a mining-impacted reservoir. Can. J. Fish. Aquat. Sci. 65(11): 2351-2366.

Stopford, W. and L.J. Goldwater. 1975. Methylmercury in the environment: A review of current understanding. Environ. Health Perspect. 12: 115-118.

Stringari, J., A.K.C. Nunes, J.L. Franco, D. Bohrer, S.C. Garcia, A.L. Dafre, D. Milatovic, D.O. Souza, J.B.T. Rocha, M. Aschner and M. Farina. 2008. Prenatal methylmercury exposure hampers glutathione antioxidant system ontogenesis and causes long-lasting oxidative stress in the mouse brain. Toxicol. Appl. Pharmacol. 227: 405-411.

Sundseth K., J.M. Pacyna, E.G. Pacyna, N. Pirrone and R.J. Thorne. 2017. Global Sources and Pathways of Mercury in the Context of Human Health. Int. J. Environ. Res. Public Health. 14(1): 105-118.

Swarzenski, C.M., S.V. Mize. B.A. Thompson and G.W. Peterson. 2004, Fish and aquatic invertebrate communities in waterways, and contaminants in fish, at the Barataria Preserve of Jean Lafitte National Historical Park and Preserve, Louisiana, 1999–2000: U.S. Geological Survey Scientific Investigations Report 2004-5065, 35 pp.

Tan, S. W., J. C. Meiller and K R. Mahaffrey. 2009. The endocrine effects of mercury in humans and wildlife. Critl. Rev. Toxicol. 39(3): 228-269.

Tanaka, Y., K. Nakamura and H. Yokomizo. 2018. Relative robustness of NOEC and ECx against large uncertainties in data. PLoS ONE. 13(11): 1-16.

Territo, P.R. and A.W. Smits. 1998. Whole-body composition of *Xenopus laevis* larvae: Implications for lean body mass during development. J. Exper. Biol. 201(7): 1013-1022.

Todd, B.D., C.M. Bergeron, M.J. Hepner and W.A. Hopkins. 2011. Aquatic and terrestrial stressors in amphibians: A test of the double jeopardy hypothesis based on maternally and trophically derived contaminants. Environ. Toxicol. Chem. 30(10): 277-2284.

Todd, B.D., J.D. Willson, C.M. Bergeron and W.A. Hopkins. 2012. Do effects of mercury in larval amphibians persist after metamorphosis? Ecotoxicol. 21(1): 87-95.

Tollefson, L. and F. Cordle. 1986. Methylmercury in fish: A review of residue levels, fish consumption and regulatory action in the United States. Environ. Health Perspect. 68: 203-208.

Tom, K.R., M.C. Newman and J. Schmerfeld. 2010. Modeling mercury biomagnification (South River, Virginia, USA) to inform river management decision making. Environ. Toxicol. Chem. 29(4): 1013-1020.

Trudel. M. and J. Rasmussen 1997. Modeling the Elimination of Mercury by fish. Environ. Sci. Technol. 31: 1716-1722.

Trudel. M. and J. Rasmussen 2006. Bioenergetics and Mercury Dynamics in Fish: A Modelling Perspective. Can. J. Fish. Aq. Sci. 63: 1890-1902.

Tsui, K.T. and W.X. Wang. 2004. Uptake and Elimination Routes of Inorganic Mercury and Methylmercury in *Daphnia magna*. Environ. Sci. Technol. 38: 808-816.

Ugarte, C.A. 2004. Human impacts on pig frog (*Rana grylio*) populations in south Florida wetlands: Harvest, water management and mercury contamination. Ph.D. Diss., Florida International University, Miami

Ullrich, S.M., T.W. Tanton and S.A. Abdrashitova. 2001. Mercury in the aquatic environment: A review of factors affecting methylation. Crit. Rev. Environ. Sci. Technol. 31(3): 241-293.

U.N. Environment Programme. 2013. Global mercury assessment 2013: Sources, emissions, releases and environmental transport. Global Mercury Assessment 2013: Sources, emissions, releases, and environmental transport (unep.org)

Unrine, J.M. and C.H. Jagoe. 2004. Dietary mercury exposure and bioaccumulation in southern leopard frog (*Rana sphenocephala*) larvae. Environ. Toxicol. Chem. 23(12): 2956-2963.

Unrine, J.M., C.H. Jagoe, W.A. Hopkins and H.A. Brant. 2004. Adverse effects of ecologically relevant dietary mercury exposure in southern leopard frog (*Rana sphenocephala*) larvae. Environ. Toxicol. Chem. 23(12): 2964-2970.

U.S. EPA (United States Environmental Protection Agency). 1985. Appendix B - Response to public comments on "Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses." Federal Regist. 50:30793-30796.

U.S. EPA (United States Environmental Protection Agency) 1991. Technical Support Document for Water Quality-based Toxics Control, Office of Water. U.S. EPA-505/2-90-001.

U.S. EPA (United States Environmental Protection Agency). 1993. Wildlife exposure factors handbook. Volume I of II. Office of Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC.

U.S. EPA (United States Environmental Protection Agency). 1995. Great Lakes Initiative

U.S. EPA (United States Environmental Protection Agency). 1997a. Mercury Study Report to Congress. Volume II: An inventory of anthropogenic mercury emissions in the United States. EPA- 452/R-97-004. Office of Air Quality Planning and Standards and Office of Research and Development, Washington, DC. https://www.epa.gov/sites/default/files/2015-09/documents/volume2.pdf

U.S. EPA (United States Environmental Protection Agency). 1997b. Mercury Study Report to Congress. Volume III: Fate and transport of mercury in the environment. EPA-452/R-97-005. Office of Air Quality Planning and Standards and Office of Research and Development, Washington, DC. https://www.epa.gov/sites/default/files/2015-09/documents/volume3.pdf

U.S. EPA (United States Environmental Protection Agency). 1997c. Mercury Study Report to Congress. Volume VI: An ecological assessment for anthropogenic mercury emissions in the United States. EPA- 452/R-97-008. Office of Air Quality Planning and Standards and Office of Research and Development, Washington, DC. https://www.epa.gov/sites/default/files/2015-09/documents/volume6.pdf

U.S. EPA (United States Environmental Protection Agency). 1997d. Mercury study report to Congress Volume VII: Characterization of human health and wildlife risks from mercury exposure in the United States. EPA-452/R-97-009. https://www.epa.gov/sites/default/files/2015-09/documents/volume7.pdf

U.S. EPA (United States Environmental Protection Agency). 1998a. Guidelines for ecological risk assessment. EPA/630/R-95/002F. Risk Assessment Forum. Office of Research and Development, Washington, D.C.

U.S. EPA (United States Environmental Protection Agency). 1998b. Method 1630 methyl mercury in water by distillation, aqueous ethylation, purge and trap, and cold vapor atomic fluorescence spectrometry. Washington, DC.

U.S. EPA (United States Environmental Protection Agency). 1998c. Method 7473 (SW-846): Mercury in solids and solutions by thermal decomposition, amalgamation, and atomic absorption spectrophotometry, Revision 0. Washington, DC.

U.S. EPA (United States Environmental Protection Agency). 1999. EPA Method 1631, Revision B: Mercury in water by oxidation, purge and trap, and cold vapor atomic fluorescence spectrometry. EPA-821-R-99-005. Washington, DC.

U.S. EPA (United States Environmental Protection Agency). 2001. Water quality criteria for the protection of human health: Methylmercury. EPA-823-R-01-001. January 2002. Office of Water, Washington, DC.

U.S. EPA (United States Environmental Protection Agency). 2002. Method 1631, Revision E: Mercury in water by oxidation, purge and trap, and cold vapor atomic fluorescence spectrometry. EPA-821-R-02-019. Office of Water, Washington, DC.

U.S.EPA (United States Environmental Protection Agency). 2008. Aquatic Life Criteria for Contaminants of Emerging Concern. Part I. General Challenges and Recommendations. Science Advisory Board 2008. https://www.epa.gov/sites/default/files/2015-08/documents/sab_advisory_on_aquatic_life_wqc_for_contaminants_of_emerging_concern.pdf

U.S. EPA (United States Environmental Protection Agency). 2010. Solid waste and emergency response glossary—Bioaccumulation: US Environmental Protection Agency.

U.S. EPA (United States Environmental Protection Agency). 2012. National emission standards for hazardous air pollutants from coal and oil-fired electric utility steam generating units and standards of performance for fossil-fuel-fired electric utility, industrial-commercial institutional, and small industrial commercial-institutional steam generating units. Federal Register, Vol. 77, No. 32: 9304-9504.

U.S. EPA (United States Environmental Protection Agency). 2013. Aquatic life ambient water quality criteria for ammonia – freshwater. EPA-822-R-13-001. Office of Water, Washington, DC.

U.S. EPA (United States Environmental Protection Agency). 2014. Draft total maximum daily load for mercury in the waters of Jordan Creek, Idaho, 2014.

U.S. EPA (United States Environmental Protection Agency). 2016a. Aquatic life ambient water quality criterion for selenium-freshwater. EPA-822-R-16-006. Office of Water, Washington, DC. https://www.epa.gov/sites/production/files/2016-07/documents/aquatic_life_awqc_for_selenium_-_freshwater_2016.pdf

U.S. EPA (United States Environmental Protection Agency). 2016b. Final Test Guidelines; OCSPP Series 850 Group A-Ecological Effects Test Guidelines; Notice of Availability. Federal Register (81 FR 95989), pp. 95989-95991.

U.S. EPA (United States Environmental Protection Agency). 2018. Application of systematic review in TSCA risk evaluations. Office of Chemical Safety and Pollution Prevention. EPA Document # 740-P1-8001. May 2018.

U.S. EPA (United States Environmental Protection Agency). 2021a. 2017 National Emissions Inventory: January 2021 Updated Release, Technical Support Document https://www.epa.gov/air-emissions-inventories/2017-national-emissions-inventory-nei-data

U.S. EPA (United States Environmental Protection Agency). 2018. Application of systematic review in TSCA risk evaluations. Office of Chemical Safety and Pollution Prevention. EPA Document # 740-P1-8001. May 2018.

U.S. EPA (United States Environmental Protection Agency). 2021b. Draft systematic review protocol supporting TSCA risk evaluations for chemical substances Version 1.0. A Generic TSCA systematic review protocol with chemical-specific methodologies. EPA-D-20-031.

U.S. EPA (United States Environmental Protection Agency). 2021c. 2021 Revision* to: Aquatic life ambient water quality criterion for selenium – freshwater 2016. EPA 822-R-21-006. Office of Water, Washington, DC.

U.S. EPA (United States Environmental Protection Agency). 2022a. Draft aquatic life ambient water quality criterion for perfluorooctanoic acid (PFOA). EPA 842-D-22-001. Office of Water, Washington, DC.

U.S. EPA (United States Environmental Protection Agency). 2022b. Draft aquatic life ambient water quality criterion for perfluorooctane sulfonic acid (PFOS). EPA 842-D-22-002. Office of Water, Washington, DC.

USGS (United States Geological Survey). 1985. Geochemistry and Hydrology of Thermal Springs in the Idaho Batholith and Adjacent areas, central Idaho. By: H. W. Young. Water-Resources Investigations Report 85-4172

USGS (United States Geological Survey). 2016. National Contaminant Biomonitoring Program (NCBP). U.S. Geological Survey. Accessed 11 August 2016. http://www.cerc.usgs.gov/data/ncbp/fish.htm

USGS (United States Geological Survey) 2022. National water information system data available on the World Wide Web (Water Data for the Nation), accessed February 16, 2022, at URL [http://waterdata.usgs.gov/nwis/].

Vacca, A.J. and K.L. Cottingham. 2019. No detectable changes in crayfish behavior due to sublethal dietary mercury. Ecotoxicol. Environ. Saf. 182:109440.

Varekamp, J.C. and P.R. Buseck. 1981. Mercury emissions from Mount St. Helen during September 1980. Nature. 293: 555-556.

Varley, T. and others, 1919, A preliminary report on the mining districts of Idaho: U. S. Bur. Mines Bull. 166, p. 1-89.

Vermeer, K., F.A.J. Armstrong and D.R.M. Hatch. 1973. Mercury in aquatic birds at Clay Lake, Western Ontario. J. Wildl. Manage. 37: 58–61.

Vidal, D.E. and A.J. Horne. 2003a. Inheritance of mercury tolerance in the aquatic oligochaete *Tubifex tubifex*. Environ. Toxicol. Chem. 22(9): 2130–2135.

Vidal, D.E. and A.J. Horne. 2003b. Mercury toxicity in the aquatic oligochaete *Sparganophilus pearsei* II: Autotomy as a novel form of protection. Arch. Environ. Contam. Toxicol. 45: 462–467.

Vieira, H.C. A.C.M. Rodrigues, A.M.V.M. Soares, S. Abreu and F. Morgado. 2021. Mercury accumulation and elimination in different tissues of zebrafish (*Danio rerio*) exposed to a mercury-supplemented diet. J. Mar. Sci. Eng. 9: 882-892.

Vijayaraghavan, K., L. Levin, L. Parker, G. Yarwood, and D. Streets. 2014. Response of fish tissue mercury in a freshwater lake to local, regional, and global changes in mercury emissions. Environ. Toxicol. Chem. 33(6): 1238–1247.

Wada, H., C.M. Bergeron, F.A. McNabb, B.D. Todd, and W. A. Hopkins. 2011. Dietary mercury has no observable effects on thyroid-mediated processes and fitness-related traits in wood frogs. Environ. Sci. Technol. 45(18): 7915-7922.

Wang, R. and W-X. Wang. 2012. Contrasting mercury accumulation patterns in tilapia (*Oreochromis niloticus*) and implications on somatic growth dilution. Aquat. Toxicol. 114-115: 23-30.

Ward, D.M., K.H. Nislow and C.L. Folt. 2010a. Bioaccumulation syndrome: Identifying factors that make some stream food webs prone to elevated mercury bioaccumulation. Annal. NY Acad. Sci. 1195: 62-83.

Ward, D.M., K.H. Nislow, C.Y. Chen and C.L. Folt. 2010b. Rapid, efficient growth reduces mercury concentrations in stream-dwelling Atlantic salmon. Trans. Amer. Fish. Soc. 139(1): 1-10.

Warne MSt.J. BGE, van Dam R.A., Chapman J.C., Fox D.R., Hickey C.W. and Stauber .J.L. 2018. Revised Method for Deriving Australian and New Zealand Water Quality Guideline Values for Toxicants – update of 2015 version. Prepared for the Council of Australian Government's Standing Council on Environment and Water (SCEW). Department of Science. Information Technology and Innovation, 2018.

Wathen, J.B., J.M. Lazorchak, A.R. Olsen and A. Batt. 2015. A national statistical survey assessment of mercury concentrations in fillets of fish collected in the U.S. EPA national rivers and streams assessment of the continental USA. Chemosphere. 122: 52-61.

Watras, C.J., R.C. Back, S. Halvorsen, R.J.M. Hudson, K.A. Morrison and S.P. Wente. 1998. Bioaccumulation of mercury in pelagic freshwater food webs. Sci. Total Environ. 219(2-3): 183-208.

Webb, M.A.H., G.W. Feist, M.S. Fitzpatrick, E.P. Foster, C.B. Schreck, M. Plumlee, C. Wong and D.T. Gundersen. 2006. Mercury Concentrations in Gonad, Liver, and Muscle of White Sturgeon *Acipenser transmontanus* in the Lower Columbia River. Arch. Environ. Contam. Toxicol. 50: 443–451.

Webber, H.M. and T.A. Haines. 2003. Mercury effects on predator avoidance behavior of a forage fish, golden shiner (*Notemigonus crysoleucas*). Environ. Toxicol. Chem. 22: 1556–1561.

Weis. M. 2004. Mercury concentrations in fish from Canadian Great Lakes areas of concern: an analysis of data from the Canadian Department of Environment database. Environ. Research. 95(3): 341-350.

Wentz, D.A., M.E. Brigham, LC. Chasar, M.A. Lutz and D.P. Krabbenhoft. 2014. Mercury in the Nation's streams - levels, trends, and implications: U.S. Geological Survey Circular 1395, 90 pp.

West, J. 2018. Importance of amphibians: A Synthesis of their environmental functions, benefits to humans, and need for conservation. In BSU Honors Program Theses and Projects.

Willacker, J.J., C.A. Eagles-Smith, J.A. Chandler, J. Naymik, R. Myers, and D.P. Krabbenhoft. 2023. Reservoir stratification modulates the influence of impoundments on fish mercury concentrations along an arid land river system. Environmental Science and Technology 57: 21313-21326.

Wobeser G. 1975. Prolonged oral administration of methylmercury chloride to rainbow trout (*Salmo gardneri*) fingerlings. J. Fish. Res. Board Can. 32: 2015–2023.

World Health Organization (WHO). 1989. Mercury - Environmental Aspects. Environmental Health Criteria 86. 114 pp.

Xie, L., J.L. Flippin, N. Deighton, D.H. Funk, D.A. Dickey and D.B. Buchwalter. 2009. Mercury(II) bioaccumulation and antioxidant physiology in four aquatic insects. Environ. Toxicol. Chem. 43: 934-940.

Yap, T.A., M.S. Koo, R.F. Ambrose and V.T. Vredenburg. 2018. Introduced bullfrog facilitates pathogen invasion in the western United States. PLoS ONE 13(4): e0188384.

Zaroban, D.W., M.P. Mulvey, T.R. Maret and R.M. Hughes. 1999. Classification of species attributes for Pacific Northwest freshwater fishes. Northwest Sci. 73(2): 81-93.

Zimmermann, L.T., D.B. Santos, A.A. Naime, R.B. Leal, J.G. Dórea, F. Barbosa, Jr., M. Aschner, J.B.T. Rocha and M. Farina. 2013. Comparative study on methyl- and ethylmercury-induced toxicity in C6 glioma cells and the potential role of LAT-1 in mediating mercurial-thiol complexes uptake. Neurotoxicol. 38:1-8.

Appendix A Data Quantitatively Used in the Mercury Criterion Derivation

A.1 Quantitative Dietary Mercury Studies

Species	Dietary Description	Exposure Duration (d)	Dietary Mercury[1]	Tissue Mercury	Endpoint(s)	Dietary Effects Concentrations	Reported Mercury Form and Units	Tissue Effects Concentrations	Reported Mercury Form and Units	Exposure Notes	Reference
Southern leopard frog (Gosner Stage 25), *Lithobates sphenocephala*	Aufwuchs from control and Hg-enriched mesocosms combined with ground, vitamin-enriched rabbit and trout pellets; 74% moisture content fed ad libitum	194	M – THg, MeHg	Whole body – THg, MeHg	Malformation rate, metamorphic success, survival	NOEC: 423 MATC: 772.0 LOEC: 1,409	THg, ng/g dw	NOEC: 95 MATC: 150.2 LOEC: 237.6	THg, ng/g dw	The measured THg concentrations in the dietary treatments were 54 ng/g dw (control; of which 22% was MeHg), 423 ng/g dw (3.4% of which was MeHg), 1,409 ng/g dw (1.9% of which was MeHg), and 3,298 ng/g dw (1.5% of which was MeHg).	Unrine and Jagoe 2004
American toad (4 dph), *Anaxyrus americanus*	Dry feed mix spiked with or without Hg [inorganic (HgII) and organic (MMHg); Alfa Aesar], suspended in an agar gelatin mixture similar to Unrine and Jagoe (2004); 58.6% moisture content fed at a ration of 6% body weight per day	116	M – THg, MeHg	Whole body – THg, MeHg	Decreased growth as mass at GS 42	NOEC: 2.5 MATC: 5.025 LOEC: 10.1	THg, µg/g dw	NOEC: 0.8 MATC: 1.200 LOEC: 1.8	THg, µg/g dw	The measured THg concentrations in dietary treatments were 0.010 ± 0.001 µg/g dw (56.7% MeHg), 2.50 ± 0.06 µg/g dw (3.19% MeHg), and 10.1 ± 2.27 µg/g dw (1.05% MeHg) for the control, low Hg, and high Hg treatments. Percent moisture of metamorphs was 90.4%. MeHg was 30% of THg in metamorphs fed high mercury diet and 53% in those fed low mercury diet.	Bergeron et al. 2011a
Fathead minnow (A-1pprox.. 3 month-old), *Pimephales promelas*	Commercial fish food mixed with reagent alcohol containing dissolved methylmercuric chloride. Ration provided was 5% of body mass per day.	Full life-cycle	M – THg	Carcass (whole body less plasma and gonads) – THg	Reproduction (reproductive capacity)	NOEC: MATC: LOEC: 0.88	THg, µg/g dw	NOEC: MATC: LOEC: 3.102	THg, µg/g dw	Mean dietary concentrations (measured as THg) were 0.060 µg/g dw (control), 0.88 µg/g dw (low), 4.11 µg/g dw (medium), and 8.46 µg/g dw (high) exposure, respectively.	Hammerschmidt et al. 2002

Species	Dietary Description	Exposure Duration (d)	Dietary Mercury[1]	Tissue Mercury	Endpoint(s)	Dietary Effects Concentrations	Reported Mercury Form and Units	Tissue Effects Concentrations	Reported Mercury Form and Units	Exposure Notes	Reference
Fathead minnow (90 dph), *Pimephales promelas*	Commercial fish food mixed with reagent alcohol containing dissolved methylmercuric chloride, similar to Hammerschmidt et al. (2002). Ration provided was 5% of body mass per day.	Full life-cycle	M – THg	Carcass (whole body less plasma and gonads) – THg	Reproduction (reproductive success)	NOEC: MATC: LOEC: 0.87	THg, µg/g dw	NOEC: MATC: LOEC: 0.8901	THg, µg/g ww	Mean dietary total mercury concentrations were 0.058, 0.87, and 3.93 µg/g dw in the control, low, and medium exposures, respectively (where "medium" is the highest treatment).	Drevnick and Sandheinrich 2003
Fathead minnow (90 dph), *Pimephales promelas*	Commercial fish food mixed with reagent alcohol containing dissolved methylmercuric chloride, similar to Hammerschmidt et al. (2002). Ration provided was 5% of body mass per day.	Full life-cycle	M – THg	Male carcass (whole body less plasma and gonads) – THg	Reproduction (reproductive success)	NOEC: MATC: LOEC: 0.87	THg, µg/g dw	NOEC: MATC: LOEC: 0.714	THg, µg/g ww	See under Drevnick and Sandheinrich (2003)	Sandheinrich and Miller 2006
Red swamp crayfish (juvenile), *Procambarus clarkii*	Farm-raised catfish (low Hg diet) or wild-caught largemouth bass (high Hg diet); fed ad libitum	142	M – THg	Abdominal muscle – THg	Survival	NOEC: MATC: LOEC: 0.278	THg, µg/g ww	NOEC: MATC: LOEC: 7,757	THg, ng/g dw	Diets consisted of a low "presumed control" one containing a mean concentration of 0.009 µg THg/g fresh weight (80% MeHg) and high mercury diet containing a mean concentration of 0.278 µg THg/g fresh weight (98% MeHg).	Brant 2004

Species	Dietary Description	Exposure Duration (d)	Dietary Mercury[1]	Tissue Mercury	Endpoint(s)	Dietary Effects Concentrations	Reported Mercury Form and Units	Tissue Effects Concentrations	Reported Mercury Form and Units	Exposure Notes	Reference
Walleye (6-month old juveniles), *Stizostedion vitreum*	Farm-raised catfish fillets injected with MeHg dissolved in distilled water, supplemented at 6 weeks with fathead minnow injected with MeHg. Ration: 1-g pieces fed 3 times per week; increased to 1.5-g at 3.5 months	180	M – THg	Whole body – THg	Growth (weight gain)	NOEC: 0.137 MATC: 0.3677 LOEC: 0.987	THg, µg/g ww	NOEC: 0.25 MATC: 0.7697 LOEC: 2.37	THg, µg/g ww	Measured dietary concentrations consisted of a control (< 0.04 µg THg/g ww), low dose (0.137 µg THg/g ww), or high dose diet (0.987 µg THg/g ww).	Friedmann et al. 1996
Tigerfish (mature; 111g), *Hoplias malabaricus*	Prey fish (Astyanax sp., tetra fish) IP injected with a solution of MeHg and fed to individual test fish at a rate of one every 5 days (approximately 10% of the wet weight). The prey item was not force-fed to individual fish.	70	U – Nominal 0.015 µg/g ww daily	Muscle – THg	Survival	NOEC: MATC: LOEC:	NA	NOEC: 1.45 MATC: >1.45 LOEC: >1.45	THg, µg/g ww	Individual experimental fish received 14 prey fish (doses) provided over the course of the experiment.	Olivera-Riberio et al. 2006; Costa et al. 2007; Mela et al. 2007
Channel catfish (juvenile; 12-15 cm), *Ictalurus punctatus*	Japanese Medaka (Oryzias latipes) with MeHgCl. Fed daily at a ration of 2% of body weight per day.	30	U – Nominal 0.1 µg/g ww daily	Muscle – THg	Growth (condition factor)	NOEC: MATC: LOEC:	NA	NOEC: 1.6 MATC: >1.6 LOEC: >1.6	THg, µg/g ww		Schlenk et al. 1997
Goldfish (pre-spawning adult females), *Carassius auratus*	Floating trout pellets mixed with 95% ethanol containing dissolved MeHg(II)Cl. The control diet was prepared by mixing food with ethanol only.	28	M – THg	Muscle – THg	Survival and growth	NOEC: 7.78 MATC: >7.78 LOEC: >7.78	THg, µg/g ww	NOEC: 2.037 MATC:>2.037 LOEC:>2.037	THg, µg/g ww	Methylmercury (measured as THg) for the pre-spawning diets were 0.035 (control), 0.69 (low), 4.48 (medium) and 7.78 (high) µg THg/g wet weight	Crump et al. 2008

Species	Dietary Description	Exposure Duration (d)	Dietary Mercury[1]	Tissue Mercury	Endpoint(s)	Dietary Effects Concentrations	Reported Mercury Form and Units	Tissue Effects Concentrations	Reported Mercury Form and Units	Exposure Notes	Reference
Beluga sturgeon (juvenile), *Huso huso*	Fish meal mixed with MeHgCl dissolved in ethanol.	70	M – THg	Muscle – THg	Specific growth rate	NOEC: MATC: LOEC: 0.76	THg, mg/kg dw	NOEC: MATC: LOEC: 3	THg, µg/g ww	Measured dietary concentrations achieved were 0.04 mg THg/kg (control); 0.76 mg THg/kg (low); 7.88 mg/kg (medium) and 16.22 m THg/kg (high)	Gharaei et al. 2008, 2011
Atlantic salmon (parr; 14.7 g), *Salmo salar*	Dry feed consisting of fishmeal (578 g/kg feed), capelin oil (119), wheat meal (160), mineral mix (10), vitamin mix (10), ground squid (95), and gelatin (28), supplemented with 0, 0.1, 0.5, 5, or 10 mg MeHgCl per kg feed. Fish were fed a ration of 2.6% of body weight the first month, 2.2% during the second month, and 2.0% during the last 2 months.	120	M – THg	Muscle	Survival and growth	NOEC: MATC: LOEC: 8.48	THg, µg/g dw	NOEC: MATC: LOEC: 3.07	THg, µg/g ww	Final mean measured dietary mercury concentrations were 0.14 (control), 1.89, 8.84 and 102.6 µg/g dw (inorganic mercury) and 0.12 (control), 0.63, 4.35 or 8.48 µg/g dw (organic [methyl] mercury). In follow-up studies, measured THg concentrations were 0.03, 4.35 and 8.48 µg/g dry weight in the control, and 10 µg THg/g supplemented diets, respectively (Berntssen et al., 2004).	Berntssen et al. 2003, 2004

Species	Dietary Description	Exposure Duration (d)	Dietary Mercury[1]	Tissue Mercury	Endpoint(s)	Dietary Effects Concentrations	Reported Mercury Form and Units	Tissue Effects Concentrations	Reported Mercury Form and Units	Exposure Notes	Reference
Rainbow trout (fingerlings; 5.5-5.7 g), *Oncorhynchus mykiss*	Experimental diets were prepared from a commercial trout food ground to a homogenous powder, then mixed, 2:1, with an aqueous solution containing the required quantities of MeHgCl. Three experiments were performed with fish fed either 1%, 2% or ad libitum rations.	84	M – THg	Whole body – THg	Survival and growth	NOEC: MATC: LOEC: 23.9	THg, µg/g dw	NOEC: MATC: LOEC:10 (ad libitum NOEC: MATC: LOEC: 9 (2% bw/d)	THg, µg/g ww	Measured mercury concentration in the diet were <0.1, 23.9, 46.9 and 94.8 µg/g THg dry weight, respectively. EPA used the THg wet weight whole body tissue LOECs from the 2% ration and ad libitum experiment.	Rodgers and Beamish 1982
Zebrafish (73 dpf females; 320 mg, 26mm), *Danio rerio*	MeHgCl dissolved in the diet lipid fraction of a formulated basal diet. Fish were fed to satiation twice daily with the prepared basal experimental diet equivalent to 3% of the estimated wet weight of fish biomass per day fed as dry weight; the percentage fed decreased to 1% as fish grew and the test continued (up to >150 dpf).	145 (from 73 to 226 dpf)	M – THg	Whole body (female fish) – THg	Survival and growth	NOEC: MATC: LOEC: 11.98	THg, mg/kg dw	NOEC: MATC: LOEC: 33.31	THg, mg/kg dw	A basal zebrafish experimental diet was formulated from casein, gelatin, vitamins, minerals and spiked with selenium (as seleno-l-methionine (SeMet)) at 0.7 or 10 mg Se/kg dw and mercury (as methylmercury chloride (MeHg)) at 0.05 or 12 mg THg/kg dw. Only the low selenium diet was considered in this assessment.	Penglase et al. 2014a,b

Species	Dietary Description	Exposure Duration (d)	Dietary Mercury[1]	Tissue Mercury	Endpoint(s)	Dietary Effects Concentrations	Reported Mercury Form and Units	Tissue Effects Concentrations	Reported Mercury Form and Units	Exposure Notes	Reference
Burrowing mayfly (nymphs), *Hexagenia* spp.	Each test beaker was provided with dried, finely ground leaves of submersed aquatic plants (curly pondweed and wild celery) every third day.	21	M – THg, MeHg	Whole body – THg, MeHg	Growth	NOEC: MATC: LOEC:	NA	NOEC: 10.819 MATC:>10.819 LOEC: >10.819	THg, µg/g dw	Concentrations of MeHg in a subsample of the plant homogenate used for food were 5.1, 1.1, 5.3, and 4.2 ng/g dry weight in tests 1, 2, 3, and 4, each test reflecting a different natural sediment type. Mean THg ranged from 880 to 22,059 ng/g dw in contaminated sediments and from 90 to 272 ng/g dw in reference sediments. Mean final concentrations of MeHg in test water were greatest (8–47 ng/L) in treatments with contaminated wetland sediments, which had mean THg ranging from 1,200 to 2,562 ng/g dw	Naimo et al. 2000
Sacramento blackfish (juvenile), *Orthodon microlepidotus*	Trout chow crumble ground mixed with MeHgCl dissolved in 100% ethanol. Gelatin (6%) was added to reduce solubility.	70	M – THg	Muscle – THg	Survival and growth	NOEC: 0.52 MATC: 3.398 LOEC: 22.2	THg, µg/g dw	NOEC: 2.3 MATC: 7.583 LOEC: 25	THg, µg/g ww	Measured THg concentrations in diets were 0.21 (control), 0.52, 22.2 and 55.5 µg/g dry weight	Houck and Cech 2004

Species	Dietary Description	Exposure Duration (d)	Dietary Mercury[1]	Tissue Mercury	Endpoint(s)	Dietary Effects Concentrations	Reported Mercury Form and Units	Tissue Effects Concentrations	Reported Mercury Form and Units	Exposure Notes	Reference
Asiatic clam (1.2-1.8 cm), *Corbicula fluminea*	Natural food from indoor experimental unit where Hg contamination levels in sediment were achieved by one-time addition from a concentrated aqueous stock solution composed of 0.5 g/L methylmercury chloride and 1 g/L mercury chloride. No external food supply was added during the experiment.	14	M – THg	Soft body – THg	Survival and growth	NOEC: MATC: LOEC:	THg, µg/g dw	NOEC: 6,000 MATC:> 6,000 LOEC:> 6,000	THg, ng/g ww	The experimental unit for the sediment compartment exposure was natural sediment (of homogenous silt, rich in clays (75-80%), and with low total organic carbon: 2% on average) collected from the banks of the Garonne River upstream of Bordeaux, France.	Inza et al. 1997
Sacramento splittail (21-dph larvae), *Pogonichthys macrolepidotus*	MeHgCl (pre-dissolved in 100% ethanol) added to a dry basal diet.	28	M – THg	Whole body – THg	Survival and growth	NOEC: MATC: LOEC:	THg, µg/g dw	NOEC: 6 MATC:> 6 LOEC:> 6	THg, µg/g ww	Measured THg concentrations in the test diets were 0.01 (control), 0.13, 4.7 and 11.7 µg/g dry weight.	Deng et al. 2008

Species	Dietary Description	Exposure Duration (d)	Dietary Mercury[1]	Tissue Mercury	Endpoint(s)	Dietary Effects Concentrations	Reported Mercury Form and Units	Tissue Effects Concentrations	Reported Mercury Form and Units	Exposure Notes	Reference
Cladoceran (3-d old), *Daphnia magna*	Green alga (*Chlamydomonas reinhardtii*) in the exponential phase spiked with $Me^{203}Hg$ at 148 kBq/L (corresponding to 28.3 nM of Hg).	5	M – THg	Whole body – THg	Survival and reproduction	NOEC: MATC: LOEC:	THg (>95% MeHg)	NOEC: MATC: LOEC: 33.3	THg, µg/g ww	After a day of growth, the percentage of MeHg associated with the green algae cells was greater than 95%. Feeding regime was repeated for a total of five days with F0 daphnids followed by 20 d of depuration. Each day, the live neonates (F1 generation) produced by individual replicates of the F0 generation were transferred to individual beakers, and their retention of maternally-transferred methylmercury and further neonate production (F2 generation) were monitored over a period of 28 d after hatching.	Tsui and Wang 2004
Green sturgeon (juvenile, 30g), *Acipenser medirostris*	MeHgCl dissolved in 100% ethanol was added to a purified diet	56	U	Muscle – THg	Survival and growth	NOEC: 25 MATC: 35.36 LOEC: 50	THg, µg/g dw	NOEC: 50.8 MATC: 76.50 LOEC: 115.2	THg, µg/g dw	Nominal THg concentrations were control, 25, 50, 100 µg /g dw	Lee et al. 2011
White sturgeon (juvenile, 30 g), *Acipenser transmontanus*	MeHgCl dissolved in 100% ethanol was added to a purified diet	56	U	Muscle – THg	Survival and growth	NOEC: 50 MATC: 70.71 LOEC: 100	THg, µg/g dw	NOEC: 104.4 MATC: 155.6 LOEC: 231.8	THg, µg/g dw	Nominal THg concentrations were control, 25, 50, 100 µg /g dw	Lee et al. 2011

1 – M: measured, U: unmeasured.

A.2 Detailed Dietary Exposure Chronic Toxicity Study Summaries

The purpose of this section was to present detailed study summaries for tests that were considered quantitatively acceptable for criterion derivation, with summaries grouped and ordered by genus sensitivity. Additional information on study conditions (diet, exposure, water quality) and other effects measured in the study are included here to help the reader understand EPA's use of the study. Finally, EPA did not independently calculate a toxicity value, but used the author reported effect concentrations to derive the criterion.

A.2.1 Southern Leopard Frog (Lithobates [Rana] sphenocephalus)

Source Document: Unrine, J.M., C.H. Jagoe, W.A. Hopkins and H.A. Brant. 2004. Adverse effects of ecologically relevant dietary mercury exposure in southern leopard frog (*Rana sphenocephala*) larvae. Environ. Toxicol. Chem. 23(12): 2964-2970.

Test Organism: Southern leopard frog (*Rana sphenocephala*)

Mercury Exposure: *Aufwuchs* samples were collected on the U.S. Department of Energy's Savannah River Site .in South Carolina. Three impoundments and a constructed wetland were chosen for aufwuchs collections to achieve a mercury concentration gradient; two impoundments were abandoned nuclear reactor cooling reservoirs, a farm pond, and a constructed wetland. The wetlands and cooling reactor impoundments received water from sources that may have been historically elevated Hg from upstream industry, but none of these sites are known to currently have significant onsite geologic or anthropogenic inputs of Hg. Aufwuchs samples were inoculated on artificial substate and placed back in the reservoirs for colonization. Samples were collected from the impoundments and constructed wetlands after one month. Five samples of aufwuchs were also collected from the sides of both control and Hg-enriched mesocosms, previously spiked with $HgCl_2$ in 1992 and open to environmental inputs and colonization by biota since construction. Experimental diets were formulated with dried aufwuchs harvested from control and Hg-enriched mesocosms, vitamin-enriched rabbit pellets (Classic Blend Rabbit

Food, L/M Animal Farms, Pleasant Plain, OH, USA) and trout pellets (Aquamax Grower 600, PMI Nutrition International, Brentwood, MO, USA), embedded in agar-gelatin matrix (Hirschfeld et al. Date). Briefly, diets were prepared by dissolving 20 g of agar and 14 g of gelatin in 750 ml of reagent water (16.7 MV deionized water) and heated to ~70ºC on a hot plate. Ground rabbit pellets, ground trout pellets, and ground mesocosm aufwuchs were ground and homogenized. The solution was poured over the dry components, mixed until homogenized, cooled, and stored in a -80ºC freezer until use. The agar-gelatin matrix prevented the diet from dissolving and fowling the exposure chambers, as well as preventing release of mercury from the aufwuchs into the water while allowing tadpoles to graze as they would in nature. Mercury concentrations were adjusted in the diets by varying the proportion of control or Hg-enriched aufwuchs.

Study Design: Southern leopard frog egg masses (as three masses) were collected from Peat Bay in Barnwell County, South Carolina. Seventy-two total tadpoles (Gosner Stage (GS) 25) from a homogenized pool of the three egg masses were assigned to a control treatment and one of three mercury-contaminated dietary treatment groups (resulting in 18 replicates per treatment). Exposure to mercury-enriched diets started at day 60 and was terminated at day 254, with a dietary mercury exposure duration of 194 days. The tadpoles were fed *ad libitum*, ensuring that each individual was provided the same size ration at each feeding. The semi-natural diets were spiked with mercury (II) chloride and methylmercury (II) chloride as the source of mercury. Methylmercury, inorganic mercury (HgII), and total mercury concentrations were determined in the diets. The measured total mercury concentrations in the dietary treatments were 54 ng/g dw (control; of which 22% was methylmercury), 423 ng/g dw (3.4% of which was methylmercury), 1,409 ng/g dw (1.9% of which was methylmercury), and 3,298 ng/g dw (1.5% of which was

methylmercury). The number of days elapsed post-hatching (DPH) at which tadpoles reached three developmental stages - complete hind-limb development (HL; GS 39), forelimb emergence (FL; GS 42), and complete tail resorption (TR; GS 46) - was also recorded and individual mass was determined at each of these stages. Complete tail resorption was considered to be completion of metamorphosis. The tadpoles were observed every one to two days for survival, food consumption, and developmental abnormalities, and external malformations were determined in both tadpoles and metamorphs.

Effects Data: Dietary mercury exposure (duration of 194 days) resulted in total and methylmercury tissue concentrations of 49 ng THg/g dw (21 ng MeHg/g dw) in the control treatment, 95 ng THg/g dw (18 ng MeHg/g dw) in the low treatment, 237.6 ng THg/g dw (20 ng MeHg/g dw) in the medium treatment, and 412 ng THg/g dw (28 ng MeHg/g dw) in the high treatment. The authors determined survival, metamorphic success, and malformation rate to be dependent on mercury treatment ($p = 0.0406$, 0.0293, and 0.0475 respectively), but did not report NOECs or LOECs. Survival was 88.2%, 100%, 72.2% and 72.2% in control, low, medium and high doses, respectively and metamorphic success was 82.4%, 100%, 66.7% and 72.2% in control, low, medium, and high doses, respectively. Malformation rates were 5.9% (1/17), 5.6% (1/18), 11.1% (2/18), and 27.8% (5/18) in control, low, medium, and high treatments, respectively. Although observed effects (malformation rate, metamorphic success, and mortality rates) were higher in the two highest dietary exposures compared to controls, mortality in the low dietary Hg treatment was lower compared to controls. This is likely due to the lower methylmercury concentration in the low dose (18 ng MeHg/g dw) compared to the control (21 ng MeHg/g dw), and relatively low total mercury concentrations (95 ng THg/g dw) as compared to the medium (237.6 ng THg/g dw) and high (412 ng THg/g dw) dietary exposures. The author

noted that this has also been observed in other studies using FETAX assays at low mercury concentrations (Prati et al. 2002).

Using these effects data, EPA estimated the low and medium treatments to be the NOEC and LOEC. Based on the whole-body accumulation data reported by Unrine and Jagoe (2004), the corresponding whole-body total mercury NOEC and LOEC for survival, malformation rate, and metamorphic success were 0.095 and 0.2376 µg THg/g dw respectively. This corresponds to an 16% difference in survival and a 15.7% difference in metamorphic success between the control and the LOEC. Since this difference is relatively small (< 20%), USEPA selected the LOEC of the study (0.2376 µg THg/g dw) as the surrogate for the EC_{10}. EPA used the average post-metamorphic stage percent moisture of 86.23% based on data for species in Bufonidae and Lithobatidae (Ranidae) as described in **Section 2.9.2** and **Appendix D**. The LOEC for survival, deformities, and metamorphic success in southern leopard frog based on whole body total mercury is 0.03272 µg THg/g ww (0.2376 µg/g dw ÷ 7.264), the value EPA selected for criterion derivation from the study. This whole-body total mercury value is equivalent to 0.03373 µg THg/g ww total mercury in muscle after applying the WB:M conversion factor of 0.97.

A.2.2 American toad (Anaxyrus americanus)

Source Document: Bergeron, C.M., W.A. Hopkins, B.D. Todd, M.J. Hepner and J.M. Unrine. 2011. Interactive effects of maternal and dietary mercury exposure have latent and lethal consequences for amphibian larvae. Environ. Sci. Tech. 45(8): 3781-3787.

Test Organism: American toads (*Anaxyrus americanus*)

Mercury Exposure: Experimental diets consisted of a dry feed mix (algae flakes were substituted for aufwuchs) spiked with or without mercury [mercury (II) chloride and methylmercury (II) chloride; Alfa Aesar] and suspended in an agar-gelatin mixture similar to the diet formulated by Unrine and Jagoe (2004). Uniform rations (6% body weight per day, wet weight basis) were

prepared by pressing the thawed diet out of a syringe and cutting into equal lengths of known masses.

Study Design: Eggs were collected from 27 reproductive pairs of American toads found in breeding pools along historic mercury-contaminated and reference stretches of the South River, Virginia. Amplexing (embracing) pairs were transferred to the laboratory, placed in bins containing dechlorinated tap water, and allowed to breed. The experiment consisted of a 2 x 3 factorial design to test the singular and interactive effects of maternally-derived and dietary mercury on larval survival, development, and swimming performance. For the purposes of deriving mercury aquatic life criterion, only the effects from dietary (trophically-derived) mercury exposure were considered as the dietary effects on the offspring, reflecting the aquatic portion of the American toad's lifecycle. Additionally, only results from offspring of adult females from reference sites were considered here.

Twenty-five individual larvae (approximately 4 days post-hatch) from eggs spawned from adult females from reference sites were fed a control (0.010 µg/g dw) or one of two mercury-contaminated diets: low (2.50 µg/g dw total mercury) or high (10.1 µg/g dw total mercury) for 26-28 days. Of the total mercury concentrations measured in the three diets, 56.7 (control), 3.19 (low Hg), and 1.05% (high Hg) were quantified as methylmercury resulting in dietary methylmercury concentrations of 0.0057, 0.0798, and 0.1061 µg/g dw, respectively.

Effects Data: Larval survival was high in all treatments until the onset of metamorphic climax (80, 92, and 96% for larvae from reference mothers fed control, low, and high Hg diets, respectively), but decreased during metamorphic climax to 60, 44, and 48%, respectively, for metamorphs fed those same diets. Therefore, only the results collected for survival, development, and swimming performance before metamorphic climax were further considered for mercury

criterion derivation. There was no effect of dietary mercury exposure on survival or average swimming speed of larvae. However, dietary exposure to mercury had a significant effect on mass at GS 42 (Component ANOVA, p = 0.004), but not on the duration of larval period (Component ANOVA, p = 0.79). Post hoc Tukey's tests showed that mass at GS 42 differed significantly between larvae fed the control diet and high Hg diet (p = 0.004). On average, animals fed the high Hg diet were 16% smaller than those fed control diet. The mean whole-body total mercury concentrations at the dietary NOEC and LOEC for mass at GS 42 were roughly 0.800 and 1.800 µg THg/g dw, respectively, resulting in an MATC of 1.2 µg THg/g dw. EPA selected the MATC as a surrogate for the EC_{10} rather than the NOEC because the percent effect between the LOEC and the control was small (16%). EPA used the average post-metamorphic stage percent moisture of 86.23% based on data for species in Bufonidae and Lithobatidae (Ranidae) as described in **Section 2.9.2**. The MATC for decreased mass at GS 42 in American toad based on whole body total mercury is 0.1653 µg/g ww (1.2 µg THg/g dw ÷ 7.26), the value EPA selected for criterion derivation from the study. This whole- body total mercury value is equivalent to 0.1704 µg THg/g ww in muscle after applying the WB:M conversion factor of 0.97.

A.2.3 Fathead minnow (Pimephales promelas)

Source Document: Hammerschmidt, C.A., M.B. Sandheinrich, J.C. Weiner and R.C. Rada. 2002. Effects of dietary methylmercury on reproduction of fathead minnows. Environ. Sci. Technol. 36: 877-883.

Test Organism: Fathead minnow (*Pimephales promelas*)

Mercury Exposure: Phase 1 and 2 diets were prepared by mixing fish food (Soft-moist fish food, Nelson and Sons, Inc.) with reagent alcohol (Fisher) containing dissolved methylmercuric chloride (Alfa Chemical). Control diets were prepared similarly by mixing fish food with alcohol only. Alcohol was evaporated from the mixtures after preparation approximately every 2 weeks.

Prepared diets were frozen until use. The dietary exposures were selected to approximate dietary MeHg concentrations of invertivorous and piscivorous species (e.g., yellow perch) from midcontinental lakes in the US. Samples of each diet from each preparation batch were analyzed for total mercury. Mean dietary concentrations (measured as total mercury) were 0.060 µg/g dw (control), 0.88 µg/g dw (low), 4.11 µg/g dw (medium), and 8.46 µg/g dw (high) exposure, respectively.

Study Design: The effects of either dietary or maternally-transferred methylmercury on fathead minnows (*Pimephales promelas*) were examined for a full life cycle. The study included four sequential phases corresponding to life stages of the fathead minnow: Phase 1 the juvenile stage until sexual maturity, Phase 2 spawning of mature fish, Phase 3 embryogenesis, and Phase 4 growth of larval progeny. For Phase 1, juvenile (~3 month-old) fathead minnows were fed one of four diets contaminated with methylmercuric chloride until sexual maturity. Phase 1 testing was terminated when fish became sexually dimorphic (~240 days). Sexually mature males and females from each dietary exposure were paired randomly for reproduction studies in Phase 2. During the 136-day period of this reproductive phase of the experiment, one set of breeding pairs (n = 50) were maintained on the same dietary MeHg exposure as Phase 1. In addition, some of the mating pairs (n = 25 pairs) from each dietary exposure were fed a control diet during Phase 2 to evaluate the effects of dietary MeHg during gametogenesis. Also, MeHg-exposed fish from Phase 1 were paired with fish (n = 25 pairs) fed the control diet in Phase 1 to examine the relative effects of either male or female exposure to dietary MeHg during Phase 1. Spawning substrates were examined daily for eggs in Phase 3. To ensure that the gametes were produced or matured while the fish were fed the Phase 2-diet, a second clutch of eggs was collected when the

Phase 2-diet was different from the Phase 1-diet of either test fish. Finally, the 7-d survival and growth of fathead minnow progeny were determined in Phase 4.

Effects Data: Several aspects of the reproductive process were negatively impacted, particularly in fish exposed in Phase 1 (from juvenile stage to sexual maturity) and as mating pairs exposed in Phase 2 to methylmercury in the diet. EPA re-evaluated study data and found that reproductive effort (defined by number of eggs laid/day and the total number of eggs laid) of fathead minnow was significantly affected (total eggs laid, $p = 0.03163$, $n = 13$; and number eggs laid/day, $p = 0.01765$, $n = 13$; Wilcoxon rank sum test). Also, dietary exposure resulted in impacts to overall spawning success of mating pairs of exposed fathead minnows. Spawning success is defined as the percentage of pairs within a dietary treatment that spawned a clutch (5 or more) eggs within 21 days after placement in breeding aquaria. Spawning success of mating pairs fed the control diet during Phase 1 and 2 was 81%, whereas pairs fed the low and medium mercury-contaminated diets was 50%, and spawning success was 36% for the high methylmercury diet. This represents a reduction in spawning success relative to control levels of 31% and 45% in low/medium and high methylmercury diets, respectively. Also, for those mating pairs that spawned successfully, the average time to spawn a clutch of 5 or more eggs was 4 days, 7.8 days, 7.6 days, and 14 days for control, low, medium, and high dietary exposures, respectively.

