

APPENDIX I

FISH DIETARY TOXICITY REFERENCE VALUES

From: [Cerise, Kathy](#)
To: [McCaig, Kris SPOK](#)
Cc: [Mills, Denise SPOK](#)
Subject: UCR - Revised Draft Final Fish Diet TRV Document (February 2019) and TAI Response to Comments on July 2018 Draft
Date: Monday, March 25, 2019 4:45:24 PM

[External email]

Kris,

We have reviewed the revised (Feb 2019) Fish TRV memo and responses to comments. We do not have any further comments, although there is one minor error that should be corrected in the final version:

In Table 3-3 Footnote C, we think the footnote probably should contain "ED20" not "ED10."

Thank you

Kathryn Cerise
UCR RPM

From: Mills Denise SPOK <Denise.Mills@teck.com>
Sent: Thursday, February 14, 2019 2:42 PM
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Subject: UCR - Revised Draft Final Fish Diet TRV Document (February 2019) and TAI Response to Comments on July 2018 Draft

Hello Kathy,

Please see the attached for your review and approval.

Please contact me or Kris if you have any questions.

Thank you,

Denise

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April 22, 2019

File No.: 01-773180-000

Kathryn Cerise
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VIA ELECTRONIC MAIL ONLY

Subject: Upper Columbia River (UCR) Remedial Investigation and Feasibility Study (RI/FS) – Final Fish Diet Toxicity Reference Values for the Baseline Ecological Risk Assessment (April 2019)

Dear Kathy:

On behalf of Teck American Incorporated (TAI) and further to your March 25, 2019 email indicating the U.S. Environmental Protection Agency's approval of the above-referenced toxicity reference values document, I am pleased to submit the final document for your records.

Should you have questions or require any additional information to assist your review, please contact me at (509) 623-4515.

Sincerely,

Teck American Incorporated

A handwritten signature in blue ink that reads "Denise" followed by a stylized flourish.

Denise Mills
Program Manager, Upper Columbia River

Enclosure: 1) *Final Fish Diet Toxicity Reference Values for the Baseline Ecological Risk Assessment (April 2019)*

cc: Dr. Jennifer Holder – ERM West, Carpinteria, CA
David DeForest – Windward Environmental, Seattle, WA
Kris McCaig – TAI, Spokane, WA
Cristy Kessel – TAI, Spokane, WA

UPPER COLUMBIA RIVER

FINAL **Fish Diet Toxicity Reference Values for the** **Baseline Ecological Risk Assessment**

Prepared for
Teck American Incorporated
P.O. Box 3087
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Prepared by



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In Association and Consultation with
Parametrix, Inc.

April 2019

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ACRONYMS AND ABBREVIATIONS

AWQC	ambient water quality criteria
BERA	baseline ecological risk assessment
CBB	critical body burden
CI	confidence interval
EC	effect concentration
EC20	20 percent effect concentration
ED	effect dose
ED20	20 percent dose concentration
EPA	U.S. Environmental Protection Agency
FIR	food ingestion rate
GSI	gonadosomatic index
LC20	concentration that is lethal to 20 percent of an exposed population
LOEC	lowest-observed-effect concentration
LOEL	lowest-observed-effect level
NOEC	no-observed-effect concentration
NOEL	no-observed-effect level
RI/FS	remedial investigation/feasibility study
SSD	species sensitivity distribution
TAI	Teck American Incorporated
TRAP	Toxicity Relationship Analysis Program
TRV	toxicity reference value
UCR	Upper Columbia River

UNITS OF MEASURE

bw	body weight
dw	dry weight
g	gram(s)
g ^{food}	gram(s) of food
g ^{food} /g bw/day	gram(s) of food per gram body weight per day
g ^{diet} /g bw/day	gram(s) of diet per gram body weight per day
g/kg	gram(s) per kilogram
km	kilometer(s)
mg	milligram(s)
mg/g	milligram(s) per gram
mg/kg	milligram(s) per kilogram
mg/kg/day	milligram(s) per kilogram per day
mg/kg ^{diet}	milligram(s) of metal per kilogram of diet (food)
mg/kg bw/day	milligram(s) of metal per kilogram body weight per day
mmol/L	millimole(s) per liter
mo	month
µg/g	microgram(s) per gram
µg/L	microgram(s) per liter
wk	week
ww	wet weight

EXECUTIVE SUMMARY

The Upper Columbia River (UCR) remedial investigation/feasibility study (RI/FS) baseline ecological risk assessment (BERA) will include an evaluation of risk to fish from diet-borne exposure to metals and metalloids (hereafter referred to as metals, for simplicity). This document presents methods used to derive toxicity reference values (TRVs) and the resulting TRVs for individual metals that will be used in the BERA for the fish dietary pathway.

Toxicity studies relevant to dietary exposure of fish to metals were compiled from the published scientific literature, government reports, and graduate theses and dissertations. The compiled studies were reviewed, and data related to acceptable toxicity endpoints (i.e., direct measures of survival, growth, and reproduction) were extracted from these studies. Toxicity exposure data from each study were expressed as concentrations in the diet (i.e., mg/kg_{diet}). In addition, if information on food ingestion rate (FIR) and body weight were available from the study to support the dose-based calculations, data were expressed as body weight-normalized dietary doses (i.e., mg/kg_{food} body weight [bw]/day). (Throughout this document, the term “dose” refers to diet-borne dose.)

If sufficient concentration- or dose-response data were available for a particular study, regression analysis was used to identify the 20 percent effect level, defined as the 20 percent effect concentration (EC20) for data expressed on a concentration basis and as the 20 percent dose concentration (ED20) for data expressed on a dose basis. If an EC20 or ED20 could not be derived for a test, the lowest test treatment that resulted in an effect greater than 20 percent relative to the controls was used to define the toxicity threshold for that test. In such cases, the toxicity threshold was identified as a “less than” value, because a lower concentration or dose would have been expected to achieve the targeted 20 percent effect level. If none of the treatments in a test resulted in an effect greater than 20 percent relative to the controls, the highest concentration or dose was used to define the toxicity threshold. In such cases, the toxicity threshold was defined as a “greater than” value, because a higher concentration or dose would have been required to achieve the targeted 20 percent effect level. If multiple toxicity thresholds were identified for a species, the species mean toxicity threshold was calculated as the geometric mean of the available toxicity thresholds within a particular endpoint category. Once concentration- and dose-based toxicity thresholds had been compiled for each metal, and species mean values had been calculated as necessary, TRVs were defined as either the 5th percentile of the species sensitivity distribution (SSD) of species mean toxicity thresholds, or as the lowest species mean toxicity threshold.

Concentration-based diet-borne fish TRVs were derived for aluminum, arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, vanadium, and zinc. Dose-based

TRVs were developed for all of these metals except aluminum, selenium, and vanadium. Neither concentration- nor dose-based TRVs were derived for iron or manganese.

To evaluate the protectiveness of individual metal TRVs, this document also includes a discussion of studies of diet-borne field toxicity, conducted with metal mixtures. Most of these studies involved feeding fish benthic invertebrates from the Clark Fork River in Montana or the Coeur d'Alene River in Idaho or benthic invertebrates exposed to sediments from these sites. The Clark Fork River studies ultimately identified arsenic as a driver of the effects observed in the field studies, indicating the importance of evaluating the dietary pathway for arsenic and fish. However, the possibility of additive or synergistic toxicity due to other metals cannot be excluded. The metals mixtures studies were not used to adjust any of the TRVs for individual metals, but the studies will be included as part of the uncertainty discussions in the BERA.

1 DIET-BORNE TOXICITY REFERENCE VALUES FOR FISH

Studies on the diet-borne toxicity of metals and metalloids (hereafter referred to as metals, for simplicity) to fish were reviewed to develop diet-borne toxicity reference values (TRVs) for use in the Upper Columbia River (UCR) remedial investigation/feasibility study (RI/FS) baseline ecological risk assessment (BERA). In the BERA work plan, the diet was identified as a complete exposure pathway to fish for metals and other chemicals (Parametrix et al. 2011). The diet-borne pathway for fish is being evaluated following the approach described in the BERA work plan, which includes the development of benchmarks (herein referred to as TRVs) that are “associated with a NOAEL [no-observed-adverse-effect level] or LOAEL [lowest-observed-adverse-effect level], or a proportional response (EC_x [effect concentrations associated with an effect level of x percent]) for growth, survival, or reproduction” (Parametrix et al. 2011).

For some chemicals, the diet-borne pathway can also be evaluated using critical body burdens (CBBs), as this approach considers the combined exposure of fish to chemicals in food and water. However, for most metals, the CBB approach is not applicable and will not be used in the BERA (Parametrix et al. 2011), except for mercury and selenium as discussed below. This is because the linkage between whole-body metal concentrations in aquatic biota and toxicity is highly variable due to the dynamic nature of metal uptake and elimination, and due to internal partitioning of bioaccumulated metal (Luoma and Rainbow 2005). As a result, it is not possible to develop universally or broadly applicable whole-body critical residues for metals (Adams et al. 2011). The exceptions are mercury and selenium, because they both have organic forms in which tissue concentrations are strong indicators of potential toxicity (Beckvar et al. 2005; USEPA 2016a).

Diet-borne toxicity data were compiled for both individual metals and metal mixtures. TRVs were developed for individual metals, but studies with metal mixtures were used as a line of evidence for evaluating the protectiveness of TRVs for individual metals. As for any metal mixture in any exposure medium—whether food, water, or sediment—toxicity (or lack of) is highly dependent on the relative concentrations of the metals present, as metals can act antagonistically (i.e., less than additive), additively (i.e., strictly additive), or synergistically (i.e., more than additive). How the BERA will address uncertainty in the assessment of diet-borne metals is beyond the scope of this TRV document; options *may* include concentration addition and response addition approaches (USEPA 2007), although it is important to note that these types of approaches have not been well tested for metals in fish diets. The most useful approach for evaluating the potential toxicity of metal mixtures will be using a combination of individual metal TRVs and metals mixture toxicity data from studies at other sites (e.g., the Clark Fork River in Montana and Coeur d’Alene River in Idaho).

The methods used to develop the diet-borne TRVs are described in Section 2, and the resulting diet-borne TRVs for individual metals are presented in Section 3. To assess the protectiveness of the diet-borne TRVs for individual metals, these TRVs are compared to diet-borne toxicity data for metal mixtures in Section 4.

2 METHODS

2.1 CHEMICALS OF INTEREST

The working list of metals evaluated for the development of diet-borne TRVs for fish includes 14 metals and metalloids: aluminum, arsenic, cadmium, chromium, copper, iron, lead, manganese, mercury, nickel, selenium, silver, vanadium, and zinc. It is possible that some of these metals will be screened out from further evaluation once the chemical of potential concern refinement is finalized.

2.2 SOURCES OF DIET-BORNE TOXICITY DATA

Diet-borne metals toxicity data were compiled based on a review of the published scientific literature, government reports, and graduate theses and dissertations. The initial source of data was a recent review by DeForest and Meyer (2015), who developed a database of diet-borne metals toxicity data for fish and other aquatic life. That database was augmented with any diet-borne toxicity data for fish that subsequently became available in the published literature or unpublished reports. Literature searches were conducted by using Google Scholar and contacting researchers and organizations known to be conducting diet-borne toxicity studies with metals of interest. The references sections of studies identified were also consulted to identify additional diet-borne toxicity studies that could be used for TRV development.

2.3 TYPES OF DATA COMPILED

The following information was compiled, as available, from each of the studies reviewed:

- Fish species
- Age at test initiation
- Exposure duration
- FIR—units of $\text{g}_{\text{food}}/\text{day}$ or $\text{g}_{\text{food}}/\text{g bw}/\text{day}$
- Body weight (bw)—typically reported at test initiation and sometimes at different points during the exposure (especially for tests that measured weight as an endpoint)
- Dose—units of $\text{mg}/\text{kg bw}/\text{day}$ (Throughout this document, the term “dose” refers to diet-borne dose.)
- Diet type—formulated or biologically incorporated
- Metal concentration in the diet

- Corresponding metal concentration in water—some tests explicitly tested the combined effects of diet- and water-borne metals exposures; others measured metals concentrations in water to evaluate the amount of metal that leached from food
- Toxicity endpoint—the biological endpoints of interest were related to survival, growth, and reproduction; the availability of other endpoints was noted, but these were not quantified
- Concentration-response data and dose-response data—these data were compiled to evaluate the compatibility of concentration-response and dose-response models for calculating 20 percent effect concentrations and effect doses (EC20s/ED20s), and to identify the levels of effect associated with individual treatment concentrations and doses
- Statistical significance—it was noted whether the biological response in each treatment/dose was statistically significant from the negative control, as reported in the original study.

There were no explicit inclusion or rejection criteria for compiling diet-borne toxicity data, although there were a few endpoints that were not included in the TRV development (see Section 2.5). For each constituent, the strengths and weaknesses of the available data were considered in developing the applicable TRV.

2.4 DIET TYPE

Most diet-borne toxicity studies with metals and fish have used formulated diets spiked with metal salts; fewer studies have used natural diets composed of live food organisms previously exposed to metals. The former type of diet might overestimate or underestimate metal bioavailability to fish relative to the latter, depending on the relative bioavailability of the metals in question. Toxicity data were compiled based on both formulated and natural diets. It was preferable if total metals concentrations in diets were measured analytically; however, in the absence of measured concentrations, nominal (i.e., unmeasured) metals concentrations were used. If metals concentrations were reported in the basal (or control) diet, these concentrations were summed with the added metals concentrations to estimate the total metal concentration.

It was important to identify diet-borne concentrations as either dry weight or wet weight. If not explicitly stated, generally assumptions were not made, and a test was not considered for TRV development; however, this situation was infrequent. The only exception was if the methods for preparing the diet explicitly noted that the diet was dried to remove water. In these cases, it was assumed that the reported concentrations were on a dry weight basis.

2.5 TOXICITY ENDPOINTS

Among the diet-borne toxicity data gathered, acceptable toxicity endpoints for diet-borne TRV development were direct measures of survival, growth, and reproduction. Consideration of these endpoints was consistent with U.S. Environmental Protection Agency (EPA) (USEPA 1985) guidelines for ambient water quality criteria (AWQC) development. Measures of survival included endpoints that could be considered precursors to mortality, such as loss of equilibrium and immobilization. Growth-related endpoints included a variety of measures, such as weight, length, growth rate (e.g., percent weight gain per day), and condition factor ($K = W/L^3$, where W is wet mass in grams and L is length in centimeters). Toxicity data for reproductive endpoints were available from only four studies; the measures of reproduction used in these studies included number of fry per female and spawning success. Any other biological endpoints (i.e., those that were not direct measures of survival, growth, and reproduction) evaluated in a study were extracted and reported (but not quantified) as well, because EPA indicated that other endpoints clearly associated with survival, growth, and reproduction might be considered for TRV development (USEPA 2016b).

2.6 CONCENTRATION- AND DOSE-RESPONSE RELATIONSHIPS

Both concentration-based toxicity data (expressed as $\text{mg/kg}_{\text{diet}} \text{ dw}$) and dose-based toxicity data (expressed as mg/kg bw/day) were compiled. Preferably, doses were calculated from FIR and body weight information reported in the original study; however, when necessary, a FIR of $0.037 \text{ g}_{\text{food}}/\text{g bw/day}$ (i.e., 3.7 percent of body weight per day) was assumed.¹ In ecological risk assessments, diet-borne TRVs for fish based on concentrations are simpler to apply than those based on doses, because fewer assumptions are required to evaluate diet-borne concentrations for the receptor species of interest. The assumptions required to derive and apply dose-based TRVs can potentially add uncertainty to the analysis. Nevertheless, both concentration-based and dose-based TRVs were derived, where possible, and both will be applied in the BERA. Cases in which one method (concentration- or dose-based) produces TRV exceedances, and the other does not, will trigger an uncertainty analysis to decide which TRV better represents the targeted threshold response.

To define the targeted threshold response, EPA (USEPA 1985) guidelines for AWQC development were again used. These guidelines state that chronic toxicity thresholds can be obtained using either regression analysis or the geometric mean of the no-observed-effect

¹ A FIR of $0.037 \text{ g}_{\text{food}}/\text{g bw/day}$ is the mean of reported FIRs from studies compiled in developing the fish diet TRVs in the present document (Tables A-1 and A-2 in Appendix A). Species mean FIRs ranged from 0.0021 to $0.056 \text{ g}_{\text{food}}/\text{g bw/day}$, with an arithmetic mean of $0.037 \text{ g}_{\text{food}}/\text{g bw/day}$.

concentration (NOEC) and lowest-observed-effect concentration (LOEC).² For each diet-borne toxicity test, it was first determined whether regression analysis could be used to identify a toxicity threshold. The targeted threshold was a 20 percent effect level, defined as EC20 for concentration-response data and ED20 for dose-response data. The 20 percent effect level was selected following EPA precedent in developing AWQC, such as the AWQC for ammonia (USEPA 2013) and cadmium (USEPA 2016c). Use of the EC20 and ED20 was a point of conceptual agreement between EPA and Teck American Incorporated (TAI) (USEPA 2016b).

To determine whether EC20s and ED20s from each test could be evaluated, concentration- and dose-response data were first compiled. EC20s and ED20s were calculated using EPA's Toxicity Relationship Analysis Program (TRAP; Version 1.30a). To evaluate concentration- or dose-response data in TRAP, data for the negative control and a minimum of two diet-borne treatments were necessary. Otherwise, no *a priori* conditions were defined. Diet-borne concentrations and doses were log transformed. With one exception, response data were entered into TRAP as raw measurements (i.e., treatment response data were not control normalized). The one exception was when data were pooled from multiple tests with similar study designs (e.g., same species and similar life stages, exposure durations, and endpoints); in that case, treatment response data were normalized to their respective negative control.

The nonlinear regression option in TRAP was used to evaluate growth and reproduction data; the tolerance distribution option was used for survival data. Each option included three types of models: logistic equation; threshold sigmoid; and piecewise linear for nonlinear regression and Gaussian distribution, triangular distribution, and rectangular distribution for tolerance distribution. The sigmoid threshold model was generally selected.³ However, the piecewise linear regression model was sometimes used when only low-growth effects were observed, as the sigmoid threshold and logistic equation models are sensitive to uncertainty in the tails of the distributions when high-effects data are lacking. TRAP results were not used if the program cautioned that the results were acceptable for exploratory analysis only; exceptions are noted and discussed in Section 3. In some cases when TRAP could not be used to derive an EC20 or ED20, linear interpolation between diet-borne concentrations bracketing a 20 percent effect level was used to calculate the EC20 or ED20.

If an EC20 or ED20 could not be derived for a test, the lowest test treatment that resulted in an effect greater than 20 percent was used to define the toxicity threshold for that test. In such cases, the toxicity threshold was identified as a "less than" value, because a lower

² The NOEC is the highest concentration tested that did not result in a statistically significant effect relative to the negative control; the LOEC is the lowest concentration tested that did result in a statistically significant effect relative to the control.

³ The majority of diet-borne metals studies evaluated a growth-related endpoint.

concentration/dose would be required to achieve the targeted 20 percent effect level. If none of the treatments in a test resulted in an effect greater than 20 percent, the highest concentration/dose was used to define the toxicity threshold. In such cases, the toxicity threshold was defined as a “greater than” value, because a higher concentration/dose would be required to achieve the targeted 20 percent effect level.

If multiple toxicity thresholds were identified for a species, the species mean toxicity threshold was calculated as the geometric mean of the available toxicity thresholds (consistent with EPA (USEPA 1985) guidance for deriving AWQC). The geometric mean was calculated only for toxicity thresholds in the same endpoint category (e.g., growth). Within a species and endpoint, only the strongest (i.e., most reliable) toxicity thresholds relative to the targeted 20 percent effect level were considered in defining the sensitivity of a species. For example, if there was a reliable EC20 from one test and a “greater than” toxicity threshold from another test, the EC20 was used to define the sensitivity of that species.

2.7 TOXICITY REFERENCE VALUE DEVELOPMENT

Once concentration- and dose-based toxicity thresholds had been compiled for each metal, and species mean values calculated as necessary, TRVs were defined as either the 5th percentile of the species sensitivity distribution (SSD) of species mean toxicity thresholds, or as the lowest species mean toxicity threshold. An SSD-based approach was used when diet-borne toxicity data were available for at least eight species. The 5th percentiles of the SSDs were derived following EPA (USEPA 1985) guidance for AWQC derivation.

2.8 UNCERTAINTIES

In this document, key uncertainties are noted in describing the TRVs for each metal; these uncertainties may include factors such as the number of species that have been tested, consistency of results among tests, and any test-specific uncertainties for those that are the drivers of the TRVs. For EC20s and ED20s calculated using TRAP, 90 percent confidence intervals (CIs) are provided to quantify uncertainty in those values. For those metals that have more extensive diet-borne toxicity data, figures comparing all concentration-response data to the concentration-based TRV provide an overview of the protectiveness of the TRV relative to all data. The risk characterization for fish in the BERA, including the diet-borne metals line of evidence, will incorporate an evaluation of uncertainty.

3 RESULTS

All compiled toxicity data for individual metals, including test information and concentration-response and dose-response data, are provided in Table A-1 in Appendix A. For those tests lacking dosing information, the data used to develop an assumed FIR of 3.7 percent of body weight per day are documented in Table A-2. As discussed, diet-borne TRVs are based on survival, growth, and reproduction endpoints; other endpoints evaluated in each study are listed in Table A-3 of Appendix A for information purposes and are summarized in Figures A-1 through A-10, along with the TRVs, for comparative purposes.

The following subsections are organized by metal. Each section begins with a summary of the available diet-borne toxicity data for that metal, and the resulting concentration- and dose-based TRVs are provided. Details on key studies and a description of how the TRVs were derived follow.

3.1 ALUMINUM

3.1.1 Summary

The diet-borne toxicity of aluminum to fish has been tested in two fish species, Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). Test information extracted from these studies are presented in Table A-1 of Appendix A. No adverse effects of diet-borne aluminum on fish have been documented at any concentrations tested to date, precluding the calculation of EC20s. The highest diet-borne aluminum concentration tested in Atlantic salmon was 2,232 mg/kg_{diet dw}, which was associated with a 0 percent growth effect (weight gain) relative to the negative control. Dosing data were not reported, so a dose-based TRV of > 82.5 mg/kg bw/day based on an assumed FIR of 0.037 g_{food}/g bw/day was calculated (Section 2.6).

Concentration-based TRV: > 2,232 mg/kg_{diet dw}

Dose-based TRV: > 82.5 mg/kg bw/day

Because this TRV is associated with a 0 percent effect level, it is useful for interpreting diet-borne aluminum concentrations that are less than this concentration. However, any diet-borne aluminum concentrations greater than this TRV would be associated with uncertain potential risk, because the aluminum concentration that might result in a 20 percent effect level is unknown.

3.1.2 Key Studies

Young Atlantic salmon (initial mean weight was 4.8 g) were provided a formulated diet with nominal aluminum chloride added, resulting in aluminum concentrations ranging from 25 to 2,000 mg/kg ww (Poston 1991). Because the diet was comprised of about 90 percent dry matter, this corresponded to an aluminum concentration range of 28 to 2,232 mg/kg dw. Fish were fed according to a hatchery constant of 6.0, with the amount of food increased every 2 weeks over the 16-week exposure duration. The hatchery constant considered the feed conversion and length of the fish, and thus could not be converted into a FIR and used to derive the aluminum dose. Neither growth (weight gain) nor survival were significantly reduced ($p > 0.05$) relative to the control in any of the aluminum treatments. The results were a NOEC of 2,232 mg/kg_{diet} dw (Figure 3-1) and an estimated NOEL of 82.5 mg/kg bw/day based on an assumed FIR of 0.037 g_{food}/g bw/day (Section 2.6).

Handy (1993a) evaluated the accumulation of diet-borne aluminum in rainbow trout (initial mean weight was 171 g) through the addition of aluminum sulfate to the diet. A single nominal aluminum concentration of 10,000 mg/kg dw in a commercial diet was tested. Fish were fed to satiation once per day for up to 42 days. The author noted that fish consumed about 5 percent of their body weight per day, although that percentage may have been overstated in the aluminum treatment, because the fish tended to regurgitate a portion of the food ingested. Although the focus of the study was on aluminum bioaccumulation in the fish, Handy (1993a) also reported that only a single fish died, and that body weights were maintained. The diet-borne NOEC for survival, therefore, was 10,000 mg/kg_{diet} dw. The associated dose-based NOEC was not calculated due to uncertainty in the true FIR of the fish. The fish retained only ≤ 1 percent of the total aluminum, thus demonstrating the low bioavailability of diet-borne aluminum.

Although empirical data on the bioavailability of aluminum in natural diets are lacking, it is reasonable that the tests using both forms of aluminum employed in the studies described above (i.e., aluminum chloride and aluminum sulfate) err on the side of higher bioavailability and thus are conservative. As noted in Wilson (2012), bioaccumulation and toxicity of aluminum to aquatic fish are unlikely based on laboratory studies, as well as field studies, in which there is a lack of aluminum biomagnification in invertebrates, even in those invertebrates that are expected to be food items for fish in waters that are acidic and have high aluminum concentrations.

3.1.3 Selection of Aluminum TRVs

Because diet-borne aluminum toxicity data were available for only two species, the diet-borne concentration- and dose-based TRVs were based on the lowest species mean toxicity

thresholds of 2,232 mg/kg_{diet} dw and 82.5 mg/kg bw/day, respectively, for Atlantic salmon. Because this concentration and dose were a NOEC and NOEL, respectively, associated with a 0 percent effect, the TRVs were defined as > 2,232 mg/kg_{diet} dw and > 82.5 mg/kg bw/day. A dose-based TRV could not be derived.

The aluminum TRV has high uncertainty due to the limited toxicity data available. However, the diet-borne pathway does not appear to be important for aluminum and fish. Poston (1991) did not observe statistical differences in aluminum concentrations in carcasses of Atlantic salmon fed nominal, added aluminum concentrations ranging from 0 to 2,232 mg/kg_{diet} dw, suggesting that aluminum bioavailability from the diet is low. There is increased uncertainty in the dose-based TRV because aluminum doses were estimated based on an assumed ingestion rate.

3.2 ARSENIC

3.2.1 Summary

All the studies on diet-borne arsenic toxicity to fish have been conducted with rainbow trout; Table A-1 in Appendix A provides information extracted from these studies. Diet-borne toxicity data were available from studies in which food items (oligochaetes) were exposed to waterborne arsenite [As(III)] and arsenate [As(V)], and from studies that used formulated diets. The concentration- and dose-response data were consistent among studies, so the data were pooled to derive concentration- and dose-based TRVs.

Concentration-based TRV: 42 mg/kg_{diet} dw

Dose-based TRV: 0.78 mg/kg bw/day

3.2.2 Key Studies

In the first of a series of four studies on diet-borne arsenic toxicity to juvenile rainbow trout, Cockell and Hilton (1988) fed juvenile rainbow trout semi-purified diets spiked with either arsenite (as arsenic trioxide) or arsenate (as disodium arsenate). They also tested diets spiked with dimethylarsenic acid (a common product of inorganic arsenic detoxification in some biological systems) and arsanilic acid (used in swine and poultry diets to promote growth). The latter two arsenic forms were not included in this review, because they were assumed to be irrelevant to forms of arsenic in the diets of UCR fish. Fish were fed—to satiation 4 times per day for 8 weeks—concentrations ranging from 180 to 1,477 mg/kg_{diet} dw of arsenite or 137 to 1,053 mg/kg_{diet} dw of arsenate. All diet-borne concentrations of arsenite and arsenate resulted in significantly reduced ($p \leq 0.05$) weight gain relative to the controls, and an EC20 of

150 mg/kg_{diet} dw (90 percent CI not available)⁴ was derived for arsenite (a reliable EC20 could not be derived for arsenate) (Figure 3-2A). Arsenic doses provided in the study resulted in an ED20 of 2.8 mg/kg bw/day (90 percent CI = 2.4 to 3.2 mg/kg bw/day) for arsenite (again, however, a reliable ED20 could not be derived for arsenate) (Figure 3-3A).

As a follow-up to Cockell and Hilton (1988), Cockell et al. (1991) evaluated the diet-borne toxicity of arsenate (as disodium arsenate) to juvenile rainbow trout over a longer exposure period of 24 weeks. A semi-purified diet was again used, and fish were fed to satiation three to four times daily. The EC20 and ED20 based on the diet-borne concentrations and doses were 44 mg/kg_{diet} dw (90 percent CI = 41 to 48 mg/kg_{diet} dw) and 0.86 mg/kg bw/day (90 percent CI = 0.79 to 0.93 mg/kg bw/day), respectively (Figures 3-2B and 3-3B).

In a third study, Cockell et al. (1992) evaluated the hepatobiliary and hematological effects of diet-borne arsenate (as disodium arsenate) on juvenile rainbow trout; as part of this study, weight gain was also reported. Fish were fed, to near satiation, a semi-purified diet amended with arsenate three to four times per day, for 12 weeks. Although only two arsenate concentrations were tested, which imparted greater uncertainty in deriving an EC20, the EC20 was 42 mg/kg_{diet} dw (90 percent CI = not available) (Figure 3-2C), which was comparable to the EC20 of 44 mg/kg_{diet} dw from Cockell et al. (1991). The dose-based ED20 was 0.41 mg/kg bw/day (90 percent CI = not available) (Figure 3-3C), approximately one-half of that derived from Cockell et al. (1991).

Cockell and Bettger (1993) conducted a fourth study that evaluated gall bladder pathology in juvenile rainbow trout exposed to arsenate (as disodium arsenate), again reporting the influence of arsenate on weight gain. Fish were fed the control diet and a diet with an arsenate concentration of 58 mg/kg dw to near satiation three to four times per day for 12 weeks. Fish fed the arsenic diet had significantly reduced ($p \leq 0.05$) weight gain relative to the control. However, an EC20 could not be derived because only a single treatment concentration was tested.

Given the similarity of responses in the studies conducted by Cockell and colleagues (Cockell et al. 1991, 1992; Cockell and Bettger 1993; Cockell and Hilton 1988), these data were pooled in order to derive a pooled EC20 and ED20. Because weight gain was reported based on different exposure durations and fish of different sizes at test initiation, weight gain in each arsenic treatment was first adjusted as a percentage of the respective controls. The pooled data resulted in consistent concentration response relationships, an EC20 of 38 mg/kg_{diet} dw

⁴ TRAP provided lower and upper 95 percent confidence limits that were equal to the EC20.

(90 percent CI = 33 to 43 mg/kg_{diet} dw) (Figure 3-2D), and an ED20 of 0.59 mg/kg bw/day (90 percent CI = 0.35 to 1.0 mg/kg bw/day) (Figure 3-3D).

More recently, Erickson et al. (2010, 2011) evaluated the diet-borne toxicity of arsenic biologically incorporated into oligochaete worms (*Lumbriculus variegatus*). Erickson et al. (2010) fed juvenile rainbow trout worms that had been exposed to either waterborne arsenite or arsenate. The resulting diet-borne arsenic concentrations were 28 and 61 mg/kg dw in oligochaetes exposed to waterborne arsenite concentrations of 1,500 and 3,200 µg/L, respectively, and 40 and 76 mg/kg dw in oligochaetes exposed to waterborne arsenate concentrations of 3,900 and 7,900 µg/L, respectively. Growth was reduced in all treatments (Figures 3-2E and 3-3E). Erickson et al. (2011) tested a greater number of diet-borne arsenic treatments, as well as combined diet-borne and waterborne arsenic exposures. No reliable EC20 or ED20 could be calculated, but growth decreased as diet-borne arsenic concentrations and doses increased (Figures 3-2F and 3-3F). The waterborne arsenic concentrations combined with the diet-borne concentrations were very high, ranging from 8,400 to 33,000 µg/L. Given that added toxicity was observed at these very high concentrations, the tests with combined diet-borne and waterborne arsenic exposures were excluded from TRV development.

3.2.3 Selection of Arsenic TRVs

The arsenate data from all four studies conducted by Cockell and colleagues (Cockell et al. 1991, 1992; Cockell and Bettger 1993; Cockell and Hilton 1988) and all data from Erickson et al. (2010, 2011) were pooled, resulting in diet-borne and dose-based TRVs of 42 mg/kg_{diet} dw (90 percent CI = 37 to 49 mg/kg_{diet} dw) and 0.78 mg/kg bw/day (90 percent CI = 0.53 to 1.1 mg/kg bw/day), respectively (Figures 3-4A and 3-4B). The diet-borne arsenite data from Cockell and Hilton (1988) were excluded, because this form of arsenic was less toxic in the semi-purified diet. In contrast, Erickson et al. (2010) observed that the form of waterborne arsenic to which oligochaetes were exposed had negligible influence on toxicity (i.e., levels of response followed a similar pattern regardless of form) when the worms were fed to rainbow trout (Figures 3-2E and 3-3E). Erickson et al. (2011) reported that absorbed arsenate was largely reduced to arsenite, with no measurable conversion to organoarsenic species. This suggests that total arsenic toxicity to a fish will be similar, regardless of the form of arsenic in the water column to which its food items are exposed.

The concentration- and dose-based arsenic TRVs have high uncertainty because data are only available for rainbow trout. However, the sensitivity of rainbow trout to diet-borne arsenic has low uncertainty due to consistency in the concentration- and dose-response data from multiple laboratories and diet types (synthetic and natural).

3.3 CADMIUM

3.3.1 Summary

Studies on diet-borne cadmium toxicity were identified for eight freshwater fish species; information from these studies is presented in Table A-1 of Appendix A. Rainbow trout was the species most extensively studied, with diet-borne cadmium toxicity data available from a total of eight individual tests. The diet-borne toxicity thresholds for rainbow trout were variable, with several tests showing no significant effects at the highest concentration tested. Ultimately, a single test from which an EC20 and ED20 could be derived was used to represent the sensitivity of rainbow trout. (Section 3.3.2 details how the results from this test relate to those from other tests with rainbow trout). A southeast Asia hybrid catfish (*Clarias macrocephalus* × *C. gariepinus*) had the lowest diet-borne cadmium toxicity threshold. Because toxicity data were available for eight fish species, the 5th percentile of the SSD was used to derive the concentration- and dose-based TRVs.

Concentration-based TRV: 8.1 mg/kg_{diet} dw

Dose-based TRV: 0.023 mg/kg bw/day

3.3.2 Key Studies

The fish species tested to date are generally insensitive to diet-borne cadmium, with toxicity thresholds in the hundreds to thousands of mg/kg dw. The key sensitive species for TRV consideration are rainbow trout and the southeast Asia hybrid catfish. The latter is obviously not native to North America, but the possibility that it could be representative of North American species cannot be dismissed (i.e., there is no *a priori* basis for why it may be more or less sensitive to diet-borne cadmium).

The sensitivity of rainbow trout to diet-borne cadmium is highly variable. In one test, significant effects were observed at a diet-borne concentration of 11.6 mg/kg_{diet} dw as cadmium nitrate, while in another test, no significant effects were observed at a diet-borne concentration of 519 mg/kg_{diet} dw as cadmium nitrate. Some of the differences may be attributable to whether the diet was formulated and spiked with cadmium, or contained biologically incorporated cadmium. However, relatively high no-effect thresholds were observed in tests using both diet types. Other differences may be attributable to the calcium concentration in the diet, because increased calcium concentrations limit the uptake of cadmium. This effect was apparent in one study that explicitly evaluated the influence of calcium, but results were not consistent with other studies of the same diet.

The lowest rainbow trout LOEC of 11.6 mg/kg_{diet} dw (associated with a 90 percent reduction in growth relative to control) was based on a study that evaluated the effects of diet-borne cadmium in a low-calcium diet (0.8 mg/g dw) versus a high-calcium diet (76.2 mg/g dw) (Ng et al. 2009). No statistically significant ($p > 0.05$) effects were observed when trout fed a diet-borne cadmium concentration of 9.6 g/kg_{diet} dw were provided the high-calcium diet (associated with a 22 percent reduction in growth relative to the control) (Table 3-1). The diet consisted of oligochaete worm pellets that had been amended with cadmium nitrate (and calcium for the high-calcium treatment). For comparison to the calcium concentrations in the test diets, calcium concentrations in potential UCR food items include means of 146 (range was 105 to 173 mg/g dw in crayfish) (Bott 2015); 191 (range was 130 to 252 mg/g dw in crayfish based on a mean moisture content of 77 percent) (Windward 2018); 52 (range was 21 to 106 mg/g dw in mussels based on a mean moisture content of 91 percent) (Windward 2018); and 30 (range was 3 to 62 mg/g dw in fish) (CH2M HILL and E&E 2007; Exponent et al. 2013). Thus, the calcium concentrations in UCR crayfish exceed the high-calcium diet tested by Ng et al. (2009), and although the calcium concentrations in UCR fish do not exceed the high-calcium diet, the concentrations are well above the low-calcium diet of concentration of 0.8 mg/g dw.

In other rainbow trout studies where cadmium was biologically incorporated into an oligochaete diet (based on oligochaete exposure to waterborne cadmium), there were no significant effect concentrations that were much greater than 11.6 mg/kg_{diet} dw (Table 3-1). One of these studies, Ng and Wood (2008), reported the only bounded NOEC and LOEC values available for rainbow trout—13.8 (0 percent effect) and 189.4 (50 percent effect) mg/kg dw, respectively. For this test, TRAP was used to derive an EC20 of 67.0 mg/kg_{diet} dw (90 percent CI = not available) and an ED20 of 0.36 mg/kg bw/day (90 percent CI = not available) (Table 3-1). Although the model cautioned that the results should only be used for exploratory analysis, these values are considered to provide the best estimate of the toxicity threshold from this test. Potential alternatives for deriving the threshold from this test would have resulted in either a similar or a less conservative threshold. For example, use of the geometric mean of the NOEC and LOEC (51 mg/kg_{diet} dw) would have resulted in a similar threshold but with an undefined level of effect, and use of the LOEC alone (189 mg/kg_{diet} dw) would have resulted in a higher threshold and a 50 percent level of effect.

The most sensitive species was the southeast Asia hybrid catfish, which had a LOEC of 16.48 mg/kg_{diet} dw and a LOEL of 0.124 mg/kg bw/day based on a 19 percent reduction in growth compared to the control (a corresponding NOEC was not available). The next highest diet-borne cadmium concentration tested was 56.6 mg/kg_{diet} dw, which resulted in a 22 percent reduction in growth relative to the control. The diet was composed of cladocerans (*Moina*

macrocopa) that had been exposed to waterborne cadmium. Using TRAP, an EC20 of 8.9 mg/kg_{diet} dw (90 percent CI = not available) was derived, but the model again cautioned that the results should only be used for exploratory analysis. Because the LOEC and LOEL were associated with a 19 percent level of effect, the LOEC of 16.48 mg/kg_{diet} dw and LOEL of 0.124 mg/kg bw/day were assumed to provide reasonable approximations of the EC20 and ED20 for the hybrid catfish.

3.3.3 Selection of Cadmium TRVs

Cadmium toxicity thresholds were available for eight species; therefore, the concentration-based TRV of 8.1 mg/kg_{diet} dw and dose-based TRV of 0.023 mg/kg bw/day were based on the 5th percentiles of their respective SSDs (Figure 3-5 data points are based on the species mean diet-borne concentrations and doses in Table 3-1.).

Uncertainty in the protectiveness of the concentration- and dose-based cadmium TRVs is considered low. With diet-borne toxicity data available for eight species, cadmium is unique among most metals analyzed, which have data for one or two species or none at all. The sensitivity of fish to diet-borne cadmium is highly variable, with toxicity thresholds ranging over two orders of magnitude (Figure 3-5). When considering all of the concentration-response data compiled, no adverse responses were observed in all of the treatments that tested cadmium concentrations less than the diet-borne TRV of 8.1 mg/kg_{diet} dw (Figure 3-6).

Furthermore, as noted, the most sensitive species was a southeast Asia hybrid catfish. A diet-borne cadmium concentration of 16.48 mg/kg_{diet} dw (dose of 0.124 mg/kg bw/day) was associated with a 19 percent reduction in wet weight, while a diet-borne concentration of 56.6 mg/kg_{diet} dw (dose of 0.425 mg/kg bw/day) was associated with a slightly higher 22 percent reduction in wet weight. For comparison, the second most sensitive species, rainbow trout, had an EC20 of 67 mg/kg_{diet} dw (dose of 0.36 mg/kg bw/day). The cadmium TRVs were based on the more conservative interpretation of the southeast Asia catfish hybrid data — the lower concentration associated with a 19 percent effect. Although the level of uncertainty pertaining to the rainbow trout dietary toxicity threshold is high, the selected TRV is sufficiently conservative to account for that uncertainty.

3.4 CHROMIUM(III)

3.4.1 Summary

For diet-borne chromium as chromium(III), toxicity data are limited to three fish species: carp (*Cyprinus carpio*), rainbow trout, and tilapia (*Oreochromis niloticus* x *O. aureus*). These studies were designed to identify a diet-borne chromium concentration to optimize growth in support

of aquaculture, but they can still be useful for identifying toxicity thresholds because they were likewise designed to identify concentrations that have a negative influence on growth. Information extracted from the chromium toxicity studies is presented in Table A-1 of Appendix A. Based on the limited diet-borne toxicity data available for chromium(III), the concentration-based TRV was set equal to a diet-borne chromium concentration of 3.9 mg/kg_{diet} dw, which was associated with a 20 percent effect level in juvenile carp; the dose-based TRV was set equal to the corresponding dose of 0.074 mg/kg bw/day.

Concentration-based TRV: 3.9 mg/kg_{diet} dw

Dose-based TRV: 0.074 mg/kg bw/day

3.4.2 Key Studies

Ahmed et al. (2012) fed juvenile carp formulated diets with chromium(III) concentrations of 1.1 (control), 2.0, and 3.9 mg/kg_{diet} dw (or 0.69, 1.2, and 2.5 mg/kg ww) (as chromium chloride) for 8 weeks. Based on a study-specific FIR of 0.03 g_{food}/g bw/day, these diet-borne chromium(III) concentrations, on a wet weight basis, resulted in doses of approximately 0.021, 0.037, and 0.074 mg/kg bw/day, respectively. The highest diet-borne concentration/dose resulted in a 20 percent reduction in the carp growth rate, which was statistically significant ($p < 0.05$) compared to the control.

