

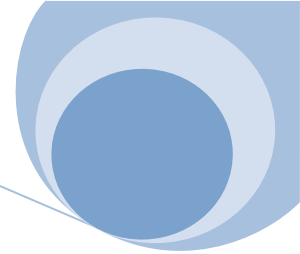


bio-met bioavailability tool

User Guide (version 5.1)

Guidance document on the use of the bio-met
bioavailability tool

**www.bio-met.net
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GLOSSARY

AA	Annual Average.
BioF	The Bioavailability Factor. The BioF is the ratio of the 'Reference HC5' value calculated from water chemistry parameters reflecting high bioavailability ("reference conditions") divided by the 'Local HC5', the value calculated from water chemistry parameters from a local site. Through the use of a BioF, differences in (bio)availability are accounted for by adjustments to the monitoring data, but the EQS-bioavailable remains the same.
BLM	Biotic Ligand Model. This is a predictive tool that can account for variation in metal toxicity and calculates a site-specific Predicted No Effect Concentration (PNEC) using information on the chemistry of local water sources, i.e. pH, dissolved organic carbon, etc.
DOC	Dissolved Organic Carbon.
EQS	An Environmental Quality Standard (EQS) is a regulatory threshold used for assessing the chemical status of waterbodies. The concentration of a chemical can be compared to its EQS in order to determine (non) compliance.
EQS-bioavailable	<p>In bio-met, the EQS-bioavailable of a metal is defined in agreement with the Technical Guidance for Deriving Environmental Quality Standards (CIS guidance nr. 27). The EQS bioavailable is derived from the median HC5, normalized to conditions of high bioavailability.</p> <p>In Europe, the EQS-bioavailable for "Priority Substances" (including Ni and Pb) are derived at a European level and apply to all Member States. Therefore, the EQS-bioavailable of these metals are fixed in bio-met. Individual Member States may develop an EQS-bioavailable for additional substances ("Specific Pollutants"), such as Cu and Zn.</p>
HC5	The HC5 is the 5 th percentile of a species sensitivity distribution. This value therefore protects 95% of the species. The HC5 is commonly used as the scientific basis for setting EQSs. If a full biotic ligand model is available, the HC5 can be normalized to account for the bioavailability conditions (see also local HC5 and reference HC5).
Local HC5	A local HC5 is a bioavailability corrected HC5. This means that the local HC5 takes the bioavailability conditions at a specific site into account.
RCR	<p>Risk Characterisation Ratio, also sometimes called the risk quotient. This is calculated by dividing the bioavailable metal concentration by the EQS-bioavailable.</p> <p>Values equal to or greater than 1 present a potential risk.</p>
Reference HC5	The HC5 under conditions of high bioavailability ("reference condition")
User-friendly BLM Tool	The User-friendly tool mimics the BLM outputs in a precautionary way. It requires relatively few inputs and can readily be used in a compliance assessment framework.

1 INTRODUCTION

1.1 bio-met.net

Metals, because of their unique properties, are critical to many of the technologies that modern society relies on. Metals are naturally present in the aquatic environment, but can also be released to it as a consequence of industrial manufacturing, consumer use and recycling. The risks posed by metals to the aquatic environment are managed in many countries. In Europe, these risks are managed by legislation including REACH¹ and the Water Framework Directive².

Metals present many challenges to those responsible for managing their safety, not least because they are naturally occurring in the freshwater environment. Accounting for the bioavailability of metals in the freshwater environment, using techniques such as the Biotic Ligand Model, resolves many of these difficulties³.

bio-met.net is a free online resource for those interested in using bioavailability-based approaches for assessing the risk of metals in the freshwater aquatic environment, particularly as either specific pollutants or priority substances under the EU Water Framework Directive. bio-met.net is intended as a "one-stop shop" of information, software, and guidance. The bio-met website is currently focussed on the compliance assessment of copper (Cu), nickel (Ni), zinc (Zn), lead (Pb) and cobalt (Co), but may be extended in the future to include other metals as and when the scientific understanding of the factors affecting their bioavailability and effects in the environment becomes sufficiently comprehensive.

bio-met.net resources include:

- The bio-met bioavailability tool⁴. A 'user friendly' software tool, based on Biotic Ligand Models, for calculating the bioavailability of copper, nickel, zinc, lead, and cobalt in different freshwaters. The tool is available as a MS Excel Spreadsheet.
- An evidence base⁵ of information on metal bioavailability and its use in the regulatory risk assessment of metals. This section also contains information on the development and validation of the bio-met bioavailability tool.
- A series of case studies⁶ that demonstrate the application of bioavailability-based approaches within the risk-management of metals in the aquatic environment.

¹ http://ec.europa.eu/environment/chemicals/reach/reach_intro.htm

² http://ec.europa.eu/environment/water/water-framework/index_en.html

³ <https://vimeo.com/user39439554>

⁴ <http://www.bio-met.net/bio-met-bioavailability-tool/>

⁵ <http://www.bio-met.net/evidence-base/>

⁶ <http://www.bio-met.net/case-studies/>

1.2 About bio-met.net

bio-met is a collaborative initiative led by the European Copper Institute⁷, International Zinc Association⁸ and NiPERA⁹ which started in 2009. The tools and resources on bio-met.net have been developed collaboratively by ARCHE¹⁰ and wca environment¹¹. The International Lead Association (ILA) developed a full chronic BLM for lead in 2015 (see <http://www.ila-lead.org/responsibility/lead-blm-tool> for more information) and has been integrated in bio-met soon after. In 2022, the Cobalt Institute (CI) joined the bio-met team, after they developed a full chronic BLM for Cobalt (publication in preparation), which has been integrated into this updated version of the bio-met tool.

Specifically for lead, it may be helpful to note that, it is currently not appropriate to use the bio-met tool (or full lead BLM) to derive bioavailable lead EQS (Environmental Quality Standard) for chemical status classifications under the Water Framework Directive (WFD). For the assessment of site-specific lead EQS under WFD, a screening tool is available for download (<http://www.wca-environment.com/models-and-downloads/Pb-EQS-Screening-Tool>) or the lead EQS can simply be calculated using this equation:

$$EQS_{\text{site}} = EQS_{\text{bioavailable}} + (1.2 \times (DOC_{\text{site}} - DOC_{\text{reference}}))$$

Where:

EQS_{site} = EQS at the site under consideration

$EQS_{\text{bioavailable}}$ = EQS for a reference condition to ensure all water bodies are protected

DOC_{site} = Dissolved Organic Carbon at the site under consideration

$DOC_{\text{reference}}$ = average Dissolved Organic Carbon (DOC) concentration in the ecotoxicity tests that the EQS-bioavailable is based upon = 1.0 mg/L.

1.3 bio-met bioavailability tool

bio-met is a 'user-friendly' bioavailability tool based on the Biotic Ligand Model (BLM) that calculates Bioavailability Factors (BioF) and Local HC5 values for metals based on site-specific information for three water quality parameters: pH, dissolved organic carbon [DOC] concentration and calcium concentration.

The HC5 values are obtained from the 'full' Biotic Ligand Models. If measured, dissolved metal concentrations are provided, the tool will also calculate the corresponding concentration of bioavailable metal and a Risk Characterisation Ratio (RCR). The RCR is calculated from the bioavailable metal concentration and the EQS-bioavailable. Where applicable (e.g., for copper and zinc), the EQS-bioavailable can be adapted by the user.

⁷ <http://www.eurocopper.org/>

⁸ <http://www.zinc.org/>

⁹ <http://www.nipera.org/>

¹⁰ <http://www.arche-consulting.be/>

¹¹ <http://www.wca-environment.com/>

The bio-met bioavailability tool can be downloaded as a Microsoft Excel macro-enabled spreadsheet. Note that macros must be enabled for the tool to operate correctly, and that the tool may not function in older versions of Microsoft Excel. The tool also works under a mac-environment but may give error-messages when running the calculations that do not appear in a Windows-environment. However, the resulting local HC5 predictions are correct.

1.4 Development of the bio-met bioavailability tool

1.4.1 Background

The currently available software tools for undertaking Biotic Ligand Model calculations are data-demanding (more than 10 physico-chemical input parameters are required to run the models) and time-consuming (around one minute per sample/site). For some metals, the available software tools are also insufficiently user-friendly (for example for Ni and Zn BLMs, a combination of WHAM chemical speciation software and species sensitivity distribution [SSD] calculations are currently required). These drawbacks are significant barriers to the regulatory acceptance and implementation of Biotic Ligand Models for routine use in metals risk assessment in Europe and elsewhere.

To address these barriers a 'user-friendly' tool (bio-met bioavailability tool) has been developed as part of the bio-met initiative. The bio-met bioavailability tool estimates site-specific bioavailability based on a limited suite of three input variables (pH, DOC and Ca) based on results obtained from 'Full' Biotic Ligand Models. The tool is also able to process large numbers of samples quickly, does not require the installation of proprietary software, and is fully compatible with the tiered approach to the implementation of EQS_{bioavailable} proposed for the WFD.

bio-met is based on the full chronic Biotic Ligand Models for calculating the bioavailability of metals in different freshwaters. The main differences of bio-met compared to the full BLMs are:

- **Faster** simulations (calculations for many sites take minutes compared to hours in case of full BLMs) at the cost of a small, acceptable decrease in precision compensated by an increase in conservatism.
- **More feasible to implement** with national/regional phys-chem monitoring practice: (less physico-chemical input parameters required (only pH, DOC and Ca), again at the cost of a small, acceptable decrease in precision compensated by an increase in conservatism).
- **More user-friendly** environment (e.g., input of physico-chemistry in the more common mg/L instead mol per litre), software (general user familiarity with MS excel commands and operation allows for more flexibility and ease-of-use compared to a stand-alone package) and output (e.g. excel tables instead of text files), **and automatic processing** for entire ecotoxicity data set / Species Sensitivity Distribution instead of normalising species by species in case of full BLM.

The main advantages of bio-met compared to other user-friendly derivatives of the full BLMs are:

- bio-met is based on the full BLMs **as originally developed by the scientists** and not based on secondary (meta-)sources such as publications and reports.
- bio-met is based on the **latest state-of-the-art ecotoxicity data sets**.
- bio-met is based on a **lookup table algorithm** selecting the best matching full BLM prediction (rather than trying to simulate with multi-linear regression models to mimic non-linear behaviour). See below for more details.
- Finally, it is **validated with independent data sets** from several EU Member States, as demonstrated in this document.

1.4.2 Principle

The basic approach behind the bio-met bioavailability tool is a large database of more than 20,000 different combinations of key input parameters (pH, Dissolved Organic Carbon [DOC] and Calcium [Ca] concentrations) and corresponding HC5 (Hazardous Concentration at 5% assuming a lognormal Species Sensitivity Distribution) calculations for various metals, using their respective BLM. The database then serves as a lookup table. The physico-chemistry of a site of interest is compared to the physico-chemistry of existing simulations in the lookup table. The minimum HC5 of the two “best-matching” lookup table entries is selected as local HC5.

1.4.3 Description of the look-up table

1.4.3.1 Development of the look-up table

In the development of the bio-met bioavailability tool the following steps were taken to construct the look-up table:

1) Of all required input parameters, the key parameters driving the HC5 calculation were identified by means of a combination of sensitivity analysis and expert judgment. The average outcome across Ni, Cu, Pb, Zn, and Co was that:

- pH, DOC and Ca (or hardness) have moderate to major impact on HC5 estimation.
- Magnesium [Mg], Sodium [Na], alkalinity, Dissolved Inorganic Carbon [DIC], iron [Fe] and aluminium [Al] have low to moderate impact on HC5 estimation (depending on the metal of concern) but can be reasonably accurately calculated from Ca or pH.
- Temperature, potassium [K], sulphate [SO₄] and chloride [Cl] have negligible to low impact on HC5 estimation.

DOC, pH and Ca were therefore selected as key input parameters. For the purposes of lookup table database construction, small incremental steps were taken for pH (0.125 pH units from pH=6 to pH=8.5) and DOC (at least 30 concentrations, encompassing the range of 0.1-30 mg

DOC/L with an interval based on the distribution of the DOC concentrations in the FOREGS database). The following values for Ca were selected: 14.125, 40 and 80. For Zn, also low competition scenarios were selected with Ca 1 and 5 mg/L.

2) The next step was to select and/or derive the relationships to calculate the low to moderate impact parameters¹²:

Relationship	Reference
$Mg \text{ (mg/L)} = 10^{(0.6113 * \log_{10}(Ca) - 0.2754)}$	Peters et al. 2011
$Na \text{ (mg/L)} = 10^{(0.7068 * \log_{10}(Ca) + 0.1029)}$	Peters et al. 2011
Alkalinity (mg CaCO ₃ /L) = $10^{(1.0439 * \log_{10}(Ca) + 0.1881)}$ (for Zn)	Peters et al. 2011
Alkalinity = $10^{(1.0665 * pH - 6.5978)}$ (for Cu, Ni)	Calculated from De Schamphelaere & Janssen, 2004
Fe and Al are calculated based on speciation (for Ni only)	De Schamphelaere et al., 2006

The negligible to low impact parameters were set at following reasonable worst-case values: temperature = 5°C, K = 25 mg/L, SO₄ = 100 mg/L, Cl = 160 mg/L.

¹² For Co, a slightly different approach of calculating the low to moderate impact parameters have been taken. These parameters have been calculated based on log-linear relationships with Ca or hardness which were derived from the geochemical monitoring database of the FOREGS database (Salminen et al. 2007). Alignment of relationships to derive physico-chemistry parameters that have a low to moderate impact on the local HC5 calculation between all metals is foreseen when updating the bio-met look-up tables to include latest updates in EQS derivations and/or effects assessments, which are currently (June 2022) under review for all metals included in bio-met.

Relationship used for Co (hardness and alkalinity in mg CaCO ₃ /L, all others in mg/L)
$\ln(Mg) = -0.943 + 0.795 * \ln(Ca)$
$\ln(Na) = -0.674 + 0.571 * \ln(Hardness)$
$\ln(K) = -2.48 + 0.639 * \ln(Hardness)$
$\ln(SO_4) = -1.466 + 0.928 * \ln(Hardness)$
$\ln(Cl) = -1.308 + 0.753 * \ln(Hardness)$
$\ln(Alkalinity) = 0.151 + 0.938 * \ln(Hardness)$

3) More than 20,000 simulations of different combinations of pH, DOC and Ca were simulated to calculate HC5 for each metal using the full BLM software. This is often referred to as the bio-met database.