The mean whole-body total mercury concentrations attained by male and female fish exposed to the same diet during Phases 1 and 2 were 0.32 and 0.48µg THg/g dw (control diet), 2.83 and 3.40 µg THg/g dw low methylmercury diet), 11.7 and 14.0 µg THg/g dw medium methylmercury diet), and 18.4 and 22.2 µg THg/g dw high methylmercury diet), respectively. The arithmetic means of the average male and female whole-body total mercury concentrations (0.40, 3.102, 12.85, and 20.3 µg THg/g dw) were used to represent effect concentrations. Dietary

methylmercury was observed to reduce reproductive capacity based on daily and total number of eggs laid by spawning female fathead minnows in the study, resulting in a 31% reduction of reproductive capacity from control levels observed in the low methylmercury diet fed in Phases 1 and 2. Hammerschmidt et al. (2002) also observed reduced gonadal development ($r^2 = 0.15$, $p = 0.005$, $n = 52$) due to mercury exposure; and, EPA notes that this could contribute to effects on reproductive capacity. For the LOEC, the whole-body mean total mercury concentration of male and female fish fed the low methylmercury diet in Phases 1 and 2 is 3.102 µg THg/g dw, or 0.7245 µg THg/g ww (3.102 ug THg/g ww ÷ 4.28) based on 76.64% moisture content in fathead minnow (USEPA, 2021). USEPA applied a LOEC:NOEC uncertainty factor of 3 (U.S. EPA 1997d) to the LOEC (0.7245 µg/g ww), yielding an estimate for the NOEC of 0.2415 µg THg/g ww based on whole body, or 0.3355 µg THg/g ww based on muscle after application of a WB:M conversion factor of 0.72. EPA recommended these values for use in deriving the mercury criterion from this study.

Source Document: Drevnick, P.E. and M.B. Sandheinrich. 2003. Effects of dietary methylmercury on reproductive endocrinology of fathead minnows. Environ. Sci. Technol. 3(7): 4390-4396.

Test Organism: Fathead minnow (*Pimephales promelas*)

Mercury exposure: Contaminated diets were prepared by mixing commercial fish food (Sterling Silver Cup Fish Food, Nelson and Sons, Inc., Murray, UT) with reagent alcohol containing dissolved methylmercuric chloride similar to dietary preparation described in Hammerschimdt et al. (2002). Mean dietary total mercury concentrations were 0.058, 0.87, and 3.93 µg/g dw in the control, low, and medium exposures, respectively (where "medium" is the highest treatment).

Study Design: Drevnick and Sandheinrich (2003) conducted a similar study as Phase 1 of Hammerschmidt et al. (2002). Juvenile fathead minnows (ninety days post-hatch) were fed a

control diet or methylmercury-contaminated diet quantified as total mercury at one of two concentrations until sexual maturity (approximately 250 days). Ration provided was 5% of body mass per day. After fathead minnows became sexually dimorphic at approximately 300-320 days post-hatch, five breeding pairs from each 180-L dietary exposure aquarium were selected and randomly assigned, within treatment, to one of fifteen 50-L breeding aquaria receiving well water and the same diet as during pre-sexual maturation for reproductive trials and subsequent blood and tissue sample collection.

Effects Data: Growth and survival of fathead minnows were not affected by the dietary methylmercury exposure, however reproductive biomarkers as well as reproductive success were impacted. Methylmercury suppressed testosterone levels in males (ANOVA, $F_{2,12} = 4.941$, $P = 0.03$), as well as estrogen levels in females (ANOVA, $F_{2,12} = 9.135$, $P < 0.01$). Dietary methylmercury also adversely affected the reproductive success (proportion of pairs spawning within 21 days) of fathead minnows in a dose-dependent manner ($X^2_{df=2} = 10.439$, $P < 0.01$). Spawning success was 32% in controls, 12% in the low treatment, and 0% in the highest treatment. The mean total mercury carcass (whole body less plasma and gonads) concentrations (µg THg/g ww) for males and females were 0.071 and 0.079 in controls, 0.864 and 0.917 in the low treatment, and 3.557 and 3.842 in the highest treatment, respectively. The arithmetic mean of the average male and female carcass total mercury concentrations was used to represent effect concentrations (0.0750, 0.8901, and 3.70 µg THg/g ww in control, low and high treatments respectively). Since there was no study concentration between the control and the lowest concentration eliciting a toxic effect (NOEC), EPA estimated the NOEC for this study by applying an uncertainty factor of 3 (U.S. EPA 1997d), to the LOEC carcass concentration of 0.8901 µg THg/g ww (the low exposure) resulting in an estimated NOEC of 0.2967 µg THg/g

ww, based on whole body concentrations, or 0.4121 µg THg/g ww based on muscle tissue after application of a WB:M conversion factor of 0.72. EPA selected these values for use in criterion derivation.

Source Document: Sandheinrich, M.B. and K.M. Miller. 2006. Effects of Dietary Methylmercury on Reproductive Behavior of Fathead Minnows (Pimephales promelas). Environ. Toxicol. Chem. 25(11): 3053-3057

Test Organism: Fathead minnow (*Pimephales promelas*)

Mercury Exposure: Similar to the Drevnick and Sandheinrich (2003) exposure setup discussed above, juveniles were exposed to dietary total mercury concentrations of 0.058 µg/g dw (control), 0.87 µg/g dw (low), and 3.93 µg/g dw ("medium" in similar previous studies, but actually highest exposure in this study). And as previously described in the Hammerschmidt (2002) and Drevnick (2003) studies, wastes and uneaten food were removed from the aquaria daily and relatively little methylmercury dissociated from the diets, minimizing the potential for confounding due to aqueous exposure.

Study Design: Expanding on the previous experiments designed to elucidate reproductive effects in fathead minnow from dietary exposure to methylmercury at ecologically-relevant concentrations, Sandheinrich and Miller (2006) used a similar study design as Hammerschmidt et al. (2002) and Drevnick and Sandheinrich (2003) to examine the effects of dietary methylmercury on the production of testosterone in and the reproductive behavior of male fathead minnows. After fathead minnows became sexually mature, one male and one female fish were selected from each aquarium and assigned randomly to a breeding aquarium receiving well water and the same diet as during pre-sexual maturation for behavioral testing. Breeding pairs were observed over the course of 21 days, or upon successful spawning, whichever came first.

Reproductive behavior was videotaped, spawning success was evaluated, and plasma testosterone was quantified at the end of the trials.

Effects Data: As shown in the related and previously discussed experiments (Hammerschmidt et al. 2002, Drevnick and Sandheinrich 2003), dietary exposure to methylmercury at ecologically-relevant concentrations did not impact growth or survival of fathead minnows. Furthermore, no significant differences were found among treatments in the amount of time spent by male fish in nest preparation ($F_{2,12} = 0.955$, $p = 0.412$) or courtship activities ($F_{2,12} = 0.287$, $p = 0.76$). However, dietary methylmercury did alter the reproductive behavior of male fathead minnows. Exposure suppressed mating behavior ($F_{2,12} = 3.263$, $p = 0.07$). Fish that were fed control, low-, and medium-methylmercury diets respectively spent an average of 5, 0.6, and 0.4% of their time in spawning behavior, resulting in the reduction of reproductive success of pairs of fish exposed at both mercury-contaminated levels (chi-square statistic = 17.5, degrees of freedom = 5, $p < 0.05$). Control fish had a spawning success of 40%, but low- and medium-treatment level fish both had spawning success of 20%. Mean male total mercury carcass (whole body less plasma) concentrations (µg/g ww) were 0.068 in controls, 0.714 in the low exposure, and 4.225 in the medium exposure. Since there was no study concentration between the control and the lowest concentration eliciting a toxic effect (NOEC), EPA estimated the NOEC for this study by applying an uncertainty factor of 3 (U.S. EPA 1997d), to the LOEC male carcass concentration of 0.714 µg/g ww, resulting in a NOEC of 0.2380 µg Hg/g ww based on whole body, or 0.3306 µg Hg/g ww, based on muscle tissue by applying a whole body to muscle conversion factor of 0.72. EPA selected those values for criterion derivation. To derive the SMCV (and GMCV) for the fathead minnow in the genus *Pimephales*, EPA calculated the geometric means of the chronic

values in this study and the previous two studies yielding a tissue-based total mercury GMCV of 0.2574 µg/g ww, as whole body, or 0.3575 µg/g ww expressed as muscle tissue equivalents.

A.2.4 Fourth Most Sensitive Genus, Crayfish (Procambarus clarkii)

Source Document: Brant, H.A, 2004. Chronic dietary methylmercury exposure on three juvenile life stages of the crayfish *Procambarus clarkii.* University of Georgia, MS Thesis, under direction of C. Jagoe.

Test Organism: Red swamp crayfish (*Procambarus clarkii*).

Mercury Exposure: Juvenile crayfish were fed one of two mercury-contaminated diets for 142 days: a low mercury diet containing a mean concentration of 0.009 mg THg/g fresh weight (80% methylmercury) and high mercury diet containing a mean concentration of 0.278 mg THg/g fresh weight (98% methylmercury). The mercury concentrations for the two diets were adjusted by the addition of fish fillets from different sources. Fish used to prepare the high mercury diet were wild-caught largemouth bass (*Micropterus salmoides*) from local reservoirs previously shown to contain elevated concentrations of mercury. Fish used to prepare the low mercury (unofficial, assumed control) diet were commercially available, farm-raised catfish (*Ictalurus punctatus*) purchased at a local grocery store. The diets were formulated from a combination of finely ground spirulina, brine shrimp, alligator chow and fish fillets, all embedded in a matrix of high-quality gelatin and agar. The agar and gelatin were brought to boiling within a liter of water. The spirulina, shrimp and shredded fish fillets were then added to the mixture and stirred to homogeneity. The solution was poured into a vessel that contained a polycarbonate grid along the bottom, spread evenly and allowed to cool and solidify at 1°C refrigeration. The grid provided multiple cubes of food that were homogeneous in size. Crayfish were fed daily *ad libitum* and checked for molting. A cube of diet was placed in each container with an individual crayfish and renewed when it had been nearly consumed.

Study Design: This study evaluated the relationship of sex and age on uptake, elimination, and potential adverse effects of dietary methylmercury on three different age classes (fourth, sixth, and eighth molt) of juvenile red swamp crayfish (*Procambarus clarkii*). Juvenile crayfish were from a stock of mature adult crayfish originally purchased from a local farmer that were acclimated, kept and bred until observation of females bearing eggs. Mean total mercury concentration in these females was 32.16 ng/g dry weight, indicating a low potential for maternal transfer. The egg-bearing females were divided according to the date when eggs were observed which was used to categorize three distinct juvenile age classes, all within 3-4 weeks of each other. Juvenile age class I, the youngest, were approximately three weeks old at the beginning of the feeding experiment and had reached the fourth molt. Juvenile age class II, the middle age group, were approximately five weeks old at the beginning of the experiment and had reached the sixth molt. Juvenile age class III, the oldest age group, were approximately eight weeks old when the feeding experiment began and had reached the eighth molt. Crayfish are considered adults after 12 molts.

The 142-d feeding experiment was conducted using a 2x2x3 randomized block design (2 dietary treatments, 2 sexes, 3 age classes) with a total of 72 crayfish. A total of 36 juvenile crayfish composed of males and females and representing all three age classes were randomly assigned a diet containing either the low or high mercury concentration and housed under flowing well-water conditions (10 liters/hour and aerated) for the duration of the test; water temperature 16 to 18°C. Survival and molting were observed daily, and growth was monitored throughout the duration of the study by taking weight measurements (grams) every seven days. Growth (mass, g) increased in juvenile crayfish of all ages and sexes throughout the duration of 142-d exposure indicating adequate nutrition, and mortality was minimal in the low mercury

diet. Behavior trials consisting of time to find and enter shelter and forced escape response from shelter area were also evaluated.

Effects Data: Chronic exposure to the high mercury diet resulted in higher mortality than in the low (presumed control) mercury diet treatment ($p=0.025$, $c^2 =5.25$), when evaluated based on all three juvenile age classes and both sexes combined. Nine of 36 crayfish died in the high mercury dietary treatment, whereas only 2 died in the low mercury treatment: 72% vs 94% survival, respectively. Crayfish weight at the end of the experiment did not differ between diet treatments in any age, but crayfish fed the high mercury diet took approximately twice the time to find refuge as those fed the low mercury diet. By the end of the experiment, surviving crayfish fed the high mercury diet accumulated a mean total mercury concentration that was orders of magnitude above the crayfish fed the low mercury diet. Although tissue concentration patterns varied slightly between the two diets, crayfish fed either the high or low mercury diet accumulated the most total mercury within the abdominal muscle: approximately 6,000 ng/g dw in the high mercury versus approximately 275 ng/g dw in the low mercury diet. Total mercury was also measured and reported in deceased crayfish; mean total mercury in the abdominal muscle of the nine crayfish killed by high dietary mercury exposure was 7,757 ng/g dw (LOEC) versus 303.3 ng/g dw in the low mercury diet. An 80.77% moisture content of abdominal muscle tissue was applied to the dry weight effect concentration, based on a relationship for crayfish established by Anastacio et al. (1999) – see Appendix D. Using the relationship, the LOEC of 7,757 ng/g dw total mercury is estimated to be 1.491 µg THg/g ww (7,757 ng/g dw or 7.757 µg THg/g ÷ 5.20). EPA divided this value by an uncertainty factor of 3 (U.S. EPA 1997d) to estimate a NOEC for the study of 0.4973 µg THg/g ww based on muscle concentrations, or 0.3581 µg THg/g ww base on whole body equivalence after application of the WB:M conversion factor of 0.72. These

values were used by EPA to represent the relative sensitivity of this species to dietary mercury exposure in the chronic criterion dataset.

A.2.5 Fifth Most Sensitive Genus, Walleye (Stizostedion [Sander] vitreus)

Source Document: Friedmann, A.S., M.C. Watzin, T. Brinck-Johnsen and J.C. Leiter. 1996. Low levels of dietary methylmercury inhibit growth and gonadal development in juvenile walleye (*Stizostedion vitreum*). Aquat. Toxicol. 35: 265-278.

Test Organism: Walleye (*Stizostedion vitreum*)

Mercury Exposure: Fish were maintained on a natural diet (farm-raised catfish fillets), prior to the study, and this same diet was used in the exposures. Fillets were injected with methylmercury (Sigma Chemical Company, St. Louis, MO) dissolved in distilled water resulting in a low mercury diet (0.1 µg Hg/g food) and a high mercury diet (1.0 µg Hg/g food). Analyses confirmed dietary concentrations to which walleye were exposed as control (< 0.04 µg THg/g ww]), low dose (0.137 µg THg/g ww), and high dose diet (0.987 µg THg/g ww). Test organisms were fed 1gram pieces, three times per week, increased to 1.5 grams at three and half months into the 6-month exposure period. Diets were supplemented with uncontaminated and MeHg-injected fathead minnow (1.3-1.5 grams) approximating the MeHg doses in the catfish fillets at 6 weeks after exposure initiation.

Study Design: Hatchery-raised juvenile (6-month-old) walleye were randomly assigned and acclimated in four 180 1 aquaria (22 animals per tank) over a period of two and a half months. Fish length (total) and weight were recorded after acclimation, then exposed to methylmercury for six months via a natural diet. At the end of the six-month exposure, mercury body burdens were determined, as well as dietary methylmercury effects on growth, gonadosomatic index (GSI), and cortisol levels. Walleye body burdens were 0.06 µg THg/g ww (control fish), 0.25 µg THg/g ww (low dose diet) and 2.37 µg THg/g ww (high dose diet).

Effects Data: Mortality in low dose (45%) and high dose (32%) were evaluated against control mortality rates (28%) using Kaplan-Meier survival statistics, and differences were not significant. EPA therefore used this study, even though control survival was slightly elevated. Elevated control mortality illustrates the difficulty in maintaining larger wild fish species for long exposure durations. Methylmercury exposure did have a significant negative effect on both fish length (r=0.82; P-C 0.004) and weight (approximately 25-30% reduction; r= 0.74; $P < 0.02$). Also, gross measurement and histological assessment of the gonads revealed effects of dietary methylmercury exposure on reproductive potential in walleye. Although the mean GSI between exposed and control fish did not show a concentration-dependent effect by ANOVA, pooled analyses of control versus exposed fish showed a significant decrease in GSI for male fish, and histological examination revealed testicular atrophy in both mercury-exposed groups, with severity being dependent on dietary dose. Also, cortisol levels were significantly lower in fish reared on the low-mercury dietary dose compared to controls, although cortisol in fish reared on the high-mercury diet was not. To evaluate the data for a possible effect of the anesthesia used on cortisol levels, plasma steroid levels and sampling order within each tank were analyzed by regression analysis. No significant correlations were detected.

Based on the approximately 25-30% reduction in weight gain at the high mercury exposure, the tissue total mercury NOEC and LOEC for walleye were determined to be 0.25 and 2.37 µg/g ww, respectively, yielding an MATC of 0.7697 µg THg/g ww as a whole-body concentration, and 1.069 µg THg/g ww as a muscle concentration equivalent based on application of a WB:M conversion factor of 0.72. These values were utilized by EPA in criterion derivation.

A.2.6 Sixth Most Sensitive Genus, Tiger fish (Hoplias malabaricus; non- resident species)

Source Data: Oliveira-Ribeiro, C.A., F. Filipak Neto, M. Mela, P.H. Silva, M.A.F. Randi, I.S. Rabitto, J.R.M. Alves Costa and E. Pelletier. 2006. Hematological findings in neotropical fish Hoplias malabaricus exposed to subchronic and dietary doses of methylmercury, inorganic lead, and tributyltin chloride. Environ. Res. 101(1): 74-80.

Costa, J.R.M.A., M. Mela, H.C. da Silva de Assis, E. Pelletier, M.A.F. Randi and C.A. Oliveira Ribeiro. 2007. Enzymatic inhibition and morphological changes in *Hoplias malabaricus* from dietary exposure to lead(II) or methylmercury. Ecotoxicol. Environ. Saf. 67: 82-88.

Mela, M., M.A.F. Randi, D.F. Ventura, C.E.V. Carvalho, E. Pelletier and C.A. Oliveira Ribeiro. 2007. Effects of dietary methylmercury on liver and kidney histology in the neotropical fish *Hoplias malabaricus*. Ecotoxicol. Environ. Saf. 68: 426–435.

Test Organism: Tigerfish (*Hoplias malabaricus*)

Mercury Exposure: Tiger fish (trajira) were fed doses of a single dietary concentration of MeHg over a period of 10 weeks with an additional fish serving as controls. The diet consisted of *Astyanax*, a genus of freshwater fish in the family Characidae, which were individually injected intraperitoneally with MeHg chloride (CH_3HgCl) to reach a nominal does of 0.075 µg/g wet weight MeHg. Control animals were fed in the same manner with prey items injected with 1 ml of distilled water. Experimental tiger fish were fed individually their own prey item (approximately 10% of the wet weight of the test fish) over a course of five days to ensure complete ingestion of prey; constituting one of the 14 doses provided over the course of the experiments. While mercury concentrations in prey items were not measured, the authors estimated each fish received a daily dose of approximately 0.015 µg/g wet weight MeHg for a period of 70 days based on the 14 doses provided.

Study Design: The collective studies conducted and reported by Olivera-Riberio et al. (2006), Costa et al. (2007), and Mela et al. (2007) examined the effects of dietary exposure on survival and multiple biochemical endpoints in tigerfish exposed to methylmercury injected into prey (*Astyanax* spp.). For each study, thirty-five mature tigerfish (H. malabaricus, ~110.8749 g) were obtained from native waters in Brazil. Before initiation of the contaminated diet, tigerfish were

held individually in 30 L tanks filled with dechlorinated tap water for 30 days to acclimate to experimental conditions (21°C, 12:12 hour photoperiod). Tigerfish in the MeHg-contaminated diet averaged 150 g in weight and 23.72 cm in length with control fish averaging 71 g and 20.05 cm. At the end of 70 days fish were sacrificed with liver and muscle tissue frozen for mercury analysis. Additionally, liver, blood and kidney samples were taken. Measured total mercury concentrations in liver and muscle for the contaminated diet group were 1.69 and > 1.45 µg/g wet weight, while control fish were 0.601 and 0.67 µg/g wet weight, respectively.

Effects Data: Although no mortality occurred throughout the experiments, histological examinations of liver and kidneys from the three studies showed signs of organ injury in tigerfish fed contaminated prey. Similarly, there were significant decreases in red blood cells counts, hemoglobin concentration, hematocrit percentages, leukocytes counts, neutrophils counts, mononuclear cells counts, and mean corpuscular volume in the blood of exposed fishes. Additionally, erythrocyte δ-aminolevulinic acid dehydratase (ALAd) activity and muscle cholinesterase (ChE) activity were inhibited in exposed individuals. For criterion derivation EPA used the NOEC for mortality of >1.45 µg THg/g ww in muscle, or >1.04 µg THg/g ww expressed as whole-body equivalence after application of the WB:M conversion factor of 0.72 representing the relative insensitivity of this species for overt toxic effects to dietary exposures to methylmercury.

A.2.7 Seventh Most Sensitive Genus, Channel catfish (Ictalurus punctatus)

Source Document: Schlenk, D., M. Chelius, L. Wolford, S. Khan and K.M. Chan. 1997. Characterization of Hepatic Metallothionein Expression in Channel Catfish (*Ictalurus punctatus*) by Reverse-Transcriptase Polymerase Chain Reaction. Biomarkers (Lond.) 2(3):161-167.

Test Organism: Channel catfish (*Ictalurus punctatus*)

Mercury Exposure: Juvenile channel catfish (12-15 cm) were fed Japanese medaka (*Oryzias latipes*) injected with solutions of methylmercuric chloride to provide a nominal daily dose of 0.1

µg/g total mercury wet weight. Channel catfish were fed medaka daily and then ARKAT catfish food at 2% of their body weight during acclimation to study conditions.

Study Design: The expression of hepatic metallothionein (MT) was investigated in juvenile channel catfish (*Ictalurus punctatus*) fed dietary methylmercury for 30 days. Channel catfish (12-15 cm) were obtained from the U.S. Department of Agriculture National Aquaculture Laboratory in Stuttgart, Arkansas and acclimated in flow-through aquarium filled with carbon-filtered dechlorinated tap water to test conditions (18-22℃). Fish were fed daily to ARKAT catfish chow during acclimation at 2% of their body weight. After acclimation, the diet for the channel catfish was prepared by lethally injecting Japanese medaka (*Oryzias latipes*) with solutions of methylmercuric chloride to provide a nominal daily dose of 0.1 µg/g total mercury wet weight. Channel catfish were fed medaka daily and then ARKAT catfish food at 2% of their body weight. After 30 days, catfish were euthanized, weighed and the liver dissected. Total mercury was measured in the axial muscle and liver. Hepatic metallothionein (MT) expression was measured and condition factors [100 x (body weight, g)/standard length, cm)^3] and liver somatic indices (LSI) (percent body weight represented by the liver) were calculated in untreated and mercury-treated fish. Total mercury concentrations were significantly greater in fish fed methylmercury-contaminated medaka with a reported range of 1.2-1.8 µg THg/g wet weight (average of 1.6 µg THg/g ww).

Effects Data: There was no effect on condition factor, LSI and MT expression between the control and mercury fed fish. The NOEC of >1.6 µg THg/g ww, or >1.15 µg THg/g (after application of the WB:M conversion factor of 0.72) based on no effect on growth was used by EPA to represent the relative sensitivity of this species to dietary mercury exposure in the chronic criterion dataset.

A.2.8 Eighth Most Sensitive Genus, Goldfish (Carassius auratus)

Document Source: Crump, K. 2008. The effects of methylmercury on the reproductive axis of goldfish (*Carassius auratus*). M.S. Thesis, University of Ottawa, Canada. 117 pp.

Test Organism: Goldfish (*Carassius auratus*)

Mercury Exposure: Contaminated diets were prepared by mixing floating trout pellets (Martin Mills Inc, Ontario, Canada) with 95% ethanol containing dissolved methylmercury (II) chloride (CH3HgCl; Sigma Aldrich, Oakville, Ontario, Canada) at nominal concentrations of 0.8 µg/g ww (low), 4.0 µg/g ww (medium) and 8.0 µg/g ww (high). After the ethanol was evaporated, prepared diets were stored in the dark at -20°C in 50 mL tubes. Methylmercury (measured as THg) for the pre-spawning diets were 0.035 (control), 0.69 (low), 4.48 (medium) and 7.78 (high) µg/g total mercury wet weight and 0.022 (control), 0.83 (low) and 8.21 (high) µg/g total mercury wet weight in post-spawning diets; the medium diet was not used in the post-spawning exposure.

Study Design: Lifetable and endocrine effects of dietary sub-chronic methylmercury exposure on adult goldfish (*Carassius auratus*) at two different periods within the annual spawning cycle. Two experiments were conducted one with pre-spawning females (March-April, 2007) and one with post-spawning females (May-June, 2006). Female adult goldfish were purchased from a commercial supplier in February 2007 (pre-spawning) or April 2006 (post-spawning) and acclimated over several weeks to tests conditions (18°C, natural photoperiod and a diet of floating trout pellets). In the pre-spawning exposure 13-15 individuals were placed in 70 L flow through tanks (dilution water not identified) and fed one of four treatment diets for 28 days; each treatment was replicated five times with additional exposure tanks. In the post-spawning exposure 13 individuals were placed in the same tanks with each treatment replicated four times. After 28 days, fish were euthanized, weighed and a blood sample was collected. Fish were sacrificed, and gonads, brain and pituitaries were dissected for RNA isolation, luteinizing

hormone (LH) analysis and total mercury concentrations. Testosterone (T) and 17β-estradiol (E2) concentrations in the blood were also measured. There was no significant mortality and growth (length and weight) effect of any dietary mercury treatment on goldfish in both the pre-spawning and post-spawning exposure. The % GSI (gonadosomatic index) in adult females was significantly inhibited in pre-spawning exposure at the high dietary treatment, but this effect was not observed in the post-spawning exposure. This is expected since goldfish undergo an annual cycle of gonadal growth reaching a maximum GSI just prior to spawning in May. In the pre-spawning exposure, control fish had a 7.2% GSI and fish in the highest treatment had a 3.2% GSI. Similarly, T and E2 concentrations in the blood was also significantly reduced in pre-spawning fish at the high dietary treatment, but not in the post-spawning exposure.

Effects Data: Average total mercury concentration in the muscle of pre-spawning goldfish were 0.02 (control), 0.201, (low), 0.949 (medium) and 2.037 (high) µg THg/g ww. No significant effects to mortality or growth of goldfish were observed in either the pre-spawning and post-spawning exposure. The NOEC of >2.037 µg THg/g wet weight measured in muscle tissue or >1.47 µg THg/g as whole-body concentration (estimate based on application of WB:M conversion factor of 0.72) were used to represent the relative sensitivity of this species to dietary mercury exposure in the chronic criterion dataset.

A.2.9 Ninth Most Sensitive Genus, (Huso) Beluga Sturgeon

Gharaei et al. (2008, 2011) Source Documents: Gharaei, A., A. Esmaili-Sari, V. Jafari-shamoshaki and M. Ghaffari. 2008. Beluga (Huso huso) bioenergetics under dietary methylmercury. Fish Phyiol. Biochem. 34: 473–482.

Gharaei, A., M. Ghaffari, S. Keyvanshokooh and R. Akrami. 2011. Changes in metabolic enzymes, cortisol and glucose concentrations of beluga (Huso huso) exposed to dietary methylmercury. Fish Physiol. Biochem 37:485–493.

Test Organism: Beluga sturgeon (*Huso huso*)

Mercury Exposure: For both studies, a fish meal (62.8 % herring powder) based diet containing sufficient nutrients to meet the sturgeons' dietary needs was prepared. The prepared diet was stabilized with gelatin to reduce dissolution of the pellets in water, minimizing methylmercury release. Then methylmercuric chloride dissolved in ethanol was combined with the fish meal preparation to achieve dietary concentrations of 0.04 mg/kg (control); 0.76 mg/kg (low mercury); 7.88 mg/kg (medium mercury) and 16.22 mg/kg (high mercury). Total mercury content in the diet was confirmed from three random samples per treatment.

Study Design: The focus of the Gharaei et al. (2008) experiment was bioenergetics where the researchers focused on the adverse effects on beluga sturgeon mortality, food consumption, and specific growth rate based on a 70 day dietary exposure, whereas the companion study, Gharaei et al., (2011), focused on the effects of dietary methylmercury exposure on several blood biochemical parameters including GLU (glucose), LDH (lactate dehydrogenase), AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase) and cortisol. One hundred juvenile beluga were transferred from the reproduction facility to the laboratory to acclimate to feeding and test conditions for 3 weeks. A flow through system was employed with aerated test water with under the following test conditions: pH 7.6-7.8, 25℃, hardness of 255 mg/L as CaCO3, alkalinity of 232 mg/L as $CaCO_3$, dissolved oxygen 6.5-7 mg/L and a photoperiod of 19:9 hour light:dark. Animals were fed an experimental diet three times per day based on fish biomass. After acclimation, 20 fish were distributed to twenty 500 L tanks each. Each treatment was replicated five times with 100 fish total per treatment.

Effects Data: Mean muscle concentrations at day 70 were <0.05, 3, and 9 µg THg/g ww for the control, 0.76 and 7.88 µg/g total mercury dw diets. While only 2-3% percent mortality was observed in the control, low, and mid-level treatment diets, 100% mortality was observed in the

highest test diet with death occurring between 40 and 42 days. The most sensitive apical endpoint from these studies was specific growth rate measured from day 36 to day 70 with the two lowest mercury supplemented test diets having SGR significantly less than the control. The control SGR in this time period averaged 2.3 g, whereas specific growth rate for the low and medium treatments were 2.06 g and 1.31 g, a 10.4% and 41% difference from the specific growth rate of the control, respectively. In Gharaei et al. (2011), the samples collected by Gharaei et al. (2008) from five beluga sturgeons at four interim time periods (day 0, 35, 42 and 70) used in the determination of mean muscle tissue concentrations were also used for assessment of biochemical parameters. By day 32, blood parameters for AST, ALT, LDH, GLU and cortisol levels remained high in all dietary treatment groups, while ALP activity decreased significantly compared to the control. The levels were almost approximately two times higher or lower in the highest test treatment compared to the control.

The most sensitive apical endpoint from these studies was specific growth rate measured from day 36 to day 70 with fish exposed to the two lowest mercury supplemented test diets having specific growth rate significantly less than the control fish (Gharaei et al. 2008). Since the percent effect of the low dietary mercury treatment approximated an EC_{10} level of effect, the EPA selected the muscle tissue concentration of 3 µg THg/g ww (or 2.16 µg THg/g ww as an estimated whole-body concentration based on application of a WB:M conversion factor of 0.72) as the values to represent the sensitivity of this species to dietary mercury exposure in the chronic dataset.

A.2.10 Tenth Most Sensitive Genus, Atlantic salmon (Salmo salar)

Document Sources: Berntssen, M.H.G., A. Aatland and R.D. Handy. 2003. Chronic dietary mercury exposure causes oxidative stress, brain lesions, and altered behaviour in Atlantic salmon (Salmo salar) parr. Aquat. Toxicol. 65: 55-72.

Berntssen, M.H.G., R. Hylland, K. Julshamn, A-K. Lundebye and R. Waagbo. 2004. Maximum limits of organic and inorganic mercury in fish feed. Aquacult. Nutrit. 10: 83-97.

Test Organism: Atlantic salmon parr (*Salmo salar*)

Mercury Exposure: Nine experimental diets (control, four graded levels of organic or inorganic mercury) were prepared (Berntssen et al. 2003) based on fish meal, wheat, capelin oil and gelatin was used, along with ground wet squid added to enhance palatability of the experimental diets. A stock of finely ground methylmercuric chloride and mercuric chloride in wheat meal was added and mixed well with the other feed ingredients. The mixture was cold pelleted after adding 12% (w/w) water in a food extruder. Pellets were dried at 50°C for 24 hours and stored at -20°C until being fed to fish. Final mean measured dietary mercury concentrations were 0.14 (control), 1.89, 8.84 and 102.6 µg/g dw (inorganic mercury) and 0.12 (control), 0.63, 4.35 or 8.48 µg/g dw (organic [methyl] mercury). In a follow-up study, measured total mercury concentrations were 0.03, 4.35 and 8.48 µg/g dry weight in the control, 5 µg THg/g, and 10 µg THg/g supplemented diets, respectively (Berntssen et al. 2004). Organic mercury was calculated from the difference between measured total and inorganic mercury concentrations measured in the food.

Study Design: Atlantic salmon (*Salmo salar* L.) parr (14.7 ±3.8 g) were exposed to several levels of dietary organic and inorganic mercury to document sublethal toxicity threshold levels in this species and assess feed-fillet transfer of dietary mercury. Atlantic salmon parr were bred locally at the station where the experiments were carried out (Matre Aquaculture Research Station, Institute of Marine Research, Matredal, Western Norway). Initially, all fish were fed a control diet, without mercury supplementation for 2 weeks to acclimate them to experimental conditions. Thereafter each of the nine experimental diets (control, four graded levels of organic or inorganic mercury) were fed to fish in duplicate tanks for 4 months according to standardized in-house growth tables for salmonids, with 2.6% of body weight being fed during the first month, 2.2%

during the second month, and 2.0% during the last 2 months. Berntssen et al. (2003) reported biochemical (metallothionein, plasma enzymes), histopathological (cell proliferation), hematological (hematocrit), physiological (plasma creatinine and blood hemoglobin), hepatic somatic index (HSI), nutritional (digestibility) and apical (growth, body composition, and survival) test endpoints. Bernstssen et al. (2004) reported on the neurotoxic effects of mercury *in vivo* and brain lipid peroxidation.

Effects Data: Mean muscle tissue concentrations (reported in Berntssen et al. 2004) were 0.09 (control), 1.09 (low MeHg), and 3.07 µg THg/g ww (high MeHg). No effects of dietary methylmercury on survival or growth (final weight) were observed in any of the test treatments. Carcass composition was not significantly affected by dietary methylmercury or inorganic mercury concentrations. The apparent digestibility of protein and glycogen was significantly inhibited in fish fed the highest and second highest levels of inorganic mercury, but not methylmercury. The most sensitive endpoints observed from dietary methylmercury exposure were decreased hematocrit and increases in liver metallothionein and increases in intestinal pathology in parr fed 8.48 µg/g dw diet. The lack of impact on survival and growth resulted in determination of a muscle tissue NOEC of > 3.07 µg/g THg ww (or >2.21 µg/g THg ww as a whole-body estimate based on application of the WB:M conversion factor of 0.72). The EPA used these values to represent the relative sensitivity of this genera for criterion derivation. Although not used quantitatively, post-feeding activity (assessed via two repeat feeding activity trials) decreased in fish exposed to the high MeHg dietary treatment compared to fish from the control treatments. The decrease was only statistically significant after the second feeding trial, resulting in a potential NOEC of 1.09 and LOEC of 3.07 µg/g wet weight total mercury based on feeding behavior (MATC = 1.829 µg/g wet weight total mercury).

A.2.11 Eleventh Most Sensitive Genus, Rainbow trout (Oncorhynchus mykiss)

Rodgers, D.W. and F.W.H Beamish. 1982. Dynamics of dietary methylmercury in rainbow trout, *Salmo gairdneri*. Aquat. Toxicol. 2: 271–290.

Test Organism: Rainbow trout (*Oncorhynchus mykiss*) fingerlings

Mercury Exposure: Diets were prepared by grinding a commercial trout food to a powder and then mixing it with a 2:1 aqueous solution with the necessary amount of methylmercuric chloride to reach the five nominal test concentrations, before being lyophilized and frozen until use. Average measured concentrations in the test diet for this experiment were <0.1 (0/ad lib) 23.9 (25/ad lib), 46.9 (45/ad lib) and 94.8 (95/ad lib) µg/g total mercury dry weight.

Study Design: The effects of dietary methylmercury on rainbow trout was investigated for exposure to one of four dietary test treatments (nominal, 24, 45, 75 or 95 µg/g, assumed dry weight) and a control for 84 days (12 weeks). Fingerlings were purchased from a commercial hatchery and held in 50 L circular tanks filled with aerated ground water for three weeks to acclimate to test conditions and diet. A partition in tanks forced rainbow trout to swim against a current of 5-10 cm/s. Water quality conditions were an average test temperature of 10.5°C, dissolved oxygen > 80% saturation, total hardness of 380-390 mg/L, and pH 7.9-8.1. The photoperiod used in the study was16 hour light:8 hour dark. Total mercury concentrations were measured in both the fish and diet. Three separate sets of experiments were conducted where the treatment levels and feeding regime varied.

In the first experiment 65 fingerlings (5.5 g) each per tank were assigned to one of four treatments and fed daily for a 5-min period for a period of twelve weeks, this exposure is considered *ad libitum*. Ten fish were sampled from each treatment before test initiation and at test days 14, 28, 56 and 84. Mercury concentrations in the whole body of fish fed *ad libitum* were estimated visually from a figure to be <0.1 (control), 10, 23, and 29 µg/g total mercury wet

weight. Fish fed mercury supplemented diets were discolored and exhibited a decrease in appetite leading to a decrease in growth (weight) in fish exposed to all three MeHg-contaminated dietary treatments. The LOEC (based on growth) for the *ad libitum* experiment is 10 µg/g total mercury wet weight whole body tissue.

In the second set of experiments 65 fingerlings (5.7 g) each per tank were assigned to one of six treatments and fed a control or MeHg-contaminated diet for a period of twelve weeks, but at either1% or 2% wet weight per day with each exposure defined as 0/1%, 25/1%, and 75/1% in the one experiment and as 0/2%, 25/2% and 75/2% in the other. There were two replicates for each of the six possible treatments. Measured mercury concentrations in the diets were <0.1, 23.2 and 76.5 µg/g total mercury dry weight for the control (0), 25 and 75 nominal diets, respectively. Fish were anesthetized and weighed for growth estimation on day 3, 14, 28, 42, 56, 70 and 84 of the experiment with rations (diets) adjusted based on fish weight. Five fish were sampled from each treatment before the test initiation and at 14, 28, 56 and 84 days of exposure.

Effects Data: Mercury concentrations in the whole body of fish were estimated visually from a figure to be <0.1 (0/1% and 0/2%), 8 (25/1%), 9 (25/2%), 29 (75/1%) and 35 (75/2%) µg THg/g wet weight. Effects of dietary mercury on fish growth varied between the two fixed ration diets. In the 2% per day ration, both final weight and growth rate of fish were significantly less than control fish, however in the 1% per day ration, these effects were not seen. Fish fed the higher ration (2%) were almost twice as big as the fish fed the smaller ration at the end of the experiment. Amongst groups the final average wet weight of fish was 28.2 (0/2%), 19.7 (25/2%), and 17.9 (75/2%) for the 2% ration diet and 12.4 (0/1%), 10.2 (25/1%) and 9.7 (75/1%) g for the 1% ration diet. The LOEC for the 2% per day ration experiment, based on growth rate and weight, is 9 µg THg/g wet weight whole body tissue and is similar to the *ad libitum* experiment.

For the 1% per day ration experiment the NOEC, based on growth, is 29 µg THg/g wet weight whole-body tissue. EPA used the LOEC of 9 µg THg/g and 10 µg THg/g wet weight whole body tissue from the 2% ration and *ad libitum* experiment which, after application of the uncertainty factor of 3 (U.S. EPA 1997d) yields an estimate for the NOEC of 3.0 µg THg/g ww and 3.333 µg THg/g ww as whole body or 4.17 µg THg/g ww and 4.63 µg THg/g ww as muscle equivalents, based on application of the WB:M conversion factor of 0.72. EPA used the geometric mean of the two studies, 3.162 µg THg/g ww whole body and 4.392 µg THg/g ww as muscle to represent the relative sensitivity of rainbow trout to dietary mercury exposure in the sensitivity distribution. EPA used the geometric mean of the two estimated NOECs from Rogers and Beamish (1982) because the fingerlings for this study were approximately 4 times smaller (5.6 g vs 20.9 g), thus younger and potentially more sensitive than fingerlings used in the Wobeser study and the exposure was approximately 3.5 times longer (84 days vs 24 days) than the Phillips and Buhler study (see summaries of the latter two studies in Appendix B).

A.2.12 Twelfth Most Sensitive Genus, Zebrafish (Danio rerio)

Source Documents: Penglase, S., K. Hamre and S. Ellingsen. 2014a. Selenium and mercury have a synergistic negative effect on fish reproduction. Aquat. Toxicol. 149: 16-24.

Penglase, S., K. Hamre and S. Ellingsen. 2014b. Selenium Prevents Downregulation of Antioxidant Selenoprotein Genes by Methylmercury. Free Radic. Biol. Med. 75:95-104.

Test Organism: Zebrafish (*Danio rerio*)

Mercury Exposure: Multiple dietary regimens were used, diet from Penglase et al. (2014a, 2014b) is reported here as results from this study were used in criterion derivation. A basal zebrafish experimental diet was formulated from casein, gelatin, vitamins, minerals and spiked with selenium (as seleno-l-methionine (SeMet)) at 0.7 or 10 mg Se/kg dw and mercury (as methylmercury chloride (MeHg)) at 0.05 or 12 mg THg/kg dw, sourced from Sigma–Aldrich (Germany). Only the low selenium diet was considered in this assessment. Fish were fed to

satiation twice daily with the prepared basal experimental diet equivalent to 3% of the estimated wet weight of fish biomass per day fed as dry weight; the percentage fed decreased to 1% as fish grew and the test continued (up to >150 dpf).

Study Design: The interactive effects of dietary selenium and methylmercury on zebrafish growth, survival, and reproduction were investigated in a 2 x 2 factorial study including two selenium (0.7 and 10 µg/g selenium dry weight) and two mercury (0.05 and 12 µg/g total mercury dry weight) diet levels added as seleno-L-methionine and methylmercury chloride, respectively. The combination of the low Se and low Hg test levels were designed by the authors to be a nutritionally-optimal diet and represents a control diet. The focus of this study (and results selected for criterion derivation) is on those diets not supplemented with selenium; identified as "(-)Se" by the authors. In-house cultures of zebrafish larvae were transferred at 12 days post fertilization (dpf) to 9 L tanks with recirculating water held under the following conditions: 28.5°C, 500 µS EC, pH 7.6, 10% daily water exchange. Photoperiod used was14:10 light:dark. At 73 dpf, 15 female fish (320 mg, 26 mm) each were transferred to into one of twelve 3 L tanks, with 3 tanks per treatment. Note that some males were inadvertently placed in the tanks, with an average of three male fish per tank. These were removed at test day 50. Measured selenium and total mercury concentrations in the test diet were 0.69 and 0.06 µg/g dry weight, respectively for the control [(-)Se/(-)Hg] and 0.74 and 11.98 µg/g dry weight, respectively for the mercury supplemented diet [(-)Se/(+)Hg]. Fish were weighed before the test diet commenced (73 dpf), 123 dpf and 266 dpf; experimental diets with elevated Hg and no selenium were discontinued at 218 dpf due to welfare concerns. At test day 50 (123 dpf), one fish per tank (3 per treatment) was sacrificed for quantification of tissue total mercury concentration. At test days 58-71 a subset of female fish from each tank were transferred to 9 L

spawning tanks which contained male fish (not treated) to determine mercury concentrations in the eggs. To study reproductive effects, 3 female fish per tank were mated individually with a single unexposed male at test day 78, 97 and 133: resulting in 27 pairings per treatment. Surviving embryos from these parings were used in further experimentation to study the maternal transfer effects on mRNA expression of selenoprotein genes and larval locomotor activity (Penglase et al. 2014b). Whole body total mercury concentrations measured in adult female fish were 0.27 and 33.31 µg/g dry weight for the control [(-)Se/(-)Hg] and mercury supplemented[(-)Se/(+)Hg] diets, respectively. Based on a moisture content of 71.3% in the fish these concentrations equate to 0.077 and 9.560µg/g wet weight whole body. Corresponding whole body total mercury concentrations measured in the F1 generation (eggs measured at ≤4 hpf) were 0.61 and 12.71 µg/g dry weight for the control and mercury supplemented diets, respectively. Based on a 75% moisture content in fish eggs these concentrations equate to 0.1524 and 3.178 µg/g wet weight.

Effects Data: At test termination there was a significant decrease in survival (73.3 % versus 97.8%), weight (0.836 g versus 1.308 g), and condition factor in fish fed mercury supplemented diets compared to the control (Penglase et al. 2014a). Dietary MeHg effects on reproduction were less conclusive, with elevated Hg diets having improved mating and overall reproductive success in the short-term (<100 days on diet), but as exposures continued these metrics were decreased (i.e., reduced reproductive success). However, after 100 days of dietary mercury exposure fish also began to show signs of lethargy and mortality, so dietary MeHg may not be targeting reproductive systems. Fish in the F1 generation also had a 20-60% reduction in various locomotor metrics and a ~80% reduction in mRNA expression of selenogenes (GPX1a and GPX4a activity) as compared to the control. The most sensitive apical endpoints in the studies by

Penglase et al. were growth (weight and condition factor) and survival in the F0 generation, with a reported LOEC of 9.560 µg/g total mercury wet weight whole body tissue. This value is used to represent the relative insensitivity of this species to dietary mercury exposure in the chronic criterion dataset. which, after application of the uncertainty factor of 3 (U.S. EPA 1997d) yields an estimate for the NOEC of 3.187 µg Hg/g ww as whole-body tissue, or 4.426 µg Hg/g ww as muscle tissue. Since these values were the lowest values of the zebrafish studies, EPA selected these values to represent the sensitivity of zebrafish relative to the other species in the dataset. The remainder of the zebrafish studies are discussed in **Appendix B**.

A.2.13 Thirteenth Most Sensitive Genera, Mayfly (Hexagenia bilineata)

Source Document: Naimo, T.J., J.G. Wiener, W.G. Cope and N.S. Bloom. 2000. Bioavailability of sediment-associated mercury in Hexagenia mayflies in a contaminated flood plain river. Can. J. Fish. Aquat. Sci. 57: 1092-1102.

Test Organism: Field-collected mayfly nymphs (almost entirely *Hexagenia bilineata*)

Mercury Exposure: To supply organic carbon to support microbial activity, each beaker was provided with dried, finely ground leaves of submersed aquatic plants. Beginning 2–3 days before day 0 of a test, 193 ± 5 mg dry weight of curly pondweed or 228 ± 5 mg dry weight of wild celery was added to each beaker every third day. Concentrations of methylmercury in a subsample of the plant homogenate were 5.1, 1.1, 5.3, and 4.2 ng Hg/g dry weight in tests 1, 2, 3, and 4, respectively. Mean total mercury ranged from 880 to 22,059 ng/g dw in contaminated sediments and from 90 to 272 ng/g dw in reference sediments. Mean final concentrations of methylmercury in test water were greatest (8–47 ng/L) in treatments with contaminated wetland sediments, which had mean total mercury ranging from 1,200 to 2,562 ng/g dw.

Study Design: Field-collected mayfly nymphs (*Hexagenia spp.*) were exposed to mercury-contaminated and reference sediments collected from Sudbury River subbasin in Massachusetts to examine differences in bioavailability and mercury transfer in the benthic food web.

Bioaccumulation tests (21 days) were conducted with sediments sampled from impoundments, flowing reaches, and a riverine lake during July (test 1) and September (test 2) in 1994, and wetland areas within the river floodplain, with sediments obtained in May (test 3) and September (test 4) in 1995. Each bioaccumulation test employed a randomized block experimental design; tests 1 and 2 included six replicates of six sediment treatments, whereas tests 3 and 4 had nine replicates of four sediment treatments. Within each area, surficial sediments (uppermost 4–6 cm) were obtained from randomly selected sampling sites, identified by latitude and longitude coordinates. The experimental unit in each bioaccumulation test was a 4-L glass beaker containing 725 mL of wet sediment from a sampling site and 2.9 L of test water, providing a 4:1 (v/v) water to sediment ratio. A temperature of 20 ± 2°C and photoperiod of 16 h light:8 h dark was maintained. The overlying test water was soft, similar in hardness and pH to water from the Sudbury River. During each test, the overlying water in each beaker was aerated to maintain dissolved oxygen >5 mg/L. The *Hexagenia* nymphs (almost entirely *Hexagenia bilineata*) were obtained within a day before the start of each bioaccumulation test from an area on the Upper Mississippi River with low mean total Hg concentrations in both sediment and resident *Hexagenia*. Each mayfly was measured (total length) before being transferred into a test beaker. Fifteen nymphs, ranging from 10 to 19 mm in total length, were randomly allocated to each beaker 7–10 days after the test sediment and water had been placed into the beaker. Day 0 of a given test was defined as the day on which mayflies were introduced into the beakers.

