ECOTOX TOOLBOX
User's Manual

v. 1.4

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November 1, 2026

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1. OVERVIEW

1.1 Toolbox Summary

The EcoTox Toolbox (the Toolbox) is an R Shiny application for modeling the bioavailability, bioaccumulation, and effects of environmental stressors, with a focus on inorganic stressors. The Toolbox includes models for aluminum, copper, nickel, selenium, and zinc (Figure 1). The stressor sections describe the available tools and their technical bases.

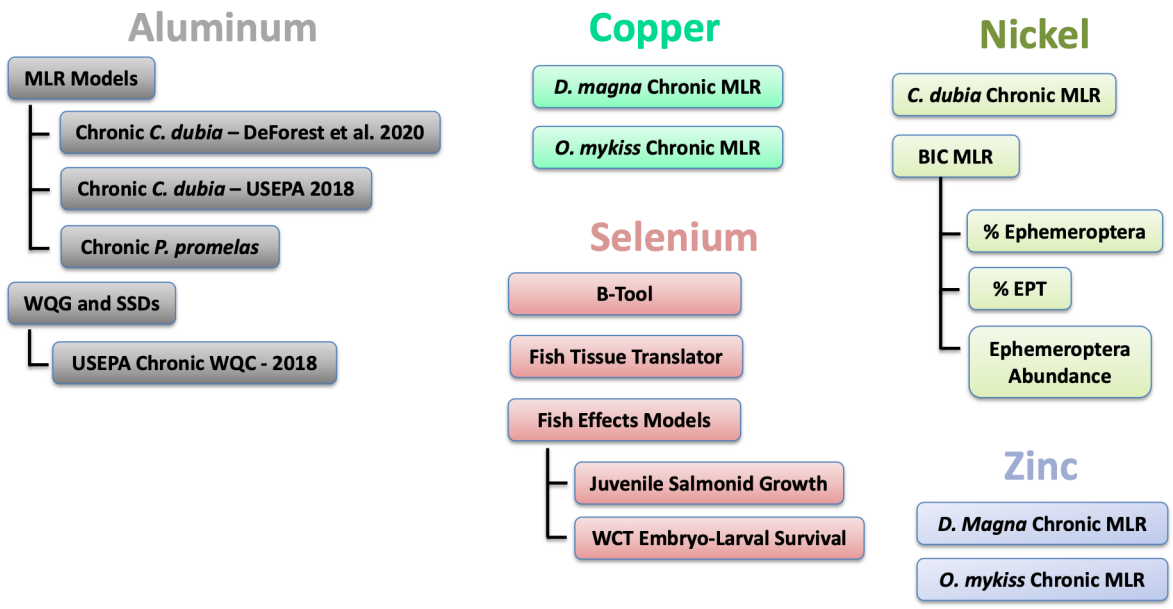


Figure 1. Overview of the EcoTox Toolbox

This manual provides information on access and citation, a Quick Start guide to the common workflow, and stressor-specific instructions, equations, parameterization, and applicability guidance. The Quick Start describes steps shared among tools; the subsequent workflows identify the inputs, outputs, and operating requirements specific to each model.

1.2 Version 1.4 Update Summary

Version 1.4 of the Toolbox includes several updates compared to Version 1.3:

1. Addition of a chronic *C. dubia* Al MLR model and chronic WQC calculator based on USEPA (2018);
2. Improvement to the Se Fish Tissue Translator functionality;
3. Addition of a chronic *O. mykiss* Zn MLR model.

2. ACCESS TO THE TOOLBOX AND CITATION

The EcoTox Toolbox is an R Shiny application that can be freely accessed through the EcoTox LLC website (https://www.ecotoxllc.com). For assistance with access or application functionality, contact KevinBrix@ecotoxllc.com.

If you use the Toolbox to support analyses in reports or peer reviewed publications, please consider citing it:

Brix, K.V. 2026. EcoTox Toolbox, v1.4. EcoTox LLC, Miami, Florida

Where a tool implements a model from the scientific literature, also cite the primary source to acknowledge the scientists who developed that model.

3. QUICK START

3.1 Selecting a Stressor and Tool from the Toolbox

When the Toolbox opens, the Title Page provides citation information. Select Main Page, then use Select Stressor and Select Tool in the sidebar to choose the analysis of interest. Additional controls allow selection of a species, endpoint, or model version where applicable. This section describes the common workflow; the stressor sections provide model-specific operating instructions and technical information.

3.2 Review Model Information

Before entering data, review the Model Information box and application note for the selected model. Confirm that the species, endpoint, exposure metric, and environmental conditions are appropriate for the intended analysis. Check the units beside each input and the meaning of the reported output. An effect concentration, such as an EC_{20}, is a concentration associated with a specified effect and should not be interpreted as a predicted percentage effect. Passing automated input checks does not, by itself, establish that a model is appropriate for a particular site or assessment.

3.3 Enter or Upload Data

For tools with an Input Mode selection, choose Single Sample to enter one set of inputs or Upload Excel File to analyze multiple samples. Enter values in the displayed units. For batch analyses, use the template for the selected tool, review its instructions, and retain the required column names and units. Use Browse to select the completed workbook. Templates and required fields differ among tools and should not be assumed to be interchangeable.

Where available, review Input Summary to confirm the selected file and inputs before running the model. File selection does not mean that predictions have been calculated. Include optional sample identifiers, sites, and dates where supported to identify results and enable the

available plots. The Fish Tissue Translator has additional species and tissue selection steps, described in Section 7.2.1.

3.4 Run the Model and Review Results

After checking the inputs, select Run Model or the corresponding run button for the tool. Review the prediction summary and any validation messages before interpreting the results. Where valid rows from a mixed batch are processed, review the rejected rows and their messages; a missing prediction should not be interpreted as zero effect. Correct the affected inputs and rerun the model where appropriate.