Metal	Software reference
Zn	Hydroqual BLM version 2.12 and ARCHE semi-automatic spreadsheet processing script version 0011gBAM
Cu	Hydroqual BLM version 2.12 and ARCHE automatic spreadsheet processing script version 1.3
Ni	WHAM version 6.0.10 and ARCHE semi-automatic spreadsheet processing script (Nys et al. 2016a).
Pb	Lead BLM SSD normalisation version 1.0 (2013), developed by ARCHE, UGent and KTH (http://www.ila-lead.org/responsibility/lead-blm-tool ; Van Sprang et al. 2016).
Co	Windward Chronic Co BLM version 3.13.2.40 using the chronic Co database of June 2021

4) The application range of the bio-met tool for each of the metals has been defined based on the application ranges of the full BLMs. The following table summarizes the physicochemical application range of the full BLM models (see Annex 2).

Metal	pH	Ca (mg/L)
Zn	5.5-8.5	5.0-160
Ni¹	6.5-8.2	2.0-88
Cu	6.0-8.5	3.1-129 ²
Pb	6.3-8.4	3.6-204
Co	6.4-8.4	8.0-100 ³

1. While the pH boundaries of the full Ni BLM have been extended to pH 8.7 (Nys et al. 2016a) and the Ca boundaries to lower Ca concentrations (0.5 mg/L; Peters et al. 2018), this research and its impact on the Ni EQS have not been discussed at the EU regulatory level. Since Ni is a Priority Substance, regulatory discussions are required before such changes can be made. Therefore, bio-met retains the previously established limits for pH and Ca.
2. Please note that in previous versions of bio-met, the upper Ca validation limit of the Cu BLMs was erroneously set at 93 mg Ca/L, while the models have been validated up to 129 mg Ca/L. The related out of boundary-flag was corrected in the updated bio-met tool.
3. Please note that the calibration and validation ranges for Ca of the Co full BLM are wider (Ca=4.0-147 mg/L) than the bio-met application range. The bio-met Ca application range for Co has been determined based on the Ca concentrations present in the look-up table (Ca 8, 14, 40 and 80 mg/L). Applying bio-met outside the defined application ranges may result in non-protective local HC5 values for some scenarios.

When the user inputs a value for pH or Ca outside its validated range, an out of boundary flag is given and a prediction using the best-fitting combination of validated pH, DOC and Ca values is returned.

5) Reference HC5 values have been established for each of the metals based on HC5 values under conditions of reasonable maximised (worst-case) bioavailability. For priority substances under the WFD (Ni and Pb), the reference HC5 has been set equal to the EU wide harmonised EQS-bioavailable derived under the WFD as priority substances. These values are not user-editable.

For specific pollutants under the WFD (Zn and Cu), the reference HC5 in bio-met has been set to 1 µg/L for Cu and 10.9 µg/L for Zn. For Cu and Zn, this value reflects “worst-case” conditions of maximised bioavailability: i.e. even in the EU Member State with the most sensitive water conditions, more than 95% of the waters had a local HC5 above this reference HC5 value. For Cu and Zn, these values are therefore also used as default EQS-bioavailable in bio-met. However, some Member States have derived other EQS-bioavailable values for Zn or Cu as Specific Pollutants under the WFD. The user can amend the EQS-bioavailable of specific pollutants in bio-met.

Metal	EQS-bioavailable (µg/L)
Cu	1*
Ni	4
Zn	10.9*
Pb	1.2
Co	1.6*

A '*' denotes the EQS-bioavailable for specific pollutants; these values can be adjusted if necessary. Ni and Pb are priority substances, these values are fixed under the WFD

1.4.3.2 What is new in bio-met version 5.1?

The only update in bio-met version 5.1 (released in 2022) is the addition of Co into the bio-met tool. All other features available in bio-met versions 5.0 remain unchanged. The most important update of bio-met version 5.0 (released in 2020) compared to bio-met version 4.0 was the enlargement of the look-up table. The same full BLM normalisation procedure as described in section 1.4.3.1 has been used to calculate HC5s for this extension. The look-up table has been enlarged on two levels:

- A) Increased DOC resolution for all metals: The original DOC resolution of the bio-met look-up table contained 20 DOC concentrations that were based on a logarithmic distribution; this has been revised to increase the number of DOC concentrations to at least 30 values, ranging between 0.63 and 29.4 mg/L for every metal. The DOC concentrations for the extension of the DOC resolution were selected based on the DOC distribution of the FOREGS database (see Figure below). The FOREGS-EuroGeoSurveys Geochemical Baseline Database represents a set of water chemistry conditions of baseline surface waters across Europe (784 entries). For every metal, additional inputs were added to the look-up table with a resolution of every 2nd percentile of the FOREGS distribution up to the 60th percentile and for every percentile

of the FOREGS distribution above the 60th percentile (open circles in Figure below). Furthermore, the applicability range of the bio-met tool v5 for DOC has been set at 0.1-30 mg/L. This range covers the <1% to 97% percentile of the DOC concentrations within the DOC distributions in the FOREGS database. When the user inputs a value for DOC outside this range, an out of boundary flag is given and a prediction using the best-fitting combination within the applicability range of the bio-met tool for DOC is returned.

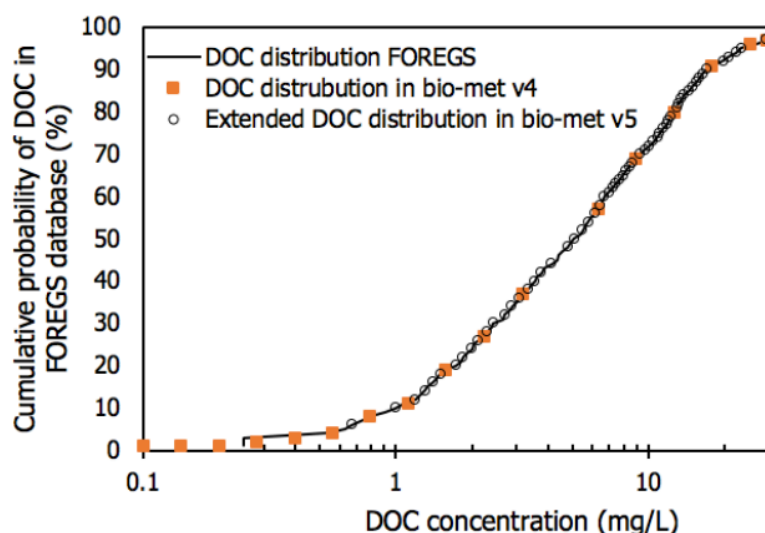
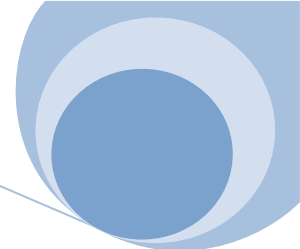


Figure 1. Overview of DOC concentrations used to extend the DOC resolution of the look-up table in bio-met v 5 (open circles) vs the DOC distribution in bio-met v4 (filled squares). The DOC distribution of the FOREGS database is plotted for reference.

- B) Addition of low competition waters for Ni and Cu: For Ni and Cu, the look-up table has also been enlarged to include more waters characterised with low ionic competition (i.e. low Ca and/or Na). For Ni, a Ca concentration of 2 mg/L was selected for this purpose, while for Cu a combination of 3 mg Ca/L and 3 mg Na/L was used (i.e. the lower validation boundary). The addition of the low competition waters increases the prediction performance of the most sensitive waters.

In addition, bio-met v5 also contains the following minor updates regarding the output of the bioavailability tool: I) the calculation of the risk characterization ratio (RCR) for Zn (removal of negative RCR's), II) local HC5 derivation for Ni (when the bioavailable Ni HC5 is lower than the $EQS_{bioavailable}$ the latter will be reported in the output table) and III), the upper Ca boundary for the Cu validation range has been corrected to 129 mg Ca/L, while the upper pH boundary for Ni validation range has been set at pH 8.2. The option to include Ambient Background Concentrations (ABC) for Zn in the bio-met calculations has been removed, as this calculation was not in line with the most recent guidance of WFD (WG Chemicals, 2021). How to deal with Ambient Background Concentrations in deriving local HC5 for Zn is discussed in Section 2.6.



1.4.3.3 Prediction performance of the bio-met v5 tool

The use of BLM predictions for regulatory purposes was underpinned by an acceptance that a factor of two between BLM predicted toxicity and observed toxicity in laboratory testing was acceptable (see Annex 1 for original BLM validation graphs for *Daphnia magna* and *Ceriodaphnia dubia* describing the performance of the full invertebrate BLMs versus the observed toxicity in field sample validation tests). Equally, this same factor can be used to validate the performance of the bio-met bioavailability tool relative to full BLM predictions. A high proportion of bio-met BLM predictions should therefore lie between a factor of two from the full BLM predictions. In order to facilitate a judgement upon the performance of bio-met, it is important to establish a set of criteria on which that judgement is to be made prior to commencement of an analysis. Therefore, the performance of the user-friendly tools is to be based upon the accuracy/precision of the predictions relative to the full BLM predictions using following criteria:

1. Predictions should largely (>95%) be within a factor of two (as used during BLM developments and assessment of ecotoxicity data, see supporting Annex A); or differently expressed, there should be less than 5% of “false positives” and “false negatives” (within the applicability domain of the full BLMs).
2. bio-met predictions are preferably on the conservative side; i.e. >50% of the acceptable predictions should be precautionary (within the applicability domain of the full BLMs).

Figure 2 below provides a pictorial representation of the assessment criteria with six zones clearly delineated and described on the right-hand portion of the figure. To achieve a perfect correspondence between the BLM and user-friendly tools, all data points should be on the 1:1 diagonal line on an x/y scatter plot of full BLM prediction HC5 prediction vs bio-met bioavailability tool HC5 prediction. Many points above the 1:1 line would suggest that the bio-met bioavailability tool was less-conservative than the full BLM. Many points below the 1:1 line would suggest that the bio-met bioavailability tool was more precautionary than the full BLM. Predictions of HC5 by the bio-met bioavailability tool greater than a factor of two above HC5 predictions by the full BLM can be considered as potential false negatives, as application of these values during compliance / risk assessment may not be protective of aquatic communities.

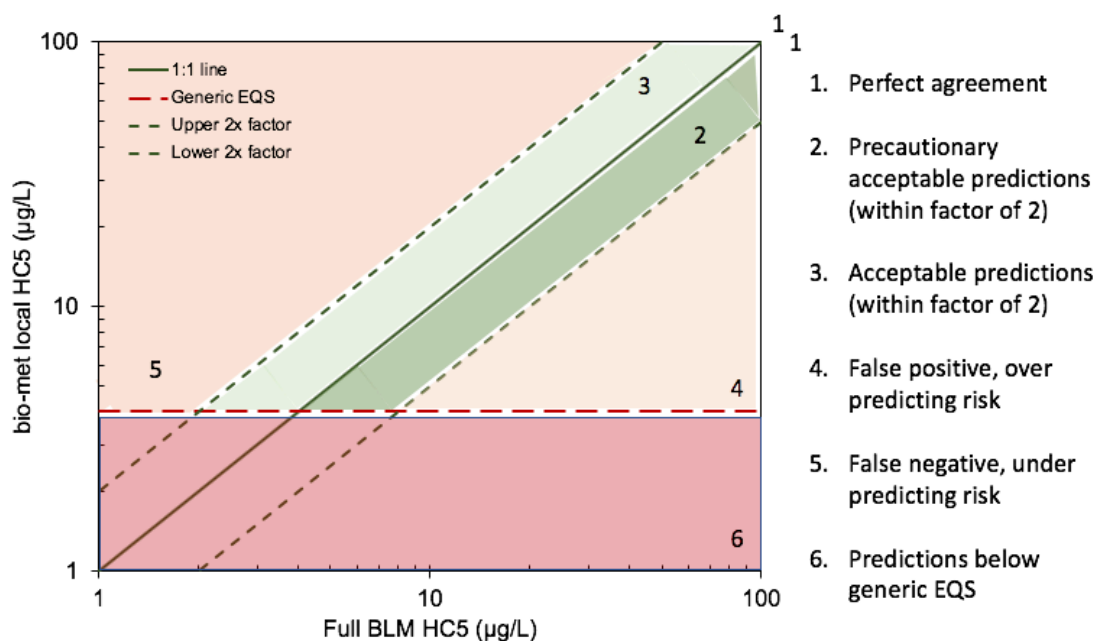


Figure 2 Pictorial representation of the criteria by which performance of the user-friendly tools is assessed.

To evaluate the performance of the bio-met v5-bioavailability tool against the full BLMs, monitoring data from the FOREGS database were used to prepare a composite “validation dataset” from which estimates of HC5 using the ‘full’ BLM/WHAM models and the bio-met v5 bioavailability tool could be compared statistically. The FOREGS-EuroGeoSurveys Geochemical Baseline Database represents a set of water chemistry conditions of baseline surface waters across Europe (784 entries; Salminen et al. 2005). The number of data points available for comparisons for each metal was variable and was dependent on the validation range of each of the respective full BLMs as only surface waters within the validated ranges of the full BLMs were included in the validation dataset. For all metals, at least 94% of the bio-met predictions were within a factor of 2 of the Full BLM predictions (Table 1). For those metals for which acceptable predictions were less than 100% (i.e. Cu and Ni), the local HC5s predicted with more than 2-fold error were conservative estimates, i.e. more than 70% of the bio-met local HC5 < full BLM simulated HC5. Hence, bio-met v5 results in sufficiently accurate predictions of local EQS compared to the full BLM simulated HC5.

Table 1: Performance of bio-met assessed against performance criteria

	Cu	Ni	Zn	Pb	Co
Number of waters within BLM application ranges	608	460	578	594	361
Acceptable predictions (% within factor 2)	94%	99%	100%	100%	100%
Conservatism (% of total predictions)	79%	71%	43%	46%	70%
Median prediction error	1.17	1.09	1.05	1.10	1.03

Figure 3 below presents the performance of bio-met versus the full BLM tools for all metals. There is a good match for all data sets between the full BLM prediction and the user-friendly bio-met tool. A more detailed study on the validation of bio-met against natural field waters is currently in preparation and will be made available on the bio-met-website once published.