In a similar study, Tacon and Beveridge (1982) fed juvenile rainbow trout formulated diets with chromium(III) concentrations of 1.7 (control), 2.7, 6.5, and 9.1 mg/kg dw (as chromium chloride) for 8 weeks. Based on the reported FIRs in each treatment, along with the median of the initial and final body weights, diet-borne chromium(III) doses of 0.04, 0.06, 0.14, and 0.22 mg/kg bw/day, respectively, were calculated. The highest concentration/dose significantly reduced ($p < 0.05$) the growth rate of the fish. The EC20 and ED20 based on diet-borne concentrations and doses were 8.1 mg/kg_{diet} dw (90 percent CI = 0.80 to 81 mg/kg_{diet} dw) and 0.17 mg/kg bw/day (90 percent CI = 0.011 to 2.7 mg/kg bw/day), respectively (Figure 3-7).

In a third 8-week growth study, Shiau and Lin (1993) fed a tilapia hybrid formulated diets containing either 40 percent glucose or 40 percent starch. A diet-borne chromium(III) concentration of 1.8 mg/kg_{diet} dw (as chromium chloride) and a corresponding chromium(III) dose of 0.09 mg/kg bw/day in each diet type increased weight gain by 18 percent and 8 percent, for the 40 percent glucose diet and 40 percent starch diet, respectively, relative to their respective controls.

3.4.3 Selection of Chromium TRVs

An EC20 of 8.1 mg/kg_{diet} dw and ED20 of 0.17 mg/kg bw/day were calculated for rainbow trout. An EC20 and ED20 could not be derived for carp due to insufficient concentration- and dose-response data, but the highest tested concentration of 3.9 mg/kg_{diet} dw (dose of 0.074 mg/kg bw/day) resulted in a 20 percent reduction in growth. Because the concentration and dose for carp were less than the EC20 and ED20 calculated for rainbow trout, and because the former were based on a 20 percent effect level, the concentration- and dose-based dietary chromium(III) TRVs were set equal to 3.9 mg/kg_{diet} dw and 0.074 mg/kg bw/day, respectively.

Diet-borne chromium toxicity data for fish are limited to three species. The tests for these three species were all conducted using laboratory-formulated diets spiked with chromium(III) chloride, so diet-borne chromium toxicity in a natural diet has not been evaluated to date. Uncertainty in the chromium TRV (3.9 mg/kg_{diet} dw and 0.074 mg/kg bw/day based on carp) is high due to the limited data available, but uncertainty in its protectiveness is considered low, because a slightly lower diet-borne concentration and dose (2.0 mg/kg_{diet} dw and 0.037 mg/kg bw/day, respectively) resulted in enhanced carp growth (Ahmed et al. 2012). Furthermore, the concentration- and dose-based TRVs are less than a chromium concentration (3.9 mg/kg_{diet} dw) and dose (0.13 mg/kg bw/day) that did not result in any effects on the growth of rainbow trout (Table A-1).

3.5 COPPER

3.5.1 Summary

Studies on diet-borne copper toxicity were identified for eight freshwater fish species; Table A-1 of Appendix A presents information derived from these studies. Several species, however, only had “greater than” NOECs that were associated with low levels of effect relative to the control (Table 3-2). Because the species with toxicity thresholds based on no-effect concentrations were the lowest in the SSD, the concentration-based TRV was based on the lowest species mean EC20 of 666 mg/kg_{diet} dw for Atlantic salmon, rather than the 5th percentile of the SSD. The dose-based copper TRV was set equal to < 21.2 mg/kg bw/day, which was the dose that resulted in an effect ≥ 20 percent in common carp (in this case, a 36 percent reduction in growth).

Concentration-based TRV: 666 mg/kg_{diet} dw

Dose-based TRV: < 21.2 mg/kg bw/day

3.5.2 Key Studies

The three lowest concentration-based toxicity thresholds for species in the SSD were all based on NOECs and NOELs (Figure 3-8 data points are based on the species mean diet-borne concentrations and doses in Table 3-2). The species with the lowest concentration-based threshold was the fathead minnow (*Pimephales promelas*), which had NOECs of 156, 156, and 216 mg/kg_{diet} dw associated with growth reductions of 0, 2, and 0 percent, respectively, relative to their respective controls (Table 3-2). In all three tests, minnows were fed an oligochaete diet in which worms had previously been exposed to waterborne copper (as copper sulfate). The species with the second-lowest concentration-based threshold in the SSD was channel catfish, which had a NOEC of 40 mg/kg_{diet} dw based on a formulated diet containing copper sulfate, and a NOEC of 246 mg/kg_{diet} dw based on a diet of oligochaetes that had been exposed to copper sulfate. These two NOECs were based on growth reductions of 2 and 3 percent, respectively, compared to their respective controls (Table 3-2). The species with the third-lowest concentration-based threshold in the SSD was a hybrid striped bass (*Morone chrysops* x *Morone saxatilis*), which had a NOEC of 571 mg/kg_{diet} dw (as copper sulfate) in both freshwater- and saltwater-acclimated fish. This NOEC was associated with growth reductions of 16 and 17 percent relative to controls in freshwater and saltwater striped bass, respectively (Table 3-2).

The most sensitive species for which effects greater than 20 percent were observed was Atlantic salmon. A concentration-based EC20 of 666 mg/kg_{diet} dw (90 percent CI = 309 to 1,434 mg/kg_{diet} dw) and a dose-based ED20 of 22.6 mg/kg bw/day (90 percent CI = 10.5 to 48.7 mg/kg bw/day) were derived from Berntssen et al. (1999a) based on exposure to copper sulfate in the diet (Figure 3-9).

Definitive dose-based toxicity thresholds (i.e., those not based on “greater than” no-observed-effect levels [NOELs]) expressed as species mean values ranged from 21.2 to 72.8 mg/kg bw/day among five fish species. The lowest toxicity threshold was for carp, which were fed formulated diets with copper concentrations of 1,000 and 2,000 mg/kg_{diet} dw (as copper sulfate) at rates of 0.0108 and 0.0106 g_{diet}/g bw/day, respectively, for 42 days (Ajani and Akpoilih 2012). This regimen resulted in doses of 10.8 and 21.2 mg/kg bw/day. The lower dose was a NOEL at which weight gain increased relative to controls, and the higher dose was a lowest-observed-effect level (LOEL) at which weight gain significantly reduced (by 36 percent) relative to controls ($p < 0.05$). Because it was not possible to estimate a 20 percent effect level from these data, the dose-based toxicity threshold from this test was set equal to the dose that resulted in a 36 percent reduction in growth relative to controls (21.2 mg/kg bw/day).

3.5.3 Selection of Copper TRVs

Concentration- and dose-based SSDs were initially developed because diet-borne toxicity data were available for eight species; however, the three lowest species mean toxicity values were all NOECs and NOELs associated with low levels of effect (Figure 3-8; Table 3-2). Because the 5th percentiles of the SSDs were driven by no-effect concentrations or doses, the concentration- and dose-based TRVs for copper were selected based on toxicity data for the most sensitive species with effects data equal to or near the targeted effect level of 20 percent. The resulting concentration-based copper TRV was based on the EC20 of 666 mg/kg_{diet} dw for Atlantic salmon, and the dose-based copper TRV was set equal to < 21.2 mg/kg bw/day based on the dose that resulted in an effect ≥ 20 percent in common carp (in this case, a 36 percent reduction in growth). The ED20 of 22.6 mg/kg bw/day for Atlantic salmon was greater than the ED20 of < 21.2 mg/kg bw/day, so the latter was conservatively identified as the dose-based copper TRV.

Diet-borne copper toxicity data were compiled for eight fish species, including two species of salmonids (Atlantic salmon and rainbow trout). The rainbow trout dataset was particularly robust, with a total of 13 studies identified. It was possible to calculate EC20 and ED20 values for both salmonid species, which were similar (Figure 3-8; Table A-1). In addition, diet-borne copper EC20 values were similar between rainbow trout provided formulated diets and those provided brine shrimp diets amended with copper. Uncertainty in the protectiveness of the concentration- and dose-based copper TRVs is considered low, given the number of species tested and the consistency of the concentration- and dose-response data.

When considering all of the concentration-response data, the only treatments with effects greater than 20 percent relative to the control at diet-borne concentrations less than the TRV of 666 mg/kg_{diet} dw were from the rainbow trout study by Julshamn et al. (1988) (Figure 3-10). The test described in Julshamn et al. (1988) was 1 of 16 growth tests with rainbow trout compiled in the present evaluation. In that study, high variability in trout growth was observed in fish fed the control and copper-amended diets, which resulted in a NOEC and LOEC of 810 and 990 mg/kg_{diet} dw, respectively. This, and all of the data compiled, indicates that the diet-borne TRV is protective of the targeted level of protection.

3.6 IRON

No diet-borne TRV was developed for iron due to the lack of data. However, as a point of comparison, iron requirements in fish range from 30 to 170 mg/kg_{diet} dw (Watanabe et al. 1997). Iron is not expected to be a constituent of concern in fish diets.

Concentration-based TRV: not available

Dose-based TRV: not available

3.7 LEAD

3.7.1 Summary

Diet-borne lead toxicity data were identified for four fish species, as presented in Table A-1 of Appendix A. Consequently, the concentration- and dose-based lead TRVs were based on the most sensitive endpoint and species, which was a 21 percent reduction in juvenile rainbow trout growth relative to the control.

Concentration-based TRV: 111 mg/kg_{diet} dw

Dose-based TRV: 1.9 mg/kg bw/day

3.7.2 Key Studies

Few studies have observed diet-borne lead toxicity in fish at the concentrations and doses tested. When evidence of toxicity has been observed, there have been conflicting data within the same species.

Alsop et al. (2016) fed juvenile rainbow trout oligochaete worms previously exposed to waterborne lead concentrations of 0.1 (control), 6.0, 11.7, 53.5, or 123.8 µg/L (as lead nitrate). In one test, rainbow trout were fed the lead diets in control water (the diet-only test); in a second test, rainbow trout were simultaneously exposed to diet-borne lead and the water to which the fish's food had been exposed (the diet+water test). The exposure duration was 7 weeks in both tests. In the diet-only test, the growth rate was reduced by 29 percent relative to the control at the highest diet-borne concentration tested (728 mg/kg_{diet} dw; dose of 12.4 mg/kg bw/day); in the diet+water test, the growth rate was reduced by 21 percent relative to the control at the same diet-borne concentration (728 mg/kg_{diet} dw) and a waterborne concentration of 119.5 µg/L (Figure 3-11). None of these growth reductions was statistically significant relative to the control ($p > 0.05$). An EC20 of 111 mg/kg_{diet} dw and ED20 of 1.9 mg/kg bw/day could be calculated from the diet-only test, but an EC20 could not be calculated from the diet+water test (Meaningful CIs on the EC20 and ED20 could not be calculated given the low level of effects across all lead treatments.). Alsop et al. (2016) noted that diet-borne lead exposure may have protected juvenile rainbow trout from bioaccumulation via waterborne exposures, and from disruption of sodium ion homeostasis.

The maximum surface water lead concentration measured in the UCR in 2009/2010 was 0.329 µg/L (Exponent and Parametrix 2013), a value less than the lowest waterborne lead concentration of 8.2 µg/L to which rainbow trout were exposed in the diet+water test (and similar to the control). As such, it was concluded that the diet-only test was more appropriate for the development of diet-borne lead toxicity thresholds for the UCR (an EC20 of

111 mg/kg_{diet} dw and an ED20 of 1.9 mg/kg bw/day). Given the nature of the concentration- and dose-response data, these EC20 and ED20 values appear to be conservative, as a diet-borne concentration of 309 mg/kg_{diet} dw and a dose of 5.4 mg/kg bw/day were associated with growth rate reductions of 14 percent (i.e., < 20 percent).

In Nile tilapia, Ayyat et al. (2003) reported statistically significant ($p < 0.001$) 9 and 17 percent reductions in weight relative to the control when fish (6.7 g) were fed formulated diets with lead concentrations of 349 and 667 mg/kg_{diet} dw (as lead oxide), respectively, for 4 months. The corresponding lead doses for these diet-borne concentrations were 3 and 6 mg/kg bw/day, respectively.

In contrast, Dai et al. (2009) did not observe any significant effects ($p > 0.05$) on Nile tilapia growth rate when fish (~32 g) were fed a formulated diet with concentrations of lead (in an unreported form) up to 800 mg/kg_{diet} dw for 60 days; weight gain was reduced by 2 to 6 percent relative to the control. It is possible that a growth effect was observed by Ayyat et al. (2003), because those fish were smaller (i.e., younger) at test initiation (6.7 g vs. ~32 g) and the duration was longer (4 months vs. 60 days). Thus, the sensitivity of Nile tilapia was set equal to the diet-borne lead concentration and dose that resulted in a 17 percent reduction in weight (667 mg/kg_{diet} dw and 6 mg/kg bw/day), because this was a close approximation of a 20 percent effect level (Table 3-3).

3.7.3 Selection of Lead TRVs

The concentration-based diet-borne lead TRV was set equal to the EC20 of 111 mg/kg_{diet} dw from the diet-only test in Alsop et al. (2016), and the dose-based TRV was set equal to the corresponding ED20 of 1.9 mg/kg bw/day (Figure 3-11). As noted, these EC20 and ED20 values are conservative relative to the empirical data due to the nature of the concentration- and dose-response curves (in which a higher concentration and dose result in effects < 20 percent). Furthermore, with one exception, the lowest diet-borne lead concentration resulting in a more than 20 percent reduction in growth relative to controls for any of the four fish species with diet-borne lead toxicity data was 728 mg/kg_{diet} dw (which was not significantly different [$p > 0.05$] from the control) (Figure 3-12). The one exception was in the rainbow trout diet+water exposure reported in Alsop et al. (2016), in which a 21 percent reduction in growth rate was observed at a diet-borne concentration of 35.3 mg/kg_{diet} dw (waterborne lead concentration was 8.2 µg/L), although no reductions in growth rate were observed at the next two highest diet-borne concentrations of 68.8 mg/kg_{diet} dw (20.2 µg lead/L) and 315 mg/kg_{diet} dw (68.7 µg/L). As such, the lead TRVs of 111 mg/kg_{diet} dw and 1.9 mg/kg bw/day are considered to be conservative.

3.8 MANGANESE

A diet-borne TRV was not developed for manganese due to a lack of data. Manganese requirements in fish ranged from 2 to 20 mg/kg_{diet} dw (Watanabe et al. 1997). As found for iron, manganese is not expected to be a constituent of concern in fish diets.

Concentration-based TRV: not available

Dose-based TRV: not available

3.9 MERCURY

3.9.1 Summary

Most of the mercury in fish is methylmercury, which most wild fish obtain from their food (Sandheinrich and Wiener 2011). As such, diet-borne TRVs for mercury were developed based on toxicity data for methylmercury. A recent review by Depew et al. (2012) provided a detailed examination of diet-borne methylmercury toxicity data for fish and a recommendation for a diet-borne methylmercury toxicity threshold. That review afforded the initial basis for the concentration- and dose-based TRVs developed for methylmercury. Readers are referred to that study for a broader review of diet-borne methylmercury toxicity data for fish; the key studies used for TRV development are discussed below.

Concentration-based TRV: 0.26 mg/kg_{diet} dw

Dose-based TRV: 0.013 mg/kg bw/day

3.9.2 Key Studies

Depew et al. (2012) compiled methylmercury toxicity data based on endpoints of survival, growth, development, reproduction, and altered biochemical and behavioral effects. Ultimately, Depew et al. (2012) determined that the highest NOEC and lowest LOEC for reproductive effects following diet-borne exposure to methylmercury were 0.04 and 0.05 mg/kg_{diet} ww, respectively. The authors recommended 0.04 mg/kg_{diet} ww as a diet-borne toxicity threshold for methylmercury.

The NOEC of 0.04 mg/kg_{diet} ww, as tabulated by Depew et al. (2012), was based on measurements of the gonadosomatic index (GSI) in walleye (*Sander vitreus*) fed methylmercury in a synthetic diet. The study was described by Friedmann et al. (1996), and the diet-borne concentration of 0.04 mg/kg_{diet} ww (as mercury) was the concentration in the control diet; thus, this concentration was not truly a NOEC as defined by Depew et al. (2012). Furthermore, 0.04 mg/kg_{diet} ww was actually a “less than” concentration, because

methylmercury was not detected at this detection limit. The methylmercury treatment concentrations tested were 0.137 and 0.987 mg/kg_{diet} ww (as mercury). None of the two methylmercury treatments resulted in a GSI value with a significantly lower mean than the control ($p > 0.05$), although the authors noted that the mean GSI value was significantly lower than the control when the two methylmercury treatments were pooled, thereby increasing statistical power. However, a reproductive toxicity threshold could not be developed, because no significant effects were observed for individual methylmercury treatments, and insufficient data were available to consider calculation of an EC20 or other effects concentration.

The basis for the reproductive LOEC of 0.05 mg/kg_{diet} ww is unclear, because a reproductive LOEC of 0.05 mg/kg_{diet} ww was not tabulated by Depew et al. (2012). Rather, a LOEC of 0.05 mg/kg_{diet} ww based on altered behavior of Atlantic croaker (*Micropogonias undulatus*) larvae from parent fish fed diet-borne methylmercury was tabulated; this LOEC was based on the study described by Alvarez et al. (2006). In that study, adults were fed diets, *ad libitum* for 30 days, with methylmercury concentrations of 0, 0.05, and 0.1 mg/kg_{diet} ww. Fish were then spawned, eggs were collected, and hatched larvae were used for behavioral tests. The behaviors analyzed were routine behavior (e.g., rate of travel, active swimming speed) and startle response to visual stimuli and vibratory stimuli (e.g., responsiveness, reactive distance, response distance, response duration). Some behavioral responses were significantly different ($p \leq 0.05$) in larvae from methylmercury-exposed parent fish compared to the control, such as activity levels and some responses to vibratory stimuli, but other responses were not ($p > 0.05$), such as responses to visual stimuli. Thus, a behavioral LOEC would be 0.20 mg/kg_{diet} dw, if a moisture content of 75 percent were assumed. (The 0.05 mg/kg_{diet} ww diet was based on blue marlin muscle.)

Because the endpoints of interest were survival, growth, and reproduction per the BERA work plan (Parametrix et al. 2011), the next lowest toxicity thresholds summarized by Depew et al. (2012) were considered for TRV development: 0.218 and 0.22 mg/kg ww for the fathead minnow based on Hammerschmidt et al. (2002), Drevnick and Sandheinrich (2003), and Sandheinrich and Miller (2006). These diet-borne concentrations were 0.87 and 0.88 mg/kg (as mercury) on a dry weight basis, as reported in the original studies. These studies were conducted in the same laboratory and had similar test designs and concentrations. Diet-borne methylmercury concentrations up to 8.5 mg/kg_{diet} dw (as mercury) did not have any adverse effects on survival and growth, but impaired reproduction (i.e., spawning success) was observed at diet-borne concentrations of 0.87 and 0.88 mg/kg_{diet} dw (as mercury). Because models in TRAP could not be fit to the data, linear interpolation was used to estimate EC20 values that ranged from 0.32 to 0.48 mg/kg_{diet} dw (as mercury), with a geometric mean of

0.39 mg/kg_{diet} dw (as mercury) (Figure 3-13). CIs on the EC20 values were not calculated because they were derived using linear interpolation.

3.9.3 Selection of Mercury TRVs

The review by Depew et al. (2012) identified studies of the reproductive or growth-related effects of diet-borne methylmercury on 23 endpoints and 10 species of freshwater and brackish/marine fish. An SSD using the fathead minnow EC20 of 0.39 mg/kg_{diet} dw (as mercury), as well as growth- and reproduction-based toxicity data for the four other freshwater species and five saltwater/brackish species reported in Depew et al. (2012), was developed and used to derive a 5th percentile of 0.26 mg/kg_{diet} dw (Figure 3-14). This value was identified as the concentration-based TRV. A dose-based TRV of 0.013 mg/kg bw/day was also calculated based on the fathead minnow ingestion rate of 5 percent of body weight from Drevnick and Sandheinrich (2003). The TRVs are conservative for other fish species, which have, for example, diet-borne toxicity thresholds that are 8- to 240-fold greater (Figure 3-14). Notably and of particular relevance in the UCR, white sturgeon is the least sensitive species tested (Figure 3-14).

3.10 NICKEL

3.10.1 Summary

Diet-borne toxicity data for nickel are limited to lake whitefish (*Coregonus clupeaformis*), three Asian species in the carp family (*Catla catla*, *Labeo rohita*, and *Cirrhina mrigala*), and zebrafish (*Danio rerio*). Information extracted from studies on nickel toxicity is presented in Table A-1 of Appendix A. The concentration- and dose-based lead TRVs were based on the most sensitive endpoint and species, which was a 59 percent reduction in carp (*C. catla*) growth relative to the control.

Concentration-based TRV: < 70.4 mg/kg_{diet} dw

Dose-based TRV: < 3.9 mg/kg bw/day

3.10.2 Key Studies

In the lake whitefish study, Ptashynski et al. (2002) fed adults a formulated diet with measured nickel concentrations ranging up to 1,100 mg/kg_{diet} dw (as nickel sulfate) for 104 days. Diets were dried to remove water, as described in Ptashynski and Klaverkamp (2002), so it was assumed that concentrations were on a dry weight basis. Diet-borne nickel concentrations had no effect on the condition factor ($[\text{final body weight}/\text{final fork length}^3] \times 100$) of exposed fish, as shown by lack of response relative to the control (Figure 3-15).

Diet-borne nickel toxicity to Asian carp species has been evaluated in a series of studies by the same research group. Shafiq et al. (2012) fed 45-, 65-, and 90-day-old *C. mrigala* formulated diets with reported diet-borne nickel concentrations of 79.1, 83.2, and 88.8 mg/kg_{diet dw} (as nickel chloride), respectively, for 12 weeks. The diet was not described and the moisture content of the diet was not provided; however, previous studies on carp from the same laboratory have used a pelleted feed (Naz and Javed 2013), so it was assumed that the formulated diet had a low moisture content and that nickel concentrations were on a dry weight basis. Along with the diet-borne exposure, 45-, 65- and 90-day-old carp were simultaneously exposed to aqueous nickel concentrations of 21,930, 26,410, and 29,430 µg/L, respectively. These nickel concentrations were defined by the authors as sub-lethal concentrations for this species, but, for perspective, these concentrations are two orders of magnitude greater than chronic AWQC for nickel. The toxicity data are difficult to interpret, as it appears that mean growth-related measurements were first tabulated based on the combined dataset for control and nickel-exposed fish. However, the mean weight increase and other growth-related parameters were also tabulated, and significant ($p \leq 0.05$) reductions in the nickel-exposed fish compared to the control fish were observed (the basis for the mean values is unclear based on the data provided).

In a subsequent study with a study design similar to the one used by Shafiq et al. (2012), Javed (2013) fed *C. catla*, *L. rohita*, and *C. mrigala* formulated diets with diet-borne nickel concentrations of 70.4, 72.0, and 79.1 mg/kg_{diet dw} (as nickel chloride). As for Shafiq et al. (2012), the diet was not described, but it was assumed that concentrations were on a dry weight basis. Weight was reduced by 56 to 68 percent in fish fed the diet-borne nickel diets compared to the control.

In the zebrafish study, Alsop et al. (2014) fed fish either a control diet containing a nickel concentration of 3.66 mg/kg_{diet dw} or a treatment diet with a nickel concentration of 115.8 mg/kg_{diet dw}. The nickel concentrations in the diets appear to have been on a wet weight basis, and the moisture contents of the diets were not reported (The analytical methods describe the measurement of nickel concentrations in both the diet and fish tissues, and fish tissue concentrations are reported on a wet weight basis.). Fish were fed to satiation two times per day for 80 days. During days 75 to 78 of the exposure, reproductive performance was evaluated by counting the number of eggs per female. The number of eggs per female was reduced ($p = 0.032$) by 65 percent in fish fed the nickel diets. In addition, after 80 days, weight of males fed the nickel diet was reduced ($p = 0.013$) by 26 percent compared to the control, but no growth-related effects were observed for females ($p = 0.74$).

3.10.3 Selection of Nickel TRVs

The concentration-based diet-borne nickel TRV was set at $> 70.4 \text{ mg/kg}_{\text{diet}} \text{ dw}$ based on the toxicity threshold for *C. catla*, which had the lowest toxicity threshold identified (Figure 3-15). Fish were fed to satiation on a daily basis, with a mean food intake of 9.39 g reported. (Details on how food intake was measured were not provided; however, it was assumed that this was the mean food intake on a daily basis, as measured once per week over the course of the 12-week test.) The mean initial body weight in the test was 83.33 g and the mean increase in weight after the 12-week exposure was 14.22 g, resulting in a mean body weight of 169 g over the duration of the test. Division of the mean food intake rate of 9.39 g by the mean body weight of 169 g results in an ingestion rate of $0.0557 \text{ g}_{\text{food}}/\text{g bw}/\text{day}$. Application of this FIR to the concentration-based TRV of $< 70.4 \text{ mg/kg}_{\text{diet}} \text{ dw}$ resulted in a corresponding dose-based TRV of $< 3.9 \text{ mg/kg bw}/\text{day}$.

There is high uncertainty in the nickel TRVs due to the limited diet-borne toxicity data available. The data for the three Asian carp species indicate that these species may be more sensitive to diet-borne nickel than are lake whitefish; however, there is greater uncertainty in the Asian carp studies because 1) nickel concentrations in the lake whitefish diets were verified analytically, while there is no mention of whether nickel concentrations in the Asian carp species study were measured; 2) preparation of the diet for the lake whitefish study was well-described, based on reference to a companion paper (Ptashynski and Klaverkamp 2002), while preparation of the diet for the Asian carp species study was poorly described (including other studies published by the same research group); and 3) a series of three diet-borne nickel concentrations was tested for lake whitefish, while a single diet-borne nickel concentration was tested in the Asian carp species studies.

3.11 SELENIUM

The potential toxicity of selenium to fish is predominantly determined by the concentrations of organo-selenium compounds (e.g., selenomethionine) in their diets (Janz et al. 2010). The strongest line of evidence for evaluating potential selenium toxicity to fish is selenium concentrations in fish tissue. Accordingly, EPA's recently updated AWQC for selenium are based on fish tissue (USEPA 2016a). EPA's recommended whole-body fish selenium criterion is 8.5 mg/kg dw . Selenium trophic transfer factors, based on the ratio of whole-body selenium to diet-borne selenium, typically average about 1.1 (Presser and Luoma 2010). Therefore, a reasonable diet-borne selenium toxicity threshold, one that is linked to EPA's whole-body fish selenium criterion, is $7.7 \text{ mg/kg}_{\text{diet}} \text{ dw}$ ($8.5 \text{ mg/kg dw} \div 1.1 = 7.7 \text{ mg/kg}_{\text{diet}} \text{ dw}$).

Most of the toxicity data used to develop EPA's fish tissue-based selenium criteria are based on field studies in which wild fish were naturally exposed to selenium in the field, over a range of selenium exposure conditions. In the typical study design, 1) eggs and milt are collected, 2) eggs are fertilized in the field, 3) fertilized eggs are reared in the laboratory through hatch, and 4) larval survival and deformities are assessed (Rudolph et al. 2008; McDonald et al. 2010). Field-based exposure studies are typically used because the critical exposure pathway is diet-borne organic selenium during vitellogenesis (Janz et al. 2010). Adults are generally insensitive to selenium, but maternal transfer of selenium to the ovaries, and thence to the eggs, can impact larval survival and development.

Laboratory studies for evaluating this exposure pathway and endpoint are limited due to logistical challenges. However, the available data indicate that a diet-borne selenium TRV of 7.7 mg/kg_{diet} dw would be protective. For example, DeForest et al. (1999) synthesized concentration-response data for bluegill sunfish (*Lepomis macrochirus*) from several laboratory maternal transfer studies (Bryson et al. 1985; Woock et al. 1987; Coyle et al. 1993) and derived diet-borne EC10 and EC20 values of 10 and 13 mg/kg_{diet} dw, respectively. Hardy et al. (2010) conducted a 2.5-year study in which cutthroat trout (*Oncorhynchus clarki bouvieri*) were provided diets with selenium concentrations up to 11.2 mg/kg dw (as seleniomethionine). No significant effects ($p > 0.05$) on larval survival or development were observed in any of the diet-borne selenium treatments. Accordingly, uncertainty in the protectiveness of the diet-borne selenium TRV of 7.7 mg/kg_{diet} dw is considered low.

3.12 SILVER

3.12.1 Summary

The dietary toxicity of silver has been tested in one fish species, rainbow trout. Information from the two studies on silver toxicity to rainbow trout is presented in Table A-1 of Appendix A. The toxicity data were anomalous, which creates a challenge for defining a TRV. However, a concentration-based TRV of $> 3,000$ mg/kg_{diet} dw and a dose-based TRV of > 70 mg/kg bw/day were derived.

Concentration-based TRV: $> 3,000$ mg/kg_{diet} dw

Dose-based TRV: > 70 mg/kg bw/day

3.12.2 Key Studies

Galvez and Wood (1999) fed juvenile rainbow trout commercial trout feed that had been amended with nominal silver concentrations of 3, 30, 300, and 3,000 mg/kg_{diet} (as silver sulfide). The nominal silver concentrations were assumed to be on a dry weight basis given the method for preparing the diet, which included drying the feed pellets for 36 hours at 50°C. Fish were fed daily to satiation for 58 days. An anomalous response was observed. While the specific growth rate (percent wet weight per day) was significantly reduced ($p < 0.05$) relative to the control by 23 percent for both the 3- and 300-mg/kg_{diet} dw treatments, the specific growth rates were higher than in the control for the 30- and 3,000-mg/kg_{diet} dw treatments (Figure 3-16). The authors noted that silver sulfide would probably not be dissociated within the gut lumen of the fish during digestion, which would prevent internalization of silver.

Galvez et al. (2001) fed juvenile rainbow trout a formulated diet that had been augmented with biologically incorporated silver. To create the diet, adult rainbow trout were first exposed to a waterborne silver thiosulfate concentration of 0.37 mmol/L for 7 days. The fish were then sacrificed and blended into an experimental diet, which had a silver concentration of 3.12 mg/kg_{diet} ww. The juvenile rainbow trout were fed daily to satiation for 126 days. Specific growth rate (percent wet weight per day) for the silver treatment (3.12 mg/kg_{diet} ww) did not differ significantly from the controls.

3.12.3 Selection of Silver TRVs

Overall, Galvez and Wood (1999), considering results from their study and preliminary results from what would be later published by Galvez et al. (2001), concluded that dietary silver exposure is physiologically benign. Although an anomalous concentration/dose-response relationship was observed, the highest diet-borne concentration of 3,000 mg/kg_{diet} dw (and the corresponding dose of 70 mg/kg bw/day) resulted in a specific growth rate greater than the control. The concentration- and dose-based TRVs were therefore set equal to $> 3,000$ mg/kg_{diet} dw and 70 mg/kg bw/day, respectively.

Uncertainty in the absolute magnitudes of the concentration- and dose-based TRVs for silver is high due to the limited data available; however, this uncertainty is not considered important, given the evidence that the diet-borne exposure route is not significant for silver and fish.

3.13 VANADIUM

Diet-borne vanadium toxicity data were identified for only rainbow trout in one study; information for this study is provided in Table A-1 of Appendix A. Hilton and Bettger (1988) fed juvenile rainbow trout vanadium-amended formulated diets for 12 weeks. Diet-borne

vanadium concentrations of 10.2, 80, and 493 mg/kg_{diet} dw (as sodium orthovanadate) resulted in statistically significant ($p < 0.05$) growth reductions of 59, 68, and 95 percent relative to the control, respectively (Figure 3-17). An EC20 could not be derived using TRAP, so one was calculated using linear interpolation (Figure 3-17); the concentration-based diet-borne vanadium TRV was set equal to this EC20. Dosing data were not reported, so a dose-based TRV of 0.16 mg/kg bw/day based on an assumed FIR of 0.037 g_{food}/g bw/day was calculated (Section 2.6).

Concentration-based TRV: 4.3 mg/kg_{diet} dw

Dose-based TRV: 0.16 mg/kg bw/day

Uncertainty in the diet-borne vanadium TRV is high, as toxicity data were only available for a single species and from only one study.

3.14 ZINC

3.14.1 Summary

Diet-borne zinc toxicity data were identified for seven fish species, as presented in Table A-1 of Appendix A. Accordingly, an SSD approach was not used, and concentration- and dose-based zinc TRVs were derived based on the most sensitive species.

Concentration-based TRV: 500 mg/kg_{diet} dw

Dose-based TRV: 38.5 mg/kg bw/day

3.14.2 Key Studies

The fish species most sensitive to diet-borne zinc was common carp (Table 3-4). Jeng and Sun (1981) fed carp fry zinc-amended diets (as zinc sulfate) for 8 weeks. Diet-borne concentrations of 294, 1,007, and 1,974 mg/kg_{diet} dw resulted in growth (weight) reductions of 21, 23, and 35 percent, respectively, which resulted in an EC20 of 500 mg/kg_{diet} dw (90 percent CI = 0.022 to 1.1E+07 mg/kg_{diet} dw) (Figure 3-18). The concentration-response data from this test have a limited range of effects. The diets with zinc concentrations of 294 and 1,007 mg/kg_{diet} dw had effect levels near 20 percent (i.e., 21 and 23 percent, respectively), and the diet with the highest zinc concentration had an effect level of 35 percent. The piece-wise regression model was selected from TRAP, as it is less sensitive to assumptions about the tails, for which there were no high-effect data. Based on dosing information provided for the study, an ED20 of 38.5 mg/kg bw/day (90 percent CI = 0.0017 to 8.7E+05 mg/kg bw/day) was also calculated (Figure 3-18).

The next highest diet-borne concentration-based toxicity thresholds were a LOEC of 1,900 mg/kg_{diet} dw (as zinc chloride) based on 54 and 66 percent growth reduction in two tests with coho salmon (*Oncorhynchus kisutch*) (Bowen et al. 2006), and a LOEC of 2,220 mg/kg_{diet} dw (as zinc sulfate) based on a 20 percent reduction in rainbow trout growth (Knox et al. 1984) (Table 3-4). For comparison, all other rainbow trout toxicity thresholds were “greater than” NOECs, ranging from 500 to 1,920 mg/kg_{diet} dw (Table 3-4). The dose-based LOEL for coho salmon, which corresponds with the concentration-based LOEC of 1,900 mg/kg_{diet} dw, is 95 mg/kg bw/day (Table 3-4).

3.14.3 Selection of Zinc TRVs

No effects have been observed in most diet-borne zinc toxicity studies with fish; however, a concentration-based TRV of 500 mg/kg_{diet} dw and a dose-based TRV of 38.5 mg/kg bw/day were identified based on the sensitivity of common carp. This TRV was less than other toxicity thresholds for salmonids, including several “greater than” NOECs for rainbow trout. Uncertainty in the protectiveness of the TRVs is considered low, as they were derived based on an EC20 and ED20 (for carp), and these TRVs are less than higher diet-borne zinc concentrations shown not to adversely affect several salmonid species (Figure 3-19).

3.15 SUMMARY OF FINAL DIET-BORNE TRVS

The final diet-borne concentration- and dose-based TRVs for fish are summarized in Table 3-5.

4 METAL MIXTURES

The diet-borne TRVs presented in the preceding sections were developed from toxicity tests with individual metals. Because metals in the environment, including the UCR, occur as mixtures, diet-borne toxicity studies with metal mixtures were used to evaluate the protectiveness of the individual metal TRVs.

A key series of studies was conducted with rainbow trout and brown trout that were fed either benthic invertebrates collected from the Clark Fork River in Montana (Woodward et al. 1994; Woodward et al. 1995), or oligochaete worms that had been exposed to sediments collected from the Clark Fork River (Hansen et al. 2004). Farag et al. (1999) also conducted a study in which cutthroat trout were fed benthic invertebrates collected from the Coeur d'Alene River in Idaho. In each of these studies, reduced trout growth, and sometimes survival, was observed in diets with elevated metals concentrations.

- **Woodward et al. (1994).** Rainbow trout that were fed benthic invertebrates collected from the Clark Fork River had an approximate 42 percent reduction in growth and approximate 55 percent reduction in survival relative to trout that were fed benthic invertebrates collected from a Snake River reference site. Arsenic and zinc concentrations in Clark Fork River invertebrates were approximately equal to the diet-borne arsenic and zinc TRVs of 42 and 500 mg/kg_{diet dw}, respectively; aluminum, cadmium, copper, and lead concentrations were all less than their respective TRVs (Figure 4-1). As discussed further below, arsenic has been implicated as a key metal in these diet-borne toxicity studies.
- **Woodward et al. (1995).** Brown trout and rainbow trout that were fed benthic invertebrates collected from two locations on the Clark Fork River with elevated metal concentrations had growth reductions of approximately 39 percent and 45 percent, compared with trout fed benthic invertebrates collected from what the authors defined as a reference site on the Clark Fork River, which was approximately 200 km downstream from metals sources at Anaconda and Butte. Arsenic concentrations in benthic invertebrates were less than the constituent's TRV, but zinc concentrations were greater (Figure 4-2). Cadmium, copper, and lead concentrations in benthic invertebrates were less than their corresponding TRVs.
- **Hansen et al. (2004).** Rainbow trout were fed oligochaete worms that had been exposed to sediment collected from the Clark Fork River and a tributary. The wet weights of these rainbow trout were reduced by approximately 30 percent and 19 percent, respectively, compared with trout that were fed invertebrates that had been exposed to a reference sediment collected from the Cache la Poudre River in Colorado. Reductions in the wet weights of fish fed the Clark Fork River and

tributary diets compared to reductions in the wet weights of fish fed the reference site diet were statistically significant; Hansen et al. (2004) did not present a comparison of the differences in wet weight gain between the two exposed groups. Arsenic concentrations in the worms exceeded the constituent's TRV, but cadmium, copper, lead, and zinc concentrations did not exceed their respective TRVs (Figure 4-3).

- **Farag et al. (1999).** Cutthroat trout were fed benthic invertebrates collected from two locations with elevated metals concentrations on the Coeur d'Alene River. Despite having similar metal concentrations, invertebrates from one site (South Fork) did not result in reduced survival and growth relative to trout that were fed invertebrates from a reference site, while invertebrates from another site (Cataldo) did (Figure 4-4).

Other diet-borne mixture studies have been conducted in the laboratory using formulated diets. Knox et al. (1982, 1984) evaluated the effects of varying combinations of diet-borne copper and zinc concentrations on rainbow trout growth. Negligible growth effects were observed, even when copper and zinc concentrations approached their respective TRVs in combination (Figure 4-5). This study provides evidence that the copper and zinc TRVs are protective against diet-borne toxicity, even when present as binary mixtures.

The Clark Fork River studies led to the study conducted by Erickson et al. (2010), who concluded that the diet-borne toxicity observed was likely more attributable to arsenic. Erickson et al. (2011) subsequently noted that assessments of risks from arsenic exposures is incomplete without considering diet-borne exposures. However, the possibility of additive or synergistic toxicity due to other metals cannot be excluded. The information from these metal mixture toxicity studies was not used to modify any of the individual metal TRVs, but it will be considered as part of uncertainty discussions in the BERA.

5 REFERENCES

- Adams, W.J., R. Blust, U. Borgmann, K.V. Brix, D.K. DeForest, A.S. Green, J.S. Meyer, J.C. McGeer, P.R. Paquin, P.S. Rainbow, and C.M. Wood. 2011. Utility of tissue residues for predicting effects of metals on aquatic organisms. *Integr. Environ. Assess. Manag.* 7(1): 75-98.
- Ahmed, A.R., A.N. Jha, and S.J. Davies. 2012. The efficacy of chromium as a growth enhancer for mirror carp (*Cyprinus carpio* L.): an integrated study using biochemical, genetic, and historical responses. *Biol. Trace. Elem. Res.* 148:187-197.
- Ajani, E.K., and B.U. Akpoilih. 2012. Growth response and ionic regulation in common carp (*Cyprinus carpio* L.) after chronic dietary copper exposure and recovery. *Intl. J. Fish. Aquacult.* 4(2):13-21.
- Alsop, D., S. Brown, and G. Van Der Kraak. 2007. The effects of copper and benzo(a)pyrene on retinoids and reproduction in zebrafish. *Aquat. Toxicol.* 82:281-295.
- Alsop, D., S.P. Lall, and C.M. Wood. 2014. Reproductive impacts and physiological adaptations of zebrafish to elevated dietary nickel. *Comp. Biochem. Physiol. Part C* 165:67-75.
- Alsop, D., TY-T. Ng, M.J. Chowdhury, and C.M. Wood. 2016. Interactions of waterborne and dietborne Pb in rainbow trout, *Oncorhynchus mykiss*: bioaccumulation, physiological responses, and chronic toxicity. *Aqua. Tox.* 177:343-354.
- 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.* 80:329-337.
- Ayyat, M.S., S.A. Fayza, and H.I. El-Marakby. 2003. Reductions of dietary lead toxicity in Nile tilapia (*Oreochromis niloticus*). The 10th Scientific Conference on Animal Nutrition, Hurghada, Egypt, October 14-18, 2003.
- Beckvar, N., T.M. Dillon, and L.B. Read. 2005. Approaches for linking whole-body fish tissue residues of mercury or DDT to biological effects thresholds. *Environ. Toxicol. Chem.* 24(8): 2094-2105.
- Berntssen, M.H.G., A-K. Lundebye, and A. Maage. 1999a. Effects of elevated dietary copper concentrations on growth, feed utilisation and nutritional status of Atlantic salmon (*Salmo salar* L.) fry. *Aquacult.* 174(1-2):167-181.
- Berntssen, M.H.G., K. Hylland, S.E. Wendelaar Bonga, and A. Maage. 1999b. Toxic levels of dietary copper in Atlantic salmon (*Salmo salar* L.) parr. *Aquat. Toxicol.* 46:87-99.

