bio-met bioavailability tool

User Guide (version 6.0)

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

www.bio-met.net
15/12/2025

CONTENTS

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) or local HC5 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.
$EQS_{bioavailable}$	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) and Technical Guidance for implementing Environmental Quality Standards (EQS) for metals (CIS guidance nr. 38). The $EQS_{bioavailable}$ is derived from the median HC5, normalized to conditions of high bioavailability.
	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, Zn and Co.
HC5	The HC5 is the 5th 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.
Prediction Range	The prediction range boundaries based on an assessment of bio-met predictions outside the validated range compared to BLM predictions and whether they are conservative
RCR	Risk Characterisation Ratio, also sometimes called the risk quotient. This is calculated by dividing the bioavailable metal concentration by the $EQS_{bioavailable}$.
	Values equal to or greater than 1 present a potential risk.
Reference HC5	The HC5 under conditions of high bioavailability ("reference condition")
User-friendly BLM Tool	The User-friendly tool mimics the BLM outputs through the use of look-up tables. It requires relatively few inputs and can readily be used in a compliance assessment framework.
Validated Range	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

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 because of industrial manufacturing, consumer use, recycling activities and normal geogenic processes. The risks posed by metals to the aquatic environment are managed in many countries. In Europe, these risks are managed by legislation including REACH[1] and the Water Framework Directive[2].

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 difficulties[3].

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 tool[4]. 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 Macro-Enabled MS Excel Spreadsheet.

- An evidence base[5] 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 studies[6] that demonstrate the application of bioavailability-based approaches within the risk-management of metals in the aquatic environment.

---

[1] https://environment.ec.europa.eu/topics/chemicals/reach-regulation_en
[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 Institute[7], International Zinc Association[8] and NiPERA[9] which started in 2009. The tools and resources on [bio-met.net](bio-met.net) have been developed collaboratively by ARCHE[10] and wca environment[11].The International Lead Association (ILA)[12] developed a full chronic BLM for lead in 2015[13] and was integrated in bio-met soon after. In 2022, the Cobalt Institute (CI)[14] joined the bio-met team, after they developed a full chronic BLM for cobalt (publication in preparation), which was integrated into version 5 of the bio-met tool. bio-met is one the bioavailability correction tools discussed in Chapter 3 of the CIS guidance No. 38 Technical Guidance for implementing Environmental Quality Standards (EQS) for metals[15].

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 assess compliance against the 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:

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

Where:
EQS_{site} = EQS at the site under consideration
$EQS_{bioavailable}$ = EQS for a reference condition to ensure all water bodies are protected
DOC_{site} = Dissolved Organic Carbon concentration at the site under consideration
$DOC_{reference}$ = average Dissolved Organic Carbon (DOC) concentration in the ecotoxicity tests
        that the EQSbioavailable is based upon = 1.0 mg L^{-1}.

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 (Ca) 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

---

[7] https://internationalcopper.org/
[8] http://www.zinc.org/
[9] http://www.nipera.org/
[10] http://www.arche-consulting.be/
[11] http://www.wca-environment.com/
[12] https://ila-lead.org/
[13] See https://arche-consulting.be/en/tools/lead-chronic-biotic-ligand-model for more information
[14] http://www.cobaltinstitute.org
[15] https://circabc.europa.eu/sd/a/a705289f-7001-4c7d-ac7c-1cf8140e2117/Guidance%20No%2038%20-%20Technical%20guidance%20for%20EQS%20for%20metals.pdf

bioavailable metal concentration and the $EQS_{bioavailable}$. Where applicable (e.g., for Cu, Zn and Co), the $EQS_{bioavailable}$ can be adapted by the user.

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. 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 takes 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 are 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** (e.g., input of physico-chemistry in the more common mg L^{-1} 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 ecotoxicity data sets[16]**.

- 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 45,000 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 the 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 a 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.