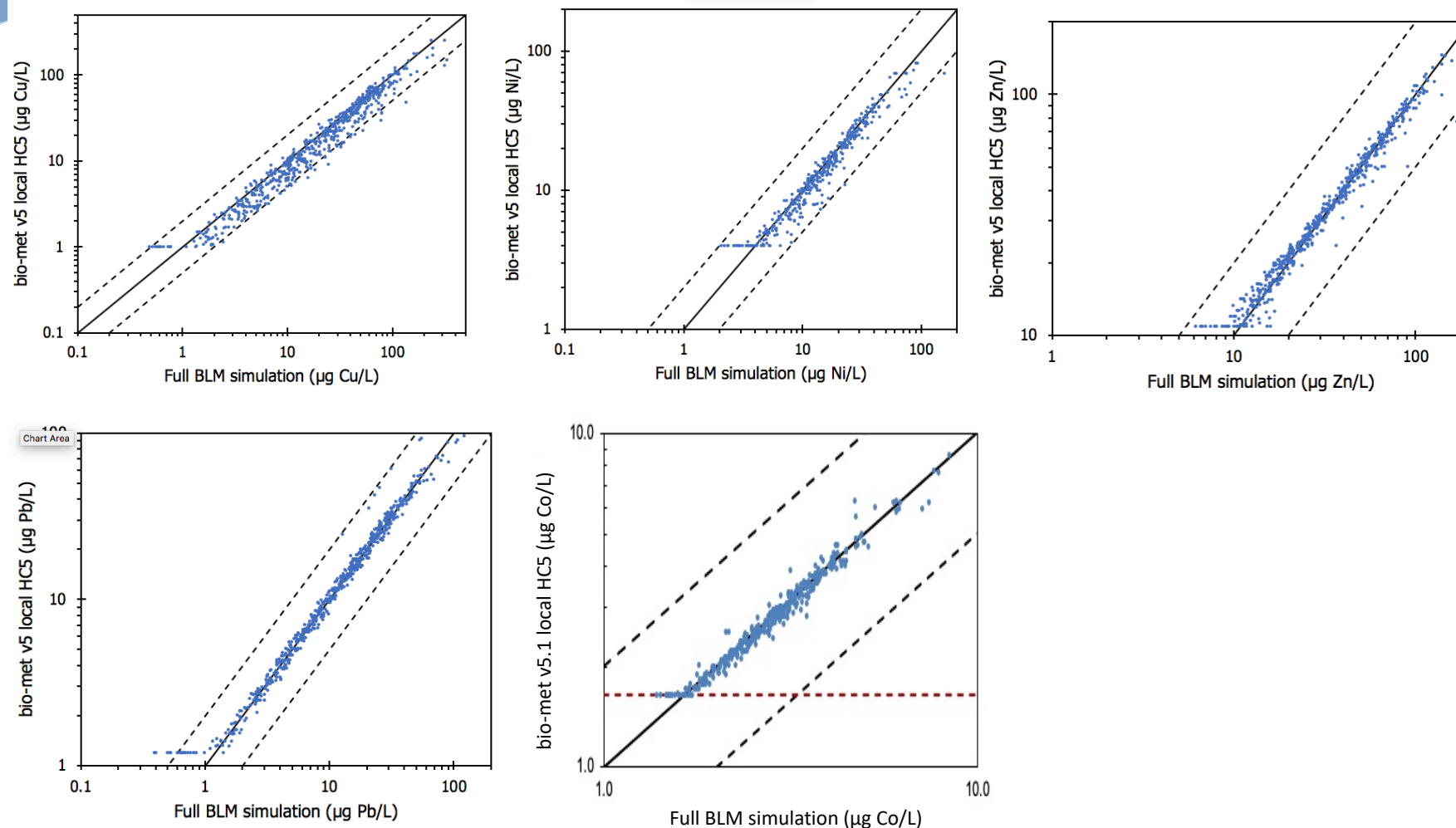


Figure 3 Results of the independent validation-exercise with the FOREGS database for Cu, Ni, Zn, Pb, and Co represented as full BLM simulated HC5 vs bio-met v5-predicted local HC5. The full line represents a perfect fit between the full BLM HC5 vs bio-met v5-predicted local HC5. The dashed lines represent a 2-fold error on the local HC5 predicted with bio-met v5. Only data of waters with pH and Ca within the BLM boundaries are shown.

2 HOW TO USE THE BIO-MET BIOAVAILABILITY TOOL

In order to make the model run, open the excel file as usual. The screen shown in Figure 2.1 will appear. To use the bio-met bioavailability software tool, you must ensure that macros are "enabled" in this workbook. Either click the **"options button"** in the security warning that may have appeared above the worksheet and select **"enable this content"** (see Figure 2.1), or click the **"Microsoft Office Button"** in the top left of the screen and select the following options:

>> *Excel Options*, >> *Trust Center*, >> *Trust Center Settings*, >> *Macro Settings*, >> *Enable all macros*.

"User-friendly" Biotic Ligand Model

Please register at www.bio-met.net to ensure you're using the most recent version of the tool

Version 5.1 - January 2022

Start

Help

EQS-bioavailable

About this tool

This software tool estimates the potential risk to the aquatic environment posed by copper, nickel, zinc, lead* and cobalt after considering bioavailability. The tool will calculate Local HCS values and Bioavailable Metal Concentrations based on information on local water physicochemistry. This tool has been developed as part of the bio-met project and has been designed to operate in Microsoft Excel 2007 and 2010, and works also in more recent versions. A fuller description of the science underpinning the tool, a description of the tool's operation and validation, case studies and comprehensive guidance on its use are available at www.bio-met.net.

This software tool is based on calculations from Biotic Ligand Models (BLM). It is currently intended to be used as part of a tiered risk assessment or as an early tier in an Environmental Quality Standard (EQS) compliance assessment.

* Specifically for lead, it may be helpful to note that, it is not currently recommended to use the Bio-met tool (or full lead BLM) to derive bioavailable lead EQS (Environmental Quality Standard) for chemical status classifications under the Water Framework Directive (WFD).

What's NEW this version?

- Version 5.1: Co has been added
- In Version 5:
 - Lower range of the Ni EQS is changed to 4 µg Ni/L
 - The DOC resolution of the underlying database with key water parameters has been increased.
 - Worst-case competition waters have been added to the underlying database
 - The option to include Ambient Background Concentrations (ABC) for Zn in the bio-met calculations has been removed, as this calculation was not in line with the most recent guidance of the WFD. See manual Section 2.6 for more details on how to deal with ambient background concentrations

Hints and Tips

You can enter data for up to 2000 samples. Make sure that each sample is entered on a separate row. You can paste data in from another spreadsheet, so long as it is laid out in the same order as in the bio-met tool.

The tool will not work if you enter blanks, zeros or text in the DOC, pH or Ca fields. You must enter positive numeric data only.

If you edit any of the input data after running the programme, the results will not adjust automatically. You will have to click **Calculate** again, even if you've only changed one row.

If you want to re-run the spreadsheet with a completely new set of input data, as if from scratch, click **Clear Data** and start again.

How to use this tool

Please read these instructions carefully before you start. Further guidance on using this tool can be obtained by visiting www.bio-met.net

1. To use this software tool, you must ensure that macros are "enabled" in this workbook. Either click the **"options button"** in the security warning that may have appeared above this worksheet and select **"enable this content"**, or click the **"Microsoft Office Button"** in the top left of the screen and select the following options:
>> *Excel Options*, >> *Trust Centre*, >> *Trust Centre Settings*, >> *Macro Settings*, >> *Enable all macros*
2. Now click the **Start** button. This will open the main Data Entry and Results sheet.
3. This sheet contains an empty table (if it isn't empty, click the **Clear Data** button to empty it).
4. The grey columns on the left can be used to enter information about each of your samples if you wish. Enter a separate row of data for each sample. Note: Variables in bold type must be entered):
 - Sample number
 - Sample name
 - Sample date
 - Sample pH
 - Sample Dissolved Organic Carbon (DOC) (mg/L C)
 - Sample Calcium (Ca) (mg/L) - Ca can be estimated using the bio-met hardness conversion tool (see <http://bio-met.net/evidence-base/>)
5. If you have measured concentrations of dissolved metal in your samples (µg/L) you can enter these values, although they are not required to calculate Local HCS or BioF values.
6. When you have entered your data, click **Calculate** to continue. A box will pop up to tell you when calculation is complete. Click **OK** to continue.
7. The results are displayed in the columns on the right-hand side of the table.
8. In all cases, the following results are shown:
 - Local HCS (dissolved) for each metal (µg/L)
 - BioF for each metal
9. Where you have entered data about the measured concentrations of the metal(s), the following results are also shown:
 - Bioavailable concentration for each metal (µg/L)
 - Risk Characterisation Ratio (RCR) for each metal. Values >1 are highlighted with **red** cells
10. The bioavailability models underpinning this software tool have been validated within certain physicochemical ranges (see below). Where local physicochemical conditions are outside of these ranges the tool will automatically use the most appropriate validated value to calculate results. Where this occurs the tool returns an 'out of boundary'-message in the notes column and provides further explanation and guidance on appropriate interpretation in comments boxes (hover your cursor over the red triangles in cells to read the comments).

Notice

Bio-met does not promise that the worksheet will provide any particular facilities or functions. You must ensure that the worksheet meets your needs. You will be solely responsible for the complete use of the worksheet and you are entirely responsible for the consequences of any use of the worksheet. Bio-met provides no warranty about the fitness for purpose or performance of any part of the worksheet. We do not promise that the media will always be free from defects, computer viruses, software locks or other similar code or that the operation of the worksheet will be uninterrupted or error free. You should carry out all necessary virus checks prior to installing on your computing system.

Guide

Macro's must be enabled before running the biomet tool.

The manual can be downloaded [here](#).

contact@bio-met.net | www.bio-met.net

Figure 2.1 The first screen of the bio-met bioavailability tool

The front page of the tool provides a description of the tool, and instruction on how to use it. In the left hand bar there are several buttons that could be helpful, including an overview of EQS-bioavailable used in the tool.

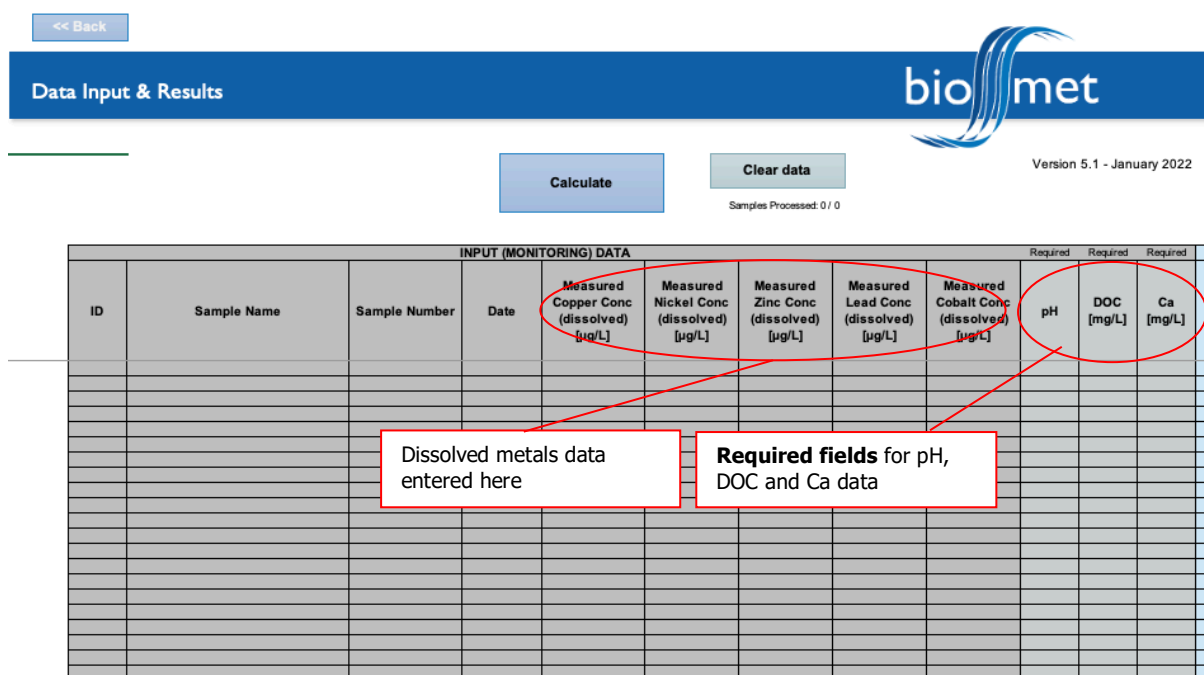


Figure 2.2 Required and optional input data to run the bio-met bioavailability tool

On clicking the 'Start' button the user is taken to the screen shown in Figure 2.2. The required fields to run the model are circled in the figure. If just these data are entered without the dissolved metal concentrations, the bio-met bioavailability tool will perform a hazard assessment. Such an assessment can be used to identify sites or waterbodies with low Local HC5 that would be sensitive to specific metal exposures.

Once the 'calculate' button has been pressed the tool begins to populate the fields to the right side of the required input fields, one row at a time. There are a series of identical columns for each metal, headed: Local EQS (dissolved) [$\mu\text{g l}^{-1}$], BioF, Bioavailable metal conc. [$\mu\text{g l}^{-1}$], RCR, Notes. When the calculations are complete a dialogue box (as shown in Figure 2.3 is shown).

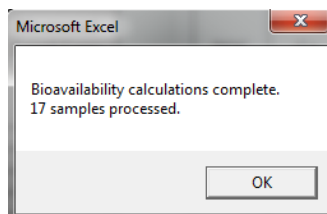


Figure 2.3 On-screen prompt once bioavailability calculations are complete

2.4 What do the outputs from the bio-met bioavailability tool mean?

Figure 2.4 shows the screen that will be seen once bioavailability calculations for each row have been completed.