Where a status indicator is provided, a green message indicates that results are current. Changing inputs makes the results stale and requires another run before current results can be exported. Review application limits as well as input validation messages, since automated checks cannot identify every situation in which a model may be unsuitable.

3.5 Inspect Plots and Export Results

Inspect the available plots alongside the tabulated results. For supported batch analyses, the X-axis Selector allows results to be displayed by Date or Site where those fields contain usable values. Plot availability and the quantities displayed differ among tools. A row without the information needed for a selected plot may still have a valid prediction in the results table or workbook.

Use the download control for the selected tool to save current results. Retain accompanying model information, field descriptions, and validation messages where supplied. Where a plot includes the Plotly toolbar, place the cursor over the plot and select the camera icon to save a PNG image. The stressor-specific workflows identify additional steps or output interpretations.

4. ALUMINUM

4.1 Chronic Aluminum MLR Models

The chronic Al MLR models estimate EC_{20}s for *C. dubia* reproduction and *P. promelas* growth while accounting for the effects of hardness, pH, and DOC on Al bioavailability. For *C. dubia*, the Toolbox includes separate models described in DeForest et al. (2020) and USEPA (2018). For *P. promelas*, these sources describe the same model, which is implemented as a single option in the Toolbox. Each model predicts an EC20 expressed as total Al under the specified water chemistry conditions.

4.1.1 Aluminum MLR Models Workflow

Select Aluminum as the stressor and the chronic MLR model of interest. Follow Section 3 for single sample inputs, batch uploads, review of results, and export. Required chemistry

inputs are hardness, pH, and DOC. Confirm the species, endpoint, and model version before running the analysis. The models report an EC_{20} expressed as total aluminum concentration.

4.1.2 Model Development and Parameterization

The chronic MLR models predict EC_{20}s for *C. dubia* reproduction and *Pimephales promelas* growth using hardness, pH, and DOC to account for differences in Al bioavailability. Model development used toxicity data from experiments spanning a range of water chemistry conditions, with EC_{20}, DOC, and hardness expressed as natural logarithms. The models differ in their coefficients and retained terms. In particular, the USEPA (2018) *C. dubia* model includes a squared pH term that is omitted from the recommended DeForest et al. (2020) *C. dubia* model. The *P. promelas* model is identical in both sources. The models have the following general form:

$$\ln(EC_{20}) = a + b\ln(DOC) + c\text{pH} + d\ln(Hard) + e\text{pH} \times \ln(Hard) + f\text{pH}^2 + g\text{pH} \times \ln(DOC)$$

where EC_{20} is the total Al concentration (µg L^{-1}) causing a 20% effect on the species and endpoint of interest, and DOC (mg L^{-1}), pH, and hardness (mg L^{-1} as $CaCO_3$) represent the water quality conditions at which the EC_{20} is being estimated. The model uses natural logarithms of EC_{20}, DOC, and hardness; pH is untransformed, with a squared pH term included where applicable. Inputs and predicted EC_{20}s are displayed as untransformed values. Terms omitted from a particular model have coefficients of zero. The coefficients for each model are provided in Table 1.

Table 1. Parameterization Summary of Chronic Aluminum MLR Models

Parameter	*C. dubia* – DeForest et al. 2020	*C. dubia* – USEPA 2018	*P. promelas*
Intercept	-9.272	-32.523	-7.371
ln DOC	0.619	0.597	2.209
pH	2.021	8.802	2.041
ln Hardness	2.581	2.089	1.862
pH x ln Hardness	-0.307	-0.230	-0.232
pH^2	0	-0.491	0
pH x ln DOC	0	0	-0.261
n	30	32	31
Adj. R^2	0.91	0.88	0.92
Predicted R^2	0.89	NR	0.87

NR = Not reported.

4.1.3 Model Applicability and Limitations

The water chemistry ranges supported the Al MLR models and the application limits enforced by the Toolbox are summarized in Table 2. All Toolbox bounds are inclusive, and predictions are not calculated if any chemistry input is outside the limits of the selected

model. This restriction also applies to the USEPA (2018) *C. dubia* model. Although USEPA permits extrapolation beyond the underlying experimental conditions when deriving water quality criteria (WQC), the accuracy of species-specific EC20 predictions outside model boundaries has not been established. The Toolbox therefore restricts predictions to the specific ranges to avoid extrapolation.

Table 2. Application Limits for Chronic Aluminum MLR Models

Model/Endpoint	Hardness (mg L^{-1} as $CaCO_3$)	pH	DOC (mg L^{-1})
***C. dubia* Reproduction**	10 – 428	6.3 – 8.7	0.1 – 12.3
***P. promelas* Growth**	10 – 422	6.0 – 8.1	0.08 – 11.6

The models predict the specified chronic endpoints for *C. dubia* and *P. promelas*. Predicted EC_{20}s are expressed as total Al; dissolved Al concentrations should not be substituted.

4.2 Chronic Aluminum WQC

The chronic Al WQC calculator implements the freshwater criterion described in USEPA (2018), accounting for the effects of hardness, pH, and DOC on Al bioavailability. The output is the Criterion Continuous Concentration (CCC), expressed in µg L^{-1} total Al. This tool calculates the chronic criterion for the specified water chemistry conditions; it does not calculate an acute criterion.

4.2.1 Chronic Aluminum WQC Calculator Workflow

Select Aluminum as the stressor, WQG and SSD Tools under Select Aluminum Tool, and USEPA 2018 Chronic WQC under Select Aluminum WQG or SSD. Enter a Sample ID, pH, hardness (mg L^{-1} as $CaCO_3$), and DOC (mg L^{-1} as C), then press Calculate. The calculator currently accepts one sample at a time.

Review the calculated criterion, water chemistry inputs, and any warning messages. The Genus Sensitivity Distribution plot shows the normalized genus mean chronic values, the genera used in the criterion calculation, and the calculated criterion. The plotted curve represents the USEPA lower-tail calculation rather than a fit to the full distribution. Press Download Excel Results to save the criterion, supporting toxicity values, plot, and calculation information. The workbook includes the unrounded criterion and any warnings. Changing an input requires another calculation before results can be exported.