---

[16] For Cu, the ecotoxicity is currently undergoing revision in the context of the EU-wide harmonisation of the Cu EQS. Until this revision at EU-level is completed, the current tool reflects the ecotoxicity database and bioavailability models of the Cu VRAR (ECI 2008). An update of the Cu look-up table is foreseen once the updated EQS is finalised and implemented.

- Temperature, potassium [K], sulphate [SO$_4$] 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, with covered pH range depending on the pH validation range of the respective metal) and DOC (58 concentrations, encompassing the range of 0.1 - 30 mg DOC L^{-1} with an interval based on the distribution of the DOC concentrations in the FOREGS database, discussed in more detail in the next paragraph). Depending on the metal, 6 to 8 Ca-levels were selected, ranging between 0.8 and 140 mg Ca L^{-1}. Selected Ca concentrations were based on the Ca validation ranges of the bioavailability model of each metal.

Table 1.1 Overview physico-chemistry inputs for populating the bio-met look-up tables.

Metal	Design used for look-up table design[a]
Co	Ca[b]= 4, 8, 14, 40, 80 and 140 mg L^{-1} (6-levels) pH[c]=6.4-8.4 (18 pH levels) DOC[d]=0-30 mg L^{-1} (58 DOC levels)
Cu	Ca[b]= 4, 8, 14, 40, 80 and 140 mg L^{-1} (6-levels) pH[c]=6.0-8.5 (21 pH levels) DOC[d]=0-30 mg L^{-1} (58 DOC levels)
Ni	Ca[b]= 0.5[e], 1.75[e] , 1.8, 8, 14, 40, 80 and 110 mg L^{-1} (8-levels) pH[c]=5.9-8.7[e] (24 pH levels) DOC[d]=0-30 mg L^{-1} (58 DOC levels)
Pb	Ca[b]= 3.6, 8, 14, 40, 80 and 140 mg L^{-1} (6-levels) pH[c]=6.3-8.4 (19 pH levels) DOC[d]=0-30 mg L^{-1} (58 DOC levels)
Zn	Ca[b]= 1.0, 4.0, 8.0, 14, 40, 80 and 140 mg L^{-1} (7-levels) pH[c]=5.7-8.4 (23 pH levels) DOC[d]=0-30 mg L^{-1} (58 DOC levels)

[a] The lookup table design represent a full-factorial design: i.e. all Ca-levels x all pH-levels x all DOC-levels have been combined to populate the lookup table..
[b] The lowest Ca-level reflects the lower Ca limit of the Ca validation range per metal
[c] pH-levels are spaced with 0.125 pH-units.
[d] DOC levels represent different percentiles of DOC-values measured in the FOREGS database.
[e] For Ni, for all Ca-levels below 1.8 mg L^{-1} the soft-water extension of the Ni full bioavailability normalization approach was used (Peters et al. 2018). For all pH-levels above 8.2, the high pH extension of the Ni full bioavailability normalization approach was used (Nys et al. 2016).

The DOC concentrations in the look-up table were selected based on the DOC distribution of the FOREGS database[17] (see Figure 1.1 below). The FOREGS-EuroGeoSurveys Geochemical Baseline Database represents a set of water chemistry conditions of baseline surface waters across Europe (784 entries). From this database, 58 DOC concentrations have been selected reflecting different percentiles of the distribution of DOC concentrations in European surface waters, and used to populate the look-up tables for all metals.
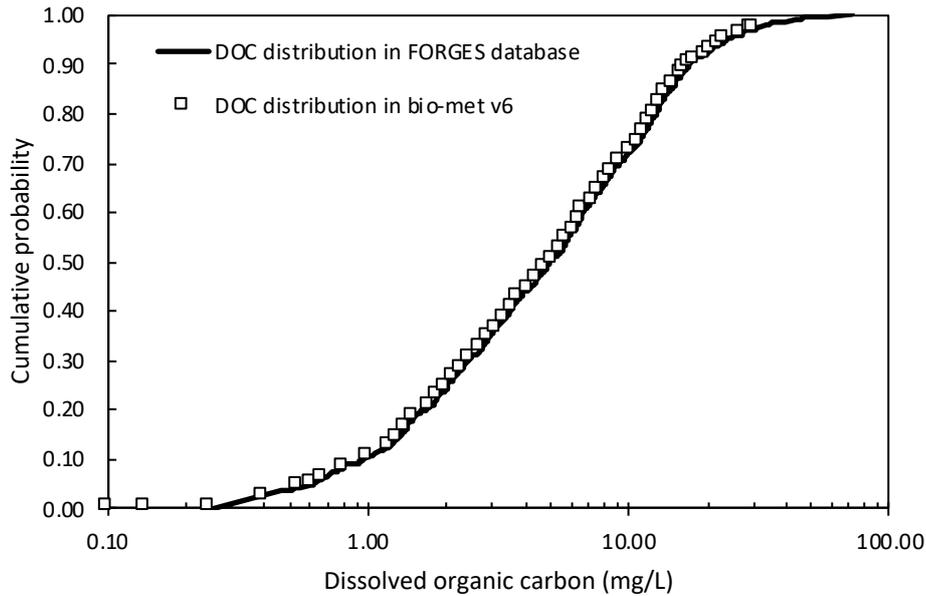
---