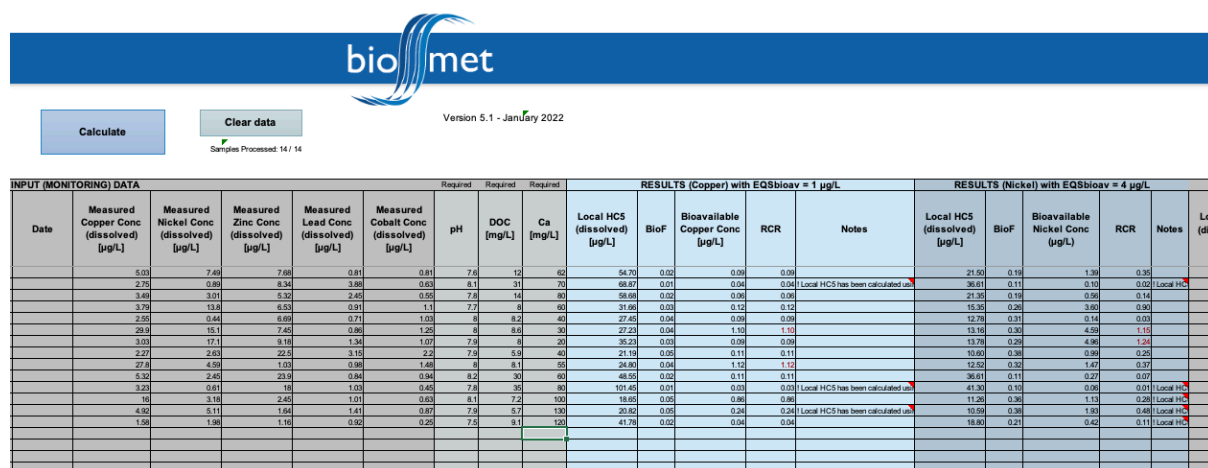


Figure 2.4 The bio-met bioavailability tool once calculations are complete

Comments boxes are used to identify where physicochemical input parameter data are outside of the validated range for a particular metal (i.e., pH, DOC and/or Ca data). Where this occurs, calculations are performed based on "best-fitting" data from within the validated ranges, and should be interpreted with caution.

Local HC5 (dissolved) [µg l⁻¹] – An HC5 is a value derived from ecotoxicological data (from a species sensitivity distribution) which aims to protect at least 95% of the species. In bio-met, the local HC5 of a metal is an HC5 which reflects the bioavailability conditions at a specific site. It is calculated based on the local water conditions at the site using a bioavailability model.

Under "sensitive conditions" (conditions of maximised bioavailability), the Local HC5 equals the reference HC5. Under these sensitive conditions, as a further measure, the use of a full BLM may be required to determine the Local HC5.

Notes for Ni:

Ni: In 2013, the EQSbioavailable for Ni was established by the European Commission at 4 µg Ni/L. This value was determined by using the Ni Biotic Ligand Models (BLMs) to normalize chronic ecotoxicity data to a combination of water chemistry conditions that were considered to maximize Ni bioavailability. The specific combination of water chemistry parameters was as follows: pH = 8.2, DOC = 2 mg l⁻¹, and Ca = 40 mg l⁻¹. These are representative of Alpine regions within Austrian water quality monitoring datasets, which were considered by the Commission and Member State representatives to be the most sensitive waters for Ni within Europe.

When the EQSbioavailable was determined, the monitoring datasets that were used to define maximum bioavailability were limited by the existing boundaries of the Ni BLMs. Notably, the upper pH boundary for the Ni BLM was 8.2. Ni toxicity increases with increasing pH, and several Member States have surface waters with pH ranges that are above 8.2. To address these issues, research was performed to increase the pH boundary to pH 8.7 in 2014, after the EQSbioavailable was established. The technical basis for the increase in the pH boundary is described by Nys et al. (2016a). The underlying calculations allowing the pH boundary to be increased within bio-met have been performed, and are available upon request. However, the increase in the pH boundary has not been incorporated into bio-met because the results of Nys et al. (2016a) and their consequences to the Ni EQS have not been addressed at the level of the European Commission and the Member States. Therefore, in situations where the pH is > 8.2, the user is informed that the pH boundary has been exceeded, and that predictions of bio-met are not binding.,

At the time of this writing (June 2022), the European Commission's Joint Research Centre has initiated a review of the Ni EQS. This review should typically consider the data supporting the extension of the pH boundary to 8.7, and any consequences this may have on the determination of the EQS bioavailable. Until that time, bio-met will default to the EQS bioavailable in situations where the pH boundary of 8.2 is exceeded, and indicate to the user that expert judgment (e.g., using the full Ni BLM) is required when making decisions about compliance.

BioF - is the metal bioavailability at a specific site, relative to the bioavailability under "sensitive conditions" (conditions of high bioavailability). It equals the ratio of the reference HC5 divided by the local HC5. This value is always 1 or less. When the value is 1 the metal, under the specific water conditions provided, is 100% bioavailable and the site is described as "sensitive conditions".

$$BioF = \frac{Reference\ HC5}{Local\ HC5}$$

Bioavailable metal concentration [$\mu\text{g l}^{-1}$] – this is the concentration of metal that is bioavailable at the site or waterbody. This value is calculated by multiplying the dissolved metal concentration for the site by the BioF. The BioF column is not available when dissolved metal data for a row are not entered.

$$Bioavailable\ metal\ concentration = Dissolved\ metal\ concentration * BioF$$

RCR - is the risk characterisation ratio for the site or waterbody under consideration. A value of 1 or greater identifies a potential risk. Under these circumstances the cell in the notes column is highlighted red. The RCR column is not available when dissolved metal data for a row are not entered. The RCR is calculated as:

$$RCR = \frac{Bioavailable\ metal\ concentration}{EQSbioavailable}$$

The above approach is the one used in bio-met. Alternatively, the RCR can also be calculated from another formula. The local EQS can be defined as an EQS at a specific site, taking the local bioavailability conditions into account. It is mathematically calculated from the EQS-bioavailable divided by BioF. The RCR can then be calculated as the dissolved metal concentration divided by the local EQS. The result is identical to the formula above. An overview of the calculation steps involved in bio-met is presented in Figure 2.5.

Bio-met calculation steps

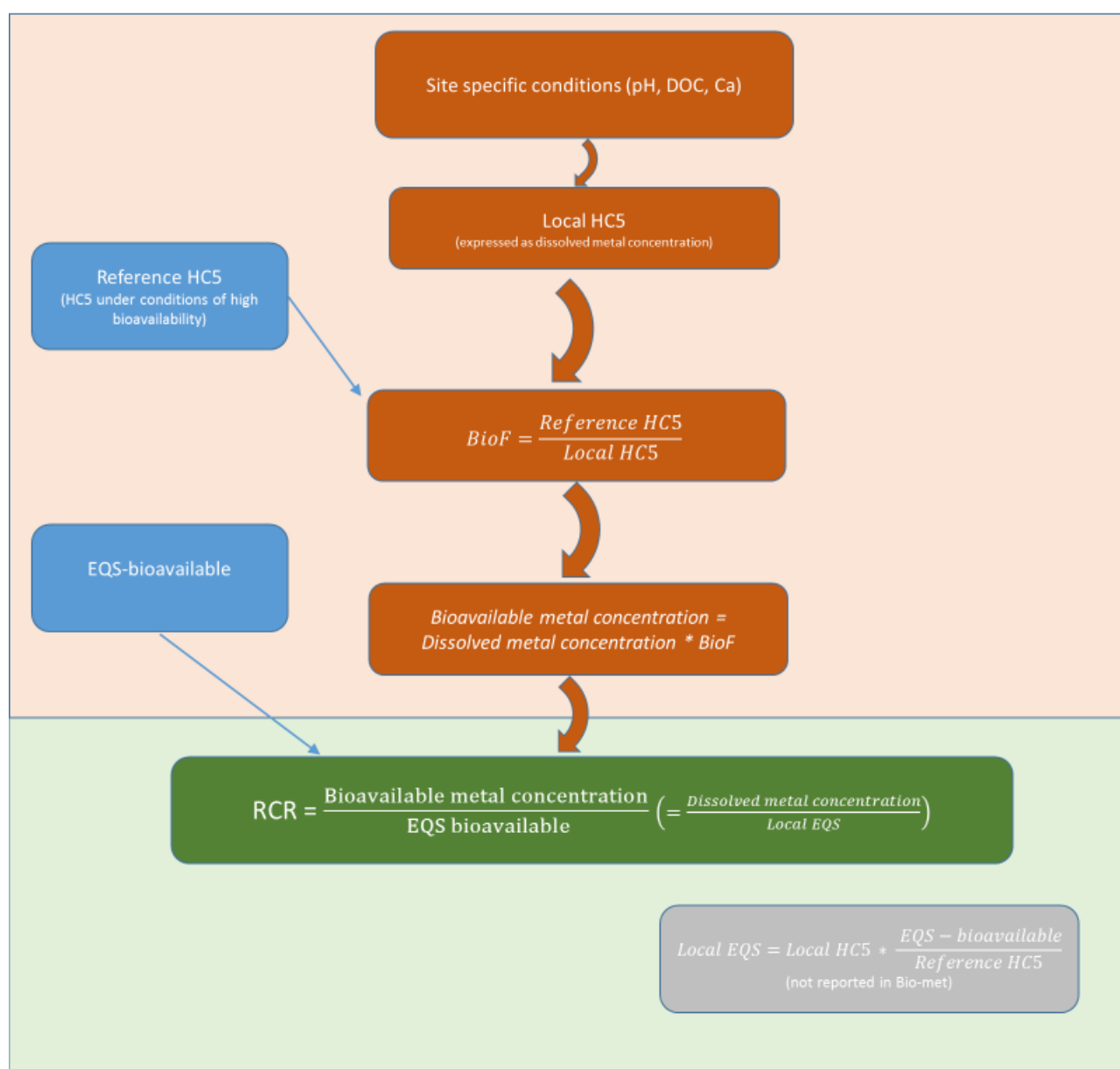


Figure 2.5 Calculation steps used in bio-met

2.2 Hardness Conversion Tool

A Hardness Conversion Tool has also been developed because the bio-met bioavailability tool uses input data for Ca, rather than for water hardness. In cases where water hardness data are available but Ca data are not the tool can be used to calculate the equivalent concentration of Ca from water hardness. This calculation takes into account the fact that both Ca and Mg contribute to hardness, and that it is only the Ca component that is used for the BLM input. A contribution from Mg is calculated assuming that the ratio between Ca and Mg concentrations is as identified by Peters et al. (2011)¹³.

The tool will convert input water hardness data, expressed in a variety of units, into an equivalent Ca concentration, in units of mg l^{-1} . This data can then be used as input data for the bio-met bioavailability tool. Basic background information on the tool and its use is provided on the introduction sheet (Figure 2.6).

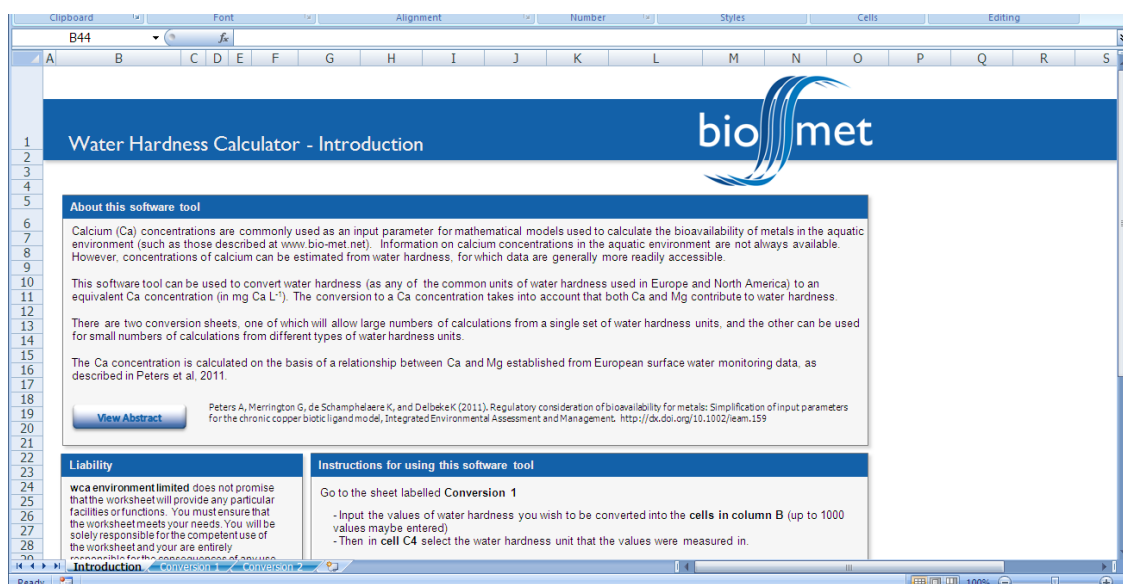


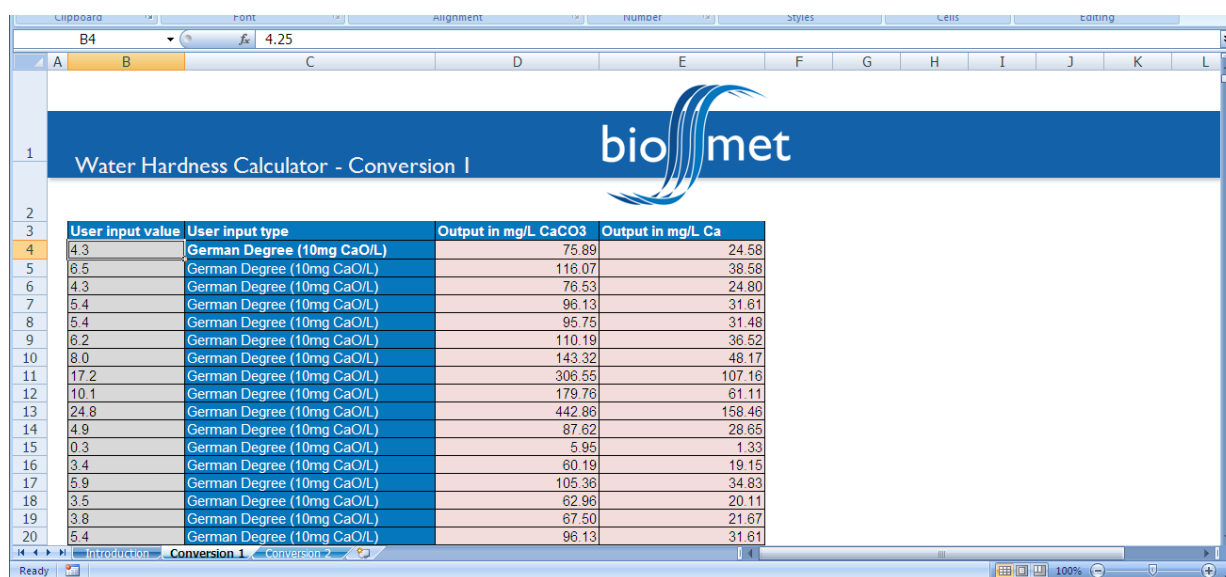
Figure 2.6 Introductory sheet of the Hardness Conversion Tool

Hardness data, expressed as mg l^{-1} of CaCO_3 , CaO , or Ca , as degrees of hardness (English, French, German, and USA), or as moles per litre can be converted into an equivalent Ca concentration (in mg l^{-1}). Large numbers of samples which are all expressed in the same hardness units can be processed on the first conversion sheet (Figure 2.7), and the second conversion sheet can be used for small numbers of samples which are expressed in different hardness units.