- Berntssen, M.H.G., R. Waagbo, H. Toften, and A-K. Lundebye. 2003. Effects of dietary cadmium on calcium homeostasis, Ca mobilization and bone deformities in Atlantic salmon (*Salmo salar* L.) parr. *Aquacult. Nutr.* 9:175-183.
- Bielmyer, G.K., D. Gatlin, J.J. Isely, J.R. Tomasso, and S.J. Klaine. 2005. Responses of hybrid striped bass to waterborne and dietary copper in freshwater and saltwater. *Compar. Biochem. Physiol. Part C* 140:131-137.
- Bott, D. 2015. Personal communication (email from D. Bott, EPA, to J. Toll, Windward, regarding attached FWS survey data for mussels and crayfish). U.S. Environmental Protection Agency, Seattle, WA. December 15, 2015.
- Bowen, L., I. Werner, and M.L. Johnson. 2006. Physiological and behavioral effects of zinc and temperature on coho salmon (*Oncorhynchus kisutch*). *Hydrobiologia* 559:161-168.
- Bryson, W.T., K.A. MacPherson, M.A. Mallin, W.E. Partin, and S.E. Woock. 1985. Hyco Reservoir 1984 bioassay report. Carolina Power and Light Company, New Hill, NC.
- CH2M HILL, and E&E. 2007. Region 10 U.S. Environmental Protection Agency. Final Phase I fish tissue sampling data evaluation, Upper Columbia River Site CERCLA RI/FS. CH2M HILL and Ecology and Environment, Inc.
- Cockell, K.A., and W.J. Bettger. 1993. Investigations of the gallbladder pathology associated with dietary exposure to disodium arsenate heptahydrate in juvenile rainbow trout (*Oncorhynchus mykiss*). *Toxicology* 77:233-248.
- Cockell, K.A., and J.W. Hilton. 1988. Preliminary investigation on the comparative chronic toxicity of four dietary arsenicals to juvenile rainbow trout (*Salmo gairdneri* R.). *Aquat. Toxicol.* 12:73-82.
- Cockell, K.A., J.W. Hilton, and W.J. Bettger. 1991. Chronic toxicity of dietary disodium arsenate heptahydrate to juvenile rainbow trout (*Oncorhynchus mykiss*). *Arch. Environ. Contam. Toxicol.* 21:518-527.
- Cockell, K.A., J.W. Hilton, and W.J. Bettger. 1992. Hepatobiliary and hematological effects of dietary disodium arsenate heptahydrate in juvenile rainbow trout (*Oncorhynchus mykiss*). *Comp. Biochem. Physiol.* 103C(3):453-458.
- Coyle, J.J., D.R. Buckler, C.G. Ingersoll, J.F. Fairchild, and T.W. May. 1993. Effect of dietary selenium on the reproductive success of bluegills (*Lepomis macrochirus*). *Environ. Toxicol. Chem.* 12:551-565.

- Dai, W., L. Fu, H. Du, and C. Jin. 2009. Changes in growth performance, metabolic enzyme activities, and content of Fe, Cu, and Zn in liver and kidney of tilapia (*Oreochromis niloticus*) exposed to dietary Pb. *Biol. Trace. Elem. Res.* 128:176-183.
- DeForest, D.K., Brix K.V., Adams W.J. 1999. Critical review of proposed residue-based selenium toxicity thresholds for freshwater fish. *Human Ecol Risk Assess* 5(6):1187-1228.
- DeForest, D.K., and J.S. Meyer. 2015. Critical review: toxicity of dietborne metals to aquatic organisms. *Crit. Rev. Environ. Sci. Tech.* 45(11):1176-1241.
- 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. Toxicol. Chem.* 31(7):1536-1547.
- Drevnick, P.E., and M.B. Sandheinrich. 2003. Effects of dietary methylmercury on reproductive endocrinology of fathead minnows. *Environ. Sci. Technol.* 37:4390-4396.
- Eid, A.E., and S.I. Ghonim. 1994. Dietary zinc requirement of fingerling *Oreochromis niloticus*. *Aquacult.* 119:259-264.
- Erickson, R.J., D.R. Mount, T.L. Highland, J.R. Hocket, E.N. Leonard, V.R. Mattson, T.D. Dawson, and K.G. Lott. 2010. Effects of copper, cadmium, lead, and arsenic in a live diet on juvenile fish growth. *Can. J. Fish. Aquat. Sci.* 67:1816-1826.
- Erickson, R.J., D.R. Mount, T.L. Highland, J.R. Hocket, and C.T. Jenson. 2011. The relative importance of waterborne and dietborne arsenic exposure on survival and growth of juvenile rainbow trout. *Aquat. Toxicol.* 104:108-115.
- Exponent, and Parametrix. 2013. Upper Columbia River final surface water data summary and data gap report. Prepared for Teck American Incorporated. Exponent, Bellevue, WA; Parametrix, Inc., Bellevue, WA.
- Exponent, Parametrix, and Integral. 2013. Upper Columbia River. Final. Fish tissue data summary and data gaps report. Prepared for Teck American Incorporated. Exponent, Bellevue, WA; Parametrix, Bellevue, WA; Integral Consulting Inc., Seattle, WA.
- Farag, A.M., W. Brumbaugh, J.N. Goldstein, E. MacConnell, C. Hogstrand, and F.T. Barrows. 1999. Dietary effects of metals-contaminated invertebrates from the Coeur d'Alene River, Idaho, on cutthroat trout. *Trans. Am. Fish. Soc.* 128:578-592.
- Franklin, N.M., C.N. Glover, J.A. Nicol, and C.M. Wood. 2005. Calcium/cadmium interactions at uptake surfaces in rainbow trout: waterborne versus dietary routes of exposure. *Environ. Toxicol. Chem.* 24(11):2954-2964.

- Friedmann, A.S., M.C. Watzin, T. Brinck-Johnson, and J.C. Leiter. 1996. Low levels of dietary methylmercury inhibit growth and gonadal development in juvenile walleye (*Stizostedion vitreum*). *Aquat. Toxicol.* 35(1996):265-278.
- Galvez, F., and C.M. Wood. 1999. Physiological effects of dietary silver sulfide exposure in rainbow trout. *Environ. Toxicol. Chem.* 18(1):84-88.
- Galvez, F., C. Hogstrand, J.C. McGeer, and C.M. Wood. 2001. The physiological effects of a biologically incorporated silver diet on rainbow trout (*Oncorhynchus mykiss*). *Aqua. Tox.* 55:95-112.
- Gatlin, D.M. III, and R.P. Wilson. 1986. Dietary copper requirement of fingerling channel catfish. *Aquaculture* 54:277-285.
- Hammerschmidt, C.R., M.B. Sandheinrich, J.G. Wiener, and R.G. Rada. 2002. Effects of dietary methylmercury on reproduction of fathead minnows. *Environ. Sci. Technol.* 36(5):877-883.
- Handy, R.D. 1993a. The accumulation of dietary aluminium by rainbow trout, *Oncorhynchus mykiss*, at high exposure concentrations. *J. Fish. Biol.* 42:603-606.
- Handy, R.D. 1993b. The effect of acute exposure to dietary Cd and Cu on organ toxicant concentrations in rainbow trout, *Oncorhynchus mykiss*. *Aquat. Toxicol.* 27:1-14.
- Handy, R.D., D.W. Sims, A. Giles, H.A. Campbell, and M.M. Musonda. 1999. Metabolic trade-off between locomotion and detoxification for maintenance of blood chemistry and growth parameters by rainbow trout (*Oncorhynchus mykiss*) during chronic dietary exposure to copper. *Aquat. Toxicol.* 47:23-41.
- Hansen, J.A., J.L. 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.
- Hardy, R.W., Oram L.L., and Möller G. 2010. Effects of dietary selenomethionine on cutthroat trout (*Oncorhynchus clarki bouvieri*) growth and reproductive performance over a life cycle. *Arch. Environ. Contam. Toxicol.* 51(1):237-245.
- Hatakeyama, S., and M. Yasuno. 1982. Accumulation and effects of cadmium on guppy (*Poecilia reticulata*) fed cadmium-dosed Cladocera (*Moina macrocopa*). *Bull. Environ. Contam. Toxicol.* 29:159-166.
- Hatakeyama, S., and M. Yasuno. 1987. Chronic effects of Cd on the reproduction of the guppy (*Poecilia reticulata*) through Cd-accumulated midge larvae (*Chironomus yoshimatsui*). *Ecotox. Environ. Saf.* 14:191-207.

- Hilton, J.W., and W.J. Bettger. 1988. Dietary vanadium toxicity in juvenile rainbow trout: a preliminary study. *Aquat. Toxicol.* 12:63-71.
- Hodson, P.V., B.R. Blunt, and D.J. Spry. 1978. Chronic toxicity of water-borne and dietary lead to rainbow trout (*Salmo gairdneri*) in Lake Ontario water. *Water. Res.* 12:869-878.
- Janz, D.M., D.K. DeForest, M.L. Brooks, P.M. Chapman, G. Gilron, D. Hoff, W.A. Hopkins, D.O. McIntyre, C.A. Mebane, V.P. Palace, J.P. Skorupa, and M. Wayland. 2010. Selenium toxicity to aquatic organisms. In: P.M. Chapman, W.J. Adams, M.L. Brooks, C.G. Delos, S.N. Luoma, W.A. Maher, H.M. Ohlendorf, T.S. Presser, and D.P. Shaw, eds, Ecological assessment of selenium in the aquatic environment. SETAC Press, Pensacola, FL, pp 141-231.
- Javed, M. 2013. Chronic effects of nickel and cobalt on fish growth. *Int. J. Agric. Biol.* 15:575-579.
- Jeng, S.S., and L.T. Sun. 1981. Effects of dietary zinc levels on zinc concentrations in tissues of common carp. *J. Nutr.* 111:134-140.
- Julshamn, K., K-J. Andersen, O. Ringdal, and J. Brenna. 1988. Effect of dietary copper on the hepatic concentration and subcellular distribution of copper and zinc in the rainbow trout (*Salmo gairdneri*). *Aquacult.* 73:143-155.
- Kamunde, C., and C.M. Wood. 2003. The influence of ration size on copper homeostasis during sublethal dietary copper exposure in juvenile rainbow trout, *Oncorhynchus mykiss*. *Aquat. Toxicol.* 62(3):235-254.
- Kamunde, C.N, M. Grosell, J.N.A. Lott, and C.M. Wood. 2001. Copper metabolism and gut morphology in rainbow trout (*Oncorhynchus mykiss*) during chronic sublethal dietary copper exposure. *Can. J. Fish. Aquat. Sci.* 58:293-305.
- Kamunde, C.N, M. Grosell, D. Higgs, and C.M. Wood. 2002. Copper metabolism in actively growing rainbow trout (*Oncorhynchus mykiss*): interactions between dietary and waterborne copper uptake. *J. Exp. Biol.* 205:279-290.
- Kjoss, V.A., M. Grosell, and C.M. Wood. 2005. The influence of dietary Na on Cu accumulation in juvenile rainbow trout exposed to combined dietary and waterborne Cu in soft water. *Arch. Environ. Contam. Toxicol.* 49:520-527.
- Kjoss, V.A., C.M. Wood, and D.G. McDonald. 2006. Effects of different ligands on the bioaccumulation and subsequent depuration of dietary Cu and Zn in juvenile rainbow trout (*Oncorhynchus mykiss*). *Can. J. Fish. Aquat. Sci.* 63:412-422.
- Knox, D., C.B. Cowey, and J.W. Adron. 1982. Effects of dietary copper and copper: zinc ratio on rainbow trout (*Salmo gairdneri*). *Aquacult.* 27:111-119.

- Knox, D., C.B. Cowey, and J.W. Adron. 1984. Effects of dietary zinc intake upon copper metabolism in rainbow trout (*Salmo gairdneri*). *Aquacult.* 40:199-207.
- Lanno, R.P., S.J. Slinger, and J.W. Hilton. 1985a. Effect of ascorbic acid on dietary copper toxicity in rainbow trout (*Salmo gairdneri* Richardson). *Aquaculture* 49:269-287.
- Lanno, R.P., S.J. Slinger, and J.W. Hilton. 1985b. Maximum tolerable and toxicity levels of dietary copper in rainbow trout (*Salmo gairdneri* Richardson). *Aquaculture* 49:257-268.
- Lapointe, D., F. Pierron, and P. Couture. 2011. Individual and combined effects of heat stress and aqueous or dietary copper exposure in fathead minnows (*Pimephales promelas*). *Aquat. Toxicol.* 104:80-85.
- Lundebye, A.K., M.H.G. Berntssen, S.E. Wendelaar Bonga, and A. Maage. 1999. Biochemical and physiological responses in Atlantic salmon (*Salmo salar*) following dietary exposure to copper and cadmium. *Mar. Pollut. Bull.* 39:137-144.
- Luoma, S.N., and P.S. Rainbow. 2005. Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. *Environ. Sci. Technol.* 39:1921-1931. Maage, A., and K. Julshamn. 1993. Assessment of zinc status in juvenile Atlantic salmon (*Salmo salar*) by measurement of whole body and tissue levels of zinc. *Aquacult.* 117:179-191.
- McDonald, B.G., A.M. deBruyn, J.R. Elphick, M. Davies, D. Bustard, and P.M. Chapman. 2010. Developmental toxicity of selenium to Dolly Varden char (*Salvelinus malma*). *Environ. Toxicol. Chem.* 29(12): 2800-2805.
- Mount, D.R., A.K. Barth, T.D. Garrison, K.A. Barten, and J.R. Hockett. 1994. Dietary and waterborne exposure of rainbow trout (*Oncorhynchus mykiss*) to copper, cadmium, lead and zinc using a live diet. *Environ. Toxicol. Chem.* 13(12):2031-41.
- Murai, T., J.W. Andrews, and R.G. Smith, Jr. 1981. Effects of dietary copper on channel catfish. *Aquacult.* 22:353-357.
- Naz, S., and M. Javed. 2013. Evaluation of acute toxicity of metals mixture and bioaccumulation in freshwater fish. *Biosci. Methods* 4(3):11-18.
- Ng, TY-T., and C.M. Wood. 2008. Trophic transfer and dietary toxicity of Cd from the oligochaete to the rainbow trout. *Aquat. Toxicol.* 87:47-59.
- Ng, TY-T., J.S. Klinck, and C.M. Wood. 2009. Does dietary Ca protect against toxicity of a low dietborne Cd exposure to the rainbow trout? *Aquat. Toxicol.* 91:75-86.
- Overnell, J., T.C. Fletcher, and R. McIntosh. 1988. Factors affecting hepatic metallothionein levels in marine flatfish. *Mar. Environ. Res.* 24:155-158.

- Parametrix, Exponent, HydroQual, Integral, and Cardwell. 2011. Upper Columbia River baseline ecological risk assessment work plan. Prepared for Teck American Incorporated. Parametrix, Inc., Bellevue, WA; Exponent, Bellevue, WA; HydroQual, Mahwah, NJ; Integral Consulting Inc., Seattle, WA; Cardwell Consulting LLC, Corvallis, OR. February.
- Poston, H.A. 1991. Effects of dietary aluminum on growth and composition of young Atlantic salmon. *Progr. Fish. Cult.* 53:7-10.
- Presser, T.S., and S.N. Luoma. 2010. A methodology for ecosystem-scale modeling of selenium. *Integr. Environ. Assess. Manage.* 6 (4):685-710.
- Ptashynski MD, Klaverkamp JF. 2002. Accumulation and distribution of dietary nickel in lake whitefish (*Coregonus clupeaformis*). *Aqua Tox* 58:249-264.
- Ptashynski, M.D., R.M. Pedlar, R.E. Evans, C.L. Baron, and J.F. Klaverkamp. 2002. Toxicology of dietary nickel in lake whitefish (*Coregonus clupeaformis*). *Aquat. Toxicol.* 58:229-247.
- Reynders, H., K. Van Campenhout, L. Bervoets, W.M. De Coen, and R. Blust. 2006. Dynamics of cadmium accumulation and effects in common carp (*Cyprinus carpio*) during simultaneous exposure to water and food (*Tubifex tubifex*). *Environ. Toxicol. Chem.* 25(6):1558-1567.
- Richardson, N.L., D.A. Higgs, R.M. Beames, and J.R. McBride. 1985. Influence of dietary calcium, phosphorus, zinc and sodium phytate level on cataract incidence, growth and histopathology in juvenile chinook salmon (*Oncorhynchus tshawytscha*). *J. Nutr.* 115(5):553-567.
- Ruangsomboon, S., and L. Wongrat. 2006. Bioaccumulation of cadmium in an experimental aquatic food chain involving phytoplankton (*Chlorella vulgaris*), zoo plankton (*Moina macrocopa*), and the predatory catfish *Clarias macrocephalus* x *C. gariapinus*. *Aquat. Toxicol.* 78:15-20.
- Rudolph, B., I. Andreller, and C.J. Kennedy. 2008. Reproductive success, early life stage development, and survival of westslope cutthroat trout (*Oncorhynchus clarki lewisi*) exposed to elevated selenium in an area of active coal mining. *Environ. Sci. Technol.* 42: 3109-3114.
- 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.

- Sandheinrich, M.B., J.G. Wiener, eds. 2011. Methylmercury in freshwater fish: recent advances in assessing toxicity of environmentally relevant exposures. Second ed. Environmental contaminants in biota: Interpreting tissue concentrations. 2nd ed, Beyer WN, Meador JP, eds. CRC Press, Boca Raton, FL.
- Shafiq, A., S. Abdullah, M. Javed, S. Hayat, and M. Batool. 2012. Effect of combined exposure of water-borne and dietary nickel on the growth performance of *Cirrhina mrigala*. *J. Anim. Plant. Sci.* 22(2):425-430.
- Shaw, B.J., and R.D. Handy. 2006. Dietary copper exposure and recovery in Nile tilapia, *Oreochromis niloticus*. *Aquat. Toxicol.* 76:111-121.
- Shiau, S-Y., and S-F. Lin. 1993. Effect of supplemental dietary chromium and vanadium on the utilization of different carbohydrates in tilapia, *Oreochromis niloticus* x *O. aureus*. *Aquaculture* 110:321-330.
- Spry, D.J., P.V. Hodson, and C.M. Wood. 1988. Relative contributions of dietary and waterborne zinc in the rainbow trout, *Salmo gairdneri*. *Can. J. Fish. Aquat. Sci.* 45:32-41.
- Szczerbik, P., T. Mikolajczyk, M. Sokolowska-Mikolajczyk, M. Socha, J. Chyb, and P. Epler. 2006. Influence of long-term exposure to dietary cadmium on growth, maturation and reproduction of goldfish (subspecies: Prussian carp *Carrassium auratus gibelio* B.). *Aquat. Toxicol.* 77:126-135.
- Tacon, A.G.J., and M.M. Beveridge. 1982. Effects of dietary trivalent chromium on rainbow trout. *Nutr. Rep. Internat.* 25(1):49-56.
- Takeda, H., and Y. Shimma Y. 1977. Effects of toxic amounts of dietary zinc on the growth and body components of rainbow trout at two levels of calcium. *Bull. Freshw. Fish. Res.* 27:103-109.
- USEPA (U.S. Environmental Protection Agency). 1985. Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses. PB85-227049. Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC.
- USEPA. 2007. Framework for metals risk assessment. EPA 120/R-07/001. US Environmental Protection Agency, Office of the Science Advisor, Risk Assessment Forum, Washington, D.C.
- USEPA. 2013. Aquatic life ambient water quality criteria for ammonia—freshwater. 2013. EPA 822-R-13-001. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

- USEPA. 2016a. Aquatic life ambient water quality criterion for selenium—freshwater. 2016. EPA 822-R-16-006. U.S. Environmental Protection Agency, Washington, DC.
- USEPA. 2016b. Personal communication (letter from L. Buelow, EPA, to K. McCaig, TAI, Upper Columbia River baseline ecological risk assessment TRV development follow up). U.S. Environmental Protection Agency, Richland, WA. June 17.
- USEPA. 2016c. Aquatic life ambient water quality criteria cadmium—2016. EPA-820-R-16-002. U.S. Environmental Protection Agency, Washington, DC.
- Watanabe, T., V. Kiron, and S. Satoh. 1997. Trace minerals in fish nutrition. *Aquacult.* 151:185-207.
- Wilson RW. 2012. Aluminum. In: Wood CM, Farrell AP, Brauner CJ, eds, Homeostasis and Toxicology of Non-essential Metals. Elsevier, Amsterdam, The Netherlands, pp 67-123.
- Windward. 2018. Upper Columbia River. Final macroinvertebrate tissue study data summary report. Windward Environmental LLC, Seattle, WA. March.
- Woock, S.E., W.R. Garrett, W.E. Partin, and W.T. Bryson. 1987. Decreased survival and teratogenesis during laboratory selenium exposures to bluegill, *Lepomis macrochirus*. *Bull. Environ. Contam. Toxicol.* 39:998-1005.
- Woodward, D.F., W.G. Brumbaugh, A.J. DeLonay, E.E. Little, and C.E. Smith. 1994. Effects on rainbow trout fry of a metals-contaminated diet of benthic invertebrates from the Clark Fork River, Montana. *Trans. Am. Fish. Soc.* 123:51-62.
- Woodward, D.F., A.M. Farag, H.L. Bergman, A.J. DeLonay, E.E. Little, C.E. Smith, and F.T. Barrows. 1995. Metals-contaminated benthic invertebrates in the Clark Fork River, Montana: effects on age-0 brown trout and rainbow trout. *Can. J. Fish. Aquat. Sci.* 52:1994-2004.

FIGURES

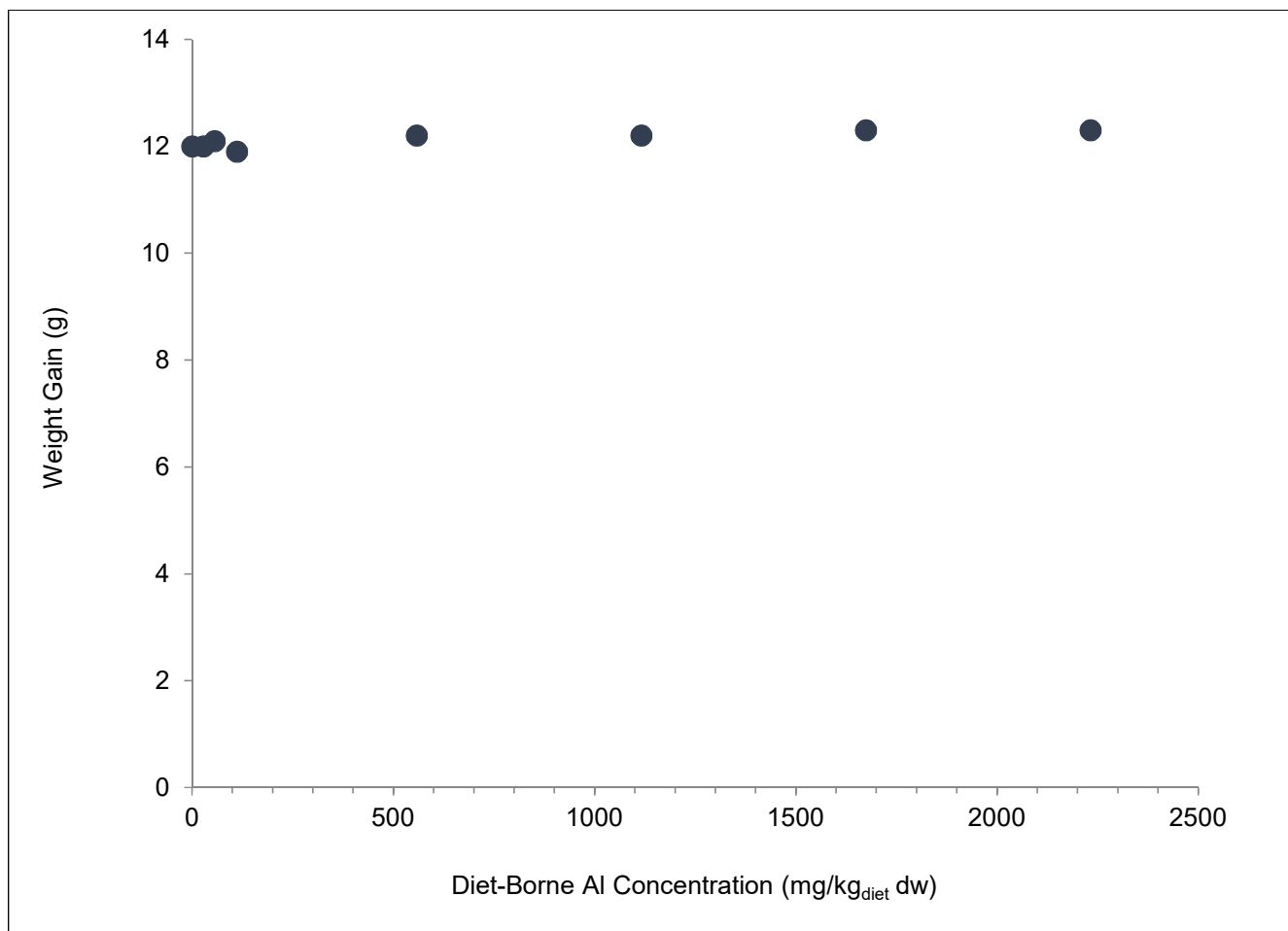


Figure 3-1. Relationship Between Diet-Borne Aluminum Concentrations and Weight Gain in Atlantic Salmon

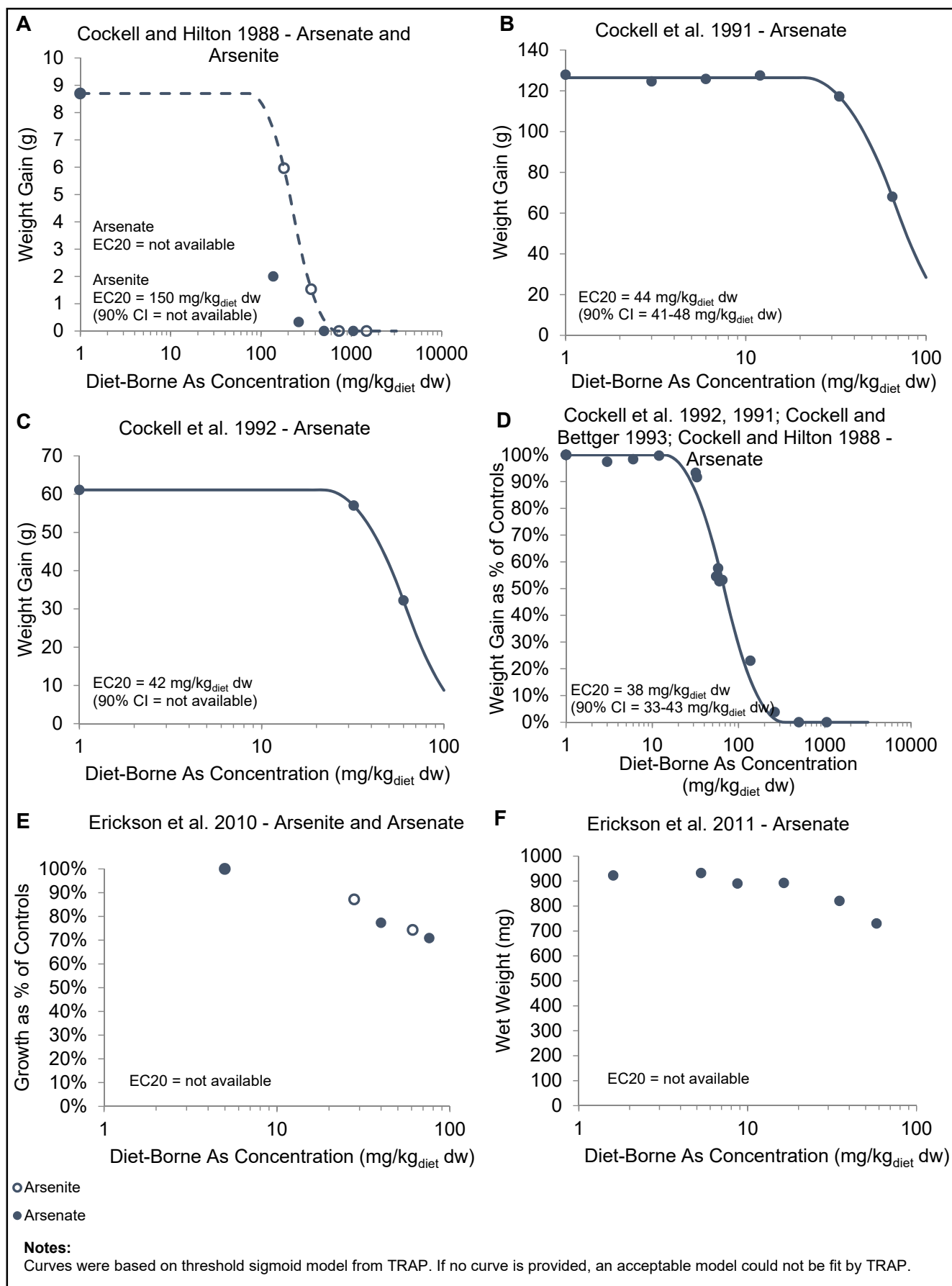


Figure 3-2. Concentration-Response Relationships for Juvenile Rainbow Trout Exposed to Diet-Borne Arsenate or Arsenite Based on Data from Individual Studies

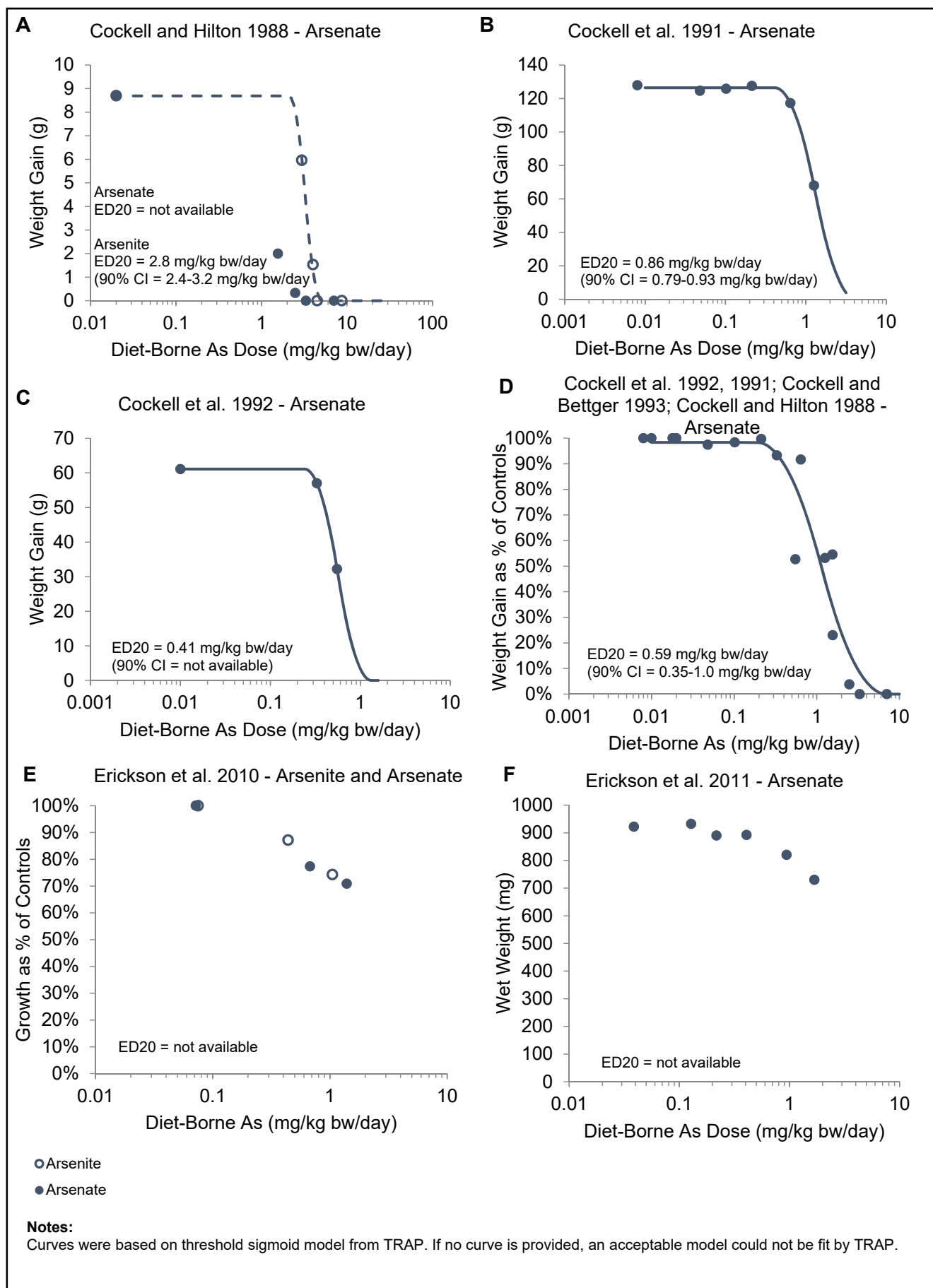


Figure 3-3. Dose-Response Relationships for Juvenile Rainbow Trout Exposed to Diet-Borne Arsenate or Arsenite Based on Data from Individual Studies

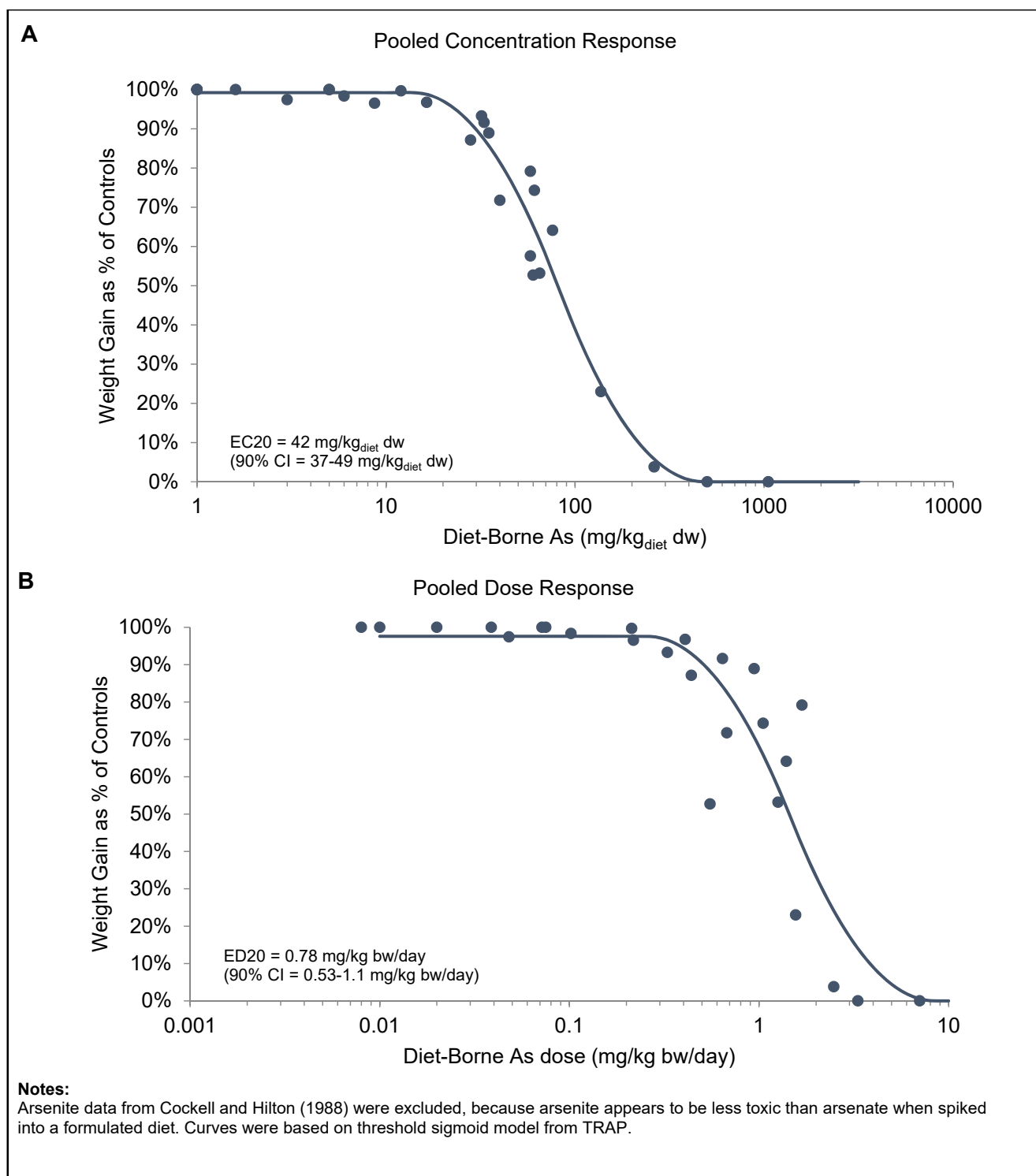


Figure 3-4. Pooled (A) Concentration-Response Relationship and (B) Dose-Response Relationship for Diet-Borne Arsenic Toxicity to Rainbow Trout

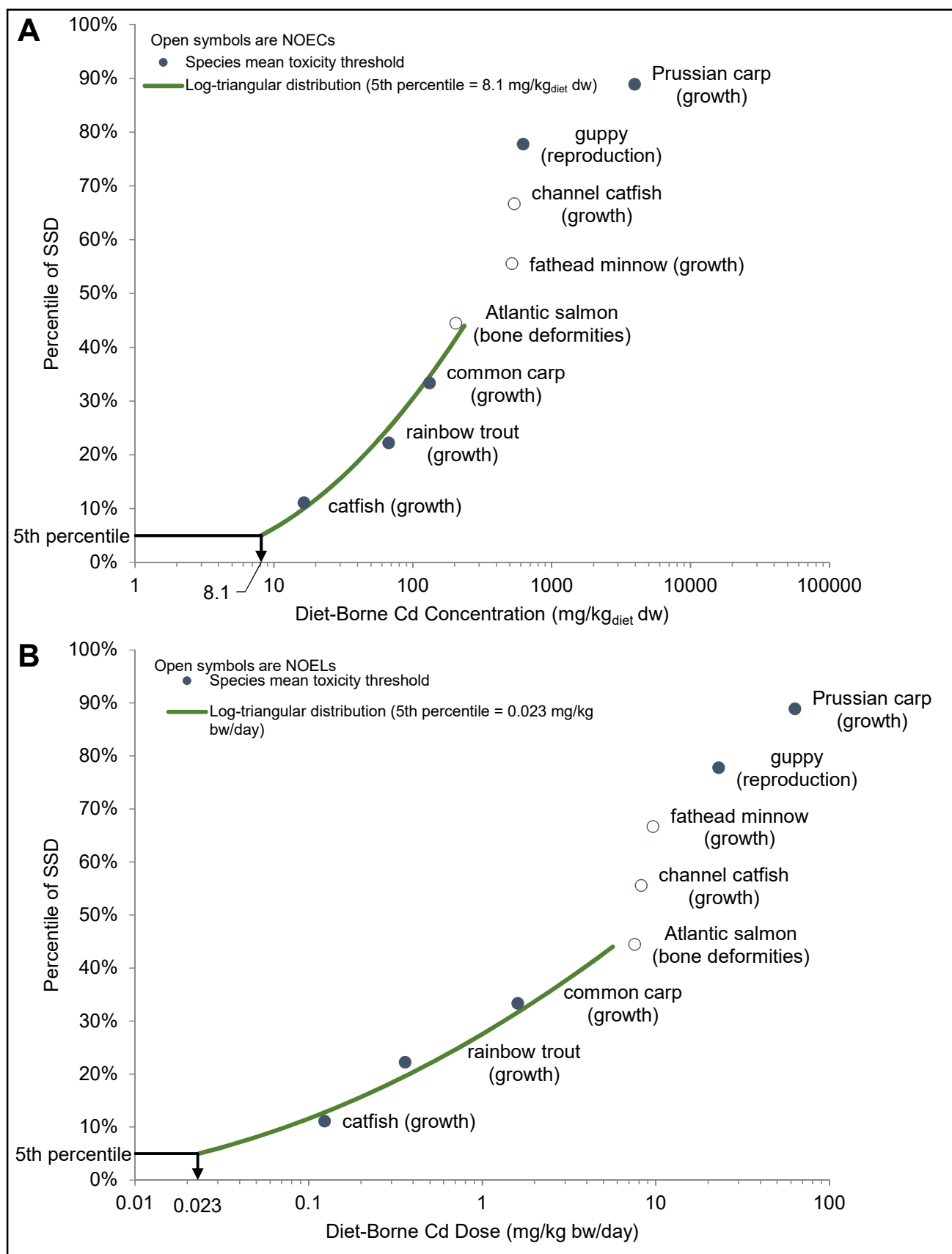


Figure 3-5. (A) Concentration-Based and (B) Dose-Based SSD for Diet-Borne Cadmium