4.2.2 Model Development and Parameterization

The calculator uses the toxicity dataset and bioavailability models adopted by USEPA (2018). Toxicity values are normalized to the entered water chemistry, combined into species and genus mean chronic values, and used to derive the chronic criterion following the USEPA procedure (USEPA 1985). The USEPA *C. dubia* MLR model is used for this calculation,

rather than the DeForest et al. (2020) alternative described in Section 4.1. Refer to USEPA (2018) for the underlying data, model parameterization, and criterion derivation.

The criterion is reported to two significant figures, consistent with the USEPA calculator. USEPA (2018) expresses the chronic criterion as a four-day average total Al concentration that should not be exceeded more than once every three years on average.

4.2.3 Model Applicability and Limitations

The Toolbox accepts pH from 5 to 10.5, hardness from 0.01 to 430 mg L^{-1} as $CaCO_3$, and DOC from 0.08 to 12 mg L^{-1} as C. All bounds are inclusive. Inputs outside these ranges are rejected rather than replaced with boundary values, as occurs for some inputs in the USEPA workbook.

These application ranges extend beyond the water chemistry conditions used to develop the underlying MLR models. Unlike the species-specific predictions in Section 4.1, the WQC calculator permits extrapolation within the USEPA application ranges. A warning is provided when the entered chemistry is outside an underlying MLR model's calibration range and is retained in the exported results. These estimates are more uncertain and should be considered carefully when applying the criterion (USEPA 2018). The criterion is expressed as total Al; dissolved Al concentrations should not be substituted.

5. COPPER

5.1 Chronic Copper MLR Models

The chronic Cu MLR models estimate EC_{20}s for *D. magna* reproduction and *O. mykiss* survival and growth while accounting for the effects of hardness, pH, and DOC on metal bioavailability (Brix et al. 2021). The predicted EC_{20} is expressed as dissolved Cu and accounts for the effects of these water chemistry variables on chronic toxicity.

5.1.1 Copper MLR Models Workflow

Select Copper as the stressor and the species or endpoint of interest. The models share the workflow in Section 3. Required chemistry inputs are hardness, pH, and DOC. Results are EC_{20} concentrations expressed as dissolved copper; confirm the selected endpoint before interpreting the output.

5.1.2 Model Development and Parameterization

The chronic MLR models for Cu predict the EC_{20} for *Daphnia magna* reproduction and *Oncorhynchus mykiss* survival and growth while accounting for the effects of hardness, pH, and DOC on Cu bioavailability (Brix et al. 2021). Model development was undertaken using ln-transformed data from multiple experiments. The models have a general form of:

$$\ln(EC20) = a + b \times \ln(DOC) + c \times pH + d \times \ln(Hard)$$

where, EC_{20} is the dissolved Cu concentration (µg L^{-1}) causing a 20% effect on the species and endpoint of interest, and DOC (mg L^{-1}), pH, and hardness (mg L^{-1} as $CaCO_3$) represent the water quality conditions at which the EC_{20} is being estimated. The EC_{20}, DOC, and hardness are ln-transformed in the model but both inputs and outputs are provided as untransformed data. The parameterization for each model is summarized below (Table 3).

Table 3. Parameterization Summary Chronic Copper MLR Models

Parameter	*D. magna*	*O. mykiss*
Intercept	0.372	-0.064
ln DOC	0.851	0.882
pH	0.175	0.284
ln Hardness	0.198	0.284
n	74	17
Adj. R^2	0.87	0.64
Predicted R^2	0.86	0.53

5.1.3 Model Applicability and Limitations

The water chemistry ranges used to develop the species-specific Cu models and the limits applied by the Toolbox are summarized in Table 4. All Toolbox bounds are inclusive. Predictions are not calculated if any chemistry input is outside its applicable range.

Table 4. Application Limits for Chronic Copper MLR Models

Model/Endpoint	Hardness (mg L^{-1} as $CaCO_3$)	pH	DOC (mg L^{-1})
D. magna Reproduction	10 – 500	5.6 – 8.6	0.2–21.6
O. mykiss Survival/Growth	11-320	5.1-8.6	0.4–11

The models describe *D. magna* reproduction and *O. mykiss* survival and growth and should be interpreted using dissolved Cu. The two species have different chemistry domains; satisfying the limits for one model does not establish applicability of the other.

6. NICKEL

6.1 Nickel Multiple Linear Regression Models

The Ni MLR models are based on studies reported in Van Geest et al. (2025) and de Bruyn et al. (2025). The models use a two-step approach. First, a laboratory derived MLR accounts for the effects of hardness, DOC, and bicarbonate on chronic Ni toxicity to *Ceriodaphnia dubia*. The predicted effect on *C. dubia* reproduction is then used as the exposure variable in field calibrated models describing changes in BIC metrics. Currently the

metrics available are % Ephemeroptera, % Ephemeroptera, Plecoptera, and Trichoptera (% EPT), and Ephemeroptera abundance. Consequently, the BIC models assume the same response to water chemistry effects as the *C. dubia* models.

6.1.1 Nickel MLR Models Workflow

Select Nickel as the stressor and the *C. dubia* reproduction or benthic invertebrate community endpoint of interest. Follow Section 3 for the common operating steps. Inputs include dissolved nickel, hardness, DOC, and bicarbonate, using the units displayed by the tool. Bicarbonate is a required input and should not be replaced by alkalinity. Review the predicted *C. dubia* effect or the selected community metric, as applicable; these outputs represent different biological responses.