¹³ Peters A, Merrington G, Delbeke K, de Schamphelaere K. 2011. Regulatory consideration of bioavailability for metals: Simplification of input parameters for the chronic copper Biotic Ligand Model. Integrated Environmental Assessment and Management 7:437-444.



Data is pasted into the column (User Input Data), and the correct unit used for the input hardness data is selected from the drop down list in cell C4 (below Use Input Type).



The tool converts the input data (expressed as hardness in German Degrees in Figure 2.8) to $\text{mg l}^{-1} \text{CaCO}_3$, and also into an equivalent Ca concentration. This equivalent Ca concentration takes account of the fact that both Ca and Mg contribute to hardness.

2.3 DOC input data

Input data for DOC is required for bioavailability calculations to be performed. DOC is a particularly important factor in the bioavailability calculations for metals in freshwaters.

Information on measured DOC concentrations is always preferable, but in some cases where no information is available for a specific site other approaches can be taken to provide an indication of bioavailability and better inform whether or not any action needs to be taken, e.g. to measure DOC concentrations to confirm bioavailability estimates.

DOC information for other sampling locations within the same waterbody, or surrounding waterbodies, may be able to provide an indication of the local concentrations. In these cases it is recommended to take a relatively low percentile, e.g. the 25th percentile, of the monitoring data in order to ensure that the resulting value is unlikely to be conservative (i.e. precautionary).

DOC concentrations can also be predicted from UV absorbance data or dissolved iron concentrations. These methods allow screening level assessment to be undertaken in the absence of measured DOC data and may be particularly useful in highlighting areas where it obtaining measured DOC data is of the greatest importance. Equations 1 and 2 relate dissolved iron concentrations to DOC concentrations from the data shown in Figure 2.9.

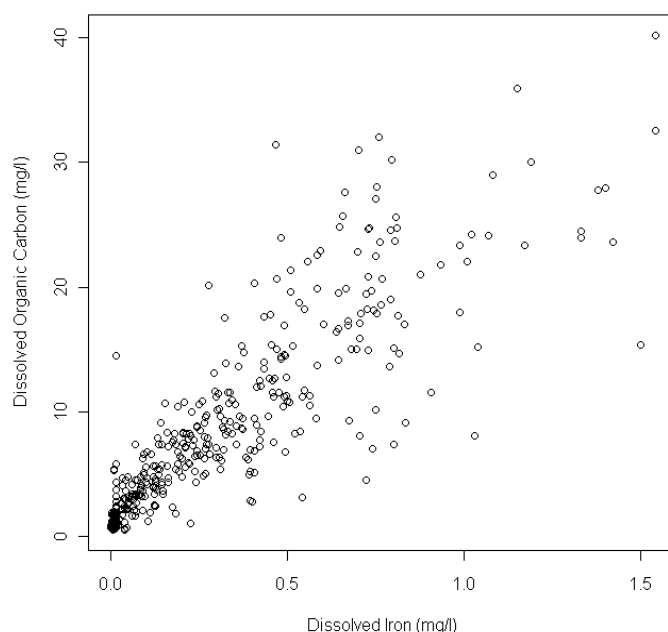


Figure 2.9 Relationship between dissolved iron and dissolved organic carbon

$$\text{DOC (mg l}^{-1}\text{)} = 20.79 \cdot \text{Fe (dissolved, mg l}^{-1}\text{)} + 2.32 \quad r^2 = 0.738 \quad \text{Eq. 1}$$

$$\log_{10}(\text{DOC, mg l}^{-1}\text{)} = 0.56 \cdot \log_{10}(\text{Fe, dissolved, mg l}^{-1}\text{)} + 1.24 \quad r^2 = 0.781 \quad \text{Eq. 2}$$

2.4 Summarising input data for calculations

The EQS-bioavailable is expressed as an annual average concentration. It is possible to calculate bioavailability for each sample and calculate the annual average of the bioavailable metal concentration, or alternatively it is possible to summarise the input data to calculate an

average bioavailability factor. In reality, the two approaches result in very similar results where the same input information is used.

Calculating bioavailable metal on each sampling occasion is preferable, but is only possible where all of the required supporting parameters are analysed alongside metals on every sampling occasion. If samples for metals and the supporting parameters are taken at different times then calculation in this way may not be appropriate.

Averaging of input parameters may be appropriate for several reasons. In some cases, samples may only be taken four times per year for some supporting parameters, and averaging data from multiple years may provide a better indication of the “average” conditions of pH, Ca, and DOC. A missing result for a single determinant may prevent calculation of the bioavailability for a given sample but is less important overall when taken as part of an average. This approach may therefore be more tolerant of minor problems in the sampling and analysis procedure which may result in the occasional loss of data.

Where average values are used for model input parameters it is appropriate to consider how the data are summarised to provide the average values. The number of samples used and time period over which they were taken need to be defined, as well as the location, or locations, which are used. In addition, whilst an arithmetic mean may be used for dissolved metal concentrations this may tend to overestimate the “average” situation if the exposure follows a log-normal distribution. The arithmetic mean is usually appropriate for pH (which is already log transformed (Boyd et al 2011)), and Ca concentrations which are often relatively consistent for a given location. It is recommended that median (50th percentile) DOC concentrations are used rather than arithmetic means because this will better represent the “average” concentration if the distribution of concentrations is log-normal, whereas an arithmetic mean may result in overestimation of the average DOC concentration.

2.5 Physicochemical conditions that are outside the applicability domain (validated ranges) of the BLMs

This subsection provides some considerations on how to assess sites where the water chemistry conditions are outside the applicability domain (validated range) of the BLMs. An important factor is whether or not bioavailability is likely to be increased outside of validated ranges, relative to boundary conditions, or if it is likely to be reduced.

Upper and Lower boundaries are set for both pH and Ca, which both affect uptake and binding at the biotic ligand, and are physiologically important for aquatic organisms. The boundaries which commonly cause surface waters to be outside of the applicability range of the BLMs are hard waters (high Ca concentrations), where the Ca concentration may exceed the BLM application range, and soft waters where the Ca concentration is below the applicability range of the BLM. Low pH may also cause waters to be outside of the validated range of the BLMs, and in some instances both low pH and low Ca may be encountered in the same waters. No boundaries are set for DOC concentrations.

Hard waters, where the Ca concentration exceeds the BLM application range, especially for the Ni BLM, can be treated relatively easily. The upper limit to the applicable range of Ca concentrations exists because there is a limit to the “protective” effect from Ca as a competitor for binding sites on the “Biotic Ligand”. Increases in Ca concentrations beyond the boundary do not result in further reductions in metal bioavailability/toxicity. This situation can be adequately handled by limiting the input data to the maximum allowable Ca concentration, and BLM predictions performed by doing so should continue to be reliable.

In contrast, the boundaries established for soft, acid waters occur because the test organisms used for BLM development are unable to survive and reproduce adequately under these relatively extreme conditions. These water quality conditions may support different species than those found in harder water or more alkaline environments. Protons (H^+) and Ca ions may both compete with metals for binding sites at the biotic ligand. As pH decreases the competition from protons will increase (resulting in lower metal bioavailability), whereas as hardness decreases the competition from Ca will decrease (resulting in higher metal bioavailability). The relative importance of competition from protons and Ca for each individual metal may affect organism responses under these conditions. A reduction in pH may also result in a reduction in metal binding to DOC, due to increased competition from protons, resulting in increased metal bioavailability. Changes in pH can also result in changes to the inorganic speciation of a metal, and the fraction which exists as bioavailable species (e.g., Cu^{2+}), although significant changes in speciation around the lower pH limit for the BLMs are unlikely for Cu, Ni, Zn, and Co.

Several options for treating conditions which are outside of the applicability range of the BLMs are available.

1. Consider 100% bioavailability of the metal (i.e. apply the EQS-bioavailable)
2. Assume model predictions still apply outside boundary conditions
3. Extrapolation (e.g. consider complexation of metal by DOC only)
4. Bioassays (including ecotox. tests, WER, and ecological community monitoring)
5. Use specific Local EQS

The first option applies the EQS-bioavailable outside the applicable conditions and can effectively result in a step change to the standard where the boundary conditions are met. The second option applies the BLMs and assumes that no boundary conditions apply and that the models can be extrapolated beyond their validated range. This approach cannot be applied with the bio-met bioavailability tool as input values for pH and Ca are limited to the allowable range, irrespective of the values entered into the tool, although this approach could be applied using the full BLMs.

Bioassays provide a means of validating any predictions or assumptions that are made about the protection of aquatic ecosystems in relatively extreme environments. Water Effect Ratios, which compare the results of toxicity tests in locally collected water and standardised water have been used in the US. These tests use a Water Effects Ratio to correct the EQS under

standard conditions to the local EQS. Such tests would need to use test species which are appropriate to the water chemistry conditions of the local surface waters. Reference-based ecological monitoring (e.g., benthic macroinvertebrate community monitoring) will be performed under the WFD and may provide a means to ensure the adequacy of any derived EQS for relatively extreme environmental conditions. The use of additional biological monitoring may be valuable in supporting the application of the standards under potentially sensitive conditions.

An example of a specific EQS would be the $PNEC_{\text{softwater}}$ for Zn which was derived for the Existing Substances Regulations Risk Assessment Report on Zinc and Zinc compounds¹⁴, although other approaches to developing specific localised standards may be more appropriate.

2.6 Dealing with ambient background concentrations (ABCs) for zinc – Added Risk Approach

Annex I, part B, of the WFD Daughter Directive on priority substances (EC 2007) suggests that Member States may consider natural backgrounds when assessing compliance for metals. In Europe there are probably very few surface waters containing only “natural” concentrations of metals, due to historical contamination. Therefore, the usual or “ambient” concentration of a metal in surface waters consists of *both* a natural geochemical fraction and an anthropogenic fraction (ISO 2005).

The Added Risk Approach (ARA) is the most widely recognised policy-based approach for regulating naturally occurring substances in environmental matrices. The ARA is a pragmatic Dutch policy solution to the issue of implementing single metal EQS in waters with variable background metal concentrations (Crommentuijn et al. 1997). It assumes that the effects of naturally occurring background metal concentrations may be desirable, i.e., the ecosystem has developed in the way that is has at least partly *because* of the metals that are present.

The $EQS_{\text{bioavailable}}$ for zinc used in the bio-met bioavailability tool has been derived as a bioavailable EQS_{add} . An EQS_{add} allows for the Ambient Background Concentrations (ABC) to be incorporated within a compliance assessment. EQS for copper and nickel have not been derived to account for ambient background concentrations (which are considered to be relatively insignificant for both these metals). ABC can be significant for zinc (as dissolved metal), as compared to the EQS_{add} . In such case, ABC can be subtracted from the input data before being processed by the bio-met bioavailability tool, to assess the anthropogenic part of the exposure.

The derivation of ABCs is not straightforward as there are currently no universally agreed “default” ABC values within the EU, either at Member States or WFD waterbodies level. ABCs should be derived at a local level for them to have the most relevance to local conditions. The WFD EQS Technical Guidance Document describes methodologies for estimating the background levels for metals for the purposes of the ARA.

¹⁴ <http://ecb.jrc.ec.europa.eu/risk-assessment/REPORT/zincmetalreport072.pdf>

Default ABC data for hydrometric areas in England and Wales have been derived from the 5th percentile of available monitoring data (Environment Agency, in press). This approach may be useful applied in other areas of the EU to derive ABCs.

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Supporting Annex 1: Predictions within a factor of two as used during BLM developments

The original validation graphs for *Daphnia magna* (Cu, Ni, and Zn) and *Ceriodaphnia dubia* (Pb), visualising the performance of the full BLM versus the observed toxicity in the field can be found below. All predictions of full BLM are within factor of around 2 of observed toxicity.

Copper (De Schamphelaere K. and C. Janssen. 2004)

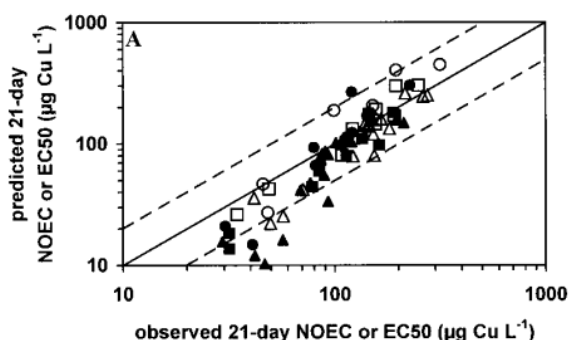


Fig. A.1. Predictive capacity of the chronic Cu biotic ligand model for *Daphnia magna* as shown by observed versus predicted 21-d no-observed-effect concentrations (NOECs; filled symbols) and 21-d 50% effective concentrations (EC50s; open symbols). Assumption for dissolved organic matter (DOM): 50% active fulvic acid Symbols: Bihain (●), Ossenkolk (△), and Ankeveen (□). The solid line is the 1:1 reference line indicating a perfect match between observed and predicted values; the dashed lines indicate an error within a factor of two between observed and predicted values. Bihain, Ossenkolk, and Ankeveen are sampling sites at which DOM was collected.