[17] http://weppi.gtk.fi/publ/foregsatlas/

Figure 1.1. Overview of DOC concentrations used to populate the look-up table in bio-met v 6 (open squares) vs DOC distribution of the FOREGS database (line).

2) The next step was to select and/or derive the relationships to calculate the low to moderate impact parameters. For all metals, except Cu, 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). For Cu, these parameters have been calculated based on log-linear relationships with Ca reported by Peters et al. (2011a) (Table 1.2). It is anticipated that the approach for Cu will be aligned with those of the other metals in the following update of bio-met.

Table 1.2 Relationships to calculate low to moderate impact parameters

	Relationships to calculate low to moderate impact parameters	
	Co, Pb, Ni[a], Zn	**Cu**
Mg (mg L^{-1})	$Ln(Mg) = -0.943 + 0.795*Ln(Ca)$	$Log_{10}(Mg) = -0.275 + 0.611*Log_{10}(Ca)$
Na (mg L^{-1})	$Ln(Na) = -0.674 + 0.571*Ln(Hardness)$	$Log_{10}(Na) = 0.103 + 0.707* Log_{10}(Ca)$
K (mg L^{-1})	$Ln(K) = -2.48 + 0.639*Ln(Hardness)$	$Log_{10}(K) = -0.539 + 0.669* Log_{10}(Ca)$
Cl (mg L^{-1})	$Ln(Cl) = -1.308 + 0.753*Ln(Hardness)$	$Log_{10}(Cl) = 0.276 + 0.718* Log_{10}(Ca)$
SO$_4$ (mg L^{-1})	$Ln(SO_4) = -1.466 + 0.928*Ln(Hardness)$	$Log_{10}(SO_4) = -0.077 + 0.954* Log_{10}(Ca)$
Alkalinity (mg CaCO$_3$ L^{-1})	$Ln(Alkalinity) = 0.151 + 0.938*Ln(Hardness)$	$Log_{10}(Alkalinity) = 0.188 + 1.044*Log_{10}(Ca)$

[a] For Ni, the activity of Fe^{3+} and Al^{2+} are also required input parameters, and have been calculated based on relationship with pH following De Schamphelaere et al. (2006).

The negligible to low impact parameters were set at following reasonable worst-case values: temperature = 15°C.

3) More than 45,000 simulations of combinations of pH, DOC and Ca were simulated to calculate HC5 for the different metals using the full BLM software. The exact full BLM approach has been detailed for each metal in Table 1.3, below. This is often referred to as the bio-met lookup table database.