Effects Data: In mayflies, final mean concentrations of methylmercury were highest in treatments with contaminated wetland sediments (122–183 ng/g dw), intermediate in treatments with contaminated sediments from reservoirs, flowing reaches, and a riverine lake (75–127 ng/g dw), and lowest in treatments with reference sediments (32–41 ng/g dw). The overall survival of

Hexagenia mayflies ranged from 90 - 96% in all treatments and growth was not correlated with mercury concentrations in test sediment. The greatest mean total mercury concentration measured in *Hexagenia* during the study was 10,819 ng THg/g dw, or 10.819 µg THg/g dw. The average percent moisture value for the mayfly families was 67.5 (Appendix D). The NOEC of 10.819 µg/g THg dw was divided by a factor of 3.08 to calculate the NOEC of > 3.516 µg/g THg ww, which represents the SMCV for the genus, *Hexagenia*; the value used by EPA to represent the relative sensitivity of these species to dietary mercury exposure in the chronic criterion dataset.

A.2.14 Fourteenth Most Sensitive Genus, Sacramento blackfish (Orthodon microlepidotus)

Document Source: Houck, A. and J.J. Cech, Jr. 2004. Effects of dietary methylmercury on juvenile Sacramento blackfish bioenergetics. Aquat. Toxicol. 69: 107–123.

Test Organism: Sacramento blackfish, juvenile (*Orthodon microlepidotus*)

Mercury Exposure: Diets consisted of commercial trout chow that was ground and thoroughly mixed with water and the appropriate concentration of methyl mercuric chloride dissolved in 100% ethanol. Gelatin (6%) was added to reduce solubility before the mixture was dried. Fish were fed, in excess, a pre-weighed amount per day. Fish were weighed and measured individually on days 0, 35 and 70.

Study Design: Houck and Cech (2004) investigated the bioenergetics of juvenile Sacramento blackfish (*Orthodon microlepidotus*) fed one of four measured diets containing MeHg [0.21 (control), 0.52, 22.2 and 55.5 µg/g total mercury dry weight] for 70 days. The experiment was extended up to 247 days to investigate MeHg accumulation and survival. Adult fish were purchased from a commercial supplier and brought to the laboratory where they spawned naturally. Larvae were collected and raised in 38 L tanks until testing. Thirty fish were each assigned to aquaria and fed one of the four test treatments. Each treatment was replicated four

times. Tanks contained aerated well water and maintained at 23°C, with dissolved oxygen levels at 7-8 mg/L, pH 7-7.5 and a 16:8 hour light:dark photoperiod. Eighteen fish per treatment (3/tank) were sampled on day 0, 35 and 70 and frozen for total mercury concentrations in the muscle. Muscle concentration at test day 247 were visually estimated from a figure to be 0.52, 2.3, 25, 33 µg/g total mercury wet weight for the control, low, medium, and high test diets. While total mercury was measured in the muscle tissue, independent MeHg analysis from an outside laboratory confirmed that total mercury muscle concentrations approximated MeHg concentrations.

Effects Data: For the first 70 days of the experiment there were no observed effects of dietary methylmercury treatment on survival with >99% survival across all groups. However, after 70 days fish in both the medium and high MeHg test diets experienced decreased survival compared to the control group, which was significant at test day 247 in fish fed the high MeHg test diet. No effects of discoloration were seen in any of the treatment groups. At test day 70 fish in the medium and high MeHg test diets weighed significantly less than the control fish, however there was no significant effect seen in the condition factor across all groups. Approximate weight (g) at test day 70 was estimated visually to be: 1.95 g (control), 1.8 g (low), 1.75 g (medium) and 1.7 g (high). A significant effect of dietary MeHg on specific growth rate (weight gain/day) was observed in the highest test treatment from day 0-35, but not test days 35-70. A significant effect on gross conversion efficiency was also observed in the highest test treatment at day 70. The MATC (geometric mean of NOEC 2.3 and LOEC 25 µg THg/g wet weight) for Sacramento blackfish based on growth (weight reduction) for tissue concentrations of 7.583 µg THg/g ww in muscle or 5.460 µg THg/g ww as whole-body equivalence after application of the WB:M

conversion factor of 0.72 was used to represent the relative sensitivity of this species to dietary mercury exposure in the chronic criterion dataset.

A.2.15 Fifteenth Most Sensitive Genus, Asiatic clam (Corbicula fluminea)

Document Source: Inza, B., E. Ribeyre, R. Mary-Brachet and A. Boudou. 1997. Tissue distribution of inorganic mercury, methylmercury, and cadmium in the Asiatic Clam (*Corbicula fluminea*) in relation to the contamination levels of the water column and sediment. Chemosphere. 35(12): 2817-2836.

Test Organism: Asiatic clam (*Corbicula fluminea*), various size classes (1.2 -1.8 cm)

Mercury Exposure: The experimental unit for the sediment compartment exposure was natural sediment (of homogenous silt, rich in clays (75-80%), and with low total organic carbon: 2% on average) collected from the banks of the Garonne River upstream of Bordeaux, France. Mercury contamination levels in sediment were achieved by one-time addition from a concentrated aqueous stock solution (0.5 g THg/L methylmercury chloride; 1 g THg/L mercury chloride).

Study Design: Asiatic clams (*Corbicula fluminea*) of various size classes (maximum range 1.2 to 1.8 cm) were exposed to mercury or methylmercury separately in either the water column or sediment compartment of indoor experimental units for 14 days. Clams were collected in the wild from the Canal du Midi, France and maintained in the laboratory on a sand substrate with feeding. Six different size classes of clams were identified and one clam from each size class was randomly allocated to each experimental unit (EU). The EU for the water compartment exposure consisted of three liters of dechlorinated tap water in glass tanks lined with plastic film and containing 50/50 natural sediment + pure sand mixture. The EU for the sediment compartment exposure was natural sediment (of homogenous silt, rich in clays (75-80%), and with low total organic carbon: 2% on average) collected from the banks of the Garonne River upstream of Bordeaux, France in plastic containers and containing overlying dechlorinated tap water (background mercury and cadmium of 85 and 240 µg/kg ww). Seven days after setting up the

EUs, six clams representing the six size classes were added to the Eus. The Eus were placed in larger tanks, which were enclosed in thermoregulated containers. Temperature was maintained at 21 ±0.2°C. Treatments consisted of five contamination levels and a control (dechlorinated tap water or 50/50 natural sediment + pure sand mixture). Two replicates were tested under each condition. Mercury contamination levels in sediment were achieved by one time addition from a concentrated aqueous stock solution (0.5 g Hg/L methylmercury chloride; 1 g Hg/L mercury chloride). Mercury contamination levels in water were achieved by constant addition from concentrated aqueous stock solution throughout the experiment, but equivalent to half the first addition of 3 mg Hg/L of mercury chloride, or two daily additions from a 1 mg Hg/L methylmercury chloride aqueous stock solution. Volume additions of stock solutions in the water compartment exposure were defined according to metal determinations made on water samples collected and analyzed after 1, 2, 3, 6, 7, 8, 10 and 13 days of the 14-d exposure, to take into account the complex processes that give rise to the decrease in mercury concentrations in the water column after each addition, due to adsorption on the tank walls, transfers to the sediment interface, volatilization, and bioaccumulation by clams. No external food supply was added during the experiment.

Mercury contamination levels in the EUs during the water exposure were estimated using concentration day equivalents (CDE), calculated based on the integration of different mercury concentrations measured in the water according to the length of time between sampling points. As mercury transfers to the sediment compartment, sediment could represent a secondary contamination source for the clams. Total mercury determinations were made on four sediment cores collected from each EU at the end of the experiment. For the water exposure, average total mercury concentrations in the water column obtained from the CDE values were close to

nominal concentrations at a ratio of 1.2 measured to nominal concentration for methylmercury exposure and 1.08 for mercury exposure. The amount of mercury transferred to the first 0-5 cm in sediment was 26% for mercury, but only 14% for methylmercury. For the sediment exposure, total mercury concentrations measured in samples collected before the sediment was introduced to the EUs were also very close to nominal values. The amount of mercury transferred to the water column from sediment exposure was negligible for both methylmercury and mercury sediment exposure (i.e., less than detection).

Effects Data: No mortality was observed during the experiments, and multiple regression analysis of the soft body weights after 14 days of exposure showed that none of the factors accounted for (water, sediment or combined total mercury contamination sources) contributed to significant differences in soft body weight compared to controls. Mercury accumulated in soft tissues of clams from methylmercury exposure in the water column was much greater than from mercury exposure from sediment, leading to soft body concentrations greater than 6,000 ng/g ww after 14 days (or 6.0 µg THg/g ww). The indeterminate NOEC of >6.000 µg THg/g ww in whole body was selected by the EPA to represent the relative sensitivity of this genera in the chronic criterion dataset.

A.2.16 Sixteenth Most Sensitive Genus, Sacramento splittail (Pogonichthys macrolepidotus)

Document Source: Deng, D.F., F.C. Teh and S.J. Teh. 2008. Effect of dietary methylmercury and seleno-methionine on Sacramento splittail larvae. Sci. Total Environ. 407(1): 197-203.

Test Organism: Sacramento splittail (*Pogonichthys macrolepidotus*), larvae (21-day post-hatch)

Mercury Exposure: A dry basal diet was mixed with methylmercuric chloride dissolved in 100% ethanol and or selenomethionine and water to form a dough. The dough was pelleted and then freeze-dried until use. Measured total mercury concentrations in the test diets were 0.01 (control), 0.13, 4.7 and 11.7 µg/g dry weight. The focus for this document is those treatments

with the low selenium concentration (0.64 µg/g dry weight; the amount present in the diet without added selenium) to avoid possible mixture effects.

Study Design: The interactive effects on Sacramento splittail (*Pogonichthys macrolepidotus*) larvae fed a dietary combination of MeHg and seleno-methionine for four weeks was investigated. Fish were fed one of twelve test treatments (a factorial design comprised of four mercury concentrations and three selenium concentrations). Forty splittail larvae (21-day post-hatch, 5.1 mg) were added to 2 L beakers with two beakers used for each treatment level. Test beakers were kept at 25°C and fish exposed using a 16:8-hour photoperiod, with average dissolved oxygen of 6.8 mg/L, hardness of 120 mg/L and pH 7.8 in the experimental water. Fish were fed twice daily a ration of 40%, 30%, 25% and 20% of body weight per day for the 1st, 2nd, 3rd, and 4th week, respectively. Water was changed daily after each feeding and mortality was recorded daily. At the end of the experiment fish were observed for abnormal swimming behavior and then sacrificed. Fish were weighed and measured individually to determine condition factor and examined for external lesions. Six fish per treatment were collected for histopathological and twenty fish per treatment were frozen for mercury and selenium analysis. Mean total mercury concentration in the whole body of fish was estimated with a figure to be 0, 0.1, 2.5 and 6 µg THg/g wet weight for the control, 0.13, 4.7 and 11.7 µg/g total mercury dietary treatments, respectively.

Effects Data: None of the mercury treatments had any effect on body weight, body length or condition factor. Limited mortality (2.5%) was only observed in fish fed diets containing 11.7 µg/g total mercury dry weight at the end of the 3rd week. Also limited swimming behavioral changes (e.g., spinning in a circular, dart-like movement, hyperactivity) was observed during the second week of feeding in the 4.7 (2.5% of fish) and 11.7 (10% of fish) µg/g total mercury dry

weight diets. Based on the apical endpoints (mortality and growth) the NOEC of > 6 µg THg/g ww whole body (or > 8.33 µg THg/g ww as muscle tissue equivalence based on application of the WB:M conversion factor of 0.72) was used to represent the relative insensitivity of this species to dietary mercury exposure in the chronic criterion dataset.

A.2.17 Seventeenth Most Sensitive Genus, Cladoceran (Daphnia magna)

Source Document: Tsui , K.T. and W.X. Wang. 2004, Uptake and Elimination Routes of Inorganic Mercury and Methylmercury in *Daphnia magna.* Environ. Sci. Technol. 38: 808-816

Test Organism: Cladoceran (*Daphnia magna*), 3-day old

Mercury Exposure: Radio-labeled methylmercury ($CH_3{}^{203}HgCl$) was synthesized from $^{203}HgCl_2$ using an established protocol at the laboratory. The experimental diet was prepared by spiking green alga (*Chlamydomonas reinhardtii*) in the exponential phase with $Me^{203}Hg$ at 148 kBq/L (corresponding to 28.3 nM of Hg). After a day of growth, the percentage of methylmercury associated with the cells was greater than 95%. The relatively high concentration of radioactive methylmercury used in this study was previously determined necessary to obtain an accurate measurement of maternal transfer efficiency in *D. magna* and for the subsequent retention of methylmercury by offspring. The resulting concentration of methylmercury in adult reproducing females was shown in preliminary experiments to induce the direct release of undeveloped eggs to the water (i.e., sublethal toxicity to the animals), thus allowing a comparison of the methylmercury content in the live neonates and undeveloped eggs.

Study Design: conducted a study to quantify the transfer efficiency of methylmercury in the diet of adult female *Daphnia magna* to their reproductive outputs under laboratory conditions for two generations. The effect of dietary methylmercury residence time in the daphnids on the efflux system also was quantified. Radiotracer technique was employed to follow the biokinetics of methylmercury throughout the study. A batch of approximately fifty 3-d old *D. magna* (F_0

generation) was collected from stock cultures and added to 500 ml of GF/C pond water. The animals were fed with the radiolabeled *C. reinhardtii* at 5 x 10^4 cells/ml for 6 h each day. Afterward, the animals were rinsed and transferred to another beaker containing the filtered pond water with unlabeled alga. This feeding regime was repeated for a total of five days. The exposed F_0 daphnids were subsequently divided into three groups, each with 15 radiolabeled individuals, and were depurated in individual feeding beakers containing 100 ml of filtered pond water plus unlabeled *C. reinhardtii* at 5 x 10^4 cells/ml. During the next 20 d of depuration, water and food were renewed daily, and animals of the F_0 generation radio-assayed each day and any live neonates and undeveloped eggs collected, counted, and also radio-assayed for methylmercury quantification. Each day, the live neonates (F_1 generation) produced by individual replicates of the F_0 generation were transferred to individual beakers, and their retention of maternally-transferred methylmercury and further neonate production (F_2 generation) were monitored over a period of 28 d after hatching. The live neonates of the F_2 generation from individual replicates of the F_1 generation were similarly radio-assayed and cultured and their survival monitored for 10 days (i.e., a 10-day survival test with the same food provided).

Effects Data: The relatively high body burden of methylmercury in *D. magna* (33.3 µg/g wet weight) after 5 d of dietary exposure resulted in a high mortality in the F_0 generation. The elevated maternal methylmercury tissue concentrations in F_0 females reduced the survival rate of the F_1 generation, but the variation in the survival rate of this generation was large (20–80%) according to brood batches produced. In the F_2 generation, the 10-d survival test indicated a generally high survival rate of neonates, with the majority of broods achieving greater than 75% survival. After ingesting the relatively high dosage of dietary methylmercury, exposed F_0 females exhibited a reduction of live neonates and an increase of undeveloped eggs (or

embryos). The number of live neonates produced (i.e., 0–1.44 neonates per female per day) was smaller than that of unexposed animals in the laboratory (5–7 neonates per female per day), indicating the sublethal toxicity of methylmercury (i.e., only one-quarter of live neonate production when compared to the normal animals). Assuming total mercury in exposed F_0 females as 100 percent methylmercury, and in comparison to normal observed daphnid laboratory culture reproduction output and survival as the control condition, the F_0 survival and reproduction LOEC for the study is estimated to be 33.3 µg total mercury (assumed 100 percent methylmercury)/g ww. EPA divided this value by an uncertainty factor of 3 (U.S. EPA 1997d) to estimate a NOEC for the study of 11.1 µg total mercury/g ww; the value used by EPA to represent the relative sensitivity of this species to dietary mercury exposure in the chronic criterion dataset.

A.2.18 Eighteenth Most Sensitive Genus, Green, and White Sturgeons (Acipenser medirostris, and Acipenser transmontanus)

Source Document: Lee, J.-W., N. De Riu, S. Lee, S.C. Bai, G. Moniello and S.S.O. Hung. 2011. Effects of dietary methylmercury on growth performance and tissue burden in juvenile green (*Acipenser medirostris*) and white sturgeon (*A. transmontanus*). Aquat. Toxicol. 105:227–234.

Test Organisms: Green Sturgeon and White Sturgeons (*Acipenser medirostris* and *Acipenser transmontanus*)

Mercury Exposure: Commercial feed diets for at least 90 days; then a purified diet one week prior to experimentation to the purified diet. The purified diet had been shown to contain sufficient nutrients to support growth in juvenile white sturgeon. A concentration of MeHg chloride dissolved in 100% ethanol was added to the purified diet mixture to constitute the four treatment levels. Up to 6 mL of ethanol was added per kg of diet, but the authors noted that most evaporated during the processes of experimental diet preparation (pelleting and fan drying overnight). Experiments with both species employed the same test treatments and tank system with one species tested consecutively after the other.

Study Design: Green sturgeon larvae were obtained from spawned captive broodstocks originating from the Klamath River, while white sturgeon larvae were obtained from a sturgeon fish farm. Both sturgeon species were reared on a commercial diet for 90 days prior to the start of the test. For each experiment 300 juvenile sturgeon (average 30 g each) were distributed to 12 circular fiberglass tanks under a flow-through system receiving aerated well water; pH was 7-8, temperature was 18-19°C and dissolved oxygen ranged from 7-9 mg/L. Juvenile sturgeon were fed one of four dietary treatments of methylmercury (nominal [MeHg] were control, 25, 50, 100 µg THg/g dw) yielding mean muscle concentrations measured at eight weeks for the green sturgeon of 0.005, 12.7 and 28.8 µg THg/g ww; and 0.005, 14.1, 26.1 and 58.0 µg THg/g ww for the white sturgeon, respectively for 8 weeks to determine and compare the effects on growth performance and mercury tissue concentrations in the two sturgeon species. Subsamples of fish were sampled from each treatment at 2, 4, 6 and 8 weeks of experiments to determine total mercury tissue burdens.

Effects Data: Mean muscle total mercury concentrations measured at eight weeks for the green sturgeon were 0.02, 50.8 and 115.2 µg THg/g dw for the control, 25, and 50 µg/g total mercury dw dietary treatments, respectively. Green sturgeon were relatively more sensitive to methylmercury than the white sturgeon, with 100% mortality of green sturgeon in the highest test concentration compared to only 38.5% mortality for the white sturgeon. The most sensitive apical endpoints observed for the green sturgeon were mortality and growth. For mortality both the control and lowest test diet experienced 7.7% mortality, while the next test diet (50 µg/g total mercury dw) had 71.7% mortality. Similarly, growth (% body weight increase/day) was significantly decreased in the 50 µg/g total mercury dw test diet compared to the control, but not

in the lowest test diet. The % body weight increase/day was 8.2% in the control, 7.0% in the 25 µg/g total mercury dw test diet, and 3.3% in the 50 µg/g total mercury dw diet.

The most sensitive apical endpoints for the white sturgeon were mortality and growth. For mortality both the control and lowest test diet experienced 0% mortality, while the next test diets had 2.6% (50 µg/g total mercury dw) and 38.5% (100 µg/g total mercury dw) mortality. Only the highest test diet was significantly different from the control. Similarly, growth (% body weight increase/day) was significantly decreased in the 100 µg/g total mercury dw test diet compared to the control, but not in the two lowest test diets. The % body weight increase/day was 4.7% in the control, 5.7% in the 25 µg/g total mercury dw test diet, 4.2% in the 50 µg/g total mercury dw diet and 1.5% in the 100 µg/g total mercury dw diet.

The average percent moisture value for fish in the Family Acipenseridae is 76.5 (see Appendix D). The NOEC and LOECs for the green and white sturgeon from Lee et al. (2011) were divided by a factor of 4.26 to convert dry weight tissue Hg concentrations to wet weight. The NOEC and LOEC for the green sturgeon are 50.8 and 115.2 µg/g THg dw, respectively, or 11.94 and 27.07 µg/g THg ww. The MATC (geometric mean of the NOEC and LOEC) of the latter values represents the SMCV for the green sturgeon, or 17.98 µg THg/g ww based on muscle tissue (or 12.94 µg THg/g ww as whole body based on application of the WB:M conversion factor of 0.72). Similarly, the white sturgeon NOEC and LOEC (104.4 and 231.8 µg/g THg dw) was divided by 4.26 and is equal to 24.53 and 54.47 µg/g THg ww, respectively. The MATC of 36.56 µg THg/g ww based on muscle tissue (or 26.32 µg THg/g ww as whole body based on application of the WB:M conversion factor of 0.72) represents the SMCV for the white sturgeon. EPA derived the GMCV for the genus Acipenser based on the geometric mean of the green and white sturgeon muscle-based SMCVs yielding a GMCV of 25.64 µg THg/g ww,

or 18.46 µg THg/g ww based on the whole body. EPA used these values to represent the relative sensitivity of this genus in the sensitivity distribution used to drive the tissue criterion for mercury.

Appendix B Data Used Qualitatively in the Criterion Derivation

B.1 Qualitative Dietary Mercury Studies

Species	Dietary Description	Exposure Duration (d)	Dietary Mercury	Tissue Mercury	Endpoint(s)	Dietary Effects Concentrations	Reported Mercury Form and Units	Tissue Effects Concentrations	Reported Mercury Form and Units	Exposure Notes	Reference
Grayling (embryos), *Thymallus thymallus*	See Exposure Notes	Embryonic with observations of latent effects after 3 years	NA	Whole body - THg	Behavioral (foraging efficiency)	NOEC: MATC: LOEC:	NA	NOEC: 0.09 MATC: 0.1559 LOEC: 0.27	THg, µg/g ww	This study involved a 13-day embryonic exposure to aqueous MeHgCl dissolved in distilled water during early embryonic development to examine latent sublethal effects on fish foraging behavior. MeHg aqueous exposure to grayling embryos in the study was not designed to be ecologically realistic, but instead produce ecologically-relevant mercury in tissues of embryos.	Fjeld et al. 1998
Rainbow trout (fingerlings; 11.7-13.8 cm, 20.9 - 31.7 g), *Oncorhynchus mykiss*	MeHgCl added to a 5:1 (by weight) mixture of ground pork liver + dry trout food. Ration equal to 3-4% of total body weight of the fish in each tank.	105	U	Muscle - THg	Survival and growth	NOEC: 16 MATC: 19.56 LOEC: 24	THg, µg/g ww	NOEC: 13 MATC: 15.30 LOEC: 18	THg, µg/g ww	Methylmercury chloride was added to the food mixture to produce one of four nominal test diets: 4 (trial I), 8 (trial II), 16 (trial III) and 24 (trial IV) µg THg/g wet weight.	Wobeser 1975
Rainbow trout (fingerlings; 3-10 g), *Oncorhynchus mykiss*	MeHgCl dissolved in the salmon oil component of the Oregon test diet prior to diet formulation.	24	M - THg	Whole body - THg	Growth rate	NOEC: 3.08 MATC: > 3.08 LOEC: > 3.08	THg, µg/g ww	NOEC: 5.67 MATC: >5.67 LOEC:>5.67	THg, µg/g ww	Measured THg in the test diet was 3.08 µg/g wet weight.	Phillips and Buhler 1978

Species	Dietary Description	Exposure Duration (d)	Dietary Mercury	Tissue Mercury	Endpoint(s)	Dietary Effects Concentrations	Reported Mercury Form and Units	Tissue Effects Concentrations	Reported Mercury Form and Units	Exposure Notes	Reference
Fathead minnow (90 dph), *Pimephales promelas*	Commercial fish food mixed with reagent alcohol containing dissolved methylmercuric chloride, similar to Hammerschmidt et al. (2002). Ration provided was 5% of body mass per day.	Full life-cycle	M - THg	Female carcass (whole body less plasma and gonads) - THg	Apoptosis in steroidogenic gonadal cells	NOEC: MATC: LOEC: 0.87	THg, µg/g dw	NOEC: MATC: LOEC: 0.917	THg, µg/g ww	Mean dietary total mercury concentrations were 0.058, 0.87, and 3.93 µg/g dw in the control, low, and medium exposures, respectively (where "medium" is the highest treatment).	Drevnick et al. 2006
Fathead minnow (90 dph), *Pimephales promelas*	Commercial fish food mixed with reagent alcohol containing dissolved methylmercuric chloride, similar to Hammerschmidt et al. (2002). Ration provided was 5% of body mass per day.	600-day multi-generation	M - THg	Female carcass (whole body less plasma and gonads) - THg	Expression of genes commonly associated with endocrine disruption	NOEC: MATC: LOEC: 0.87	THg, µg/g dw	NOEC: MATC: LOEC: 0.917	THg, µg/g ww	Mean dietary total mercury concentrations were 0.058, 0.87, and 3.93 µg/g dw in the control, low, and medium exposures, respectively (where "medium" is the highest treatment).	Klaper et al. 2008
Golden shiner (50–70 mm total length), *Notemigonus crysoleucas*	MeHgCl dissolved in reagent-grade ethanol added to a fish meal-casein based diet. The food was fed at a ration of 2 percent body weight per day.	90	M - THg	Whole body - THg	Predator avoidance behavior	NOEC: 0.455 MATC: 0.6606 LOEC: 0.959	THg, µg/g ww	NOEC: 0.230 MATC: 0.351 LOEC: 0.536	THg, µg/g ww	Measured concentrations of THg in the diet were: control diet (0.012 µg/g ww), low-Hg diet (0.455 µg/g ww), and high-Hg diet (0.959 µg/g ww).	Webber and Haines 2003
Zebrafish (adult males 0.88 g, 3.63 cm), *Danio rerio*	Diet prepared by mixing artificial fish food with an ethanolic solution of MeHgCl. Fish were fed an artificial food equal to 5% of fish wet weight twice a day.	63	M - THg	Muscle - THg	Survival	NOEC: 13.5 MATC:>13.5 LOEC: >13.5	THg, µg/g dw	NOEC: 32.7 MATC: LOEC: NOEC: 34.20 MATC: LOEC:	THg, µg/g dw	THg concentrations in the diet were measured every two weeks over the duration of the experiment and were 0.08 µg/g dry weight (control), 5 µg/g dry weight (low) or 13.5 µg/g dry weight (high).	Gonzalez et al. 2005; Oliviera-Ribeiro et al. 2008

Species	Dietary Description	Exposure Duration (d)	Dietary Mercury	Tissue Mercury	Endpoint(s)	Dietary Effects Concentrations	Reported Mercury Form and Units	Tissue Effects Concentrations	Reported Mercury Form and Units	Exposure Notes	Reference
Zebrafish (adult males 0.88 g, 3.63 cm), *Danio rerio*	Diet prepared by mixing artificial fish food with an ethanolic solution of MeHgCl. Fish were fed an artificial food equal to 5% of fish wet weight twice a day.	25-49	M - THg	Muscle - THg	Survival	NOEC: 13.5 MATC:>13.5 LOEC: >13.5	THg, µg/g dw	NOEC: 25.4 MATC: LOEC: NOEC: 35.5 MATC: LOEC:	THg, µg/g dw	THg concentrations in the diet were measured every two weeks over the duration of the experiment and were 0.08 µg/g dry weight (control) or 13.5 µg/g dry weight (high).	Cambier et al. 2009, 2010
Zebrafish (adult males 0.88 g, 3.63 cm), *Danio rerio*	Diet prepared by mixing artificial fish food with an ethanolic solution of MeHgCl. Fish were fed an artificial food equal to 2.5% of fish wet weight twice a day.	50	M - THg	Muscle - THg	DNA damage	NOEC: MATC: LOEC: 13.5	THg, µg/g dw	NOEC: MATC: LOEC: 36	THg, µg/g dw	THg concentrations in the diet were measured every two weeks over the duration of the experiment and were 0.08 µg/g dry weight (control) or 13.5 µg/g dry weight (high).	Lerebours et al. 2013
Zebrafish (adult), *Danio rerio*	A stock solution of MeHgCl was mixed with a stock solution of cysteine dissolved in water in a 1:1.2 molar mixture. The experimental diets were produced by adding aqueous solutions of the MeHg-cysteine mixture to a commercial pelleted zebra fish diet.	56	M - THg	Muscle - THg	Survival and growth	NOEC: 9.8 MATC: > 9.8 LOEC: > 9.8	THg, µg/g dw	NOEC: 6.4 MATC: >6.4 LOEC: >6.4	THg, µg/g ww	Measured THg concentrations in the diet were 0.08, 5.2 and 9.8 µg/g dry weight for the control, low and high Hg test diets, respectively	Amlund et al. 2015
White sturgeon (field-caught 110 to 137 cm fork length; 14-20 year-old), *Acipenser transmontanus*	Natural diet from the Columbia River estuary, and the Bonneville, The Dalles, and John Day Reservoirs	Lifetime	U	Muscle - THg	Sex steriods and GSI of immature male sturgeon	NOEC: MATC: LOEC:	NA	NOEC: MATC: LOEC: 0.176	THg, µg/g ww	Since the observed effects on reproduction in immature males were observed in more than one waterbody in the study, EPA used the average mercury concentration in the muscle (0.176 µg THg/g ww) reported for the Columbia River Basin.	Webb et al. 2006

B.2 Salmonidae

B.2.1 Grayling (Thymallus thymallus)

Document Source: Fjeld, E., L. Haugenb and A. Vøllestad. 1998. Permanent impairment in the feeding behavior of grayling *Thymallus thymallus* exposed to methylmercury during embryogenesis. Sci. Total Environ. 213: 247-254.

Fjeld et al. (1998) exposed grayling (*Thymallus thymallus*) embryos to different concentrations of methylmercury during early embryonic development to examine latent sublethal effects on fish foraging behavior. MeHg aqueous exposure to grayling embryos in the study was not designed to be ecologically realistic, but instead produce ecologically-relevant mercury in tissues of embryos. Eggs were stripped and fertilized in the laboratory from sexually mature male and female grayling collected from a subalpine lake in southern Norway. The fertilized eggs (~1800 eggs per group) were divided and randomly assigned to a control and four different exposure groups (treatments A-E) in aquaria containing 40 L of water from Lake Maridalsvannet, a drinking water reservoir (<1 ng/L Hg; assumed total mercury) in Norway. Embryos were exposed for 13 days to methylmercury chloride dissolved in distilled water to achieve nominal concentrations of 0.16, 0.8, 4.0 and 20 µg/L. The onset of hatching occurred 10 days after fertilization and by day 13, more than 90% of the viable eggs in all groups had hatched, except for the high (20 µg/L) exposure group (Group E). Observation of viable eggs in Group E revealed numerous hatch failures as well as several malformations (scoliosis). By day 15, this group had only achieved an 80% hatch rate and so the exposure phase of the experiment was terminated.

A sample of 100 live embryos from each exposure group were analyzed for total mercury yielding tissue concentrations of 0.01 (Group A, control) and 0.09, 0.27, 0.63 and 3.8 µg/g ww for groups B-E, respectively. Remaining free-living normal embryos were then transferred to larger aquaria for exogenous feeding and grow out. After three years, when the fish had reached

a modal length of 10 - 16 cm, feeding experiments were started. Two foraging efficiency studies were conducted to assess the latent effects of embryonic exposure to methylmercury. First, the feeding efficiency of exposed fish was assessed in single fish feeding trials, and then competitive foraging efficiency was tested for groups of up to eight MeHg-exposed fish vs control fish for five minutes, with cladoceran prey (*Daphnia magna*) introduced to test aquaria 10 at a time every 30 seconds. Control feeding efficiency was monitored closely to ensure stability of experimental conditions. The mean number of prey caught decreased in dose-response fashion from control response with increasing total mercury tissue concentration three years post-embryonic aqueous methyl mercury exposure (ANOVA $F = 9.62$, d.f. = 4,47, $P < 0.001$). The percent reduction from control response for Group C-E was 15.2, 13.8, and 23.9%, respectively. The NOEC for this phase of the study was 0.09 µg/g ww and the LOEC was 0.27 µg/g ww, yielding an MATC of 0.1559 µg Hg/g ww.

The second phase of the study consisted of competitive feeding trials where a group of mercury exposed fish were combined with control fish and the latent effect of mercury on feeding efficiency measured. Control fish (group A) exhibited prey consumption rates approximately twice as high as group C (60.3 vs 30.5; $F = 10.41$, d.f. = 1,46, $p = 0.002$) and group D (62.8 vs 25.8; $F= 6.49$, d.f. = 1,30, $P = 0.016$). Although statistically significant, an effect concentration (MATC) could not be calculated since group B (0.09 µg/g ww) was not used in this phase of the feeding trial and could not be compared to the control, therefore a meaningful NOEC could not be determined. However, the magnitude of the effect and statistical significance of the difference in competitive feeding efficiency of group C versus the control group (A) support the use of the MATC derived from the first feeding efficiency trial as the chronic value for this study.

B.2.2 Rainbow trout (Oncorhynchus mykiss)

Document Source: Wobeser. 1975. Prolonged Oral Administration of Methyl Mercury Chloride to Rainbow Trout (*Salmo gairdneri*) Fingerlings. J. Fish. Board Canada. 32(11): 2015–2023.

Wobeser (1975) investigated the prolonged effects of dietary methylmercury fed to rainbow trout (*Oncorhynchus mykiss*) over a period of 15 weeks (105 days). Rainbow trout fingerlings were obtained from a commercial supplier. Not all treatments were started at the same time, so rainbow trout sizes varied between experiments. In the first series (trial I-II) fish averaged 11.7 cm in length and 20.9 g in weight. In the second series of experiments (trial III-IV) fish averaged 13.8 cm in length and 31.7 g in weight. All fish were held for a minimum for 14 days to acclimate to test conditions. Each series of experiments employed a flow-through test design using four aquaria (containing 450 L of dechlorinated tap water) with 30 fish per tank. Reported water conditions average 10°C with a dissolved oxygen range from 7.3-8.3 mg/L. Rainbow trout were fed a commercial dry trout food during acclimation. During testing the experimental diet consisted of ground pork liver plus the dry trout food mixed at a ratio of 5:1 by weight. Background mercury concentrations in this basal ration <0.1 µg/g total mercury. Methylmercury chloride was added to this ration to produce one of four nominal test diets: 4 (trial I), 8 (trial II), 16 (trial III) and 24 (trial IV) µg/g total mercury wet weight. Concentrations of mercury in the test diet were not measured. Fish were fed control or test diet at 3-4% of total body weight of the fish in each tank. In each set of experiments, 30 control fish received the basal ration while two replicate sets of 30 fish each were fed the test diets. Total weight of the fish was determined weekly in each group by and two fish removed and sacrificed for blood and tissue samples to determine histopathology and mercury content over the course of the experiment.

Concentrations of total mercury in the axial muscle of fish from the various trials were estimated (visually from a figure) to be: 7, 13, 18, 27 µg/g total mercury wet weight for trials I-

IV, respectively. Authors reported mercury concentrations in the controls were <0.2 µg/g total mercury wet weight. No mortality was attributed to the mercury-supplemented diets in the study. There was also no observed difference in appetite, vision, or escape behavior between groups, but fish in trial III and IV tended to occupy the middle of the tank instead of upstream of the tank like the control fish. Over the duration of the experiment growth (weight) of treated fish was not significantly different from the control, however in the final five weeks of the experiments fish from trial III and IV exhibited significantly lower growth compared to the control group. Therefore, the MATC (geometric mean of NOEC 13 and LOEC 18 µg/g total mercury wet weight, based on growth (weight) is 15.30 µg/g total mercury wet weight and is used to represent the relative insensitivity of rainbow trout to dietary mercury exposure in the study.

Document Source: Phillips, G.R. and D.R. Buhler. 1978. The Relative Contributions of Methylmercury from Food or Water to Rainbow Trout (*Salmo gairdneri*) in a Controlled Laboratory Environment. Trans. Amer. Fish. Soc. 107(6): 853-861.

Phillips and Buhler (1978) examined the effects of methylmercury on rainbow trout over 24 days via the water column, diet, and the combination of the two. The focus of this study for mercury criterion development for the protection of aquatic life was on the dietary portion of the study. Fingerlings (3-10 g) were obtained from the Oregon Department of Fish and Wildlife Hatchery and acclimated to the laboratory conditions and experimental diet for at least two weeks before experimentation. A flow-through regime delivered dechlorinated tap water to 30 L tanks at 14.8-15.5°C, dissolved oxygen of 9.6-10.1 mg/L, and pH of 7.3-7.6. A photoperiod of 16:8 hour light:dark was used during the exposure. The number of fish per tank and the number of replicates per treatment were not reported. Rainbow trout were fed (adjusted by weight) either an Oregon test diet or a test diet where methylmercuric chloride was dissolved in the salmon oil fraction of the Oregon test diet. Measured total mercury in the test diet was 3.08 µg/g total

mercury wet weight. Mean whole body total mercury concentrations measured in the control and fish treated with MeHg-contaminated diets in the study were 0.0008-0.00012 (control) and 5.67 µg/g total mercury wet weight, respectively. At the end of the experiment there was no difference between growth rates of fish from the control versus the MeHg contaminated diet. The NOEC of 5.67 µg/g total mercury wet weight whole-body tissue, based on growth, was used to represent the relative insensitivity of rainbow trout to dietary mercury exposure in the study.

B.3 Cyprinidae

B.3.1 Fathead minnow (Pimephales promelas)

Document Source: Drevnick, P.E., M.B. Sandheinrich and J.T. Oris. 2006. Increased ovarian follicular apoptosis in fathead minnows (*Pimephales promelas*) exposed to dietary methylmercury. Aquat. Toxicol. 79: 49-54.

Test Organism: Fathead minnow (*Pimephales promelas*)

Mercury Exposure: The dietary exposure setup was that described by Drevnick and Sandheinrich (2003), consisting of three concentrations: 0.06 µg/g dw (control), 0.87 µg/g dw (low), and 3.93 µg/g dw (medium).

Study Design: Following up on the above-described studies, Drevnick et al. (2006) sought to uncover the specific mechanisms associated with the observed reproductive impairment. The authors hypothesized that methylmercury induces apoptosis in steroidogenic gonadal cells in fish, thereby interfering with the synthesis of sex steroid hormones critical for the regulation of reproduction.

Effects Data: Apoptosis was evaluated histologically in ovaries of female fathead minnows. Methylmercury significantly increased the number of apoptotic follicular cells in primary growth and cortical alveolus stage ovarian follicles. Ovarian follicular cells (i.e., granulosa, theca) are responsible for the production of 17β-estradiol and other sex steroid hormones. Increased ovarian follicular apoptosis was related to suppressed 17β-estradiol concentrations and smaller ovary size

of female fathead minnows exposed to dietary methylmercury. The authors suggest increased apoptosis of steroidogenic gonadal cells as a possible mechanism for the suppression of sex steroid hormones and ultimately the impairment of reproduction in fish exposed to methylmercury. Mean female total mercury carcass (whole body less plasma and gonad) concentrations were those reported in Drevnick and Sandheinrich (2003): 0.079 µg/g ww (control), 0.917 µg/g ww (low), and 3.842 µg/g ww (medium). Since there was no study concentration between the control and the LOEC, EPA estimated the NOEC for this study by applying an uncertainty factor of 3 (U.S. EPA 1997d), to the female carcass concentration of 0.917 µg/g ww (the LOEC) to obtain a NOEC of 0.3057 µg Hg/g ww. This study was not used quantitatively since the effect concentration was not based on an apical endpoint, however, the discussion was included here as it is a related study and provides supporting information for the GMCV based on reproductive effects.

Document Source: Klaper, R., B.J. Carter, C.A. Richter, P.E. Drevnick, M.B. Sandheinrich and D.E. Tillitt. 2008. Use of a 15k gene microarray to determine gene expression changes in response to acute and chronic methylmercury exposure in the fathead minnow *Pimephales promelas* Rafinesque. J. Fish Biol. 72(9): 2207-2280.

Test Organism: Fathead minnow (*Pimephales promelas*)

Mercury Exposure: The juvenile fathead minnows were exposed to the same dietary methylmercury concentrations as Drevnick and Sandheinrich (2003): reported as 0.058 µg/g dw (control), 0.87 µg/g dw (low), and 3.93 µg/g dw (medium), but exposure was for 600 days, a longer duration than the previous work.

Study Design: As a final experimental investigation in the series of similar studies, Klaper et al. (2006) conducted a dietary methylmercury study aimed at identifying alterations in gene expression associated with previously observed changes in reproduction and reproductive

biomarkers in fathead minnows. A commercial microarray was used in conjunction with quantitative polymerase chain reaction to examine gene expression in fish in relation to exposure to the environmentally relevant doses of methylmercury.

Effects: Expression of genes commonly associated with endocrine disruption was altered with dietary methylmercury exposure. A significant up-regulation in vitellogenin mRNA in individual mercury-exposed males and a significant decline in vitellogenin gene expression in females was observed with increasing dietary concentrations. Other genes identified by the microarray experiment included those associated with egg fertilization and development, sugar metabolism, apoptosis, and electron transport. Differences in expression patterns between male and female fish not related to genes specifically associated with reproduction were also observed, indicating a potential physiological difference in the reaction of males and females to methylmercury.

Similar to the findings of Drevnick et al. (2006), this study was not used quantitatively by EPA as it yielded no apical endpoints for the assessment. However, the findings in the study support the results from the previously discussed studies, which yielded a tissue-based total mercury SMCV of 0.2574 µg/g ww based on whole body tissue, calculated as the geometric mean of the three previously described fathead minnow NOEC values in **Appendix A**.

B.3.2 Golden shiner (Notemigonus crysoleucas)

Document Source: Webber. H. and T.A. Haines. 2003. Mercury effects on predator avoidance behavior of a forage fish, golden shiner (*Notemigonus crysoleucas*). Environ. Toxicol. Chem. 22(7): 1556-61.

Webber and Haines (2003) examined the effects of dietary methylmercury exposure at environmental levels to golden shiner (*Notemigonus crysoleucas*; 50–70 mm total length) collected from a man-made pond in Maine. The researchers fed fish a nutritionally complete fish meal (casein-based diet with and without addition of methylmercuric chloride) to the shiners for 90 days. A 0.01 mg/ml solution of methylmercury was made by dissolving methylmercuric

chloride (solid, 95+%; Alfa Aesar, Ward Hill, MA, USA) in reagent-grade ethanol. The stock solution was mixed with deionized water, brought up to 40% weight:volume of the dry material, then extruded through a meat grinder. The diets were dried, then double bagged in precleaned plastic bags and stored at -18°C until they were ground. Three diets were produced: control (no added MeHg), low Hg (target final MeHg concentration 0.5 µg/g ww), and high Hg (target concentration 1 µg/g ww). The food was fed at a ration of 2 percent body weight per day. Measured concentrations of total Hg (all in the form of methylmercury) in the diet were: control diet (0.012 µg/g ww), low-Hg diet (0.455 µg/g ww), and high-Hg diet (0.959 µg/g ww). Expressed as total mercury, corresponding mean whole body tissue concentrations in fish were: 0.041 µg/g ww (control), 0.230 µg/g ww (low Hg), and 0.536 µg/g ww (high Hg). These concentrations were within the range found in this species in northern U.S. lakes at the time of the study.

Investigators assessed apical endpoints (growth, survival), as well as brain acetylcholinesterase (ACHe) levels and predator avoidance behavior (endpoints related to the neurotoxic mode of action of methylmercury). There was no mortality during the 90-day dietary exposure, nor was there mortality during the post-exposure behavioral testing period (9 days). Fish growth over the 90-day exposure period averaged between 32.8% and 42.7%, with no significant difference between control and mercury exposed treatments. Mean Hg brain concentrations were approximately 10-fold and 23-fold higher than control in low-Hg and high-Hg treatments, respectively (ANOVA, $p < 0.0001$). However, there was no significant difference in ACHe activity in brain tissue between control and mercury exposed shiners.

Predator avoidance behavior of shiners was tested following the exposure. Researchers used a model of a belted kingfisher made from balsa wood and Styrofoam as the predator. The

time required to respond to the bird model did not differ significantly among treatment and control groups. But fish fed the high-Hg diet exhibited significantly greater shoal vertical dispersal following predator exposure (57 cm at high exposure versus 7.7 cm at low exposure and 5.9 cm control), took longer to return to pre-exposure activity level (58.5 sec at high exposure versus 8.7 sec at low exposure and 7.4 sec control), and had greater shoal area after return to pre-exposure activity than did the other treatments. The whole-body Hg concentrations attained by the fish in the present study for the low-Hg (0.230 µg/g ww; NOEC) and high-Hg (0.536 µg/g ww; LOEC) diets are similar to those found in wild golden shiners. An increase in movement as well as shoaling area elicited by dietary exposure to MeHg increased the susceptibility of golden shiner to a model avian predator, the belted kingfisher. Therefore, because of the ecological relevance of the dietary exposure and the neurotoxic mode of action of methylmercury, EPA selected the tissue-based MATC of 0.351 µg Hg/g ww as the chronic value for this study based on the effect on predator avoidance behavior of the golden shiner.

B.3.3 Zebrafish (Danio rerio)

Document Sources: Gonzalez, P., Y. Dominique, J.C. Massabuau, A. Boudou and J.P. Boourdineaud. 2005. Comparative effects of dietary methylmercury on gene expression in liver, skeletal muscle, and brain of the zebrafish (*Danio rerio*). Environ. Sci. Technol. 39: 3972–3980.

Oliviera-Ribeiro, C.A.D., N. Mesmer-Dudons, P. Gonzalez, Y. Dominique, J.P. Bourdineaud, A. Boudou and J.C. Massabuau. 2008. Effects of dietary methylmercury on zebrafish skeletal muscle fibres. Environ. Toxicol. Pharmacol. 25: 304–309**.**

Gonzalez et al. (2005) and Oliviera-Riberio et al. (2008) describe two toxicity studies with zebrafish (*Danio rerio*) with a similar test design but reported different effect measures. Gonzalez et al. examined the dietary effects of MeHg on gene expression in the liver, skeletal muscle, and brain tissue and Oliviera-Riberio et al. examined the histological and ultrastructural changes in skeletal muscle fibers. In Gonzalez et al. (2005) thirty-six adult male fish (0.88 g, 3.63 cm) each were placed in three tanks containing 100 L of dechlorinated tap water held at

24℃. Fish were fed a diet equal to 5% of fish wet weight twice a day. Each tank was fed either a control diet or diet supplemented with 5 or 13.5 µg/g total mercury dry weight (95% ethanol with dissolved MeHg chloride). Total mercury concentrations in the diet (measured every two weeks over the duration of the experiment) indicated no significant change in treatment levels. Control diets contained 0.08 µg/g total mercury dry weight. Every two days water was replaced in each tank and cleaned of remaining food and feces. In Oliviera-Riberio et al. (2008) fish were only fed the high mercury test diet and the number of fish was not defined, but all other test conditions were the same as in Gonzalez et al. (2005). Twelve fish per tank were sacrificed on day 7, 21 and 63 for brain liver and skeletal muscle in Gonzalez et al., and five fish were sacrificed on the same test days and harvested for skeletal muscle in Olivera-Riberio et al. Gonzalez et al. reported measured mercury concentrations in the skeletal muscle of <0.7, 15 and 32.7 µg/g total mercury dry weight for the control, 5 and 13.5 µg/g Hg diets; assuming a moisture content for zebrafish of 71.3% (see Appendix D), these concentrations equate to 0.2011, 4.310 and 9.397 µg/g total mercury wet weight in skeletal muscle tissue. Olivera-Riberio et al. reported average total mercury concentrations in the skeletal muscle of 1.01 and 34.20 µg/g dry weight for the control and test diet, this equating to 0.2902 and 9.828 µg/g total mercury wet weight. Dietary mercury had no effect on mortality, mobility, injury, or discoloration throughout both experiments, but the lowest test diet had a significant change in the gene expression in the skeletal muscle (Gonzalez et al. 2005). The highest test treatment also caused a significant change in the gene expression in the liver (Gonzalez et al. 2005) and increase in mitochondrial pathology (Oliviera-Riberio et al. 2008). The NOECs, based on lack of mortality, for the two studies are 9.397 and 9.828 µg/g total mercury wet weight skeletal muscle, and are used to represent the relative insensitivity of zebrafish to dietary mercury exposure in the two similar studies.

Document Sources: Cambier, S., G. Bénard, N. Mesmer-Dudons, P. Gonzalez, R. Rossignol, D. Brèthes and J-P. Bourdineaud. 2009. At environmental doses, dietary methylmercury inhibits mitochondrial energy metabolism in skeletal muscles of the zebra fish (*Danio rerio*). Internat. J. Biochem. Cell Biol. 41: 791–799.

Cambier, S., P. Gonzalez, G. Durrieu, R. Maury-Brachet, A. Boudou and J-P Bourdineaud. 2010. Serial Analysis of Gene Expression in the Skeletal Muscles of Zebrafish Fed with a Methylmercury-Contaminated Diet. Environ. Sci. Technol. 44: 469–475.