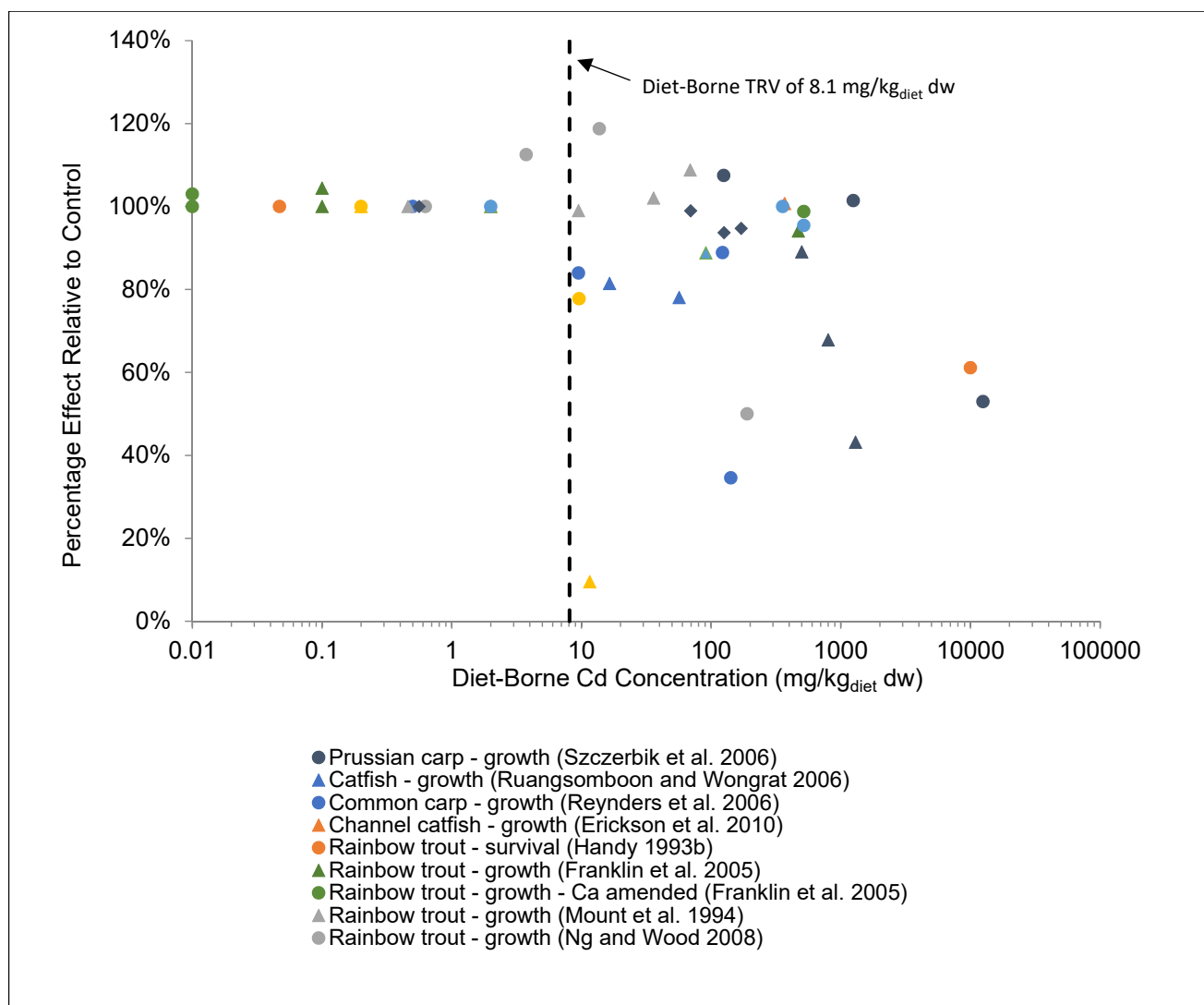


Figure 3-6. Comparison of All Concentration-Response Data for Cadmium, as a Percentage of Control Response, to Diet-Borne TRV

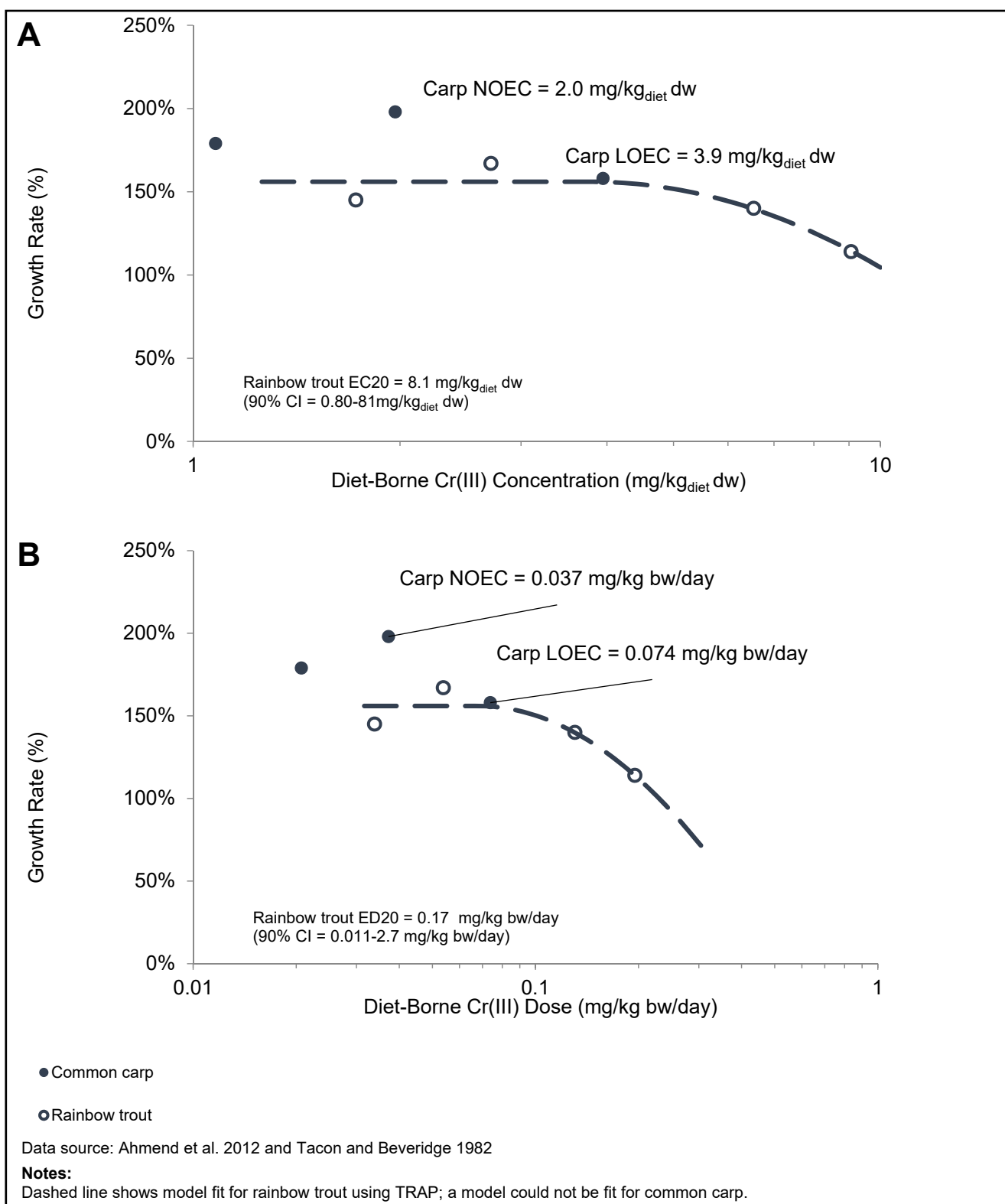


Figure 3-7. (A) Concentration-Response and (B) Dose-Response Relationships for Common Carp and Rainbow Trout Fed Diet-Borne Chromium(III)

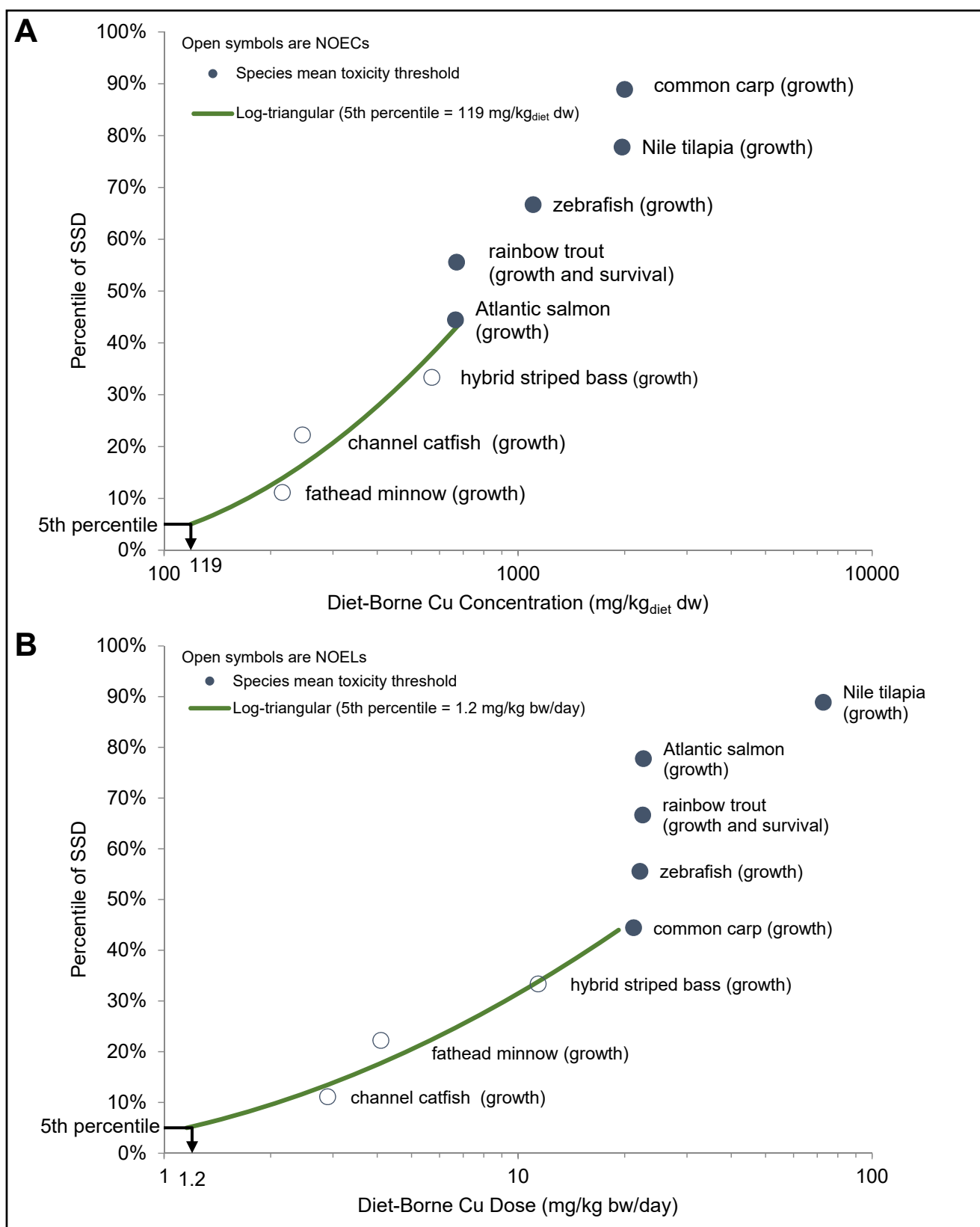


Figure 3-8. (A) Concentration-Based and (B) Dose-Based SSD for Diet-Borne Copper

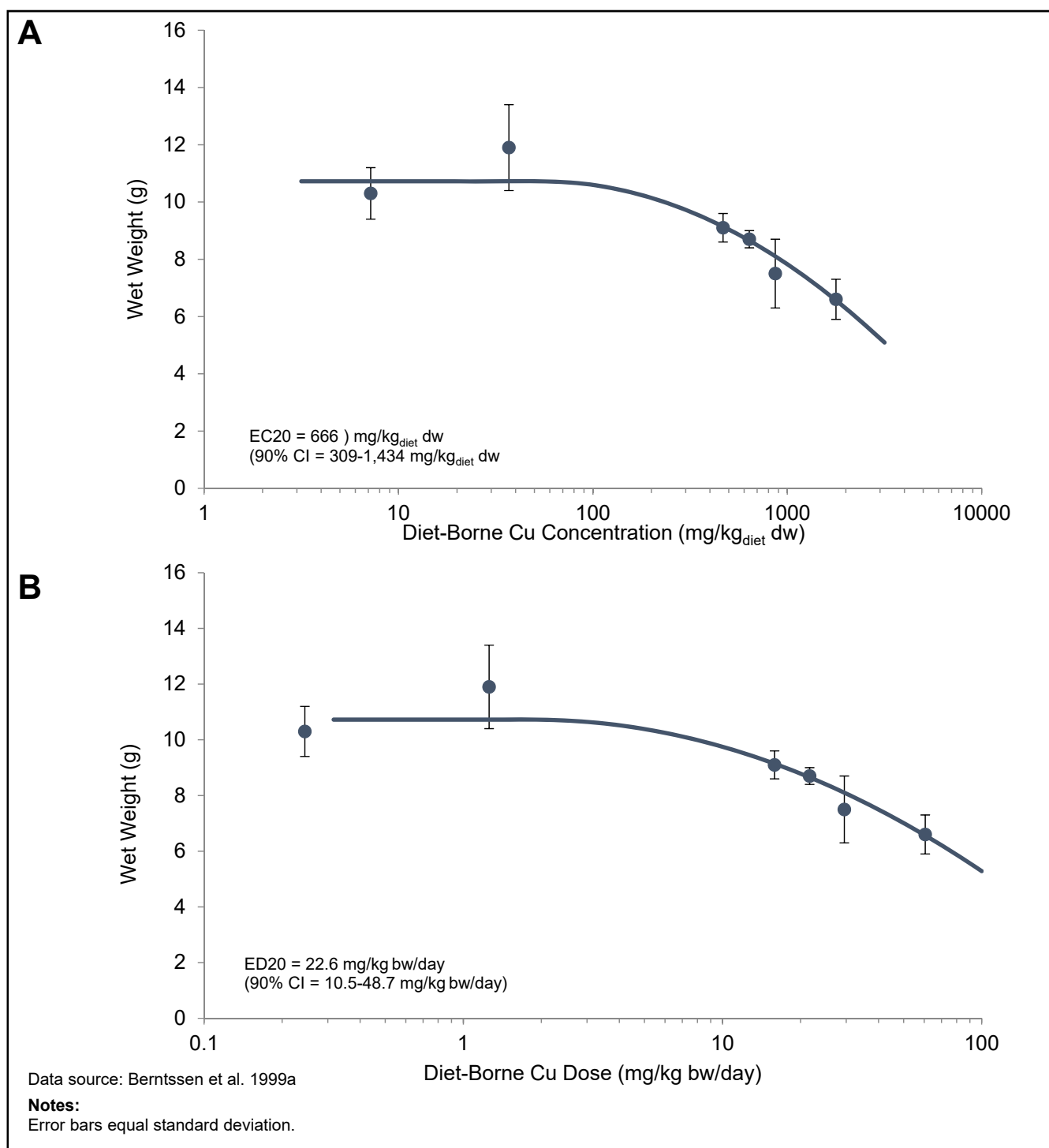


Figure 3-9. (A) Concentration-Response and (B) Dose-Response Relationships for Atlantic Salmon Fed Diet-Borne Copper in a Formulated Diet

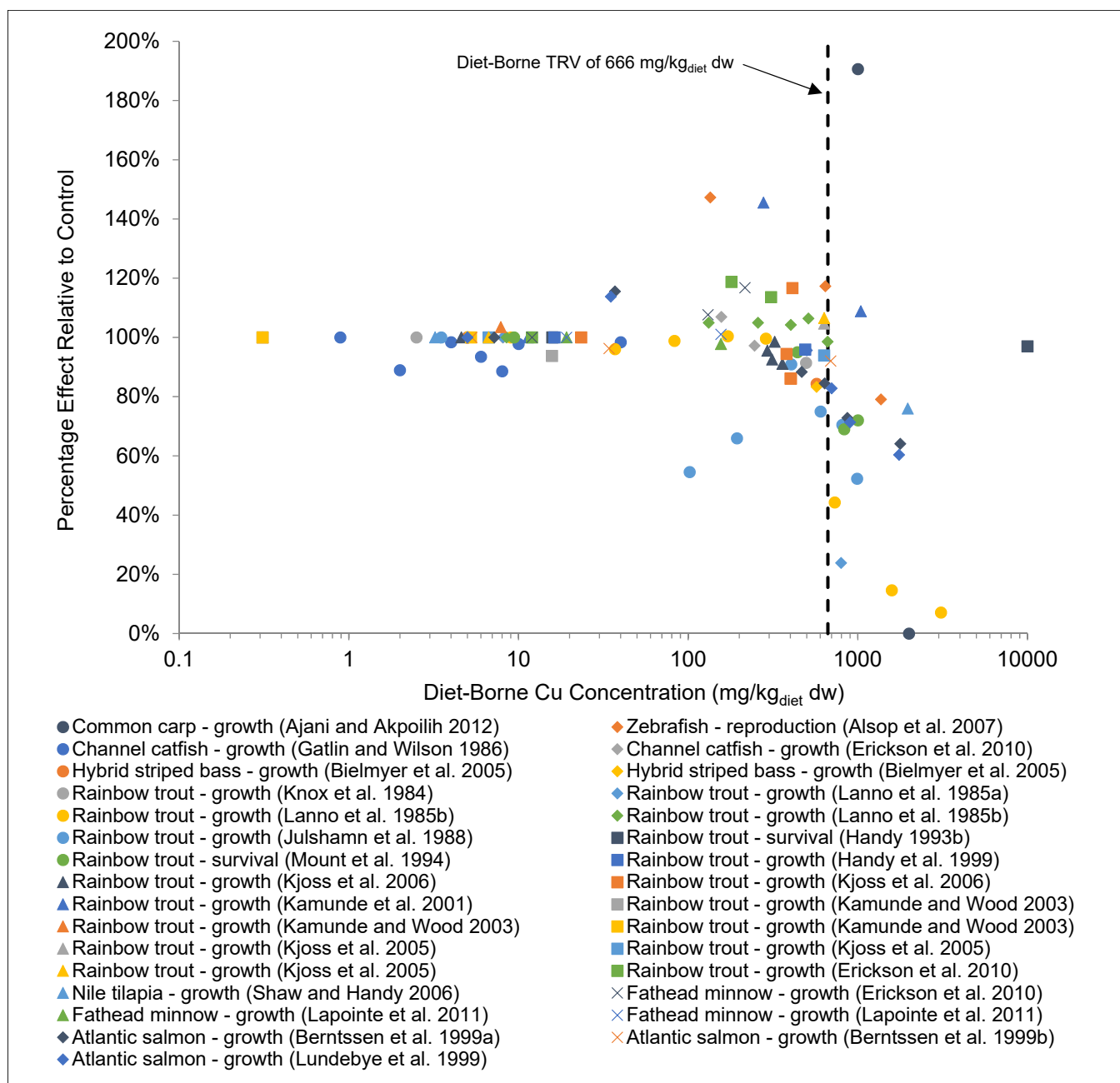


Figure 3-10. Comparison of All Concentration-Response Data for Copper, as a Percentage of Control Response, to Diet-Borne TRV

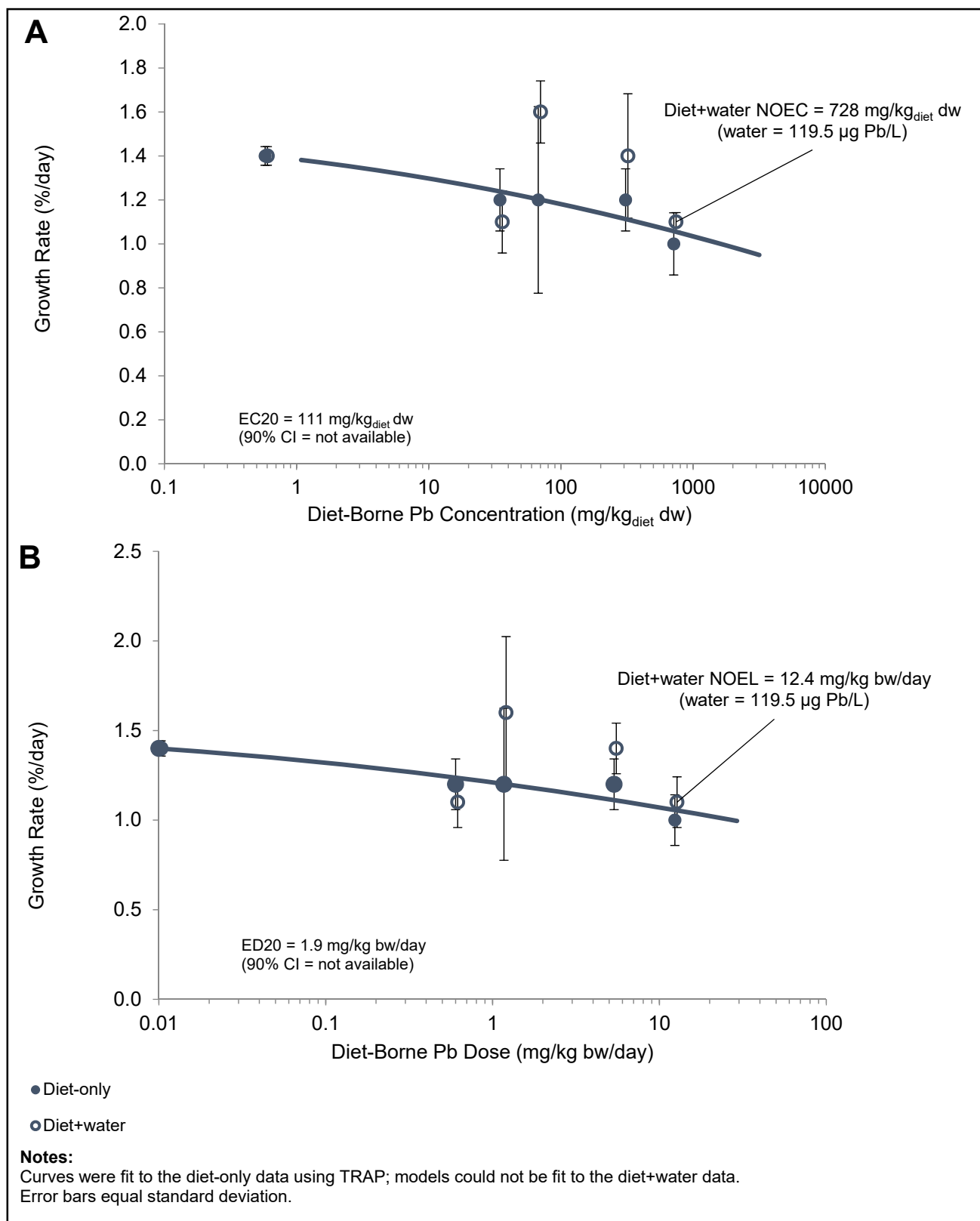


Figure 3-11. (A) Concentration-Response Data and (B) Dose-Response Data for Effects of Diet-Only and Diet+Water Lead Exposures on the Growth Rate of Juvenile Rainbow Trout

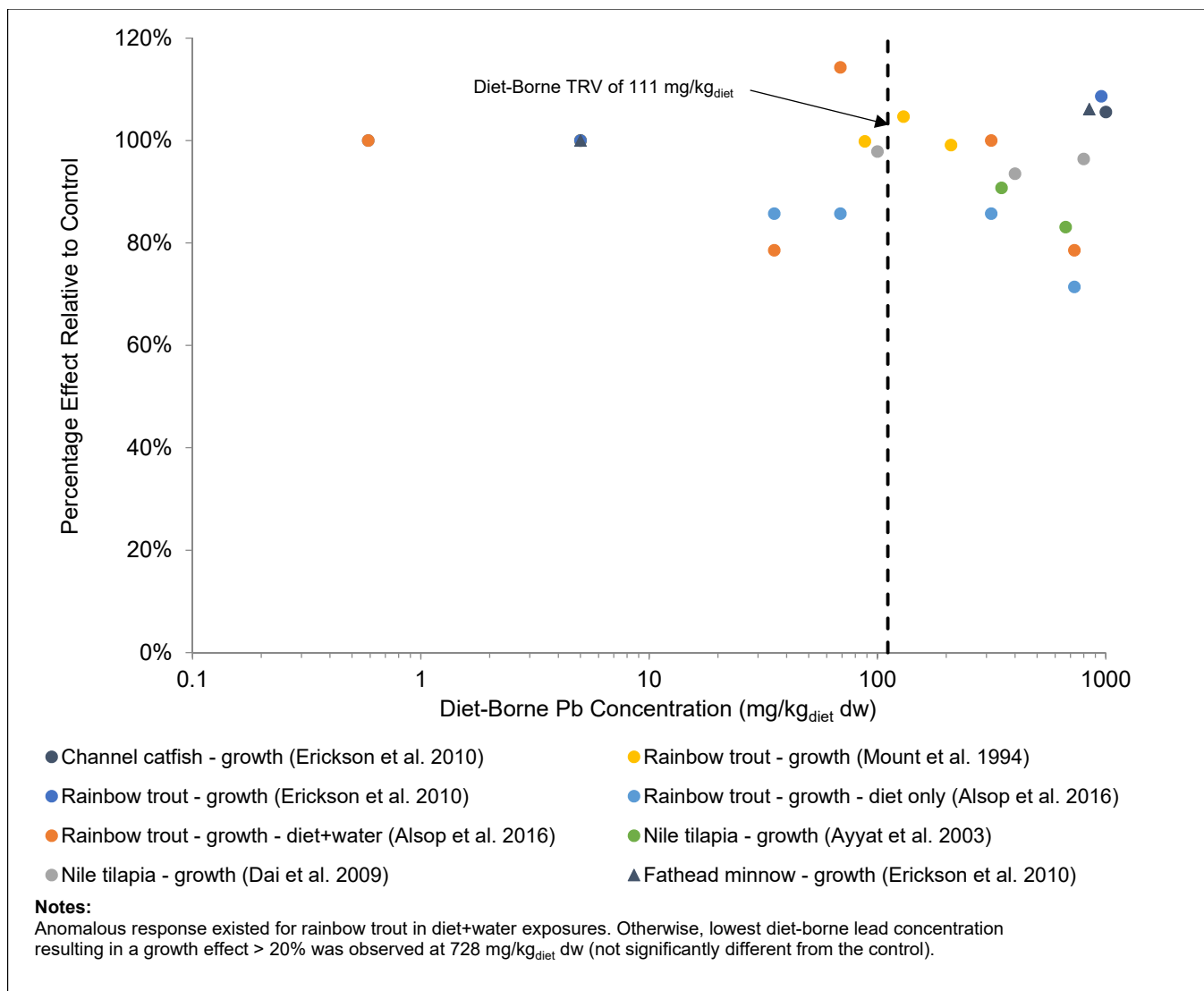


Figure 3-12. Comparison of All Concentration-Response Data for Lead, as a Percentage of Control Response, to Diet-Borne TRV

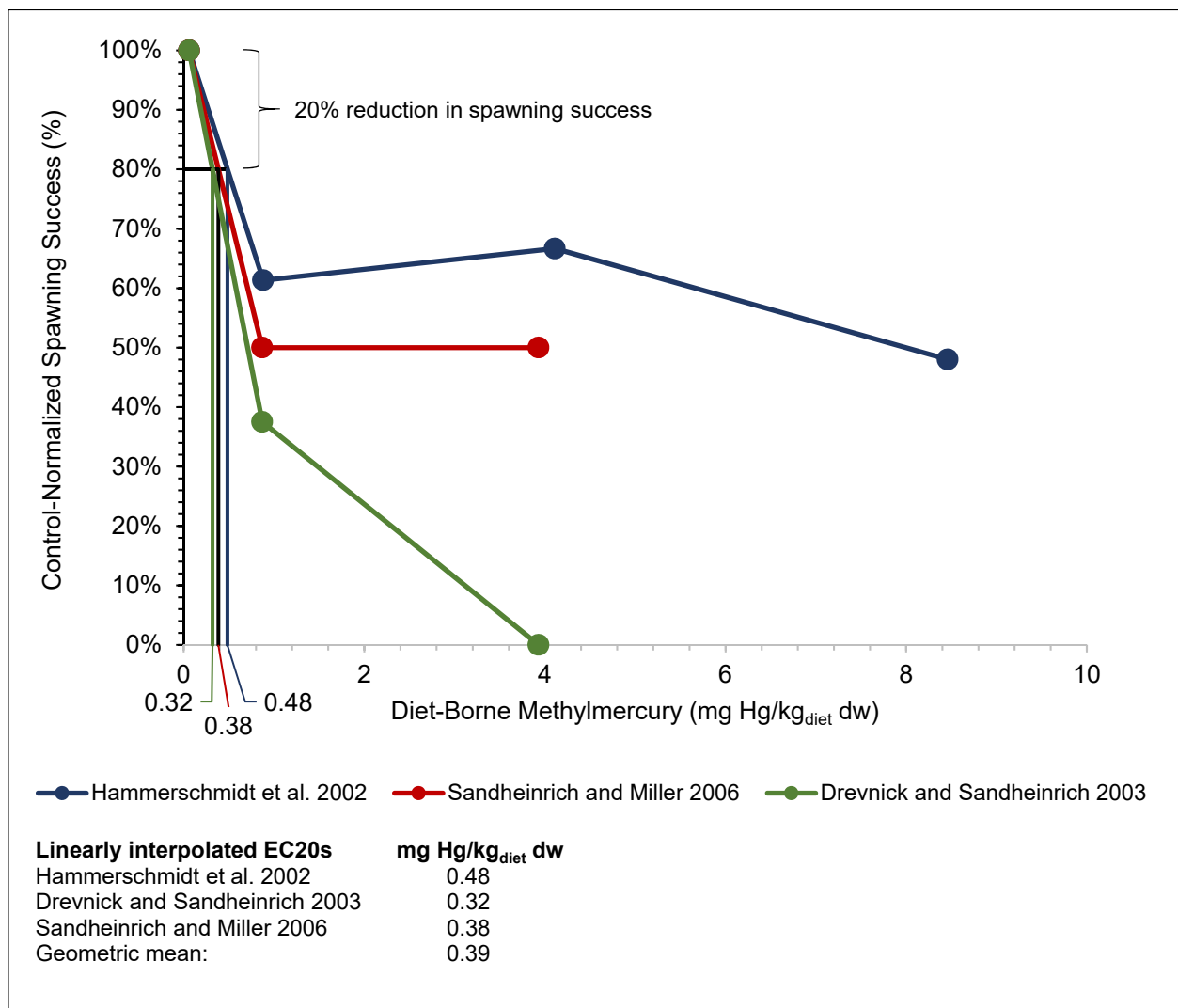


Figure 3-13. Relationship Between Diet-Borne Methylmercury Concentrations and Fathead Minnow Spawning Success

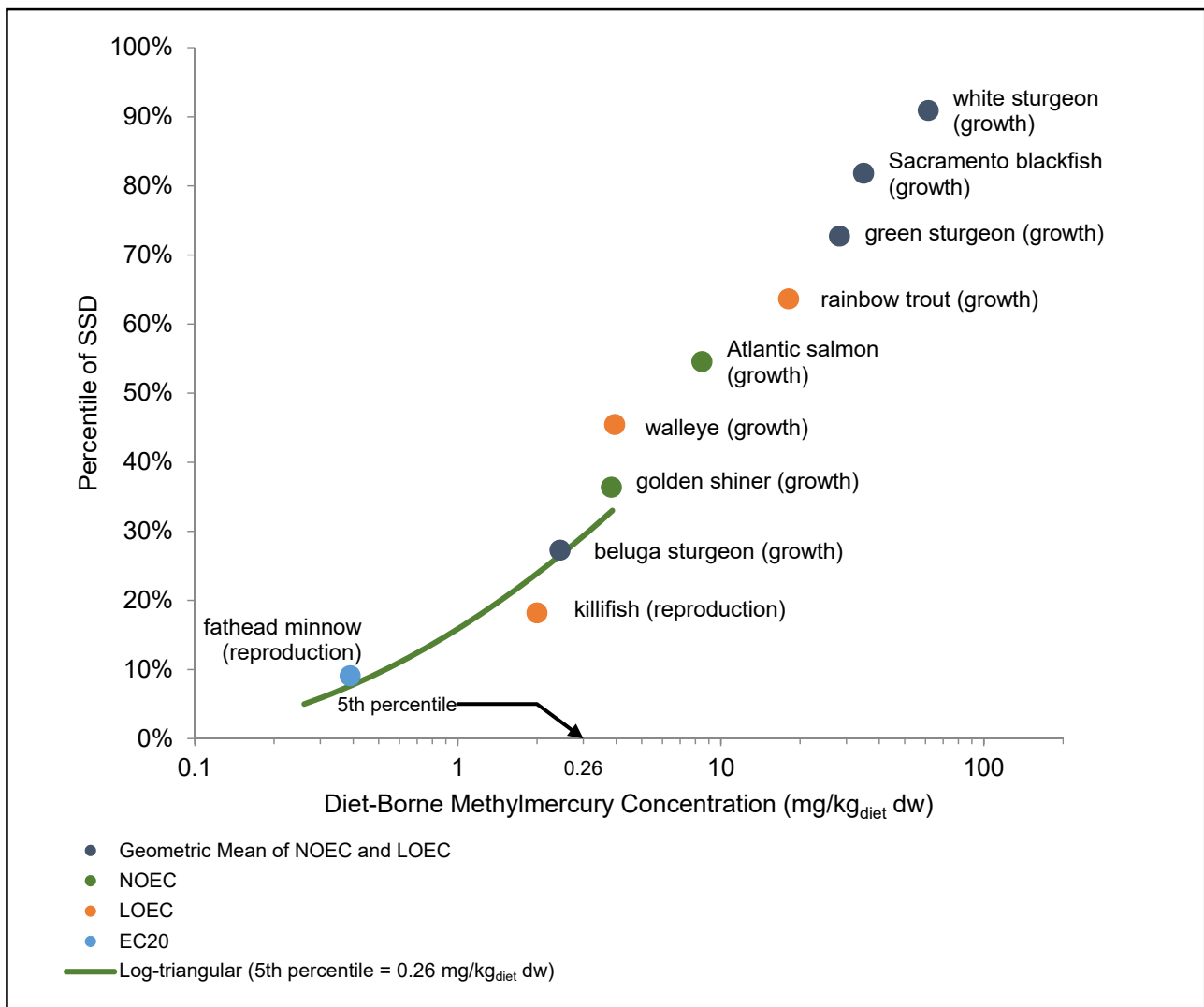


Figure 3-14. Concentration-Based SSD for Diet-Borne Methylmercury

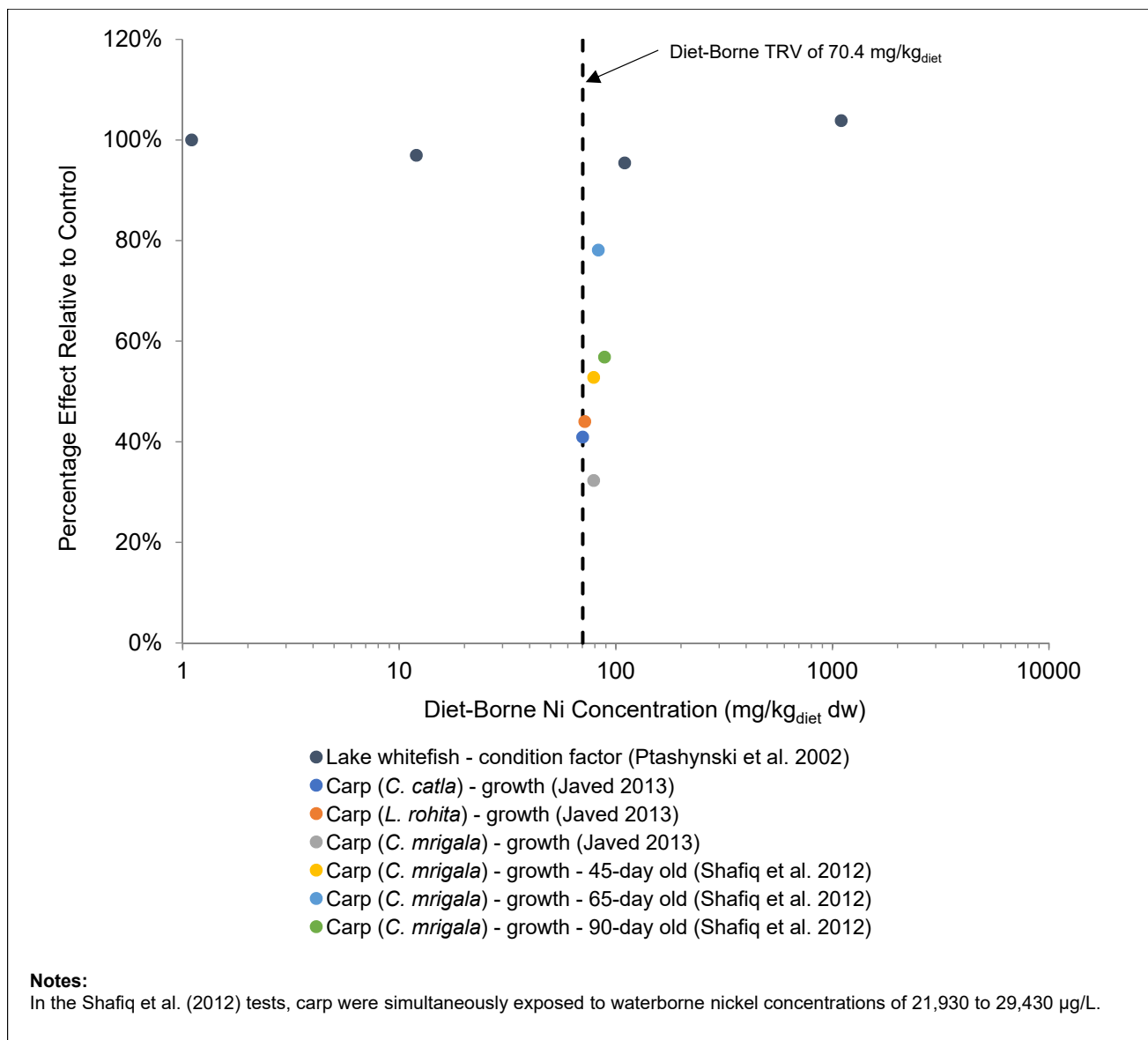


Figure 3-15. Comparison of All Concentration-Response Data for Nickel, as a Percentage of Control Response, to Diet-Borne TRV

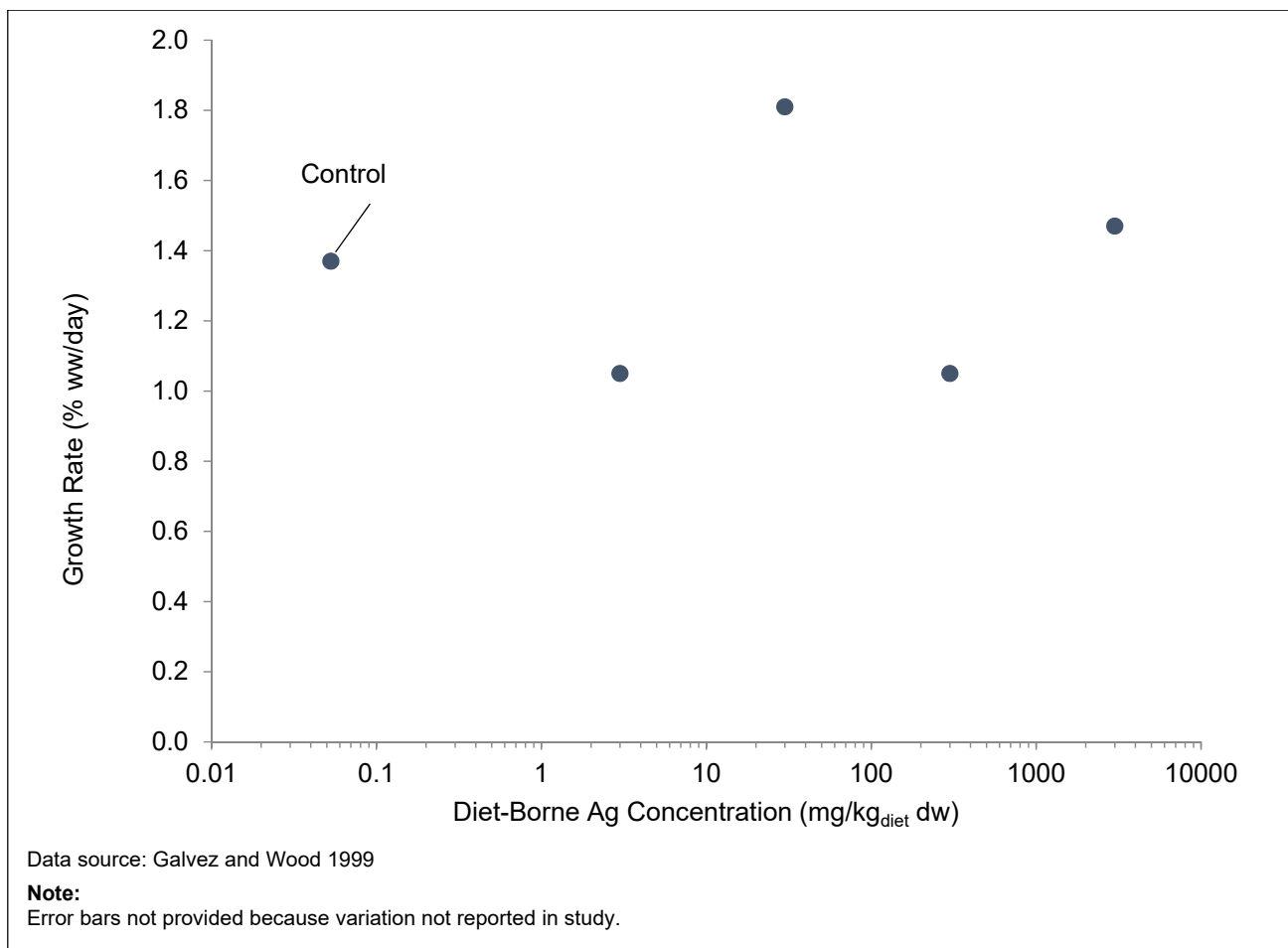
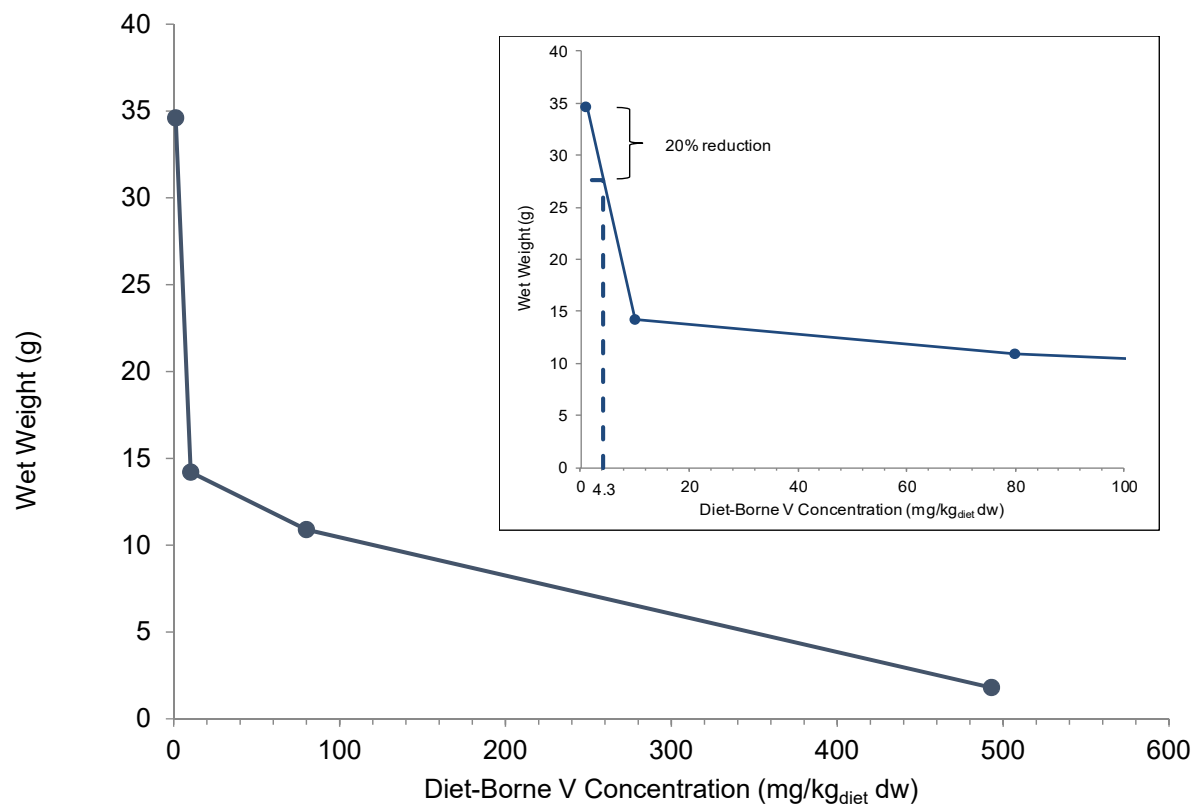


Figure 3-16. Relationship Between Diet-Borne Silver Concentrations and Rainbow Trout Growth Rate



Data source: Hilton and Bettger 1988

Note:

Error bars not provided because basis unclear in original paper.