Table 1.3 Full BLM versions used for simulations for each metal

Metal	Software reference
Co	Windward Chronic Co BLM version 3.15.2.41 using the chronic Co database of June 2021
Cu	Hydroqual BLM version 2.12 and ARCHE automatic spreadsheet processing script version 1.3
Ni	WHAM version 6.0.10 and semi-automatic spreadsheet processing script (Peters et al. 2023).
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) coupled with the PNEC database of 2023-update (Van Sprang 2023)
Zn	2023 Zn bioavailability models coupled with WHAM VII using semi-automatic excel spreadsheet processing script (Nys & Van Sprang 2023, Van Sprang & Nys 2024; publication in preparation)

4) The validated range of the bio-met tool for each of the metals has been defined based on the validation ranges of the full BLMs. The Table 1.4 summarizes the physicochemical validation range of the full BLM models (see Annex 2). The applicability range of the bio-met tool for DOC has been set at 0.1-30 mg L^{-1}. This range covers the <1% to 97% percentile of the DOC concentrations within the DOC distributions in the FOREGS database.

Table 1.4 Validated range of full BLMs

Metal	pH	Ca (mg L^{-1})
Co	6.4-8.4	4.0-147
Cu	6.0-8.5	3.1-129
Ni[1]	5.9-8.7	0.5-110
Pb	6.3-8.4	3.6-200
Zn	5.5-8.5	0.8-160

[1]Following Peters et al. (2023), the pH boundaries of the full Ni BLM have been extended to pH 8.7 (based on Nys et al. 2016a) and the Ca boundaries to lower Ca concentrations (0.5 mg L^{-1}; based on Peters et al. 2018)[18].

---

[18] The validated range presented for Ni in Table 1.4 differs from the validated range presented in CIS TGD No. 38 as the increased pH range and lower Ca range validation is included in bio-met, though in the TGD it is only stated and not included as the application ranges in the TGD reflect the state of the science when the Ni EQS was determined.

When the user inputs a value for pH, DOC and Ca outside its validated range, an out of boundary flag is given. A prediction using the best-fitting combination of validated pH, DOC and Ca values is returned only where analysis of the full BLM shows the prediction is conservative (further information available in Section 2.5 and Annex 4). If a prediction is not provided, further information on potential next steps is given.

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. Reference HC5 values for priority substances are not user-editable.

For specific pollutants under the WFD (Cu, Zn and Co), the reference HC5 in bio-met has been set to 1 µg L^{-1} for Cu, 10.9 µg L^{-1} for Zn and 1.64 µg L^{-1} for Co (Table 1.5). For Cu, Zn and Co, these values reflect "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. However, some Member States have derived other EQS$_{bioavailable}$ values for Zn, Co or Cu as Specific Pollutants under the WFD. The user can amend the EQS$_{bioavailable}$ of specific pollutants in bio-met.

Table 1.5 Default EQS$_{bioavailable}$ included in bio-met

Metal	EQS$_{bioavailable}$ (µg L^{-1})
Co	1.64*
Cu	1*
Ni	4
Pb	1.2
Zn	10.9*

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 6.0?

The updates in bio-met version 6.0 (released in 2025) included a revision of the look-up tables for Co, Ni, Pb and Zn to ensure that the included HC5-values, and as such the bio-met output, reflect the most up-to-date effects assessment methodologies for these metals (see 1.4.3.1 for references). In addition, the validation ranges of the different metals have also been adapted to reflect these of the latest bioavailability models.

The major usability changes performed to bio-met version 6.0 were to allow users to select the metal(s) to be assessed allowing for only information of interest to be generated, and to improve the processing time. Additional features include an export function for easier review of the data, and a built-in hardness-to-Ca converter. All the warning flags and guidance have been updated and embedded within the tool to better assist the user and the results of an assessment as to when bio-met provides predictions, based on results from the full BLMs have been incorporated.

1.4.3.3   Prediction performance of the bio-met v6 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 <u>largely (>95%) be within a factor of two</u> (as used during BLM developments and assessment of ecotoxicity data, see supporting Annex 1); or differently expressed, there should be less than 5% of "false positives" and "false negatives" (<u>within the applicability domain of the full BLMs</u>).

2. bio-met predictions are <u>preferably on the conservative side</u>; i.e. >50% of the acceptable predictions should be precautionary (<u>within the applicability domain of the full BLMs</u>).