The studies by Cambier et al. (2009, 2010) are a continuation of work by Gonzalez et al. (2005) and Oliviera-Riberio et al. (2008), keeping a similar test design but where duration of the exposure was varied in the experiments. Again, adult male fish (0.88 g, 3.63 cm) each were placed in one of two tanks containing 100 L of dechlorinated tap water held at 24°C. Fish were fed a diet equal to 5% of fish wet weight twice a day. Each tank was fed either a control diet or diet supplemented with 13.5 µg/g total mercury dry weight (95% ethanol with dissolved MeHg chloride). Every two days water was replaced in each tank and cleaned of remaining food and feces. Skeletal muscle was harvested from sacrificed fish in both experiments. Cambier et al. (2009) continued the experiment for an additional 14 days and reported the same mercury concentrations at day 25 in the skeletal muscle fiber as Cambier et al. (2010). At day 25 skeletal muscle mercury concentrations were 1.77 and 25.4 µg/g total mercury dry weight for the control and test diet, respectively (Cambier et al. 2009, 2010). Assuming a 71.3% moisture content for zebrafish (Appendix D), the concentrations equate to 0.5086 and 7.299 µg/g total mercury wet weight skeletal muscle. At day 49 total mercury concentrations in skeletal muscle of zebrafish were 1.93 and 35.5 µg/g total mercury dry weight for the control and test diet, respectively (Cambier et al. 2009), or approximately 0.5546 and 10.20 µg/g total mercury wet weight. At test termination (day 49) there were no effects on mortality or mobility, or after another 14 days of continued observation to 63 days (Cambier et al. 2009). At day 49 there was a significant

decrease in mitochondrial oxygen consumption in zebrafish fed diets supplemented with MeHg (approximately 1.8 versus 0.4 ng O/min/mg fiber) (Cambier et al. 2009). At day 25, the test diet also altered the gene expression in the skeletal muscle with 60 genes up-regulated and 15 down regulated by more than two times (Cambier et al. 2010). Based on these studies the 25-day (Cambier et al. 2009) and 49-day (Cambier et al. 2010) NOECs based on lack of significant mortality were 7.299 and 10.20 µg/g total mercury wet weight skeletal muscle, respectively, and were the values used to represent the relative insensitivity of zebrafish to dietary mercury exposure in the two similar studies.

Document Source: Lerebours, A., S. Cambier, L. Hislop, C. Adam-Guillermina and J-P Bourdineaud. 2013. Genotoxic effects of exposure to waterborne uranium, dietary methylmercury and hyperoxia in zebrafish assessed by the quantitative RAPD-PCR method. Mutation Res. 755: 55-60.

Lerebours et al. (2013) investigated the genotoxic effects of water concentrations of uranium, dietary MeHg and hyperoxia on zebrafish using the RAPD-PCR quantitative method. The focus of this assessment is on the dietary MeHg exposure which is similar to the other test designs (Gonzalez et al. 2005; Oliviera-Ribeiro et al. 2008; Cambier et al. 2009, 2010). Adult male fish (0.88 g, 3.63 cm) each were placed in one of two flow-through exposure tanks held at 24°C for two weeks to acclimate to test conditions. Fish were fed a diet equal to 2.5% of fish wet weight twice a day. Each tank was fed either a control diet or diet supplemented with 13.5 µg/g total mercury dry weight (95% ethanol with dissolved MeHg chloride). Ten fish from each exposure were removed after 50 days and sacrificed. The authors noted that no macroscopic health effects were observed during the exposure. Muscle concentrations at test termination were 1.9 and 36 µg/g total mercury dry weight for the control and test diet, respectively (note: this is similar to values reported in Cambier et al. 2009); assuming a 71.3% moisture content for zebrafish these

skeletal muscle values equate to 0.5460 and 10.34 µg/g total mercury wet weight. At test termination there was no change in the number of hybridization sites using OPB7 and hybridization temperatures of 50°C and 60°C, and using OPB11 and a hybridization temperature of 50°C, but there was a significant increase and a significant decrease in the frequency of appearance of PCR products using OPB7 and a hybridization temperature of 60°C in the temperature intervals (75–76) and (76–77), respectively. A significant decrease was found in the interval (74–75) using OPB11 and a hybridization temperature of 50°C was also observed. An LOEC, based on DNA damage, of 10.34 µg/g total mercury wet weight skeletal muscle tissue was used to represent the relative insensitivity of zebrafish to dietary mercury exposure in the study.

Document Source: Amlund. H., A-K. Lundebyea, D. Boyleb and S. Ellingsen. 2015. Dietary selenomethionine influences the accumulation and depuration of dietary methylmercury in zebrafish (*Danio rerio*). Aquat. Toxicol. 158: 211-217.

Amlund et al. (2015) fed adult zebrafish MeHg (as methyl mercury-cysteine) with or without selenium (as selenomethionine) for 8 weeks to study MeHg toxicokinetics. Zebrafish were from an in-house culture where fish were kept in filtered dechlorinated tap water held at 28.5°C, 500 µS/cm, pH 7.5, and 10% daily water exchange. Photoperiod was 14:10 light:dark. Thirty-three to fifty-three male and female fish (0.32 g) were placed in 9 L tanks with three replicate tanks per treatment. Zebrafish were fed one of two MeHg enriched diets (5 and 10 µg/g dry weight) or control diet (commercial pelleted zebrafish diet) three times per day for 8 weeks at a total ratio of 1.0% of their body weight. A subset (11-15 fish per tank) was held for another 4 weeks and fed a control diet for depuration observations. Measured total mercury concentrations in the diet were 0.08, 5.2 and 9.8 µg/g dry weight for the control, low and high Hg test diets, respectively. Three fish per tank were pooled for mercury tissue analysis at test initiation, week 2 and week 8 of the

exposure period, and at week 12 during the depuration period. Mean measured total mercury muscle concentrations at the end of 8 weeks were <0.005, 3.4 and 6.4 µg/g wet weight, for the control, low and high MeHg test diets, respectively. The dietary MeHg treatment did not have a significant effect on zebrafish growth (weight) or survival after 8 weeks of exposure. Fish weight in the control, low and high treatments were 0.34 g, 0.33g and 0.35 g at 8 weeks, with no significant decrease observed in either control or MeHg treated fish after the 4-week depuration period. Mortality was low (zero to few fish died) throughout the experiment. The NOEC, based on growth and survival, was 6.4 µg THg/g ww muscle tissue and was used to represent the relative insensitivity of zebrafish to dietary mercury exposure in the study. Taken together, these studies provide supporting evidence for the GMCV of 4.426 µg THg/g ww in muscle tissue (Penglase et al. 2014a, 2014b) that represents the relative sensitivity of this species in the family Cyprinidae.

B.4 Acipenseridae

B.4.1 White Sturgeon *(Acipenser transmontanus)*

Document Source: Webb, M.A.H., G.W. Feist, M.S. Fitzpatrick, E.P. Foster, C.B. Schreck, M. Plumlee, C. Wong and D.T. Gundersen. 2006. Mercury concentrations in gonad, liver, and muscle of white sturgeon *Acipenser transmontanus* in the lower Columbia River. Arch. Environ. Contam. Toxicol. 50(3): 443-51.

Webb et al. (2006) previously collected 57 “legal size” (slot limit - 110 to 137 cm fork length; 14-20 year-old) white sturgeon (*Acipenser transmontanus*) from the Columbia River estuary, and the Bonneville, The Dalles, and John Day Reservoirs to assess the relationship between tissue mercury concentrations and various physiological parameters. All of the female fish (n = 26), and 29/31 male sturgeon were sexually immature. Total mercury (THg) was quantified in liver, gonad, and cheek muscle tissue and condition factor (CdF), relative weight (Wr), and gonadosomatic index (GSI) and plasma sex steroid concentrations were determined.

Condition Factor (CdF) and Wr were both significantly lower ($p < 0.0001$) in sturgeon from the Bonneville Reservoir, though this effect was attributed to intraspecific competition (Beamesderfer et al., 1995) rather than dietary mercury exposure. However, mercury did have an impact on the reproductive physiology of immature sturgeon in the Columbia River Basin. Reproductive staging was evaluated based on observing correlations between tissue mercury concentration and circulating testosterone (T) and 11-Ketotestosterone (KT) concentrations in immature Stage 2 males. Webb observed that 21/29 (72%) male fish had circulating [T] <4 ng/ml, and that no male fish with muscle [THg] > 0.187 µg/g ww had plasma T concentrations >4 ng/ml ($p = 0.0122$, $R^2 = 0.26$). Significant reductions in KT (ng/L) with increasing muscle concentrations ($p = 0.0024$, $R^2 = 0.16$) was also observed. Also, Webb and co-investigators observed negative correlations gonad and liver mercury, and immature male sturgeon had decrease GSI negatively correlated with increased gonadal mercury ($p = 0.0014$, $R^2 = 0.21$). The decrease in sex steroids and GSI in sturgeon with increased muscle and liver mercury content suggests negative effects of mercury on steroidogenesis and development of reproductive organs, although the mechanism(s) were not studied further. The reduction in the GSI of immature male sturgeon correlating to elevated tissue mercury concentrations was similar to observations in juvenile male walleye (Friedmann et.al. 1996), and in juvenile female fathead minnows (Hammerschmidt et al. 2002; Drevnick and Sandheinrich. 2003, discussed in **Section 3.3**) following long term dietary exposures (≥ 6 months). Since the observed effects on reproduction in immature males were observed in more than one waterbody in the study, EPA used the average mercury concentration in the muscle (0.176 µg THg/g ww) reported for the Columbia River Basin to represent the relative sensitivity of this species. This study is important because a listed subpopulation of the white sturgeon is present in the Kootenai River in Idaho.

Appendix C Data Not Acceptable for Use in Criterion Derivation

C.1 Unacceptable Dietary Mercury Studies

C.1.1 American Toad (Anaxyrus americanus)

Using the same 2 x 3 factorial experimental design as Bergeron et al. (2011a), Todd et al. (2011) examined the individual and interactive effects of maternally-derived and dietary mercury on fitness-related traits of American toad larvae. As described above, for the purposes of deriving the mercury aquatic life criterion, only the effects from dietary mercury exposure were considered and only for offspring from reference mothers. Eggs from reference mothers were allowed to hatch and larvae were fed diets of either no added Hg or 2.5 or 10 µg/g total Hg (dry wt.). Preparation of the experimental diets was consistent with those described above in Bergeron et al. (2011a), as were the measured total and methylmercury concentrations in the prepared diets. In this study, the control and two dietary treatments were replicated six times, each in a polypropylene bin containing approximately 60 L dechlorinated tap water and fifty approximately 4-day old post hatchlings. Ten larvae from each of the bins were randomly drawn every 9 days and weighed to adjust food rations to account for growth. Food rations were also adjusted to account for reduced density resulting from mortality or metamorphosing animals. Each bin was supplied with rations equivalent to 9% of the total larval mass in each bin per day (wet wt. basis) every 3 days.

All bins were inspected daily for dead individuals and checked at 12-h intervals as larvae neared metamorphosis for front limb emergence at Gosner stage (GS) 42, where upon emergence of the front limbs, larvae were removed, weighed, measured, and placed in individual 500-ml cups too allow observation of the presence of any gross spinal malformations at this stage and prior to the animals beginning tail resorption. Metamorphosing larvae in cups were also checked at 12-h intervals for completion of tail resorption (GS 46) or mortality. In addition to quantifying

the proportion of individuals that successfully completed metamorphosis in each treatment, mass and size at GS 42 and 46 were also determined, as well as the duration of the larval period to GS 42 and the time required for complete tail resorption (time between GS 42 and GS 46). During the peak frequency of metamorphosis, three to six individuals were randomly selected from each replicate for hopping performance trials. Within 24 hours of completing tail resorption, each recently metamorphosed toad was placed on a clean, dry platform and gently nudged on the urostyle to elicit a flight response. Length of the first four hops was marked and measured, and mean hop length for each individual was calculated. The mean hop lengths of each individual within a replicate bin were averaged to produce a representative mean hop length for each replicate bin. All surviving metamorphosed toads were euthanized and then frozen for later total mercury and methylmercury tissue analyses.

In this study, no statistically significant effects were observed on survival, growth, development, malformation, or hopping performance in tadpoles fed up to 10.13 µg/g total mercury dw. The study authors note that most of the effects from dietary mercury could be attributed to the variance properties of the combined endpoints rather than to a single effect. The total mercury NOEC for dietary mercury exposure in this study was predicted to be greater than 10.13 µg/g dw based on survival, development, and hopping performance at metamorphosis. Based on the mercury accumulation level at metamorphosis presented in the follow-up work by Todd et al. (2012), the corresponding whole-body total mercury NOEC would be >3.25 µg/g dw. Assuming 75% moisture content of larval American toad, the NOEC for whole body total mercury is >0.8125 µg/g ww (3.250 µg/g dw ÷ 4), the value EPA selected for criterion derivation from the study.

Todd et al. (2012) observed the persistent effects of maternally-derived and dietary mercury exposure on American toads after metamorphosis. Recently metamorphosed toads from the study described previously (see Todd et al. 2011) were placed in terrestrial, outdoor mesocosms to examine the latent effects of mercury exposure following exposure in a 2 x 2 experimental design consisting of juveniles from reference and mercury-exposed mothers either fed a control (0.01 µg/g dw) or high total mercury (10.1 µg/g dw) diet during the larval stage in Todd et al. (2011). Juvenile toads were released into the outdoor enclosures in late June 2009 within 48 hours of metamorphosis. No additional dietary mercury was provided to the animals at any point after being released into the mesocosms. Enclosures were searched throughout 2009 and again in May 2010, upon which time captured individuals were identified, and snout-vent length and mass measurements were collected. Total mercury concentrations in toads fed dietary mercury were 3 - 6 times greater after metamorphosis than those fed the control diet. Dietary mercury did not affect overall survival or growth of toads after one year. As with Todd et al. (2011), an author-reported total mercury NOEC for trophically-derived (dietary) mercury was predicted to be greater than 10.13 µg/g dw in diet based on survival after metamorphosis. Whole-body (WB) total mercury concentrations dropped 13.6-fold during the course of the study. EPA estimated the time-averaged WB concentration for the NOEC as the geometric mean of the initial level, 3.25 µg/g dw (at metamorphosis), and final level, 0.2388 µg/g dw (at 1 year): 0.881 µg/g dw (where the fraction methylmercury was not reported). Assuming 75% moisture content of larval American toad, the WB total mercury NOEC, a greater than value since no effects were observed, is >0.220 µg/g ww (0.881 µg/g dw ÷ 4), the value EPA selected for criterion derivation from the study.

C.1.2 Wood frog (Lithobates sylvaticus)

Wada et al. (2011) examined the effects of dietary mercury on the thyroid hormone concentrations, development, growth, performance, and survival of wood frog (*Lithobates sylvaticus*) tadpoles. Five recently laid egg masses were collected from a forested wetland in Montgomery County, Virginia. After being brough back to the laboratory and hatching after a little over a week, 216 free-swimming stage tadpoles (Gosner Stage (GS) 21-23) were arbitrarily chosen and individually placed in polypropylene containers (2.2 L) filled with dechlorinated tap water. These tadpoles were evenly distributed among the control (0.006 µg/g dw) and two dietary mercury treatment groups of 2.5 and 10.13 µg/g dw of mercury measured as total mercury, with 2.75% measured as methylmercury for the low Hg diet and 1.05% for the high Hg diet. Preparation of the experimental diets was consistent with those described above for Bergeron et al. (2011a). Tadpoles were subsequently fed 6% of their body weight per day on a wet-weight basis. Fresh diet was provided every 2-3 days, after which uneaten food was suctioned out and water was exchanged. Twenty-five percent of the tadpoles (18 tadpoles/treatment) were weighed every 8-9 days to determine the effects of Hg treatment on growth rate and to adjust diet portions to accommodate larval growth. The exposures lasted until the last tadpole completed metamorphosis (GS 46), resulting in an exposure duration of up to 84 days.

During the experiment tadpoles were analyzed for thyroid hormone concentrations at three different developmental stages according to Gosner stage: 36-37, 42 (front limb emergence, and 46 (completion of tail resorption). Individuals at GS 46 were euthanized within one day of testing hopping performance as previously described under Todd et al. (2011). Mercury concentrations (inorganic mercury (HgII) and methylmercury) were determined for individuals at GS 42 and 46. In addition to thyroid hormone and mercury concentrations, mortality, growth,

and time to reach GS 42 and 46 were recorded. Tadpoles were checked once a day for mortality and onset of metamorphic climax (GS 42) and checked twice a day for completion of tail resorption (GS 46). When animals were euthanized for final thyroid hormone or Hg analysis, they were weighed, and their snout-vent length (SVL) was measured.

Control survival of tadpoles was 94.4%. There were no observed differences in survival, metamorphic success, or growth between the control and any of the dietary mercury treatment groups ($p \geq 0.09$). Additionally, dietary mercury treatment did not alter the whole-body thyroid hormone concentrations in wood frog tadpoles at any of the developmental stages sampled. The total mercury NOEC for dietary mercury exposure to wood frog tadpoles was predicted to be greater than 10.13 µg/g dw in diet based on survival, development and performance after metamorphosis. The corresponding whole-body total mercury concentration at the dietary NOEC was 3.54 µg/g dw at GS 42 and 2.57 µg/g dw at GS 46. Assuming 75% moisture content of larval wood frog, the whole-body total mercury NOECs, which are greater-than values because no effects were observed, are >0.885 µg/g ww (GS 42) and >0.643 µg/g ww (GS 46), the latter value EPA selected for criterion derivation.

C.1.3 Finescale dace (Phoxinus neogaeus)

Hall et al. (1997) conducted a field experiment to examine the relative importance of food and water to methylmercury uptake in fish at natural concentrations. Differences in the uptake and accumulation of methylmercury via aqueous and dietary pathways were determined using finescale dace (*Phoxinus neogaeus*) obtained commercially and held in 2000 L enclosed pens floating in an undisturbed, oligotrophic lake (Lake 240, Experimental Lakes Area) in northwestern Ontario. A 2x2 factorial design was used to expose dace to water containing either low (0.10–0.40 ng/L) or high (0.80–2.1 ng/L) methylmercury, and zooplankton with either low (0.16–0.18 µg/g dw) or high (0.28–0.76 µg/g dw) methylmercury added daily to each pen. Water

from natural sources, consisting of either high or low MeHg concentrations, was used to fill the pens. Pens holding low MeHg water were from Lake 240 (chosen because of its low methylmercury water concentrations and location of experimental pens) and high methylmercury water was taken from nearby Lake 470 (L470), a lake surrounded by wetlands. Twenty percent of the water in each of the pens was renewed three times a week. Zooplankton with low concentrations of MeHg were collected from Lake 304 (L304), a small fishless lake. Lake 979 (L979), an experimentally flooded wetland pond, was the source of the high MeHg zooplankton. Zooplankton community structure differed in the two lakes. To ensure fish were receiving similar amounts of food daily, dry/wet weight relationships were determined weekly and used to calculate the quantity of live zooplankton added to each pen on a dry weight basis. On a given day, all pens received the same dry weight of zooplankton. An increase in water MeHg concentrations with the addition of high MeHg zooplankton resulted in the fish being exposed to a third (intermediate) water concentration of 0.45-1.30 ng/L. The unexpected elevated MeHg concentrations in the water resulted from either leaching of MeHg during decomposition of dead zooplankton, or equilibration of levels of MeHg in living zooplankton with the water.

Following a 32-day exposure, fish survival, weight, and mercury accumulation were assessed at the end of the exposure. Survival was highest in the low water, low dietary methylmercury treatment at 96% (23 of 24 fish surviving), while all other combinations exhibited 79% survival (19 of 24 fish surviving). Finescale dace maintained their weight in one replicate of one treatment (high MeHg food, intermediate MeHg water), however most treatments lost between approximately 0.4 and 1.1 g (-8.4% to -22.8%) over the course of the experiment. Weight loss was not dependent on the treatment (one-way ANOVA, $p = 0.982$). Fish fed zooplankton with high concentrations of methylmercury had significantly higher

concentrations of total mercury in muscle than fish fed zooplankton with low concentrations of methylmercury (ANCOVA, $p<0.0001$). The total mercury concentrations of fish that fed on zooplankton with low concentrations of methylmercury were not significantly different from those in fish at the start of the experiment, indicating that food was the dominant pathway of methylmercury uptake by fish. The authors estimate that direct absorption of methylmercury from the water may have been responsible for approximately 15% of the mercury uptake in fish muscle. One-way ANOVAs revealed that differences between average Hg concentrations of fish from duplicate pens were not significant. The mean total mercury concentration in dace exposed to high methylmercury in water and food was 0.240 µg/g ww. Given the lack of significant reduction in growth of fish as this tissue concentration, for purposes of criterion derivation the NOEC is defined as >0.240 µg Hg/g ww.

Recently, Martins et al. (2021) conducted a thorough review of the peer-reviewed literature (23 papers) examining methylmercury bioaccumulation in freshwater invertebrates, focused principally on aquatic insects. The data selection criterion required information on mean values of methylmercury, total mercury, percentage of total mercury in the form of methylmercury (%MeHg), stable isotopes of nitrogen (δ15N) and carbon (δ13C) in invertebrates, as well as characteristics of waterbodies where the invertebrates were collected (e.g., pH, total organic carbon [TOC], and total nitrogen [TN]) that could influence mercury exposure.
The researchers found that across studies, trophic position had the greatest influence on both total mercury and highest percentage of mercury that was quantified as methylmercury. Most of these taxa are predatory, so both their higher total and methylmercury concentration is expected to biomagnification in the food web similar to fish. In contrast, and similar to data from Xie et.al. (2009), ambient measurements from primary consumers (freshwater mussels, caddisflies,

mayflies and zooplankton) had among the lowest reported total and methylmercury content (average range of 0.01 – 0.025 µg THg/g ww), with median %MeHg ranging anywhere from approximately 40 to 47% for mayflies and caddisflies, specifically. It was also clear that many individual invertebrates, including aquatic insect larvae of all trophic positions, are capable of acquiring and withstanding elevated MeHg concentrations. For example, the maximum THg concentration reported for caddisflies was 0.1364 µg THg/g ww. The highest mercury concentrations observed in invertebrates have been from crayfish collected from river systems contaminated by chloralkali plants and pulp mills ranging from 0.56 µg THg/g ww in the Wisconsin River (Sheffy 1978) to 4.7 to 9.6 µg THg/g ww from a river in Canada (Vermeer et al. 1973).

Appendix D Idaho Mercury Conversion Factors

D.1 Background

EPA derived a tissue-based criterion element for the protection of aquatic life in the State of Idaho due to the importance of the dietary route for mercury exposure in aquatic life. A tissue-based criterion for the receptor organisms was determined to be a better approach than a dietary-based criterion due the wide variability in diet types used for mercury exposures found in scientific publications, and some uncertainty with the composition and form of mercury in diets. An important implementation issue raised in the mercury aquatic life criterion (ALC) development effort for the State of Idaho is the expression of the criterion as a wet weight (ww) concentration, and expression of the criterion as either whole body or muscle concentration equivalents. EPA recognizes that it is important to be able to compare EPA's proposed tissue-based values (whole body and muscle) to monitoring data for aquatic life collected as muscle (fillet or muscle plug) in fish or as whole-body tissue concentrations for fish or other aquatic life. Therefore, EPA collected available data for derivation of wet weight (ww) to dry weight (dw) and whole body to muscle conversion factors, described below.

D.2 Percent Moisture Conversion

The current Hg dataset for Idaho includes 19 species (13 fish species, two amphibian species, one insect species, two non-insect arthropod species, and one mollusk species). While the majority of the 22 chronic values used to calculate the species mean chronic values reported tissue total Hg concentrations on a wet weight basis, several values required conversion from dry weight to wet weight. The associated species are displayed in **Table D-1**. The list includes two frog species, four fish species, one insect and one invertebrate.

Table D-1. Species with chronic Hg tissue values reported as dry weight.

Family	Species	Endpoint	Reference
Ranidae	Southern leopard frog, *Lithobates sphenocephala*	Survival and metamorphic success	Unrine et al. 2004
Bufonidae	American toad, *Anaxyrus americanus*	Decreased growth as mass at Gosner Stage (GS) 42	Bergeron et al. 2011a
Acipenseridae	Green sturgeon, *Acipenser medirostris*	Survival and growth	Lee et al. 2011
Acipenseridae	White sturgeon, *Acipenser transmontanus*	Survival and growth	Lee et al. 2011
Cyprinidae	Fathead minnow, *Pimephales promelas*	Reproductive endpoints (reproductive success, delay spawning, etc.)	Hammerschmidt et al. 2002
Cyprinidae	Zebrafish, *Danio rerio*	Survival and growth	Penglase et al. 2014a, 2014b
Cambaridae	Red swamp crayfish, *Procambarus clarkia*,	Survival	Brant 2004
Ephemeridae	Mayfly, *Hexagenia sp*.	Survival and growth	Naimo et al. 2000

EPA conducted a literature search to find percent moisture values for the several species requiring this conversion (**Table D-1**). Available percentages for each species or their surrogates up to the family level were available for all species. The percentages are displayed in **Table D-2**. Dry weight tissue concentrations were converted to wet weight based on the following equation:

$$Wet\ weight\ tissue\ concentration = \frac{Dry\ weight\ tissue\ concentration}{[100 \div (100 - Perecent\ Moisture)]}$$

Details regarding conversion, and how percent moisture was calculated for each taxonomic group is described below. A summary of converted tissue concentrations and conversion factors is provided in **Table D-4**. Additional percent moisture values not used for conversion are provided in **Table D-5** as a resource for other research needs.

Table D-2. Species-specific percent moisture values used to convert tissue Hg concentrations from dry weight to wet weight.

Family	Common Name	Tissue	Count	Min. % Moisture	Max. % Moisture	Avg. % Moisture	Reference
Ranidae	European common frog, *Rana temporaria*	Whole body[a]	NR	-	-	86.25	Fletcher and Myant 1959
Ranidae	Wood frog, *Lithobates sylvaticus*	Whole body[a]	NR	-	-	83.5	Wada et al. 2011
Bufonidae	Toad, *Bufo arenarum*	Whole body[a]	NR	-	-	87.1	Bergeron et al. 2011a
Bufonidae	Toad, *Bufo arenarum*	Whole body[a]	3	-	-	90.4	Bergeron et al. 2011b
Bufonidae	Toad, *Bufo arenarum*	Whole body[a]	NR	-	-	89.3	Todd et al. 2011
Acipenseridae	Shortnose sturgeon, *Acipenser brevirostrum*	Skinless, boneless filet	9	70.5	82.5	76.1	Mierzykowski 2012
Acipenseridae	Atlantic sturgeon, *Acipenser oxyrinchus*	Skinless, boneless filet	2	75.6	77.0	76.3	Mierzykowski 2012
Acipenseridae	Shovelnose sturgeon, *Scaphirhynchus platorynchus*	Fish fillet	13	67.1	81.6	77.1	May et al. 2009
Cyprinidae	Zebrafish, *Danio rerio*	Whole Body	3	70.0	73.0	71.3	Lin et al. 2022
Cyprinidae	Fathead minnow	Whole Body	298	0	84.7	76.64	GEI 2014; U.S. EPA 2016a
Cambaridae	Crayfish, *Procambarus clarkii*	Whole body	-	-	-	-[b]	Anastacio et al. 1999
Ephemeridae	Mayfly, *Hexagenis bilineata*	Whole body (emergent)	30	40	74	60.9	Steingraeber et al. 1994
Ephemeroptera	Mayfly, (species not identified)	Whole body (immature)	18	72.2	76.6	74.1	Saiki et al. 2001

NR: not reported
a Post-metamorphosis
b Expressed as equation dependent on body size: Wet Weight = 5.28607 x Dry Weight$^{0.937422}$

D.2.1 Amphibians

A total of five percent moisture values representing three species from two families were available for the development of a dry weight to wet weight conversion factor for amphibians. All values were for the post-metamorphic life stage and ranged from 83.5 to 90.4 (**Table D-2**). To calculate the conversion factor for amphibians, the percent moisture was averaged for each species and then the grand average of the three species was determined. This approach was chosen since values had a narrow range, were not dissimilar, and were from the same families as the species needing conversion. The grand average percent moisture value used for conversion of

amphibian tissue Hg concentrations from dry weight to wet weight was 86.23; the average of *Bufo arenarum* (88.93), *Rana temporaria* (86.25), and *Lithobates sylvaticus* (83.5). The dry weight tissue values for the southern leopard frog (*Lithobates sphenocephala*) and the American toad (*Anaxyrus americanus*) were subsequently divided by a factor of 7.26 to convert to wet weight. The LOEC of 0.2376 µg/g THg dw from Unrine et al. (2004) divided by 7.26 is equal to 0.03272 µg/g THg dw and is used as the SMCV for the southern leopard frog. The NOEC and LOEC of 0.800 and 1.800 µg/g THg dw, respectively from Bergeron et al. (2011a) were divided by 7.26 and are equal to 0.1102 and 0.2479 µg/g THg ww, respectively. The geometric mean of these two values (0.1653 µg/g THg ww) represents the MATC and SMCV for the species, *Anaxyrus americanus*.

D.2.2 Fish

Three average percent moisture values were available for three different fish species in the Family Acipenseridae (**Table D-2**). These values were averaged to convert the two dry weight tissue values for two other related sturgeon species (green sturgeon, *Acipenser medirostris* and white sturgeon, *Acipenser transmontanus*) in the same family. The average percent moisture value for fish in the Family Acipenseridae is 76.5. The NOEC and LOECs for the green and white sturgeon from Lee et al. (2011) were divided by a factor of 4.26 to convert dry weight tissue Hg concentrations to wet weight. The NOEC and LOEC for the green sturgeon are 50.8 and 115.2 µg/g THg dw muscle, respectively, or 11.94 and 27.07 µg/g THg ww. The MATC (geometric mean of the NOEC and LOEC) of the latter values represents the SMCV for the green sturgeon based on muscle tissue, or 17.98 µg/g THg ww. Similarly, the white sturgeon NOEC and LOEC (104.4 and 231.8 µg/g THg dw muscle) was divided by 4.26 and is equal to 24.53 and 54.47 µg/g THg ww, respectively. The MATC of 36.56 µg/g THg ww represents the SMCV for the white sturgeon based on muscle tissue.

Only one of the three studies used to calculate the SMCV for the fathead minnow reported mercury tissue concentrations as dry weight: Hammerschmidt et al. (2002). An average percent moisture value (76.64) for the species from close to 300 whole body samples was used to convert the chronic value from the test to wet weight. The LOEC of 3.102 µg/g THg dw whole body tissue from the study was divided by a factor of 4.28 and is equal to 0.7246 µg/g THg ww. This value was subsequently divided by a factor of three to represent a NOEC (0.2415 µg/g THg ww) for the study and subsequently used with two other chronic whole body tissue Hg values in the SMCV calculation for the fathead minnow.

Likewise, the chronic tissue Hg concentration from Penglase et al. (2014a, 2014b) for the zebrafish, *Danio rerio*, was reported on a dry weight basis. Three different percent moisture values estimated from Figure 1C in Lin et al. (2022) were averaged to convert the tissue Hg value from dry to wet weight. A factor of 3.48 based on an average percent moisture value of 71.3 for zebrafish was used to convert the LOEC of 33.31 µg/g THg dw whole body tissue to 9.560 µg/g THg ww. This value was then divided by a factor of three to represent a NOEC of 3.187 µg/g THg ww whole body tissue for the test and is the SMCV for the zebrafish.

D.2.3 Crayfish

The relationship between wet weight and dry weight of the red swamp crayfish, *Procambarus clarkii*, was previously described in Anastacio et al. (1999). Based on this relationship percent muscle moisture decreases as crayfish grow. To translate the chronic tissue Hg value for red swamp crayfish from Brant (2004), the wet weight of crayfish that died during the test was estimated from Figure 3.5 and Figure 3.6 of the publication. These weights were than translated to dry weight using the equation presented by Anastacio: Wet Weight = 5.28607 x Dry Weight$^{0.937422}$. The percent moisture of the deceased crayfish from Brant (2004) ranged from 80.55 to 81.51, with an average value of 80.77 (**Table D-3**). There was very little variation

in the percent moisture for the crayfish despite the range in sizes of deceased organisms (~3.75 – 8 g ww). The average abdominal muscle tissue Hg concentration of the deceased crayfish was divided by a factor of 5.20 and is equal to 1492 ng/g THg ww or 1.492 µg/g THg ww abdominal muscle tissue. This value was then divided by a factor of three to represent a NOEC of 0.4973 µg/g THg ww for the test and is the SMCV for the crayfish.

Table D-3. Crayfish Percent Moisture from Brant 2004

Exp. Day of death	Sex	Age	Muscle THg (ng/g dw)	Estimated WW (g) from Figures	Calculated DW (g) from Equation	% Moisture
90	F	2	6544.92	6.7	1.29	80.78
100	F	2	7820.54	6.9	1.33	80.74
114	M	2	8435.2	7.25	1.40	80.68
129	F	2	6764.27	7.75	1.50	80.59
133	F	2	6818.74	7.5	1.45	80.64
136	M	1	7777.87	3.75	0.69	81.51
136	F	1	10128.56	6.25	1.20	80.87
141	M	2	7173.53	8	1.56	80.55
142	M	2	8353.16	8	1.56	80.55
			AVG = 7757			**AVG = 80.77**

D.2.4 Mayfly

Two different percent moisture values are available for mayflies in two different families, Ephemeridae and Ephemeroptera (**Table D-2**). While the tissue value in the criterion dataset needing conversion is for mayfly (*Hexagenia* sp.) nymphs from the Family Ephemeridae, the average percent moisture value for the family is based on emergent mayflies. A second average percent moisture value for a different family (Ephemeroptera) is based on immature mayflies. The chronic tissue Hg value from Naimo et al. (2000) was based on whole body concentrations in mayfly nymphs. Therefore, the two average percent moisture values from the two families, one based on emergent mayflies in the same family and one on immature mayflies from a different family, were averaged to convert the dry tissue concentration. The average percent moisture value for the two mayfly families was 67.5. The NOEC of 10.819 µg/g THg dw whole

body tissue was divided by a factor of 3.08 to calculate the NOEC of 3.516 µg/g THg ww, which represents the SMCV for the genus, *Hexagenia*.

Table D-4. Summary of Converted Tissue Concentrations.

Species	Reported NOEC (µg/g THg dw)	Reported LOEC (µg/g THg dw)	Percent Moisture Used / Conversion Factor	Calculated NOEC (µg/g THg ww	Calculated LOEC (µg/g THg ww
Southern leopard frog, *Lithobates sphenocephala*	-	0.2376	86.23 / 7.26	0.01308	0.03272
American toad, *Anaxyrus americanus*	0.8	1.8	86.23 / 7.26	0.1102	0.2479
Green sturgeon, *Acipenser medirostris*	50.8	115.2	76.5 / 4.26	11.94	27.07
White sturgeon, *Acipenser transmontanus*	104.4	231.8	76.5 / 4.26	24.53	54.47
Fathead minnow, *Pimephales promelas*	-	3.102	76.64 / 4.28	-	0.7246
Zebrafish, *Danio rerio*	-	33.31	71.3 / 3.48	-	9.560
Red swamp crayfish, *Procambarus clarkii*,	-	7.757	80.77 / 5.20	-	1.492
Mayfly, *Hexagenia sp.*	10.819	-	67.5 / 3.08	3.516	-

Table D-5. Percent Moisture Values for Other Taxa.

Class	Family	Common Name	Genus	Species	Tissue	Count	Min. % Moisture	Max. % Moisture	Avg. % Moisture	Reference
FISH TAXON										
	-	Mixed species			Whole Body	3	69.0	74.2	71.3	USGS NCBP 2016
Actinopterygii	Acipenseridae	Shortnose sturgeon	*Acipenser*	*brevirostrum*	Skinless, boneless filet	9	70.5	82.5	76.1	Mierzykowski 2012
Actinopterygii	Acipenseridae	Atlantic sturgeon	*Acipenser*	*oxyrinchus*	Skinless, boneless filet	2	75.6	77.0	76.3	Mierzykowski 2012
Actinopterygii	Acipenseridae	Shovelnose sturgeon	*Scaphirhynchus*	*platorynchus*	Fish fillet	13	67.1	81.6	77.1	May et al. 2009
Actinopterygii	Amiidae	Bowfin	*Amia*	*calva*	Whole Body	2	70.5	74.3	72.4	USGS NCBP 2016
Actinopterygii	Amiidae	Bowfin	*Amia*	*calva*	Whole Body	2	-	-	79.0	Swarzenski et al. 2004
Actinopterygii	Catostomidae	River carpsucker	*Carpiodes*	*carpio*	Whole Body	13	60.7	80.7	69.2	USGS NCBP 2016
Actinopterygii	Catostomidae	Quillback carpsucker	*Carpiodes*	*cyprinus*	Whole Body	6	63.5	69.2	66.2	USGS NCBP 2016
Actinopterygii	Catostomidae	Carpsucker	*Carpiodes*	*sp.*	Whole Body	18	67.2	77.2	71.5	USGS NCBP 2016
Actinopterygii	Catostomidae	Longnose sucker	*Catostomus*	*catostomus*	Whole Body	21	64.1	80.7	73.2	USGS NCBP 2016
Actinopterygii	Catostomidae	Bridgelip sucker	*Catostomus*	*columbianus*	Whole Body	4	70.1	76.4	74.1	USGS NCBP 2016
Actinopterygii	Catostomidae	White sucker	*Catostomus*	*commersoni*	Whole Body	114	62.7	81.3	74.8	USGS NCBP 2016
Actinopterygii	Catostomidae	White sucker	*Catostomus*	*commersoni*	Whole Body	246	71.6	83.5	77.4	GEI 2014; U.S. EPA 2016a
Actinopterygii	Catostomidae	Flannelmouth sucker	*Catostomus*	*latipinnis*	Whole Body	-	-	-	-	USGS NCBP 2016
Actinopterygii	Catostomidae	Largescale sucker	*Catostomus*	*macrocheilus*	Whole Body	59	63.5	78.7	73.5	USGS NCBP 2016
Actinopterygii	Catostomidae	Klamath sucker	*Catostomus*	*snyderi*	Whole Body	7	68.5	75.6	72.5	USGS NCBP 2016
Actinopterygii	Catostomidae	Tahoe sucker	*Catostomus*	*tahoensis*	Whole Body	6	69.3	77.7	72.8	USGS NCBP 2016

Class	Family	Common Name	Genus	Species	Tissue	Count	Min. % Moisture	Max. % Moisture	Avg. % Moisture	Reference
Actinopterygii	Catostomidae	Smallmouth buffalo	*Ictiobus*	*bubalus*	Whole Body	25	58.2	75.9	68.5	USGS NCBP 2016
Actinopterygii	Catostomidae	Bigmouth buffalo	*Ictiobus*	*cyprinellus*	Whole Body	8	61.6	72.2	68.3	USGS NCBP 2016
Actinopterygii	Catostomidae	Spotted sucker	*Minytrema*	*melanops*	Whole Body	22	67.9	75.6	72.3	USGS NCBP 2016
Actinopterygii	Catostomidae	Redhorse	*Moxostoma*	*sp.*	Whole Body	36	58.8	79.2	71.9	USGS NCBP 2016
Actinopterygii	Catostomidae	River redhorse	*Moxostoma*	*carinatum*	Whole Body	1	-	-	79.2	GEI 2014; U.S. EPA 2016a
Actinopterygii	Catostomidae	Northern hogsucker	*Hypentelium*	*nigricans*	Whole Body	113	61	83	76.1	GEI 2014; U.S. EPA 2016a
Actinopterygii	Catostomidae	Northern hogsucker	*Hypentelium*	*nigricans*	Fish fillet	3	78.5	78.8	78.6	May et al. 2009
Actinopterygii	Centrarchidae	Rock bass	*Ambloplites*	*rupestris*	Whole Body	8	71.0	74.7	73.0	USGS NCBP 2016
Actinopterygii	Centrarchidae	Warmouth	*Chaenobryttus*	*gulosus*	Whole Body	-	-	-	-	USGS NCBP 2016
Actinopterygii	Centrarchidae	Redbreast sunfish	*Lepomis*	*auritus*	Whole Body	-	-	-	-	USGS NCBP 2016
Actinopterygii	Centrarchidae	Green sunfish	*Lepomis*	*cyanellus*	Whole Body	7	68.5	78.6	73.4	USGS NCBP 2016
Actinopterygii	Centrarchidae	Green sunfish	*Lepomis*	*cyanellus*	Whole Body	150	71	92.1	76.1	GEI 2014; U.S. EPA 2016a
Actinopterygii	Centrarchidae	Pumpkinseed	*Lepomis*	*gibbosus*	Whole Body	4	64.4	79.8	73.7	USGS NCBP 2016
Actinopterygii	Centrarchidae	Orangespotted sunfish	*Lepomis*	*humilis*	Whole Body	1	-	-	72.0	USGS NCBP 2016
Actinopterygii	Centrarchidae	Bluegill	*Lepomis*	*macrochirus*	Whole Body	8	71.0	77.7	74.8	USGS NCBP 2016
Actinopterygii	Centrarchidae	Bluegill	*Lepomis*	*macrochirus*	Whole Body	4	-	-	82.0	Swarzenski et al. 2004
Actinopterygii	Centrarchidae	Longear sunfish	*Lepomis*	*megalotis*	Whole Body	1	-	-	76.0	USGS NCBP 2016
Actinopterygii	Centrarchidae	Redear sunfish	*Lepomis*	*microlophus*	Whole Body	1	-	-	79.0	USGS NCBP 2016
Actinopterygii	Centrarchidae	Smallmouth bass	*Micropterus*	*dolomieui*	Whole Body	28	60.0	76.4	71.9	USGS NCBP 2016

Class	Family	Common Name	Genus	Species	Tissue	Count	Min. % Moisture	Max. % Moisture	Avg. % Moisture	Reference
Actinopterygii	Centrarchidae	Smallmouth bass	*Micropterus*	*dolomieui*	Whole Body	12	71.9	77.3	74.2	GEI 2014; U.S. EPA 2016a
Actinopterygii	Centrarchidae	Spotted bass	*Micropterus*	*punctulatus*	Whole Body	2	73.5	77.6	75.6	USGS NCBP 2016
Actinopterygii	Centrarchidae	Largemouth bass	*Micropterus*	*salmoides*	Whole Body	109	63.0	79.0	72.9	USGS NCBP 2016
Actinopterygii	Centrarchidae	Largemouth bass	*Micropterus*	*salmoides*	Whole Body	64	71.2	79.4	75.7	GEI 2014; U.S. EPA 2016a
Actinopterygii	Centrarchidae	Largemouth bass	*Micropterus*	*salmoides*	Whole Body	3	-	-	80.0	Swarzenski et al. 2004
Actinopterygii	Centrarchidae	Largemouth bass	*Micropterus*	*salmoides*	Fish fillet	6	78.2	79.1	78.8	May et al. 2009
Actinopterygii	Centrarchidae	White crappie	*Pomoxis*	*annularis*	Whole Body	30	69.3	77.7	73.4	USGS NCBP 2016
Actinopterygii	Centrarchidae	Black crappie	*Pomoxis*	*annularis*	Fish fillet	3	79.2	80.3	79.8	May et al. 2009
Actinopterygii	Centrarchidae	Black crappie	*Pomoxis*	*nigromaculatus*	Whole Body	24	60.9	77.5	72.6	USGS NCBP 2016
Actinopterygii	Centrarchidae	Black crappie	*Pomoxis*	*nigromaculatus*	Fish fillet	3	80.2	80.8	80.6	May et al. 2009
Actinopterygii	Centrarchidae	Rock bass	*Ambloplites*	*rupestris*	Whole Body	24	70.7	78.8	75.0	GEI 2014; U.S. EPA 2016a
Actinopterygii	Centrarchidae	Sunfish	*Lepomis*	*sp.*	Whole Body	1	-	-	76.8	GEI 2014; U.S. EPA 2016a
Actinopterygii	Cichlidae	Convict cichlid	*Cichlasoma*	*nigrofasciatum*	Whole Body	1	-	-	68.4	USGS NCBP 2016
Actinopterygii	Cichlidae	Mozambique tilapia	*Oreochromis*	*mossambicus*	Whole Body	7	69.0	74.4	70.7	USGS NCBP2016
Actinopterygii	Clariidae	Chinese catfish	*Clarias*	*fuscus*	Whole Body	-	-	-	-	USGS NCBP 2016
Actinopterygii	Clupeidae	Skipjack herring	*Alosa*	*chrysochloris*	Whole Body	-	-	-	-	USGS NCBP 2016
Actinopterygii	Clupeidae	Gizzard shad	*Dorosoma*	*cepedianum*	Whole Body	36	62.4	77.7	71.5	USGS NCBP 2016
Actinopterygii	Clupeidae	Threadfin shad	*Dorosoma*	*petenense*	Whole Body	1	-	-	73.9	USGS NCBP 2016
Actinopterygii	Cyprinidae	Chiselmouth	*Acrocheilus*	*alutaceus*	Whole Body	6	65.7	74.5	70.8	USGS NCBP 2016

Class	Family	Common Name	Genus	Species	Tissue	Count	Min. % Moisture	Max. % Moisture	Avg. % Moisture	Reference
Actinopterygii	Cyprinidae	Goldfish	*Carassius*	*auratus*	Whole Body	5	61.8	72.2	67.7	USGS NCBP 2016
Actinopterygii	Cyprinidae	Redside dace	*Clinostomus*	*elongatus*	Whole Body	1	-	-	73.4	USGS NCBP 2016
Actinopterygii	Cyprinidae	Common carp	*Cyprinus*	*carpio*	Whole Body	333	62.4	85.6	72.2	USGS NCBP 2016
Actinopterygii	Cyprinidae	Common carp	*Cyprinus*	*carpio*	Whole Body	62	57	82.6	75.6	GEI 2014; U.S. EPA 2016a
Actinopterygii	Cyprinidae	Common carp	*Cyprinus*	*carpio*	Whole Body	2	-	-	79.0	Swarzenski et al. 2004
Actinopterygii	Cyprinidae	Zebrafish	*Danio*	*rerio*	Whole Body	3	70.0	73.0	71.3	Lin et al. 2022
Actinopterygii	Cyprinidae	Peamouth	*Mylocheilus*	*caurinus*	Whole Body	4	71.1	78.6	74.6	USGS NCBP 2016
Actinopterygii	Cyprinidae	River chub	*Nocomis*	*micropogon*	Whole Body	-	-	-	-	USGS NCBP 2016
Actinopterygii	Cyprinidae	River chub	*Nocomis*	*micropogon*	Whole Body	4	72.7	77.1	75.2	GEI 2014; U.S. EPA 2016a
Actinopterygii	Cyprinidae	Golden shiner	*Notemigonus*	*crysoleucas*	Whole Body	-	-	-	-	USGS NCBP 2016
Actinopterygii	Cyprinidae	Sacramento blackfish	*Orthodon*	*microlepidotus*	Whole Body	6	70.0	78.4	75.3	USGS NCBP 2016
Actinopterygii	Cyprinidae	Northern pikeminnow	*Ptychocheilus*	*oregonensis*	Whole Body	15	68.5	80.6	74.1	USGS NCBP 2016
Actinopterygii	Cyprinidae	Blacknose dace	*Rhinichthys*	*sp.*	Whole Body	44	68.8	78.7	73.8	GEI 2014; U.S. EPA 2016a
Actinopterygii	Cyprinidae	Bluenose minnow	*Pimephales*	*notatus*	Whole Body	3	74.1	76.2	74.8	GEI 2014; U.S. EPA 2016a
Actinopterygii	Cyprinidae	Carp			Whole Body	6	77.2	78.9	78.2	GEI 2014; U.S. EPA 2016a
Actinopterygii	Cyprinidae	Central Stoneroller	*Campostoma*	*anomalum*	Whole Body	174	66.3	82.8	74.6	GEI 2014; U.S. EPA 2016a
Actinopterygii	Cyprinidae	Creek chub	*Semotilus*	*atromaculatus*	Whole Body	306	70.7	83.5	76.7	GEI 2014; U.S. EPA 2016a
Actinopterygii	Cyprinidae	Fathead minnow	*Pimephales*	*promelas*	Whole Body	298	0	84.7	76.6	GEI 2014; U.S. EPA 2016a
Actinopterygii	Cyprinidae	Longnose dace	*Rhinichthys*	*cataractae*	Whole Body	17	68.7	76.6	73.3	GEI 2014; U.S. EPA 2016a