Bicarbonate can be entered directly or estimated from alkalinity, pH and, optionally, temperature. For single sample inputs, select **Estimate from alkalinity** and enter alkalinity (mg L^{-1} as $CaCO_3$) and pH. Temperature (°C) is optional with 25 °C used as a default if no value is provided. The estimated bicarbonate concentration is displayed in the sidebar and used in the selected Ni model. For batch uploads, each sample may contain either a supplied bicarbonate concentration or alkalinity, pH, and optionally temperature for estimation. Supplied bicarbonate takes precedence over alkalinity and pH if both are provided. The calculation and its assumptions are described in Section 6.1.2.

Once data are entered or uploaded, press **Run Model** and review the **Model Outputs** table for the selected endpoint.

6.1.2 Estimating Bicarbonate Concentrations from Alkalinity, pH, and Temperature

The Toolbox estimates bicarbonate from measured alkalinity, pH, and optionally temperature using the equilibrium relationships described by USGS (2012). The calculation accounts for the contributions of bicarbonate, carbonate, hydroxide, and hydrogen ions to alkalinity. Is assume dilute freshwater conditions, with activity coefficients equal to one and negligible contributions from other acid-base constituents. Under these assumptions, bicarbonate concentration is calculated as:

$$[HCO_3^-] = \frac{A - \frac{K_w}{H} + H}{1 + \frac{2K_2}{H}}$$

where $[HCO_3^-]$ is the bicarbonate concentration in mol L^{-1}, (A) is alkalinity in equivalents L^{-1}, $H = 10^{-pH}$, K_w is the water dissociation constant, and K_2 is the second disassociation constant of carbonic acid. Alkalinity reported in mg L^{-1} as $CaCO_3$ is divided by 50,044 to obtain (A). The calculated bicarbonate concentration is multiplied by 61,071.1 to obtain mg L^{-1} as HCO_3^- (USGS 2012).

Temperature affects both dissociation constants. The Toolbox calculates these constants using the following relationship and coefficients obtain from the USGS Alkalinity Calculator:

$$log_{10}K = a_1 + a_2T + \frac{a_3}{T} + a_4log_{10}T + \frac{a_5}{T^2}$$

where T is temperature in Kelvin and model coefficients are provided in Table 5.

Table 5. Summary of Bicarbonate Calculator Constants for Temperature

Constant	a_1	a_2	a_3	a_4	a_5
K_w	-283.9710	-0.05069842	13,323.0	102.24447	-1,119,669
K_2	-107.8871	-0.03252849	5,151.79	38.92561	-563,713.9

Temperature is optional; the Tooblox uses 25 °C when no value is provided and accepts temperatures from 0 – 50 °C.

The estimates do not correct for ionic strength or account for alkalinity contributed by compounds such as phosphate, silicate, ammonia, or organic acids. These assumptions may reduce accuracy in waters where such contributions or activity corrections are significant. Estimated bicarbonate is subject to the same Ni model applicability range as supplied bicarbonate: 8 – 366 mg L^{-1} as HCO_3^-.

6.1.3 *Ceriodaphnia dubia* MLR Development and Parameterization

The *C. dubia* MLR is based on compiled chronic data from previous studies and supplemental testing with Ni-spiked site and formulated waters. The combined data set includes 101 EC50s for *C. dubia* reproduction and covers a broad range of water chemistry conditions. The model predicts the dissolved Ni EC50 for *C. dubia* reproduction as:

$$log_{10}(EC50) = \beta_0 + \beta_H log_{10}(H) + \beta_B log_{10}(B) + \beta_{DOC} log_{10}(DOC)$$

where (EC50) is expressed in µg L^{-1} dissolved Ni, (H) is hardness in mg L^{-1} as CaCO3, (B) is bicarbonate in mg L^{-1}, and (DOC) is dissolved organic carbon in mg L^{-1}. Model coefficients are summarized in Table 6.

Table 6. Summary of *C. dubia* Bicarbonate MLR and Response Model Parameters

Parameter	Estimate
β_0	1.124
β_H	0.411
β_B	-0.520
β_{DOC}	0.547

A concentration-response model converts predicted EC50 into an EC_x using a mean slope of 2.05 (95% CI = 1.79 – 2.31) from all site-specific experiments. This allows for calculation of the dissolved Ni concentration associated with a specific ECx:

$$EC_x = 10^{\left[log_{10}(EC50) - \frac{log_{10}\left(\frac{1}{x}-1\right)}{s}\right]}$$

or an EC_x associated with a specified dissolved Ni concentration:

$$\%\ Effect = 100\left[\frac{1}{1+10^{s[log_{10}(EC50)\ -\ log_{10}(Ni)]}}\right]$$

6.1.4 BIC Models Development and Parameterization

The BIC models were derived by applying the *C. dubia* MLR model to paired water quality and BIC monitoring data from Elk Valley, British Columbia. The data set was screened to remove sampling locations/events with significant effects from other mine-associated stressors. The resulting data set contains 434 sampling events from 77 mine-influenced and 43 reference locations. Dissolved Ni concentrations ranged from <0.5 µg L^{-1} to 30 µg L^{-1}, corresponding to predicted effects of up to 92% on *C. dubia* reproduction.

BIC responses were evaluated using limiting factor analysis. This approach recognizes that many environmental factors can reduce BIC metrics at low Ni exposure, producing substantial variation in observed values. If Ni becomes limiting as exposure increases, the upper bound of the response distribution should decline even when variation caused by other factors remains. Quantile regression was therefore used to characterize changes in the upper portion of the response distribution rather than changes in its mean or median.

Logistic curves were fit to quantiles 0.80–0.95 in increments of 0.01:

$$y_q(x) = \frac{Asym_q}{1 + exp\left(\frac{Mid_q - x}{Scal_q}\right)}$$

where, $y_q(x)$ is the fitted value of the BIC metric at quantile (q), (x) is the predicted effect of Ni on *C. dubia* reproduction, $Asym_q$ is the upper asymptote, Mid_q is the value of (x) at the inflection point, and $Scal_q$ is the scale parameter. Negative values of $Scal_q$ describe a declining BIC response with increasing predicted Ni toxicity.