Figure 3-17. Relationship Between Diet-Borne Vanadium Concentrations and Rainbow Trout Growth

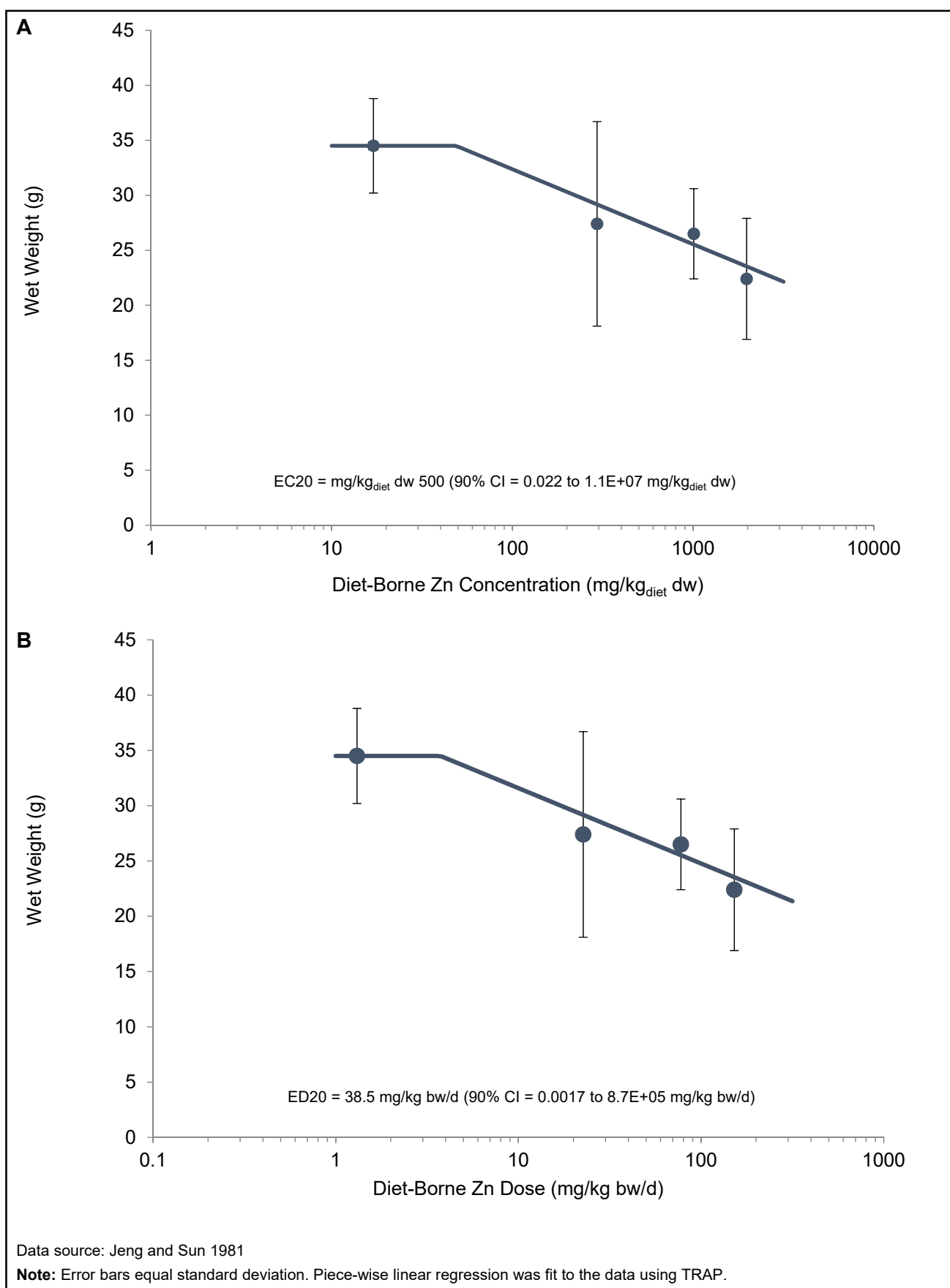


Figure 3-18. Effects of Diet-Borne Zinc in a Formulated Diet for Common Carp

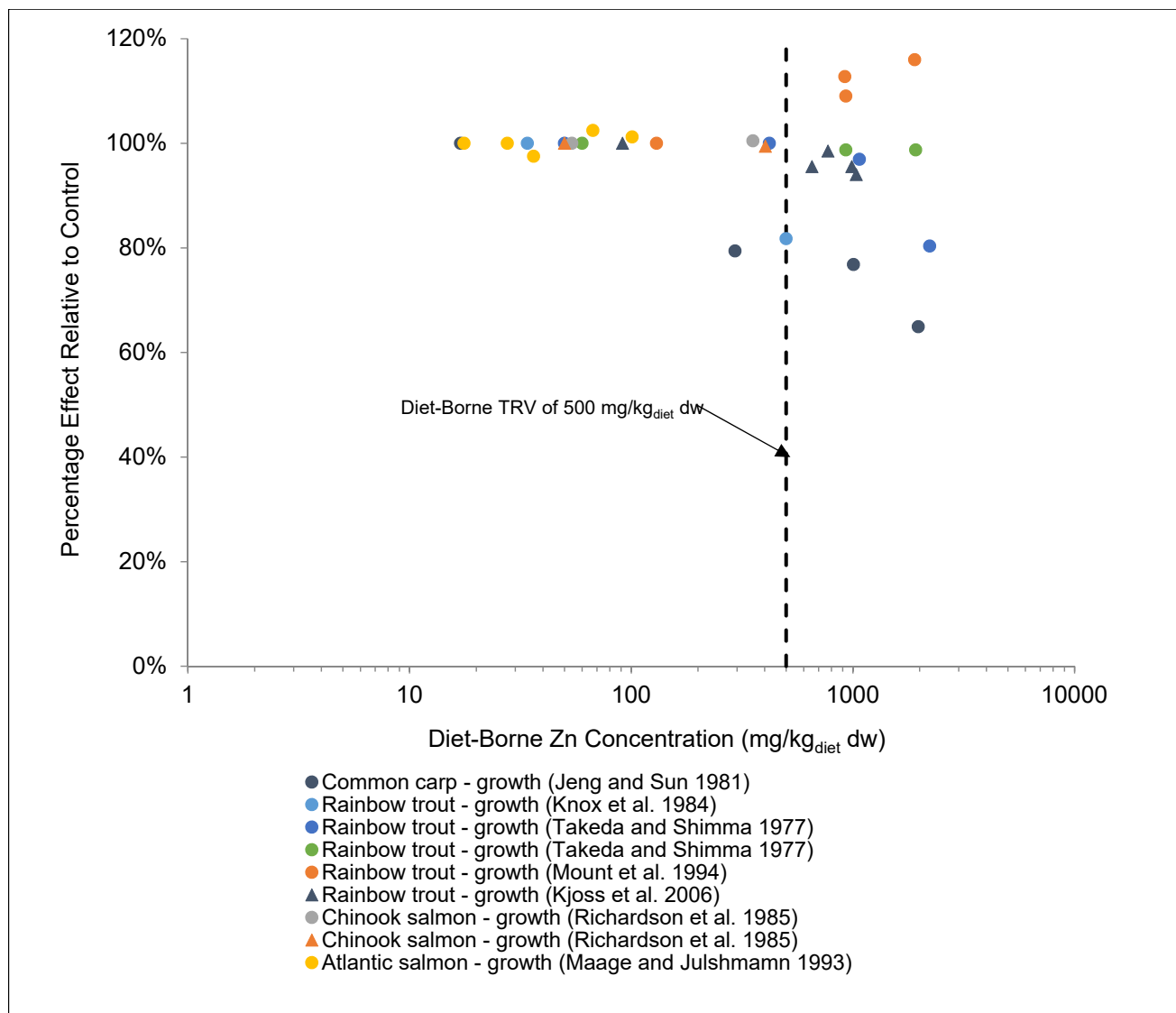
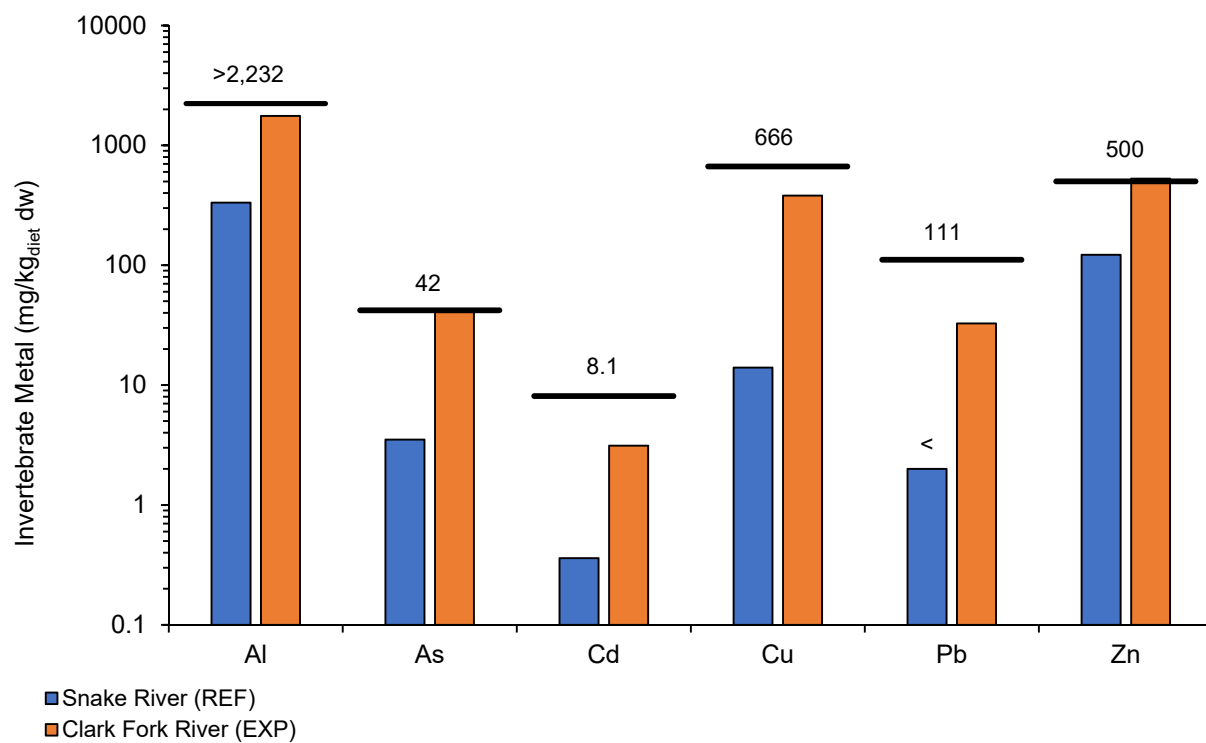


Figure 3-19. Comparison of All Concentration-Response Data for Zinc, as a Percentage of Control Response, to Diet-Borne TRV



Data source: Woodward et al. 1994

Notes:

Snake River (REF) samples represent a reference site; Clark Fork River (EXP) samples represent a site with elevated metal concentrations. Growth and survival were reduced by approximately 42% and 55% in trout fed Clark Fork River invertebrates compared with trout fed Snake River invertebrates. Horizontal black lines denote metal TRVs, and numeric TRVs are presented above those lines.

Figure 4-1. Concentrations of Metals in Field-Collected Benthic Invertebrates Fed to Rainbow Trout Fry

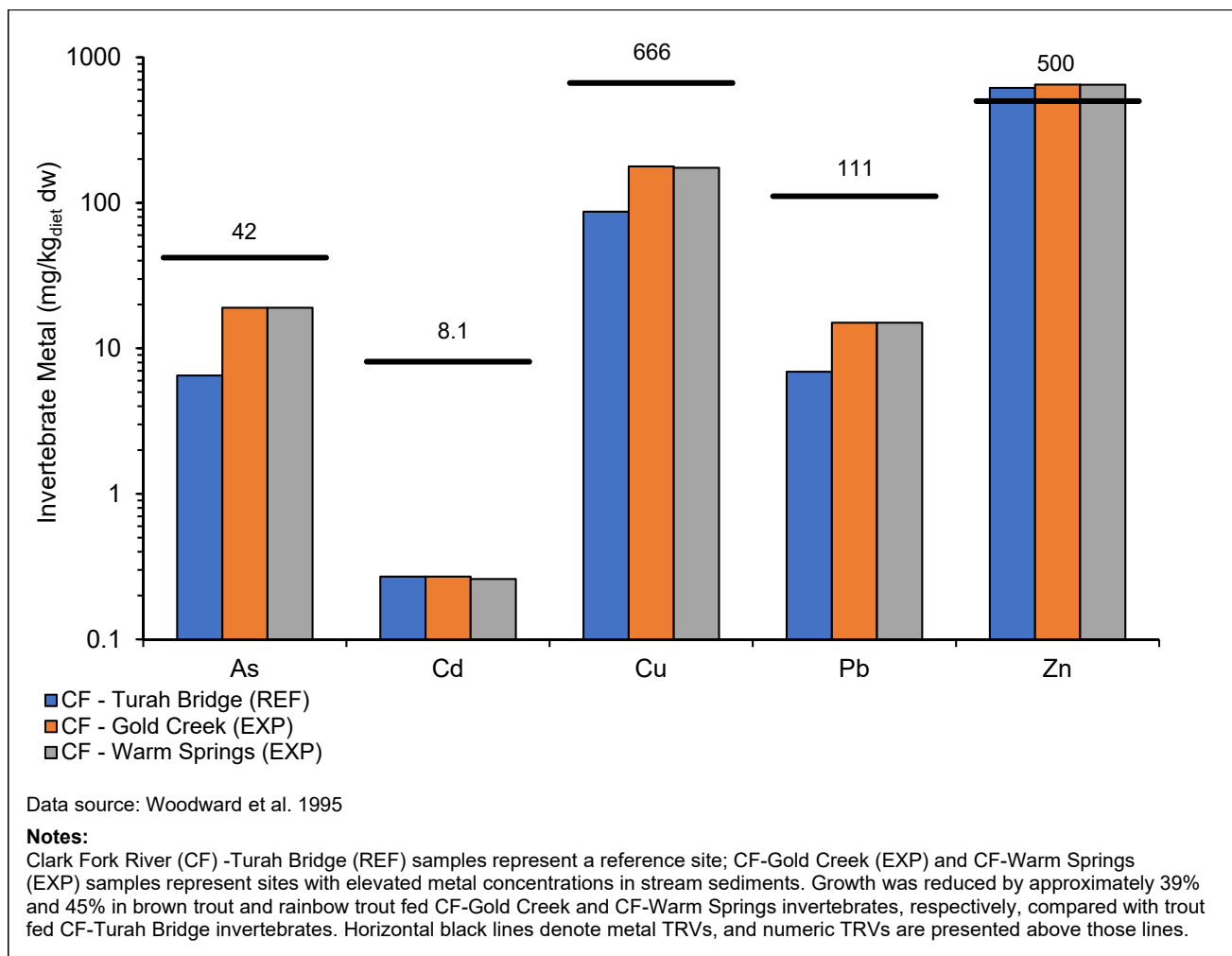


Figure 4-2. Concentrations of Metals in Field-Collected Benthic Invertebrates Fed to Brown Trout and Rainbow Trout Fry

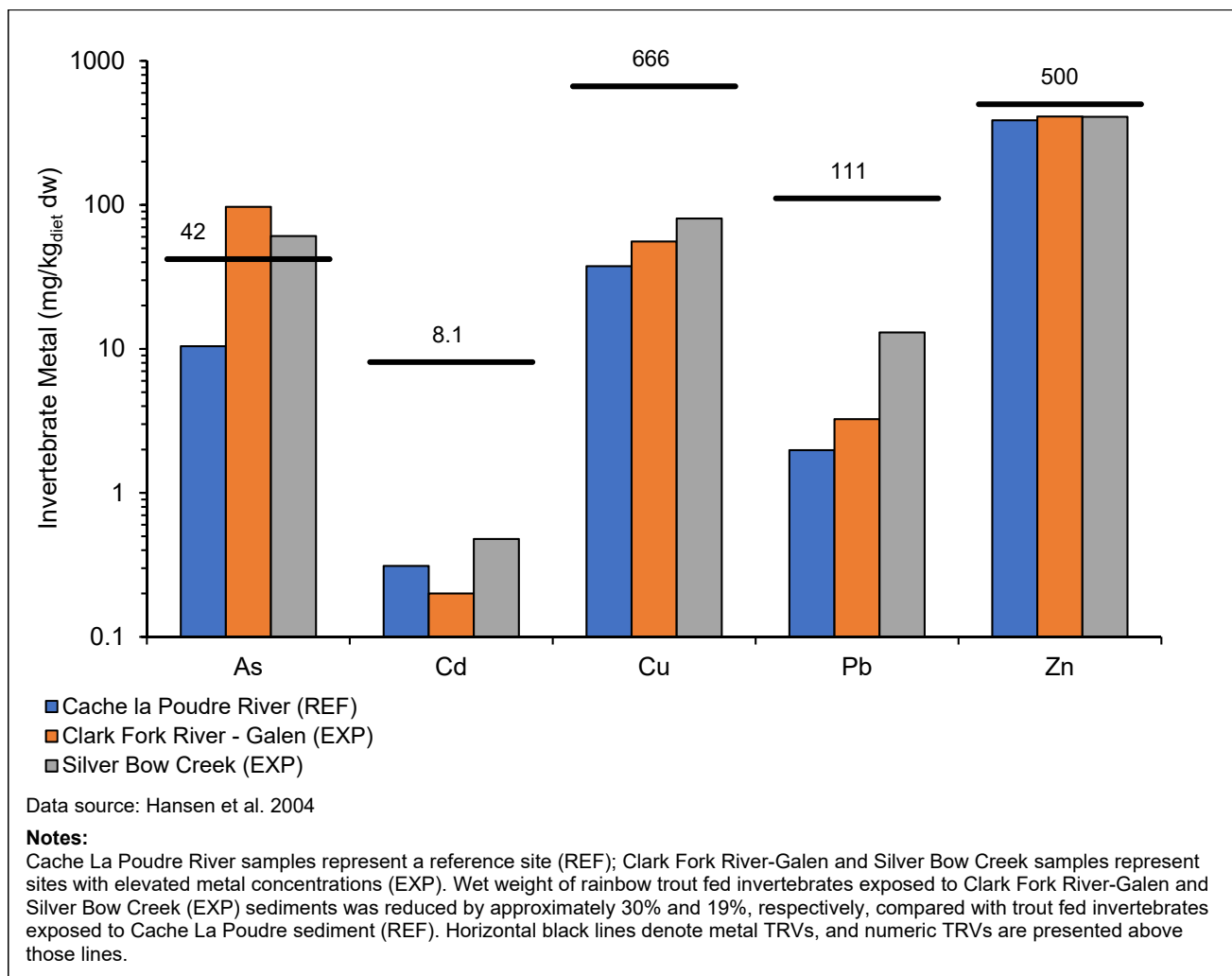


Figure 4-3. Concentrations of Metals in Benthic Invertebrates Exposed to Field-Collected Sediments and Fed to Juvenile Rainbow Trout

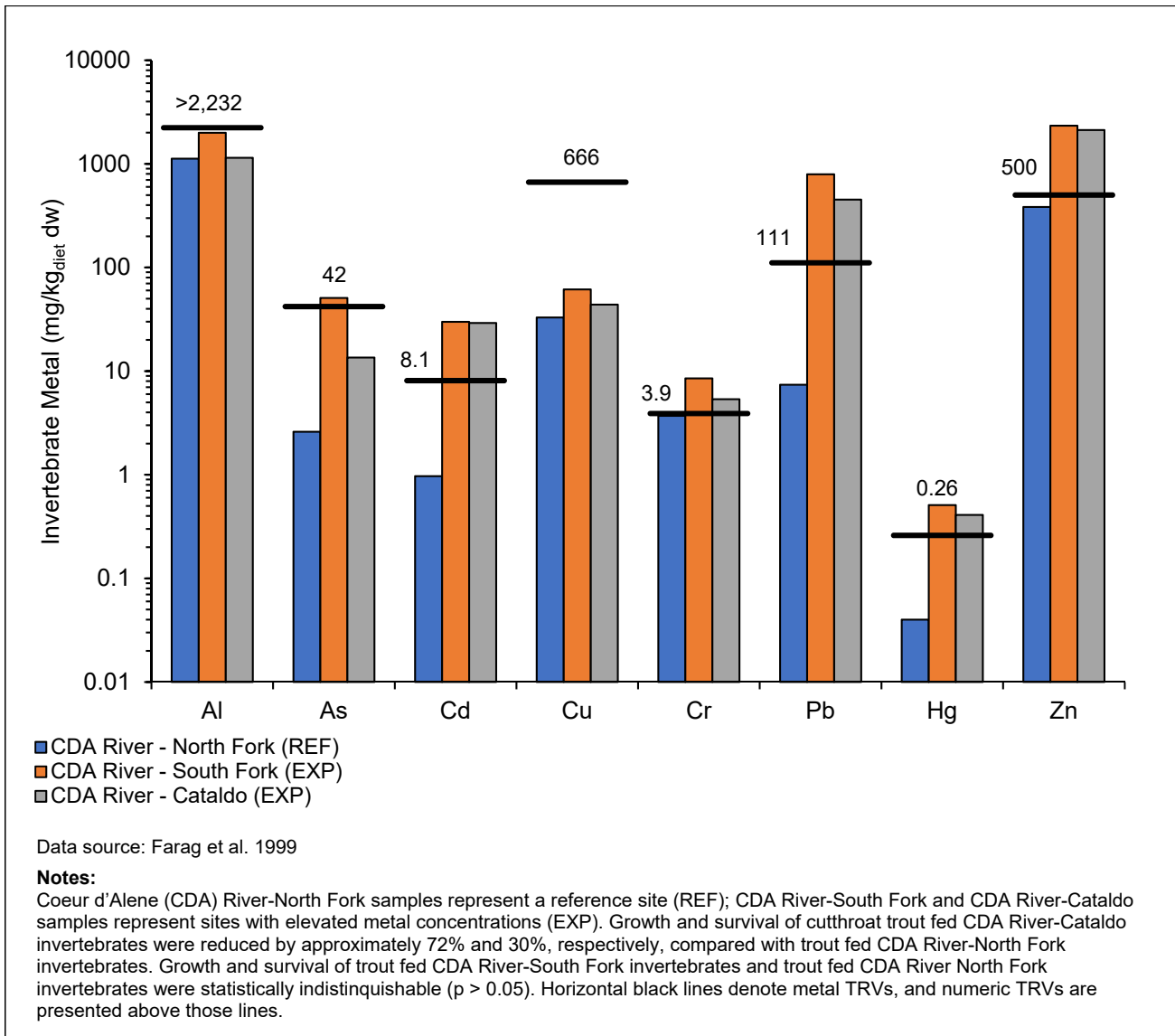


Figure 4-4. Concentrations of Metals in Field-Collected Benthic Invertebrates Fed to Cutthroat Trout Fry

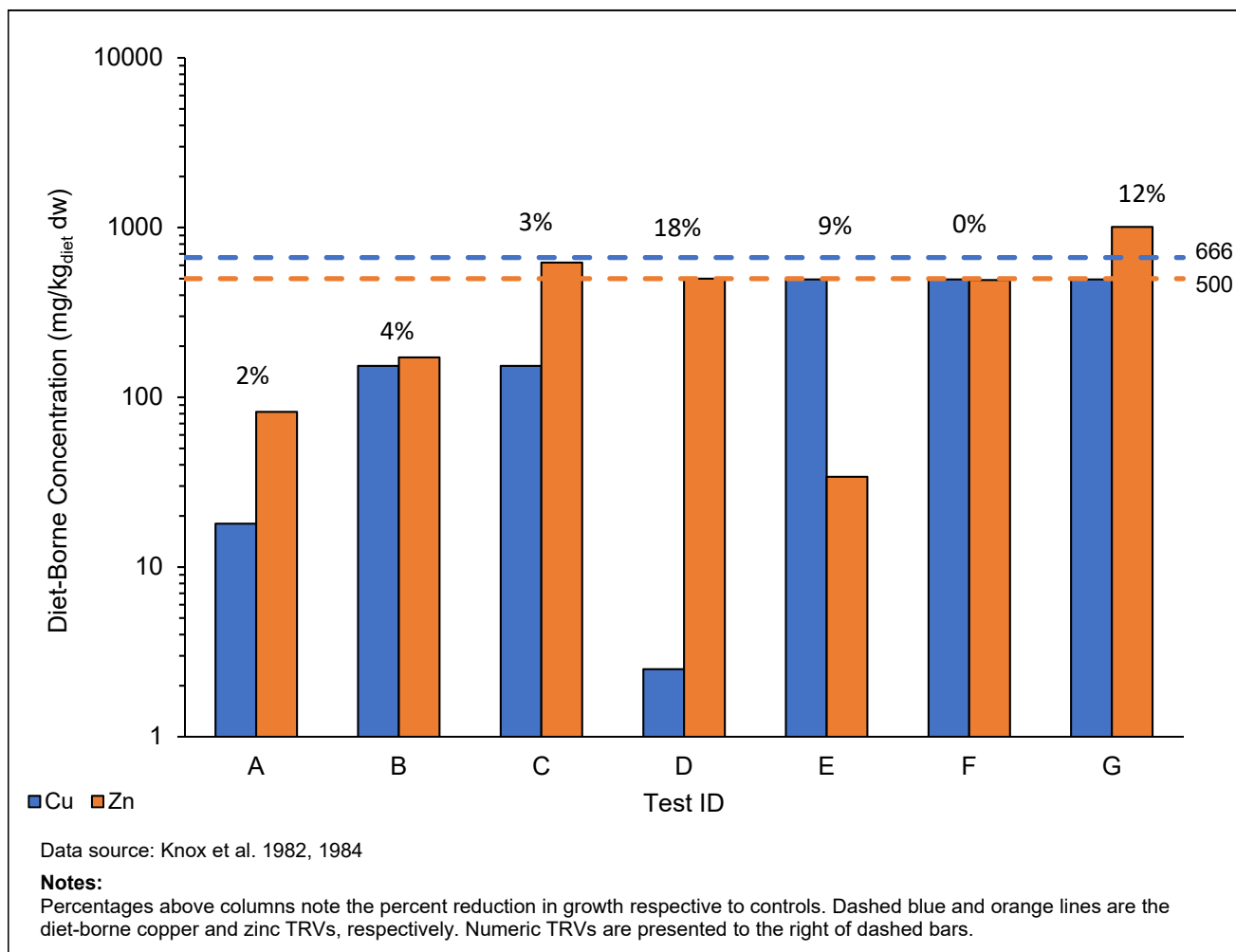


Figure 4-5. Mixtures of Diet-Borne Copper and Zinc Concentrations in Formulated Diets Fed to Rainbow Trout in Seven Tests

TABLES

Table 3-1. Concentration- and Dose-Based Diet-Borne Toxicity Data Used to Develop SSDs for Cadmium

Species	Endpoint	Duration	Diet ^a	Cadmium Form	Statistical Endpoint Basis (% Effect Relative to Control)	Diet-Borne Concentration		Diet-Borne Dose		Reference
						Cadmium (mg/kg _{diet} dw)	Species Mean Cadmium (mg/ kg _{diet} dw)	Cadmium (mg/kg bw/day)	Species Mean Cadmium (mg/kg bw/day)	
Prussian carp (<i>Carassius auratus gibelio</i>)	wet weight	~32 months	formulated	CdCl ₂	Geomean of NOEC/LOEC and NOEL/LOEL (0%, 47%)	3,953	3,953	63	63	Szczerbik et al. 2006
Catfish (<i>Clarias</i> spp.)	wet weight	60 days	cladoceran (<i>M. macrocopa</i>)	CdNO ₃	LOEC/LOEL (19%)	16.48	16.48	0.124	0.124	Ruangsomboon and Wongrat 2006
Common carp (<i>Cyprinus carpio</i>)	weight gain	28 days	worm (<i>T. tubifex</i>)	CdCl ₂	EC20/ED20	132 (90% CI = 19-942)	132	1.6 (90% CI = 0.15-19)	1.6	Reynders et al. 2006
Channel catfish (<i>Ictalurus punctatus</i>)	dry weight	30 days	worm (<i>L. variegatus</i>)	CdCl ₂	NOEC/NOEL (0%)	538	538	8.2	8.2	Erickson et al. 2010
Rainbow trout (<i>Oncorhynchus mykiss</i>)	mortality	28 days	formulated	CdSO ₄	LOEC/LOEL (39%)	10,000 ^b	67	68.2 ^b	0.36	Handy 1993b
	growth rate	28 days	formulated - 22 mg Ca/g dw	CdNO ₃	NOEC/NOEL (6%)	471 ^c		9.42 ^c		Franklin et al. 2005
	growth rate	28 days	formulated - 60 mg Ca/g dw	CdNO ₃	NOEC/NOEL (0%)	519 ^c		10.38 ^c		Franklin et al. 2005
	wet weight	60 days	brine shrimp (<i>Artemia</i> sp.)	CdCl ₂	NOEC/NOEL(0%)	69 ^c		4.1 ^c		Mount et al. 1994
	growth rate	1 month	worm (<i>L. variegatus</i>)	CdNO ₃	EC20/ED20	67 (90% CI = na)		0.36 (90% CI = na)		Ng and Wood 2008
	growth rate	1 month	oligochaete worm pellets - 0.8 mg Ca/g dw	CdNO ₃	LOEC/LOEL (90%)	11.6 ^d		0.069 ^d		Ng et al. 2009
	growth rate	1 month	oligochaete worm pellets - 76.2 mg Ca/g dw	CdNO ₃	NOEC/NOEL (22%)	9.6 ^c		0.066 ^c		Ng et al. 2009
	dry weight	30 days	worm (<i>L. variegatus</i>)	CdCl ₂	NOEC/NOEL (11%)	91 ^c		1.5 ^c		Erickson et al. 2010
Fathead minnow (<i>Pimephales promelas</i>)	dry weight	30 days	worm (<i>L. variegatus</i>)	CdCl ₂	NOEC/NOEL (5%)	518	518	9.7	9.7	Erickson et al. 2010
Guppy (<i>Poecilia reticulata</i>)	total fry/female	30 days	midge larvae	CdCl ₂	EC20/ED20	622 (90% CI = 278-1,392)	622	23.0 ^e (90% CI = 10.3-51.5)	23.0	Hatakeyama and Yasuno 1987
Atlantic salmon (<i>Salmo salar</i>)	bone deformities	4 months	formulated	CdSO ₄	NOEC/NOEL (NA)	204.1	204.1	7.6 ^e	7.6	Berntssen et al. 2003

Notes:

^a Calcium concentration in the diet is reported where available.

^b Excluded from species mean because the mortality endpoint was less sensitive than the growth endpoint based on observations from other tests.

^c Excluded from species mean because a 20% effect concentration/20% dose concentration (EC20/ED20) could be calculated from a test with a natural diet (Ng and Wood 2008).

^d Excluded from species mean because the magnitude of reduced growth was inconsistent with that of other studies (Ng and Wood 2008; Erickson et al. 2010) in which rainbow trout were fed an oligochaete diet that was not amended with calcium.

^e Dose-based toxicity threshold is based on an assumed food ingestion rate of 0.037 g_{food}/g bw/day (Section 2.6).

CI – confidence interval

LOEC – lowest-observed-effect concentration

LOEL – lowest-observed-effect level

NA – not applicable

na – not available

NOEC – no-observed-effect concentration

NOEL – no-observed-effect level

SSD – species sensitivity distribution

Table 3-2. Concentration- and Dose-Based Diet-Borne Toxicity Data Used to Develop SSDs for Copper

Species	Endpoint	Duration	Diet	Copper Form	Simultaneous Water Exposure?	Statistical Endpoint Basis (% Effect Relative to Control)	Diet-Borne Concentration		Diet-Borne Dose		Reference
							Copper (mg/kg _{diet} dw)	Species Mean Copper (mg/kg _{diet} dw)	Copper (mg/kg bw/day)	Species Mean Copper (mg/kg bw/day)	
Common carp (<i>Cyprinus carpio</i>)	weight gain	42 days	formulated	CuSO ₄	no	LOEC/LOEL (36%)	2,000	2,000	21.2	21.2	Ajani and Akpoilih 2012
Zebrafish (<i>Danio rerio</i>)	GSI	260 days	formulated	CuSO ₄	yes	EC20/ED20	1,103 (90% CI = 0.13-9.0E+06)	1,103	22.1	22.1 (0.0027-1.8E+05)	Alsop et al. 2007
Channel catfish (<i>Ictalurus punctatus</i>)	weight gain	13 weeks	formulated	CuSO ₄	no	NOEC/NOEL (2%)	40 ^a	246	1.2 ^a	2.9	Gatlin and Wilson 1986
	dry weight	30 days	worm (<i>L. variegatus</i>)	CuSO ₄	no	NOEC/NOEL (3%)	246		2.9		Erickson et al. 2010
Hybrid striped bass (<i>Morone chrysops</i> × <i>M. saxatilis</i>)	growth – freshwater acclimated	42 days	formulated	CuSO ₄	no ^b	NOEC/NOEL (16%)	571	571	11.4	11.4	Bielmyer et al. 2005
	growth – saltwater acclimated	42 days	formulated	CuSO ₄	no ^b	NOEC/NOEL (17%)	571		11.4		
Rainbow trout (<i>Oncorhynchus mykiss</i>)	weight gain	20 weeks	formulated	CuSO ₄	no	NOEC/NOEL (9%)	495 ^c	671	8.5 ^c	22.5	Knox et al. 1984
	wet weight	16 weeks	formulated	CuSO ₄	no	LOEC/LOEL (76%)	796 ^c		29.5 ^{c,d}		Lanno et al. 1985a
	wet weight	8 weeks	formulated	CuSO ₄	no	EC20/ED20	430 (90% CI = 306-604)		15.9 ^d (90% CI = 11.3-22.3)		Lanno et al. 1985b
	wet weight	24 weeks	formulated	CuSO ₄	no	NOEC/NOEL (1%)	664 ^c		24.6 ^d		Lanno et al. 1985b
	weight gain	9 weeks	formulated	CuSO ₄	no	EC20/ED20	947		16.2		Julshamn et al. 1988
	survival	28 days	formulated	CuSO ₄	no	NOEC/NOEL (3%)	10,000 ^c		69.2 ^c		Handy 1993b
	survival	60 days	Brine shrimp (<i>Artemia</i> sp.)	CuCl ₂	yes	EC20/ED20	740 (90% CI = 644-850)		44.4 (90% CI = 38.6-51.0)		Mount et al. 1994
	growth rate	3 months	formulated	CuSO ₄	no	NOEC/NOEL (4%)	490 ^c		8.3 ^c		Handy et al. 1999
	growth rate	15 days	formulated	Cu-lysine	no	NOEC/NOEL (9%)	359 ^c		28.7 ^c		Kjoss et al. 2006
	growth rate	4 weeks	formulated	CuO	no	NOEC/NOEL (0%)	412 ^c		33.0 ^c		Kjoss et al. 2006
	weight gain	28 days	formulated	CuSO ₄	no	NOEC/NOEL (0%)	1,042 ^c		41.7 ^c		Kamunde et al. 2001
	weight gain	50 days	formulated	CuSO ₄	yes	NOEC/NOEL (0%)	282 ^c		11.3 ^c		Kamunde et al. 2002
	growth rate	35 days	formulated (1.5% ration)	CuSO ₄	no	NOEC/NOEL (6%)	15.75 ^c		0.24 ^c		Kamunde and Wood 2003
	growth rate	35 days	formulated (3.0% ration)	CuSO ₄	no	NOEC/NOEL (0%)	7.87 ^c		0.24 ^c		Kamunde and Wood 2003
	growth rate	35 days	formulated (4.5% ration)	CuSO ₄	no	NOEC/NOEL (0%)	5.24 ^c		0.24 ^c		Kamunde and Wood 2003
	condition factor	28 days	formulated (1.5% sodium)	CuSO ₄	no	NOEC/NOEL (0%)	633 ^c		23.2 ^c		Kjoss et al. 2005
	condition factor	28 days	formulated (3.0% sodium)	CuSO ₄	no	NOEC/NOEL (6%)	633 ^c		23.2 ^c		Kjoss et al. 2005
	condition factor	28 days	formulated (4.5% Na)	CuSO ₄	no	NOEC/NOEL (0%)	633 ^c		23.2 ^c		Kjoss et al. 2005
	dry weight	30 days	worm (<i>L. variegatus</i>)	CuSO ₄	no	NOEC/NOEL (0%)	308 ^c		4.0 ^c		Erickson et al. 2010
Nile tilapia (<i>Oreochromis niloticus</i>)	growth rate	42 days	formulated	CuSO ₄	no	LOEC/LOEL (24%)	1,968	1,968	72.8 ^d	72.8	Shaw and Handy 2006
Fathead minnow (<i>Pimephales promelas</i>)	dry weight	30 days	worm (<i>L. variegatus</i>)	CuSO ₄	no	NOEC/NOEL (0%)	216	216	4.1	4.1	Erickson et al. 2010
	condition factor	28 days	worm (<i>L. variegatus</i>)	CuSO ₄	yes	NOEC/NOEL (2%)	156 ^a		1.56 ^a		Lapointe et al. 2011
	condition factor	28 days	worm (<i>L. variegatus</i>)	CuSO ₄	yes	NOEC/NOEL (0%)	156 ^a		1.56 ^a		Lapointe et al. 2011
Atlantic salmon (<i>Salmo salar</i>)	wet weight	3 months	formulated	CuSO ₄	yes	EC20/ED20	666 (90% CI = 309-1,434)	666	22.6 (90% CI = 10.5-48.7)	22.6	Berntssen et al. 1999a
	wet weight	4 weeks	formulated	CuSO ₄	no	NOEC/NOEL (8%)	691 ^c		16.6 ^c		Berntssen et al. 1999b

Notes:

- ^a Excluded from species mean because there was a higher no-observed-effect concentration/no-observed-effect level (NOEC/NOEL) available for this species.
- ^b Bielmyer et al. (2005) also tested a combined water- and diet-borne copper exposure, but the copper concentration was not developed based on the corresponding waterborne copper concentration, which was based on the 96-hr copper concentration that is lethal to 20% of an exposed population (LC20) for striped bass.
- ^c Excluded from species means because 20% effect concentrations/20% dose concentrations (EC20s/ED20s) were available for this species.
- ^d Dose-based toxicity threshold is based on an assumed food ingestion rate of 0.037 g_{food}/g bw/day (Section 2.6).