Class	Family	Common Name	Genus	Species	Tissue	Count	Min. % Moisture	Max. % Moisture	Avg. % Moisture	Reference
Actinopterygii	Cyprinidae	Mimic shiner	*Notropis*	*volucellus*	Whole Body	2	74.2	76	75.1	GEI 2014; U.S. EPA 2016a
Actinopterygii	Cyprinidae	Red shiner	*Cyprinella*	*lutrensis*	Whole Body	46	65.2	79.1	73.1	GEI 2014; U.S. EPA 2016a
Actinopterygii	Cyprinidae	Redside shiner	*Richardsonius*	*balteatus*	Whole Body	8	73.1	78.2	75.6	GEI 2014; U.S. EPA 2016a
Actinopterygii	Cyprinidae	Rosyface shiner	*Notropis*	*rubellus*	Whole Body	2	67.1	72.4	69.8	GEI 2014; U.S. EPA 2016a
Actinopterygii	Cyprinidae	Rosyside shiner	*Notropis*	*rubellus*	Whole Body	5	67.1	72.4	75.5	GEI 2014; U.S. EPA 2016a
Actinopterygii	Cyprinidae	Sand shiner	*Notropis*	*stramineus*	Whole Body	83	69.3	79.3	74.0	GEI 2014; U.S. EPA 2016a
Actinopterygii	Cyprinidae	Silver shiner	*Notropis*	*photogenis*	Whole Body	7	75.4	77.7	76.6	GEI 2014; U.S. EPA 2016a
Actinopterygii	Cyprinidae	Speckled dace	*Rhinichthys*	*osculus*	Whole Body	35	68.8	79	74.0	GEI 2014; U.S. EPA 2016a
Actinopterygii	Cyprinidae	Striped shiner	*Luxilus*	*chrysocephalus*	Whole Body	64	71.2	81.8	77.1	GEI 2014; U.S. EPA 2016a
Actinopterygii	Erythrinidae	Tiger fish	*Hoplias*	*malabaricus*	Muscle	32	-	-	77.7	Santos et al. 2001
Actinopterygii	Esocidae	Redfin pickerel	*Esox*	*americanus*	Whole Body	1	-	-	76.9	USGS NCBP 2016
Actinopterygii	Esocidae	Northern pike	*Esox*	*lucius*	Whole Body	12	72.5	79.4	76.9	USGS NCBP 2016
Actinopterygii	Esocidae	Chain pickerel	*Esox*	*niger*	Whole Body	-	-	-	-	USGS NCBP 2016
Actinopterygii	Fundulidae	Plains killifish	*Fundulus*	*zebrinus*	Whole Body	9	73.9	76.7	75.5	GEI 2014; U.S. EPA 2016a
Actinopterygii	Gadidae	Burbot	*Lota*	*lota*	Whole Body	1	-	-	78.7	USGS NCBP 2016
Actinopterygii	Gasterosteidae	Brook stickleback	*Culaea*	*inconstans*	Whole Body	57	72.2	80.7	75.8	GEI 2014; U.S. EPA 2016a
Actinopterygii	Hiodontoidea	Goldeye	*Hiodon*	*alosoides*	Whole Body	15	60.4	73.0	67.2	USGS NCBP 2016
Actinopterygii	Hiodontoidea	Mooneye	*Hiodon*	*tergisus*	Whole Body	2	68.3	71.0	69.7	USGS NCBP 2016
Actinopterygii	Ictaluridae	White catfish	*Ameiurus*	*catus*	Whole Body	20	69.5	86.2	74.8	USGS NCBP 2016

Class	Family	Common Name	Genus	Species	Tissue	Count	Min. % Moisture	Max. % Moisture	Avg. % Moisture	Reference
Actinopterygii	Ictaluridae	Black bullhead	*Ameiurus*	*melas*	Whole Body	3	80.5	81.4	80.9	USGS NCBP 2016
Actinopterygii	Ictaluridae	Black bullhead	*Ameiurus*	*melas*	Whole Body	6	73	81.6	76.8	GEI 2014; U.S. EPA 2016a
Actinopterygii	Ictaluridae	Yellow bullhead	*Ameiurus*	*natalis*	Whole Body	6	68.2	78.3	74.9	USGS NCBP 2016
Actinopterygii	Ictaluridae	Brown bullhead	*Ameiurus*	*nebulosus*	Whole Body	7	75.9	80.1	77.9	USGS NCBP 2016
Actinopterygii	Ictaluridae	Blue catfish	*Ictalurus*	*furcatus*	Whole Body	7	67.0	80.1	74.3	USGS NCBP 2016
Actinopterygii	Ictaluridae	Blue catfish	*Ictalurus*	*furcatus*	Fish fillet	3	76.0	79.6	78.2	May et al. 2009
Actinopterygii	Ictaluridae	Channel catfish	*Ictalurus*	*punctatus*	Whole Body	69	59.0	81.6	72.8	USGS NCBP 2016
Actinopterygii	Ictaluridae	Channel catfish	*Ictalurus*	*punctatus*	Fish fillet	3	77.3	79.5	78.4	May et al. 2009
Actinopterygii	Ictaluridae	Flathead catfish	*Pylodictis*	*olivaris*	Whole Body	1	-	-	75.6	USGS NCBP 2016
Actinopterygii	Ictaluridae	Flathead catfish	*Pylodictis*	*olivaris*	Fish fillet	3	72.2	79.0	76.0	May et al. 2009
Actinopterygii	Lepisosteidae	Alligator gar	*Actractosteus*	*spatula*	Whole Body	2	50.3	61.6	56.0	USGS NCBP 2016
Actinopterygii	Lepisosteidae	Longnose gar	*Lepisosteus*	*osseus*	Whole Body	4	64.0	72.4	67.7	USGS NCBP 2016
Actinopterygii	Lepisosteidae	Spotted gar	*Lepisosteus*	*productus*	Whole Body	1	-	-	66.0	USGS NCBP 2016
Actinopterygii	Moronidae	White perch	*Morone*	*americana*	Whole Body	6	65.9	77.7	71.2	USGS NCBP 2016
Actinopterygii	Moronidae	White bass	*Morone*	*chrysops*	Whole Body	19	67.7	79.7	72.5	USGS NCBP 2016
Actinopterygii	Moronidae	Striped bass	*Morone*	*saxatilis*	Whole Body	6	62.4	78.0	68.8	USGS NCBP 2016
Actinopterygii	Moronidae	Whiper	*Morone*	*sp.*	Whole Body	3	66.6	73.3	70.4	USGS NCBP 2016
Actinopterygii	Mugilidae	Striped mullet	*Mugil*	*cephalus*	Whole Body	8	60.5	73.8	64.8	USGS NCBP 2016
Actinopterygii	Percidae	Yellow perch	*Perca*	*flavescens*	Whole Body	39	66.0	77.6	73.6	USGS NCBP 2016

Class	Family	Common Name	Genus	Species	Tissue	Count	Min. % Moisture	Max. % Moisture	Avg. % Moisture	Reference
Actinopterygii	Percidae	Yellow perch	*Perca*	*flavescens*	Whole Body	5	71.6	76	74.0	GEI 2014; U.S. EPA 2016a
Actinopterygii	Percidae	Sauger	*Stizostedion*	*canadense*	Whole Body	24	67.4	77.4	71.8	USGS NCBP 20106
Actinopterygii	Percidae	Sauger	*Stizostedion*	*canadense*	Whole Body	1	-	-	77.0	GEI 2014; U.S. EPA 2016a
Actinopterygii	Percidae	Walleye	*Sander*	*vitreus*	Whole Body	29	65.4	77.9	70.7	USGS NCBP 2016
Actinopterygii	Percidae	Walleye	*Sander*	*vitreus*	Muscle plug	13	77.3	78.7	78.0	May et al. 2009
Actinopterygii	Percidae	Missouri Saddeled Darter	*Etheostoma*	*tetrazonum*	Whole Body	27	66.5	80.6	69.7	May et al. 2009
Actinopterygii	Percidae	Fantail darter	*Etheostoma*	*flabellare*	Whole Body	15	27.7	80.5	72.3	GEI 2014; U.S. EPA 2016a
Actinopterygii	Percidae	Greenside darter	*Etheostoma*	*blennioides*	Whole Body	11	73	78.3	74.5	GEI 2014; U.S. EPA 2016a
Actinopterygii	Percidae	Johnny darter	*Etheostoma*	*nigrum*	Whole Body	1	-	-	71.7	GEI 2014; U.S. EPA 2016a
Actinopterygii	Percidae	Logperch	*Percina*	*sp.*	Whole Body	2	76.2	77.7	77.0	GEI 2014; U.S. EPA 2016a
Actinopterygii	Percidae	Rainbow darter	*Etheostoma*	*caeruleum*	Whole Body	85	66.7	88	72.8	GEI 2014; U.S. EPA 2016a
Actinopterygii	Percidae	Variegate darter	*Etheostoma*	*variatum*	Whole Body	13	69.7	78.3	72.6	GEI 2014; U.S. EPA 2016a
Actinopterygii	Poeciliidae	Cuban limia	*Poecilia*	*vittata*	Whole Body	16	66.7	77.7	70.7	USGS NCBP 2016
Actinopterygii	Poeciliidae	Mosquitofish	*Gambusia*	*sp.*	Whole Body	8	76	77.5	76.0	GEI 2014; U.S. EPA 2016a
Actinopterygii	Salmonidae	Lake herring	*Coregonus*	*artedii*	Whole Body	2	73.2	79.7	76.5	USGS NCBP 2016
Actinopterygii	Salmonidae	Lake whitefish	*Coregonus*	*clupeaformis*	Whole Body	12	62.1	75.9	70.6	USGS NCBP 2016
Actinopterygii	Salmonidae	Bloater	*Coregonus*	*hoyi*	Whole Body	39	43.5	76.5	65.0	USGS NCBP 2016
Actinopterygii	Salmonidae	Humpback whitefish	*Coregonus*	*pidschian*	Whole Body	2	74.5	74.7	74.6	USGS NCBP 2016
Actinopterygii	Salmonidae	Rainbow trout	*Oncorhynchus*	*mykiss*	Whole Body	8	64.2	76.8	71.8	USGS NCBP 2016

Class	Family	Common Name	Genus	Species	Tissue	Count	Min. % Moisture	Max. % Moisture	Avg. % Moisture	Reference
Actinopterygii	Salmonidae	Chinook salmon	*Oncorhynchus*	*tshawtyscha*	Whole Body (juvenile)	13	76.1	81.2	79.4	Saiki et al. 2001
Actinopterygii	Salmonidae	Round whitefish	*Prosopium*	*cylindraceum*	Whole Body	2	67.5	69.4	68.5	USGS NCBP 2016
Actinopterygii	Salmonidae	Mountain whitefish	*Prosopium*	*williamsoni*	Whole Body	4	70.5	77.2	74.3	USGS NCBP 2016
Actinopterygii	Salmonidae	Brown trout	*Salmo*	*trutta*	Whole Body	12	69.6	77.3	73.0	USGS NCBP 2016
Actinopterygii	Salmonidae	Dolly Varden	*Salvelinus*	*malma*	Whole Body	2	64.1	65.7	64.9	USGS NCBP 2016
Actinopterygii	Salmonidae	Lake trout	*Salvelinus*	*namaycush*	Whole Body	36	46.0	74.9	65.0	USGS NCBP 2016
Actinopterygii	Salmonidae	Arctic grayling	*Thymallus*	*arcticus*	Whole Body	2	76.3	76.9	76.6	USGS NCBP 2016
Actinopterygii	Sciaenidae	Freshwater drum	*Aplodinotus*	*grunniens*	Whole Body	17	64.8	75.7	71.1	USGS NCBP 2016
AMPHIBIAN TAXON										
Amphibia	Ranidae	Pickerel frog	*Rana*	*palustris*	Whole body[a]	18	91.04	94.41	92.72	Etkin 1932
Amphibia	Ranidae	Pickerel frog	*Rana*	*palustris*	Whole body[c]	13	85.41	90.73	88.27	Etkin 1932
Amphibia	Ranidae	Pickerel frog	*Rana*	*palustris*	Whole body[e]	8	81.17	86.24	83.38	Etkin 1932
Amphibia	Ranidae	Pickerel frog	*Rana*	*palustris*	Whole body[h]	3	77.95	79.37	78.79	Etkin 1932
Amphibia	Ranidae	Green frog	*Rana*	*clamitans*	Whole body[a]	10	88.07	91.48	89.39	Etkin 1932
Amphibia	Ranidae	Green frog	*Rana*	*clamitans*	Whole body[c]	13	85.64	87.95	86.59	Etkin 1932
Amphibia	Ranidae	Green frog	*Rana*	*clamitans*	Whole body[e]	5	81.21	86.57	84.54	Etkin 1932
Amphibia	Ranidae	Green frog	*Rana*	*clamitans*	Whole body[h]	3	81.76	86.89	83.60	Etkin 1932
Amphibia	Ranidae	Bullfrog	*Rana*	*catesbeiana*	Whole body[a]	8	87.90	90.46	89.70	Etkin 1932
Amphibia	Ranidae	Bullfrog	*Rana*	*Catesbeiana*	Whole body[c]	8	85.52	91.02	88.28	Etkin 1932

Class	Family	Common Name	Genus	Species	Tissue	Count	Min. % Moisture	Max. % Moisture	Avg. % Moisture	Reference
Amphibia	Ranidae	Bullfrog	*Rana*	*Catesbeiana*	Whole body[e]	6	81.80	86.74	84.51	Etkin 1932
Amphibia	Ranidae	Bullfrog	*Rana*	*Catesbeiana*	Whole body[h]	3	81.00	82.64	81.99	Etkin 1932
Amphibia	Ranidae	Bullfrog	*Rana*	*Catesbeiana*	Whole body[a]	8	-	-	89.81	Brown et al. 1986
Amphibia	Ranidae	Bullfrog	*Rana*	*Catesbeiana*	Whole body[a]	8	-	-	84.93	Brown et al. 1989
Amphibia	Ranidae	Bullfrog	*Rana*	*Catesbeiana*	Tail Tissue[b]	6	-	-	90.96	Eddy 1979
Amphibia	Ranidae	Bullfrog	*Rana*	*Catesbeiana*	Whole body[a]	10	-	-	80.00	Burger and Snodgrass 1998
Amphibia	Ranidae	Mountain yellow-legged frog	*Rana*	*Muscosa*	Whole body[b]	NR	-	-	89.30	Bradford 1984
Amphibia	Ranidae	European common frog	*Rana*	*Temporaria*	Whole body[b]	NR	-	-	93.65	Fletcher and Myant 1959
Amphibia	Ranidae	European common frog	*Rana*	*Temporaria*	Whole body[c]	NR	-	-	91.41	Fletcher and Myant 1959
Amphibia	Ranidae	European common frog	*Rana*	*Temporaria*	Whole body[d]	NR	-	-	90.96	Fletcher and Myant 1959
Amphibia	Ranidae	European common frog	*Rana*	*Temporaria*	Whole body[e]	NR	-	-	88.82	Fletcher and Myant 1959
Amphibia	Ranidae	European common frog	*Rana*	*Temporaria*	Whole body[f]	NR	-	-	87.07	Fletcher and Myant 1959
Amphibia	Ranidae	European common frog	*Rana*	*Temporaria*	Whole body[g]	NR	-	-	86.25	Fletcher and Myant 1959
Amphibia	Ranidae	Leopard frog	*Rana*	*Pipiens*	Whole body[i]	34	-	-	81.35	Gillis 1979
Amphibia	Ranidae	Leopard frog	*Rana*	*Blairi*	Whole body[i]	33	-	-	80.68	Gillis 1979
Amphibia	Ranidae	Wood frog	*Lithobates*	*Sylvaticus*	Whole body[g]	NR	-	-	83.50	Wada et al. 2011
Amphibia	Hylidae	Sierra chorus frog	*Pseudacris*	*Sierra*	Whole body[a]	NR	84.4	96.9	92.30	Bradford et al. 2012
Amphibia	Hylidae	Sierra chorus frog	*Pseudacris*	*Sierra*	Whole body[b]	NR	86.6	94.1	89.30	Bradford et al. 2012

Class	Family	Common Name	Genus	Species	Tissue	Count	Min. % Moisture	Max. % Moisture	Avg. % Moisture	Reference
Amphibia	Hylidae	Western chorus frog	*Pseudacris*	*Triseriata*	Whole body[i]	18	-	-	82.3	Farrell and MacMahon 1969
Amphibia	Hylidae	Southern Cricket Frog	*Acris*	*gryllus*	Whole body[i]	20	-	-	80.2	Farrell and MacMahon 1969
Amphibia	Hylidae	Northern Cricket Frog	*Acris*	*Crepitans*	Whole body[i]	16	-	-	80.7	Farrell and MacMahon 1969
Amphibia	Hylidae	Green treefrog	*Hyla*	*Cinerea*	Whole body[i]	20	-	-	81.0	Farrell and MacMahon 1969
Amphibia	Hylidae	Spring peeper	*Hyla*	*crucifer*	Whole body[i]	15	-	-	81.2	Farrell and MacMahon 1969
Amphibia	Hylidae	Squirell treefrog	*Hyla*	*Squirella*	Whole body (females)[i]	5	-		77.60	Farrell and MacMahon 1969
Amphibia	Hylidae	Squirell treefrog	*Hyla*	*Squirella*	Whole body (males)[i]	10	-		82.40	Farrell and MacMahon 1969
Amphibia	Hylidae	Cope's gray treefrog	*Hyla*	*versicolor*	Whole body[i]	12	-		80.2	Farrell and MacMahon 1969
Amphibia	Hylidae	Barking treefrog	*Hyla*	*Gratiosa*	Whole body[i]	11	-		82.3	Farrell and MacMahon 1969
Amphibia	Bufonidae	Toad	*Bufo*	*Arenarum*	Whole body[a]	192	95.13	95.63	95.36	Ferrari et al. 1995
Amphibia	Bufonidae	Toad	*Bufo*	*Arenarum*	Whole body[g]	NR	-	-	87.10	Bergeron et al. 2011a
Amphibia	Bufonidae	Toad	*Bufo*	*Arenarum*	Whole body[i]	NR	-	-	77.80	Bergeron et al. 2011a
Amphibia	Bufonidae	Toad	*Bufo*	*Arenarum*	Whole body[g]	3	-	-	90.40	Bergeron et al. 2011b
Amphibia	Bufonidae	Toad	*Bufo*	*Arenarum*	Whole body[g]	NR	-	-	89.30	Todd et al. 2011
Amphibia	Pipidae	South African Clawed frog	*Xenopus*	*laevis*	Whole body[b]	6	-	-	94.0	Bender et al. 2018
Amphibia	Pipidae	South African Clawed frog	*Xenopus*	*laevis*	Whole body[c]	6	-	-	92.0	Bender et al. 2018
Amphibia	Pipidae	South African Clawed frog	*Xenopus*	*laevis*	Whole body[d]	6	-	-	90.6	Bender et al. 2018
Amphibia	Pipidae	South African Clawed frog	*Xenopus*	*laevis*	Whole body[e]	6	-	-	88.7	Bender et al. 2018
Amphibia	Pipidae	South African Clawed frog	*Xenopus*	*laevis*	Whole body[g]	6	-	-	87.6	Bender et al. 2018

Class	Family	Common Name	Genus	Species	Tissue	Count	Min. % Moisture	Max. % Moisture	Avg. % Moisture	Reference
Amphibia	Pipidae	South African Clawed frog	*Xenopus*	*laevis*	Whole body[b]	NR	-	-	95.10	Fletcher and Myant 1959
Amphibia	Pipidae	South African Clawed frog	*Xenopus*	*laevis*	Whole body[c]	NR	-	-	94.70	Fletcher and Myant 1959
Amphibia	Pipidae	South African Clawed frog	*Xenopus*	*laevis*	Whole body[d]	NR	-	-	93.00	Fletcher and Myant 1959
Amphibia	Pipidae	South African Clawed frog	*Xenopus*	*laevis*	Whole body[e]	NR	-	-	90.80	Fletcher and Myant 1959
Amphibia	Pipidae	South African Clawed frog	*Xenopus*	*laevis*	Whole body[f]	NR	-	-	88.50	Fletcher and Myant 1959
Amphibia	Pipidae	South African Clawed frog	*Xenopus*	*laevis*	Whole body[g]	NR	-	-	87.90	Fletcher and Myant 1959
Amphibia	Pipidae	South African Clawed frog	*Xenopus*	*laevis*	Whole body[h]	6	-	-	78.30	Nybroe et al. 1985
Amphibia	Pipidae	South African Clawed frog	*Xenopus*	*laevis*	Whole body[a]	504	92.90	92.98	92.93	Territo and Smits 1998
Amphibia	Ambystomatidae	Tiger salamander	*Ambystoma*	*Tigrinum*	Tail Tissue[i]	8	-	-	90.70	Platt and Christopher 1977
INVERTEBRATE TAXON										
Clitellata	Lumbriculidae	Oligochaete worm	*Lumbriculus*	*variegatus*	Whole body	2	84	85	84.5	Elissen et al. 2010; Hansen et al. 2004
-	-	Bivalves (without shell)	-	-	Whole body	3	-	-	82	U.S. EPA 1993
Bivalvia	Corbiculidae	Asian clam	*Corbicula*	*Fluminea*	Muscle	6	80.4	81.1	80.8	Sarazudin 2019
Bivalvia	Corbiculidae	Asian clam	*Corbicula*	*Fluminea*	Muscle	15	79.2	80.9	80.1	Rak et al. 2020
-	-	Crabs (with shell)	-	-	Whole body	5	-	-	74	U.S. EPA 1993
-	-	Shrimp	-	-	Whole body	7	-	-	78	U.S. EPA 1993
-	-	Isopods, Amphipods	-	-	Whole body	2	71	80	75.5	U.S. EPA 1993
Malacostraca	Cambaridae	Crayfish	*Procambarus*	*Clarkii*	Whole body	-	-	-	-[j]	Anastacio et al. 1999
-	-	Cladocerans	-	-	Whole body	2	79	87	83	U.S. EPA 1993

Class	Family	Common Name	Genus	Species	Tissue	Count	Min. % Moisture	Max. % Moisture	Avg. % Moisture	Reference
Branchiopoda	Daphniidae	Cladoceran	*Daphnia*	*Magna*	Whole body	1	-	-	95.7	Manar et al. 2009
Insecta	Ephemeridae	Mayfly	*Hexagenia*	*Bilineata*	Whole body (emergent)	30	40	74	60.9	Steingraeber et al. 1994
Insecta	Ephemeroptera	Mayfly	-	-	Whole body (immature)	18	72.2	76.6	74.1	Saiki et al. 2001
Insecta	Chironomidae	Midge	-	-	Whole body (immature)	18	68.9	82.2	78	Saiki et al. 2001
Insecta	Trichoptera	Caddisfly	-	-	Whole body (immature)	15	59.5	77.6	70.9	Saiki et al. 2001

NR: not reported18

a Pre - premetamorphic

b L Pre - E Pro - Late Prematorphosis and Early Prometamorphosis

c Pro - Prometamorphosis

d "L Pro - E MC - Late Prometamorphosis and Early metamorphic climax"

e Metamorphic climax

f Late metamorphic climax

g Post -M - Post-metamorphosis

h Froglet - Newly metamorphosed Anuran

i Adult - Adult form

j Expressed as equation dependent on body size: Wet Weight = 5.28607 x Dry Weight$^{0.937422}$

D.3 Mercury Muscle to Whole Body Conversion Factor

Studies considered for the tissue-based criterion development were searched for relevant information needed to derive a whole-body:muscle (WB:M) conversion factor (CF) for the various taxa. The necessary information was provided in only a few studies and determined to be too limited in scope to be useful. EPA performed an additional online literature search for other studies that could be used for deriving a WB:M CF for mercury. The following summarizes the relevant information for the three relevant taxa (amphibians, crayfish, and fish). Other invertebrate whole-body concentrations were not converted. Preliminary WB:M CFs are based on values reported by the authors without further analysis of the raw data.

D.3.1 Summary and Recommendation for a Preliminary WB:M CF for Amphibians

EPA conducted a literature search for information regarding paired whole body and muscle total mercury concentrations in amphibians, with emphasis on aquatic life stages and or fully aquatic amphibians. No such information was found specific to these life stages via preliminary search. Hothem et al. (2009) provides results of paired muscle (hind leg) and total body mercury in bullfrog tissues from Bear Creek in the Cache Creek Watershed, Northern California. The mean WB:M CF for a mix of 10 juvenile and adult bullfrogs of mixed gender is 0.97, which is substantially higher than the mean value for fish of 0.72. It is currently unknown whether this CF is representative of larval (aquatic life stages) of Anuran and other amphibians, however, for purpose of implementation whole body concentrations are likely the tissue to be sampled in practice. The authors report that the mean THg concentration in adults was 142% greater than that in larvae, and mean concentration of THg for juveniles was 76% greater than in larvae.

D.3.2 Summary and Recommendation for a Preliminary WB:M CF for Crayfish

No studies were identified that could be used to determine a WB:M CF for the crayfish. Given the lack of data, the abdominal muscle concentrations for the crayfish were converted to whole body concentrations based on the 0.72 WB:M CF recommended for fish.

D.3.3 Summary and Recommendation for a Preliminary WB:M CF for Fish

Seven studies were identified and reviewed for utility and developing a WB:M CF to support implementation of a future tissue-based mercury ALC for the State of Idaho. Six of the studies contained either equations to calculate mean and median WB:M CFs, or WB:M CFs that can be used directly for EPA purposes (**Table D-6**). Of the six studies, Eagles-Smith et al. (2016) reported a WB:M CF of 0.74 calculated as the average ratio of whole body to muscle concentration from three studies where both tissue types were measured on the same individuals: Bevelhimer et al. (1997); Boalt et al. (2012); and Goldstein et al. (1996). These studies are included separately in the current analysis (**Table D-6**). For this preliminary analysis, and since EPA cannot recalculate the WB:M CF of 0.74 from Eagles-Smith et al. (2016) as reported, the published value has been retained and used by EPA for the derivation of the current recommended WB:M CF. EPA is aware of the "double-counting" of the WB:M CFs between Eagles-Smith et al. (2016) and Bevelhimer et al. (1997); Boalt et al. (2012); and Goldstein et al. (1996), and will revisit this decision at a later date upon further analysis. Additionally, EPA is aware of the mixed dataset of WB:M CFs given the inclusion of the two values reported for marine species in Boalt et al. (2012), which were also used by Eagles-Smith et al. (2016). EPA's preliminary WB:M CF for fish is 0.72 based on the grand mean of average WB:M CF values reported in **Table D-6**, below.

Table D-6. Summary and Whole-body: Muscle Conversion Factor (WB:M CF) for Fish used by EPA HECD to support implementation of the tissue-based mercury ALC for State of Idaho.

Species	*CF* (C_{wb}/C_m)	n	Location	Notes	Reference
Herring, *Clupea harengus*	0.86	20	Bothnian Sea (Sweden)	paired muscle and whole body; marine	Boalt et al. 2012
Perch, *Perca fluviatilis*	0.74	20	Bothnian Sea (Sweden)	paired muscle and whole body; marine	Boalt et al. 2012
Largemouth bass, *Micropterus salmoides* Spotted bass, *M. punctulatis*	0.70	12	Tennessee, Ohio (USA); multiple sites	paired fillet and whole body minus fillet	Bevelhimer et al. 1997
Several species (13 total)*	0.67	210	Various rivers & streams **	paired muscle plug and whole body; CF (mean) derived from regression equation provided	Peterson et al. 2005
Unspecified	0.77	3	South Fork of the Humboldt River near Elko in the Te-Moak Indian Reservation, Nevada	paired muscle plug and whole body	May and Brumbaugh 2007
Carp, *Cyprinus carpio* Channel catfish, *Ictalurus punctatu*s	0.57	-	Red River from Wahpeton, North Dakota, and Breckenridge, Minnesota, to the Canadian border	paired dorsal muscle and whole body	Goldstein et al. 1996
See notes column	0.74	-	NA	CF value reported as the mean of CFs from Bevelhimer et al. (1997); Boalt et al. (2014); and Goldstein et al. (1996)	Eagles-Smith et al. 2016
Grand Mean - All	**0.72**				

* Species: Brook trout (*Salvelinus fontinalis*); Brown trout (*Salmo trutta*); Channel catfish (*Ictalurus punctatus*); Cutthroat trout (*Oncorhynchus clarkii*); Rainbow trout (*O. mykiss*; White sucker (*Catostomus commersoni*); Largemouth bass (*Micropterus salmoides*); Smallmouth bass (*M. dolomieu*); Northern pike (*Esox lucius*); Northern pikeminnow (*Ptychocheilus oregonensis*); Sauger (*Sander canadensis*); Walleye (*S. vitreus*); Yellow perch (*Perca flavescens*)

** Rivers and streams: Arizona, California, Colorado, Idaho, Montana, Nevada, North Dakota, Oregon, South Dakota, Utah, Washington, Wyoming

D.3.4 Study-by-Study Summary and Analysis of Available Information for Fish

The following section is organized beginning with earliest publication to the most recent publication. All values below are reported on the basis of total mercury (Hg) in tissue.

Goldstein, R.M., M.E. Brigham and J.C. Stauffer. 1996. Comparison of mercury concentrations in liver, muscle, whole bodies, and composites of fish from the Red River of the North. Can. J. Fish. Aquat. Sci. 53: 244–252.

Goldstein and co-investigators collected carp (*Cyprinus carpio*) from four sites and channel catfish (*Ictalurus punctatus*) from one site in the Red River for analysis of total mercury content in liver, muscle, and whole bodies. A portion of the liver, skinless muscle tissue from below the dorsal fin and the remainder of the whole body was collected from each fish and analyzed for mercury concentration. The ratio of mercury in whole bodies to mercury in muscle was similar for both carp and channel catfish. The mean and median WB:M CFs were calculated to be 0.57 and 0.59. Historical data indicate that this ratio may be applicable to other species and locations, which supports the use of a WB:M CF for EPA's stated intent and purpose.

location	size group	n	Hg µg/g ww		WB/M ratio
			M	WB	
Wahpeton, ND; Breckenridge, MN	large	7	0.35	0.16	0.46
	small	7	0.33	0.2	0.61
Fargo, ND; Moorhead, MN	large	7	0.3	0.19	0.63
	small	7	0.24	0.15	0.63
Grand Forks, ND; East Grand Forks, MN	large	7	0.38	0.19	0.50
	small	7	0.31	0.19	0.61
Drayton, ND	large	7	0.32	0.17	0.53
	small	7	0.3	0.17	0.57
All	large	28	0.34	0.18	
	small	28	0.29	0.18	
total		56	0.31	0.18	
				mean	**0.57**
				median	**0.59**

Bevelhimer, M.S., J.J. Beauchamp, B.E. Sample and G.R. Southworth. 1997. Estimation of whole-fish contaminant concentrations from fish fillet data. ES/ER/TM-202. Prepared by the Risk Assessment Program, Oak Ridge National Laboratory, Oak Ridge, TN. 23 pp.

This technical memorandum presents the results of an investigation of the relationship between fillet and whole-fish contaminant concentrations in a mix of 12 largemouth

(*Micropterus salmoides*) and spotted bass (*M. punctulatis*) collected from several sites in Ohio and Tennessee. Mercury analyses were conducted on fillet portions as well as the remaining carcasses of each fish. Equations were developed for the estimation of whole-fish concentrations for mercury and several analytes. Using the equation provided for mercury in their Table 2 (Cwb=exp[-0.84+0.74•ln(Cf)]) with the raw data given, the mean WB:M CF was calculated to be 0.70. Raw data was provided in tabular form in Appendix A.2 of the memorandum in Peterson et al. 2005.

Peterson, S.A., J. Van Sickle, R.M. Hughes, J.A. Schacher and S.F. Echols. 2005. A biopsy procedure for determining filet and predicting whole-fish mercury concentration. Arch. Environ. Contam. Toxicol. 48: 99–107.

Mercury concentrations were evaluated in 210 fillet biopsies from 65 sites in 12 western states relative to whole-body mercury concentration in the same fish. A highly significant relationship ($r^2 = 0.96$) was found between biopsy muscle plugs and whole-fish mercury concentrations for 13 piscivorous and non-piscivorous fish species. Using the equation provided in the publication [log10 [whole-body mercury] = -0.2712+0.9005 log10 [biopsy mercury])] the mean and median WB:M CF were calculated as 0.67. Based on raw data visually estimated from Figure 2 in the publication, the mean and median WB:M CF were calculated to be 0.70 and 0.63, respectively. The mean WB:M CF of 0.67 calculated from the regression model was used for the overall WB:M CF calculation. It was concluded that relative to conventional fish-tissue sampling and analysis procedures for whole fish or fillets, the biopsy procedure for mercury in fish tissue is non-lethal, less cumbersome, more likely to be permitted by fisheries agencies, and a precise and accurate means for determining both fillet and whole-fish mercury concentrations.

May, T.W. and W.G. Brumbaugh. 2007. Determination of total mercury in whole-body fish and fish muscle plugs collected from the South Fork of the Humboldt River, Nevada, September 2005: U.S. Geological Survey Open-File Report 2007–1059, 4 pp.

In this study, investigators determined mercury concentrations in muscle plugs and whole body from the same fish collected from the South Fork of the Humboldt River near Elko in the Te-Moak Indian Reservation. A single muscle plug was collected from beneath the dorsal fin area in each of the three whole-body fish samples (species not given). The muscle to whole body ratio was similar for the three fish samples (see following table).

fish ID	wt, g	TL, mm	%moisture WB	%moisture M	Hg, µg/g WB	Hg, µg/g M	ratio WB/M
LCCSIF002	70.4	184	73.5	77.3	0.048	0.061	0.79
LCCSIF0023	148	239	73.8	79.4	0.061	0.082	0.74
LCCSIF0024	475	351	71.8	75.1	0.053	0.068	0.78
						mean	**0.77**
						median	**0.78**

Boalt, E., H. Dahlgren and A. Miller. 2012. Cadmium, lead, and mercury concentrations in whole-fish, liver, and muscle of herring (*Clupea harengus*) and perch (*Perca fluviatilis*). Report NR 6:2012. Swedish Museum of Natural History, Department of Contaminant Research, Stockholm, Sweden. 11 pp.

In this study, concentrations of cadmium, lead, and mercury in herring and perch are compared between liver, muscle, and whole-fish to create conversion factors that can be used to convert metal concentrations between tissues and organs. Twenty herring and 20 perch, both marine species, were collected from Öviksfjärden, located south of Umeå in the northern part of the Bothnian Sea. There was a strong relationship between muscle concentrations and concentrations in whole fish, indicating that creation of a conversion factor between muscle and whole body tissue is suitable. The conversion factor between muscle and whole-fish concentrations for mercury were 0.86 and 0.74 for herring and perch, respectively. Conversion

factors levels differed significantly between herring and perch, indicating that species-specific conversion factors are necessary.

Eagles-Smith, C.A., J.T. Ackerman, J.J. Willacker, M.T. Tate, M.A. Lutz, J.A. Fleck, A.R. Stewart, J.G. Wiener, D.C. Evers, J.M. Lepak, J.A. Davis and C.F. Pritz. 2016. Spatial and temporal patterns of mercury concentrations in freshwater fish across the Western United States and Canada. Sci. Total Environ. 568: 1171–1184. Supplemental data available on-line.

A database was compiled with total mercury concentrations in 96,310 fish that comprised 206 species from 4,262 locations and used to evaluate the spatial distribution of fish total mercury (THg) across the region and effects of species, foraging guilds, habitats, and ecoregions. Areas of elevated THg exposure were identified by developing a relativized estimate of fish mercury concentrations at a watershed scale that accounted for the variability associated with fish species, fish size, and site effects. Total Hg concentrations in the original dataset were reported as skinless boneless fillet (76.8% of data rows), whole body (19.9% of data rows), or skin-on fillet (3.3% of data rows). All whole-body concentrations were converted to skinless boneless fillet equivalents by dividing by a WB:M CF of 0.74, the average ratio of whole body to muscle concentration from studies where both tissue types were measured on the same individuals (Bevelhimer et al. 1997; Boalt et al. 2012; Goldstein et al. 1996).

Appendix E Translation of the Chronic Muscle Tissue Criterion to a Water Column Criterion using Bioaccumulation Factors (BAF)

E.1 Calculation of Fish BAFs

EPA derived the chronic water column total mercury criterion element for Idaho waters by translating the total mercury tissue criterion to an equivalent water concentration using bioaccumulation factors (BAFs) (Equation E-1).

$$Bioaccumulation\ Factor\ \left(\frac{L}{kg}\right) = \frac{Fish\ Tissue\ [\frac{\mu g}{g}\ THg{-}ww]}{Water\ [\frac{\mu g}{L}]} \qquad \text{(Equation E-1)}$$

Because mercury bioaccumulation varies across different taxa, and because the mercury tissue species sensitivity distribution (SSD) is comprised of a wide range of taxa, including frogs, invertebrates, and fish, an approach was developed to apply BAFs representing taxonomic or trophic magnitude categories that were most appropriate for each species in the tissue SSD.

The majority of BAFs were for fish, which were calculated from a database of Idaho fish tissue and water samples provided by EPA Region 10. The methods for calculating fish species and fish trophic magnitude category BAFs are described in detail in **Section 3.6** and are summarized below. The initial dataset consisted of fish tissue and water mercury measurements for waterbodies across the state of Idaho. Tissue and water measurements collected at the same site within one year were paired, and an initial set of 474 BAFs were calculated. Next, the dataset was censored to remove 84 BAFs from seven sites across five watersheds (Cinnabar Creek, Jordan Creek, Orofino Creek, Portneuf River, and Sugar Creek) with high water total mercury concentrations (13.3-92.7 ng/L), resulting in 390 fish BAFs. The fish BAF dataset, before and after censoring, is summarized in **Table 3-1**.

Next, the 390 BAFs were reduced to 119 BAFs representing every unique fish species by location by year combination (**Table E-1**). If more than one individual fish tissue sample for the same species during the same year was available, then those tissue samples were averaged, and the BAF representing the species-location-year combination was represented by the arithmetic average tissue concentration divided by the spatially and temporally paired water concentration. This step was followed to ensure that sampling events for a given species represented as individual samples was evaluated in the same way as sampling events where multiple individuals of the same species were composited.

When more than one fish species by location combination was sampled during more than one year, the median of those inter-annual BAFs was calculated to represent the BAF for that fish species by location combination. Following this step, the set of 119 BAFs for all species-site-year combinations were reduced to a set of 101 BAFs for all species-site combinations. Finally, when more than one BAF for a particular species was available at more than one location, the median of those BAFs was calculated to represent the BAF for that fish species, resulting in a total of 30 fish species BAFs (**Table 3-2**).

Three of the 30 fish species (the sucker species) were assigned to the low trophic magnitude category, 21 were assigned to the medium trophic magnitude category, and 6 were assigned to the high trophic magnitude category. These categories largely correspond to the trophic level 2, 3, and 4 designations reported in Essig (2010), with the exceptions of the Kokanee salmon being assigned to the medium trophic magnitude category to better reflect their diet of zooplankton (assigned to trophic level 2 by Essig 2010), bull trout being assigned to the medium trophic magnitude category based on an assumption of a largely invertebrate diet for that size, the subdivision of brook trout and northern pikeminnow into medium and high trophic

magnitude categories based on dietary assumption based on size, and suckers being assigned to the low trophic magnitude category (assigned to trophic level 3 by Essig 2010). Suckers were reassigned because they were the most appropriate taxa to represent the low trophic magnitude category, which would otherwise not be represented. For each trophic magnitude category, a representative BAF was calculated as the 80th centile fish species BAF within each category, or as the maximum (75th centile) for the low trophic magnitude category, which only had three species (**Table 3-12**). Finally, sculpin species were not assigned to a trophic level in Essig (2010) but were assigned to the medium trophic magnitude category based on their diet (Zaroban et al. 1999). These trophic magnitude category BAFs were used as surrogate BAFs for fish species in the tissue dataset for which a species- or genus-level BAF was not available.

Table E-1. Fish Muscle THg BAFs (L/kg) for all unique species by location by year combinations.

Tissue concentrations represent either a single individual, or when more than one individual fish tissue sample for the same species during the same year was available, the average tissue concentration.

Waterbody Name	Site	Year	Latitude	Longitude	Waterbody Type	Fish Length (mm)[a]	Fish Weight (g)[a]	Fish Common Name	TL[b]	Trophic Magnitude Category	Muscle THg (mg/kg-ww)	Water THg (ng/L)	THg BAF (L/kg)
Bear River	Bear River	2008	42.36	-111.74	River	570	2370	Common carp	3	medium	0.252	0.93	270,968
Big Wood River	Big Wood River, U	2008	43.78	-114.54	River	280	239	Rainbow trout	3	medium	0.029	0.28	103,571
Big Wood River	Big Wood River, L	2008	43.43	-114.26	River	330	295	Rainbow trout	3	medium	0.044	0.37	118,919
Big Wood River	Big Wood River, L	2008	43.43	-114.26	River	360	500	Brown trout	4	high	0.094	0.37	254,054
Blackfoot River	Blackfoot R	2008	43.21	-112.20	River	440	1050	Utah sucker	2	Low	0.032	0.7	45,714
Blackfoot River	Blackfoot R-2	2008	42.80	-111.49	River	44	970	Bridgelip sucker	2	Low	0.086	0.59	144,915
Blackfoot River	Blackfoot R-2	2008	42.80	-111.49	River	300	250	Cutthroat trout	3	medium	0.056	0.59	94,915
Boise River	Boise River NR Twin Springs	2008	43.67	-115.73	River	NA	NA	Mountain whitefish	3	medium	0.405	0.69	586,957
Boise River	Boise River at Eckert Rd near Boise	2013	43.57	-116.13	River	393	634	Mountain whitefish	3	medium	0.185	0.73	253,425
Boise River	Boise River at Eckert Rd near Boise	2017	43.57	-116.13	River	369	496	Mountain whitefish	3	medium	0.119	1.13	105,310
Boise River	Boise River at Eckert Rd near Boise	2015	43.57	-116.13	River	291	221	Rainbow trout	3	medium	0.022	0.77	28,571
Boise River	Boise River at Glenwood Bridge Near Boise	2008	43.66	-116.28	River	NA	NA	Mountain whitefish	3	medium	0.199	0.91	218,681
Boise River	Boise River near Middleton	2013	43.68	-116.57	River	306	266	Mountain whitefish	3	medium	0.175	0.89	196,629
Boise River	Boise River near Middleton	2014	43.68	-116.57	River	263	269	Mountain whitefish	3	medium	0.173	0.989444	174,846
Boise River	Boise River near Middleton	2015	43.68	-116.57	River	297	263	Mountain whitefish	3	medium	0.113	1.1	102,727

Waterbody Name	Site	Year	Latitude	Longitude	Waterbody Type	Fish Length (mm)[a]	Fish Weight (g)[a]	Fish Common Name	TL[b]	Trophic Magnitude Category	Muscle THg (mg/kg-ww)	Water THg (ng/L)	THg BAF (L/kg)
Boise River	Boise River near Middleton	2016	43.68	-116.57	River	329	353	Mountain whitefish	3	medium	0.133	1.1	120,909
Boise River	Boise River near Middleton	2017	43.68	-116.57	River	297	229	Mountain whitefish	3	medium	0.221	1.35	163,704
Boise River	Boise River near Parma	2013	43.82	-117.02	River	594	2184	Channel catfish	3	medium	0.326	1.2	271,667
Boise River	Boise River near Parma	2015	43.82	-117.02	River	625	3033	Channel catfish	3	medium	0.225	1.6	140,625
Boise River	Boise River near Parma	2017	43.82	-117.02	River	230	158	Smallmouth bass	4	high	0.223	1.48	150,676
Camas Creek	Camas Creek #2	2008	44.82	-114.49	River	310	296	Mountain whitefish	3	medium	0.061	0.68	89,706
Cane Creek	Cane Creek	2016	44.95	-115.29	River	176	57	Bull trout	3	medium	0.051	0.49	103,265
Cane Creek	Cane Creek	2016	44.95	-115.29	River	0	4	Sculpin	3	medium	0.040	0.49	82,449
Clearwater River	Clearwater River at Riverside	2006	46.49	116.30	River	NA	NA	Salmonidae sp.	3	medium	0.134	1.58	84,810
Coeur d'Alene River	Cd'A R-1	2008	47.48	-116.74	River	250	220	Black crappie	3	medium	0.280	6.21	45,089
Coeur d'Alene River	Cd'A R-1	2008	47.48	-116.74	River	500	1500	Largemouth bass	4	high	0.572	6.21	92,110
Henry's Fork River	Henry's Fork R	2008	43.80	-111.93	River	NA	NA	Mountain whitefish	3	medium	0.153	0.799125	191,460
Henry's Fork River	Henry's Fork R	2008	43.80	-111.93	River	530	1600	Cutthroat trout	3	medium	0.275	0.799125	344,127
Lemhi River	Lemhi Nr Lemhi	2008	44.94	-113.64	River	NA	NA	Mountain whitefish	3	medium	0.316	1.005982	314,121
Lochsa River	Lochsa R	2008	46.93	-115.04	River	300	278	Cutthroat trout	3	medium	0.048	0.54	88,889
Lochsa River	Lochsa R	2008	46.93	-115.04	River	350	373	Mountain whitefish	3	medium	0.052	0.54	96,296
North Fork Big Lost River	NF Big Lost R	2008	43.93	-114.19	River	250	170	Small Brook trout	3	medium	0.064	0.96	66,667
North Fork Clearwater River	NF Clearwater R	2008	46.73	-115.29	River	340	380	Cutthroat trout	3	medium	0.066	0.23	286,957
North Fork Clearwater River	NF Clearwater R	2008	46.73	-115.29	River	320	278	Kokanee salmon	3	medium	0.113	0.23	491,304

Waterbody Name	Site	Year	Latitude	Longitude	Waterbody Type	Fish Length (mm)[a]	Fish Weight (g)[a]	Fish Common Name	TL[b]	Trophic Magnitude Category	Muscle THg (mg/kg-ww)	Water THg (ng/L)	THg BAF (L/kg)
North Fork Clearwater River	NF Clearwater R	2008	46.73	-115.29	River	350	406	Mountain whitefish	3	medium	0.085	0.23	369,565
North Fork Payette River	NF Payette R	2008	44.21	-116.11	River	380	500	Rainbow trout	3	medium	0.132	0.7	188,571
North Fork Payette River	NF Payette R	2008	44.21	-116.11	River	230	138	Yellow perch	3	medium	0.108	0.7	154,286
Pahsimeroi River	Pahsimeroi @ Ellis	2008	44.69	-114.05	River	NA	NA	Mountain whitefish	3	medium	0.250	0.422493	590,543
Payette River	Payette R	2008	44.00	-116.80	River	550	1650	Bridgelip sucker	2	Low	0.234	1.08	216,667
Payette River	Payette R	2008	44.00	-116.80	River	290	363	Smallmouth bass	4	high	0.123	1.08	113,889
Payette River	Payette R	2008	44.00	-116.80	River	510	1525	Largescale sucker	3	medium	0.186	1.08	172,222
Payette River	Payette R	2008	44.00	-116.80	River	320	250	Mountain whitefish	3	medium	0.050	1.08	46,296
Payette River	Payette R-2	2008	43.90	-116.63	River	540	1680	Largescale sucker	3	medium	0.276	0.95	290,526
Payette River	Payette R-2	2008	43.90	-116.63	River	280	231	Mountain whitefish	3	medium	0.041	0.95	43,158
Portneuf River	Portneuf R	2008	42.85	-112.44	River	380	518	Utah sucker	2	Low	0.192	1.89	101,587
Portneuf River	Portneuf R--Croney Road Reach	2007	42.86	-112.06	River	362	NA	Rainbow trout	3	medium	0.332	0.21	1,582,011
Portneuf River	Portneuf R--Croney Road Reach	2007	42.86	-112.06	River	408	NA	Cutthroat trout	3	medium	0.675	0.21	3,214,286
Priest River	Priest R	2008	48.24	-116.88	River	410	705	Largescale sucker	3	medium	0.278	0.17	1,635,294
Priest River	Priest R	2008	48.24	-116.88	River	260	244	Smallmouth bass	4	high	0.156	0.17	917,647
Saint Joe River	Saint Joe R	2008	47.14	-115.41	River	255	172	Cutthroat trout	3	medium	0.044	0.22	197,727
Saint Joe River	Saint Joe R	2008	47.14	-115.41	River	320	318	Mountain whitefish	3	medium	0.040	0.22	181,818
Saint Joe River	Saint Joe R	2008	47.14	-115.41	River	430	728	Large Brook trout	4	high	0.174	0.22	790,909
Salmon Falls Creek Reservoir	Salmon Falls Creek Reservoir at Grey's Landing	2005	42.13	-114.73	Reservoir	457	NA	Walleye	4	high	0.753	2.208	341,033

Waterbody Name	Site	Year	Latitude	Longitude	Waterbody Type	Fish Length (mm)[a]	Fish Weight (g)[a]	Fish Common Name	TL[b]	Trophic Magnitude Category	Muscle THg (mg/kg-ww)	Water THg (ng/L)	THg BAF (L/kg)
Salmon Falls Creek Reservoir	Salmon Falls Creek Reservoir at Grey's Landing	2006	42.13	-114.73	Reservoir	442	NA	Walleye	4	high	1.250	2.208	566,123
Salmon Falls Creek Reservoir	Salmon Falls Creek Reservoir at Grey's Landing	2006	42.13	-114.73	Reservoir	495	NA	Largescale sucker	3	medium	0.489	2.208	221,467
Salmon Falls Creek Reservoir	Salmon Falls Creek Reservoir at Grey's Landing	2006	42.13	-114.73	Reservoir	355	NA	Rainbow trout	3	medium	0.357	2.208	161,685
Salmon Falls Creek Reservoir	Salmon Falls Creek Reservoir at Grey's Landing	2006	42.13	-114.73	Reservoir	339	NA	Smallmouth bass	4	high	1.020	2.208	461,957
Salmon Falls Creek Reservoir	Salmon Falls Creek Reservoir at Grey's Landing	2006	42.13	-114.73	Reservoir	264	NA	Yellow perch	3	medium	0.587	2.208	265,851
Salmon River	Salmon R-3	2008	45.41	-116.19	River	290	353	Smallmouth bass	4	high	0.380	1.09	348,624
Salmon River	Salmon R-2	2008	45.79	-116.32	River	330	400	Mountain whitefish	3	medium	0.142	0.88	161,364
Salmon River	Salmon R-2	2008	45.79	-116.32	River	300	300	Smallmouth bass	4	high	0.548	0.88	622,727
Salmon River	Salmon R-1	2008	45.46	-115.77	River	320	300	Mountain whitefish	3	medium	0.097	0.98	98,980
Salmon River	Salmon R-1	2008	45.46	-115.77	River	330	299	Large Northern pikeminnow	4	high	0.674	0.98	687,755
Salmon River	Salmon R-1	2008	45.46	-115.77	River	270	300	Smallmouth bass	4	high	0.253	0.98	258,163
Selway River	Selway R	2008	46.05	-115.30	River	320	232	Cutthroat trout	3	medium	0.053	0.4	132,500
Selway River	Selway R	2008	46.05	-115.30	River	310	267	Mountain whitefish	3	medium	0.083	0.4	207,500
Selway River	Selway R	2008	46.05	-115.30	River	400	500	Large Brook trout	4	high	0.153	0.4	382,500
Snake River	Snake R-2	2008	43.61	-116.91	River	610	4040	Common carp	3	medium	0.138	1.71	80,702