Figure 1.2 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.
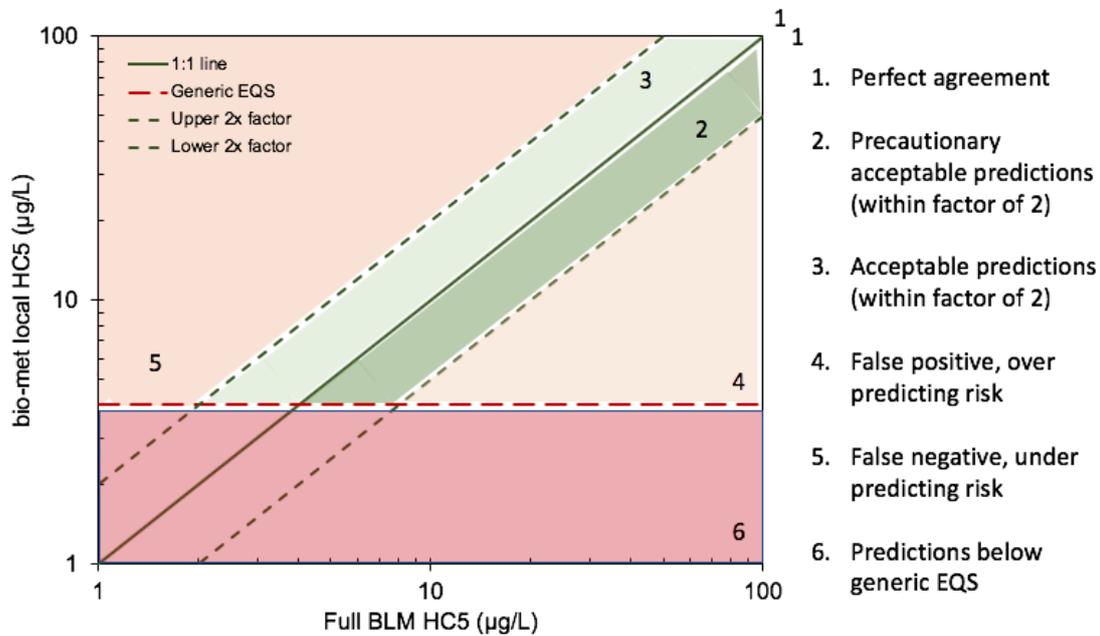
Figure 1.2 Pictorial representation of the criteria by which performance of the user-friendly tools is assessed.

To evaluate the performance of the bio-met v6-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 v6 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 1.6). For those metals for which acceptable predictions were less than 95% (i.e. Cu), the local HC5s predicted with more than 2-fold error where conservative estimates, i.e. 79% of the bio-met local HC5<full BLM simulated HC5. Hence, bio-met v6 results in sufficiently accurate predictions of local EQS compared to the full BLM simulated HC5.

Table 1.6: Performance of bio-met v6[a] assessed against performance criteria

	Co	Cu	Ni	Pb	Zn
Number of waters within BLM validation ranges	549	609	619	590	667
Acceptable predictions (% within factor 2)	100%	94%	99%	100%	100%
Conservatism (% of total predictions)	69%	79%	45%	70%	60%
Median prediction error	1.03	1.17	1.10	1.04	1.09

[a] Evaluated using the independent monitoring dataset, FOREGS (Salminen et al. 2005), only waters with physico-chemistry within the validation ranges have been included in the performance assessment. Full BLM HC5 were compared to bio-met v6.0 predicted HC5.