CI – confidence interval
GSI – gonadosomatic index
LOEC – lowest-observed-effect concentration
LOEL – lowest-observed-effect level
SSD – species sensitivity distribution

Table 3-3. Concentration- and Dose-Based Diet-Borne Toxicity Data Used to Derive TRVs for Lead

Species	Endpoint	Duration	Diet	Lead Form	Simultaneous Water Exposure?	Statistical Endpoint Basis (% Effect Relative to Control)	Diet-Borne Concentration		Diet-Borne Dose		Reference
							Lead (mg/kg _{diet} dw)	Species Mean Lead (mg/ kg _{diet} dw)	Lead (mg/kg bw/day)	Species Mean Lead (mg/kg bw/day)	
Channel catfish (<i>Ictalurus punctatus</i>)	dry weight	30 days	worm (<i>L. variegatus</i>)	PbNO ₃	no	NOEC/NOEL (0%)	1,000	1,000	16.2	16.2	Erickson et al. 2010
Rainbow trout (<i>Oncorhynchus mykiss</i>)	growth	32 weeks	formulated	PbNO ₃	no	NOEC/NOEL (NA)	40.1 ^a	728	0.78 ^a	12.4	Hodson et al. 1978
	wet weight	60 days	brine shrimp (<i>Artemia</i> sp.)	PbNO ₃	yes	NOEC/NOEL (1%)	210 ^a		12.6 ^a		Mount et al. 1994
	dry weight	30 days	worm (<i>L. variegatus</i>)	PbNO ₃	no	NOEC/NOEL (0%)	956 ^a		12.2 ^a		Erickson et al. 2010
	growth rate	7 weeks	worm (<i>L. variegatus</i>)	PbNO ₃	no	EC20/ED20	111 ^{b,c}		1.9 ^{b,c}		Alsop et al. 2016
	growth rate	7 weeks	worm (<i>L. variegatus</i>)	PbNO ₃	yes	NOEC/NOEL (21%)	728		12.4		Alsop et al. 2016
Nile tilapia (<i>Oreochromis niloticus</i>)	wet weight	4 months	formulated	PbO	no	LOEC/LOEL (17%)	667	667	6	6	Ayyat et al. 2003
	growth rate	60 days	formulated	NR	no	NOEC (4%)	800 ^a		29.6 ^d		Dai et al. 2009
Fathead minnow (<i>Pimephales promelas</i>)	dry weight	30 days	worm (<i>L. variegatus</i>)	PbNO ₃	no	NOEC/NOEL (0%)	846	846	16.1	16.1	Erickson et al. 2010

Notes:

^a Excluded from species mean because there is a better estimate of the 20% effect concentration/20% dose concentration (EC20/ED20) for this species.

^b This test evaluated the effects of diet-only lead exposures; the test below it in the table evaluated the effects of diet+water exposures. Although it was possible to derive an EC20 of 111 mg/kg_{diet} dw and an ED20 of 1.9 mg/kg bw/day for the diet-only test using Toxic Relationship Analysis Program (TRAP), growth reduction was 14% at diet-borne concentrations up to 315 mg/kg_{diet} dw and doses up to 5.4 mg/kg bw/day (i.e., less than the targeted 20% effect level).

The next highest diet-borne concentration of 728 mg/kg_{diet} dw (dose of 12.4 mg/kg bw/day) resulted in a 29% reduction in growth relative to the control, which was not statistically significant (p > 0.05) from the control. This same concentration and dose were associated with a 21% reduction in growth when rainbow trout underwent combined diet+water lead exposures. The diet-borne concentration of 728 mg/kg_{diet} dw and dose of 12.4 mg/kg bw/day were, therefore, used to define the sensitivity of rainbow trout to lead.

^c Extreme 90% confidence intervals: 1.1E-10 to 1.1E+14 for the 10% effect concentration (EC10) and 1.1E-11 to 3.6E+12 for the ED20.

^d Dose-based toxicity threshold is based on an assumed food ingestion rate of 0.037 g_{food}/g bw/day (Section 2.6).

LOEC – lowest-observed-effect concentration

LOEL – lowest-observed-effect level

NA – not applicable

NOEC – no-observed-effect concentration

NOEL – no-observed-effect level

NR – not reported

TRV – toxicity reference value

Table 3-4. Concentration- and Dose-Based Diet-Borne Toxicity Data Used to Derive TRVs for Zinc

Species	Endpoint	Duration	Diet	Zinc Form	Simultaneous Water Exposure?	Statistical Endpoint Basis (% Effect Relative to Control)	Diet-Borne Concentration		Diet-Borne Dose		Reference
							Zinc (mg/kg dw)	Species Mean Zinc (mg/kg dw)	Zinc (mg/kg/day)	Species Mean Zinc (mg/kg/day)	
Common carp (<i>Cyprinus carpio</i>)	wet weight	8 weeks	formulated	ZnSO ₄	no	EC20	500 (90% CI = 0.022 to 1.1E+07)	500	38.5 (90% CI = 0.0017 to 8.7E+05)	38.5	Jeng and Sun 1981
Coho salmon (<i>Oncorhynchus kisutch</i>)	length (10°C)	21 days	formulated	ZnCl ₂	no	LOEC/LOEL (54%)	1,900	1,900	95	95	Bowen et al. 2006
	length (15°C)	21 days	formulated	ZnCl ₂	no	LOEC/LOEL (66%)	1,900		95		
Rainbow trout (<i>Oncorhynchus mykiss</i>)	weight gain	20 weeks	formulated	ZnSO ₄	yes	NOEC/NOEL (18%)	500 ^a	2,220	8.6 ^a	114	Knox et al. 1984
	weight	16 weeks	formulated	ZnSO ₄	yes ^b	NOEC (NA)	590 ^a		21.8 ^{a,e}		Spry et al. 1988
	weight gain	6 weeks	formulated (basal)	ZnO	no	LOEC (20%) ^c	2,220		82.1 ^{a,e}		Takeda and Shimma 1977
	weight gain	6 weeks	formulated (calcium amended)	ZnO	no	NOEC (1%)	1,920 ^a		71.0 ^{a,e}		
	wet weight	60 days	brine shrimp (<i>Artemia</i> sp.)	ZnCl ₂	yes	NOEC/NOEL (0%)	1,900 ^a		114		Mount et al. 1994
	growth rate	15 days	formulated (zinc oxide)	ZnO	no	NOEC/NOEL (1%)	772 ^a		61.8 ^a		Kjoss et al. 2006
	growth rate	15 days	formulated (zinc sulfate)	ZnSO ₄	no	NOEC/NOEL (6%)	1,036 ^a		82.9 ^a		
	growth rate	15 days	formulated (zinc proteinate)	Zn-proteinate	no	NOEC/NOEL (4%)	653 ^a		52.3 ^a		
	growth rate	15 days	formulated (zinc methionate)	Zn-methionate	no	NOEC/NOEL (4%)	989 ^a		79.1 ^a		
Chinook salmon (<i>Oncorhynchus tshawytscha</i>)	growth rate	105 days	formulated	ZnSO ₄	no	NOEC/NOEL (0%)	354 ^a	403	5.8 ^a	6.8	Richardson et al. 1985
	growth rate	105 days	formulated	ZnSO ₄	no	NOEC/NOEL (1%)	403		6.8		
Nile tilapia (<i>Oreochromis niloticus</i>)	weight gain	70 days	formulated	ZnSO ₄	no	NOEC/NOEL ^d	100	100	3.0	3.0	Eid and Ghonim 1994
Atlantic salmon (<i>Salmo salar</i>)	weight gain	8 weeks	formulated	ZnSO ₄	no	NOEC (0%)	101	101	3.7 ^e	3.7	Maage and Julshamn 1993
Turbot (<i>Scophthalmus maximus</i>)	growth rate	250 days	formulated	ZnSO ₄	no	NOEC (NA)	1,000	1,000	37 ^e	37	Overnell et al. 1988

Notes:

^a Excluded from species mean because there is a better estimate of the 20% effect concentration/20% dose concentration (EC20/ED20) for this species.

^b This study evaluated the influence of three dietary zinc concentrations, the lowest of which (1 mg/kg_{diet} dw) was deficient, combined with one of four different waterborne zinc concentrations. The highest dietary zinc concentration of 590 µg/g dw did not result in any growth effects at any waterborne zinc concentration tested.

^c A no-observed-effect concentration (NOEC) of 1,070 mg/kg_{diet} dw was also available from this study; however, since a 20% effect was associated with the lowest-observed-effect concentration (LOEC), the LOEC of 2,220 mg/kg_{diet} dw was identified as the toxicity threshold for this test.

^d This study evaluated added zinc concentrations of 0, 5, 10, 20, 30, 40, 50, 60, 80, and 100 mg/kg_{diet} dw. The authors concluded that the optimum diet-borne zinc concentration for Nile tilapia was 30 mg/kg_{diet} dw, which resulted in the greatest growth of tilapia. At higher diet-borne zinc concentrations, growth was reduced relative to the optimum, but it was still greater than that observed at diet-borne zinc concentrations of < 30 mg/kg_{diet} dw.

^e Dose-based toxicity threshold is based on an assumed food ingestion rate of 0.037 g_{food} /g bw/day (Section 2.6).

CI – confidence interval
LOEL – lowest-observed-effect level
NA – not applicable
NOEL – no-observed-effect level
TRV – toxicity reference value

Table 3-5. Final Diet-Borne Concentration- and Dose-Based Metal TRVs for Fish

Metal	Diet-Borne Concentration-Based TRV (mg/kg _{diet} dw) ^a	Diet-Borne Dose-Based TRV (mg/kg bw/day) ^a
Aluminum	> 2,232 (0%)	> 82.5 (0%)
Arsenic	42	0.78
Cadmium	8.1	0.023
Chromium	3.9	0.074
Copper	666	< 21.2 (36%)
Iron	na	na
Lead	111	1.9
Manganese	na	na
Mercury	0.26	0.013
Nickel	< 70.4 (59%)	< 3.9 (59%)
Selenium	7.7	na
Silver	> 3,000 (0%) ^b	> 70 (0%) ^b
Vanadium	4.3	0.16
Zinc	500	38.5

Notes:

^a Concentrations or doses with a less than (<) or greater than (>) symbol denote whether a lower or higher concentration would be predicted to achieve a 20% effect level. The levels of effect relative to corresponding negative controls are provided in parentheses.

^b An anomalous response was observed: growth rate reductions of 23, 0, 23, and 0% at diet-borne silver concentrations of 3, 30, 300, and 3,000 mg/kg_{diet} dw, respectively.

na – not available

TRV – toxicity reference value

APPENDIX A

DIET-BORNE TOXICITY DATA

APPENDIX A-1

FIGURES

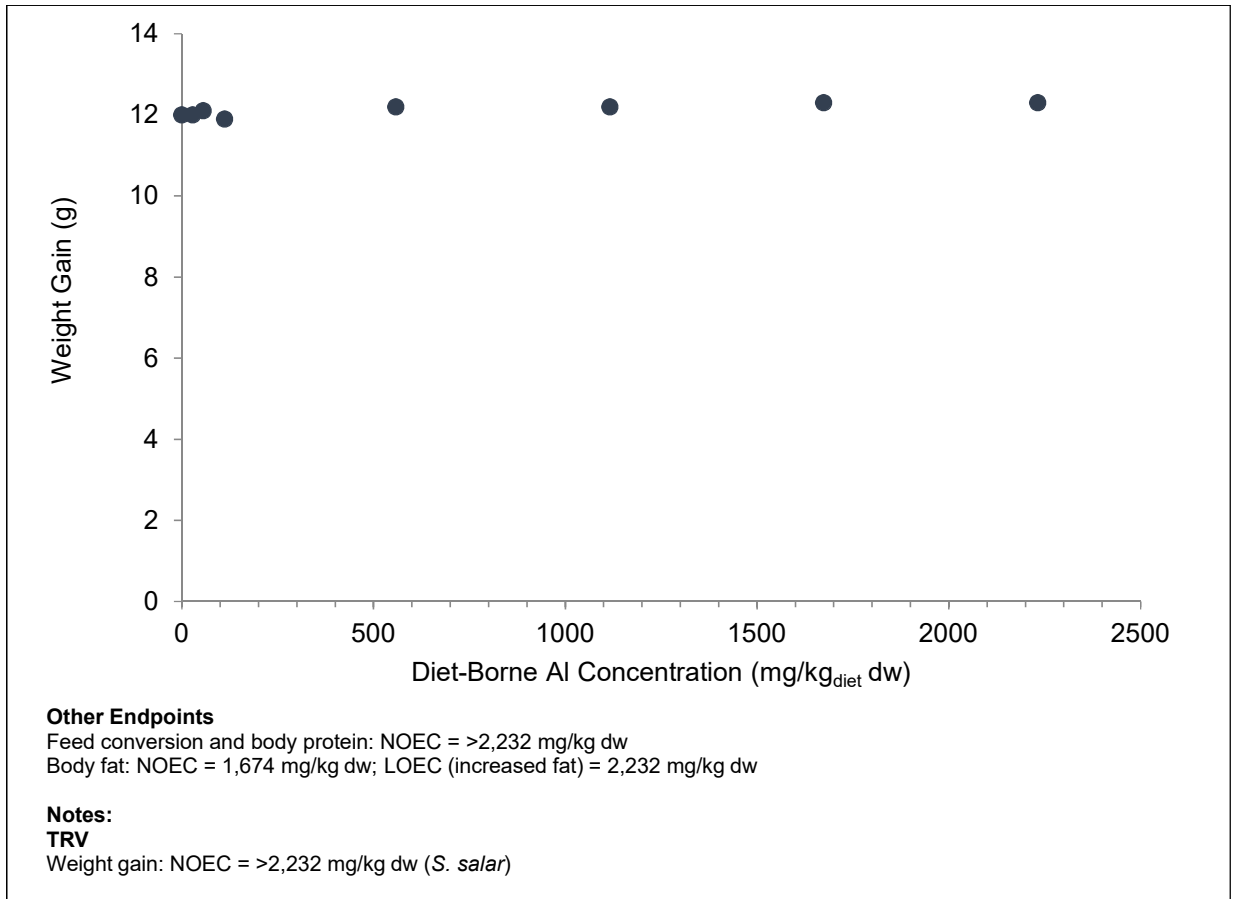
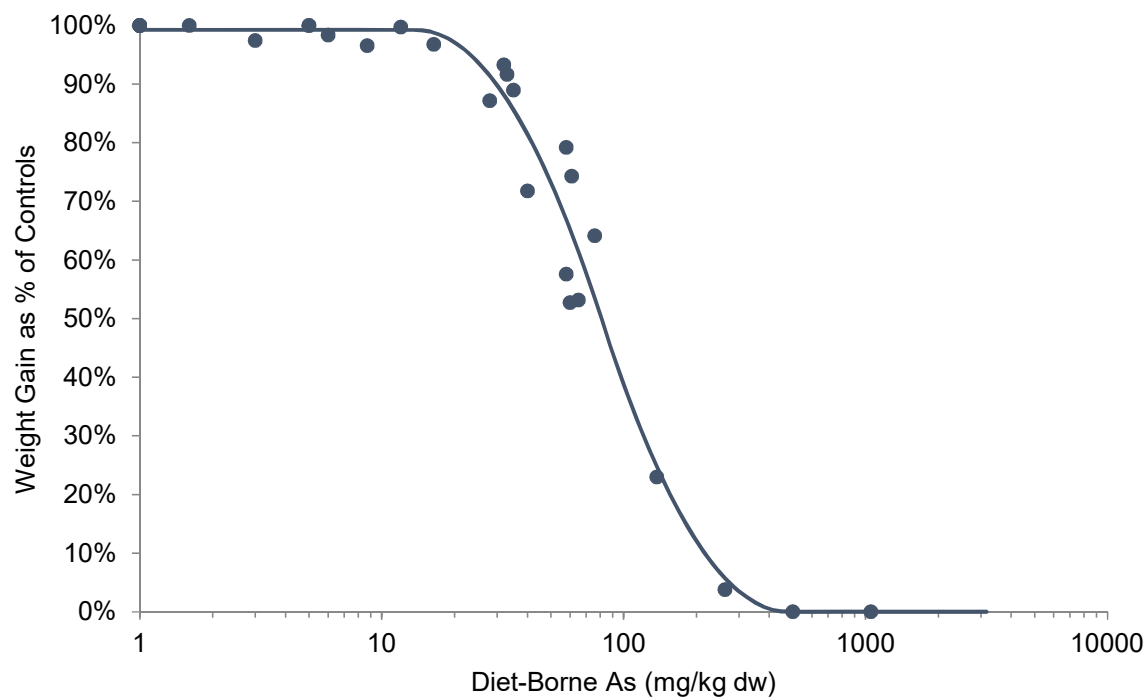


Figure A-1. Comparison of Diet-Borne Aluminum TRV to Toxicity Thresholds for Other Endpoints



Other Endpoints

Feed consumption: NOEC = 32 to 33 mg/kg dw

Feed:gain ratio: NOEC = 32 to 180 mg/kg dw; LOEC = 58 to 360 mg/kg dw

Hematology: NOEC = 32 mg/kg dw; LOEC = 60 mg/kg dw

Histopathology: LOEC = 58 mg/kg dw

Notes:

TRV

Weight gain: EC20 = 42 mg/kg dw (*O. mykiss*)

Figure A-2. Comparison of Diet-Borne Arsenic TRV to Toxicity Thresholds for Other Endpoints

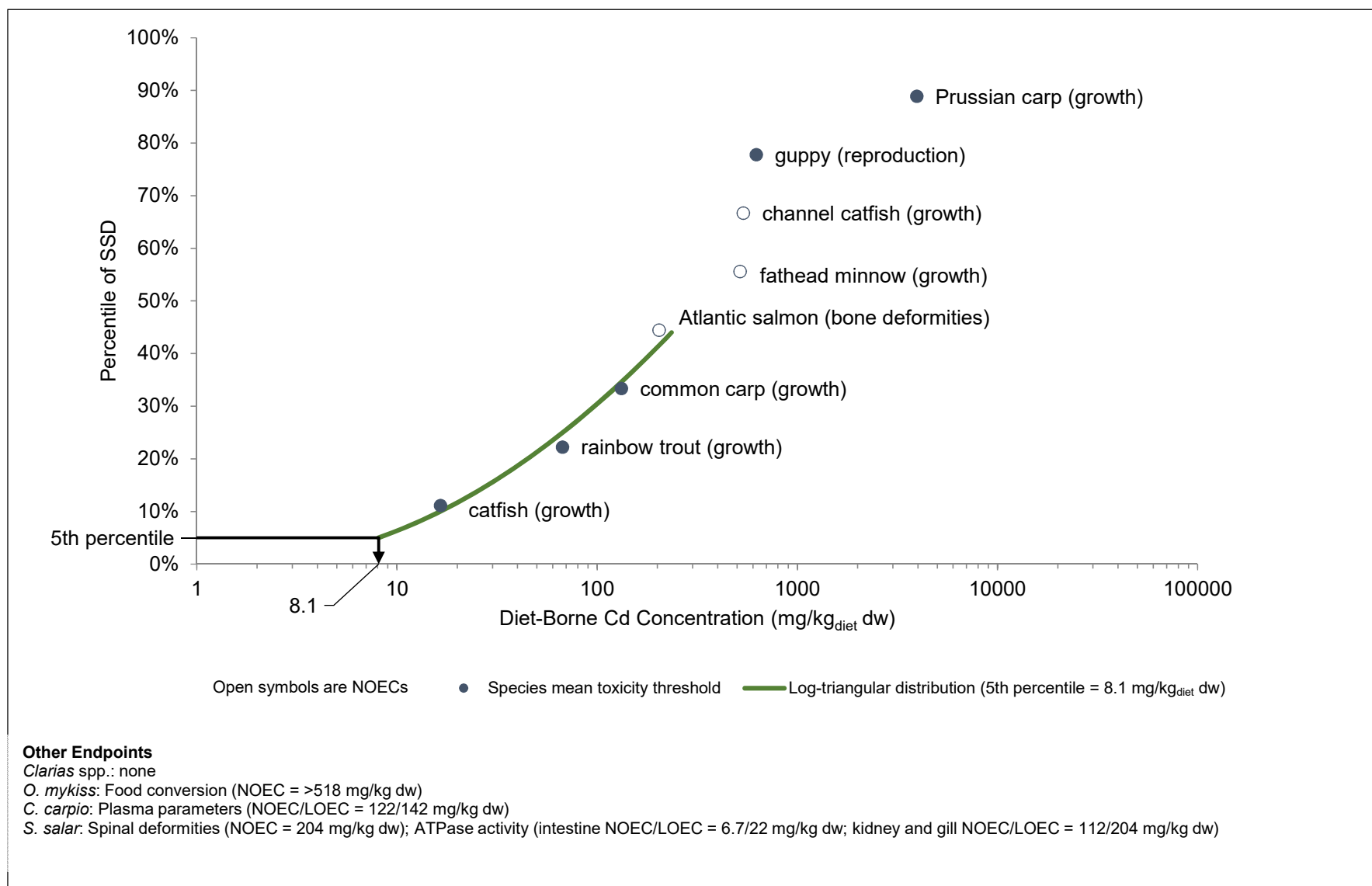


Figure A-3. Comparison of Diet-Borne Cadmium TRV to Toxicity Thresholds for Other Endpoints

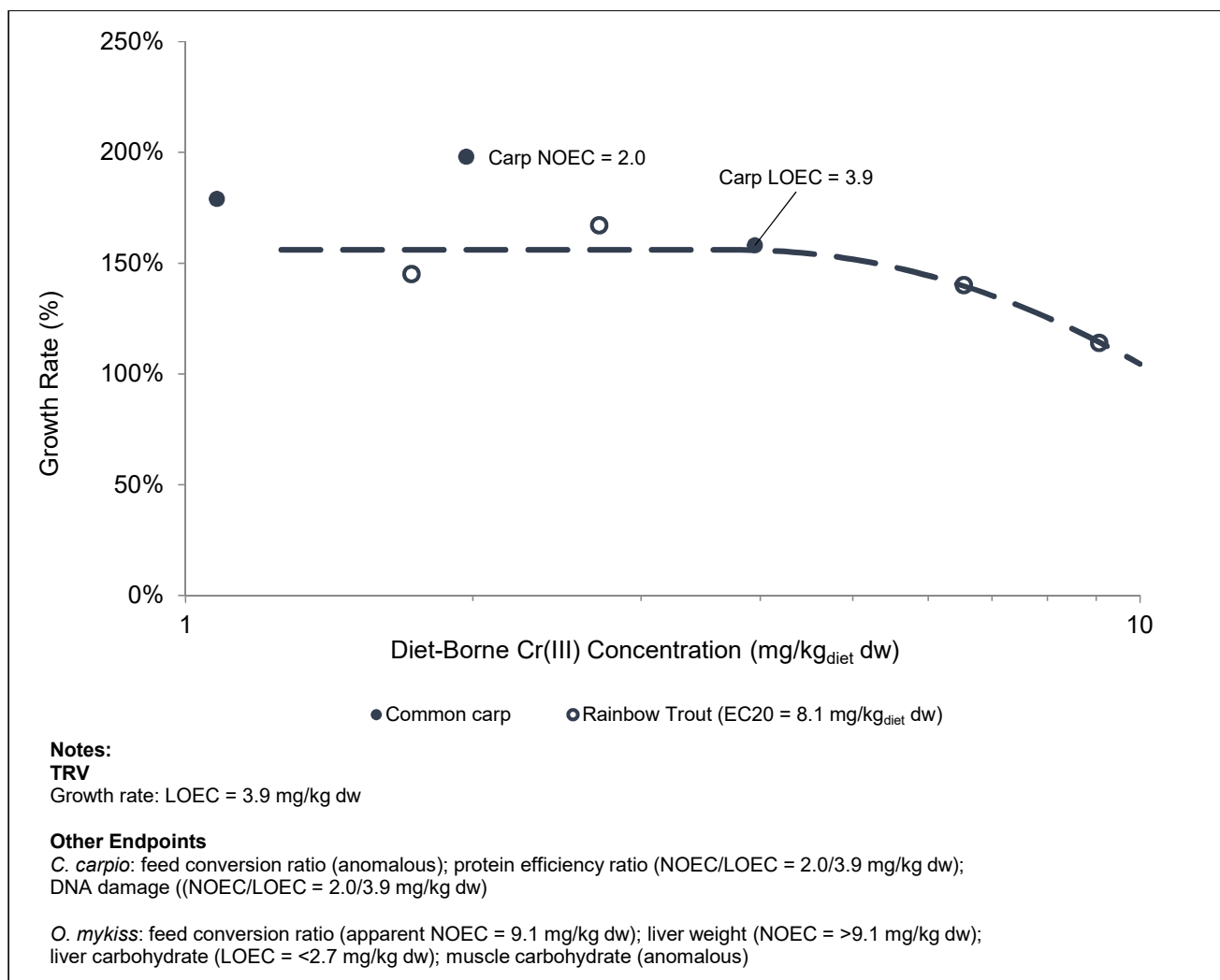
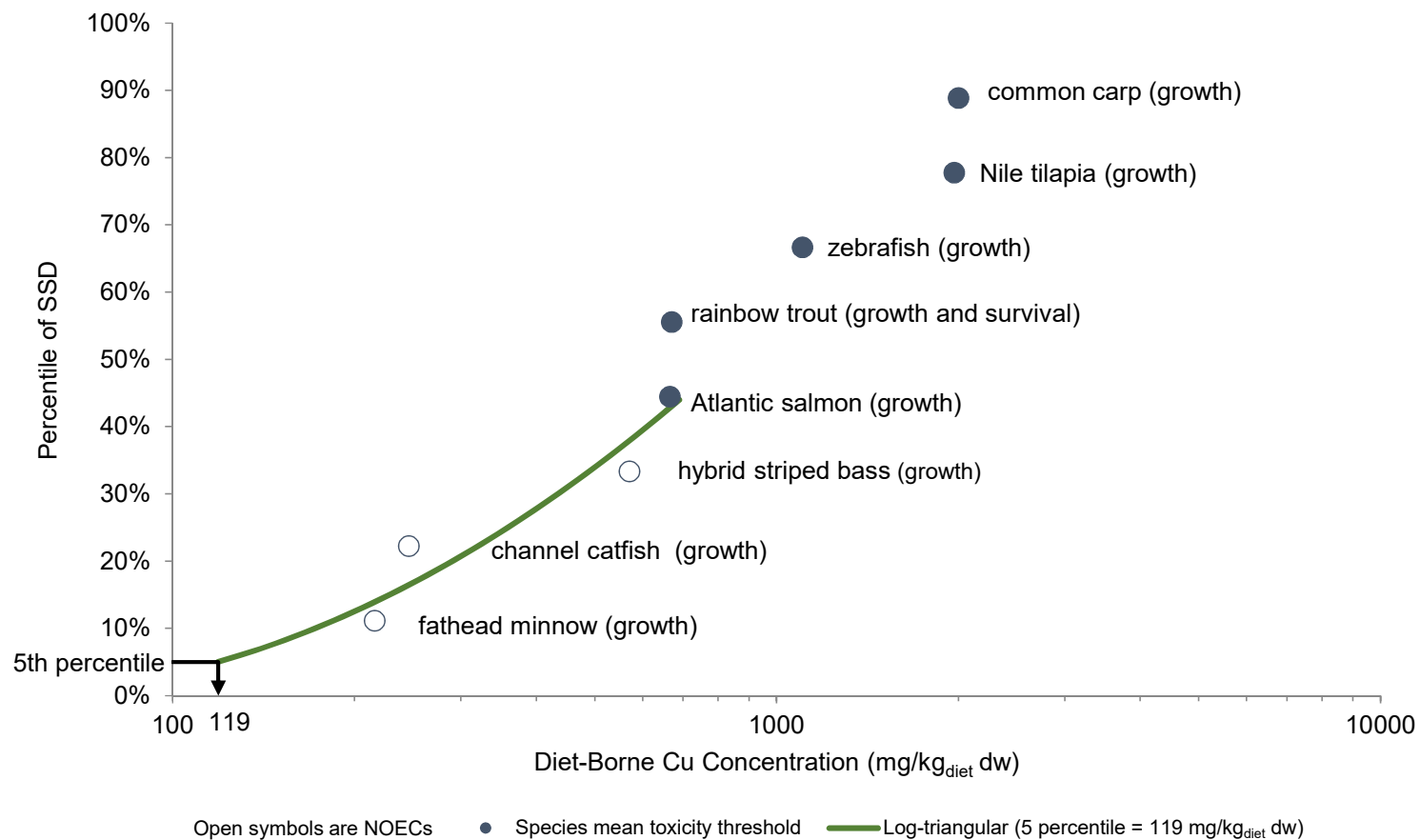


Figure A-4. Comparison of Diet-Borne Chromium(III) TRV to Toxicity Thresholds for Other Endpoints



Other Endpoints

P. promelas: none

I. punctatus: feed conversion efficiency (NOEC = >40 mg/kg dw) [Gatlin and Wilson 1986];
percent food conversion (NOEC = >246 mg/kg dw [Erickson et al. 2010])

Figure A-5. Comparison of Diet-Borne Copper TRV to Toxicity Thresholds for Other Endpoints

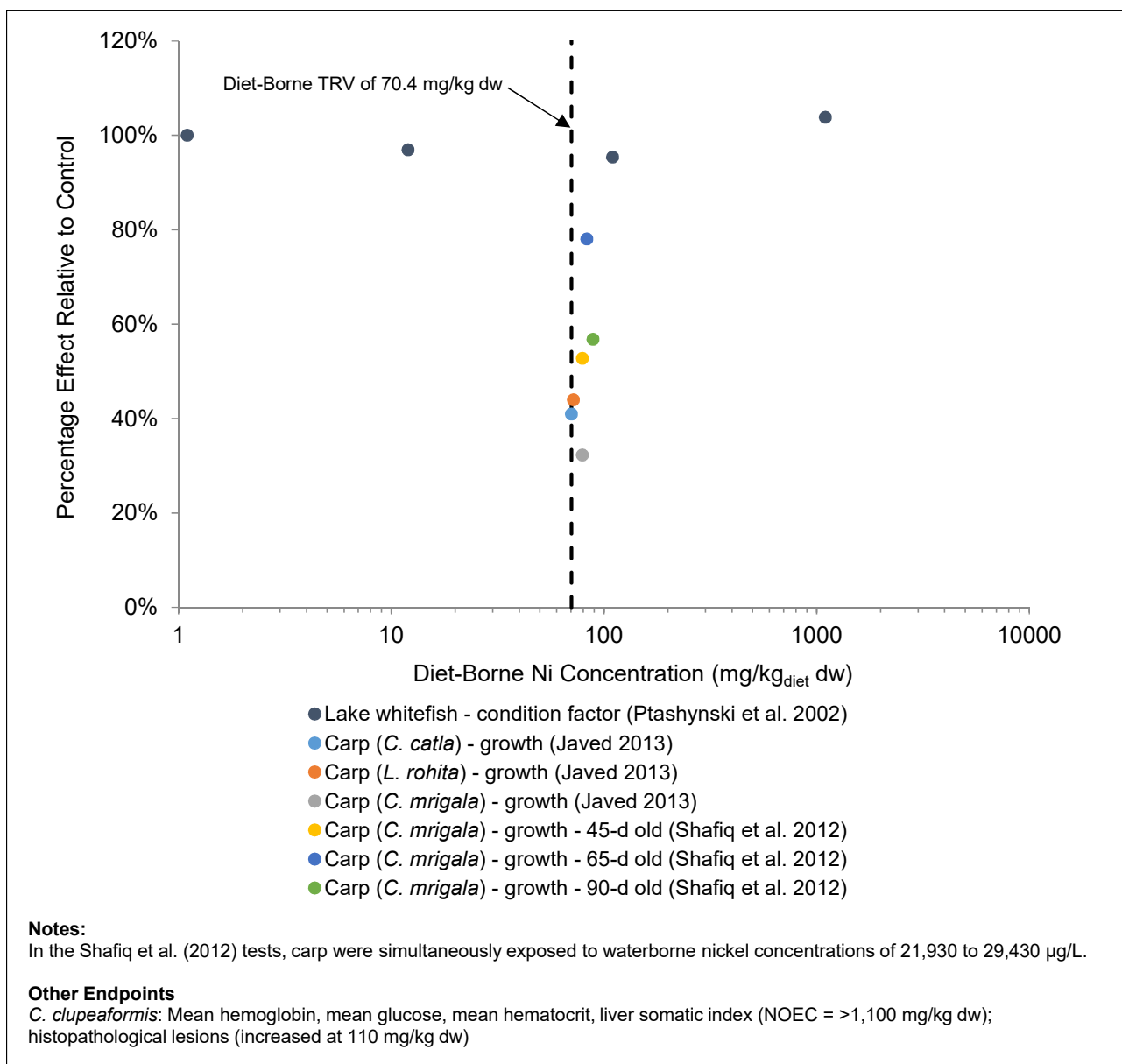


Figure A-6. Comparison of Diet-Borne Nickel TRV to Toxicity Thresholds for Other Endpoints

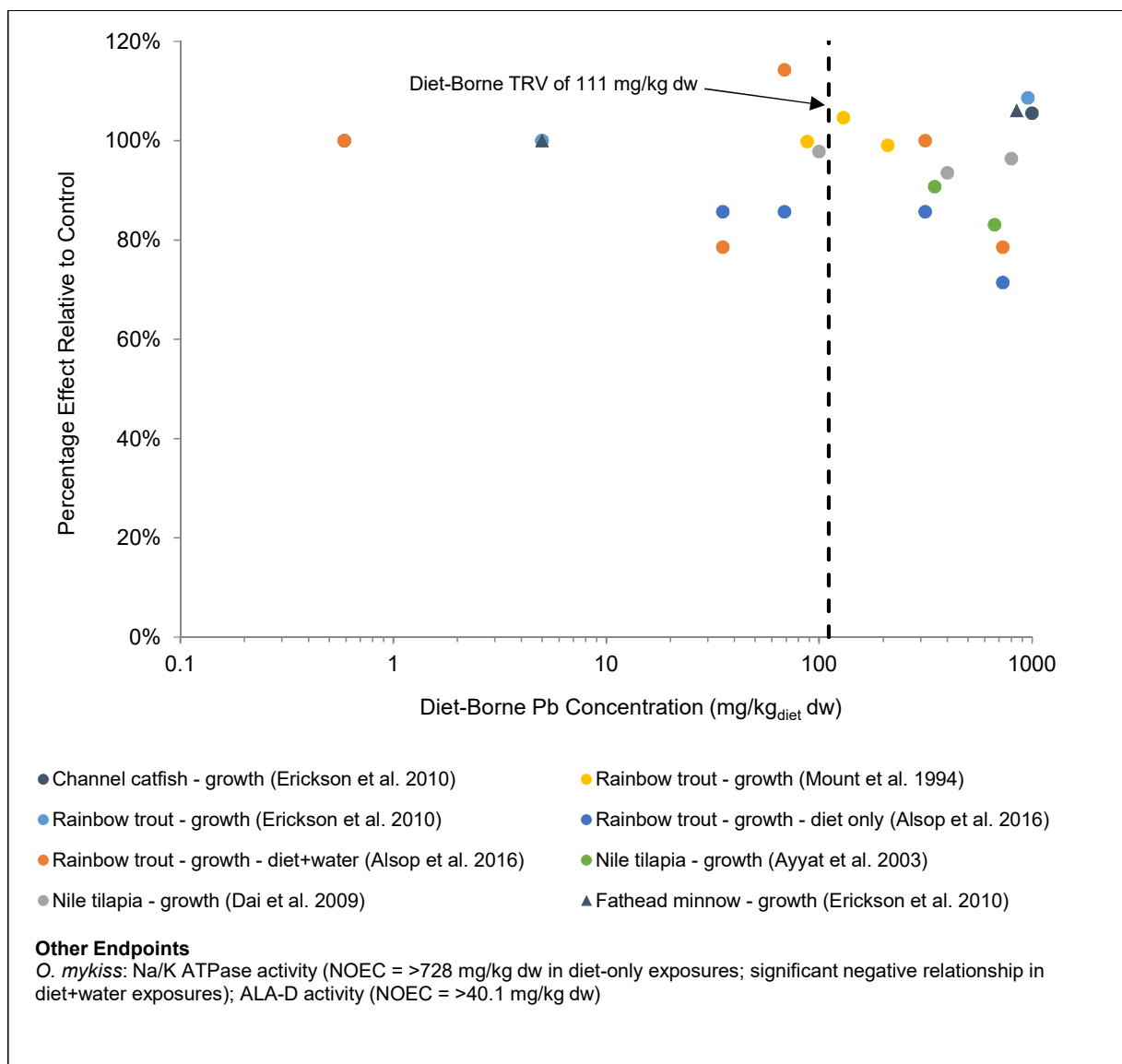


Figure A-7. Comparison of Diet-Borne Lead TRV to Toxicity Thresholds for Other Endpoints

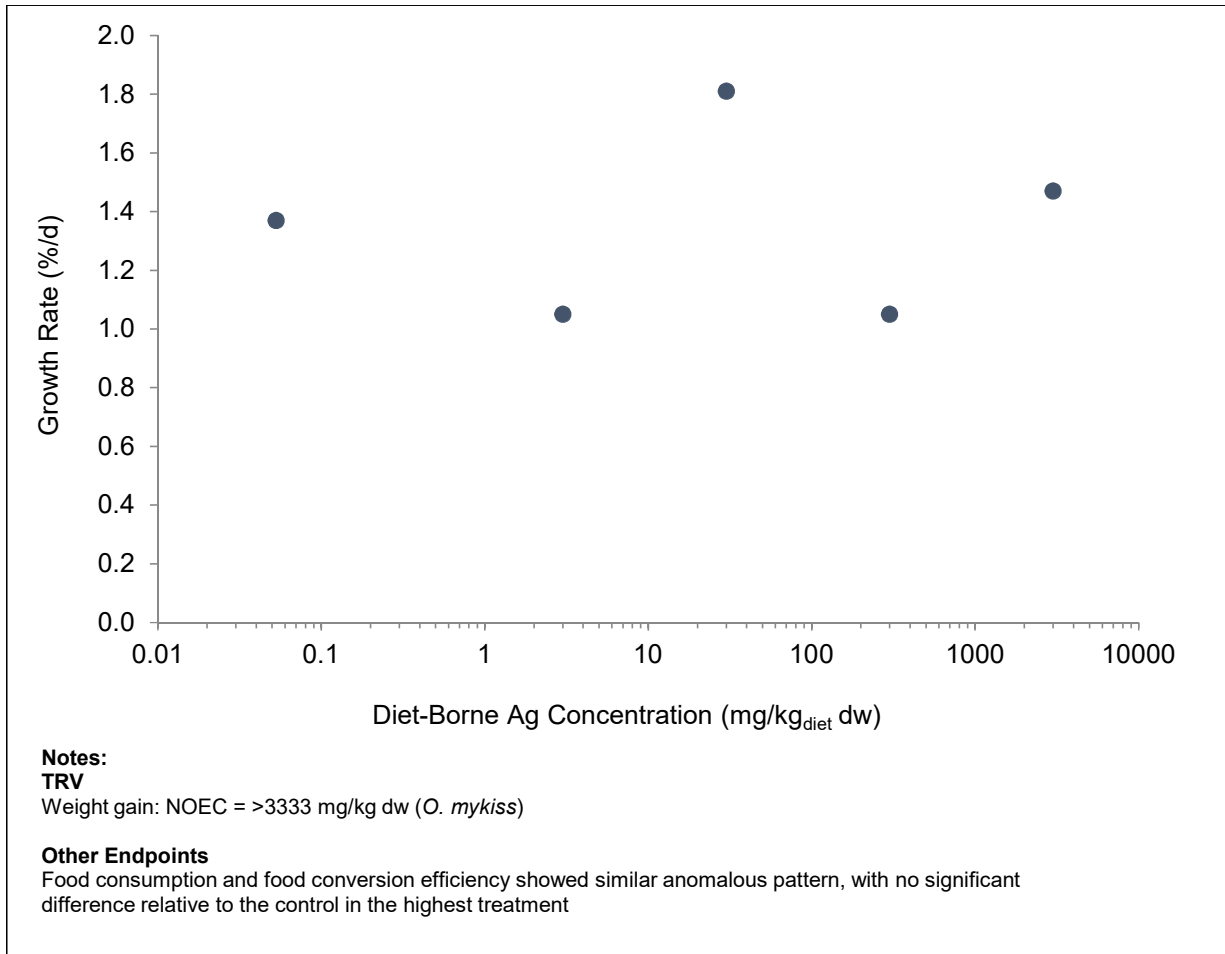


Figure A-8. Comparison of Diet-Borne Silver TRV to Toxicity Thresholds for Other Endpoints

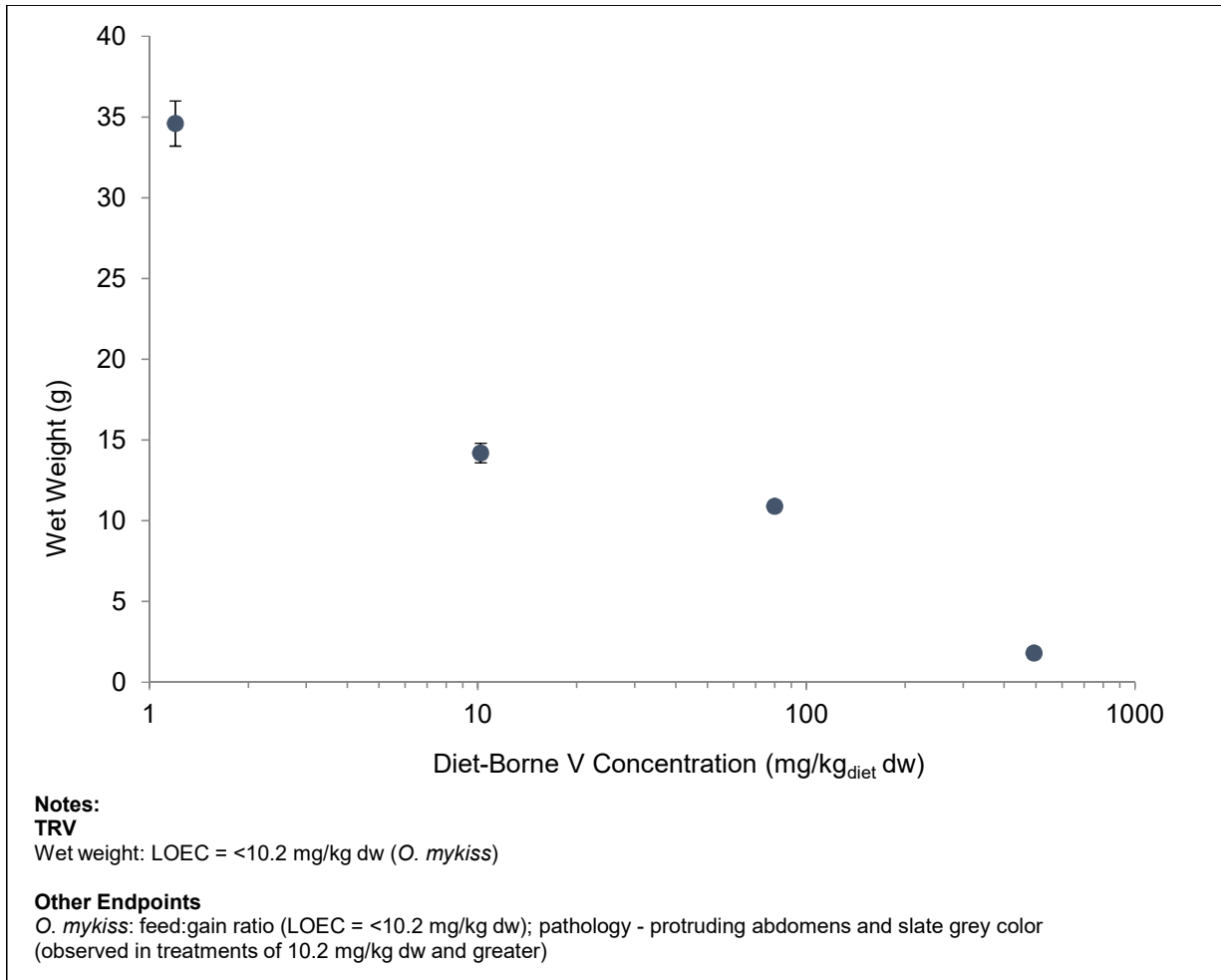


Figure A-9. Comparison of Diet-Borne Vanadium TRV to Toxicity Thresholds for Other Endpoints