Waterbody Name	Site	Year	Latitude	Longitude	Waterbody Type	Fish Length (mm)[a]	Fish Weight (g)[a]	Fish Common Name	TL[b]	Trophic Magnitude Category	Muscle THg (mg/kg-ww)	Water THg (ng/L)	THg BAF (L/kg)
Snake River	Snake R-2	2008	43.61	-116.91	River	330	550	Smallmouth bass	4	high	0.088	1.71	51,462
Snake River	Snake R-1	2008	43.01	-116.13	River	550	1870	Largescale sucker	3	medium	0.198	0.94	210,638
Snake River	Snake R-1	2008	43.01	-116.13	River	350	665	Smallmouth bass	4	high	0.200	0.94	212,766
Snake River	Snake R-3	2008	42.64	-114.56	River	450	1025	Largescale sucker	3	medium	0.190	1.82	104,396
Snake River	Snake R-3	2008	42.64	-114.56	River	400	1000	Smallmouth bass	4	high	0.318	1.82	174,725
Snake River	Snake River near Murphy	2013	43.29	-116.42	River	631	2613	Channel catfish	3	medium	0.206	0.17	1,211,765
Snake River	Snake River near Murphy	2015	43.29	-116.42	River	625	2970	Channel catfish	3	medium	0.163	0.19	857,895
Snake River	Snake River near Murphy	2017	43.29	-116.42	River	592	2266	Channel catfish	3	medium	0.108	0.41	263,415
Snake River	Snake River near Murphy	2013	43.29	-116.42	River	344	639	Smallmouth bass	4	high	0.173	0.17	1,017,647
Snake River	Snake River near Murphy	2015	43.29	-116.42	River	328	501	Smallmouth bass	4	high	0.164	0.19	863,158
Snake River	Snake River near Murphy	2017	43.29	-116.42	River	348	648	Smallmouth bass	4	high	0.192	0.41	468,293
Snake River	Snake River at Nyssa	2013	43.88	-116.98	River	599	1978	Channel catfish	3	medium	0.143	1.2	119,167
Snake River	Snake River at Nyssa	2015	43.88	-116.98	River	590	2303	Channel catfish	3	medium	0.127	0.61	208,197
Snake River	Snake River at Nyssa	2017	43.88	-116.98	River	608	2419	Channel catfish	3	medium	0.141	1.04	135,577
Brownlee Reservoir	Brownlee Reservoir at Burnt River	2013	44.37	-117.23	Reservoir	370	792	Smallmouth bass	4	high	0.324	0.67	483,582
Brownlee Reservoir	Brownlee Reservoir at Burnt River	2017	44.37	-117.23	Reservoir	341	668	Smallmouth bass	4	high	0.227	1.86	122,043
Brownlee Reservoir	Brownlee Reservoir at Burnt River	2015	44.37	-117.23	Reservoir	637	3140	Channel catfish	3	medium	0.219	1.073	204,101
Brownlee Reservoir	Brownlee Reservoir at multiple locations	2015	44.80	-116.93	Reservoir	194	NA	Smallmouth bass	4	high	0.189	1.138	166,463
Brownlee Reservoir	Brownlee Reservoir at multiple locations	2017	44.80	-116.93	Reservoir	177	NA	Smallmouth bass	4	high	0.171	1.861	92,007

Waterbody Name	Site	Year	Latitude	Longitude	Waterbody Type	Fish Length (mm)[a]	Fish Weight (g)[a]	Fish Common Name	TL[b]	Trophic Magnitude Category	Muscle THg (mg/kg-ww)	Water THg (ng/L)	THg BAF (L/kg)
Brownlee Reservoir	Brownlee Reservoir at multiple locations	2018	44.80	-116.93	Reservoir	185	NA	Smallmouth bass	4	high	0.217	1.861	116,342
Brownlee Reservoir	Brownlee Reservoir at multiple locations	2017	44.80	-116.93	Reservoir	484	NA	Channel catfish	3	medium	0.296	1.861	159,081
Brownlee Reservoir	Brownlee Reservoir at multiple locations	2017	44.80	-116.93	Reservoir	244	NA	Crappie sp.	3	medium	0.214	1.861	115,224
Brownlee Reservoir	Brownlee Reservoir at multiple locations	2017	44.80	-116.93	Reservoir	537	NA	Flathead catfish	3	medium	0.477	1.861	256,008
Brownlee Reservoir	Brownlee Reservoir at multiple locations	2017	44.80	-116.93	Reservoir	306	NA	Largescale sucker	3	medium	0.083	1.861	44,433
Brownlee Reservoir	Brownlee Reservoir at multiple locations	2017	44.80	-116.93	Reservoir	228	NA	Small Northern pikeminnow	3	medium	0.205	1.861	110,302
Brownlee Reservoir	Brownlee Reservoir at multiple locations	2017	44.80	-116.93	Reservoir	209	NA	Sucker sp.	2	Low	0.066	1.861	35,385
Brownlee Reservoir	Brownlee Reservoir at multiple locations	2017	44.80	-116.93	Reservoir	226	NA	Yellow perch	3	medium	0.202	1.861	108,291
Brownlee Reservoir	Brownlee Reservoir at multiple locations	2017	44.80	-116.93	Reservoir	57	NA	Banded killifish	3	medium	0.075	1.861	40,069
Brownlee Reservoir	Brownlee Reservoir at multiple locations	2017	44.80	-116.93	Reservoir	117	NA	Bluegill	3	medium	0.181	1.861	97,447
Brownlee Reservoir	Brownlee Reservoir at multiple locations	2018	44.80	-116.93	Reservoir	101	NA	Bluegill	3	medium	0.165	1.861	88,738

Waterbody Name	Site	Year	Latitude	Longitude	Waterbody Type	Fish Length (mm)[a]	Fish Weight (g)[a]	Fish Common Name	TL[b]	Trophic Magnitude Category	Muscle THg (mg/kg-ww)	Water THg (ng/L)	THg BAF (L/kg)
Brownlee Reservoir	Brownlee Reservoir at multiple locations	2017	44.80	-116.93	Reservoir	138	NA	Pumpkinseed	3	medium	0.167	1.861	89,846
Hells Canyon Reservoir	Hells Canyon Reservoir	2015	45.24	-116.70	Reservoir	191	NA	Smallmouth bass	4	high	0.251	1.715	146,453
Hells Canyon Reservoir	Hells Canyon Reservoir	2017	45.24	-116.70	Reservoir	157	NA	Smallmouth bass	4	high	0.262	2.347	111,578
Hells Canyon Reservoir	Hells Canyon Reservoir	2017	45.24	-116.70	Reservoir	235	NA	Bridgelip sucker	2	Low	0.040	2.347	16,991
Hells Canyon Reservoir	Hells Canyon Reservoir	2017	45.24	-116.70	Reservoir	310	NA	Channel catfish	3	medium	0.738	2.347	314,297
Hells Canyon Reservoir	Hells Canyon Reservoir	2017	45.24	-116.70	Reservoir	183	NA	Crappie sp.	3	medium	0.203	2.347	86,564
Hells Canyon Reservoir	Hells Canyon Reservoir	2017	45.24	-116.70	Reservoir	257	NA	Largescale sucker	3	medium	0.096	2.347	41,071
Hells Canyon Reservoir	Hells Canyon Reservoir	2017	45.24	-116.70	Reservoir	207	NA	Yellow perch	3	medium	0.249	2.347	106,275
Hells Canyon Reservoir	Hells Canyon Reservoir	2017	45.24	-116.70	Reservoir	52	NA	Banded killifish	3	medium	0.066	2.347	28,041
Hells Canyon Reservoir	Hells Canyon Reservoir	2017	45.24	-116.70	Reservoir	82	NA	Bluegill	3	medium	0.147	2.347	62,757
Hells Canyon Reservoir	Hells Canyon Reservoir	2017	45.24	-116.70	Reservoir	83	NA	Small Northern pikeminnow	3	medium	0.067	2.347	28,392
Hells Canyon Reservoir	Hells Canyon Reservoir	2017	45.24	-116.70	Reservoir	104	NA	Pumpkinseed	3	medium	0.089	2.347	37,758
Hells Canyon Reservoir	Hells Canyon Reservoir	2017	45.24	-116.70	Reservoir	98	NA	Warmouth	3	medium	0.128	2.347	54,616
Oxbow Reservoir	Oxbow Reservoir	2015	44.97	-116.84	Reservoir	207	NA	Smallmouth bass	4	high	0.288	0.723	398,729
South Fork Payette River	SF Payette R - SF Snake R	2008	43.44	-111.36	River	380	588	Cutthroat trout	3	medium	0.081	0.72	111,806

Waterbody Name	Site	Year	Latitude	Longitude	Waterbody Type	Fish Length (mm)[a]	Fish Weight (g)[a]	Fish Common Name	TL[b]	Trophic Magnitude Category	Muscle THg (mg/kg-ww)	Water THg (ng/L)	THg BAF (L/kg)
South Fork Payette River	SF Payette R - SF Snake R	2008	43.44	-111.36	River	360	396	Mountain whitefish	3	medium	0.090	0.72	125,000
South Fork Payette River	SF Payette R - SF Snake R	2008	43.44	-111.36	River	450	875	Brown trout	4	high	0.253	0.72	351,389
South Fork Payette River	SF Payette R - SF Snake R	2008	43.44	-111.36	River	460	700	Cutthroat trout x Rainbow trout	3	medium	0.240	0.72	333,333
South Fork Payette River	SF Payette R - SF Snake R	2008	43.44	-111.36	River	420	550	Rainbow trout	3	medium	0.175	0.72	243,056
Sugar Creek	Sugar Creek--Upstream	2016	44.95	-115.29	River	199	76	Bull trout	3	medium	0.080	0.7	113,571
Sugar Creek	Sugar Creek--Upstream	2016	44.95	-115.29	River	NA	4	Sculpin	3	medium	0.071	0.7	101,299

[a] Average species length and/or weight for all samples at that site where length and weight were reported.
[b] As reported in Essig (2010). See **Section E.1** for additional details.

E.2 Calculation of the Wood Frog (*Lithobates sylvaticus*) BAF

Paired tissue and water data for the wood frog (*Lithobates sylvaticus*) were obtained from two field studies (Loftin et al. 2012; Faccio et al. 2019). For both studies, all possible bioaccumulation factors (BAFs) were calculated by dividing total mercury THg tissue concentrations by THg water concentrations collected at the same site within 0-3 months of corresponding tissue concentrations. Tissue concentrations reported as whole body (WB) were converted to muscle (M) using an amphibian WB:M conversion factor (CF) of 0.97 (**Appendix D**). All wood frog BAFs were calculated for larval life stages. Loftin et al. (2012) reported tissue data for three different life stages (Gosner stages 24-33, 29, and 34-37, respectively) at three sites. Faccio et al. (2019) collected early larval (Gosner stages 22-25) and late larval (Gosner stages 26-39) wood frogs at six sites.

The representative wood frog BAF was calculated as the median of the two study values, as follows. For Loftin et al. (2012), the median BAF at each of the three sites (U1, U2, B1) was calculated, and then the median of the three site values was calculated to represent the study-level BAF from Loftin et al. (2012). Faccio et al. (2019) reported THg tissue concentrations as life stage averages across all six sites. Tissue concentrations were highest in late larval tadpoles, so this life stage was used to calculate a conservative BAF. At each site, the average tissue concentration was divided by the paired water concentration, and the median of those BAFs was used to represent the Faccio et al. (2019) study level BAF. The final wood frog BAF of 8,222 L.kg was the median of the Loftin et al. (2012) and Faccio et al. (2019) BAFs. This value is similar to, but slightly more conservative, than the BAF of 7,822 L/kg calculated as the median of the nine site BAFs across the two studies. Site information, tissue and water concentrations, and BAFs for the wood frog are reported in **Table E-2**.

Faccio et al. (2019) also reported paired water and tissue data for a second amphibian species, the spotted salamander (*Ambystoma maculatum*) collected at the same times and locations as the wood frog samples. Spotted salamander samples were also collected for early and late larval stages, and total mercury concentrations were averaged across sites for each life stage (**Table E-3**). A study-level spotted salamander BAF was calculated following the same approach described above for wood frogs using the most sensitive life stage, which was the early larval life stage for this species. The resulting spotted salamander BAF of 9,320 L/kg was not used in the translation calculations but is shown here because it suggests a similar degree of mercury biomagnification for a second amphibian species.

Table E-2. Data used to calculate the wood frog (*L. sylvaticus*) BAF used to represent frog species in the calculation of the translated water column criterion value.

Faccio et al. (2019) study specific BAF based on the most sensitive (late larval) life stage.

Study	Common Name	Scientific Name	Stage	Site	Measured Tissue	Tissue Date	THg-tissue	Tissue Units	% Moisture	WB / M	Final Tissue	THg-final tissue (ng/g-ww)	THg (ng/L)	Water Date	BAF (L/kg)
Loftin et al. 2012	Wood frog	*Lithobates sylvaticus*	GS 24-33	U1	Whole body	Jun-08	21.23	ng/g-ww	NA	0.97	Muscle	21.89	5.16	Jun-08	4,242
Loftin et al. 2012	Wood frog	*Lithobates sylvaticus*	GS 24-33	U1	Whole body	Jun-08	25.11	ng/g-ww	NA	0.97	Muscle	25.89	5.16	Jun-08	5,017
Loftin et al. 2012	Wood frog	*Lithobates sylvaticus*	GS 24-33	U1	Whole body	Jun-08	28.3	ng/g-ww	NA	0.97	Muscle	29.18	5.16	Jun-08	5,654
Loftin et al. 2012	Wood frog	*Lithobates sylvaticus*	GS 24-33	U1	Whole body	Jun-08	36.45	ng/g-ww	NA	0.97	Muscle	37.58	5.16	Jun-08	7,282
Loftin et al. 2012	Wood frog	*Lithobates sylvaticus*	GS 24-33	U1	Whole body	Jun-08	41.87	ng/g-ww	NA	0.97	Muscle	43.16	5.16	Jun-08	8,365
Loftin et al. 2012	Wood frog	*Lithobates sylvaticus*	GS 24-33	U1	Whole body	Jun-08	48.76	ng/g-ww	NA	0.97	Muscle	50.27	5.16	Jun-08	9,742
Loftin et al. 2012	Wood frog	*Lithobates sylvaticus*	GS 24-33	U1	Whole body	Jun-08	54.18	ng/g-ww	NA	0.97	Muscle	55.86	5.16	Jun-08	10,825
Loftin et al. 2012	Wood frog	*Lithobates sylvaticus*	GS 29	U2	Whole body	Jun-08	15.12	ng/g-ww	NA	0.97	Muscle	15.59	9.64	Jun-08	1,617
Loftin et al. 2012	Wood frog	*Lithobates sylvaticus*	GS 29	U2	Whole body	Jun-08	29.37	ng/g-ww	NA	0.97	Muscle	30.28	9.64	Jun-08	3,141
Loftin et al. 2012	Wood frog	*Lithobates sylvaticus*	GS 29	U2	Whole body	Jun-08	30.15	ng/g-ww	NA	0.97	Muscle	31.08	9.64	Jun-08	3,224
Loftin et al. 2012	Wood frog	*Lithobates sylvaticus*	GS 29	U2	Whole body	Jun-08	33.05	ng/g-ww	NA	0.97	Muscle	34.07	9.64	Jun-08	3,534
Loftin et al. 2012	Wood frog	*Lithobates sylvaticus*	GS 29	U2	Whole body	Jun-08	39.16	ng/g-ww	NA	0.97	Muscle	40.37	9.64	Jun-08	4,188
Loftin et al. 2012	Wood frog	*Lithobates sylvaticus*	GS 34-37	B1	Whole body	Jun-08	16.87	ng/g-ww	NA	0.97	Muscle	17.39	4.47	Jun-08	3,891
Loftin et al. 2012	Wood frog	*Lithobates sylvaticus*	GS 34-37	B1	Whole body	Jun-08	17.06	ng/g-ww	NA	0.97	Muscle	17.59	4.47	Jun-08	3,935
Loftin et al. 2012	Wood frog	*Lithobates sylvaticus*	GS 34-37	B1	Whole body	Jun-08	28.21	ng/g-ww	NA	0.97	Muscle	29.08	4.47	Jun-08	6,506
Loftin et al. 2012	Wood frog	*Lithobates sylvaticus*	GS 34-37	B1	Whole body	Jun-08	28.5	ng/g-ww	NA	0.97	Muscle	29.38	4.47	Jun-08	6,573
Loftin et al. 2012	Wood frog	*Lithobates sylvaticus*	GS 34-37	B1	Whole body	Jun-08	30.92	ng/g-ww	NA	0.97	Muscle	31.88	4.47	Jun-08	7,131
Loftin et al. 2012	Wood frog	*Lithobates sylvaticus*	GS 34-37	B1	Whole body	Jun-08	34.6	ng/g-ww	NA	0.97	Muscle	35.67	4.47	Jun-08	7,980
Loftin et al. 2012	Wood frog	*Lithobates sylvaticus*	GS 34-37	B1	Whole body	Jun-08	36.64	ng/g-ww	NA	0.97	Muscle	37.77	4.47	Jun-08	8,450
Loftin et al. 2012	Wood frog	*Lithobates sylvaticus*	GS 34-37	B1	Whole body	Jun-08	40.23	ng/g-ww	NA	0.97	Muscle	41.47	4.47	Jun-08	9,278

Study	Common Name	Scientific Name	Stage	Site	Measured Tissue	Tissue Date	THg-tissue	Tissue Units	% Moisture	WB / M	Final Tissue	THg-final tissue (ng/g-ww)	THg (ng/L)	Water Date	BAF (L/kg)
Faccio et al. 2019	Wood frog	*Lithobates sylvaticus*	GS 22-25	KWN467	Whole body	May 12 - June 6, 2015	70.03	ng/g-dw	0.862	0.97	Muscle	9.94	3.36	April - July 2015	2,960
Faccio et al. 2019	Wood frog	*Lithobates sylvaticus*	GS 22-25	SDF509	Whole body	May 12 - June 6, 2015	70.03	ng/g-dw	0.862	0.97	Muscle	9.94	2.55	April - July 2015	3,900
Faccio et al. 2019	Wood frog	*Lithobates sylvaticus*	GS 22-25	SDF791	Whole body	May 12 - June 6, 2015	70.03	ng/g-dw	0.862	0.97	Muscle	9.94	3.76	April - July 2015	2,645
Faccio et al. 2019	Wood frog	*Lithobates sylvaticus*	GS 22-25	NEW110	Whole body	May 12 - June 6, 2015	70.03	ng/g-dw	0.862	0.97	Muscle	9.94	6.63	April - July 2015	1,500
Faccio et al. 2019	Wood frog	*Lithobates sylvaticus*	GS 22-25	SDF516	Whole body	May 12 - June 6, 2015	70.03	ng/g-dw	0.862	0.97	Muscle	9.94	5.15	April - July 2015	1,931
Faccio et al. 2019	Wood frog	*Lithobates sylvaticus*	GS 22-25	SDF951	Whole body	May 12 -June 6, 2015	70.03	ng/g-dw	0.862	0.97	Muscle	9.94	5.33	April - July 2015	1,866
Faccio et al. 2019	Wood frog	*Lithobates sylvaticus*	GS 26-39	KWN467	Whole body	July 6-8, 2015	293.58	ng/g-dw	0.862	0.97	Muscle	41.69	3.36	April - July 2015	12,407
Faccio et al. 2019	Wood frog	*Lithobates sylvaticus*	GS 26-39	SDF509	Whole body	July 6-8, 2015	293.58	ng/g-dw	0.862	0.97	Muscle	41.69	2.55	April - July 2015	16,349
Faccio et al. 2019	Wood frog	*Lithobates sylvaticus*	GS 26-39	SDF791	Whole body	July 6-8, 2015	293.58	ng/g-dw	0.862	0.97	Muscle	41.69	3.76	April - July 2015	11,087
Faccio et al. 2019	Wood frog	*Lithobates sylvaticus*	GS 26-39	NEW110	Whole body	July 6-8, 2015	293.58	ng/g-dw	0.862	0.97	Muscle	41.69	6.63	April - July 2015	6,288
Faccio et al. 2019	Wood frog	*Lithobates sylvaticus*	GS 26-39	SDF516	Whole body	July 6-8, 2015	293.58	ng/g-dw	0.862	0.97	Muscle	41.69	5.15	April - July 2015	8,095
Faccio et al. 2019	Wood frog	*Lithobates sylvaticus*	GS 26-39	SDF951	Whole body	July 6-8, 2015	293.58	ng/g-dw	0.862	0.97	Muscle	41.69	5.33	April - July 2015	7,822

Table E-3. Data used to calculate the spotted salamander (*A. maculatum*) BAF.

Faccio et al. (2019) study specific BAF based on the most sensitive (early larval) life stage.

Study	Common Name	Scientific Name	Stage	Site	Measured Tissue	Tissue Date	THg-tissue	Tissue Units	% Moisture	WB / M	Final Tissue	THg-final tissue (ng/g-ww)	THg (ng/L)	Water Date	BAF (L/kg)
Faccio et al. 2019	Spotted salamander	*Ambystoma maculatum*	early larval	KWN467	Whole body	May 12- June 6, 2015	285.3	ng/g-dw	0.862	0.97	Muscle	38.12	40.51	April - July 2015	12,057
Faccio et al. 2019	Spotted salamander	*Ambystoma maculatum*	early larval	SDF509	Whole body	May 12 - June 6, 2015	285.3	ng/g-dw	0.862	0.97	Muscle	38.12	40.51	April - July 2015	15,886
Faccio et al. 2019	Spotted salamander	*Ambystoma maculatum*	early larval	SDF791	Whole body	May 12- June 6, 2015	285.3	ng/g-dw	0.862	0.97	Muscle	38.12	40.51	April - July 2015	10,774
Faccio et al. 2019	Spotted salamander	*Ambystoma maculatum*	early larval	NEW110	Whole body	May 12 - June 6, 2015	285.3	ng/g-dw	0.862	0.97	Muscle	38.12	40.51	April - July 2015	6,110
Faccio et al. 2019	Spotted salamander	*Ambystoma maculatum*	early larval	SDF516	Whole body	May 12- June 6, 2015	285.3	ng/g-dw	0.862	0.97	Muscle	38.12	40.51	April - July 2015	7,866
Faccio et al. 2019	Spotted salamander	*Ambystoma maculatum*	early larval	SDF951	Whole body	May 12 - June 6, 2015	285.3	ng/g-dw	0.862	0.97	Muscle	38.12	40.51	April - July 2015	7,600
Faccio et al. 2019	Spotted salamander	*Ambystoma maculatum*	late larval	KWN467	Whole body	July 6-8. 2015	241.1	ng/g-dw	0.862	0.97	Muscle	32.21	34.24	April - July 2015	10,189
Faccio et al. 2019	Spotted salamander	*Ambystoma maculatum*	late larval	SDF509	Whole body	July 6-8. 2015	241.1	ng/g-dw	0.862	0.97	Muscle	32.21	34.24	April - July 2015	13,426
Faccio et al. 2019	Spotted salamander	*Ambystoma maculatum*	late larval	SDF791	Whole body	July 6-8. 2015	241.1	ng/g-dw	0.862	0.97	Muscle	32.21	34.24	April - July 2015	9,105
Faccio et al. 2019	Spotted salamander	*Ambystoma maculatum*	late larval	NEW110	Whole body	July 6-8. 2015	241.1	ng/g-dw	0.862	0.97	Muscle	32.21	34.24	April - July 2015	5,164
Faccio et al. 2019	Spotted salamander	*Ambystoma maculatum*	late larval	SDF516	Whole body	July 6-8. 2015	241.1	ng/g-dw	0.862	0.97	Muscle	32.21	34.24	April - July 2015	6,648
Faccio et al. 2019	Spotted salamander	*Ambystoma maculatum*	late larval	SDF951	Whole body	July 6-8. 2015	241.1	ng/g-dw	0.862	0.97	Muscle	32.21	34.24	April - July 2015	6,423

E.3 Calculation of Crayfish BAF

The crayfish BAF was calculated using crayfish (unidentified species) THg tail muscle tissue concentrations collected from the Boise River in 2021 that were available from the Idaho Crayfish Project (https://crayfish.nkn.uidaho.edu/wp-content/uploads/2022/02/Crayfish-Infographic-_FINAL.pdf). Tissue concentrations were paired with water data from the Boise River from USGS monitoring studies in 2020-2021. The final BAF of 128,414 L/kg was calculated as the 2021 average tissue concentration divided by the geometric mean THg concentration of the three water samples collected between late 2020 and early 2021 (**Table E-4**). The crayfish BAF was used to translate invertebrate tissue SMCVs to water column SMCVs. Although there is some uncertainty in the application of the crayfish BAF to non-crayfish species, it is the only available invertebrate BAF, and is most likely a conservative value given the likelihood of omnivory in field-collected crayfish.

Table E-4. Data used to calculate the crayfish BAF used to represent invertebrate species in the calculation of the translated water column criterion value.

Sampling Location	Sampling Date	Muscle THg (µg/kg-ww)	Water THg (ng/L)	BAF (L/kg)
Lower Boise River	Summer 2021	103.4		
Boise River near Glenwood	9/1/2020		1.45	
Boise River near Glenwood	9/28/2020		0.95	
Boise River near Glenwood	10/26/2020		0.38	
Geometric Mean Water		103.4	0.81	128,414

E.4 Calculation of Ecoregional Water Concentrations

As an alternative to calculating fish BAFs based on spatially and temporally paired water and tissue samples, an alternative BAF calculation approach was examined based on pairing tissue samples with geometric mean level III ecoregion water concentrations. Water total mercury (THg) concentrations can show large variability throughout the year, and many of the water samples in the Idaho mercury fish tissue and water dataset are based on a single surface water grab sample at one location in the waterbody. In addition, the methylmercury (MeHg) that accumulates in aquatic food webs that the fish consume is spatially and temporally disconnected from the single THg grab sample of water that was collected when fish were being sampled.

It was also noted that some of the BAFs based on spatially-paired tissue and water were visually (not statistically) identified to be outliers, and that while the fish tissue concentrations appeared similar to statewide averages, the water THg concentrations were more variable. Finally, mercury methylation varies depending on several ecosystem characteristics (organic carbon levels, presence of wetlands, nutrient loading, etc.) that are related to ecoregions.

Ecoregional THg water concentrations were calculated as follows. First, the level III ecoregion was determined for all locations in the Idaho fish and tissue database where THg water measurements were available. Next, all locations impacted by Hg point sources of contamination (downstream Coeur d'Alene River, Jordan Creek, Cinnabar Creek, and downstream Sugar Creek) were removed from the analysis. Finally, the geometric mean THg concentrations were calculated for each of the level III ecoregions in the dataset as the representative ecoregional water concentrations. **Table E-5** summarizes the ecoregional THg water concentration data, and **Table E-6** shows the location-level data used for the calculations. Data were available for six of the eight level III ecoregions in Idaho.

Table E-5. Summary of Level III Ecoregional Total Mercury (THg) Concentrations in Idaho.

Concentrations (ng/L) represent geometric means of THg measurements across all locations (n) within an ecoregion.

Level III Ecoregion	THg (ng/L)	n
11	1.30	10
12	0.95	34
15	0.38	10
16	0.64	12
17	0.65	6
80	1.32	4

Following the calculation of ecoregional water concentrations, the relationship between THg and MeHg was separately examined for all data with paired THg and MeHg measurements examined as individual sites (**Figure E-1**) and averaged across ecoregions (**Figure E-2**). Data from Hg contaminated sites were excluded. Results of this analysis demonstrate there is a positive relationship between THg and MeHg in the dataset, and that the relationship is similar when examined as individual sites or averaged across ecoregions. Because these relationships were observed, it was determined that the recalculation of fish BAFs based on ecoregional averages was an option worth further exploration. Results of these recalculations are described in the following section.

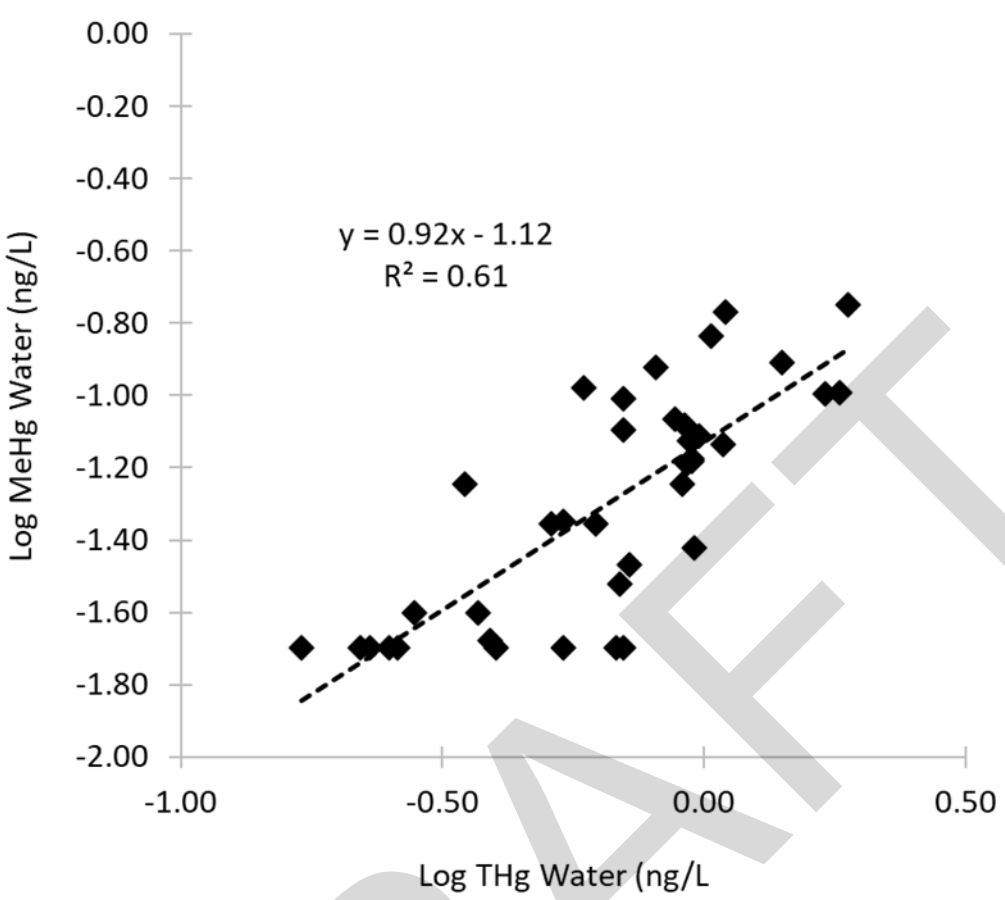


Figure E-1. THg versus MeHg for all locations in the Idaho fish tissue and water database with paired measurements.
All locations impacted by Hg point sources of contamination removed from analysis.

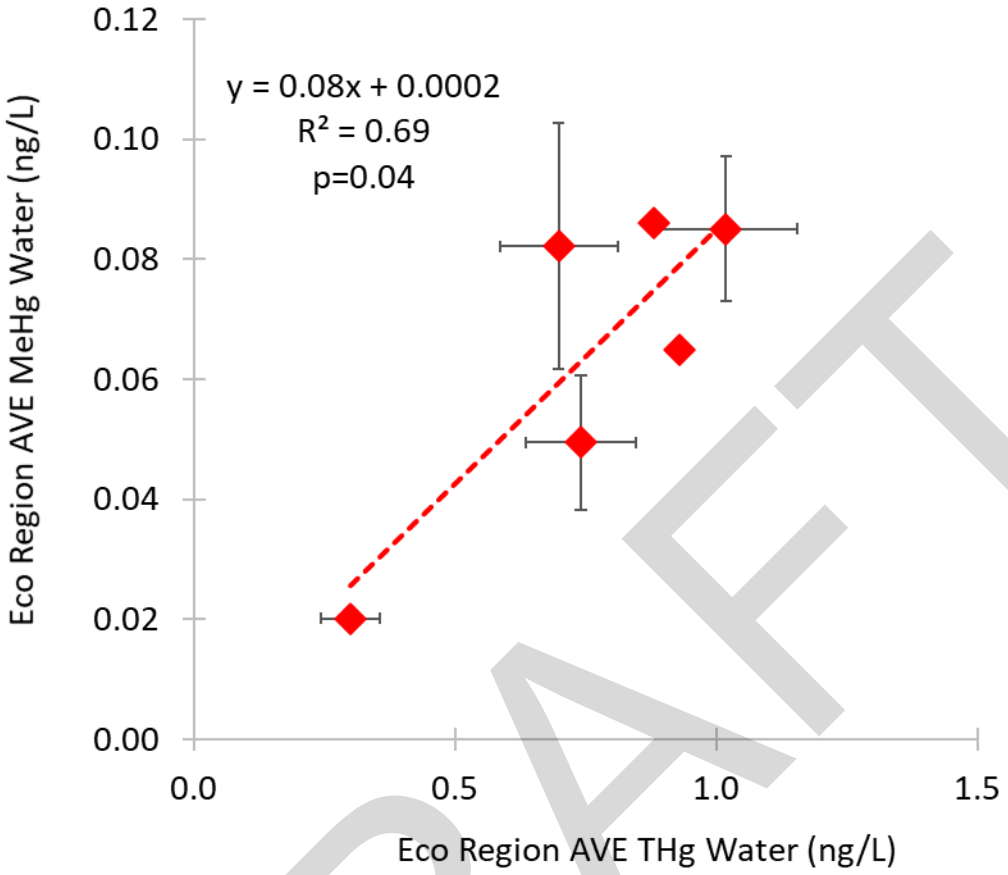


Figure E-2. THg versus MeHg for all locations in the Idaho fish tissue and water database with paired measurements, averaged across level III ecoregions.
All locations impacted by Hg point sources of contamination were removed from analysis.

Table E-6. Water samples used to calculate the ecoregional total mercury water concentrations for Idaho.
Ecoregional water concentrations were calculated as the geometric mean of all water samples for a given ecoregion.

Site Name	Site ID (num)	LAT	LON	THg (ng/L)	n	Collector	Study info
Blue Mountains (Ecoregion 11)							
Salmon R #2	28	45.79	-116.32	0.88	1	IDEQ	Essig 2010
Brownlee Reservoir at Burnt River	4422221171355	44.37	-117.23	0.67	7	USGS	Baldwin et al. 2020
Brownlee Reservoir at Burnt River	4422221171355	44.37	-117.23	1.073	23	USGS	MacCoy and Mebane 2018
Brownlee Reservoir at Burnt River	4422221171355	44.37	-117.23	1.86	1	USGS	MacCoy and Mebane 2018
Brownlee Reservoir at multiple locations	Unknown	44.80	-116.93	1.138	145	USGS	Poulin et al. 2020
Brownlee Reservoir at multiple locations	Unknown	44.80	-116.93	2.552	97	USGS	Poulin et al. 2020
Brownlee Reservoir at multiple locations	Unknown	44.80	-116.93	1.357	66	USGS	Poulin et al. 2020
Oxbow Reservoir	Unknown	44.97	-116.84	0.723	15	USGS	Poulin et al. 2020
Hells Canyon Reservoir	Unknown	45.24	-116.70	1.715	28	USGS	Poulin et al. 2020
Hells Canyon Reservoir	Unknown	45.24	-116.70	2.347	22	USGS	Poulin et al. 2020
Snake River Plain (Ecoregion 12)							
Big Wood River #2	91	43.43	-114.26	0.37	1	IDEQ	Essig 2010
Blackfoot	5	43.21	-112.20	0.7	1	IDEQ	Essig 2010
Boise R @ Glenwood	13206000	43.66	-116.28	0.91	1	USGS	Essig 2010
Boise R. at Ann Morrison Park	x99	43.61	-116.21	1.33	1	IDEQ	Essig 2010
Boise River at Eckert Rd near Boise	13203760	43.57	-116.13	0.73	1	City of Boise	MacCoy and Mebane 2018
Boise River at mouth, near Parma	13213030	43.82	-117.02	1.2	1	City of Boise	MacCoy and Mebane 2018
Boise River near Middleton	13210050	43.68	-116.57	0.89	1	City of Boise	MacCoy and Mebane 2018
Boise River at Eckert Rd near Boise	13203760	43.57	-116.13	0.77	1	City of Boise	MacCoy and Mebane 2018

Site Name	Site ID (num)	LAT	LON	THg (ng/L)	n	Collector	Study info
Boise River at mouth, near Parma	13213030	43.82	-117.02	1.6	1	City of Boise	MacCoy and Mebane 2018
Boise River near Middleton	13210050	43.68	-116.57	1.1	1	City of Boise	MacCoy and Mebane 2018
Boise River at Eckert Rd near Boise	13203760	43.57	-116.13	1.13	1	City of Boise	MacCoy and Mebane 2018
Boise River at mouth, near Parma	13213030	43.82	-117.02	1.48	1	City of Boise	MacCoy and Mebane 2018
Boise River near Middleton	13210050	43.68	-116.57	1.35	1	City of Boise	MacCoy and Mebane 2018
Boise River at Glenwood Bridge near Boise	13206000	43.66	-116.28	0.986	1	USGS	
Anderson Ranch Res at Anderson Ranch Dam (South Fork Boise River)	13190000			0.63925	12	USGS	
Lucky Peak Lake near Boise (Boise River)	13190500			0.8301	10	USGS	
Boise River at Glenwood Bridge near Boise	13206000			2.0315	8	USGS	
Bruneau River	51	42.79	-115.72	0.81	1	IDEQ	Essig 2010
Camas Creek	61	43.88	-112.35	0.95	1	IDEQ	Essig 2010
Henry's Fk Nr Rexburg	13056500	43.83	-111.91	0.62	1	USGS	Essig 2010
Henry's Fork	77	43.80	-111.93	1.03	1	IDEQ	Essig 2010
Payette River #2	99	43.90	-116.63	0.95	1	IDEQ	Essig 2010
Payette River	63	44.00	-116.80	1.08	1	IDEQ	Essig 2010
Portneuf River	85	42.85	-112.44	1.89	3	IDEQ	Essig 2010
Snake River #1	83	43.01	-116.13	0.94	1	IDEQ	Essig 2010
Snake River #2	47	43.61	-116.91	1.71	1	IDEQ	Essig 2010
Snake River #3	95	42.64	-114.56	1.82	1	IDEQ	Essig 2010
Snake River at Nyssa	13213100	43.88	-116.98	1.2	1	City of Boise	MacCoy and Mebane 2018
Snake River at Nyssa	13213100	43.88	-116.98	0.61	1	City of Boise	MacCoy and Mebane 2018
Snake River near Murphy	13172500	43.29	-116.42	0.41	1	City of Boise	MacCoy and Mebane 2018
Snake River at Nyssa	13213100	43.88	-116.98	1.04	1	City of Boise	MacCoy and Mebane 2018

Site Name	Site ID (num)	LAT	LON	THg (ng/L)	n	Collector	Study info
Snake River near Murphy	13172500	43.29	-116.42	0.93	1	City of Boise	MacCoy and Mebane 2018
Snake River near Murphy	13172500	43.29	-116.42	0.48	1	City of Boise	MacCoy and Mebane 2018
Weiser River	31	44.63	-116.59	0.54	1	IDEQ	Essig 2010
Northern Rockies (Ecoregion 15)							
Kootenai River near Crossport, ID	12308500	48.70	-116.24	0.18	1	USGS	MacCoy and Mebane 2018
Kootenai River downstream of the Yaak River in MT	12305000	48.59	-116.00	0.13	1	USGS	MacCoy and Mebane 2018
Coeur d'Alene R #2	38	-116.23	48.01	0.25	1	IDEQ	Essig 2010
Coeur d'Alene R #3	54	-116.29	48.02	0.39	1	IDEQ	Essig 2010
Lochsa River	74	46.93	-115.04	0.54	1	IDEQ	Essig 2010
NF Clearwater R	26	46.73	-115.29	0.23	1	IDEQ	Essig 2010
Priest River	50	48.24	-116.88	0.17	1	IDEQ	Essig 2010
Saint Joe River	86	47.14	-115.41	0.22	1	IDEQ	Essig 2010
Clearwater River at Riverside	LRO Merc - 2	46.49	-116.30	1.580	1	IDEQ	IDEQ 2007a
Orofino Creek at Cow Creek	LRO Merc - 1	46.50	-115.93	4.25	1	IDEQ	IDEQ 2007a
Idaho Batholith (Ecoregion 16)							
Big Wood River	11	43.78	-114.54	0.28	1	IDEQ	Essig 2010
Boise R @ Twn Spr	13185000	43.66	-115.73	0.69	1	USGS	Essig 2010
Camas Creek #2	68	44.82	-114.49	0.68	1	IDEQ	Essig 2010
Cane Creek	16ID-007	44.95	-115.29	0.49	1	USGS	Mcgee et al. 2020
Johnson Creek @ YP	13313000	44.96	-115.50	0.7	1	USGS	Essig 2010
NF Big Lost	27	43.93	-114.19	0.96	1	IDEQ	Essig 2010
NF Payette	55	44.21	-116.11	0.7	1	IDEQ	Essig 2010
Salmon R #1	40	45.46	-115.77	0.98	1	IDEQ	Essig 2010
Salmon R #3	12	45.41	-116.19	1.09	1	IDEQ	Essig 2010

Site Name	Site ID (num)	LAT	LON	THg (ng/L)	n	Collector	Study info
Selway River	88	46.05	-115.30	0.4	1	IDEQ	Essig 2010
SF Payette	87	44.17	-115.23	0.26	1	IDEQ	Essig 2010
SF Salmon	84	44.70	-115.70	1.41	1	IDEQ	Essig 2010
Middle Rockies (Ecoregion 17)							
Blackfoot River #2	37	42.80	-111.49	0.59	1	IDEQ	Essig 2010
Lemhi Nr Lemhi	13305000	44.94	-113.64	0.92	1	USGS	Essig 2010
Lemhi River	94	45.10	-113.73	1.1	1	IDEQ	Essig 2010
Pahsimeroi	44	44.66	-114.02	0.35	1	IDEQ	Essig 2010
Pahsimeroi @ Ellis	13302005	44.69	-114.05	0.51	1	USGS	Essig 2010
SF Snake	97	43.44	-111.36	0.72	1	IDEQ	Essig 2010
Northern Basin and Range (Ecoregion 80)							
Bear River	17	42.36	-111.74	0.93	1	IDEQ	Essig 2010
Portneuf R--Croney Road Reach	NA	42.86	-112.06	0.21	1	IDEQ	IDEQ 2007c
Portneuf R--Topaz Reach	NA	42.62	-112.03	6.98	1	IDEQ	IDEQ 2007c
Salmon Falls Cr. Res. at Grey's Landing	SFCRGL	42.13	-114.73	2.208	17[a]	IDEQ	IDEQ 2007b

[a] Plus Gray and Hines (2009) data.

E.5 Alternative to the Criterion Water Concentration Approach – Fish Taxa-Specific BAFs based on 80th Centiles

In addition to the derivation of the mercury water column criterion (2.1 ng/L) described in **Section 3.6**, EPA explored seven additional approaches that focused on protecting specific Idaho fish taxa. The approach described in this section followed the criterion approach with one exception. Instead of using median taxa specific BAFs when available, 80th centile taxa-specific BAFs were used.

In the translation approach used to derive the mercury water column criterion, species- or genus-level (taxon-specific) fish BAFs were used when available, and when they were not available, trophic magnitude category fish BAFs were used as surrogate BAFs. Trophic magnitude category BAFs were calculated as the 80th centile fish species BAF within that category, while fish species BAFs were calculated as the median BAF across all locations where a BAF for that species was available. As an alternative to that approach, the taxon-specific 80th centile BAFs (or maximum, when an 80th centile cannot be calculated) are used in this translation procedure.

Taxon-specific fish BAFs were available for channel catfish, rainbow trout, walleye, and brown trout (genus-level surrogate for Atlantic salmon). Channel catfish and rainbow trout BAFs were available at more than four locations, so 80th centile species BAFs could be calculated. Brown trout BAFs were available at two locations, so the maximum (67th centile) BAF was used as the Salmo BAF. A walleye BAF was only available at one site; however, walleye and water were collected at that site for two years, so the larger of the two BAFs was used. Taxon-specific BAFs used in this alternate approach are shown below in **Table E-7**. The frog, crayfish, and fish trophic magnitude category BAFs described in the original translation approach (**Table 3-12**) were also used here.

Table E-7. Taxon Specific 80th Centile BAFs Used in the Tissue to Water Translation Procedure (Additional Approach 1).

Trophic Magnitude Category	Common Name Scientific Name	Median THg (ug/kg ww)	BAF (L/kg muscle-ww)
Low		NA	144,915
Medium		NA	199,646
High		NA	647,335
	L. sylvaticus	NA	8,222
	Crayfish (sp.)	NA	128,414
	Walleye (*Sander vitreus*)	1.002	566,123 (maximum)
	Channel Catfish (*Ictalurus punctatus*)	0.247	640,456 (80th centile)
	Rainbow trout (*Oncorhynchus mykiss*)	0.132	778,638 (80th centile)
	Brown Trout *(Salmo trutta)* Surrogate for Atlantic salmon	0.174	351,389 (maximum)

Aside from the application of 80th centile taxon-specific fish BAFs, the translation procedure was identical to the water column criterion derivation approach. Each muscle tissue SMCV was multiplied by the most appropriate BAF to calculate a distribution of SMCVs expressed as water column concentrations using Equation 2 (**Table E-8**). SMCVs were grouped into GMCVs and a water column FCV and CCC was calculated as was done for the muscle tissue-based criterion element (**Table E-9**). The distribution of translated water GMCVs ranked by sensitivity centile is shown in **Figure E-3**. The translated water FCV calculated using the four lowest definitive GMCVs (see **Sections 3.6)** for this approach is 1.806 ng/L.

The overall effect was a decrease in the SMCVs of walleye, channel catfish, rainbow trout, and Atlantic salmon, resulting in a lower FCV. Walleye remained the most sensitive species in this approach, but because a larger BAF is used, the SMCV is lower. The relative sensitivity of channel catfish increased from being the 7th most sensitive species to the 3rd most sensitive species, reflecting the influence of larger fish with higher tissue THg on the channel

catfish BAF; however, it was not included in the FCV calculations because it was a low greater than value. The lower SMCVs for rainbow trout and Atlantic salmon did not affect the FCV because they were not among the four most sensitive genera.

Table E-8. Ranked Freshwater Genus Mean Chronic Values based on Muscle Concentrations Translated to Water Concentrations using Bioaccumulation Factors (Additional Approach 1).
80th centile (or maximum) species- and genus-specific fish BAFs, when available.

Rank[a]	MDR Group[b]	Genus	Species	Muscle SMCV[c] (µg THg/g ww)	BAF (L/kg ww)	Water SMCV (ng THg/ L)	Water GMCV (ng THg/ L)	BAF Source[d]
1	B	*Sander*	Walleye (*Sander vitreus*)	1.069	566,123	1.888	1.888	*S. vitreus*
2	B	*Hoplias*	Tigerfish (*Hoplias malabaricus*)	>1.45	647,335	>2.240	>2.240	High trophic magnitude
3	B	*Pimephales*	Fathead minnow (*Pimephales promelas*)	0.3575	144,915	2.467	2.467	Low trophic magnitude
4	B	*Ictalurus*	Channel catfish (*Ictalurus punctatus*)	>1.6	640,456	>2.498	>2.498	*I. punctatus*
5	E	*Procambarus*	Red swamp crayfish (*Procambarus Clarkii)*	0.4973	128,414	3.873	3.873	Crayfish
6	C	*Lithobates*	Southern leopard frog (*Lithobates sphenocephala*)	0.03373	8,222	4.103	4.103	Anura
7	B	*Huso*	Beluga sturgeon (*Huso huso*)	3.0	647,335	4.634	4.634	High trophic magnitude
8	A	*Oncorhynchus*	Rainbow trout (*Oncorhynchus mykiss*)	4.392	778,638	5.641	5.641	*O. mykiss*
9	A	*Salmo*	Atlantic Salmon (*Salmo salar*)	>3.07	351,389	>8.737	>8.737	Salmo
10	B	*Carassius*	Goldfish (*Carassius auratus*)	>2.037	144,915	>14.06	>14.06	Low trophic magnitude
11	C	*Anaxyrus*	American toad (*Anaxyrus americanus*)	0.1704	8,222	20.73	20.73	Anura
12	C	*Danio*	Zebrafish (*Danio rerio*)	4.426	199,646	22.17	22.17	Medium trophic magnitude

Rank[a]	MDR Group[b]	Genus	Species	Muscle SMCV[c] (µg THg/g ww)	BAF (L/kg ww)	Water SMCV (ng THg/ L)	Water GMCV (ng THg/ L)	BAF Source[d]
13	F	*Hexagenia*	Mayfly (*Hexagenia sp.*)	>3.516	128,414	27.38	>27.38	Crayfish
14	G	*Corbicula*	Asiatic clam (*Corbicula fluminea*)	>6.0	128,414	46.72	>46.72	Crayfish
15	B	*Orthodon*	Sacramento blackfish (*Orthodon microlepidotus*)	7.583	144,915	52.33	52.33	Low trophic magnitude
16	B	*Pogonichthys*	Sacramento splittail (*Pogonichthys macrolepidotus*)	>8.33	144,915	57.48	>57.48	Low trophic magnitude
17	B	*Acipenser*	Green sturgeon (*Acipenser medirostris*)	17.98	647,335	27.78	71.32	High trophic magnitude
			White sturgeon (*Acipenser transmontanus*)	36.56	199,646	183.1		Medium trophic magnitude
18	D	*Daphnia*	Cladoceran (*Daphnia magna)*	11.1	128,414	86.44	86.44	Crayfish

[a] Ranked from the most to least sensitive based on Genus Mean Chronic Value.
[b] MDR Groups identified by list provided in Section 2.6 above.
[c] From Table 3-7 above.
[d] From Table E-7 above.