Nickel (Deleebeeck N., K. De Schamphelaere, C. Janssen, 2008)

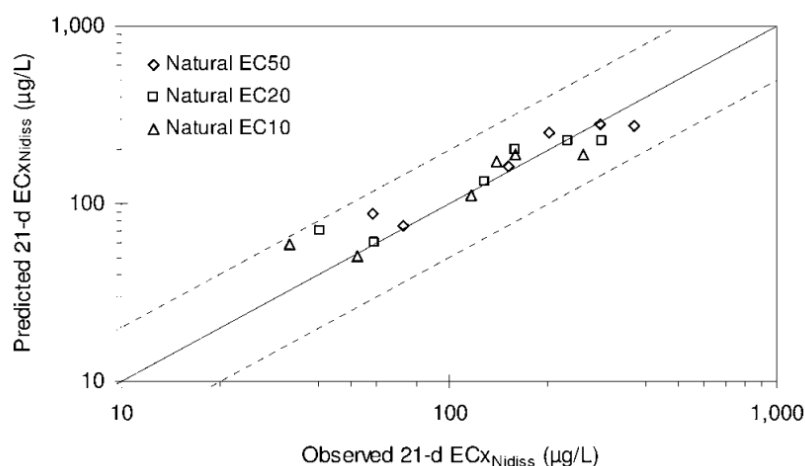


Fig. A.2. Predicted versus observed effect concentrations resulting in x% reduction of reproduction expressed as dissolved Ni concentration: predictions for all natural waters using optimized sensitivity parameters (Q50, Q20, and Q10, respectively). The solid line indicates a perfect match between predicted and observed ECx values; the dashed lines indicate ratios of 0.5 and 2 between observed and predicted ECx values.

Zinc (De Schamphelaere K., S. Lofts, C. Janssen. 2005)

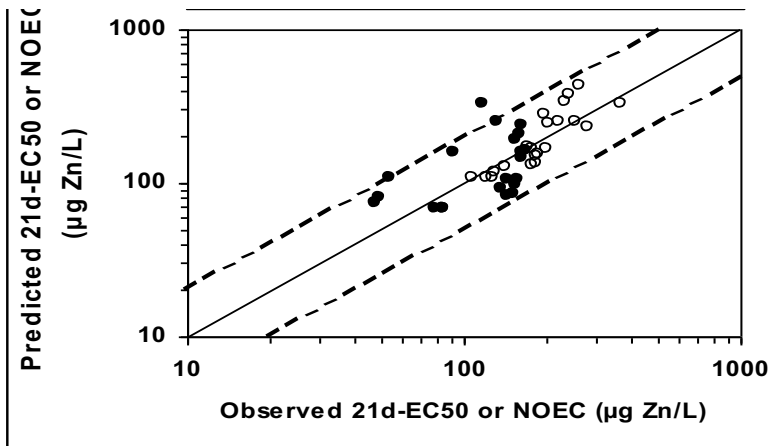


Fig. A.3. Predicted versus observed effect concentrations resulting in x% reduction of reproduction expressed as dissolved Zn concentration (21-d no-observed-effect concentrations (NOECs; filled symbols) and 21-d 50% effective concentrations (EC50s; open symbols)). The solid line indicates a perfect match between predicted and observed ECx values; the dashed lines indicate ratios of 0.5 and 2 between observed and predicted ECx values.

Lead (Nys et al., 2014.)

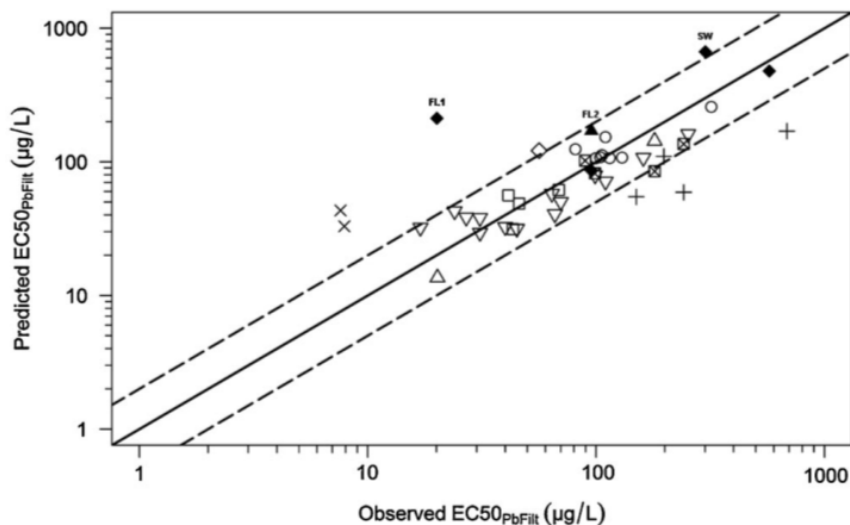


Figure A.4. Predicted versus observed median effective concentration (EC50, expressed as mg filtered Pb/L) for the chronic Pb *Ceriodaphnia dubia* biotic ligand model (BLM) calibrated with the developed BLM calibrated with the clone-specific intrinsic sensitivities: predictions for the data used for the BLM development and the validation. Dashed line represents a difference of a factor of 2 between the observed and predicted data. Full line represents a perfect fit between observed and predicted data. Open data points are from synthetic media, and filled points are from natural waters. Crossed symbols represent data points where precipitation is predicted by speciation calculations.

Cobalt (Stubblefield et al., in prep)

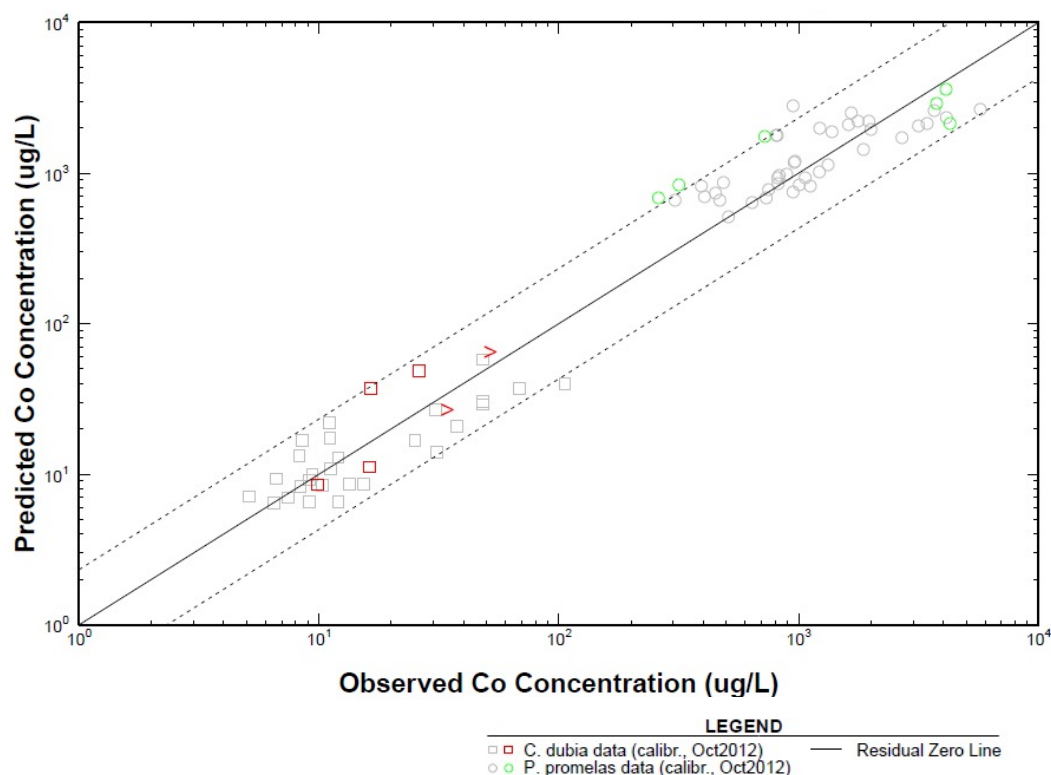
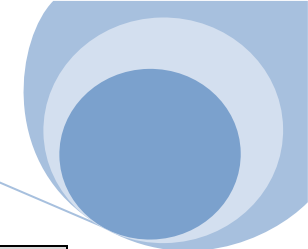


Figure A.5. Predicted versus observed median effective concentration (EC50, expressed as mg filtered Co/L) of *Ceriodaphnia dubia* (squares) predicted with the chronic Co *Ceriodaphnia dubia* biotic ligand model (BLM) and of *Pimephales promelas* (circles) predicted with the chronic Co *Pimephales promelas* biotic ligand model: predictions for the data used for the BLM development and the validation. Dashed line represents a difference of a factor of 2 between the observed and predicted data. Full line represents a perfect fit between observed and predicted data. (Source OSU 2013a,b,c,d)

Supporting Annex 2: Calibration and validation ranges for bio-met

The application range of the bio-met tool for each of the metals has been defined based on the application ranges of the full BLMs. The application range of a BLM model represents the range of water chemistry parameters for which the predictive ability of the model has been demonstrated. The application range of the full BLMs encompasses both calibration and validation ranges of the models. The calibration ranges of the BLMs represent the range of water chemistry parameters for the experiments used in the development of the model. Most of the BLMs for Ni, Zn and Cu have been developed based on synthetic or natural waters in which water chemistry parameters were univariately varied (following the approach of De Schamphelaere & Janssen 2002). The validation ranges represent the physico-chemical ranges in which the predictive performance of a model (developed based on synthetic waters) has been evaluated in natural waters or for non-model species.

The following tables summarize the calibration, validation, and application ranges for the different full BLM models for pH, DOC and Ca.


Table A2.1 Calibration, validation and application ranges of pH for the full BLMs that underlie the bio-met tool

	Type of BLM	pH range for Nickel (Reference)	pH range for Copper (Reference)	pH range for Zinc (Reference)	pH range for Lead (Reference)	pH range for Cobalt (Reference)
Calibration range	Algae	5.7 - 8.7 (Deleebeeck et al. 2009a)	5.7 - 8.8 (De Schamphelaere et al. 2003b)	5.6 - 8.0 (De Schamphelaere et al. 2003a)	6.0 - 8.0 (De Schamphelaere et al. 2014)	6.1 - 8.4 (OSU 2015, OSU 2020a)
	Invert.	5.9 - 8.7 ^A 6.5 - 8.7 ^B (Deleebeeck et al. 2008; De Schamphelaere et al. 2006)	5.6 - 8.7 (De Schamphelaere and Janssen, 2004a)	5.5 - 8.0 (De Schamphelaere et al. 2003a)	6.3 - 8.2 (Nys et al. 2014 ; Nys et al. 2016b)	6.4-8.5 (Parametrix 2005; Parametrix 2010a; OSU 2013a; OSU 2020b)
	Fish	5.4 - 8.5 (Deleebeeck et al. 2007a)	6.6 - 8.7 (De Schamphelaere and Janssen, 2004c)	5.7 - 8.1 (De Schamphelaere et al. 2003a)	6.3 - 8.2 (Van Sprang et al. 2016)	6.1 – 8.5 (Parametrix 2009a,b; Parametrix 2010c ; OSU 2013c)
Validation range	Algae	5.7 - 8.0 (Worms et al. 2007; Deleebeeck et al. 2009a)	5.5 - 8.7 (De Schamphelaere et al., 2003b, ECI 2008)	5.7 - 8.5 (De Schamphelaere et al. 2005; Van Regenmortel et al. 2017)	6.0 - 8.4 (De Schamphelaere et al. 2014)	7.0 – 7.8 (Heijerick et al. 2007)
	Invert.	5.9 - 8.7 ^A 6.5 - 8.7 ^B (Nys et al. 2016a ; Peters et al. 2018)	5.5 - 8.5 (De Schamphelaere and Janssen 2004a, ECI 2008)	6.0 - 8.4 (U Gent 2015 unpublished report. De Schamphelaere et al. 2005; Van Regenmortel et al. 2017)	6.0 - 8.6 (Nys et al. 2014 ; Nys et al. 2016b)	6.8 - 8.5 (Parametrix 2010b; OSU 2013b; OSU 2017)
	Fish	5.4 - 8.5 (Deleebeeck et al. 2007a; Peters et al. 2018)	6.0 - 8.6 (De Schamphelaere and Janssen 2004c, ECI 2008)	6.2 - 8.1 (De Schamphelaere et al. 2005)	6.4 - 8.0 (Van Sprang et al. 2016)	6.6 - 8.1 (Parametrix 2010d ; OSU. 2013d)
Application range	“Full BLM”	6.5 - 8.2 ***	6.0 - 8.5	5.5 - 8.5	6.3 - 8.4	6.4-8.4

^A : *Daphnia magna* BLM

^B : *Ceriodaphnia dubia* BLM

***: The Ni BLM models have been validated to higher pH ranges (Nys et al. 2016a) and lower Ca ranges (Peters et al. 2018) than those reflected in the Application Ranges shown in the table. The Application Ranges reflect the state of the science when the Ni EQS was determined.

Table A2.2 Calibration, validation and application ranges of DOC for the full BLMs that underlie the bio-met tool

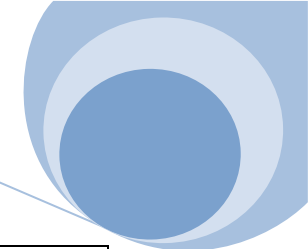
	Type of BLM	DOC range for Nickel in mg/L (Reference)	DOC range for Copper in mg/L (Reference)	DOC range for Zinc in mg/L (Reference)	DOC range for Lead in mg/L (Reference)	DOC range for Cobalt in mg/L (Reference)
Calibration range	Algae	0.1 * (Deleebeeck et al. 2009a)	1.3 - 20 (De Schampelaere et al., 2003b)	0.3 - 22.3 (De Schampelaere et al. 2003a)	2.1 - 10.6 (De Schampelaere et al. 2014)	0.3 – 12.7 (OSU 2015)
	Invert.	0.1 * (Deleebeeck et al. 2008)	1.7 - 18 (De Schampelaere and Janssen, 2004a)	0.3 - 17.3 (De Schampelaere et al. 2003a)	1.1 - 18.6 (Nys et al. 2014 ; Nys et al. 2016b)	0.5 - 12.7 (Parametrix 2005; Parametrix 2010a; OSU 2013a; OSU 2022)
	Fish	0.1 * (Deleebeeck et al. 2007a)	1 ** (De Schampelaere and Janssen, 2004c)	0.3 - 22.9 (De Schampelaere et al. 2003a)	1.2 - 10.5 (Van Sprang et al. 2016)	0.5 – 12.3 (Parametrix 2009a,b; Parametrix 2010c ; OSU 2013c)
Validation range	Algae	2.5 - 25.8 (Worms et al. 2007; Deleebeeck et al. 2009b; Peters et al. 2018)	0 - 20 (De Schampelaere et al. 2003b, ECI 2008)	2.3 - 22.3 (Van Regenmortel et al. 2017; De Schampelaere et al., 2005)	2.1- 22.4 (De Schampelaere et al. 2014)	4.1 – 22.6 (Heijerick et al. 2007)
	Invert.	2.5 - 25.8 ^A 3.2 - 23.6 ^B (Peters et al. 2018)	0 - 20 (De Schampelaere and Janssen, 2004a, ECI 2008)	0.3 – 17.3 (Van Regenmortel et al. 2017; De Schampelaere et al. 2005)	0.4 - 31.5 (Nys et al. 2014 ; Nys et al. 2016b)	0.7-19.8 (Parametrix 2010b; OSU 2013b; OSU 2017)
	Fish	3.8 - 18.4 (Deleebeeck et al. 2007a; Peters et al. 2018)	0 - 18 (De Schampelaere and Janssen. 2004c, ECI 2008)	2.8 - 22.9 (De Schampelaere et al., 2005)	0.5 – 12 (Van Sprang et al. 2016)	0.1 – 19.8 (Parametrix 2010d ; OSU. 2013d)
Application range	"Full BLM"	0.1 - 30	0.1 - 30	0.3 - 22.9	0.4 - 27.3	0.5 - 19.8

^A: *Daphnia magna* BLM

^B: *Ceriodaphnia dubia* BLM

*: Tests were performed in synthetic laboratory water, which contains no added dissolved organic carbon (DOC). For the purposes of speciation modeling, the DOC in these waters is operationally defined as 0 mg/L. Since no natural water will include 0 mg/L, 0.1 mg/L is used as a reasonable alternative.