Models were considered credible when the fitted parameters were reasonably consistent or varied monotonically among adjacent quantiles. Credible models were obtained for all 16 tested quantiles for percent Ephemeroptera, for 14 quantiles for Ephemeroptera abundance (all but q89 and q90), and for only 6 quantiles (q80–q85) for %EPT. Parameter ranges for the curves retained in the Toolbox are summarized in Table 7.

Table 7. Summary of BIC Model Parameters

BIC Metric	Credible Quantiles	Asym Range	Mid Range	Scal Range
% Ephemeroptera	q80 – q95	0.632 – 0.828	55.0 – 67.0	-34.3 – -19.7
% EPT	q80 – q85	89.5 – 94.9	82.8 – 91.3	-30.6 – -19.9
Ephemeroptera Abundance	q80 – q88, q91 – q95	3.778 – 4.057	108 – 134	-19.3 – -9.2

For each credible quantile, the model calculates the relative reduction in the fitted BIC response as it relates to the predicted effect on *C. dubia* reproduction:

$$Effect_q(x) = 100\left[1 - \frac{y_q(x)}{y_q(0)}\right]$$

where, $Effect_q(x)$ is the percent reduction in the fitted upper quantile response. The model summarizes these responses as the arithmetic mean and sample standard deviation. This output is interpreted as the mean modeled reduction in the upper quantile BIC response. Representative effects thresholds for the available BIC endpoints expressed as predicted effects on *C. dubia* reproduction are provided in Table 8.

Table 8. Summary of BIC Effect Thresholds Expressed as Percent Effect on *C. dubia* Reproduction (mean ± SD)

BIC Metric	EC10 (%)	EC20 (%)	EC50 (%)
% Ephemeroptera	21.8 ± 3.6	35.8 ± 3.3	67.4 ± 1.9
% EPT	40.1 ± 2.1	56.3 ± 0.9	86.7 ± 3.8
Ephemeroptera Abundance	56.4 ± 5.8	66.7 ± 4.2	83.0 ± 3.0

6.1.5 Model Applicability and Limitations

The water chemistry ranges used to develop the species-specific Ni models and the limits applied by the Toolbox are summarized in Table 9. All Toolbox bounds are inclusive. Predictions are not calculated if any chemistry input is outside its applicable range. The Ni BIC models share the water chemistry limits of the *C. dubia* MLR, with an additional exposure limit for the BIC models (Table 9). In add to these application limits for toxicity modifying factors, the BIC models are based on quantile regressions extended to a maximum predicted effect of 92% on *C. dubia* reproduction. The Toolbox will therefore not calculate BIC effects for dissolved Ni concentrations predicted to exceed a 92% effect on *C. dubia* reproduction. This do not mean that BIC effects do not extend beyond this range.

Table 9. Application Limits for Chronic Nickel MLR Models

Model/Endpoint	Hardness (mg L^{-1} as $CaCO_3$)	Bicarbonate (mg L^{-1})	DOC (mg L^{-1})
C. dubia Reproduction	15 – 1,020	8 – 366	0.4 – 40
BIC Models	15 – 1,020	8 – 366	0.4 – 40

The applicability of these models to other watersheds has not been tested but is expected to be reasonably predictive for first through fourth order streams in temperate zones at a similar elevation (~1,300 – 2,300 m).

7. SELENIUM

7.1 B-Tool

The B-Tool predicts Se concentrations in benthic invertebrates from water Se speciation and sulphate concentrations. It represents steady-state bioaccumulation in streams and provides an estimate of Se exposure in a food source for fish and other consumers.

7.1.1 B-Tool Workflow

Select Selenium as the stressor and B-Tool as the tool. Follow Section 3 for entering or uploading data, running the model, and exporting results. The B-Tool uses water selenium speciation and sulphate concentrations to predict benthic invertebrate selenium concentrations. Review the required selenium species and their units before running the model; total water selenium alone does not provide the speciation inputs.

7.1.2 Model Development and Parameterization

The B-Tool is a steady-state model based on a large dataset of paired analyses of water Se speciation and composite macroinvertebrate samples. The data were collected in the Elk Valley drainage of British Columbia that has elevated selenium concentrations associated with coal mining operations. The model also considers the inhibitory effects of sulphate on selenate uptake (Williams et al. 1994, Lo et al. 2015, Ponton et al. 2018). In taking this simple one-step approach and bypassing periphyton (Se concentrations in macroinvertebrates implicitly account for Se uptake by periphyton), the model eliminates much of the uncertainty associated with sampling this intermediate compartment in the food chain (Adams et al. 1998). Heterogeneity in Se speciation in water in the dataset facilitated a sequential analysis of subsets of the data to model the individual contribution of each Se species to observed invertebrate Se (de Bruyn et al. 2025). The general model form is:

$$Inv_{Se} = [SeVI]\left(a_{[SeVI]}[SeVI]^{b_{(SeVI)}}[SO_4]^{b_{SO_4}}\right) + [Se(IV)]\left(a_{[SeIV]}[Se(IV)]^{b_{Se(IV)}}\right) + [DMSeO](a_{DMSeO}) + [MeSeIV](a_{MeSeIV})$$

where, Inv_{Se} is the invertebrate Se concentration (mg kg^{-1} dw), each of the Se species are in expressed in µg L^{-1} and SO_4 is in mg L^{-1}.

The B-Tool parameterization was derived in de Bruyn et al. (2025a)(2025) (Table 10).

Table 10. Summary of B-Tool Model Parameters

Se Species	Parameter	de Bruyn et al. 2025a
Selenate	$a_{Se(VI)}$	6.56
	$b_{Se(VI)}$	-0.936
	b_{SO4}	-0.052
Selenite	$a_{Se(IV)}$	3.16
	$b_{Se(IV)}$	-0.373

DMSeO	a_{DMSeO}	13.1
MeSeIV	$a_{MeSe(IV)}$	104

7.1.3 Model Applicability and Limitations

The B-Tool is a steady-state model developed from stream data in the Elk Valley drainage. It is intended for lotic systems and should not be applied to lentic systems. It predicts invertebrate selenium concentration rather than a biological effect.