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Table E-9. Freshwater Final Translated Water Column Chronic Value (Criterion Continuous Concentration) (Additional Approach 1).

80th centile (or maximum) species- and genus-specific fish BAFs, when available. Four lowest definitive translated water GMCVs.

Genus	*N*	Rank	GMCV	ln(GMCV)	ln(GMCV)^2	P=R/(N+1)	sqrt(P)
Sander	18	1	1.888	0.64	0.40	0.053	0.229
Pimephales		2	2.467	0.90	0.82	0.105	0.324
Procambarus		3	3.873	1.35	1.83	0.158	0.397
Lithobates		4	4.103	1.41	1.99	0.211	0.459
		Sum:		**4.30**	**5.05**	**0.53**	**1.41**
				S^2 =	14.04		
				L =	-0.250		
				A =	0.591		
				FCV =	**1.806**		

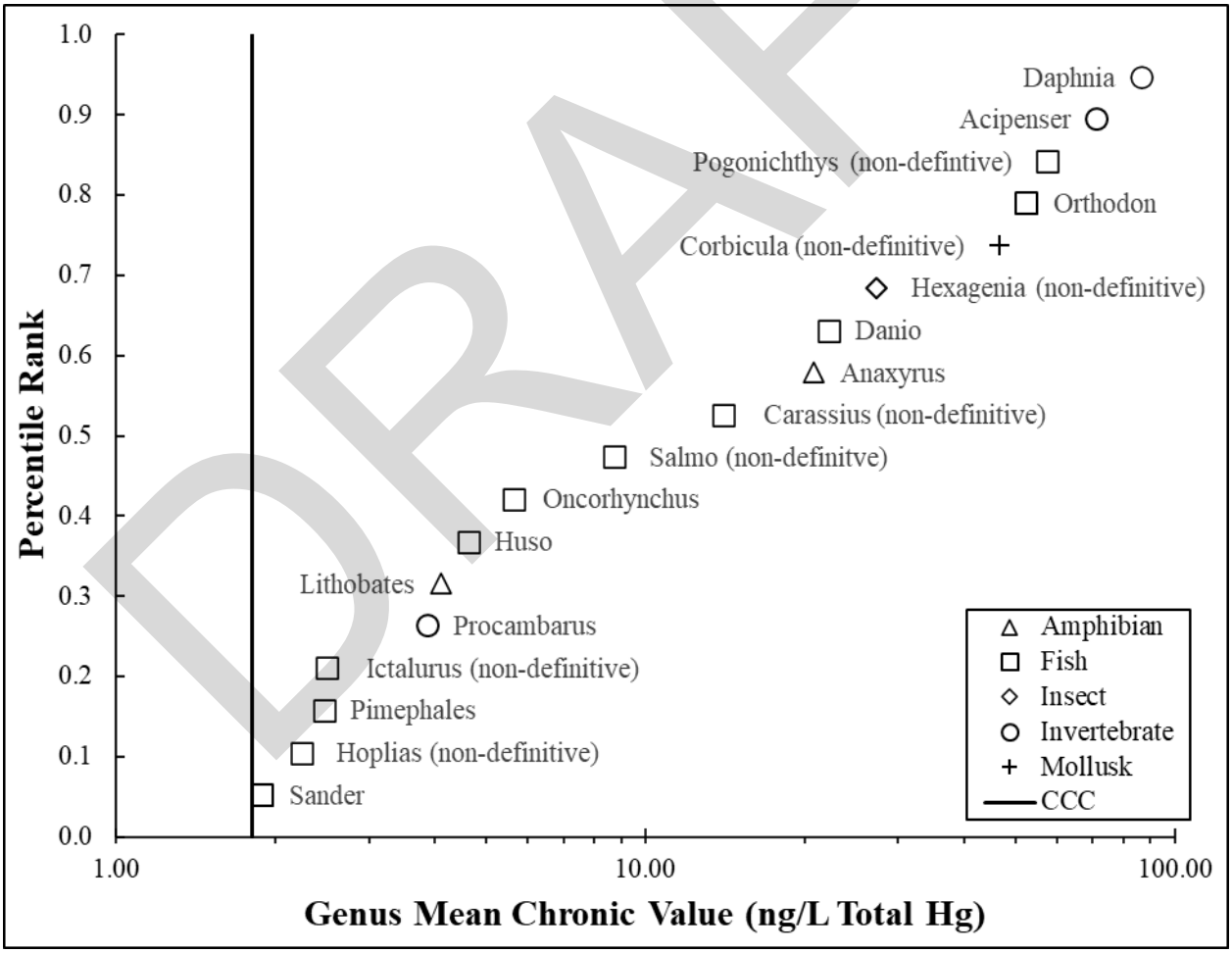

Figure E-3. Distribution of Mercury Water Column GMCVs (THg, ng/L) Translated from Measured Dietary Mercury Effect GMCVs Expressed as Muscle (THg, µg/g ww).

80th centile (or maximum) species- and genus-specific fish BAFs, when available (Additional Approach 1).

E.6 Translation of the Chronic Tissue Criterion Element to Water Column Criterion Element to integrate exposures to Largemouth Bass and across sites within a waterbody

In addition to the derivation of the mercury water column criterion (2.1 ng/L) presented in **Section 3.6**, EPA explored seven additional approaches that focused on protecting specific Idaho fish taxa. The first additional approach was described in **Section E.5**. The two approaches described in this section examine the effects of recalculating BAFs in a waterbody with variable water THg concentrations, in order to characterize exposures to certain fish species based on water measurements throughout the waterbody, rather than at a single site.

The water column criterion concentration (**Section 3.6**) was calculated using a fish BAF dataset where BAFs were calculated based on temporally paired fish tissue and water concentrations collected at the same location (site) within a waterbody. However, fish are mobile, and mercury concentrations can vary both spatially and temporally within a waterbody. The mercury tissue concentration within a fish, particularly larger fish species with larger home ranges (e.g., piscivores, salmonids) integrates the exposure history of a toxicant over its lifetime, and may not be adequately represented by a single water measurement at one site within a larger waterbody.

In the censored Idaho fish BAF dataset, water concentrations at different locations within a waterbody are relatively similar, with the exception of the Coeur d'Alene River, which has one downstream site (confluence with lake Coeur d'Alene) with a THg concentration of 6.21 ng/L, and two upstream sites with THg concentrations of 0.25 ng/L and 0.39 ng/L, respectively. Two fish species, largemouth bass (*Micropterus salmoides*) and black crappie (*Pomoxis nigricans*), were sampled at the site with a water THg concentration of 6.21 ng/L, and this is the only site

where a BAF for either of these species was available. All samples were collected by Essig (2010) in 2008.

In the translation approaches previously described in the Effects Analysis (**Section 3.6**), the largemouth bass and black crappie BAFs were calculated as the tissue concentration divided by the paired water concentration at the lake confluence site, resulting in relatively small BAFs compared to expected BAFs based on the trophic ecology and ambient tissue concentrations of these two species (0.572 and 0.280 mg/kg ww respectively). In the approaches described below, the possibility that the tissue concentrations in largemouth bass and black crappie from the Coeur d'Alene River reflect THg water concentrations for the entire river are examined by calculating those BAFs using the geometric mean of the three water concentrations sampled within that waterbody (0.85 ng/L). The resulting updated largemouth bass BAF is 676,131 L/kg, and the updated black crappie BAF is 330,973 L/kg.

Fish trophic magnitude categories were recalculated using these revised largemouth bass and black crappie BAFs. Both the medium and high trophic magnitude BAFs increased, because the updated largemouth bass and black crappie BAFs were both greater than the 80th centile of their respective trophic magnitude categories (**Table E-10**). All other BAFs used were the same as those used in **Section 3.6**. The two options considered here combine the BAFs calculated from the geometric mean Coeur d'Alene River THg concentration with taxon-specific fish BAFs based on medians and 80th centiles, respectively, to derive the corresponding FCVs.

E.6.1 Updated Trophic Magnitude Category BAFs with Median Taxa Specific BAFs (Additional Approach 2)

The BAFs used in the translation procedure are identical to those used in the translation described in **Section 3.6**, except for the higher medium and high trophic magnitude fish BAFs resulting from the larger largemouth bass and black crappie BAFs (**Table E-10**).

Table E-10. BAFs Used in the Tissue to Water Translation Procedure Including fish BAFs integrating multiple sites in a waterbody and median taxa-specific BAFs (Additional Approach 2).

Trophic Magnitude Category	Scientific Name	Median THg (ug/g ww)	BAF (L/kg muscle-ww)
Low		NA	144,915
Medium		NA	235,654
High		NA	683,105
	L. sylvaticus	NA	8,222
	Crayfish (sp.)	NA	128,414
	Walleye (*Sander vitreus*)	1.002	453,578 (median)
	Channel catfish (*Ictalurus punctatus*)	0.247	205,123 (median)
	Rainbow trout (*Oncorhynchus mykiss*)	0.132	161,685 (median)
	Brown trout (*Salmo trutta*) Surrogate for Atlantic salmon.	0.174	302,721 (median)

The FCV resulting from this option was 2.118 ng/L, which is identical to the corresponding water column criterion concentration of 2.118 ng/L described in **Section 3.6**. The relative rankings and translated GMCVs of the four most sensitive genera remained the same, because they were all translated from either taxon-specific BAFs, or in the case of Pimephales, the low trophic magnitude fish BAF, whereas the effect of the water THg averaging was an increase in the medium and high trophic magnitude BAFs. The translated value for Hoplias decreased from >2.240 ng/L to >2.123 ng/L, but it was not included in the FCV calculation because it was a low greater than value. Ranked translated GMCVs (**Table E-11**), FCV calculations (**Table E-12**), and plotted GSD (**Figure E-4**) for this approach are shown below.

Table E-11. Ranked Freshwater Genus Mean Chronic Values based on Muscle Concentrations Translated to Water Concentrations using Bioaccumulation Factors (Additional Approach 2).

Coeur d'Alene River largemouth bass and black crappie BAFs based on geometric mean water concentrations. Median species- and genus-specific fish BAFs, when available.

Rank[a]	MDR Group[b]	Genus	Species	Muscle SMCV[c] (µg THg/g ww)	BAF (L/kg ww)	Water SMCV (ng THg/ L)	Water GMCV (ng THg/ L)	BAF Source[c]
1	B	*Hoplias*	Tigerfish (*Hoplias malabaricus*)	>1.45	683,105	>2.123	>2.123	High trophic magnitude
2	B	*Sander*	Walleye (*Sander vitreus*)	1.069	453,578	2.357	2.357	*S. vitreus*
3	B	*Pimephales*	Fathead minnow (*Pimephales promelas*)	0.3575	144,915	2.467	2.467	Low trophic magnitude
4	E	*Procambarus*	Red swamp crayfish (*Procambarus clarkii)*	0.4973	128,414	3.873	3.873	Crayfish
5	C	*Lithobates*	Southern leopard frog (*Lithobates sphenocephala*)	0.03373	8,222	4.103	4.103	Anura
6	B	*Huso*	Beluga sturgeon (*Huso huso*)	3.0	683,105	4.392	4.392	High trophic magnitude
7	B	*Ictalurus*	Channel catfish (*Ictalurus punctatus*)	>1.6	205,123	>7.800	>7.800	*I. punctatus*
8	A	*Salmo*	Atlantic Salmon (*Salmo salar*)	>3.07	302,721	>10.14	>10.14	Salmo
9	B	*Carassius*	Goldfish (*Carassius auratus*)	>2.037	144,915	>14.06	>14.06	Low trophic magnitude
10	C	*Danio*	Zebrafish (*Danio rerio*)	4.426	235,654	18.78	18.78	Medium trophic magnitude
11	C	*Anaxyrus*	American toad (*Anaxyrus americanus*)	0.1704	8,222	20.73	20.73	Anura
12	A	*Oncorhynchus*	Rainbow trout (*Oncorhynchus mykiss*)	4.392	161,685	27.16	27.16	*O. mykiss*

Rank[a]	MDR Group[b]	Genus	Species	Muscle SMCV[c] (µg THg/g ww)	BAF (L/kg ww)	Water SMCV (ng THg/ L)	Water GMCV (ng THg/ L)	BAF Source[c]
13	F	*Hexagenia*	Mayfly (*Hexagenia sp.*)	>3.516	128,414	>27.38	>27.38	Crayfish
14	G	*Corbicula*	Asiatic clam (*Corbicula fluminea*)	>6.0	128,414	>46.72	>46.72	Crayfish
15	B	*Orthodon*	Sacramento blackfish (*Orthodon microlepidotus*)	7.583	144,915	52.33	52.33	Low trophic magnitude
16	B	*Pogonichthys*	Sacramento splittail (*Pogonichthys macrolepidotus*)	>8.33	144,915	>57.48	>57.48	Low trophic magnitude
17	B	*Acipenser*	Green sturgeon (*Acipenser medirostris*)	17.98	683,105	26.32	63.90	High trophic magnitude
			White sturgeon (*Acipenser transmontanus*)	36.56	235,654	155.1		Medium trophic magnitude
18	D	*Daphnia*	Cladoceran (*Daphnia magna)*	11.1	128,414	86.44	86.44	Crayfish

[a] Ranked from the most to least sensitive based on Genus Mean Chronic Value.
[b] MDR Groups identified by list provided in Section 2.6 above.
[c] From Table 3-7 above.
[d] From Table E-10 above.

Table E-12. Freshwater Final Translated Water Column Chronic Value (Additional Approach 2).

Coeur d'Alene River largemouth bass and black crappie BAFs based on geometric mean water concentrations. Median species- and genus-specific fish BAFs, when available.

Genus	*N*	Rank	GMCV	ln(GMCV)	ln(GMCV)^2	P=R/(N+1)	sqrt(P)
Sander	18	1	2.357	0.86	0.73	0.053	0.229
Pimephales		2	2.467	0.90	0.82	0.105	0.324
Procambarus		3	3.873	1.35	1.83	0.158	0.397
Lithobates		4	4.103	1.41	1.99	0.211	0.459
		Sum:				**0.53**	**1.41**
				S^2 =	8.73		
				L =	0.090		
				A =	0.751		
				FCV =	**2.118**		

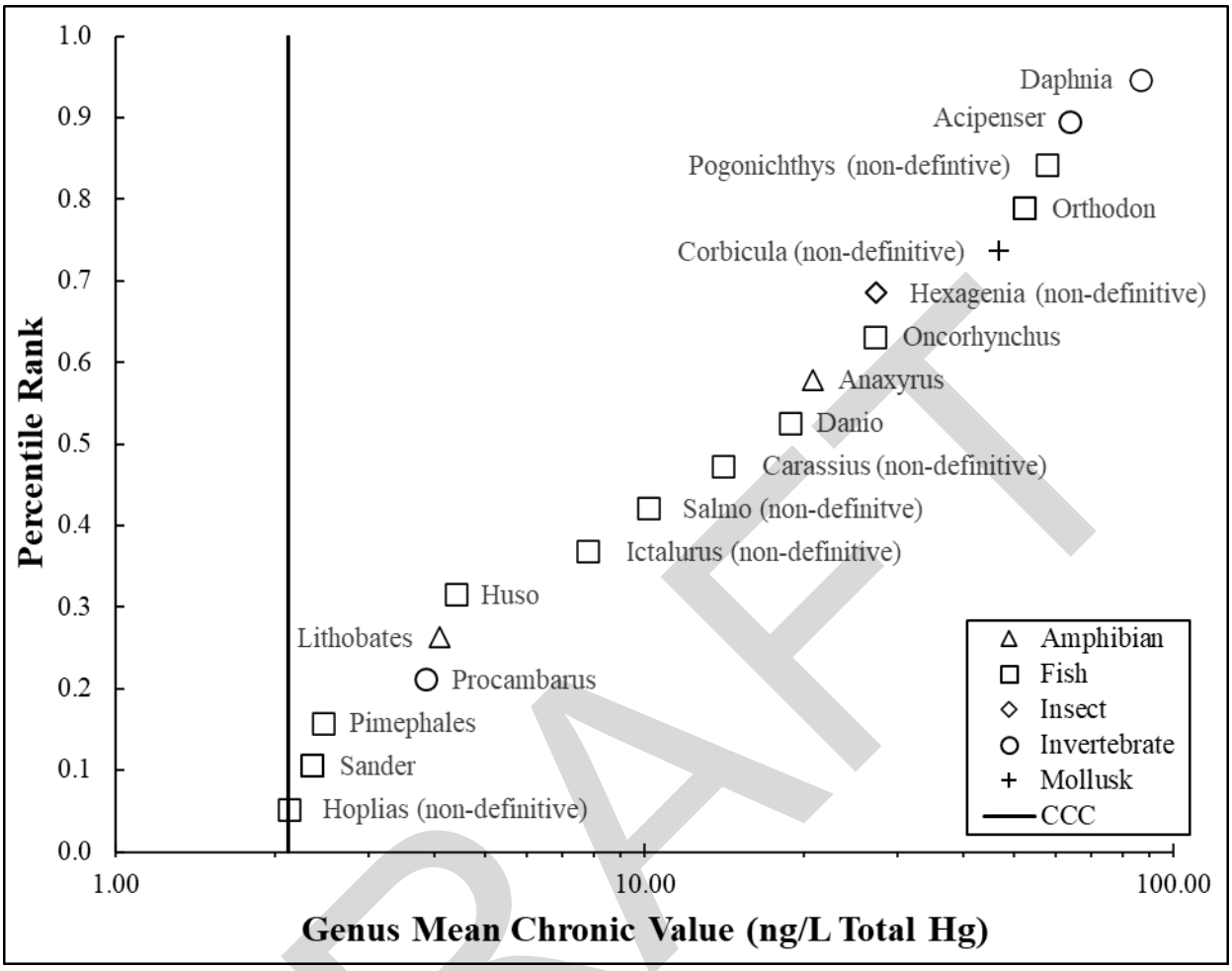

Figure E-4. Distribution of mercury water column GMCVs (THg, ng/L) translated from measured dietary mercury effect GMCVs expressed as Muscle (THg, µg/g ww) (Additional Approach 2).

Coeur d'Alene River largemouth bass and black crappie BAFs based on geometric mean water concentrations. Median species- and genus-specific fish BAFs, when available.

E.6.2 Updated Trophic Magnitude Category BAFs with 80th Centile Taxa Specific BAFs (Additional Approach 3)

The BAFs used in the translation procedure are identical to those used in the approach described in **Section E.5**, except for the higher medium and high trophic magnitude fish BAFs resulting from the larger largemouth bass and black crappie BAFs (**Table E-13**).

Table E-13. BAFs Used in the Tissue to Water Translation Procedure Including Sites with High Water THg (Additional Approach 3).

Trophic Magnitude Category	Scientific Name	Median THg (ug/g ww)	BAF (L/kg muscle-ww)
Low		NA	144,915
Medium		NA	235,654
High		NA	683,105
	L. sylvaticus	NA	8,222
	Crayfish (sp.)	NA	128,414
	Walleye (*Sander vitreus*)	1.002	566,123 (maximum)
	Channel catfish (*Ictalurus punctatus*)	0.247	640,456 (80th centile)
	Rainbow trout (*Oncorhynchus mykiss*)	0.132	778,638 (80th centile)
	Brown trout (*Salmo trutta*) Surrogate for Atlantic salmon	0.174	351,389 (maximum)

The final chronic value resulting from this option was 1.806 ng/L, which is identical to the corresponding water column criterion concentration of 1.838 ng/L described in **Section E.5**. As with the scenario described in **Section E.5**, the lower FCV is the result of a larger translated GMCV for Sander. The GMCV for Ictalurus decreases, but it is a non-definitive value and is not included in the FCV calculation. The second-fourth lowest definitive GMCVs use BAFs that are not affected by the Coeur d'Alene River THg averaging or the higher taxon-specific centiles. Ranked translated GMCVs (**Table E-14**), FCV calculations (**Table E-15**), and plotted GSD (**Figure E-5**) for this approach are shown below.

Table E-14. Ranked Freshwater Genus Mean Chronic Values based on Muscle Concentrations Translated to Water Concentrations using Bioaccumulation Factors (Additional Approach 3).

Coeur d'Alene River largemouth bass and black crappie BAFs based on geometric mean water concentrations. 80th centile (or maximum) species- and genus-specific fish BAFs, when available.

Rank[a]	MDR Group[b]	Genus	Species	Muscle SMCV[c] (µg THg/g ww)	BAF (L/kg ww)	Water SMCV (ng THg/ L)	Water GMCV (ng THg/ L)	BAF Source[d]
1	B	*Sander*	Walleye (*Sander vitreus*)	1.069	566,123	1.888	1.888	*S. vitreus*
2	B	*Hoplias*	Tigerfish (*Hoplias malabaricus*)	>1.45	683,105	>2.123	>2.123	High trophic magnitude
3	B	*Pimephales*	Fathead minnow (*Pimephales promelas*)	0.3575	144,915	2.467	2.467	Low trophic magnitude
4	B	*Ictalurus*	Channel catfish (*Ictalurus punctatus*)	>1.6	640,456	>2.498	>2.498	*I. punctatus*
5	E	*Procambarus*	Red swamp crayfish (*Procambarus clarkii)*	0.4973	128,414	3.873	3.873	Crayfish
6	C	*Lithobates*	Southern leopard frog (*Lithobates sphenocephala*)	0.03373	8,222	4.103	4.103	Anura
7	B	*Huso*	Beluga sturgeon (*Huso huso*)	3.0	683,105	4.392	4.392	High trophic magnitude
8	A	*Oncorhynchus*	Rainbow trout (*Oncorhynchus mykiss*)	4.392	351,389	5.641	5.641	*O. mykiss*
9	A	*Salmo*	Atlantic Salmon (*Salmo salar*)	>3.07	351,389	>8.737	>8.737	Salmo
10	B	*Carassius*	Goldfish (*Carassius auratus*)	>2.037	144,915	>14.06	>14.06	Low trophic magnitude
11	C	*Danio*	Zebrafish (*Danio rerio*)	4.426	235,654	18.78	18.78	Medium trophic magnitude
12	C	*Anaxyrus*	American toad (*Anaxyrus americanus*)	0.1704	8,222	20.73	20.73	Anura

Rank[a]	MDR Group[b]	Genus	Species	Muscle SMCV[c] (µg THg/g ww)	BAF (L/kg ww)	Water SMCV (ng THg/ L)	Water GMCV (ng THg/ L)	BAF Source[d]
13	F	*Hexagenia*	Mayfly (*Hexagenia sp.*)	>3.516	128,414	>27.38	>27.38	Crayfish
14	G	*Corbicula*	Asiatic clam (*Corbicula fluminea*)	>6.0	128,414	>46.72	>46.72	Crayfish
15	B	*Orthodon*	Sacramento blackfish (*Orthodon microlepidotus*)	7.583	144,915	52.33	52.33	Low trophic magnitude
16	B	*Pogonichthys*	Sacramento splittail (*Pogonichthys macrolepidotus*)	>8.33	144,915	>57.48	>57.48	Low trophic magnitude
17	B	*Acipenser*	Green sturgeon (*Acipenser medirostris*)	17.98	683,105	26.32	63.90	High trophic magnitude
			White sturgeon (*Acipenser transmontanus*)	36.56	235,654	155.1		Medium trophic magnitude
18	D	*Daphnia*	Cladoceran (*Daphnia magna)*	11.1	128,414	86.44	86.44	Crayfish

[a] Ranked from the most to least sensitive based on Genus Mean Chronic Value.
[b] MDR Groups identified by list provided in Section 2.6 above.
[c] From Table 3-7 above.
[d] From Table E-13 above.
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Table E-15. Freshwater Final Translated Water Column Chronic Value (Additional Approach 3).

Coeur d'Alene River largemouth bass and black crappie BAFs based on geometric mean water concentrations. 80th centile (or maximum) species- and genus-specific fish BAFs, when available.

Genus	***N***	**Rank**	**GMCV**	**ln(GMCV)**	**ln(GMCV)^2**	**P=R/(N+1)**	**sqrt(P)**
Sander	18	1	1.888	0.64	0.40	0.053	0.229
Pimephales		2	2.467	0.90	0.82	0.105	0.324
Procambarus		3	3.873	1.35	1.83	0.158	0.397
Lithobates		4	4.103	1.41	1.99	0.211	0.459
		Sum:		**4.30**	**5.05**	**0.53**	**1.41**
			S^2 =	14.04			
			L =	-0.250			
			A =	0.591			
			FCV =	**1.806**			

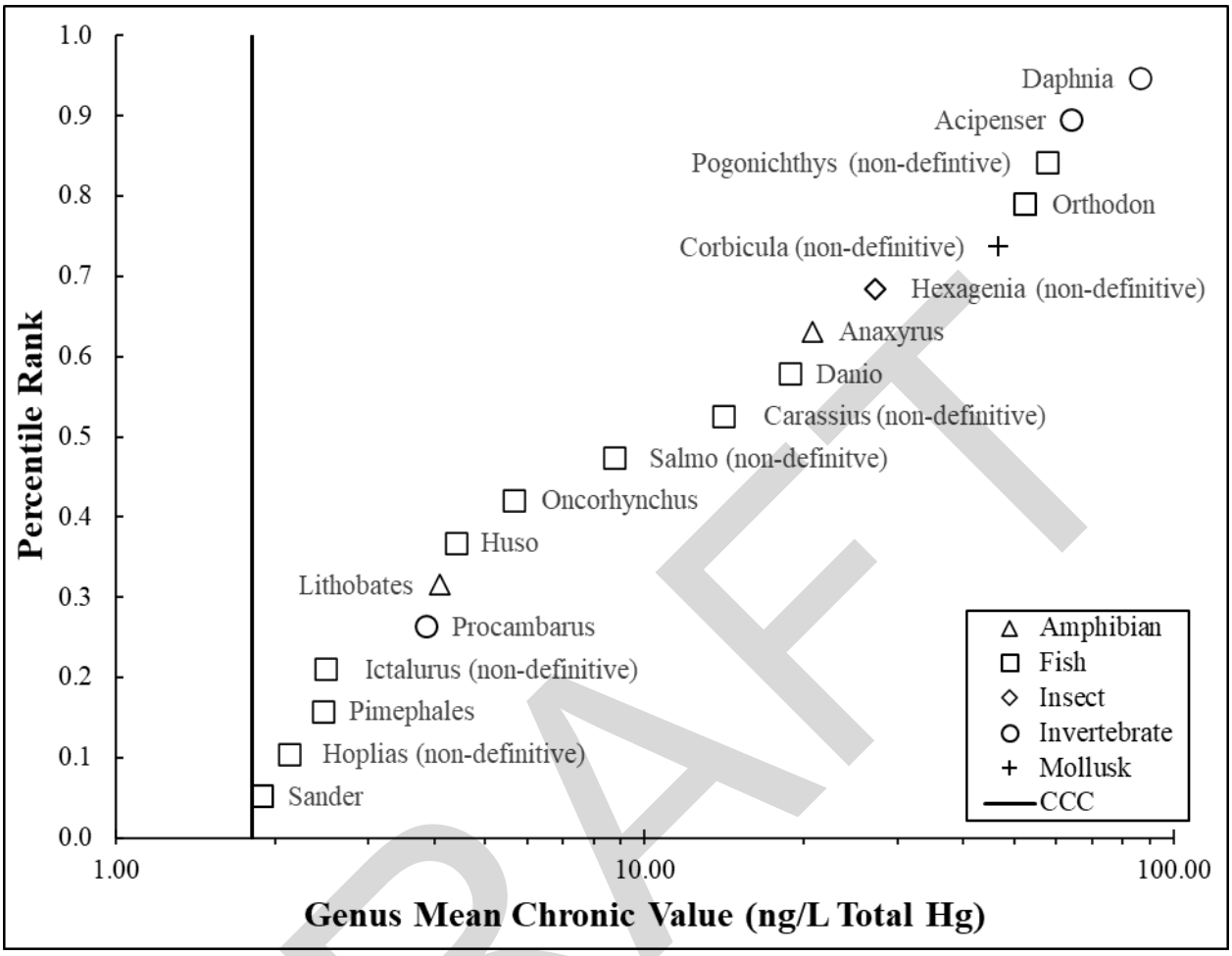

Figure E-5. Distribution of Mercury Water Column GMCVs (THg, ng/L) Translated from Measured Dietary Mercury Effect GMCVs Expressed as Muscle (THg, µg/g ww).
Coeur d'Alene River largemouth bass and black crappie BAFs based on geometric mean water concentrations. 80th centile (or maximum) species- and genus-specific fish BAFs, when available.

E.7 Ecoregional Water Concentration Translation Scenarios

The BAFs calculated using ecoregional water concentrations were used to examine four additional tissues to water translation scenarios. The four alternate approaches described below parallel the approach used to derive the tissue based criteria, the approach described in **Section E.5**, and the two approaches described in **Section E.6**, with the only difference being that the fish species BAFs, and subsequent fish trophic magnitude category BAFs, were based on geometric mean ecoregional water THg concentrations, rather than water THg concentrations collected at the same site as the paired tissue concentration.

Fish BAFs were calculated using ecoregional water concentrations following the same approach as for BAFs calculated using paired water and tissue data (see **Section 3.1.1**). A total of 474 BAFs was calculated and then reduced to 390 BAFs after censoring high THg sites. The 390 BAFs were reduced to 119 BAFs representing every unique fish species by location by year combination (**Table E-16**), which were further reduced to 101 "location by species" BAFs, which were used to calculate a set of 30 fish species BAFs based on ecoregional water concentrations (**Table E-17**). The 30 fish species were grouped into trophic magnitude categories as previously described (see **Section 3.6.1**), and low-, medium-, and high- trophic level BAFs were calculated as the 80th centile fish species BAF within each category, or as the maximum (75th centile) for the low trophic magnitude category, which only had three species. As before, these trophic magnitude category BAFs were used as surrogate BAFs for fish species in the tissue dataset for which a species- or genus-level BAF was not available.

Although the approaches described here were worth examining, for reasons described in **Section E.4**, FCV water concentrations translated based on ecoregional water concentration averages were not used for the final criterion, as uncertainties associated with paired water concentrations were less pronounced than those associated with ecoregional average water

concentrations. This is particularly evident for fish tissue collected in waters with THg concentrations that were notably different from corresponding ecoregional averages. However, results of this analysis are included here to illustrate the results of the ecoregional water concentration methodology.

Table E-16. Fish Muscle THg BAFs (L/kg) for all unique species by location by year combinations.

Tissue concentrations represent either a single individual, or when more than one individual fish tissue sample for the same species during the same year was available, the average tissue concentration. Water concentrations are the geometric mean of all water concentrations within the respective level III ecoregions.

Waterbody Name	Site	Year	Latitude	Longitude	Waterbody Type	Fish Length (mm)[a]	Fish Weight (g)[a]	Fish Common Name	TL[b]	Trophic Magnitude Category	Muscle THg (mg/kg-ww)	Level III Ecoregion	Water THg (ng/L)	THg BAF (L/kg)
Bear River	Bear River	2008	42.36	-111.74	River	570	2370	Common carp	3	medium	0.252	80	1.32	190,909
Big Wood River	Big Wood River, U	2008	43.78	-114.54	River	280	239	Rainbow trout	3	medium	0.029	16	0.64	45,313
Big Wood River	Big Wood River, L	2008	43.43	-114.26	River	330	295	Rainbow trout	3	medium	0.044	12	0.95	46,316
Big Wood River	Big Wood River, L	2008	43.43	-114.26	River	360	500	Brown trout	4	high	0.094	12	0.95	98,947
Blackfoot River	Blackfoot R	2008	43.21	-112.20	River	440	1050	Utah sucker	2	low	0.032	12	0.95	33,684
Blackfoot River	Blackfoot R-2	2008	42.80	-111.49	River	44	970	Bridgelip sucker	2	low	0.086	17	0.65	131,538
Blackfoot River	Blackfoot R-2	2008	42.80	-111.49	River	300	250	Cutthroat trout	3	medium	0.056	17	0.65	86,154
Boise River	Boise River NR Twin Springs	2008	43.67	-115.73	River	NA	NA	Mountain whitefish	3	medium	0.405	16	0.64	632,813
Boise River	Boise River at Eckert Rd near Boise	2013	43.57	-116.13	River	393	634	Mountain whitefish	3	medium	0.185	12	0.95	194,737
Boise River	Boise River at Eckert Rd near Boise	2017	43.57	-116.13	River	369	496	Mountain whitefish	3	medium	0.119	12	0.95	125,263
Boise River	Boise River at Eckert Rd near Boise	2015	43.57	-116.13	River	291	221	Rainbow trout	3	medium	0.022	12	0.95	23,158
Boise River	Boise River at Glenwood Bridge Near Boise	2008	43.66	-116.28	River	NA	NA	Mountain whitefish	3	medium	0.199	12	0.95	209,474
Boise River	Boise River near Middleton	2013	43.68	-116.57	River	306	266	Mountain whitefish	3	medium	0.175	12	0.95	184,211
Boise River	Boise River near Middleton	2014	43.68	-116.57	River	263	269	Mountain whitefish	3	medium	0.173	12	0.95	182,105
Boise River	Boise River near Middleton	2015	43.68	-116.57	River	297	263	Mountain whitefish	3	medium	0.113	12	0.95	118,947
Boise River	Boise River near Middleton	2016	43.68	-116.57	River	329	353	Mountain whitefish	3	medium	0.133	12	0.95	140,000
Boise River	Boise River near Middleton	2017	43.68	-116.57	River	297	229	Mountain whitefish	3	medium	0.221	12	0.95	232,632

Waterbody Name	Site	Year	Latitude	Longitude	Waterbody Type	Fish Length (mm)[a]	Fish Weight (g)[a]	Fish Common Name	TL[b]	Trophic Magnitude Category	Muscle THg (mg/kg-ww)	Level III Ecoregion	Water THg (ng/L)	THg BAF (L/kg)
Boise River	Boise River near Parma	2013	43.82	-117.02	River	594	2184	Channel catfish	3	medium	0.326	12	0.95	343,158
Boise River	Boise River near Parma	2015	43.82	-117.02	River	625	3033	Channel catfish	3	medium	0.225	12	0.95	236,842
Boise River	Boise River near Parma	2017	43.82	-117.02	River	230	158	Smallmouth bass	4	high	0.223	12	0.95	234,737
Camas Creek	Camas Creek #2	2008	44.82	-114.49	River	310	296	Mountain whitefish	3	medium	0.061	16	0.64	95,313
Cane Creek	Cane Creek	2016	44.95	-115.29	River	176	57	Bull trout	3	medium	0.051	16	0.64	79,063
Cane Creek	Cane Creek	2016	44.95	-115.29	River	0	4	Sculpin	3	medium	0.040	16	0.64	63,125
Clearwater River	Clearwater River at Riverside	2006	46.49	116.30	River	NA	NA	Salmonidae sp.	3	medium	0.134	15	0.38	352,632
Coeur d'Alene River	Cd'A R-1	2008	47.48	-116.74	River	250	220	Black crappie	3	medium	0.280	15	0.38	736,842
Coeur d'Alene River	Cd'A R-1	2008	47.48	-116.74	River	500	1500	Largemouth bass	4	high	0.572	15	0.38	1,505,263
Henry's Fork River	Henry's Fork R	2008	43.80	-111.93	River	NA	NA	Mountain whitefish	3	medium	0.153	12	0.95	161,053
Henry's Fork River	Henry's Fork R	2008	43.80	-111.93	River	530	1600	Cutthroat trout	3	medium	0.275	12	0.95	289,474
Lemhi River	Lemhi Nr Lemhi	2008	44.94	-113.64	River	NA	NA	Mountain whitefish	3	medium	0.316	17	0.65	486,154
Lochsa River	Lochsa R	2008	46.93	-115.04	River	300	278	Cutthroat trout	3	medium	0.048	15	0.38	126,316
Lochsa River	Lochsa R	2008	46.93	-115.04	River	350	373	Mountain whitefish	3	medium	0.052	15	0.38	136,842
North Fork Big Lost River	NF Big Lost R	2008	43.93	-114.19	River	250	170	Small Brook trout	3	medium	0.064	16	0.64	100,000
North Fork Clearwater River	NF Clearwater R	2008	46.73	-115.29	River	340	380	Cutthroat trout	3	medium	0.066	15	0.38	173,684
North Fork Clearwater River	NF Clearwater R	2008	46.73	-115.29	River	320	278	Kokanee salmon	3	medium	0.113	15	0.38	297,368
North Fork Clearwater River	NF Clearwater R	2008	46.73	-115.29	River	350	406	Mountain whitefish	3	medium	0.085	15	0.38	223,684
North Fork Payette River	NF Payette R	2008	44.21	-116.11	River	380	500	Rainbow trout	3	medium	0.132	16	0.64	206,250
North Fork Payette River	NF Payette R	2008	44.21	-116.11	River	230	138	Yellow perch	3	medium	0.108	16	0.64	168,750

Waterbody Name	Site	Year	Latitude	Longitude	Waterbody Type	Fish Length (mm)[a]	Fish Weight (g)[a]	Fish Common Name	TL[b]	Trophic Magnitude Category	Muscle THg (mg/kg-ww)	Level III Ecoregion	Water THg (ng/L)	THg BAF (L/kg)
Pahsimeroi River	Pahsimeroi @ Ellis	2008	44.69	-114.05	River	NA	NA	Mountain whitefish	3	medium	0.250	17	0.65	383,846
Payette River	Payette R	2008	44.00	-116.80	River	550	1650	Bridgelip sucker	2	low	0.234	12	0.95	246,316
Payette River	Payette R	2008	44.00	-116.80	River	290	363	Smallmouth bass	4	high	0.123	12	0.95	129,474
Payette River	Payette R	2008	44.00	-116.80	River	510	1525	Largescale sucker	3	medium	0.186	12	0.95	195,789
Payette River	Payette R	2008	44.00	-116.80	River	320	250	Mountain whitefish	3	medium	0.050	12	0.95	52,632
Payette River	Payette R-2	2008	43.90	-116.63	River	540	1680	Largescale sucker	3	medium	0.276	12	0.95	290,526
Payette River	Payette R-2	2008	43.90	-116.63	River	280	231	Mountain whitefish	3	medium	0.041	12	0.95	43,158
Portneuf River	Portneuf R	2008	42.85	-112.44	River	380	518	Utah sucker	2	low	0.192	12	0.95	202,105
Portneuf River	Portneuf R--Croney Road Reach	2007	42.86	-112.06	River	362	NA	Rainbow trout	3	medium	0.332	80	1.32	251,684
Portneuf River	Portneuf R--Croney Road Reach	2007	42.86	-112.06	River	408	NA	Cutthroat trout	3	medium	0.675	80	1.32	511,364
Priest River	Priest R	2008	48.24	-116.88	River	410	705	Largescale sucker	3	medium	0.278	15	0.38	731,579
Priest River	Priest R	2008	48.24	-116.88	River	260	244	Smallmouth bass	4	high	0.156	15	0.38	410,526
Saint Joe River	Saint Joe R	2008	47.14	-115.41	River	255	172	Cutthroat trout	3	medium	0.044	15	0.38	114,474
Saint Joe River	Saint Joe R	2008	47.14	-115.41	River	320	318	Mountain whitefish	3	medium	0.040	15	0.38	105,263
Saint Joe River	Saint Joe R	2008	47.14	-115.41	River	430	728	Large Brook trout	4	high	0.174	15	0.38	457,895
Salmon Falls Creek Reservoir	Salmon Falls Creek Reservoir at Grey's Landing	2005	42.13	-114.73	Reservoir	457	NA	Walleye	4	high	0.753	80	1.32	570,455
Salmon Falls Creek Reservoir	Salmon Falls Creek Reservoir at Grey's Landing	2006	42.13	-114.73	Reservoir	442	NA	Walleye	4	high	1.250	80	1.32	946,970
Salmon Falls Creek Reservoir	Salmon Falls Creek Reservoir at Grey's Landing	2006	42.13	-114.73	Reservoir	495	NA	Largescale sucker	3	medium	0.489	80	1.32	370,455

Waterbody Name	Site	Year	Latitude	Longitude	Waterbody Type	Fish Length (mm)[a]	Fish Weight (g)[a]	Fish Common Name	TL[b]	Trophic Magnitude Category	Muscle THg (mg/kg-ww)	Level III Ecoregion	Water THg (ng/L)	THg BAF (L/kg)
Salmon Falls Creek Reservoir	Salmon Falls Creek Reservoir at Grey's Landing	2006	42.13	-114.73	Reservoir	355	NA	Rainbow trout	3	medium	0.357	80	1.32	270,455
Salmon Falls Creek Reservoir	Salmon Falls Creek Reservoir at Grey's Landing	2006	42.13	-114.73	Reservoir	339	NA	Smallmouth bass	4	high	1.020	80	1.32	772,727
Salmon Falls Creek Reservoir	Salmon Falls Creek Reservoir at Grey's Landing	2006	42.13	-114.73	Reservoir	264	NA	Yellow perch	3	medium	0.587	80	1.32	444,697
Salmon River	Salmon R-3	2008	45.41	-116.19	River	290	353	Smallmouth bass	4	high	0.380	16	0.64	593,750
Salmon River	Salmon R-2	2008	45.79	-116.32	River	330	400	Mountain whitefish	3	medium	0.142	11	1.30	109,231
Salmon River	Salmon R-2	2008	45.79	-116.32	River	300	300	Smallmouth bass	4	high	0.548	11	1.30	421,538
Salmon River	Salmon R-1	2008	45.46	-115.77	River	320	300	Mountain whitefish	3	medium	0.097	16	0.64	151,563
Salmon River	Salmon R-1	2008	45.46	-115.77	River	330	299	Large Northern pikeminnow	4	high	0.674	16	0.64	1,053,125
Salmon River	Salmon R-1	2008	45.46	-115.77	River	270	300	Smallmouth bass	4	high	0.253	16	0.64	395,313
Selway River	Selway R	2008	46.05	-115.30	River	320	232	Cutthroat trout	3	medium	0.053	16	0.64	82,813
Selway River	Selway R	2008	46.05	-115.30	River	310	267	Mountain whitefish	3	medium	0.083	16	0.64	129,688
Selway River	Selway R	2008	46.05	-115.30	River	400	500	Large Brook trout	4	high	0.153	16	0.64	239,063
Snake River	Snake R-2	2008	43.61	-116.91	River	610	4040	Common carp	3	medium	0.138	12	0.95	145,263
Snake River	Snake R-2	2008	43.61	-116.91	River	330	550	Smallmouth bass	4	high	0.088	12	0.95	92,632
Snake River	Snake R-1	2008	43.01	-116.13	River	550	1870	Largescale sucker	3	medium	0.198	12	0.95	208,421
Snake River	Snake R-1	2008	43.01	-116.13	River	350	665	Smallmouth bass	4	high	0.200	12	0.95	210,526
Snake River	Snake R-3	2008	42.64	-114.56	River	450	1025	Largescale sucker	3	medium	0.190	12	0.95	200,000
Snake River	Snake R-3	2008	42.64	-114.56	River	400	1000	Smallmouth bass	4	high	0.318	12	0.95	334,737
Snake River	Snake River near Murphy	2013	43.29	-116.42	River	631	2613	Channel catfish	3	medium	0.206	12	0.95	216,842

Waterbody Name	Site	Year	Latitude	Longitude	Waterbody Type	Fish Length (mm)[a]	Fish Weight (g)[a]	Fish Common Name	TL[b]	Trophic Magnitude Category	Muscle THg (mg/kg-ww)	Level III Ecoregion	Water THg (ng/L)	THg BAF (L/kg)
Snake River	Snake River near Murphy	2015	43.29	-116.42	River	625	2970	Channel catfish	3	medium	0.163	12	0.95	171,579
Snake River	Snake River near Murphy	2017	43.29	-116.42	River	592	2266	Channel catfish	3	medium	0.108	12	0.95	113,684
Snake River	Snake River near Murphy	2013	43.29	-116.42	River	344	639	Smallmouth bass	4	high	0.173	12	0.95	182,105
Snake River	Snake River near Murphy	2015	43.29	-116.42	River	328	501	Smallmouth bass	4	high	0.164	12	0.95	172,632
Snake River	Snake River near Murphy	2017	43.29	-116.42	River	348	648	Smallmouth bass	4	high	0.192	12	0.95	202,105
Snake River	Snake River at Nyssa	2013	43.88	-116.98	River	599	1978	Channel catfish	3	medium	0.143	12	0.95	150,526
Snake River	Snake River at Nyssa	2015	43.88	-116.98	River	590	2303	Channel catfish	3	medium	0.127	12	0.95	133,684
Snake River	Snake River at Nyssa	2017	43.88	-116.98	River	608	2419	Channel catfish	3	medium	0.141	12	0.95	148,421
Brownlee Reservoir	Brownlee Reservoir at Burnt River	2013	44.37	-117.23	Reservoir	370	792	Smallmouth bass	4	high	0.324	11	1.30	249,231
Brownlee Reservoir	Brownlee Reservoir at Burnt River	2017	44.37	-117.23	Reservoir	341	668	Smallmouth bass	4	high	0.227	11	1.30	174,615
Brownlee Reservoir	Brownlee Reservoir at Burnt River	2015	44.37	-117.23	Reservoir	637	3140	Channel catfish	3	medium	0.219	11	1.30	168,462
Brownlee Reservoir	Brownlee Reservoir at multiple locations	2015	44.80	-116.93	Reservoir	194	NA	Smallmouth bass	4	high	0.189	11	1.30	145,662
Brownlee Reservoir	Brownlee Reservoir at multiple locations	2015	44.80	-116.93	Reservoir	177	NA	Smallmouth bass	4	high	0.171	11	1.30	131,737
Brownlee Reservoir	Brownlee Reservoir at multiple locations	2018	44.80	-116.93	Reservoir	185	NA	Smallmouth bass	4	high	0.217	11	1.30	166,579
Brownlee Reservoir	Brownlee Reservoir at multiple locations	2017	44.80	-116.93	Reservoir	484	NA	Channel catfish	3	medium	0.296	11	1.30	227,774
Brownlee Reservoir	Brownlee Reservoir at multiple locations	2017	44.80	-116.93	Reservoir	244	NA	Crappie sp.	3	medium	0.214	11	1.30	164,978

Waterbody Name	Site	Year	Latitude	Longitude	Waterbody Type	Fish Length (mm)[a]	Fish Weight (g)[a]	Fish Common Name	TL[b]	Trophic Magnitude Category	Muscle THg (mg/kg-ww)	Level III Ecoregion	Water THg (ng/L)	THg BAF (L/kg)
Brownlee Reservoir	Brownlee Reservoir at multiple locations	2017	44.80	-116.93	Reservoir	537	NA	Flathead catfish	3	medium	0.477	11	1.30	366,555
Brownlee Reservoir	Brownlee Reservoir at multiple locations	2017	44.80	-116.93	Reservoir	306	NA	Largescale sucker	3	medium	0.083	11	1.30	63,619
Brownlee Reservoir	Brownlee Reservoir at multiple locations	2017	44.80	-116.93	Reservoir	228	NA	Small Northern pikeminnow	3	medium	0.205	11	1.30	157,932
Brownlee Reservoir	Brownlee Reservoir at multiple locations	2017	44.80	-116.93	Reservoir	209	NA	Sucker sp.	2	low	0.066	11	1.30	50,664
Brownlee Reservoir	Brownlee Reservoir at multiple locations	2017	44.80	-116.93	Reservoir	226	NA	Yellow perch	3	medium	0.202	11	1.30	155,053
Brownlee Reservoir	Brownlee Reservoir at multiple locations	2017	44.80	-116.93	Reservoir	57	NA	Banded killifish	3	medium	0.075	11	1.30	57,372
Brownlee Reservoir	Brownlee Reservoir at multiple locations	2017	44.80	-116.93	Reservoir	117	NA	Bluegill	3	medium	0.181	11	1.30	139,525
Brownlee Reservoir	Brownlee Reservoir at multiple locations	2018	44.80	-116.93	Reservoir	101	NA	Bluegill	3	medium	0.165	11	1.30	127,056
Brownlee Reservoir	Brownlee Reservoir at multiple locations	2017	44.80	-116.93	Reservoir	138	NA	Pumpkinseed	3	medium	0.167	11	1.30	128,643
Hells Canyon Reservoir	Hells Canyon Reservoir	2015	45.24	-116.70	Reservoir	191	NA	Smallmouth bass	4	high	0.251	11	1.30	193,150
Hells Canyon Reservoir	Hells Canyon Reservoir	2017	45.24	-116.70	Reservoir	157	NA	Smallmouth bass	4	high	0.262	11	1.30	204,415
Hells Canyon Reservoir	Hells Canyon Reservoir	2017	45.24	-116.70	Reservoir	235	NA	Bridgelip sucker	2	low	0.040	11	1.30	30,672
Hells Canyon Reservoir	Hells Canyon Reservoir	2017	45.24	-116.70	Reservoir	310	NA	Channel catfish	3	medium	0.738	11	1.30	567,355
Hells Canyon Reservoir	Hells Canyon Reservoir	2017	45.24	-116.70	Reservoir	183	NA	Crappie sp.	3	medium	0.203	11	1.30	156,262

Waterbody Name	Site	Year	Latitude	Longitude	Waterbody Type	Fish Length (mm)[a]	Fish Weight (g)[a]	Fish Common Name	TL[b]	Trophic Magnitude Category	Muscle THg (mg/kg-ww)	Level III Ecoregion	Water THg (ng/L)	THg BAF (L/kg)
Hells Canyon Reservoir	Hells Canyon Reservoir	2017	45.24	-116.70	Reservoir	257	NA	Largescale sucker	3	medium	0.096	11	1.30	74,140
Hells Canyon Reservoir	Hells Canyon Reservoir	2017	45.24	-116.70	Reservoir	207	NA	Yellow perch	3	medium	0.249	11	1.30	191,843
Hells Canyon Reservoir	Hells Canyon Reservoir	2017	45.24	-116.70	Reservoir	52	NA	Banded killifish	3	medium	0.066	11	1.30	50,618
Hells Canyon Reservoir	Hells Canyon Reservoir	2017	45.24	-116.70	Reservoir	82	NA	Bluegill	3	medium	0.147	11	1.30	113,287
Hells Canyon Reservoir	Hells Canyon Reservoir	2017	45.24	-116.70	Reservoir	83	NA	Small Northern pikeminnow	3	medium	0.067	11	1.30	51,253
Hells Canyon Reservoir	Hells Canyon Reservoir	2017	45.24	-116.70	Reservoir	104	NA	Pumpkinseed	3	medium	0.089	11	1.30	68,159
Hells Canyon Reservoir	Hells Canyon Reservoir	2017	45.24	-116.70	Reservoir	98	NA	Warmouth	3	medium	0.128	11	1.30	98,590
Oxbow Reservoir	Oxbow Reservoir	2015	44.97	-116.84	Reservoir	207	NA	Smallmouth bass	4	high	0.288	11	1.30	221,755
South Fork Payette River	SF Payette R - SF Snake R	2008	43.44	-111.36	River	380	588	Cutthroat trout	3	medium	0.081	17	0.65	123,846
South Fork Payette River	SF Payette R - SF Snake R	2008	43.44	-111.36	River	360	396	Mountain whitefish	3	medium	0.090	17	0.65	138,462
South Fork Payette River	SF Payette R - SF Snake R	2008	43.44	-111.36	River	450	875	Brown trout	4	high	0.253	17	0.65	389,231
South Fork Payette River	SF Payette R - SF Snake R	2008	43.44	-111.36	River	460	700	Cutthroat trout x Rainbow trout	3	medium	0.240	17	0.65	369,231
South Fork Payette River	SF Payette R - SF Snake R	2008	43.44	-111.36	River	420	550	Rainbow trout	3	medium	0.175	17	0.65	269,231
Sugar Creek	Sugar Creek--Upstream	2016	44.95	-115.29	River	199	76	Bull trout	3	medium	0.080	16	0.64	124,219
Sugar Creek	Sugar Creek--Upstream	2016	44.95	-115.29	River	NA	4	Sculpin	3	medium	0.071	16	0.64	110,795

[a] Average species length and/or weight for all samples at that site where length and weight were reported.
[b] As reported in Essig (2010). See Section E.1 for additional details.