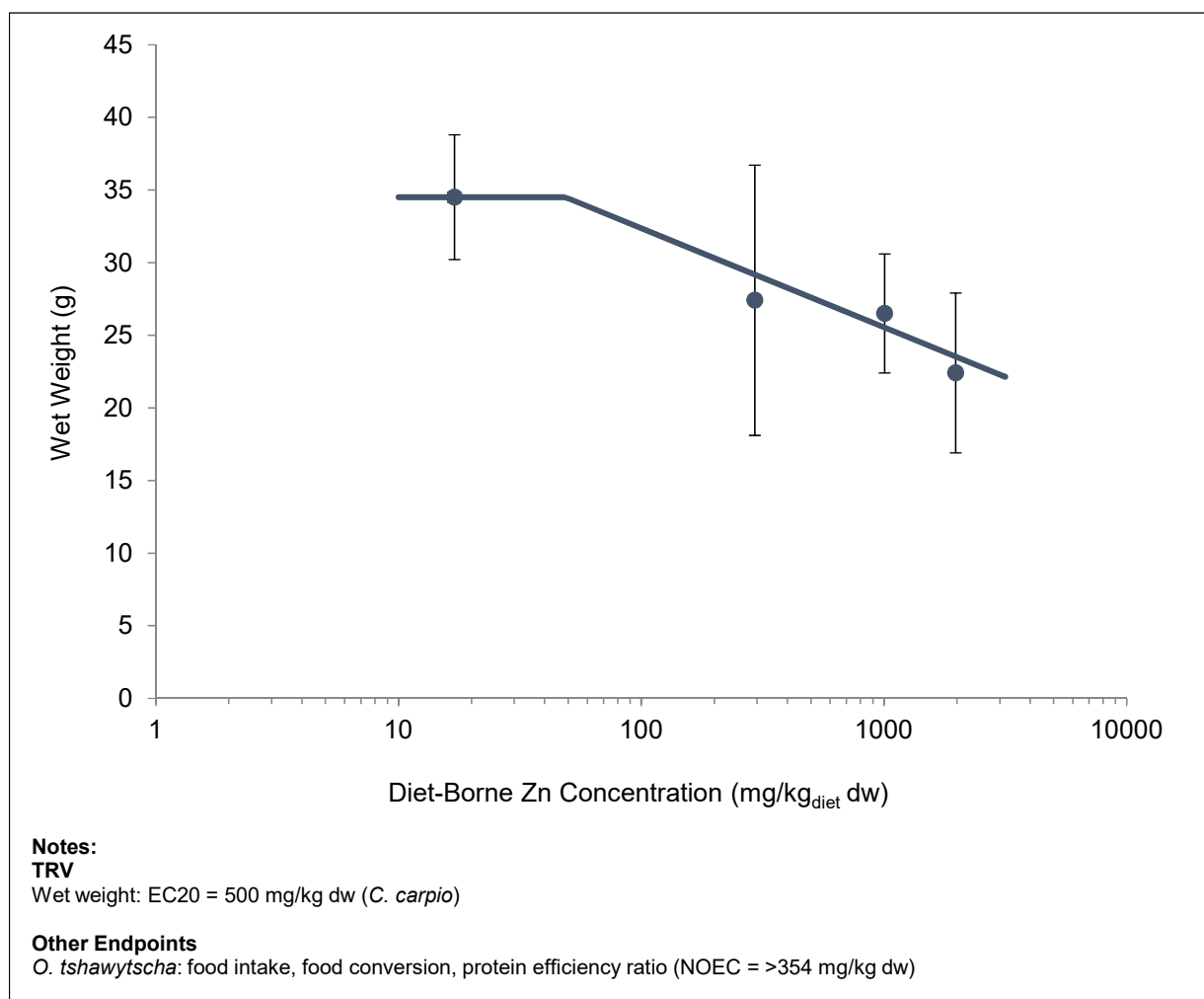


Figure A-10. Comparison of Diet-Borne Zinc TRV to Toxicity Thresholds for Other Endpoints

APPENDIX A-2

TABLES

									Exposure Information						Toxicity Information							Concentration (mg/kg dw)			Dose (mg/kg/d)					
									Diet-Borne Concentration and Dose			Water-Borne Concentration																		
Metal	Species	Chemical Form	Water Type	Age/Size at Initiation	Exposure Duration	FIR (g/d)	BW (g)	FIR (bw/gw/d) ^a	Control or Treatment?	Diet Type	mg/kg ww	Moisture	mg/kg dw	mg/kg bw/d	µg/L	Measured?	Total or Dissolved?	DOC (mg/L)	Endpoint Type	Endpoint ^b	Measure	% Effect (Control-Normalized)	NOEC or LOEC?	EC10	EC20	EC50	EC10	EC20	EC50	Reference
Aq	rainbow trout (<i>Oncorhynchus mykiss</i>)	NA	FW	juveniles	58-day	na	na	0.0282	control	formulated	0	~0	0	0	na	na	na	na	growth	growth rate (%/day)	1.37	na	control							
Aq	rainbow trout (<i>Oncorhynchus mykiss</i>)	silver sulfide	FW	juveniles	58-day	na	na	0.0222	treatment	formulated	3	~0	3	0.067	na	na	na	na	growth	growth rate (%/day)	1.05	23%	anomalous							
Aq	rainbow trout (<i>Oncorhynchus mykiss</i>)	silver sulfide	FW	juveniles	58-day	na	na	0.0242	treatment	formulated	30	~0	30	0.73	na	na	na	na	growth	growth rate (%/day)	1.81	-32%	anomalous	NA	NA	NA	NA	NA	NA	Galvez and Wood 1999
Aq	rainbow trout (<i>Oncorhynchus mykiss</i>)	silver sulfide	FW	juveniles	58-day	na	na	0.0226	treatment	formulated	300	~0	300	6.8	na	na	na	na	growth	growth rate (%/day)	1.05	23%	anomalous							
Aq	rainbow trout (<i>Oncorhynchus mykiss</i>)	silver sulfide	FW	juveniles	58-day	na	na	0.0233	treatment	formulated	3000	~0	3,000	70	na	na	na	na	growth	growth rate (%/day)	1.47	-7%	anomalous							
Aq	rainbow trout (<i>Oncorhynchus mykiss</i>)	NA	FW	juveniles	128-day	na	na	0.0136	control	formulated	0.048	na	na	0.00065	na	na	na	na	growth	growth rate (%/day)	1.03	na	control							
Aq	rainbow trout (<i>Oncorhynchus mykiss</i>)	silver thiosulphate	FW	juveniles	128-day	na	na	0.0148	treatment	formulated	3.12	na	na	0.046	na	na	na	na	growth	growth rate (%/day)	1.03	0%	NOEC*	NA	NA	NA	NA	NA	NA	Galvez et al. 2001
Al	Atlantic salmon (<i>Salmo salar</i>)	NA	FW	4.8 g	16-wk	na	4.8 (init.)	na	control	formulated	0	10%	0	na	~20	yes	na	na	growth	weight gain (g)	12.0	na	control							
Al	Atlantic salmon (<i>Salmo salar</i>)	aluminum chloride hexahydrate	FW	4.8 g	16-wk	na	4.8 (init.)	na	treatment	formulated	25	10%	28	na	~20	yes	na	na	growth	weight gain (g)	12.0	0%	NOEC(-)							
Al	Atlantic salmon (<i>Salmo salar</i>)	aluminum chloride hexahydrate	FW	4.8 g	16-wk	na	4.8 (init.)	na	treatment	formulated	50	10%	56	na	~20	yes	na	na	growth	weight gain (g)	12.1	-1%	NOEC(+)							
Al	Atlantic salmon (<i>Salmo salar</i>)	aluminum chloride hexahydrate	FW	4.8 g	16-wk	na	4.8 (init.)	na	treatment	formulated	100	10%	112	na	~20	yes	na	na	growth	weight gain (g)	11.9	1%	NOEC(-)	NA	NA	NA	NA	NA	NA	Poston 1991
Al	Atlantic salmon (<i>Salmo salar</i>)	aluminum chloride hexahydrate	FW	4.8 g	16-wk	na	4.8 (init.)	na	treatment	formulated	500	10%	558	na	~20	yes	na	na	growth	weight gain (g)	12.2	-2%	NOEC(-)							
Al	Atlantic salmon (<i>Salmo salar</i>)	aluminum chloride hexahydrate	FW	4.8 g	16-wk	na	4.8 (init.)	na	treatment	formulated	1000	10%	1,116	na	~20	yes	na	na	growth	weight gain (g)	12.2	-2%	NOEC(-)							
Al	Atlantic salmon (<i>Salmo salar</i>)	aluminum chloride hexahydrate	FW	4.8 g	16-wk	na	4.8 (init.)	na	treatment	formulated	1500	10%	1,674	na	~20	yes	na	na	growth	weight gain (g)	12.3	-3%	NOEC(-)							
Al	Atlantic salmon (<i>Salmo salar</i>)	aluminum chloride hexahydrate	FW	4.8 g	16-wk	na	4.8 (init.)	na	treatment	formulated	2000	10%	2,232	na	~20	yes	na	na	growth	weight gain (g)	12.3	-3%	NOEC*							
Al	rainbow trout (<i>Oncorhynchus mykiss</i>)	NA	FW	171 g	42-day	na	171 (init.)	0.05	control	formulated	na	na	na	na	<5	yes	NR	na	survival	mortality	na	na	control	NA	NA	NA	NA	NA	NA	Handy 1993a
Al	rainbow trout (<i>Oncorhynchus mykiss</i>)	aluminum sulfate	FW	171 g	42-day	na	171 (init.)	0.05	treatment	formulated	na	na	10,000																	

Table A-1. Concentration- and Dose-Response Data for Exposure of Aquatic Organisms to Diet-Borne Individual Metals

Metal	Species	Chemical Form	Water Type	Age/Size at Initiation	Exposure Duration	FIR (g/d)	BW (g)	FIR (g _{food} /g _{BW} /d) ^a	Control or Treatment?	Exposure Information						Toxicity Information						Concentration (mg/kg dw)			Dose (mg/kg/d)			Reference		
										Diet Type	Diet-Borne Concentration and Dose		Water-Borne Concentration		Endpoint Type	Toxicity Information		Measure	% Effect (Control-Normalized)	NOEC or LOEC?	EC10	EC20	EC50	EC10	EC20	EC50				
											mg/kg ww	Moisture	mg/kg dw	mg/kg bw/dw		µg/L	Measured?										Total or Dissolved?		DOC (mg/L)	
Cd	Atlantic salmon (<i>Salmo salar</i>)	NA	FW	parr	4-mo	na	na	na	control	formulated	na	na	0.1	na	0.12	yes	total	na	deformity	bone deformities (%)	na	na	control	NA	NA	NA	NA	NA	NA	Berttssen et al. 2003
Cd	Atlantic salmon (<i>Salmo salar</i>)	cadmium sulfate octahydrate	FW	parr	4-mo	na	na	na	treatment	formulated	na	na	0.8	na	na	na	na	deformity	bone deformities (%)	na	na	NOEC(-)								
Cd	Atlantic salmon (<i>Salmo salar</i>)	cadmium sulfate octahydrate	FW	parr	4-mo	na	na	na	treatment	formulated	na	na	6.7	na	na	na	na	deformity	bone deformities (%)	na	na	NOEC(-)								
Cd	Atlantic salmon (<i>Salmo salar</i>)	cadmium sulfate octahydrate	FW	parr	4-mo	na	na	na	treatment	formulated	na	na	21.6	na	na	na	na	deformity	bone deformities (%)	na	na	NOEC(-)								
Cd	Atlantic salmon (<i>Salmo salar</i>)	cadmium sulfate octahydrate	FW	parr	4-mo	na	na	na	treatment	formulated	na	na	112.2	na	na	na	na	deformity	bone deformities (%)	na	na	NOEC(-)								
Cr(III)	common carp (<i>Cyprinus carpio</i>)	NA	FW	juveniles	8-wk	na	na	0.03	control	formulated	0.69	36.0	1.1	0.021	na	yes	total	na	deformity	bone deformities (%)	na	na	NOEC*							
Cr(III)	common carp (<i>Cyprinus carpio</i>)	chromium chloride hexahydrate	FW	juveniles	8-wk	na	na	0.03	treatment	formulated	1.24	37.0	2.0	0.037	na	na	na	na	growth	growth rate (%/day)	1.98	-11%	NOEC	NA	NA	NA	NA	NA	Ahmed et al. 2012	
Cr(III)	common carp (<i>Cyprinus carpio</i>)	chromium chloride hexahydrate	FW	juveniles	8-wk	na	na	0.03	treatment	formulated	2.46	37.7	3.9	0.074	na	na	na	na	growth	growth rate (%/day)	1.58	12%	LOEC	6.5	8.1	12.5	0.13	0.17	0.29	Tacon and Beveridge 1982
Cr(III)	rainbow trout (<i>Oncorhynchus mykiss</i>)	NA	FW	juveniles	8-wk	0.1796	8.27	0.022	control	formulated	1.56	9.58	1.7	0.03	na	na	na	na	growth	growth rate (%/day)	1.45	na	control							
Cr(III)	rainbow trout (<i>Oncorhynchus mykiss</i>)	chromium chloride hexahydrate	FW	juveniles	8-wk	0.2258	10.28	0.022	treatment	formulated	2.45	9.67	2.7	0.05	na	na	na	na	growth	growth rate (%/day)	1.67	-15%	NOEC(-)							
Cr(III)	rainbow trout (<i>Oncorhynchus mykiss</i>)	chromium chloride hexahydrate	FW	juveniles	8-wk	0.1917	8.69	0.022	treatment	formulated	5.91	9.63	6.5	0.13	na	na	na	na	growth	growth rate (%/day)	1.40	3%	NOEC							
Cr(III)	rainbow trout (<i>Oncorhynchus mykiss</i>)	chromium chloride hexahydrate	FW	juveniles	8-wk	0.1827	7.65	0.024	treatment	formulated	8.17	9.88	9.1	0.20	na	na	na	na	growth	growth rate (%/day)	1.14	21%	LOEC	NA	NA	NA	NA	NA	NA	Shiau and Lin 1993
Cr(III)	tilapia (<i>Oreochromis niloticus</i> x <i>O. aureus</i>)	NA	FW	123 g	8-wk	na	na	0.05	control	formulated	0.36	9.0-13.7	0.41	0.021	0.31	yes	total	na	growth	weight gain (%)	591.5	na	control							
Cr(III)	tilapia (<i>Oreochromis niloticus</i> x <i>O. aureus</i>)	chromium chloride	FW	123 g	8-wk	na	na	0.05	treatment	formulated	1.6	9.0-13.7	1.78	0.089	na	na	na	na	growth	weight gain (%)	127.4	-18%	NOEC*							
Cr(III)	tilapia (<i>Oreochromis niloticus</i> x <i>O. aureus</i>)	NA	FW	123 g	8-wk	na	na	0.05	control	formulated	0.37	9.0-13.7	0.42	0.021	0.31	yes	total	na	growth	weight gain (%)	896.8	na	control							
Cu	common carp (<i>Cyprinus carpio</i>)	NA	FW	juveniles	42-day	na	na	0.0084	control	formulated	na	na	5	0.042	na	na	na	na	growth	weight gain (g)	8.5	na	control	NA	NA	NA	NA	NA	NA	Ajani and Akpolih 2012
Cu	common carp (<i>Cyprinus carpio</i>)	copper sulfate pentahydrate	FW	juveniles	42-day	na	na	0.0108	treatment	formulated	na	na	1,000	10.8	na	na	na	na	growth	weight gain (g)	16.2	-91%	NOEC							
Cu	common carp (<i>Cyprinus carpio</i>)	copper sulfate pentahydrate	FW	juveniles	42-day	na	na	0.0106	treatment	formulated	na	na	2,000	21.2	na	na	na	na	growth	weight gain (g)	-1.67	120%	LOEC							
Cu	zebrafish (<i>Danio rerio</i>)	NA	FW	~0.25 g	260-day	na	na	0.02	control	formulated	na	na	8	0.16	7.5	yes	total	na	reproduction	GSI	11.0	na	control							
Cu	zebrafish (<i>Danio rerio</i>)	copper sulfate pentahydrate	FW	~0.25 g	260-day	na	na	0.02	treatment	formulated	na	na	135	2.7	26.7	yes	total	na	reproduction	GSI	16.2	-47%	NOEC(-)	890	1103	1688	17.8	22.1	33.8	Alsop et al. 2007
Cu	zebrafish (<i>Danio rerio</i>)	copper sulfate pentahydrate	FW	~0.25 g	260-day	na	na	0.02	treatment	formulated	na	na	644	12.9	38.9	yes	total	na	reproduction	GSI	12.9	-17%	NOEC(-)							
Cu	zebrafish (<i>Danio rerio</i>)	copper sulfate pentahydrate	FW	~0.25 g	260-day	na	na	0.02	treatment	formulated	na	na	1,368	27.4	49.3	yes	total	na	reproduction	GSI	8.7	21%	NOEC*							
Cu	channel catfish (<i>Ictalurus punctatus</i>)	NA	FW	fingerlings	16-wk	na	14.5	na	control	formulated	1.5	na	na	na	0.33	yes	na	na	growth	weight gain (g)	67.7	na	control							
Cu	channel catfish (<i>Ictalurus punctatus</i>)	copper sulfate	FW	fingerlings	16-wk	na	14.5	na	treatment	formulated	3.5	na	na	na	0.33	yes	na	na	growth	weight gain (g)	74.1	-9%	NOEC(-)	na	na	na	na	na	na	Murai et al. 1981
Cu	channel catfish (<i>Ictalurus punctatus</i>)	copper sulfate	FW	fingerlings	16-wk	na	14.5	na	treatment	formulated	5.5	na	na	na	0.33	yes	na	na	growth	weight gain (g)	70.9	-5%	NOEC(-)							
Cu	channel catfish (<i>Ictalurus punctatus</i>)	copper sulfate	FW	fingerlings	16-wk	na	14.5	na	treatment	formulated	9.5	na	na	na	0.33	yes	na	na	growth	weight gain (g)	62.2	8%	NOEC							
Cu	channel catfish (<i>Ictalurus punctatus</i>)	copper sulfate	FW	fingerlings	16-wk	na	14.5	na	treatment	formulated	17.5	na	na	na	0.33	yes	na	na	growth	weight gain (g)	43.2	36%	LOEC							
Cu	channel catfish (<i>Ictalurus punctatus</i>)	copper sulfate	FW	fingerlings	16-wk	na	14.5	na	treatment	formulated	33.5	na	na	na	0.33	yes	na	na	growth	weight gain (g)	40.7	40%	LOEC(+)	NA	NA	NA	NA	NA	NA	Gatlin and Wilson 1986
Cu	channel catfish (<i>Ictalurus punctatus</i>)	NA	FW	fingerlings	13-wk	na	na	0.03	control	formulated	na	na	0.89	0.027	na	na	na	na	growth	weight gain (%)	8.1	na	control							
Cu	channel catfish (<i>Ictalurus punctatus</i>)	copper sulfate pentahydrate	FW	fingerlings	13-wk	na	na	0.03	treatment	formulated	na	na	2	0.060	na	na	na	na	growth	weight gain (%)	7.2	11%	NOEC(-)							
Cu	channel catfish (<i>Ictalurus punctatus</i>)	copper sulfate pentahydrate	FW	fingerlings	13-wk	na	na	0.03	treatment	formulated	na	na	4	0.12	na	na	na	na	growth	weight gain (%)	8.0	2%	NOEC(-)							
Cu	channel catfish (<i>Ictalurus punctatus</i>)	copper sulfate pentahydrate	FW	fingerlings	13-wk	na	na	0.03	treatment	formulated	na	na	6	0.18	na	na	na	na	growth	weight gain (%)	7.6	7%	NOEC(-)	NA	NA	NA	NA	NA	NA	Erickson et al. 2010
Cu	channel catfish (<i>Ictalurus punctatus</i>)	copper sulfate pentahydrate	FW	fingerlings	13-wk	na	na	0.03	treatment	formulated	na	na	8	0.24	na	na	na	na	growth	weight gain (%)	7.2	11%	NOEC(-)							
Cu	channel catfish (<i>Ictalurus punctatus</i>)	copper sulfate pentahydrate	FW	fingerlings	13-wk	na	na	0.03	treatment	formulated	na	na	10	0.30	na	na	na	na	growth	weight gain (%)	8.0	2%	NOEC(-)							
Cu	channel catfish (<i>Ictalurus punctatus</i>)	copper sulfate pentahydrate	FW	fingerlings	13-wk	na	na	0.03	treatment	formulated	na	na	40	1.2	na	na	na	na	growth	weight gain (%)	8.0	2%	NOEC**							
Cu	channel catfish (<i>Ictalurus punctatus</i>)	NA	FW	juveniles	30-day	na	0.807	na	control	worm (<i>L. variegatus</i>)	na	na	12	0.19	na	na	na	na	growth	dry weight (g)	0.144	na	control	NA	NA	NA	NA	NA	NA	Erickson et al. 2010
Cu	channel catfish (<i>Ictalurus punctatus</i>)	copper sulfate pentahydrate	FW	juveniles	30-day	na	0.845	na	treatment	worm (<i>L. variegatus</i>)	na	na	157	1.8	na	na	na	na	growth	dry weight (g)	0.154	-7%	NOEC(-)							
Cu	channel catfish (<i>Ictalurus punctatus</i>)	copper sulfate pentahydrate	FW	juveniles	30-day	na	0.802	na	treatment	worm (<i>L. variegatus</i>)	na	na	246	2.9	na	na	na	na	growth	dry weight (g)	0.140	3%	NOEC*							
Cu	channel catfish (<i>Ictalurus punctatus</i>)	NA	FW	adults	42-day	na	na	0.02	control	formulated	na	na	5	0.10	BDL	yes	total	na	growth	growth (%)	88.2	na	control							
Cu	hybrid striped bass (<i>Morone chrysops</i> × <i>M. saxatilis</i>)	copper sulfate pentahydrate	FW	adults	42-day	na	na	0.02	treatment	formulated	na	na	571	11.42	BDL	yes	total	na	growth	growth (%)	74.4	16%	NOEC*	NA	NA	NA	NA	NA	NA	Bielmyer et al. 2005
Cu	hybrid striped bass (<i>Morone chrysops</i> × <i>M. saxatilis</i>)	copper sulfate pentahydrate	SW	adults	42-day	na	na	0.02	treatment	formulated	na	na	5	0.1	BDL	yes	total	na	growth	growth (%)	60.5	na	control	NA	NA	NA	NA	NA	NA	Bielmyer et al. 2005
Cu	hybrid striped bass (<i>Morone chrysops</i> × <i>M. saxatilis</i>)	copper sulfate pentahydrate	SW	adults	42-day	na	na	0.02	treatment	formulated	na	na	571	11.4	BDL	yes	total	na	growth	growth (%)	50.4	17%	NOEC*							
Cu	rainbow trout (<i>Oncorhynchus mykiss</i>)	NA	FW	8 g	20-wk	na	8	0.017	control	formulated	na	na	2.5	0.043	4.0	yes	total	na	growth	weight gain (g)	85	na	control							
Cu	rainbow trout (<i>Oncorhynchus mykiss</i>)	copper sulfate	FW	8 g	20-wk	na	8	0.017	treatment	formulated	na	na	495	8.5	4.0	yes	total	na	growth	weight gain (g)	77.7	9%	NOEC**							
Cu	rainbow trout (<i>Oncorhynchus mykiss</i>)	NA	FW	juveniles	16-wk	na	3.1	na	control	formulated	na	na	8.1	na	3-10	yes	na	na	growth	wet weight (kg/100 fish)	63.3	na	control	NA	NA	NA	NA	NA	NA	Lanno et al. 1985a
Cu	rainbow trout (<i>Oncorhynchus mykiss</i>)	copper sulfate pentahydrate	FW	juveniles	16-wk	na	3.1	na	treatment	formulated	na	na	796	na	3-10	yes	na	na	growth	wet weight (kg/100 fish)	15.1	76%	LOEC							
Cu	rainbow trout (<i>Oncorhynchus mykiss</i>)	copper sulfate pentahydrate	FW	juveniles	8-wk	na	3.8	na	control	formulated	na	na	9	na	6.5-9.6	yes	na	na	growth	wet weight (g)	29.0	na	control							
Cu	rainbow trout (<i>Oncorhynchus mykiss</i>)	copper sulfate pentahydrate	FW	juveniles	8-wk	na	3.8	na	treatment	formulated	na	na	37	na	6.5-9.6	yes	na	na	growth	wet weight (g)	24.3	4%	NOEC(-)							
Cu	rainbow trout (<i>Oncorhynchus mykiss</i>)	copper sulfate pentahydrate	FW	juveniles	8-wk	na	3.8	na	treatment	formulated	na	na	83	na	6.5-9.6															

Table A-1. Concentration- and Dose-Response Data for Exposure of Aquatic Organisms to Diet-Borne Individual Metals

Metal Species		Chemical Form	Water Type	Age/Size at Initiation	Exposure Duration	FIR (g/d)	BW (g)	FIR (g _{food} /g _{BW} /d) ^a	Control or Treatment?	Exposure Information						Toxicity Information					Concentration (mg/kg dw)			Dose (mg/kg/d)			Reference			
										Diet-Borne Concentration and Dose				Water-Borne Concentration		Endpoint Type	Toxicity Information			EC10	EC20	EC50	EC10	EC20	EC50					
										mg/kg ww	Moisture	mg/kg bw/day	µg/L	Measured?	Total or Dissolved?		DOC (mg/L)	Measure	% Effect (Control-Normalized)							NOEC or LOEC?				
Cu	fathead minnow (<i>Pimephales promelas</i>)	NA	FW	juveniles	30-day	na	na	na	control	worm (<i>L. variegatus</i>)	na	na	12	0.20	na	na	na	growth	dry weight (g)	0.131	na	control							Erickson et al. 2010	
Cu	fathead minnow (<i>Pimephales promelas</i>)	copper sulfate pentahydrate	FW	juveniles	30-day	na	na	na	treatment	worm (<i>L. variegatus</i>)	na	na	131	1.9	na	na	na	growth	dry weight (g)	0.141	-8%	NOEC(-)	NA	NA	NA	NA	NA	NA		
Cu	fathead minnow (<i>Pimephales promelas</i>)	copper sulfate pentahydrate	FW	juveniles	30-day	na	na	na	treatment	worm (<i>L. variegatus</i>)	na	na	216	4.1	na	na	na	growth	dry weight (g)	0.153	-17%	NOEC*								
Cu	fathead minnow (<i>Pimephales promelas</i>)	NA	FW	adults	28-day	na	na	0.05	control	worm (<i>L. variegatus</i>)	na	na	19.2	0.19	0.73	yes	dissolved	na	growth	condition factor	1.33	na	control	NA	NA	NA	NA	NA	NA	Lapointe et al. 2011
Cu	fathead minnow (<i>Pimephales promelas</i>)	copper sulfate	FW	adults	28-day	na	na	0.05	treatment	worm (<i>L. variegatus</i>)	na	na	156	1.6	1.88	yes	dissolved	na	growth	condition factor	1.30	2%	NOEC*							
Cu	fathead minnow (<i>Pimephales promelas</i>)	NA	FW	adults	28-day	na	na	0.05	control	worm (<i>L. variegatus</i>)	na	na	19.2	0.19	0.73	yes	dissolved	na	growth	condition factor	0.97	na	control	NA	NA	NA	NA	NA	NA	
Cu	fathead minnow (<i>Pimephales promelas</i>)	copper sulfate	FW	adults	28-day	na	na	0.05	treatment	worm (<i>L. variegatus</i>)	na	na	156	1.6	1.88	yes	dissolved	na	growth	condition factor	0.98	-1%	NOEC*							Lapointe et al. 2011
Cu	Atlantic salmon (<i>Salmo salar</i>)	NA	FW	fry	3-mo	na	na	0.034	control	formulated	na	na	7.2	0.24	0.6	yes	total	na	growth	wet weight (g)	10.3	na	control							
Cu	Atlantic salmon (<i>Salmo salar</i>)	copper sulfate pentahydrate	FW	fry	3-mo	na	na	0.034	treatment	formulated	na	na	37	1.3	0.6	yes	total	na	growth	wet weight (g)	11.9	-36%	NOEC(-)							
Cu	Atlantic salmon (<i>Salmo salar</i>)	copper sulfate pentahydrate	FW	fry	3-mo	na	na	0.034	treatment	formulated	na	na	467	15.9	1.2	yes	total	na	growth	wet weight (g)	9.1	12%	NOEC(-)	320	666	2858	10.9	22.6	97.2	Bertnssen et al. 1999a
Cu	Atlantic salmon (<i>Salmo salar</i>)	copper sulfate pentahydrate	FW	fry	3-mo	na	na	0.034	treatment	formulated	na	na	638	21.7	1.6	yes	total	na	growth	wet weight (g)	8.7	16%	NOEC							
Cu	Atlantic salmon (<i>Salmo salar</i>)	copper sulfate pentahydrate	FW	fry	3-mo	na	na	0.034	treatment	formulated	na	na	868	29.5	1.6	yes	total	na	growth	wet weight (g)	7.5	27%	LOEC							
Cu	Atlantic salmon (<i>Salmo salar</i>)	copper sulfate pentahydrate	FW	fry	3-mo	na	na	0.034	treatment	formulated	na	na	1,780	60.5	1.4	yes	total	na	growth	wet weight (g)	6.6	36%	LOEC(+)							Bertnssen et al. 1999b
Cu	Atlantic salmon (<i>Salmo salar</i>)	NA	FW	parr	4-wk	na	na	0.024	control	formulated	na	na	5.3	0.13	na	na	na	na	growth	wet weight (g)	80.3	na	control	NA	NA	NA	NA	NA	NA	
Cu	Atlantic salmon (<i>Salmo salar</i>)	copper sulfate pentahydrate	FW	parr	4-wk	na	na	0.024	treatment	formulated	na	na	34.2	0.82	na	na	na	na	growth	wet weight (g)	77.3	4%	NOEC(-)							
Cu	Atlantic salmon (<i>Salmo salar</i>)	copper sulfate pentahydrate	FW	parr	4-wk	na	na	0.024	treatment	formulated	na	na	691.3	16.6	na	na	na	na	growth	wet weight (g)	73.9	8%	NOEC*							Lundebye et al. 1999
Cu	Atlantic salmon (<i>Salmo salar</i>)	NA	FW	fingerlings	12-wk	na	na	na	control	formulated	na	na	5	na	na	na	na	na	growth	relative growth (%)	966	na	control							
Cu	Atlantic salmon (<i>Salmo salar</i>)	NR	FW	fingerlings	12-wk	na	na	na	treatment	formulated	na	na	35	na	na	na	na	na	growth	relative growth (%)	1,099	-14%	NOEC(-)							
Cu	Atlantic salmon (<i>Salmo salar</i>)	NR	FW	fingerlings	12-wk	na	na	na	treatment	formulated	na	na	500	na	na	na	na	na	growth	relative growth (%)	924	4%	NOEC							Lundebye et al. 1999
Cu	Atlantic salmon (<i>Salmo salar</i>)	NR	FW	fingerlings	12-wk	na	na	na	treatment	formulated	na	na	700	na	na	na	na	na	growth	relative growth (%)	800	17%	LOEC							
Cu	Atlantic salmon (<i>Salmo salar</i>)	NR	FW	fingerlings	12-wk	na	na	na	treatment	formulated	na	na	900	na	na	na	na	na	growth	relative growth (%)	689	29%	LOEC(+)							
Cu	Atlantic salmon (<i>Salmo salar</i>)	NR	FW	fingerlings	12-wk	na	na	na	treatment	formulated	na	na	1,750	na	na	na	na	na	growth	relative growth (%)	583	40%	LOEC(+)							Hammerschmidt et al. 2002
MeHg ^d	fathead minnow (<i>Pimephales promelas</i>)	methylmercuric chloride	FW	juveniles	195-day	na	na	na	control	formulated	na	na	0.06	na	na	na	na	na	reproduction	spawning success (%)	75	na	control	0.27	0.48	1.1	NA	NA	NA	
MeHg ^d	fathead minnow (<i>Pimephales promelas</i>)	methylmercuric chloride	FW	juveniles	195-day	na	na	na	treatment	formulated	na	na	0.88	na	na	na	na	na	reproduction	spawning success (%)	46	39%	LOEC							Drevnick and Sandheinrich 2003
MeHg ^d	fathead minnow (<i>Pimephales promelas</i>)	methylmercuric chloride	FW	juveniles	195-day	na	na	na	treatment	formulated	na	na	4.11	na	na	na	na	na	reproduction	spawning success (%)	50	33%	LOEC(+)							
MeHg ^d	fathead minnow (<i>Pimephales promelas</i>)	methylmercuric chloride	FW	juveniles	250-day	na	na	0.05	control	formulated	na	na	0.06	0.0030	na	na	na	na	reproduction	spawning success (%)	36	52%	LOEC(+)	0.19	0.32	0.71	NA	NA	NA	
MeHg ^d	fathead minnow (<i>Pimephales promelas</i>)	methylmercuric chloride	FW	juveniles	250-day	na	na	0.05	treatment	formulated	na	na	0.87	0.044	na	na	na	na	reproduction	spawning success (%)	12	63%	LOEC							
MeHg ^d	fathead minnow (<i>Pimephales promelas</i>)	methylmercuric chloride	FW	juveniles	250-day	na	na	0.05	treatment	formulated	na	na	3.93	0.20	na	na	na	na	reproduction	spawning success (%)	0	100%	LOEC(+)							Sandheinrich and Miller 2006
MeHg ^d	fathead minnow (<i>Pimephales promelas</i>)	methylmercuric chloride	FW	juveniles	na	na	na	na	control	formulated	na	na	0.06	na	na	na	na	na	reproduction	spawning success (%)	40	na	control	0.22	0.38	0.87	NA	NA	NA	
MeHg ^d	fathead minnow (<i>Pimephales promelas</i>)	methylmercuric chloride	FW	juveniles	na	na	na	na	treatment	formulated	na	na	0.87	na	na	na	na	na	reproduction	spawning success (%)	20	50%	LOEC							
MeHg ^d	fathead minnow (<i>Pimephales promelas</i>)	methylmercuric chloride	FW	juveniles	na	na	na	na	treatment	formulated	na	na	3.93	na	na	na	na	na	reproduction	spawning success (%)	20	50%	LOEC(+)							
Ni	lake whitefish (<i>Coregonus clupeaformis</i>)	NA	FW	adults	104-day	na	na	0.0021	control	formulated	na	na	1.1	0.0024	<2	yes	total	na	growth	condition factor	1.31	na	control	NA	NA	NA	NA	NA	NA	Ptashynski et al. 2002
Ni	lake whitefish (<i>Coregonus clupeaformis</i>)	NR	FW	adults	104-day	na	na	0.0021	treatment	formulated	na	na	12	0.026	<2	yes	total	na	growth	condition factor	1.27	3%	NOEC(-)							
Ni	lake whitefish (<i>Coregonus clupeaformis</i>)	NR	FW	adults	104-day	na	na	0.0021	treatment	formulated	na	na	110	0.24	<2	yes	total	na	growth	condition factor	1.25	5%	NOEC(-)	NA	NA	NA	NA	NA	NA	
Ni	lake whitefish (<i>Coregonus clupeaformis</i>)	NR	FW	adults	104-day	na	na	0.0021	treatment	formulated	na	na	1,100	2.4	<2	yes	total	na	growth	condition factor	1.36	-4%	NOEC*							Javed 2013
Ni	carp (<i>Carlia carlia</i>)	NA	FW	120 d	12-wk	15.4	289.2	0.053	control	formulated	na	na	na	na	na	na	na	na	growth	weight increase (g)	34.75	na	control	NA	NA	NA	NA	NA	NA	
Ni	carp (<i>Carlia carlia</i>)	nickel chloride hexahydrate	FW	120 d	12-wk	9.39	168.7	0.056	treatment	formulated	na	na	70.4	3.9	na	na	na	na	growth	weight increase (g)	14.22	59%	LOEC*							Javed 2013
Ni	rohu, carp family (<i>Labeo rohita</i>)	NA	FW	120 d	12-wk																									

Table A-1. Concentration- and Dose-Response Data for Exposure of Aquatic Organisms to Diet-Borne Individual Metals

Metal	Species	Chemical Form	Water Type	Age/Size at Initiation	Exposure Duration	FIR (g/d)	BW (g)	FIR (g _{food} /g _{BW} /d) ^a	Control or Treatment?	Exposure Information								Toxicity Information						Concentration (mg/kg dw)			Dose (mg/kg/d)			Reference
										Diet-Borne Concentration and Dose				Water-Borne Concentration				Endpoint Type	Endpoint ^b	Measure	% Effect (Control-Normalized)	NOEC or LOEC?	EC10	EC20	EC50	EC10	EC20	EC50		
										Diet Type	mg/kg ww	Moisture	mg/kg dw	mg/kg bw/dw	µg/L	Measured?	Total or Dissolved?												DOC (mg/L)	
Zn	rainbow trout (<i>Oncorhynchus mykiss</i>)	NA	FW	fry	60-day	na	na	na	control	brine shrimp (<i>Artemia</i> sp.)	na	na	130	7.8	2.1	na	total	na	growth	wet weight (g)	2.203	na	control	NA	NA	NA	NA	NA	NA	Mount et al. 1994
Zn	rainbow trout (<i>Oncorhynchus mykiss</i>)	zinc chloride	FW	fry	60-day	na	na	na	treatment	brine shrimp (<i>Artemia</i> sp.)	na	na	920	55.2	46.3	na	total	na	growth	wet weight (g)	2.484	-13%	NOEC(-)							
Zn	rainbow trout (<i>Oncorhynchus mykiss</i>)	zinc chloride	FW	fry	60-day	na	na	na	treatment	brine shrimp (<i>Artemia</i> sp.)	na	na	930	55.8	46.3	na	total	na	growth	wet weight (g)	2.402	-9%	NOEC(-)							
Zn	rainbow trout (<i>Oncorhynchus mykiss</i>)	zinc chloride	FW	fry	60-day	na	na	na	treatment	brine shrimp (<i>Artemia</i> sp.)	na	na	1,900	114	46.3	na	total	na	growth	wet weight (g)	2.555	-16%	NOEC*							
Zn	rainbow trout (<i>Oncorhynchus mykiss</i>)	NA	FW	juveniles	15-day	na	na	0.08	control	formulated	na	na	91.3	7.3	na	na	na	na	growth	growth rate (%/day)	6.7	na	control	NA	NA	NA	NA	NA	NA	Kjoss et al. 2006
Zn	rainbow trout (<i>Oncorhynchus mykiss</i>)	zinc oxide	FW	juveniles	15-day	na	na	0.08	treatment	formulated (ZnO)	na	na	771.9	61.8	na	na	na	na	growth	growth rate (%/day)	6.6	1%	NOEC(-)							
Zn	rainbow trout (<i>Oncorhynchus mykiss</i>)	zinc sulfate heptahydrate	FW	juveniles	15-day	na	na	0.08	treatment	formulated (ZnSO4)	na	na	1,035.8	82.9	na	na	na	na	growth	growth rate (%/day)	6.3	6%	NOEC*							
Zn	rainbow trout (<i>Oncorhynchus mykiss</i>)	zinc proteinate	FW	juveniles	15-day	na	na	0.08	treatment	formulated (Zn proteinate)	na	na	653.3	52.3	na	na	na	na	growth	growth rate (%/day)	6.4	4%	NOEC(-)	NA	NA	NA	NA	NA	NA	Richardson et al. 1985
Zn	rainbow trout (<i>Oncorhynchus mykiss</i>)	zinc methionine	FW	juveniles	15-day	na	na	0.08	treatment	formulated (Zn methionine)	na	na	988.7	79.1	na	na	na	na	growth	growth rate (%/day)	6.4	4%	NOEC(-)							
Zn	Chinook salmon (<i>Oncorhynchus tshawytscha</i>)	NA	FW	juveniles	105-day	na	na	0.0210	control	formulated	na	na	54	1.1	na	na	na	na	growth	growth rate (%/day)	2.14	na	control							
Zn	Chinook salmon (<i>Oncorhynchus tshawytscha</i>)	zinc sulfate heptahydrate	FW	juveniles	105-day	na	na	0.0164	treatment	formulated	na	na	354	5.8	na	na	na	na	growth	growth rate (%/day)	2.15	0%	NOEC*	NA	NA	NA	NA	NA	NA	Richardson et al. 1985
Zn	Chinook salmon (<i>Oncorhynchus tshawytscha</i>)	NA	FW	juveniles	105-day	na	na	0.0212	control	formulated	na	na	50	1.1	na	na	na	na	growth	growth rate (%/day)	1.72	na	control							
Zn	Chinook salmon (<i>Oncorhynchus tshawytscha</i>)	zinc sulfate heptahydrate	FW	juveniles	105-day	na	na	0.0168	treatment	formulated	na	na	403	6.8	na	na	na	na	growth	growth rate (%/day)	1.71	1%	NOEC*							
Zn	Nile tilapia (<i>Oreochromis niloticus</i>)	zinc sulfate heptahydrate	FW	fingerlings	70-day	na	na	0.03	treatment ^c	formulated	na	na	1	0.03	na	na	na	na	growth	weight gain (%)	90.47	na	Zn-deficient	NA	NA	NA	NA	NA	NA	Eid and Ghonim 1994
Zn	Nile tilapia (<i>Oreochromis niloticus</i>)	zinc sulfate heptahydrate	FW	fingerlings	70-day	na	na	0.03	treatment ^c	formulated	na	na	5	0.15	na	na	na	na	growth	weight gain (%)	98.79	na	Zn-deficient							
Zn	Nile tilapia (<i>Oreochromis niloticus</i>)	zinc sulfate heptahydrate	FW	fingerlings	70-day	na	na	0.03	treatment ^c	formulated	na	na	10	0.3	na	na	na	na	growth	weight gain (%)	104.76	na	Zn-deficient							
Zn	Nile tilapia (<i>Oreochromis niloticus</i>)	zinc sulfate heptahydrate	FW	fingerlings	70-day	na	na	0.03	treatment ^c	formulated	na	na	20	0.6	na	na	na	na	growth	weight gain (%)	114.28	na	Zn-deficient	NA	NA	NA	NA	NA	NA	Eid and Ghonim 1994
Zn	Nile tilapia (<i>Oreochromis niloticus</i>)	NA	FW	fingerlings	70-day	na	na	0.03	control ^d	formulated	na	na	30	0.9	na	na	na	na	growth	weight gain (%)	171.76	na	optimum							
Zn	Nile tilapia (<i>Oreochromis niloticus</i>)	zinc sulfate heptahydrate	FW	fingerlings	70-day	na	na	0.03	treatment ^c	formulated	na	na	40	1.2	na	na	na	na	growth	weight gain (%)	124.70	na	NOEC(-)							
Zn	Nile tilapia (<i>Oreochromis niloticus</i>)	zinc sulfate heptahydrate	FW	fingerlings	70-day	na	na	0.03	treatment ^c	formulated	na	na	50	1.5	na	na	na	na	growth	weight gain (%)	148.23	na	NOEC(-)	NA	NA	NA	NA	NA	NA	Maage and Julshmann 1993
Zn	Nile tilapia (<i>Oreochromis niloticus</i>)	zinc sulfate heptahydrate	FW	fingerlings	70-day	na	na	0.03	treatment ^c	formulated	na	na	60	1.8	na	na	na	na	growth	weight gain (%)	117.85	na	NOEC(-)							
Zn	Nile tilapia (<i>Oreochromis niloticus</i>)	zinc sulfate heptahydrate	FW	fingerlings	70-day	na	na	0.03	treatment ^c	formulated	na	na	80	2.4	na	na	na	na	growth	weight gain (%)	120.00	na	NOEC(-)							
Zn	Nile tilapia (<i>Oreochromis niloticus</i>)	zinc sulfate heptahydrate	FW	fingerlings	70-day	na	na	0.03	treatment ^c	formulated	na	na	100	3.0	na	na	na	na	growth	weight gain (%)	125.00	na	NOEC*	NA	NA	NA	NA	NA	NA	Maage and Julshmann 1993
Zn	Atlantic salmon (<i>Salmo salar</i>)	NA	FW	juveniles	8-wk	na	na	na	control	formulated	na	na	17.6	na	na	na	na	na	growth	weight gain (g)	81	na	control							
Zn	Atlantic salmon (<i>Salmo salar</i>)	zinc sulfate heptahydrate	FW	juveniles	8-wk	na	na	na	treatment	formulated	na	na	27.6	na	na	na	na	na	growth	weight gain (g)	81	0%	NOEC(-)							
Zn	Atlantic salmon (<i>Salmo salar</i>)	zinc sulfate heptahydrate	FW	juveniles	8-wk	na	na	na	treatment	formulated	na	na	36.3	na	na	na	na	na	growth	weight gain (g)	79	2%	NOEC(-)	NA	NA	NA	NA	NA	NA	Maage and Julshmann 1993
Zn	Atlantic salmon (<i>Salmo salar</i>)	zinc sulfate heptahydrate	FW	juveniles	8-wk	na	na	na	treatment	formulated	na	na	67.2	na	na	na	na	na	growth	weight gain (g)	83	-2%	NOEC(-)							
Zn	Atlantic salmon (<i>Salmo salar</i>)	zinc sulfate heptahydrate	FW	juveniles	8-wk	na	na	na	treatment	formulated	na	na	101	na	na	na	na	na	growth	weight gain (g)	82	-1%	NOEC*							
Zn	turbot (<i>Scophthalmus maximus</i>)	NA	SW	450-750 g	250-day	na	na	na	control	formulated	na	na	0	na	na	na	na	na	growth	growth rate	na	na	control	NA	NA	NA	NA	NA	NA	Overnell et al. 1988
Zn	turbot (<i>Scophthalmus maximus</i>)	zinc sulfate	SW	450-750 g	250-day	na	na	na	treatment	formulated	na	na	1,000	na	na	na	na	na	growth	growth rate	*2X controls*	na	NOEC*							

Notes:

^a Food ingestion rate (FIR) is only provided if test-specific information was provided.