7.2 Fish Tissue Translator

The Selenium Fish Tissue Translator Tool allows you to estimate Se concentrations in one fish tissue using data from another tissue (e.g., estimate ROE (Ripe Ovary/Egg) Se concentrations from muscle Se data). Relationships between these tissues are species-specific and so only species-specific models are available to make these estimates.

7.2.1 Fish Tissue Translator Workflow

Most models are based on a total least squares (TLS) regression between the two tissues of interest using log-transformed data. For mass spawners, ripe ovary and egg are assumed to have equivalent Se concentrations are pooled for modeling. Models that include extrapolation to or from ROE are based on carefully curated data sets where there is high confidence the samples are either ripe ovaries or eggs. This is important as unripe ovaries tend to have higher Se concentrations than ripe ovaries or eggs and have the potential to systematically bias models (Brix et al. 2025, Detering et al. 2025). Spawning Capable Ovary (ovary only) and Egg (egg only) are separate tissue categories restricted to fractional spawners in this tool. The model for redside shiner (*Richardsonius balteatus*) uses separate Spawning Capable Ovary and Egg data. Further details on the technical basis for this species-specific model are provided in Section 6.3.2. The data for each model have been loaded into the **Database** and are largely from the dataset described in Detering et al. (2025) with some further refinement. Always confirm tissue identity before uploading data for modelling. **Never use data on unripe ovary or ovary of unknown ripeness in this tool.**

To run the Fish Tissue Translator, select **Selenium** under **Select Stressor** and **Fish Tissue Translator** under **Select Tool** on the **Main Page** UI. Review the Model Information box and Application Note, then select the species you are interested in from the drop-down menu. Select the source tissue (i.e., the tissue for which you have data) and the target tissue (i.e., the tissue for which you want to estimate Se concentrations). Select ROE for mass-spawner ripe ovary or egg data. For fractional spawners (currently only applicable to redside shiner), select Spawning Capable Ovary for ovary data or Egg for egg data, according to the tissue identity and available species-specific relationship. If there are data available for that species and tissue combination, the Toolbox will automatically derive the appropriate TLS or

OLS regression and present a plot with summary statistics. This allows you to evaluate the model and whether it is sufficiently robust for your application.

To upload data for modelling, press **Download Fish Tissue Translator Upload Template** and enter your data on the Data sheet. Use this upload template for predictions; the database import template is provided separately in the Database Interface. Press Browse under Upload source-tissue data and select your file. For an Excel workbook, select the Data sheet. Then press **Run** translator to run the analysis. Output from the model can be accessed by pressing **Download Excel** export, which provides an Excel spreadsheet of the predictions with confidence and prediction intervals.

7.2.2 Model Development and Parameterization

The Fish Translator Tool uses available data from the scientific literature and grey literature reports on Se concentrations in fish eggs, ripe ovaries, muscle, and whole body. Data for unripe ovaries or ovaries of unknown ripeness have been excluded to avoid potential effects of oocyte development on Se concentrations. There is evidence that in many species ovary Se concentrations are inversely related to oocyte development stage (Brix et al. 2025, Detering et al. 2025). Relationships between tissues are generally modeled using total least squares (TLS) regression (Golub and Van Loan 1980). This model structure is selected for most models over ordinary least squares (OLS) regression because from a mechanistic perspective, the Se concentration in one tissue is generally independent or only weakly dependent on the concentration in another tissue. Using log transformed data, the general form for the TLS regression models is:

$$y_{is} = a_s + b_s x_{is} + \varepsilon_{is},$$
$$x_{is} = log_{(10)}(Data_{is}),$$
$$y_{is} = log_{(10)}(Predicted_{is}),$$
$$\varepsilon_{is} \sim \mathcal{N}(0, \sigma_s^2)$$

Where x_{is} and y_{is} are the Se concentrations in the tissue with data and tissue to be predicted, respectively; a_s and b_s denote the species-specific intercept and slope, respectively; and σ represents residual variation around the fitted relationship, used here as a descriptive error term rather than the estimation criterion.

For mass spawners, ripe ovary and egg data have been pooled as ROE on the assumption that they are equivalent representations of egg Se. This is likely to be approximately true for fish that are mass spawners as oocytes within the ovary are all at the same stage of development. However, in fractional spawners, ovaries contain multiple cohorts of oocytes at different stages of development. Consequently, ovaries may overpredict egg Se concentrations because they are dominated by oocytes at earlier stages of development. Redside shiner is the only species with paired spawning capable ovary and egg Se data available, and these data support the hypothesis that spawning capable ovaries will

overpredict egg Se concentrations (de Bruyn et al. 2023, Brix et al. 2025). Consequently, for this species (and likely other fractional spawners once data become available) muscle Se is used to predict egg (only) Se and there is an additional model for predicting Egg Se from Spawning Capable Ovary data. Unlike other tissue models, there is a clear and direct mechanistic link between spawning capable ovary Se and egg Se concentrations. Consequently, the Spawning Capable Ovary to Egg relationship is characterized using a directional OLS rather than TLS regression model. The reverse Egg to Spawning Capable Ovary relationship is not provided. The OLS model structure is the same as the TLS model described above but the fitted relationship minimizes squared deviations in the target tissue rather than orthogonal deviations from the fitted line.

Uncertainty in tissue predictions is characterized using a bootstrap approach following standard resampling methods (Efron and Tibshirani 1993). For each of 5,000 bootstrap iterations, paired calibration observations are sampled with replacement, the applicable TLS or OLS model is refit, and the target tissue concentration is predicted for the user-supplied source tissue concentration.