Table E-17. Fish species BAFs used in the tissue to water translation procedure.
Water concentrations were geometric mean THg concentrations from Level III ecoregions.

Fish Common Name	Trophic Magnitude Category	Median THg (ug/g ww)	THg BAF (L/kg)
Banded killifish	Medium	0.070	53,995
Black crappie	Medium	0.280	736,842
Bluegill	Medium	0.160	123,289
Bridgelip sucker	Low	0.086	131,538
Small brook trout	Medium	0.064	100,000
Large brook trout	High	0.164	348,479
Brown trout	High	0.174	244,089
Bull trout	High	0.065	101,641
Channel catfish	Medium	0.247	199,676
Common carp	Medium	0.195	168,086
Crappie sp.	Medium	0.209	160,620
Cutthroat trout	Medium	0.061	125,081
Cutthroat trout x Rainbow trout	Medium	0.240	369,231
Flathead catfish	Medium	0.477	366,555
Kokanee salmon	Medium	0.113	297,368
Largemouth bass	High	0.572	1,505,263
Largescale sucker	Medium	0.194	204,211
Mountain whitefish	Medium	0.097	151,563
Small northern pikeminnow	Medium	0.136	104,592
Large northern pikeminnow	High	0.674	1,053,125
Pumpkinseed	Medium	0.128	98,401
Rainbow trout	Medium	0.132	206,250
Salmonidae sp.	Medium	0.134	352,632
Sculpin	Medium	0.056	86,960
Smallmouth bass	High	0.253	221,755
Sucker sp.	Low	0.066	50,664
Utah sucker	Low	0.112	117,895
Walleye	High	1.002	758,712
Warmouth	Medium	0.128	98,590
Yellow perch	Medium	0.225	180,296

E.7.1 Scenario 1: Ecoregional water THg concentrations, fish taxa-specific BAFs based on medians.

The BAFs used in this scenario (**Table E-18**) are identical to those used in the translation described in **Section 3.6.1**, except the fish species and trophic magnitude category BAFs were based on ecoregional water concentrations (**Section E.4**). The low- and medium trophic magnitude fish trophic level BAFs were similar to the original approach, but the high trophic magnitude BAF is 2.05 times larger than the high trophic magnitude category based on paired data. With six fish species in this category, the 80th centile is based on an extrapolation between the two species with the largest BAFs. In the original approach, the 80th centile BAF falls between the BAF of 586,705 L/kg for large brook trout, and the BAF of 687,755 L/kg for large northern pikeminnow (**Table 3-2**). In the ecoregional water approach, the high trophic magnitude 80th centile BAF falls between the BAF of 1,053,125 L/kg for large northern pikeminnow, and the BAF of 1,505,263 L/kg for largemouth bass. Both large northern pikeminnow and largemouth bass were collected from sites with measured THg water concentrations that were notably higher than the ecoregional water concentrations, resulting in larger BAFs for those species following the ecoregional water approaches. This was particularly true for largemouth bass, which was collected at a downstream site in the Coeur d'Alene River with a water concentration of 6.21 ng/L, compared to the level III ecoregional water concentration of 0.38 ng/L. This resulted in a 16-fold increase in the calculated largemouth bass BAF for the ecoregional approach, as this was the only site where a BAF for this species was available. Ranked translated GMCVs are listed in **Table E-19**, and a plot of the GSD is shown in **Figure E-6**.

Table E-18. BAFs Used in the Tissue to Water Translation Procedure based on ecoregional water concentrations for calculations of fish BAFs. Fish taxa-specific BAFs were based on medians.

Trophic Magnitude Category	Scientific Name	BAF (L/kg muscle-ww)
Low		131,538
Medium		330,526
High		1,324,408
	L. sylvaticus	8,222
	Crayfish (sp.)	128,414
	Sander vitreus	758,712 (median)
	Ictalurus punctatus	199,676 (median)
	Oncorhynchus mykiss	206,250 (median)
	Salmo sp.	244,089 (median)

The final chronic value resulting from this option was 1.390 ng/L (**Table E-20**), about 65% as large as the corresponding water column criterion concentration of 2.118 ng/L based on paired water concentrations described in **Section 3.6.1**. *Hoplias* (tigerfish) is the most sensitive genera, with a GMCV of 1.095 ng/L, because of the large high trophic magnitude BAF used. However, it was not included in the calculation because it was non-definitive. The two lowest definitive GMCVs were for *Sander* and *Huso*, and those GMCVs were lower than the paired water translated GMCVs. The relative ranking of Sander remained the same, but the relative sensitivity ranking of Huso decreased from fifth to second because of the large increase in the high trophic magnitude BAF for the ecoregional water approach. Finally, the relative rankings of *Pimephales* and *Procambarus* decreased from second and third to third and fourth, respectively.

Table E-19. Ranked Freshwater Genus Mean Chronic Values based on Muscle Concentrations Translated to Water Concentrations using Bioaccumulation Factors.

Fish BAFs were calculated using ecoregional water concentrations for calculations of fish BAFs. Fish taxa-specific BAFs were based on medians.

Rank[a]	MDR Group[b]	Genus	Species	Muscle SMCV[c] (µg THg/g ww)	BAF (L/kg ww)	Water SMCV (ng THg/ L)	Water GMCV (ng THg/ L)	BAF Source[d]
1	B	*Hoplias*	Tigerfish (*Hoplias malabaricus*)	>1.45	1,324,408	>1.095	>1.095	High trophic magnitude
2	B	*Sander*	Walleye (*Sander vitreus*)	1.069	758,712	1.409	1.409	*S. vitreus*
	B	*Huso*	Beluga sturgeon (*Huso huso*)	3.0	1,324,408	2.265	2.265	High trophic magnitude
3	B	*Pimephales*	Fathead minnow (*Pimephales promelas*)	0.3575	131,538	2.718	2.718	Low trophic magnitude
5	E	*Procambarus*	Red swamp crayfish (*Procambarus clarkii)*	0.4973	128,414	3.873	3.873	Crayfish
6	C	*Lithobates*	Southern leopard frog (*Lithobates sphenocephala*)	0.03373	8,222	4.103	4.103	Anura
7	B	*Ictalurus*	Channel catfish (*Ictalurus punctatus*)	>1.6	199,676	>8.013	>8.013	*I. punctatus*
8	A	*Salmo*	Atlantic Salmon (*Salmo salar*)	>3.07	244,089	>12.58	>12.58	Salmo
9	C	*Danio*	Zebrafish (*Danio rerio*)	4.426	330,526	13.39	13.39	Medium trophic magnitude
10	B	*Carassius*	Goldfish (*Carassius auratus*)	>2.037	131,538	>15.49	>15.49	Low trophic magnitude
11	C	*Anaxyrus*	American toad (*Anaxyrus americanus*)	0.1704	8,222	20.73	20.73	Anura
12	A	*Oncorhynchus*	Rainbow trout (*Oncorhynchus mykiss*)	4.392	206,250	21.29	21.29	*O. mykiss*

Rank[a]	MDR Group[b]	Genus	Species	Muscle SMCV[c] (µg THg/g ww)	BAF (L/kg ww)	Water SMCV (ng THg/ L)	Water GMCV (ng THg/ L)	BAF Source[d]
13	F	*Hexagenia*	Mayfly (*Hexagenia sp.*)	>3.516	128,414	>27.38	>27.38	Crayfish
14	B	*Acipenser*	Green sturgeon (*Acipenser medirostris*)	17.98	1,324,408	13.58	38.75	High trophic magnitude
			White sturgeon (*Acipenser transmontanus*)	36.56	330,526	110.6		Medium trophic magnitude
15	G	*Corbicula*	Asiatic clam (*Corbicula fluminea*)	>6.0	128,414	>46.72	>46.72	Crayfish
16	B	*Orthodon*	Sacramento blackfish (*Orthodon microlepidotus*)	7.583	131,538	57.65	57.65	Low trophic magnitude
17	B	*Pogonichthys*	Sacramento splittail (*Pogonichthys macrolepidotus*)	>8.33	131,538	>63.33	>63.33	Low trophic magnitude
18	D	*Daphnia*	Cladoceran (*Daphnia magna)*	11.1	128,414	86.44	86.44	Crayfish

[a] Ranked from the most to least sensitive based on Genus Mean Chronic Value.
[b] MDR Groups identified by list provided in Section 2.6 above.
[c] From Table 3-7 above.
[d] From Table E-18 above

Table E-20. Freshwater Final Translated Water Column Chronic Value.
Fish BAFs were calculated using ecoregional water concentrations for calculations of fish BAFs. Fish taxa-specific BAFs were based on medians.

Genus	*N*	Rank	GMCV	ln(GMCV)	ln(GMCV)^2	P=R/(N+1)	sqrt(P)
Sander	18	1	1.409	0.34	0.12	0.053	0.229
Huso		2	2.265	0.82	0.67	0.105	0.324
Pimephales		3	2.718	1.00	1.00	0.158	0.397
Procambarus		4	3.837	1.35	1.83	0.211	0.459
		Sum:		**3.51**	**3.62**	**0.53**	**1.41**
				S^2 =	18.16		
				L =	-0.624		
				A =	0.329		
				FCV =	**1.390**		

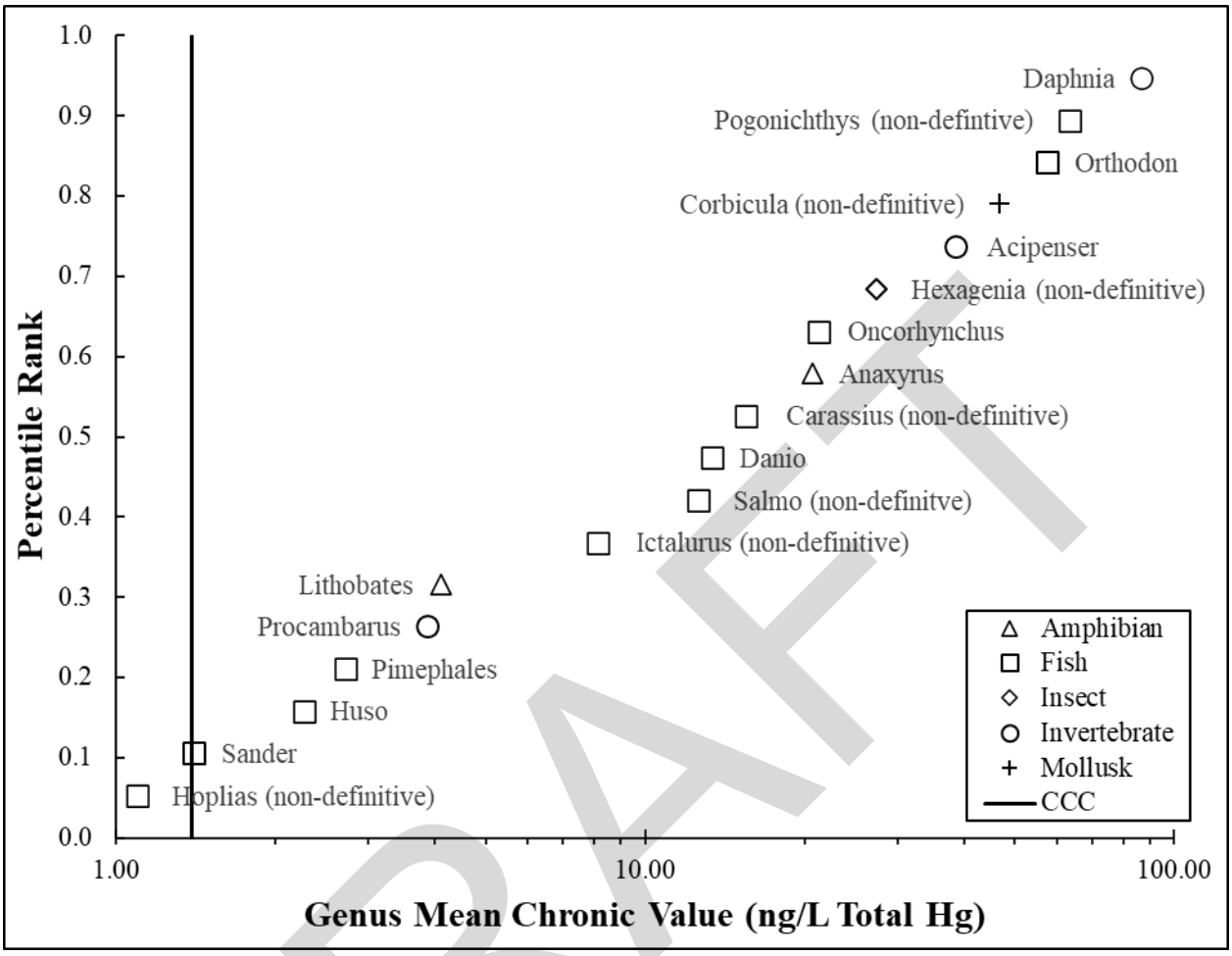

Figure E-6. Distribution of mercury water column GMCVs (THg, ng/L) translated from measured dietary mercury effect GMCVs expressed as Muscle (THg, µg/g ww).
Fish BAFs were calculated using ecoregional water concentrations for calculations of fish BAFs. Fish taxa-specific BAFs were based on medians.

E.7.2 Scenario 2: Ecoregional water THg concentrations, fish taxa-specific BAFs based on 80th centiles or maximums.

The BAFs used in this scenario (**Table E-21**) are identical to those used in the alternate translation described in **Section E.5**, except the fish species and trophic magnitude category BAFs were based on ecoregional water concentrations. Ranked translated GMCVs are listed in **Table E-22**, and a plot of the GSD is shown in **Figure E-7**.

Table E-21. BAFs Used in the Tissue to Water Translation Procedure based on ecoregional water concentrations for calculations of fish BAFs.
Fish taxa-specific BAFs were based on 80th centiles (or maximums).

Trophic Magnitude Category	Scientific Name	BAF (L/kg muscle-ww)
Low		131,538
Medium		330,526
High		1,324,408
	L. sylvaticus	8,222
	Crayfish (sp.)	128,414
	Sander vitreus	946,970 (maximum)
	Ictalurus punctatus	456,413 (80th centile)
	Oncorhynchus mykiss	269,720 (80th centile)
	Salmo sp.	389,231 (maximum)

The final chronic value resulting from this option was 1.158 ng/L (**Table E-23**), about sixty four percent as large as the corresponding water column criterion concentration of 1.806 ng/L based on paired water concentrations described in **Section E.5**. *Sander* was the most sensitive genera with a definitive GMCV, followed by *Huso, Pimephales, and Procambarus*. Huso was the most sensitive genera, but it was non-definitive. Because of its relatively large taxa specific BAF, *Ictalurus* was the fifth most sensitive genera. However, it was not included in the GMCV calculations because like *Hoplias*, it was a small greater than value.

Table E-22. Ranked Freshwater Genus Mean Chronic Values based on Muscle Concentrations Translated to Water Concentrations using Bioaccumulation Factors.

Fish BAFs were calculated using ecoregional water concentrations for calculations of fish BAFs. Fish taxa-specific BAFs were based on 80th centiles (or maximums).

Rank[a]	MDR Group[b]	Genus	Species	Muscle SMCV[c] (µg THg/g ww)	BAF (L/kg ww)	Water SMCV (ng THg/ L)	Water GMCV (ng THg/ L)	BAF Source[d]
1	B	*Hoplias*	Tigerfish (*Hoplias malabaricus*)	>1.45	1,324,408	>1.095	>1.095	High trophic magnitude
2	B	*Sander*	Walleye (*Sander vitreus*)	1.069	946,970	1.129	1.129	*S. vitreus*
3	B	*Huso*	Beluga sturgeon (*Huso huso*)	3.0	1,324,408	2.265	2.265	High trophic magnitude
3	B	*Pimephales*	Fathead minnow (*Pimephales promelas*)	0.3575	131,538	2.718	2.718	Low trophic magnitude
5	B	*Ictalurus*	Channel catfish (*Ictalurus punctatus*)	>1.6	456,413	>3.506	>3.506	*I. punctatus*
6	E	*Procambarus*	Red swamp crayfish (*Procambarus clarkii)*	0.4973	128,414	3.873	3.873	Crayfish
7	C	*Lithobates*	Southern leopard frog (*Lithobates sphenocephala*)	0.03373	8,222	4.103	4.103	Anura
8	A	*Salmo*	Atlantic Salmon (*Salmo salar*)	>3.07	389,231	>7.887	>7.887	Salmo
9	C	*Danio*	Zebrafish (*Danio rerio*)	4.426	330,526	13.39	13.39	Medium trophic magnitude
10	B	*Carassius*	Goldfish (*Carassius auratus*)	>2.037	131,538	>15.49	>15.49	Low trophic magnitude
11	A	*Oncorhynchus*	Rainbow trout (*Oncorhynchus mykiss*)	4.392	269,720	16.28	16.28	*O. mykiss*
12	C	*Anaxyrus*	American toad (*Anaxyrus americanus*)	0.1704	8,222	20.73	20.73	Anura

Rank[a]	MDR Group[b]	Genus	Species	Muscle SMCV[c] (µg THg/g ww)	BAF (L/kg ww)	Water SMCV (ng THg/ L)	Water GMCV (ng THg/ L)	BAF Source[d]
13	F	*Hexagenia*	Mayfly (*Hexagenia sp.*)	>3.516	128,414	>27.38	>27.38	Crayfish
14	B	*Acipenser*	Green sturgeon (*Acipenser medirostris*)	17.98	1,324,408	13.58	38.75	High trophic magnitude
			White sturgeon (*Acipenser transmontanus*)	36.56	330,526	110.6		Medium trophic magnitude
15	G	*Corbicula*	Asiatic clam (*Corbicula fluminea*)	>6.0	128,414	>46.72	>46.72	Crayfish
16	B	*Orthodon*	Sacramento blackfish (*Orthodon microlepidotus*)	7.583	131,538	57.65	57.65	Low trophic magnitude
17	B	*Pogonichthys*	Sacramento splittail (*Pogonichthys macrolepidotus*)	>8.33	131,538	>63.33	>63.33	Low trophic magnitude
18	D	*Daphnia*	Cladoceran (*Daphnia magna)*	11.1	128,414	86.44	86.44	Crayfish

[a] Ranked from the most to least sensitive based on Genus Mean Chronic Value.
[b] MDR Groups identified by list provided in Section 2.6 above.
[c] From Table 3-7 above.
[d] From Table E-21 above

Table E-23. Freshwater Final Translated Water Column Chronic Value.
Fish BAFs were calculated using ecoregional water concentrations for calculations of fish BAFs.
Fish taxa-specific BAFs were based on 80th centiles (or maximums).

Genus	*N*	Rank	GMCV	ln(GMCV)	ln(GMCV)^2	P=R/(N+1)	sqrt(P)
Sander	18	1	1.129	0.12	0.01	0.053	0.229
Huso		2	2.265	0.82	0.67	0.105	0.324
Pimephales		3	2.718	1.00	1.00	0.158	0.397
Procambarus		4	3.873	1.35	1.83	0.211	0.459
		Sum:		**2.48**	**2.20**	**0.53**	**1.41**
				S^2 =	27.54		
				L =	-1.027		
				A =	0.147		
				FCV =	**1.158**		

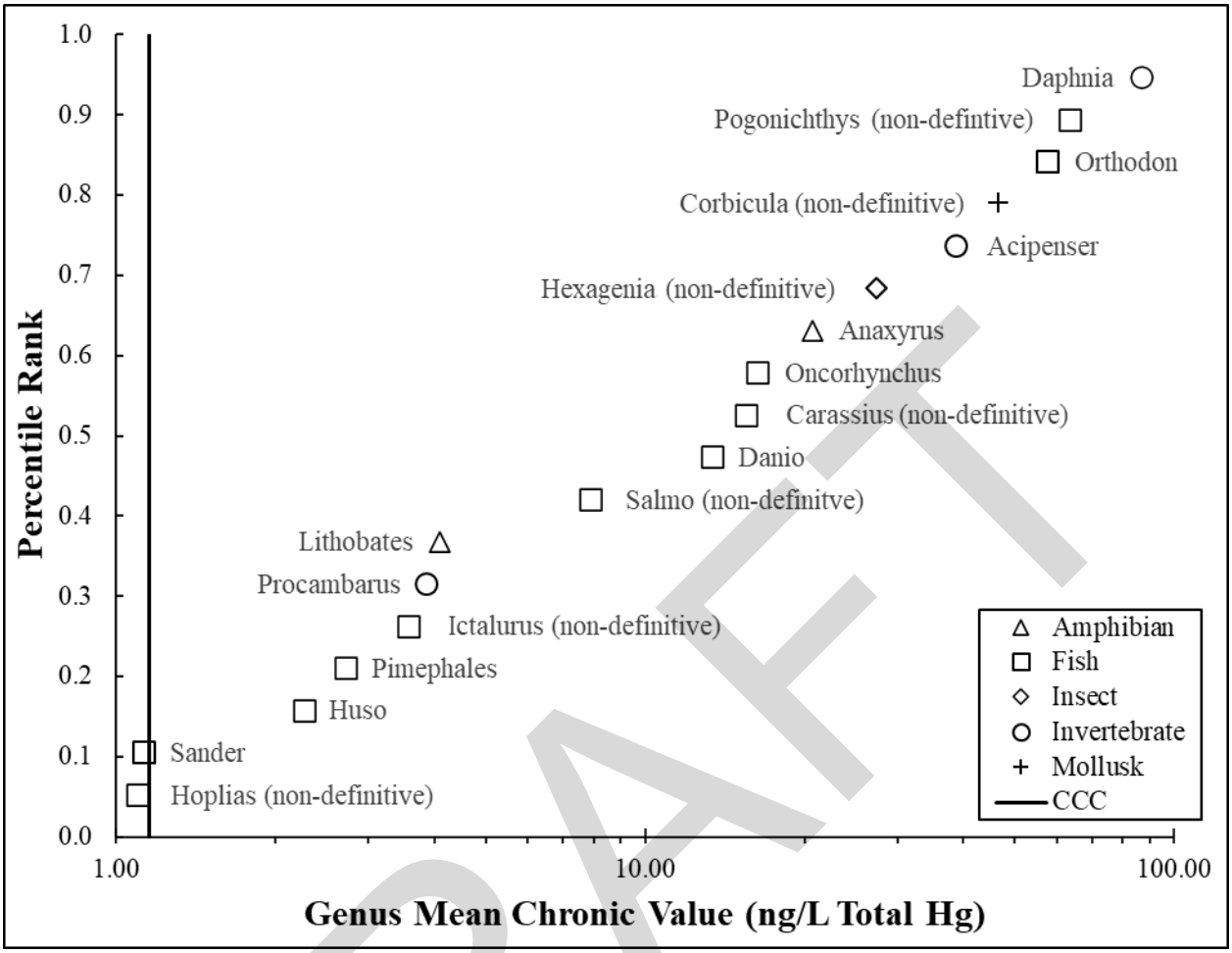

Figure E-7. Distribution of mercury water column GMCVs (THg, ng/L) translated from measured dietary mercury effect GMCVs expressed as Muscle (THg, µg/g ww).
Fish BAFs were calculated using ecoregional water concentrations for calculations of fish BAFs. Fish taxa-specific BAFs were based on 80th centiles (or maximums).

E.7.3 Scenario 3: Ecoregional water THg concentrations, fish taxa-specific BAFs based on medians. Coeur d'Alene River largemouth bass and black crappie BAFs based on geometric mean water concentrations.

The BAFs used in this scenario are identical to those used in the translation described in **Section E.6.1**, except that the majority of fish species and trophic magnitude category BAFs were based on ecoregional water concentrations. As described in **Section E.6**, the effects of assuming the observed tissue concentrations in largemouth bass and black crappie from the Coeur d'Alene River reflect THg water concentrations measured at all sites along the entire river were examined by calculating those BAFs using the geometric mean of the three water concentrations sampled within that waterbody (0.85 ng/L). The resulting updated largemouth bass BAF was 676,131 L/kg, and the updated black crappie BAF was 330,973 L/kg (**Section E.6**). All other fish BAFs were calculated using ecoregional water concentrations.

The effects of pairing the Coeur d'Alene River tissue concentrations to geometric mean water THg concentrations (as opposed to using the ecoregional water THg concentration) was a slight decrease in the medium trophic magnitude category BAF, and a larger decrease in the high trophic magnitude category BAF. This is because the geometric mean Coeur d'Alene River water THg concentration of 0.85 ng/L is higher than the Ecoregion 15 THg concentration of 0.30 ng/L, resulting in lower BAFs for black crappie (medium trophic magnitude) and largemouth bass (high trophic magnitude). Because there are fewer high trophic magnitude category species, this scenario has a greater effect on the high trophic magnitude BAF (**Table E-24**). Ranked translated GMCVs are listed in **Table E-25**, and a plot of the GSD is shown in **Figure E-8**.

Table E-24. BAFs Used in the Tissue to Water Translation Procedure based on ecoregional water concentrations for calculations of fish BAFs. Fish taxa-specific BAFs were based on medians.

Trophic Magnitude Category	Scientific Name	BAF (L/kg muscle-ww)
Low		131,538
Medium		317,531
High		935,360
	L. sylvaticus	8,222
	Crayfish (sp.)	128,414
	Sander vitreus	758,712 (median)
	Ictalurus punctatus	199,676 (median)
	Oncorhynchus mykiss	206,250 (median)
	Salmo sp.	244,089 (median)

The final chronic value resulting from this option was 1.479 ng/L, about seventy percent as large as the corresponding water column criterion concentration of 2.118 ng/L based on paired water concentrations described in **Section E.6.1**. The four most sensitive definitive genera are the same as in the parallel scenario based on paired water concentrations, but the GMCV for *Sander* is considerably lower in this scenario because of the larger BAF for walleye. The non-definitive GMCV for *Hoplias* is also lower because of the larger high trophic magnitude category BAF, although it switches positions with *Sander*.

Table E-25. Ranked Freshwater Genus Mean Chronic Values based on Muscle Concentrations Translated to Water Concentrations using Bioaccumulation Factors.

Fish BAFs were calculated using ecoregional water concentrations for calculations of fish BAFs. Coeur d'Alene River largemouth bass and black crappie BAFs based on geometric mean water concentrations. Median species- and genus-specific fish BAFs, when available.

Rank[a]	MDR Group[b]	Genus	Species	Muscle SMCV[c] (µg THg/g ww)	BAF (L/kg ww)	Water SMCV (ng THg/ L)	Water GMCV (ng THg/ L)	BAF Source[d]
1	B	*Sander*	Walleye (*Sander vitreus*)	1.069	758,712	1.409	1.409	*S. vitreus*
2	B	*Hoplias*	Tigerfish (*Hoplias malabaricus*)	>1.45	935,360	>1.550	>1.550	High trophic magnitude
3	B	*Pimephales*	Fathead minnow (*Pimephales promelas*)	0.3575	131,538	2.718	2.718	Low trophic magnitude
4	B	*Huso*	Beluga sturgeon (*Huso huso*)	3.0	935,360	3.207	3.207	High trophic magnitude
5	E	*Procambarus*	Red swamp crayfish (*Procambarus E-67larkia)*	0.4973	128,414	3.873	3.873	Crayfish
6	C	*Lithobates*	Southern leopard frog (*Lithobates sphenocephala*)	0.03373	8,222	4.103	4.103	Anura
7	B	*Ictalurus*	Channel catfish (*Ictalurus punctatus*)	>1.6	197,676	>8.103	>8.103	*I. punctatus*
8	A	*Salmo*	Atlantic Salmon (*Salmo salar*)	>3.07	244,089	>12.58	>12.58	Salmo
9	C	*Danio*	Zebrafish (*Danio rerio*)	4.426	317,531	13.94	13.94	Medium trophic magnitude
10	B	*Carassius*	Goldfish (*Carassius auratus*)	>2.037	131,538	>15.49	>15.49	Low trophic magnitude
11	C	*Anaxyrus*	American toad (*Anaxyrus americanus*)	0.1704	8,222	20.73	20.73	Anura

Rank[a]	MDR Group[b]	Genus	Species	Muscle SMCV[c] (µg THg/g ww)	BAF (L/kg ww)	Water SMCV (ng THg/ L)	Water GMCV (ng THg/ L)	BAF Source[d]
12	A	*Oncorhynchus*	Rainbow trout (*Oncorhynchus mykiss*)	4.392	206,250	21.29	21.29	*O. mykiss*
13	F	*Hexagenia*	Mayfly (*Hexagenia sp.*)	>3.516	128,414	>27.38	>27.38	Crayfish
14	G	*Corbicula*	Asiatic clam (*Corbicula fluminea*)	>6.0	128,414	>46.72	>46.72	Crayfish
15	B	*Acipenser*	Green sturgeon (*Acipenser medirostris*)	17.98	935,360	19.22	47.05	High trophic magnitude
			White sturgeon (*Acipenser transmontanus*)	36.56	317,531	115.1		Medium trophic magnitude
16	B	*Orthodon*	Sacramento blackfish (*Orthodon microlepidotus*)	7.583	131,538	57.65	57.65	Low trophic magnitude
17	B	*Pogonichthys*	Sacramento splittail (*Pogonichthys macrolepidotus*)	>8.33	131,538	>63.33	>63.33	Low trophic magnitude
18	D	*Daphnia*	Cladoceran (*Daphnia magna)*	11.1	128,414	86.44	86.44	Crayfish

[a] Ranked from the most to least sensitive based on Genus Mean Chronic Value.
[b] MDR Groups identified by list provided in Section 2.6 above.
[c] From Table 3-7 above.
[d] From Table E-24 above

Table E-26. Freshwater Final Translated Water Column Chronic Value.
Fish BAFs were calculated using ecoregional water concentrations for calculations of fish BAFs. Coeur d'Alene River largemouth bass and black crappie BAFs based on geometric mean water concentrations. Median species- and genus-specific fish BAFs, when available.

Genus	*N*	Rank	GMCV	ln(GMCV)	ln(GMCV)^2	P=R/(N+1)	sqrt(P)
Sander	18	1	1.409	0.34	0.12	0.053	0.229
Pimephales		3	2.718	1.00	1.00	0.105	0.324
Huso		2	3.207	1.17	1.36	0.158	0.397
Procambarus		4	3.873	1.35	1.83	0.211	0.459
		Sum:		**3.41**	**3.46**	**0.53**	**1.41**
				S^2 =	19.82		
				L =	-0.604		
				A =	0.392		
				FCV =	**1.479**		

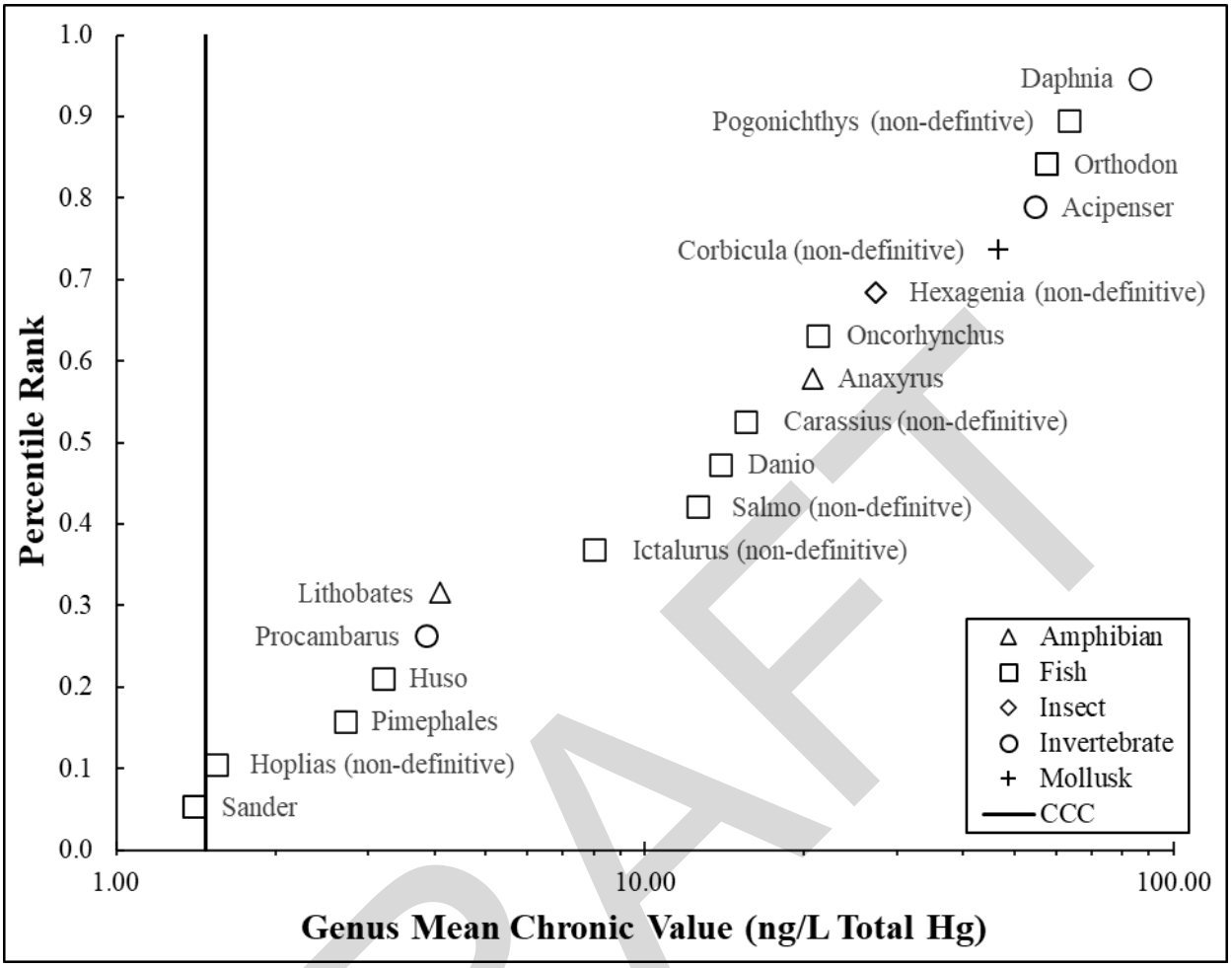

Figure E-8. Distribution of mercury water column GMCVs (THg, ng/L) translated from measured dietary mercury effect GMCVs expressed as Muscle (THg, µg/g ww).
Fish BAFs were calculated using ecoregional water concentrations for calculations of fish BAFs. Coeur d'Alene River largemouth bass and black crappie BAFs based on geometric mean water concentrations. Median species- and genus-specific fish BAFs, when available.

E.7.4 Scenario 4: Ecoregional water THg concentrations, fish taxa-specific BAFs based on 80th centiles or maximums. Coeur d'Alene River largemouth bass and black crappie BAFs based on geometric mean water concentrations.

The BAFs used in this scenario are identical to those used in the translation described in **Section E.6.2**, except the majority of fish species and trophic magnitude category BAFs were based on ecoregional water concentrations (**Table E-27**). As described above in **Section E.7.3**, the effects of assuming the tissue concentrations in largemouth bass and black crappie from the Coeur d'Alene River reflect THg water concentrations for the entire river were examined by calculating those BAFs using the geometric mean of the three water concentrations sampled within that waterbody (0.85 ng/L). The resulting updated largemouth bass BAF was 676,131 L/kg, and the updated black crappie BAF was 330,973 L/kg. Ranked translated GMCVs are listed in **Table E-28**, and a plot of the GSD is shown in **Figure E-9**.

Table E-27. Used in the Tissue to Water Translation Procedure based on ecoregional water concentrations for calculations of fish BAFs. Fish taxa-specific BAFs were based on medians.

Trophic Magnitude Category	Scientific Name	BAF (L/kg muscle-ww)
Low		131,538
Medium		317,531
High		935,360
	L. sylvaticus	8,222
	Crayfish (sp.)	128,414
	Sander vitreus	946,970 (maximum)
	Ictalurus punctatus	456,413 (80th centile)
	Oncorhynchus mykiss	269,720 (80th centile)
	Salmo sp.	389,231 (maximum)

The final chronic value resulting from this option was 1.219 ng/L (**Table E-29**), about sixty percent as large as the corresponding water column criterion concentration of 1.806 ng/L based on paired water concentrations described in **Section E.6.2**. The four most sensitive genera

are the same as in the parallel scenario based on paired water concentrations, but the FCV is largely the result of the lower GMCV for *Sander*, which has the lowest GMCV.

Table E-28. Ranked Freshwater Genus Mean Chronic Values based on Muscle Concentrations Translated to Water Concentrations using Bioaccumulation Factors.

Fish BAFs were calculated using ecoregional water concentrations for calculations of fish BAFs. Coeur d'Alene River largemouth bass and black crappie BAFs based on geometric mean water concentrations. 80th centile (or maximum) species- and genus-specific fish BAFs, when available.

Rank[a]	MDR Group[b]	Genus	Species	Muscle SMCV[c] (µg THg/g ww)	BAF (L/kg ww)	Water SMCV (ng THg/ L)	Water GMCV (ng THg/ L)	BAF Source[d]
1	B	*Sander*	Walleye (*Sander vitreus*)	1.069	946,970	1.129	1.129	*S. vitreus*
2	B	*Hoplias*	Tigerfish (*Hoplias malabaricus*)	>1.45	935,360	>1.550	>1.550	High trophic magnitude
3	B	*Pimephales*	Fathead minnow (*Pimephales promelas*)	0.3575	131,538	2.718	2.718	Low trophic magnitude
4	B	*Huso*	Beluga sturgeon (*Huso huso*)	3.0	935,3603	3.207	3.207	High trophic magnitude
5	B	*Ictalurus*	Channel catfish (*Ictalurus punctatus*)	>1.6	456,413	>3.506	>3.506	*I. punctatus*
6	E	*Procambarus*	Red swamp crayfish (*Procambarus clarkii)*	0.4973	128,414	3.873	3.873	Crayfish
7	C	*Lithobates*	Southern leopard frog (*Lithobates sphenocephala*)	0.03373	8,222	4.103	4.103	Anura
8	A	*Salmo*	Atlantic Salmon (*Salmo salar*)	>3.07	389,231	>7.887	>7.887	Salmo
9	C	*Danio*	Zebrafish (*Danio rerio*)	4.426	317,531	13.94	13.94	Medium trophic magnitude
10	B	*Carassius*	Goldfish (*Carassius auratus*)	>2.037	131,538	>15.49	>15.49	Low trophic magnitude
11	A	*Oncorhynchus*	Rainbow trout (*Oncorhynchus mykiss*)	4.392	269,720	16.28	16.28	*O. mykiss*

Rank[a]	MDR Group[b]	Genus	Species	Muscle SMCV[c] (µg THg/g ww)	BAF (L/kg ww)	Water SMCV (ng THg/ L)	Water GMCV (ng THg/ L)	BAF Source[d]
12	C	*Anaxyrus*	American toad (*Anaxyrus americanus*)	0.1704	8,222	20.73	20.73	Anura
13	F	*Hexagenia*	Mayfly (*Hexagenia sp.*)	>3.516	128,414	>27.38	>27.38	Crayfish
14	G	*Corbicula*	Asiatic clam (*Corbicula fluminea*)	>6.0	128,414	>46.72	>46.72	Crayfish
15	B	*Acipenser*	Green sturgeon (*Acipenser medirostris*)	17.98	935,360	19.22	47.05	High trophic magnitude
			White sturgeon (*Acipenser transmontanus*)	36.56	317,531	115.1		Medium trophic magnitude
16	B	*Orthodon*	Sacramento blackfish (*Orthodon microlepidotus*)	7.583	131,538	57.65	57.65	Low trophic magnitude
17	B	*Pogonichthys*	Sacramento splittail (*Pogonichthys macrolepidotus*)	>8.33	131,538	>63.33	>63.33	Low trophic magnitude
18	D	*Daphnia*	Cladoceran (*Daphnia magna)*	11.1	128,414	86.44	86.44	Crayfish

[a] Ranked from the most to least sensitive based on Genus Mean Chronic Value.
[b] MDR Groups identified by list provided in Section 2.6 above.
[c] From Table 3-7 above.
[d] From Table E-27 above

Table E-29. Freshwater Final Translated Water Column Chronic Value.
Fish BAFs were calculated using ecoregional water concentrations for calculations of fish BAFs. Coeur d'Alene River largemouth bass and black crappie BAFs based on geometric mean water concentrations. 80th centile (or maximum) species- and genus-specific fish BAFs, when available.

Genus	*N*	Rank	GMCV	ln(GMCV)	ln(GMCV)^2	P=R/(N+1)	sqrt(P)
Sander	18	1	1.129	0.12	0.01	0.053	0.229
Pimephales		2	2.718	1.00	1.00	0.105	0.324
Huso		3	3.207	1.17	1.36	0.158	0.397
Procambarus		4	3.873	1.35	1.83	0.211	0.459
		Sum:		**3.64**	**4.21**	**0.53**	**1.41**
				S^2 =	30.51		
				L =	-1.037		
				A =	0.198		
				FCV =	**1.219**		

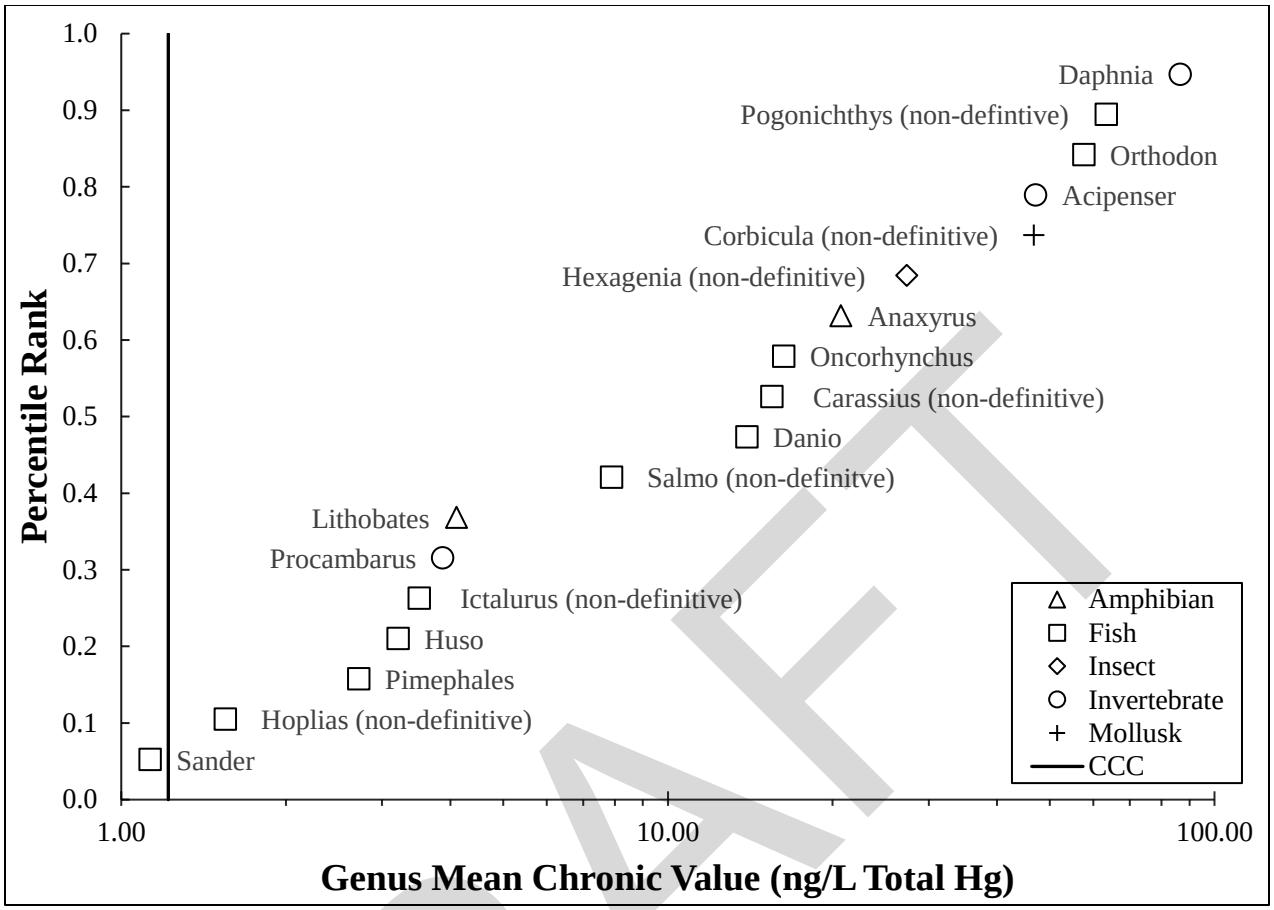

Figure E-9. Distribution of mercury water column GMCVs (THg, ng/L) translated from measured dietary mercury effect GMCVs expressed as Muscle (THg, µg/g ww).
Fish BAFs were calculated using ecoregional water concentrations for calculations of fish BAFs. Coeur d'Alene River largemouth bass and black crappie BAFs based on geometric mean water concentrations. 80th centile (or maximum) species- and genus-specific fish BAFs, when available.