**: Estimated value of DOC concentration in Lake Superior dilution water


Table A2.3 Calibration, validation and application ranges of Ca for the full BLMs that underlie the bio-met tool

	Type of BLM	Ca range for Nickel in mg/L (Reference)	Ca range for Copper in mg/L (Reference)	Ca range for Zinc in mg/L (Reference)	Ca range for Lead in mg/L (Reference)	Ca range for Cobalt in mg/L (Reference)
Calibration range	Algae	2.4 - 144 (Deleebeeck et al. 2009a)	5 - 160 (De Schampelaere et al., 2003b)	5.0 - 65.4 (De Schampelaere et al. 2003a)	4.7 – 120 (De Schampelaere et al. 2014)	4.0 -161 (OSU 2015))
	Invert.	1.3 - 88 ^{A,B} (Deleebeeck et al. 2008 De Schampelaere et al 2006)	7 - 179 (De Schampelaere and Janssen, 2004a)	5.0 - 160.3 (De Schampelaere et al. 2005; Heijerick et al. 2005)	9.6 - 98.8 (Nys et al. 2014 ; Nys et al. 2016b)	4.2 - 147 (Parametrix 2005; Parametrix 2010a; OSU 2013a; OSU 2022)
	Fish	3.8 - 110 (Deleebeeck et al. 2007a)	14 - 94 (De Schampelaere and Janssen, 2004c)	7.8 - 155.8 (De Schampelaere et al. 2003a; De Schampelaere and Janssen, 2004b)	5.7 – 83 (Van Sprang et al. 2016)	5.0 – 155 (Parametrix 2009a,b; Parametrix 2010c ; OSU 2013c)
Validation range	Algae	2.4 - 144 (Worms et al. 2007; Deleebeeck et al. 2009b)	2.5 - 179 (De Schampelaere et al. 2003b, ECI 2008)	0.8 – 159.1 (Van Regenmortel et al. 2017)	4.7 - 120 (De Schampelaere et al. 2014)	8.8 – 65.5 (Heijerick et al. 2007)
	Invert.	3.0 - 72.7 ^A 1.3 - 88 ^B (Deleebeeck et al. 2007b ; Nys et al. 2016a ; Schlekat et al. 2010)	2.5 - 179 (De Schampelaere and Janssen 2004a, ECI 2008)	4.8 - 155.9 (Van Regenmortel et al. 2017)	3.5 - 202 (Nys et al. 2014 ; Nys et al. 2016b)	2.6 – 77.0 (Parametrix 2010b; OSU 2013b; OSU 2017)
	Fish	3.8 - 83.0 (Deleebeeck et al. 2007a; Peters et al. 2018)	3.1 - 129 (De Schampelaere and Janssen 2004c, ECI 2008)	1.4 - 55.1 (De Schampelaere et al. 2003a ; De Schampelaere et al., 2005)	3.6 – 84 (Van Sprang et al. 2016)	2.6 – 77.0 (Parametrix 2010d ; OSU. 2013d)
Application range	"Full BLM"	2 - 88 ***	3.1 - 129	5.0 - 160.3	3.6 - 204	4.0 - 147

^A : *Daphnia magna* BLM

^B : *Ceriodaphnia dubia* BLM

***: The Ni BLM models have been validated to higher pH ranges (Nys et al. 2016a) and lower Ca ranges (Peters et al. 2018) than those reflected in the Application Ranges shown in the table. The Application Ranges reflect the state of the science when the Ni EQS was determined.

Supportive ANNEX 3: Frequently asked questions accompanying the use of the bio-met tool

This short note provides advice related to alters provided in the form of comment boxes in the output section of bio-met. Additional, generic guidance and advice are available in the bio-met users guide, as well as in documents drafted for EU Member States and the Technical guidance to implement bioavailability-based environmental quality standards for metals.

1) Question regarding flags/alerts for being outside validated ranges

The validation boundaries of the BLMs (and so too bio-met) represent the extremes of water quality conditions at which the validation chronic tests were undertaken (see Annex 2 of the bio-met Guidance manual and also shown on the 'flags' sheet of bio-met). However, this does not necessarily mean that the relationships developed do not hold where water conditions are outside of these ranges or that the derived local EQS is underprotective. It does mean that the certainty associated with calculations performed for water quality conditions outside of the validated boundaries is not as high as if the waters were within the range of validation conditions. Hence, bio-met does give calculations for waters that are outside of the validated ranges. But the physico-chemical parameter(s) that is outside the range is 'held' at the limit of the validation range. This introduces an additional level of uncertainty in the calculated result. How this level of uncertainty might be interpreted is described below. Please note that an alert message may also be a combination of the alert messages of Table 1 if more than one of the water chemistry parameters (pH, DOC and Ca) is outside the validated range.

Table 1. Overview of flags and alerts for being outside the validated ranges and their implication

Metal + parameter	Alert	Advice
All metals + DOC	<i>! Local HC5 has been calculated using an appropriate value from within the validated range despite DOC is above the higher end of the validated range for the BLMs. See the Guidance Manual, Section 2.5, for more information.</i>	The applicability range of the bio-met tool is set at 0.1-30 mg DOC/L. Therefore, the local HC5-calculations are held at an upper ceiling of 30 mg DOC/L when measured DOC concentrations entered are greater than 30 mg/L. <u>Implication:</u> When this flag is shown it is likely to mean that the calculated PNEC is relatively precautionary compared to the real situation. If the RCR gives a marginal fail, it may actually be a pass. If the RCR is a pass then no further action is necessary.
All metals + DOC	<i>! Local HC5 has been calculated using an appropriate value from within the validated range despite DOC is below the lower end of the validated range for the BLMs. See</i>	The applicability range of the bio-met tool is set a 0.1-30 mg DOC/L. Therefore, the local HC5-calculations are held at a lower ceiling of 0.1 mg DOC/L when measured DOC

	the Guidance Manual, Section 2.5, for more information.	<p>concentrations entered are lower than 0.1 mg/L.</p> <p><u>Implication:</u> When this flag is shown it is likely to mean that the calculated PNEC is underprotective compared to the real situation. If the RCR shows a marginal pass, the local HC5 requires further investigation. Several approaches are given in the bio-met Guidance Manual Section 2.5.</p>
Cu + low pH	<i>! Local HC5 has been calculated using an appropriate value from within the validated range despite pH is below the lower end of the validated range for Cu. See the Guidance Manual, Section 2.5, for more information.</i>	<p>The pH of the sample(s) under investigation is below the lower limit of the validated range for the CuBLM (pH 6.0-8.5), and therefore also for bio-met.</p> <p><u>Implication:</u> The bio-met-local HC5 has been calculated using a pH value of 6. The actual HC5 could be lower. If the RCR shows a marginal pass, the local HC5 requires further investigation. Several approaches are given in the bio-met Guidance Manual Section 2.5.</p>
Cu + low Ca	<i>! Local HC5 has been calculated using an appropriate value from within the validated range despite Ca is below the lower end of the validated range for Cu. See the Guidance Manual, Section 2.5, for more information.</i>	<p>The calcium concentration in the sample(s) under investigation is below the lower limit of the validated range for the CuBLM (Ca 3-129 mg/L), and therefore also for bio-met.</p> <p><u>Implication:</u> The bio-met-local HC5 has been calculated using a Ca concentration of 3 mg/L. The actual HC5 could be higher. If the RCR shows a marginal fail, the local HC5 requires further investigation. Several approaches are given in the bio-met Guidance Manual Section 2.5.</p>
Cu + high pH	<i>! Local HC5 has been calculated using an appropriate value from within the validated range despite pH is above the higher end of the validated range for Cu. See the Guidance Manual, Section 2.5, for more information.</i>	<p>The pH of the sample(s) under investigation is above the upper limit of the validated range for the CuBLM (pH 6.0-8.5), and therefore also for bio-met.</p> <p><u>Implication:</u> The bio-met-local HC5 has been calculated using a pH value of 8.5. The actual HC5 could be lower. If the RCR shows a marginal pass, the local HC5 requires further investigation. Several approaches are given in the bio-met Guidance Manual Section 2.5.</p>
Cu + high Ca	<i>! Local HC5 has been calculated using an appropriate value from within the validated range despite Ca is above the higher end of the validated range for Cu. See the Guidance Manual, Section 2.5, for more information.</i>	<p>The calcium concentration in the sample(s) under investigation is above the upper limit of the validated range for the CuBLM (Ca 3-129 mg/L), and therefore also for bio-met.</p> <p><u>Implication:</u> The bio-met-local HC5 has been calculated using a Ca concentration within the validated range. The actual HC5 could be lower. If the RCR shows a marginal pass, the local HC5 requires further</p>

		investigation. Several approaches are given in the bio-met Guidance Manual Section 2.5.
Zn + low Ca	For Zinc: <i>! Local HC5 has been calculated using an appropriate value from within the validated range despite Ca is below the lower end of the validated range for Zn. See the Guidance Manual, Section 2.5, for more information.</i>	The calcium concentration in the sample(s) under investigation is below the lower limit of the validated range for the ZnBLM (Ca 5-160 mg/L), and therefore also for bio-met. <u>Implication:</u> The bio-met-local HC5 has been calculated using a Ca concentration within the validated range. The actual HC5 could be lower. If the RCR shows a marginal pass, the local HC5 requires further investigation. Several approaches are given in the bio-met Guidance Manual Section 2.5.
Zn + low pH	<i>! Local HC5 has been calculated using an appropriate value from within the validated range despite pH is below the lower end of the validated range for Zn. See the Guidance Manual, Section 2.5, for more information.</i>	The pH of the sample(s) under investigation is below the lower limit of the validated range for the ZnBLM (pH 5.5-8.5), and therefore also for bio-met. <u>Implication:</u> The bio-met-local HC5 has been calculated using a pH value within the validated range. The actual HC5 could be higher. If the RCR shows a marginal fail, the local HC5 requires further investigation. Several approaches are given in the bio-met Guidance Manual Section 2.5.
Zn + high Ca	<i>! Local HC5 has been calculated using an appropriate value from within the validated range despite Ca is above the higher end of the validated range for Zn. See the Guidance Manual, Section 2.5, for more information.</i>	The calcium concentration in the sample(s) under investigation is above the upper limit of the validated range for the ZnBLM (Ca 5-160 mg/L), and therefore also for bio-met. <u>Implication:</u> The bio-met-local HC5 has been calculated using a Ca concentration within the validated range. The actual HC5 could be higher. If the RCR shows a marginal fail, the local HC5 requires further investigation. Several approaches are given in the bio-met Guidance Manual Section 2.5.
Zn + high pH	<i>! Local HC5 has been calculated using an appropriate value from within the validated range despite pH is above the higher end of the validated range for Zn. See the Guidance Manual, Section 2.5, for more information.</i>	The pH of the sample(s) under investigation is above the upper limit of the validated range for the ZnBLM (pH 5.5-8.5), and therefore also for bio-met. <u>Implication:</u> The bio-met-local HC5 has been calculated using a pH value within the validated range. The actual HC5 could be higher. If the RCR shows a marginal fail, the local HC5 requires further investigation. Several approaches are given in the bio-met Guidance Manual Section 2.5.
Ni + low pH	<i>! Local HC5 has been calculated using an appropriate value from within the validated range despite pH is below the lower end of the validated range for Ni. See the Guidance Manual, Section 2.5, for more information.</i>	The pH of the sample(s) under investigation is below the lower limit of the validated range for the NiBLM (pH 6.5-8.2), and therefore also for bio-met. <u>Implication:</u> The bio-met-local HC5 has been calculated using a pH value of 6.5. The

		actual HC5 could be higher. If the RCR shows a marginal fail, the local HC5 requires further investigation. Several approaches are given in the bio-met Guidance Manual Section 2.5.
Ni + low Ca	<i>! Local HC5 has been calculated using an appropriate value from within the validated range despite Ca is below the lower end of the validated range for Ni. See the Guidance Manual, Section 2.5, for more information.</i>	<p>The calcium concentration in the sample(s) under investigation is below the lower limit of the validated range for the NiBLM (Ca 2-88 mg/L), and therefore also for bio-met.</p> <p><u>Implication:</u> The bio-met-local HC5 has been calculated using a Ca concentration within the validated range. The actual HC5 could be lower. If the RCR shows a marginal pass, the local HC5 requires further investigation. Several approaches are given in the bio-met Guidance Manual Section 2.5.</p>
Ni + high pH	<i>! Local HC5 has been calculated using an appropriate value from within the validated range despite pH is above the higher end of the validated range for Ni. See the Guidance Manual, Section 2.5, for more information.</i>	<p>The pH of the sample(s) under investigation is above the upper limit of the validated range for the NiBLM (pH 6.5-8.2), and therefore also for bio-met.</p> <p><u>Implication:</u> The bio-met-local HC5 has been calculated using a pH value within the validated range. The actual HC5 could be lower. If the RCR shows a marginal pass, the local HC5 requires further investigation. Several approaches are given in the bio-met Guidance Manual Section 2.5.</p>
Ni + high Ca	<i>! Local HC5 has been calculated using an appropriate value from within the validated range despite Ca is above the higher end of the validated range for Ni. See the Guidance Manual, Section 2.5, for more information.</i>	<p>The calcium concentration in the sample(s) under investigation is above the upper limit of the validated range for the NiBLM (Ca 2-88 mg/L), and therefore also for bio-met.</p> <p><u>Implication:</u> The bio-met-local HC5 has been calculated using a Ca concentration within the validated range. The actual HC5 could be higher. If the RCR shows a marginal fail, the local HC5 requires further investigation. Several approaches are given in the bio-met Guidance Manual Section 2.5.</p>
Pb + low pH	<i>! Local HC5 has been calculated using an appropriate value from within the validated range despite pH is below the lower end of the validated range for Pb. See the Guidance Manual, Section 2.5, for more information.</i>	<p>The pH of the sample(s) under investigation is below the lower limit of the validated range for the PbBLM (pH 6.3-8.4), and therefore also for bio-met.</p> <p><u>Implication:</u> The bio-met-local HC5 has been calculated using a pH value within the validated range. The actual HC5 could be higher. If the RCR shows a marginal fail, the local HC5 requires further investigation. Several approaches are given in the bio-met Guidance Manual Section 2.5.</p>
Pb + low Ca	<i>! Local HC5 has been calculated using an appropriate value from within the validated range despite Ca is below the lower end of the</i>	The calcium concentration in the sample(s) under investigation is below the lower limit of the validated range for the Pb BLM (Ca 2-204 mg/L), and therefore also for bio-met.