Two types of uncertainty intervals are reported. The 95% confidence interval characterizes uncertainty in the fitted mean tissue relationship. For each bootstrap iteration, the refitted model is used to estimate the expected target tissue concentration at the specified source tissue concentration. The confidence interval is calculated as the 2.5th and 97.5th percentiles of the resulting bootstrap distribution of fitted mean predictions. The 95% prediction interval characterizes uncertainty for an individual tissue prediction. For each bootstrap iteration, residual variation in $\log_{10}$ target tissue is added to the bootstrap model prediction. Prediction intervals are therefore wider than confidence intervals because they include uncertainty in both the fitted tissue relationship and residual variation around the relationship. Both intervals are calculated in $\log_{10}$ space and then back-transformed, so they are generally asymmetric in normal space.

The available models and summary statistics describing their performance are summarized in Table 11. Note that the R^2 for some models is low and users should carefully consider whether a selected model is sufficiently robust for the intended use.

Table 11. Summary of Fish Tissue Translator

Species	Common Name	Model	N	R^2
Acipenser transmontanus	White Sturgeon	Muscle-ROE	6	0.93
Catostomus catastomus	Longnose Sucker	Muscle-Whole Body	11	0.84
Catostomus discobolus	Bluehead Sucker	Muscle-Whole Body	10	0.95
Catostomus latipinnis	Flannelmouth Sucker	Muscle-Whole Body	12	0.60
Catostomus macrocheilus	Largescale Sucker	Muscle-Whole Body	32	0.57
Coregonus clupeaformis	Lake Whitefish	Muscle-Whole Body	16	0.26
Cyprinus carpio	Common Carp	Muscle-Whole Body	9	0.85
Esox lucius	Northern Pike	Muscle-ROE	14	0.92
Gila robusta	Roundtail Chub	Muscle-Whole Body	10	0.65

Species	Common Name	Model	N	R^2
Ictalurus melas	Black Bullhead	Muscle-Whole Body	14	0.10
Ictalurus punctatus	Channel Catfish	Muscle-Whole Body	8	0.39
Lepomis macrochirus	Bluegill Sunfish	Muscle-ROE	4	0.99
Lota lota	Burbot	Muscle-Whole Body	31	0.66
Oncorhynchus clarkii bouvieri	Yellowstone Cutthroat Trout	Whole Body-ROE	15	0.76
Oncorhynchus clarkii lewisii	Westslope Cutthroat Trout	Muscle-ROE	54	0.91
Oncorhynchus mykiss	Rainbow Trout	Muscle-ROE Muscle-Whole Body	46 51	0.94 0.82
Oncorhynchus nerka	Kokanee, Sockeye Salmon	Muscle-Whole Body*	35	0.08
Perca flavescens	Yellow Perch	Muscle-Whole Body	11	0.75
Prosopium williamsoni	Mountain Whitefish	Muscle-ROE* Muscle-Whole Body*	31 7	0.09 0.11
Ptychochelius oregonenis	Northern Pikeminnow	Muscle-ROE	64	0.48
Richardsonius balteatus	Redside Shiner	Muscle-Egg Spawning Capable Ovary-Egg	56 56	0.89 0.87
Salmo trutta	Brown Trout	Whole Body-ROE	31	0.85
Salvelinus fontinalis	Brook Trout	Muscle-ROE	17	0.88
Salvelinus malma	Dolly Varden	Muscle-ROE Whole Body-ROE Whole Body-Muscle	16 10 10	0.92 0.97 0.81
Stizostedion vitreum	Walleye	Muscle-ROE*	8	<0.01
Thymallus arcticus	Arctic Grayling	Muscle-ROE Whole Body-ROE Whole Body-Muscle	34 34 34	0.76 0.91 0.93
Xyrauchen texanus	Razorback Sucker	Muscle-ROE	34	0.88

* Models considered unsuitable for use. ROE = Ripe Ovary/Egg, used for mass spawners. Spawning Capable Ovary and Egg are separate tissue categories used for fractional spawners.

7.2.3 Model Applicability and Limitations

Use the species and tissue combination represented by the selected relationship and review the fitted data range and any application messages. Low model fit or limited calibration data may restrict suitability for the intended assessment. Confidence intervals describe uncertainty in the fitted mean relationship, whereas prediction intervals also include variation among individual tissue predictions. Neither interval accounts for applying the model to an inappropriate species or tissue type.

7.3 Fish Effects Models

The Fish Effects Models estimate biological responses to Se exposure using relationships derived from toxicity studies. Each model is defined by its exposure metric, species or life stage, and effect endpoint. The Juvenile Salmonid Growth Model estimates growth reduction from dietary Se concentrations. The Westslope Cutthroat Trout (WCT) Embryo-Larval Survival Model estimates survival reduction from egg Se concentrations. The workflow

below describes use of these models; the subsequent subsections provide their technical bases and parameterization.

7.3.1 Fish Effects Models Workflow

Select Selenium as the stressor, Fish Effects Models as the tool, and Juvenile Salmonid Growth or WCT Embryo-Larval Survival as the model. Follow Section 3 for single sample inputs, batch uploads, validation review, and export. Enter dietary selenium for the growth model or egg selenium for the survival model, in mg kg^{-1} dry weight. The growth model accepts finite concentrations greater than or equal to zero; the survival model requires finite concentrations greater than zero.

Both models report percent reduction and the remaining response relative to the control or fitted baseline. Single sample plots show the concentration-response curve, entered concentration, and EC_{10}, EC_{20}, and EC_{50}. Batch plots are available by Site or Date where usable values are supplied; the WCT model also permits plotting against egg selenium concentration. Exported workbooks include Results, Metadata, and Dictionary worksheets.