^b Endpoint shown for each study was the most sensitive endpoint if effects were observed or a representative endpoint if no effects were observed.

^c Arsenic species that was either spiked into formulated diets or to which oligochaete diets were exposed.

^d Test details are provided here for the three fathead minnow studies that provided the basis for the diet-borne mercury TRV. See Depew et al. (2012) for a comprehensive review of diet-borne mercury toxicity studies for fish.

^e This study evaluated the influence of three dietary Zn concentrations combined with one of four different water-borne Zn concentrations, with the lowest dietary Zn concentration (1 µg/g dw) being deficient. The highest dietary Zn concentration of 590 µg/g dw did not result in any growth effects at any water-borne Zn concentration tested.

EC10 - 10% effect concentration

EC20 - 20% effect concentration

EC50 - 50% effect concentration

NOEC - no observed effect concentration

LOEC - lowest observed effect concentration

NOEC(-) - treatments below the NOEC

LOEC(+) - treatments above the LOEC

NOEC* - unbounded (i.e., no significant effects observed at highest concentration tested)

LOEC* - unbounded (i.e., significant effects observed at lowest concentration tested)

NOEC** - assumed (no statistics)

LOEC** - assumed (no statistics)

GSI - gonadal somatic index

anomalous - NOEC and LOEC could not be determined due to inconsistent concentration-response relationship

wk - week

mo - month

init. - initial

BDL - below detection limit

BW - body weight

DOC - dissolved organic carbon

FW - fresh water

SW - salt water

na - not available

NA - not applicable

NR - not reported

Table A-2. Summary of FIR Information from Tests Summarized in Table A-1.

Species	Species Mean		Reference
	FIR (g _{food} /g bw/d)	FIR (g _{food} /g bw/d)	
Atlantic salmon (<i>Salmo salar</i>)	0.024	0.029	Berntssen et al. 1999b
Atlantic salmon (<i>Salmo salar</i>)	0.034		Berntssen et al. 1999a
Carp (<i>Catla catla</i>)	0.053	0.054	Javed 2013
Carp (<i>Catla catla</i>)	0.056		
Catfish (<i>Clarias</i> spp.)	0.03	0.03	Ruangsomboon and Wongrat 2006
Channel catfish (<i>Ictalurus punctatus</i>)	0.03	0.03	Gatlin and Wilson 1986
Chinook salmon (<i>Oncorhynchus tshawytscha</i>)	0.0210	0.0189	Richardson et al. 1985
Chinook salmon (<i>Oncorhynchus tshawytscha</i>)	0.0164		
Chinook salmon (<i>Oncorhynchus tshawytscha</i>)	0.0212		
Chinook salmon (<i>Oncorhynchus tshawytscha</i>)	0.0168		
Coho salmon (<i>Oncorhynchus kisutch</i>)	0.05	0.05	Bowen et al. 2006
Common carp (<i>Cyprinus carpio</i>)	0.03	0.05	Ahmed et al. 2012
Common carp (<i>Cyprinus carpio</i>)	0.077		Jeng and Sun 1981
Fathead minnow (<i>Pimephales promelas</i>)	0.05	0.05	Lapointe et al. 2011
Fathead minnow (<i>Pimephales promelas</i>)	0.05		Drevnick and Sandheinrich 2003
Fathead minnow (<i>Pimephales promelas</i>)	0.05		
Hybrid striped bass (<i>Morone chrysops</i> × <i>M. saxatilis</i>)	0.02	0.02	Bielmyer et al. 2005
Hybrid striped bass (<i>Morone chrysops</i> × <i>M. saxatilis</i>)	0.02		
Lake whitefish (<i>Coregonus clupeaformis</i>)	0.0021	0.0021	Ptashynski et al. 2002
Mrigal carp (<i>Cirrhina mrigala</i>)	0.048	0.047	Javed 2013
Mrigal carp (<i>Cirrhina mrigala</i>)	0.046		
Nile tilapia (<i>Oreochromis niloticus</i>)	0.03	0.03	Ayyat et al. 2003
Nile tilapia (<i>Oreochromis niloticus</i>)	0.03		Eid and Ghonim 1994
Rainbow trout (<i>Oncorhynchus mykiss</i>)	0.0241	0.04	Galvez and Wood 1999
Rainbow trout (<i>Oncorhynchus mykiss</i>)	0.0142		Galvez et al. 2001
Rainbow trout (<i>Oncorhynchus mykiss</i>)	0.0500		Handy 1993a
Rainbow trout (<i>Oncorhynchus mykiss</i>)	0.022		Tacon and Beveridge 1982
Rainbow trout (<i>Oncorhynchus mykiss</i>)	0.017		Knox et al. 1984
Rainbow trout (<i>Oncorhynchus mykiss</i>)	0.017		Julshamn et al. 1988
Rainbow trout (<i>Oncorhynchus mykiss</i>)	0.0071		Handy 1993b
Rainbow trout (<i>Oncorhynchus mykiss</i>)	0.0166		Handy et al. 1999
Rainbow trout (<i>Oncorhynchus mykiss</i>)	0.08		Kjoss et al. 2006
Rainbow trout (<i>Oncorhynchus mykiss</i>)	0.08		
Rainbow trout (<i>Oncorhynchus mykiss</i>)	0.04		Kamunde et al. 2001
Rainbow trout (<i>Oncorhynchus mykiss</i>)	0.04		Kamunde et al. 2002
Rainbow trout (<i>Oncorhynchus mykiss</i>)	0.015		Kamunde and Wood 2003
Rainbow trout (<i>Oncorhynchus mykiss</i>)	0.03		
Rainbow trout (<i>Oncorhynchus mykiss</i>)	0.045		Kjoss et al. 2005
Rainbow trout (<i>Oncorhynchus mykiss</i>)	0.04		
Rainbow trout (<i>Oncorhynchus mykiss</i>)	0.04		Hodson et al. 1978
Rainbow trout (<i>Oncorhynchus mykiss</i>)	0.03		
Rainbow trout (<i>Oncorhynchus mykiss</i>)	0.017		Knox et al. 1984
Rainbow trout (<i>Oncorhynchus mykiss</i>)	0.08		Kjoss et al. 2006
Rohu, carp family (<i>Labeo rohita</i>)	0.060	0.056	Javed 2013
Rohu, carp family (<i>Labeo rohita</i>)	0.052		
Tilapia (<i>Oreochromis niloticus</i> × <i>O. aureus</i>)	0.05	0.05	Shiau and Lin 1993
Overall Mean:		0.037	

Table A-3. Additional Endpoints Evaluated in Diet-Borne Toxicity Studies Reviewed

Metal	Species	Other Endpoints	Reference
Ag	rainbow trout (<i>Oncorhynchus mykiss</i>)	food consumption rate; food conversion efficiency	Galvez and Wood 1999
Ag	rainbow trout (<i>Oncorhynchus mykiss</i>)	food consumption rate; food conversion efficiency; plasma Na and Na influx; hematocrit; plasma protein; whole blood hemoglobin; plasma glucose; ammonia-N and urea-N excretion rates; Na/K-ATPase and intestinal amylase	Galvez et al. 2001
Al	rainbow trout (<i>Oncorhynchus mykiss</i>)	no others	Handy 1993a
Al	Atlantic salmon (<i>Salmo salar</i>)	feed conversion; body fat; body protein	Poston 1991
As(III)	rainbow trout (<i>Oncorhynchus mykiss</i>)	feed: gain ratio; mortality	Cockell and Hilton 1988
As(III)	rainbow trout (<i>Oncorhynchus mykiss</i>)	percent lipid; percent protein	Erickson et al. 2010
As(V)	rainbow trout (<i>Oncorhynchus mykiss</i>)	feed consumption; feed:gain ratio; histopathology	Cockell and Bettger 1993
As(V)	rainbow trout (<i>Oncorhynchus mykiss</i>)	feed consumption; feed:gain ratio	Cockell et al. 1991
As(V)	rainbow trout (<i>Oncorhynchus mykiss</i>)	feed consumption; feed:gain ratio; hematology	Cockell et al. 1992
As(V)	rainbow trout (<i>Oncorhynchus mykiss</i>)	conversion efficiency	Erickson et al. 2011
Cd	Prussian carp (<i>Carassius auratus gibelio</i>)	ovarian development, luteinizing hormone (LH) secretion; response to hormonal stimulation of spawning	Szczerbik et al. 2006
Cd	catfish (<i>Clarias</i> spp.)	no others	Ruangsomboon and Wongrat 2006
Cd	common carp (<i>Cyprinus carpio</i>)	plasma ions; alanine transaminase (ALT); total protein content	Reynders et al. 2006
Cd	rainbow trout (<i>Oncorhynchus mykiss</i>)	no others	Franklin et al. 2005
Cd	rainbow trout (<i>Oncorhynchus mykiss</i>)	no others	Handy 1993b
Cd	rainbow trout (<i>Oncorhynchus mykiss</i>)	no others	Mount et al. 1994
Cd	rainbow trout (<i>Oncorhynchus mykiss</i>)	no others	Ng and Wood 2008
Cd	rainbow trout (<i>Oncorhynchus mykiss</i>)	no others	Ng et al. 2009
Cd	guppy (<i>Poecilia reticulata</i>)	no others	Hatakeyama and Yasuno 1982
Cd	guppy (<i>Poecilia reticulata</i>)	no others	Hatakeyama and Yasuno 1987
Cd	Atlantic salmon (<i>Salmo salar</i>)	Ca-ATPase and Na/K-ATPase	Berntssen et al. 2003
Cd	Atlantic salmon (<i>Salmo salar</i>)	histology	Lundebye et al. 1999
Cr(III)	common carp (<i>Cyprinus carpio</i>)	plasma glucose, enzyme activity, comet assay, histology	Ahmed et al. 2012
Cr(III)	rainbow trout (<i>Oncorhynchus mykiss</i>)	food conversion ratio, liver weight, liver carbohydrate, muscle carbohydrate	Tacon and Beveridge 1982
Cr(III)	tilapia (<i>Oreochromis niloticus</i> x <i>O. aureus</i>)	protein deposition, energy deposition, liver glycogen	Shiau and Lin 1993
Cu	common carp (<i>Cyprinus carpio</i>)	feed conversion ratio; specific growth rate; condition factor; hepatosomatic index	Ajani and Akpoilih 2012
Cu	zebrafish (<i>Danio rerio</i>)	feeding, growth, mortality, oxidative stress	Alsop et al. 2007
Cu	channel catfish (<i>Ictalurus punctatus</i>)	feed efficiency, mortality, superoxide dismutase (SOD) and cytochrome c oxidase (CCO) activities	Gatlin and Wilson 1986
Cu	channel catfish (<i>Ictalurus punctatus</i>)	hematology	Murai et al. 1981
Cu	hybrid striped bass (<i>Morone chrysops</i> x <i>M. saxatilis</i>)	no others	Bielmyer et al. 2005

Table A-3. Additional Endpoints Evaluated in Diet-Borne Toxicity Studies Reviewed

Metal	Species	Other Endpoints	Reference
Cu	rainbow trout (<i>Oncorhynchus mykiss</i>)	condition factor, feed conversion ratio, hematology, swimming speed	Handy et al. 1999
Cu	rainbow trout (<i>Oncorhynchus mykiss</i>)	feed efficiency ratio, hepatosomatic index, mortality	Julshamn et al. 1988
Cu	rainbow trout (<i>Oncorhynchus mykiss</i>)	food conversion efficiency, somatic indices, Na/K-ATPase activities	Kamunde and Wood 2003
Cu	rainbow trout (<i>Oncorhynchus mykiss</i>)	food conversion efficiency, midintestinal morphology	Kamunde et al. 2001
Cu	rainbow trout (<i>Oncorhynchus mykiss</i>)	food conversion efficiency	Kamunde et al. 2002
Cu	rainbow trout (<i>Oncorhynchus mykiss</i>)	somatic indices, Na flux	Kjoss et al. 2005
Cu	rainbow trout (<i>Oncorhynchus mykiss</i>)	no others	Kjoss et al. 2006
Cu	rainbow trout (<i>Oncorhynchus mykiss</i>)	feed efficiency, mortality, hematology, hepatosomatic index	Knox et al. 1984
Cu	rainbow trout (<i>Oncorhynchus mykiss</i>)	feed efficiency, mortality, hematology, hepatosomatic index	Lanno et al. 1985a
Cu	rainbow trout (<i>Oncorhynchus mykiss</i>)	feed:gain ratio; mortality	Lanno et al. 1985b
Cu	Nile tilapia (<i>Oreochromis niloticus</i>)	survival, hematology, food conversion ratio, somatic indices, pathology	Shaw and Handy 2006
Cu	fathead minnow (<i>Pimephales promelas</i>)	heat stress response	Lapointe et al. 2011
Cu	Atlantic salmon (<i>Salmo salar</i>)	K-factor, gross feed conversion efficiency, mortality	Berntssen et al. 1999a
Cu	Atlantic salmon (<i>Salmo salar</i>)	K-factor, cell proliferation, apoptosis	Berntssen et al. 1999b
Ni	lake whitefish (<i>Coregonus clupeaformis</i>)	no others	Ptashynski et al. 2002
Pb	rainbow trout (<i>Oncorhynchus mykiss</i>)	lipid, carbohydrates, blood Na, gut Na/K-ATPase	Alsop et al. 2016
Pb	rainbow trout (<i>Oncorhynchus mykiss</i>)	ALA-D activity	Hodson et al. 1978
Pb	rainbow trout (<i>Oncorhynchus mykiss</i>)	feed efficiency, hematology, mortality	Ayyat et al. 2003
Pb	rainbow trout (<i>Oncorhynchus mykiss</i>)	feed conversion ratio; survival; enzyme activities	Dai et al. 2009
V	rainbow trout (<i>Oncorhynchus mykiss</i>)	feed: gain ratio; mortality; pathology	Hilton and Bettger 1988
Zn	common carp (<i>Cyprinus carpio</i>)	no others	Jeng and Sun 1981
Zn	coho salmon (<i>Oncorhynchus kisutch</i>)	behavior; Hsp-70	Bowen et al. 2006
Zn	rainbow trout (<i>Oncorhynchus mykiss</i>)	hematocrit and plasma protein	Spry et al. 1988
Zn	rainbow trout (<i>Oncorhynchus mykiss</i>)	feed efficiency, survival	Takeda and Shimma 1977
Zn	Chinook salmon (<i>Oncorhynchus tshawytscha</i>)	food conversion, protein efficiency ratio, survival	Richardson et al. 1985
Zn	Nile tilapia (<i>Oreochromis niloticus</i>)	feed efficiency, survival	Eid and Ghonim 1994
Zn	Atlantic salmon (<i>Salmo salar</i>)	hematology, survival, alkaline phosphatase activities	Maage and Julshamn 1993
Zn	turbot (<i>Scophthalmus maximus</i>)	metallothionein levels	Overnell et al. 1988

Notes:

ALA-D = δ-amino levulinic acid dehydratase activity

Hsp-70 = heat-shock protein (70 kilodalton family)

Na/K-ATPase = sodium-potassium adenosine triphosphatase

Ca-ATPase = calcium adenosine triphosphatase

K-factor = condition factor calculated as $100 \times \text{weight (g)} / \text{length}^3 \text{ (cm)}$

UPPER COLUMBIA RIVER

FINAL **Fish Diet Toxicity Reference Values for the** **Baseline Ecological Risk Assessment**

Addendum No. 1 **Fish Diet No-Observed-Effect Concentrations and Doses for the** **Evaluation of Bull Trout**

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Table 1	"No-Effect" Fish Diet Concentrations and Doses and Comparison to 2019 Fish Diet TRVs
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ACRONYMS AND ABBREVIATIONS

BERA	baseline ecological risk assessment
bw	body weight
COPC	chemical of potential concern
dw	dry weight
EC10	10 percent effect concentration
EC20	20 percent effect concentration
ED20	20 percent effect dose
EPA	U.S. Environmental Protection Agency
SSD	species sensitivity distribution
TRV	toxicity reference value
TTF	trophic transfer factor
WB	whole-body

UNITS OF MEASURE

mg/L

milligram(s) per liter

1 INTRODUCTION

Fish diet toxicity reference values (TRVs) were previously developed to support evaluation of the dietary exposure pathway for fish in the Upper Columbia River remedial investigation/feasibility study baseline ecological risk assessment (BERA). These fish diet TRVs were approved by the U.S. Environmental Protection Agency (EPA) in 2019 and incorporated in the Draft Aquatic BERA (TAI 2024). The fish diet TRVs, hereinafter referred to as the 2019 TRVs, are described and presented in Appendix I of the Final Aquatic BERA, to which this document is an addendum.

The levels of effect and statistical endpoints for the fish diet TRVs varied depending on the types and amounts of toxicity data available for each chemical. For example, the preferred level of effect was 20 percent, expressed as a concentration-based EC20 or dose-based ED20 (i.e., 20 percent effect concentration and 20 percent effect dose, respectively). If an EC20 or ED20 could not be derived, the TRVs were expressed as no-observed-effect concentrations or levels (NOECs or NOELs, respectively) or lowest-observed-effect concentrations or levels (LOECs or LOELs, respectively) associated with varying levels of effect.

In its review of the Draft Aquatic BERA, EPA commented that bull trout (*Salvelinus confluentus*) should be evaluated at the individual level. To address this comment, fish diet no-effect concentrations and doses based on NOECs and NOELs, respectively, were developed as possible. Because NOECs and NOELs represent concentrations and doses for which no statistically significant effects were observed relative to the control, these values were assumed to provide reasonable estimates of no effects at the individual level. It is recognized that there are more rigorous approaches for defining “no-effect” thresholds in toxicity tests, such as model-based no-effect concentrations (Fox 2008), but these more rigorous approaches are generally less applicable to dietary toxicity tests, which are typically more limited in design than are aqueous exposure tests.

2 METHODS AND RESULTS

The bases for the fish diet TRVs previously approved and applied in the Draft Aquatic BERA were first reviewed. For some chemicals of potential concern (COPCs) those TRVs were already based on NOECs and NOELs, and it was thus not necessary to identify additional no-effect concentrations and doses. For the remaining COPCs, “no-effect” concentrations and doses based on NOECs and NOELs were identified as possible. The no-effect concentrations and doses are summarized by COPC in the subsections that follow. These no-effect concentrations and doses are provided and compared to the previously developed fish diet TRVs (Appendix I) in Table 1.

2.1 ALUMINUM

The 2019 fish diet TRVs for aluminum are based on a NOEC of $> 2,322 \text{ mg/kg}_{\text{diet}}$ dry weight (dw) and a NOEL of $> 82.5 \text{ mg/kg}$ body weight [bw]/day for weight gain in Atlantic salmon (*Salmo salar*) (Poston 1991). The NOEC and NOEL are expressed as “greater than” values because no effects were observed at the dietary concentration and dose tested (i.e., there was a 0 percent effect on weight gain relative to the control). The 2019 fish diet TRVs of $> 2,322 \text{ mg/kg}$ dw and $> 82.5 \text{ mg/kg}$ bw/day were therefore also defined as the “no-effect” dietary concentration and dose for aluminum (Table 1).

2.2 ARSENIC

The 2019 concentration-based TRV of $42 \text{ mg/kg}_{\text{diet}}$ dw was based on the EC20 from the pooled concentration-response data from seven rainbow trout toxicity tests with growth as the endpoint (see Section 3.2 in Appendix I for details). The lowest dietary arsenic concentration with a statistically significant ($p < 0.05$) effect relative to its respective control was $28 \text{ mg/kg}_{\text{diet}}$ dw. The geometric mean of the NOECs from all tests was $33 \text{ mg/kg}_{\text{diet}}$ dw, which was greater than the minimum LOEC of $28 \text{ mg/kg}_{\text{diet}}$ dw. The highest dietary arsenic treatment that did not cause a statistically significant effect relative to its respective control, while still being less than $28 \text{ mg/kg}_{\text{diet}}$ dw, was $16 \text{ mg/kg}_{\text{diet}}$ dw. Accordingly, the “no-effect” dietary concentration for arsenic was defined as $16 \text{ mg/kg}_{\text{diet}}$ dw (Table 1).

Like the existing concentration-based TRV, the existing dose-based TRV of 0.78 mg/kg bw/day was based on the ED20 from the pooled dose-response data from seven rainbow trout toxicity tests. The lowest dietary arsenic dose with a statistically significant ($p < 0.05$) effect relative to its respective control was 0.44 mg/kg bw/day. The geometric mean NOEL was 0.58 mg/kg bw/day, which was greater than 0.44 mg/kg bw/day. Based on all tests, the highest dietary arsenic

treatment that did not cause a statistically significant effect relative to its respective control, while still being less than 0.44 mg/kg bw/day, was 0.41 mg/kg bw/day. The “no-effect” dietary dose was therefore defined as 0.41 mg/kg bw/day (Table 1).

2.3 CADMIUM

The 2019 concentration-based TRV of 8.1 mg/kg_{diet} dw was based on the 5th percentile of a species sensitivity distribution (SSD) composed of EC20s and NOECs (when an EC20 was not available for a species). The 5th percentile of the SSD was extrapolated below the sensitivity of the most sensitive species tested, which was an unbounded LOEC of 16 mg/kg_{diet} dw (see Section 3.3 in Appendix I for details). Because there were no dietary cadmium concentrations from any test that resulted in a statistically significant effect at a concentration less than 8.1 mg/kg_{diet} dw, the 2019 TRV of 8.1 mg/kg_{diet} dw was likewise defined as the “no-effect” dietary concentration for cadmium (Table 1). Based on the same logic, the dose-based TRV of 0.023 mg/kg bw/day was defined as the “no-effect” dietary dose for cadmium (Table 1).

2.4 CHROMIUM

The 2019 concentration-based TRV of 3.9 mg/kg_{diet} dw was based on a LOEC associated with a 12 percent reduction in growth rate for juvenile carp (*Cyprinus carpio*) relative to the control (Ahmed et al. 2012). In addition, the dietary chromium concentration of 3.9 mg/kg_{diet} dw resulted in a 20 percent reduction in growth rate relative to the lowest dietary chromium concentration of 2.0 mg/kg_{diet} dw, in which enhanced growth was observed. This dietary chromium concentration of 2.0 mg/kg_{diet} dw was the NOEC in the test, as there was no reduction in growth relative to the control. Thus, the “no-effect” dietary concentration was defined as 2.0 mg/kg_{diet} dw (Table 1). The corresponding “no-effect” dietary dose from the same test was defined as 0.037 mg/kg bw/day (Table 1).

2.5 COPPER

The 2019 concentration-based TRV of 666 mg/kg_{diet} dw was based on an EC20 for Atlantic salmon (*S. salar*) growth (wet weight) (Berntssen et al. 1999). The NOEC from the test used to derive the EC20 was 638 mg/kg_{diet} dw. There were no dietary copper concentrations less than 638 mg/kg_{diet} dw, for any fish species, that resulted in a statistically significant effect relative to its respective control. Accordingly, the NOEC of 638 mg/kg_{diet} dw was defined as the “no-effect” dietary concentration for copper (Table 1).

The 2019 dose-based TRV of < 21.2 mg/kg bw/day was based on a LOEL for weight gain in common carp (*C. carpio*) (Ajani and Akpoilih 2012). This was identified as the 2019 dose-based

TRV because it was less than the ED20 of 22.6 mg/kg dw for Atlantic salmon (from the test used to derive the concentration-based TRV). The associated NOEL from the common carp test was 10.8 mg/kg dw/day, which was defined as the “no-effect” dietary dose (Table 1).

2.6 IRON

No dietary TRVs were previously developed for iron due to the lack of data. Accordingly, no “no-effect” dietary concentration or dose could be defined for iron.

2.7 LEAD

The 2019 concentration-based TRV of 111 mg/kg_{diet} dw was based on an EC20 for reduced growth rate in rainbow trout (*Oncorhynchus mykiss*) (Alsop et al. 2016). None of the dietary lead treatments in the test used to derive the EC20 resulted in a statistically significant effect relative to the control, and no dietary lead concentrations less than 111 mg/kg_{diet} dw for any species resulted in statistically significant effects relative to their respective controls (see Section 3.7 in Appendix I). As such, 111 mg/kg_{diet} dw was retained and defined as the “no-effect” dietary concentration for lead (Table 1). Based on the same logic and observation used for the concentration-based TRV, the 2019 dose-based TRV of 1.9 mg/kg bw/day was retained and defined as the “no-effect” dietary dose for lead (Table 1).

2.8 MANGANESE

No dietary TRVs were previously developed for manganese due to the lack of data. Accordingly, no “no-effect” dietary concentration or dose could be defined for manganese.

2.9 METHYL MERCURY

The 2019 concentration-based TRV of 0.26 mg/kg_{diet} dw was based on the 5th percentile of an SSD developed and reported in Depew et al. (2012). The 5th percentile of the SSD was an extrapolated value less than the toxicity threshold for the most sensitive species, which was fathead minnow (*Pimephales promelas*). Based on three dietary toxicity tests with fathead minnow, there were no treatments that did not result in a statistically significant effect relative to the control. The lowest dietary methyl mercury concentration for the three tests was 0.87 mg/kg_{diet} dw, which was greater than the 2019 concentration-based TRV of 0.26 mg/kg_{diet} dw. In the absence of a NOEC, the 2019 TRV of 0.26 mg/kg_{diet} dw was retained and defined as the “no-effect” dietary concentration for methyl mercury (Table 1). The same logic was applied

to the dose-based TRV of 0.013 mg/kg bw/day, which was retained and defined as the “no-effect” dietary dose for methyl mercury (Table 1).

2.10 NICKEL

The 2019 concentration-based TRV of $< 70.4 \text{ mg/kg}_{\text{diet}} \text{ dw}$ is from a study with carp (*Catla catla*), in which the lowest dietary concentration tested resulted in a statistically significant ($p < 0.05$) effect (weight gain) relative to the control (Javed 2013). The level of effect associated with the dietary nickel concentration of $70.4 \text{ mg/kg}_{\text{diet}} \text{ dw}$ was 59 percent. The highest NOEC for any species that was less than $70.4 \text{ mg/kg}_{\text{diet}} \text{ dw}$ was $12 \text{ mg/kg}_{\text{diet}} \text{ dw}$ for lake whitefish (*Coregonus clupeaformis*); however, dietary nickel concentrations up to $1,100 \text{ mg/kg}_{\text{diet}} \text{ dw}$ did not result in statistically significant ($p > 0.05$) effects on lake whitefish. For conservatism, the dietary nickel concentration of $12 \text{ mg/kg}_{\text{diet}} \text{ dw}$ was defined as the “no-effect” dietary concentration for nickel (Table 1).

The 2019 dose-based TRV of $< 3.9 \text{ mg/kg bw/day}$ was developed based on the same logic and test used to derive the 2019 concentration-based TRV. The dietary nickel dose associated with the dietary nickel concentration of $12 \text{ mg/kg}_{\text{diet}} \text{ dw}$ was $0.026 \text{ mg/kg bw/day}$; this value was defined as the “no-effect” dietary dose for nickel (Table 1).

2.11 SELENIUM

The concentration-based TRV for selenium was based on EPA's whole-body (WB) selenium criterion of $8.5 \text{ mg/kg}_{\text{WB}} \text{ dw}$ (USEPA 2016) divided by a diet-to-WB selenium trophic transfer factor (TTF) of 1.1 (i.e., $8.5 \text{ mg/kg}_{\text{WB}} \text{ dw} \div 1.1 = 7.7 \text{ mg/kg}_{\text{diet}} \text{ dw}$). The TTF of 1.1 is an average value recommended by Presser and Luoma (2010). The EPA's WB selenium criterion is based on the 5th percentile of a genus sensitivity distribution, for which the most sensitive genus is *Acipenser* (as represented by white sturgeon [*A. transmontanus*]). While selenium toxicity data for bull trout (*S. confluentus*) are not available, data for the closely related Dolly Varden (*Salvelinus malma*) are available. The WB selenium EC10 (i.e., 10 percent effect concentration) for Dolly Varden, which is based on dietary selenium exposures, is $34.9 \text{ mg/kg}_{\text{WB}} \text{ dw}$ (USEPA 2016). Because this WB EC10 is much greater than $8.5 \text{ mg/kg}_{\text{WB}} \text{ dw}$, which was the WB criterion used to derive the 2019 dietary selenium TRV, it was assumed that the 2019 concentration-based dietary TRV of $7.7 \text{ mg/kg}_{\text{diet}} \text{ dw}$ is protective of bull trout at the individual level (Table 1).

2.12 SILVER

The 2019 fish diet TRVs for silver are based on a test in which a dietary silver concentration of $3,000 \text{ mg/kg}_{\text{diet}} \text{ dw}$ and a corresponding dose of 70 mg/kg bw/day were associated with a

0 percent effect on a specific growth rate in juvenile rainbow trout (*O. mykiss*) (Galvez and Wood 1999). An anomalous concentration-response relationship was observed in that test, in which the specific growth rate was significantly reduced ($p < 0.05$) relative to the control in fish fed dietary silver concentrations of 3 and 300 mg/kg_{diet} dw, while fish fed dietary silver concentrations of 30 and 3,000 mg/kg_{diet} dw had specific growth rates greater than the control. Overall, Galvez and Wood (1999), considering results from their study and preliminary results from what would be later published by Galvez et al. (2001), concluded that dietary silver exposure is physiologically benign. Accordingly, the 2019 concentration- and dose-based TRVs of 3,000 mg/kg_{diet} dw and 70 mg/kg bw/day, respectively – which were associated with a 0 percent effect on specific growth rate relative to the control – were retained as the “no-effect” concentration and dose for dietary silver (Table 1).

2.13 VANADIUM

The 2019 concentration-based TRV is an EC20 of 4.3 mg/kg_{diet} dw for rainbow trout (*O. mykiss*) growth (wet weight) (Hilton and Bettger 1988). All dietary treatments in the test resulted in a statistically significant ($p < 0.05$) effect relative to the control, so a NOEC was not available. As an alternative, an EC10 of 2.7 mg/kg_{diet} dw was calculated for the rainbow trout test and defined as the “no-effect” concentration (Table 1). This concentration is nearly within a factor of two of the vanadium concentration in the control diet (1.2 mg/kg_{diet} dw). The same approach was applied to the dose-based TRV, which resulted in a “no-effect” dietary dose of 0.10 mg/kg bw/day (Table 1).

2.14 ZINC

The 2019 concentration-based TRV of 500 mg/kg_{diet} dw was based on an EC20 for common carp (*C. carpio*) growth (wet weight) (Jeng and Sun 1981). Based on all studies, the lowest dietary zinc concentration resulting in a statistically significant ($p < 0.05$) effect was 294 mg/kg_{diet} dw, and the highest dietary zinc concentration less than 294 mg/kg_{diet} dw that did not result in a statistically significant effect was 101 mg/kg_{diet} dw for Atlantic salmon (*S. salar*). As such, the “no-effect” dietary concentration was defined as 101 mg/kg_{diet} dw (Table 1).

The 2019 dose-based TRV of 38.5 mg/kg dw/day was an ED20 developed from the same test used to derive the EC20 of 500 mg/kg dw. Based on all studies, the lowest dietary dose resulting in a statistically significant ($p < 0.05$) effect was 22.6 mg/kg bw/day, and the highest dietary zinc dose lower than 22.6 mg/kg bw/day that did not result in a statistically significant effect was 8.6 mg/kg bw/day for rainbow trout. The “no-effect” dose-based TRV was therefore defined as 8.6 mg/kg bw/day (Table 1).

3 REFERENCES

- Ahmed, A.R., A.N. Jha, and S.J. Davies. 2012. The efficacy of chromium as a growth enhancer for mirror carp (*Cyprinus carpio* L.): an integrated study using biochemical, genetic, and historical responses. *Biol. Trace. Elem. Res.* 148:187–197.
- Ajani, E.K., and B.U. Akpoilih. 2012. Growth response and ionic regulation in common carp (*Cyprinus carpio* L.) after chronic dietary copper exposure and recovery. *Intl. J. Fish. Aquacult.* 4(2):13–21.
- Alsop, D., TY-T. Ng, M.J. Chowdhury, and C.M. Wood. 2016. Interactions of waterborne and dietborne Pb in rainbow trout, *Oncorhynchus mykiss*: bioaccumulation, physiological responses, and chronic toxicity. *Aqua. Tox.* 177:343–354.
- Berntssen, M.H.G., A-K. Lundebye, and A. Maage. 1999. Effects of elevated dietary copper concentrations on growth, feed utilisation and nutritional status of Atlantic salmon (*Salmo salar* L.) fry. *Aquacult.* 174(1-2):167–181.
- 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. Toxicol. Chem.* 31(7):1536–1547.
- Fox, D.R. 2008. NECS, NOECS and the ECx. *Australas. J. Ecotoxicol.* 14:7–9.
- Galvez, F., and C.M. Wood. 1999. Physiological effects of dietary silver sulfide exposure in rainbow trout. *Environ. Toxicol. Chem.* 18(1):84–88.
- Galvez, F., C. Hogstrand, J.C. McGeer, and C.M. Wood. 2001. The physiological effects of a biologically incorporated silver diet on rainbow trout (*Oncorhynchus mykiss*). *Aqua. Tox.* 55:95–112.
- Hilton, J.W., and W.J. Bettger. 1988. Dietary vanadium toxicity in juvenile rainbow trout: a preliminary study. *Aquat. Toxicol.* 12:63–71.
- Javed, M. 2013. Chronic effects of nickel and cobalt on fish growth. *Int. J. Agric. Biol.* 15:575–579.
- Jeng, S.S., and L.T. Sun. 1981. Effects of dietary zinc levels on zinc concentrations in tissues of common carp. *J. Nutr.* 111:134–140.
- Poston, H.A. 1991. Effects of dietary aluminum on growth and composition of young Atlantic salmon. *Progr. Fish. Cult.* 53:7–10.

- Presser, T.S., and S.N. Luoma. 2010. A methodology for ecosystem-scale modeling of selenium. *Integr. Environ. Assess. Manage.* 6 (4):685–710.
- TAI (Teck American Incorporated). 2024. Upper Columbia River: Aquatic Baseline Ecological Risk Assessment. Draft. Prepared for TAI by ERM, Carpinteria, CA, in association with Windward Environmental LLC, Exponent, and Parametrix, Inc.
- USEPA (U.S. Environmental Protection Agency). 2016. Aquatic life ambient water quality criterion for selenium—freshwater. 2016. EPA 822-R-16-006. U.S. Environmental Protection Agency, Washington, DC.

TABLES

Table H-1. "No-Effect" Fish Diet Concentrations and Doses and Comparison to 2019 Fish Diet TRVs

Constituent	"No-Effect" Concentrations and Doses		2019 Concentration- and Dose-Based TRVs	
	Concentration-Based (mg/kg _{diet} dw)	Dose-Based (mg/kg bw/day)	Concentration-Based TRV (mg/kg _{diet} dw) ^{a,b}	Dose-Based TRV (mg/kg bw/day) ^{a,b}
Aluminum	2,232	82.5	> 2,232	> 82.5
Arsenic	16	0.41	42	0.78
Cadmium	8.1	0.023	8.1	0.023
Chromium	2.0	0.037	3.9	0.074
Copper	638	10.8	666	< 21.2
Iron	na	na	na	na
Lead	111	1.9	111	1.9
Manganese	na	na	na	na
Methyl mercury	0.26	0.013	0.26	0.013
Nickel	12	0.026	< 70.4	< 3.9
Selenium	7.7	na	7.7	na
Silver	3,000 ^c	70 ^c	> 3,000 ^c	> 70 ^c
Vanadium	2.7	0.10	4.3	0.16
Zinc	101	8.6	500	38.5

Notes:

Bold text - "No effect" toxicity reference value (TRV) differs from the original fish diet TRV finalized in April 2019 (Appendix I)

^a Concentrations or doses with a less than (<) or greater than (>) symbol denote whether a lower or higher concentration would be predicted to achieve a 20% effect level.

^b The 2019 TRVs are based on EC20s and ED20s where possible. Otherwise, NOEC/LOECs and NOEL/LOELs were used. See main body of Appendix I for details.

^c An anomalous response was observed: growth rate reductions of 23, 0, 23, and 0% at diet-borne silver concentrations of 3, 30, 300, and 3,000 mg/kg_{diet} dw, respectively.

na - not available

NOEC - no-observed-effect concentration

NOEL - no-observed-effect level

LOEC - lowest-observed-effect concentration

LOEL - lowest-observed-effect level