	<i>validated range for Pb. See the Guidance Manual, Section 2.5, for more information.</i>	<u>Implication:</u> The bio-met-local HC5 has been calculated using a Ca concentration within the validated range. The actual HC5 could be higher. If the RCR shows a marginal fail, the local HC5 requires further investigation. Several approaches are given in the bio-met Guidance Manual Section 2.5.
Pb + high pH	<i>! Local HC5 has been calculated using an appropriate value from within the validated range despite pH is above the higher end of the validated range for Pb. See the Guidance Manual, Section 2.5, for more information.</i>	<p>The pH of the sample(s) under investigation is above the upper limit of the validated range for the PbBLM (pH 6.3-8.4), and therefore also for bio-met.</p> <p><u>Implication:</u> The bio-met-local HC5 has been calculated using a pH value within the validated range. The actual HC5 could be higher. If the RCR shows a marginal fail, the local HC5 requires further investigation. Several approaches are given in the bio-met Guidance Manual Section 2.5.</p>
Pb + high Ca	<i>! Local HC5 has been calculated using an appropriate value from within the validated range despite Ca is above the higher end of the validated range for Pb. See the Guidance Manual, Section 2.5, for more information.</i>	<p>The calcium concentration in the sample(s) under investigation is above the upper limit of the validated range for the PbBLM, and therefore also for bio-met.</p> <p><u>Implication:</u> The bio-met-local HC5 has been calculated using a Ca concentration within the validated range. The actual HC5 could be lower. If the RCR shows a marginal pass, the local HC5 requires further investigation. Several approaches are given in the bio-met Guidance Manual Section 2.5.</p>
Co + low pH	<i>! Local HC5 has been calculated using an appropriate value from within the validated range despite pH is below the lower end of the validated range for Co. See the Guidance Manual, Section 2.5, for more information.</i>	<p>The pH of the sample(s) under investigation is below the lower limit of the validated range for the CoBLM (pH 6.2-8.5), and therefore also for bio-met.</p> <p><u>Implication:</u> The bio-met-local HC5 has been calculated using a pH value within the BLM boundaries. The actual HC5 could be higher. If the RCR shows a marginal fail, the local HC5 requires further investigation. Several approaches are given in the bio-met Guidance Manual Section 2.5.</p>
Co + low Ca	<i>! Local HC5 has been calculated using an appropriate value from within the validated range despite Ca is below the lower end of the validated range for Co. See the Guidance Manual, Section 2.5, for more information.</i>	<p>The calcium concentration in the sample(s) under investigation is below the lower limit of the validated range for the CoBLM (Ca 3-148 mg/L), and therefore also for bio-met.</p> <p><u>Implication:</u> The bio-met-local HC5 has been calculated using a Ca concentration within the validated range. The actual HC5 could be lower. If the RCR shows a marginal fail, the local HC5 requires further investigation. Several approaches are given in the bio-met Guidance Manual Section 2.5.</p>

Co + high pH	<i>! Local HC5 has been calculated using an appropriate value from within the validated range despite pH is above the higher end of the validated range for Co. See the Guidance Manual, Section 2.5, for more information.</i>	<p>The pH of the sample(s) under investigation is above the upper limit of the validated range for the CoBLM (pH 6.2-8.5), and therefore also for bio-met.</p> <p>Implication: The bio-met-local HC5 has been calculated using a pH value within the validated range. The actual HC5 could be lower. If the RCR shows a marginal pass, the local HC5 requires further investigation. Several approaches are given in the bio-met Guidance Manual Section 2.5.</p>
Co + high Ca	<i>! Local HC5 has been calculated using an appropriate value from within the validated range despite Ca is above the higher end of the validated range for Co. See the Guidance Manual, Section 2.5, for more information.</i>	<p>The calcium concentration in the sample(s) under investigation is above the upper limit of the validated range for the CoBLM (Ca 3-88 mg/L), and therefore also for bio-met.</p> <p>Implication: The bio-met-local HC5 has been calculated using a Ca concentration within the validated range. The actual HC5 could be higher. If the RCR shows a marginal fail, the local HC5 requires further investigation. Several approaches are given in the bio-met Guidance Manual Section 2.5.</p>
All metals	<i>High bioavailability conditions resulting in Local HC5 < Reference HC5. See the Guidance Manual, Section 2.1, for further measures</i>	In situations representing high bioavailability conditions, the local HC5 is lower than the reference HC5. In those situations, bio-met will return the reference HC5 in the output. Under these sensitive conditions, as a further measure, the use of a full BLM may be required to determine the Local HC5.

2) Other questions

Other FAQ	
Why is the RCR red coloured?	The RCR column characterises the potential risk. A RCR value of 1 or greater identifies a potential risk. In that case bio-met will return a red coloured RCR. If the RCR is red coloured the bioavailable metal concentration exceeds the local HC5. This indicates an exceedance of the local HC5.
How is a "marginal" pass or failure defined?	A marginal pass, or a marginal failure is considered to be a case where the Risk Characterisation Ratio (RCR) lies between 0.5 (marginal pass) and 2.0 (marginal failure).
What are the application ranges for pH and Ca of the bio-met tool?	In the table below, an overview of the validation ranges for the different metals in bio-met are given. The application range of the bio-met tool for each of the metals has been defined based on the application ranges of the full BLMs. The application range of the full BLMs encompasses both the calibration and validation ranges of the models. The calibration ranges of the BLMs represent the range of water chemistry parameters for the experiments used in the development of the model. The validation ranges represent the physico-chemical ranges in which the predictive performance of a model (developed based on synthetic waters) has been evaluated in natural waters or for non-model species.

	<table><tr><th>Metal</th><th>pH</th><th>Ca (mg/L)</th></tr><tr><td>Zn</td><td>5.5-8.5</td><td>5-160</td></tr><tr><td>Ni</td><td>6.5-8.2¹</td><td>2.0-88</td></tr><tr><td>Cu</td><td>6-8.5</td><td>3.1-129²</td></tr><tr><td>Pb</td><td>6.3-8.4</td><td>3.6-204</td></tr><tr><td>Co</td><td>6.4-8.4</td><td>8.0-100</td></tr></table>	Metal	pH	Ca (mg/L)	Zn	5.5-8.5	5-160	Ni	6.5-8.2 ¹	2.0-88	Cu	6-8.5	3.1-129 ²	Pb	6.3-8.4	3.6-204	Co	6.4-8.4	8.0-100
Metal	pH	Ca (mg/L)																	
Zn	5.5-8.5	5-160																	
Ni	6.5-8.2 ¹	2.0-88																	
Cu	6-8.5	3.1-129 ²																	
Pb	6.3-8.4	3.6-204																	
Co	6.4-8.4	8.0-100																	
How important are the validation boundaries of the bio-met tool?	In cases where one or more of the key water chemistry input parameters is outside of the application range of the relevant BLM it is important to consider whether this applies to one or more of the parameters, and also how far the water chemistry of the water is from the validation limit for the BLM. Clearly, for waters which lie only slightly outside the BLM validation limit for a single parameter the results provided by bio-met will be more reliable than those provided for a water for which two parameters lie significantly outside the validation range of the BLM.																		
Are the full BLMs useful outside of their validation boundaries?	Full BLM calculations, which extrapolate the range of the models, may be useful for sites which fall slightly outside the validation boundaries. This is because they are mechanistically based, so it can be assumed that the same mechanisms continue to operate to some extent. Predictions made outside of the validation boundaries will always be uncertain, and need to be treated with caution.																		
If the models are not valid how can the water quality at the site be classified according to the EQS?	Ecological monitoring data is likely to be particularly important for sites which lie outside the BLM boundary conditions, and for which potential risks due to one or more BLM metals (Cu, Ni, Zn, Pb, and Co) are anticipated. Sites with very low metal exposures (i.e. with dissolved concentrations below the reference EQS _{bioavailable}) are not expected to be impacted.																		
How will metal toxicity be affected in very soft waters?	Hardness cations (Ca and Mg) can reduce the toxicity of metals, so lower hardness waters will tend to be more sensitive. However, for metals which bind very strongly to DOC (e.g. Cu and Pb) they can also reduce Me-DOC complexation so softer waters may not be more sensitive for copper and lead.																		
How will toxicity be affected in acidic waters?	Acidity (protons) can reduce metal toxicity in a similar manner to that caused by hardness cations, so lower pH conditions can tend to reduce toxicity. Low pH can also increase the chemical availability of metals in the solution, which could increase toxicity in some circumstances. In natural waters low pH is often associated with low hardness and these waters are often considered to be relatively sensitive.																		
How will toxicity be affected in soft, acid waters?	Soft acid waters can be very sensitive to metal toxicity, and any predictions made for such waters would represent an extrapolation for more than one parameter, consequently making the results more uncertain.																		
How will toxicity be affected in very hard waters?	Hardness cations (Ca and Mg) can reduce the toxicity of metals, so higher hardness waters will tend to be less sensitive. However, there is often a limit to the protective effect at high water hardness. The validation boundaries of the BLMs are usually within the protective range, and predictions which are made with the calcium concentration held at the upper validation limit (as is the case with																		

	<p>bio-met) will generally be reliable, provided that the hardness is not extreme (i.e. more than twice as high as the upper validation threshold).</p> <p>For metals which bind very strongly to DOC (e.g. Cu and Pb), high hardness can also reduce Me-DOC complexation so harder waters may not be necessarily less sensitive for copper and lead.</p>
How will toxicity be affected in high pH waters?	<p>Acidity (protons) can reduce metal toxicity in a similar manner to that caused by hardness cations, so higher pH conditions can tend to increase toxicity. High pH can also promote the formation of inorganic-metal complexes, which decreases toxicity. Which process dominates toxicity, and thus affects the direction of toxicity when pH increases, is dependent on the metal.</p>
How will toxicity be affected in high pH hard waters?	<p>High pH hard waters lie outside of the validation boundaries of the models, and can be sensitive waters for some of the metals, especially where DOC concentrations are low.</p>
How does DOC affect metal toxicity	<p>Because of the co-variation which is commonly observed between pH and hardness in surface waters the dominant bioavailability modifying effect is usually that of DOC. DOC complexes dissolved metals and therefore reduces their toxicity. This effect is greatest for copper and lead. Sites with low DOC concentrations will always be more sensitive than similar sites with higher DOC concentrations.</p>
How will toxicity be affected in high DOC waters.	<p>A validation limit has been set for the models at the highest DOC concentrations which were used for their validation (DOC concentration of 30 mg/L). Higher DOC concentrations will reduce toxicity, although the degree of any additional effect depends upon the metal in question.</p>
What type of water chemistry conditions are most sensitive for copper toxicity?	<p>Waters with low DOC concentrations are sensitive to copper toxicity. Extremes of pH can also increase toxicity.</p>
What type of water chemistry conditions are most sensitive for nickel toxicity?	<p>Waters with low DOC concentrations are sensitive to nickel toxicity. The combination of high pH and low hardness also increases toxicity.</p>
What type of water chemistry conditions are most sensitive for zinc toxicity?	<p>Waters with low DOC concentrations are sensitive to zinc toxicity. Extremes of pH, either high or low, and low hardness, also increase toxicity.</p>
What type of water chemistry conditions are most sensitive for lead toxicity?	<p>Waters with low DOC concentrations are sensitive to lead toxicity.</p>
What type of water chemistry conditions are most sensitive for cobalt toxicity?	<p>Waters with low DOC concentrations are sensitive to cobalt toxicity. High pH also increases toxicity.</p>
What about lead?	<p>For lead there is a full BLM, which has been implemented in the bio-met tool. However, the EQS for lead under the the Water Framework Directive (WFD) is based on a DOC correction. Therefore, it is currently not appropriate to use the bio-met tool (or full lead BLM) to derive bioavailable lead EQS (Environmental Quality Standard) for chemical status classifications under the Water Framework Directive (WFD). For the assessment of site-specific lead EQS under WFD, a screening tool is available for download (http://www.wca-environment.com/models-and-downloads/Pb-EQS-Screening-Tool) or the lead EQS can simply be calculated using this equation:</p> $EQS_{site} = EQS_{bioavailable} + (1.2 \times (DOC_{site} - DOC_{reference}))$ <p>Where:</p> <p>EQS_{site} = EQS at the site under consideration</p>

	<p>EQS_{bioavailable} = EQS for a reference condition to ensure all water bodies are protected</p> <p>DOC_{site} = Dissolved Organic Carbon at the site under consideration</p> <p>DOC_{reference} = average Dissolved Organic Carbon (DOC) concentration in the ecotoxicity tests that the EQS-bioavailable is based upon = 1.0 mg/L.</p>
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