7.3.2 Juvenile Salmonid Growth Model Development and Parameterization

The Juvenile Salmonid Growth Model estimates growth reduction from dietary Se concentration based on data for Chinook salmon (*Oncorhynchus tshawytscha*) reported by Hamilton et al. (1990) and the analysis of these data by DeForest et al. (1999). The model expresses the response as weight relative to a control and converts this to percent growth reduction. The concentration-response relationship implemented in the Toolbox is:

$$W = \frac{100}{1 + (C \,/\, EC50)^{b}}$$

$$G = 100 - W$$

where W is predicted weight as a percentage of the control, G is predicted growth reduction (%), C is dietary Se concentration (mg kg^{-1} dw), EC50 is the dietary concentration producing a 50% reduction in growth, and b is the unitless slope parameter. The current parameterization uses an EC50 of 30 mg kg^{-1} dw and a slope of 2.19. These parameters are supplied by the model and are not entered by the user.

Effect concentrations are calculated by rearranging the same relationship. The resulting EC_{10}, EC_{20}, and EC_{50} are 11.0, 15.9, and 30.0 mg kg-1 dw, respectively, rounded to one decimal place. These values describe 10%, 20%, and 50% growth reduction relative to the control.

7.3.3 WCT Embryo-Larval Survival Model Development and Parameterization

The WCT Embryo-Larval Survival Model estimates the reduction in survival to swim-up following maternal transfer of Se to eggs. It is based on a single study of Westslope cutthroat

trout reported by Nautilus Environmental and Interior Reforestation Company (2011). The Toolbox implements the logistic relationship adopted in Annex E of the Elk Valley Water Quality Plan (Teck 2014), normalized to express survival relative to the fitted baseline. The concentration-response relationship implemented in the Toolbox is:

$$S = \frac{100}{1 + (C / EC50)^b}$$
$$R = 100 - S$$

where S is predicted relative survival as a percentage of the fitted baseline, R is predicted survival reduction (%), C is egg Se concentration (mg kg^{-1} dw), EC50 is the egg concentration producing a 50% reduction in survival relative to baseline, and b is the unitless slope parameter. The current parameterization uses an EC50 of 33.3 mg kg^{-1} dw and a slope of 7.8.

The original logistic relationship had a fitted upper asymptote of 0.87, representing baseline survival under the study conditions. The Toolbox scales this baseline to 100% so that the predicted effect describes the reduction in survival associated with Se. Relative survival is therefore not an estimate of absolute survival in the laboratory or field.

Effect concentrations are calculated by rearranging the same relationship. The resulting EC10, EC20, and EC50 are 25.1, 27.9, and 33.3 mg kg^{-1} dw, respectively, rounded to one decimal place. These values describe 10%, 20%, and 50% reductions in survival relative to baseline.

7.3.4 Model Applicability and Limitations

Both models are deterministic effects models and do not simulate the time course of selenium uptake, growth, development, or recovery. They do not provide confidence or prediction intervals. Inputs must represent the exposure metric used by the model: dietary Se for juvenile growth and egg Se for embryo-larval survival. Water selenium or other fish tissue concentrations cannot be substituted directly.

The growth model is based on Chinook salmon data, and the survival model is based on Westslope cutthroat trout data. Application to other species or life stages requires consideration of that extrapolation. WCT predictions describe survival to swim-up relative to the fitted baseline, rather than absolute survival, growth, deformities, or population-level effects.

8. ZINC

8.1 Chronic Zinc MLR Model

The chronic Zn MLR models estimates the EC_{20} for *D. magna* reproduction or *O. mykiss* survival/growth while accounting for the effects of water hardness, pH, and DOC on Zn

bioavailability (DeForest et al. 2023). The predicted EC_{20}s are expressed as dissolved Zn and provides a measure of chronic toxicity under the specified water chemistry conditions.

8.1.1 Zinc MLR Model Workflow

Select Zinc as the stressor and the chronic *D. magna* or *O. mykiss* MLR model. Follow Section 3 for single sample inputs, batch uploads, review of results, and export. Required chemistry inputs are hardness, pH, and DOC. The model reports an EC_{20} for reproduction expressed as dissolved zinc concentration.

8.1.2 Model Development and Parameterization

The chronic MLR models for Zn predicts the EC_{20} for *Daphnia magna* reproduction or *O. mykiss* survival/growth while accounting for the effects of hardness, pH, and DOC on Zn bioavailability (DeForest et al. 2023). Model development was undertaken using ln-transformed data from multiple experiments. The models have a general form of:

$$\ln(EC20) = a + b \times \ln(DOC) + c \times pH + d \times \ln(Hard)$$

where, EC_{20} is the dissolved Zn concentration (µg L^{-1}) causing a 20% effect on the species and endpoint of interest, and DOC (mg L^{-1}), pH, and hardness (mg L^{-1} as $CaCO_3$) represent the water quality conditions at which the EC_{20} is being estimated. The EC_{20}, DOC, and hardness are ln-transformed in the model but both inputs and outputs are provided as untransformed data. The parameterization for each model is summarized below (Table 12).

Table 12. Parameterization Summary Chronic Zinc MLR Models

Parameter	*D. magna*	*O. mykiss*
Intercept	4.672	7.627
ln DOC	0.422	0.301
pH	-0.226	-0.884
ln Hardness	0.296	1.093
n	48	14
Adj. R^2	0.30	0.563
Predicted R^2	0.22	0.373

8.1.3 Model Applicability and Limitations

The water chemistry ranges used to develop the Zn model and the limits applied by the Toolbox are summarized in Table 13. All Toolbox bounds are inclusive. Predictions are not calculated if any chemistry input is outside its applicable range.

Table 13. Application Limits for Chronic Zinc MLR Models

Model/Endpoint	Hardness (mg L^{-1} as $CaCO_3$)	pH	DOC (mg L^{-1})
D. magna Reproduction	7 – 608	5.5 – 8.4	2 – 17

The Zn model describes chronic *D. magna* reproduction and should be interpreted using dissolved Zn. The development dataset accounted for DOC contributed by food in chronic daphnid tests, including an assumed DOC concentration of 2 mg L^{-1} where appropriate (DeForest et al. 2023). The Toolbox uses the entered DOC concentration without a food adjustment. The 2 mg L^{-1} lower bound is therefore an application limit based on the model-development dataset, not a biological threshold.

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