Figure 1.3 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 previous version of bio-met (v5.0) has been validated in more detail against natural field waters (see Peters et al. 2020)..
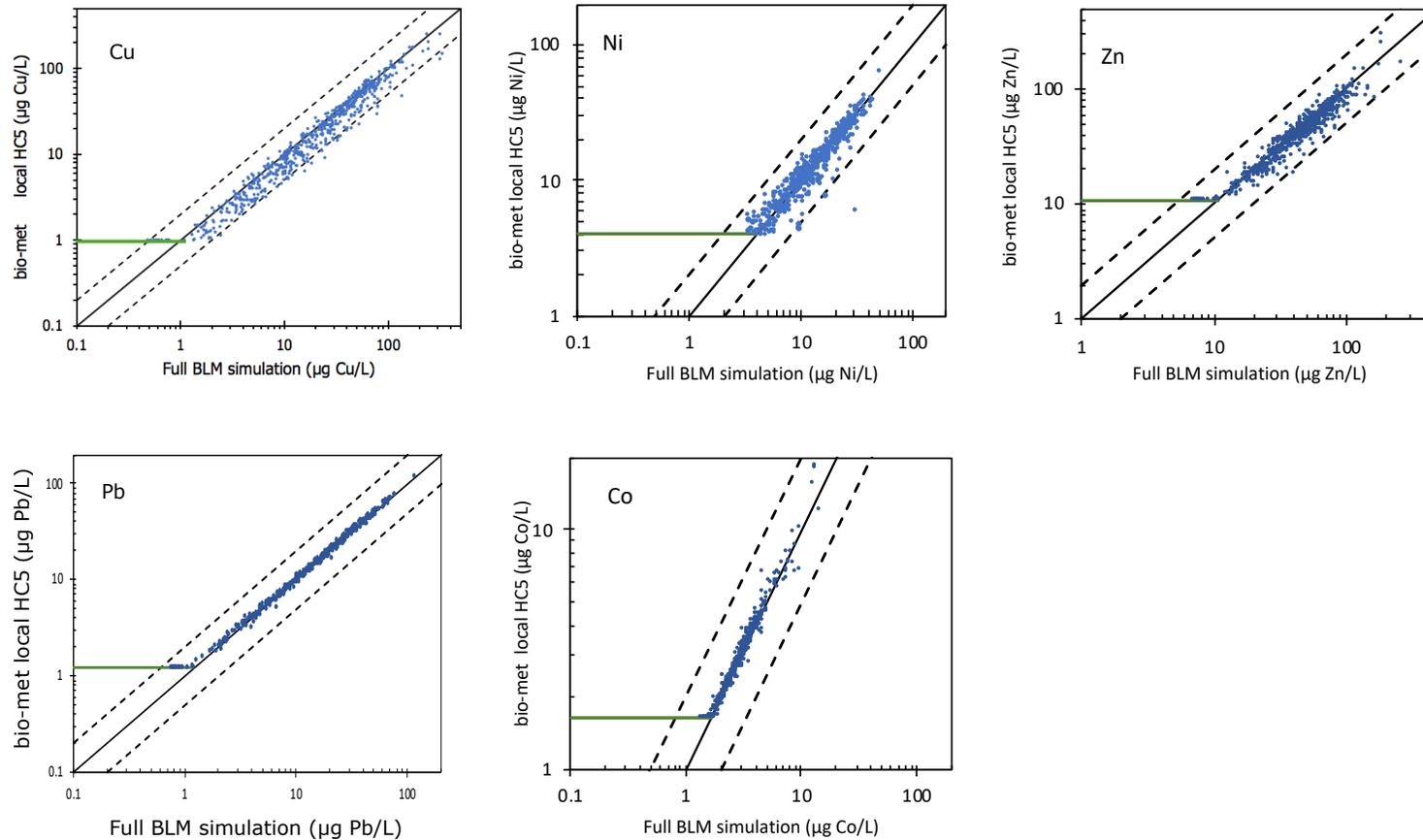
Figure 1.3 Results of the <u>independent validation</u>-exercise with the FOREGS database for Cu, Ni, Zn, Pb, and Co represented as full BLM simulated HC5 vs bio-met v6-predicted local HC5. The full line represents a perfect fit between the full BLM HC5 vs bio-met v6-predicted local HC5. The dashed lines 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[19]. 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.

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

---

[19] All screenshot figures of the tool have been generated using the Microsoft Windows operating system. There may be some minor difference in the appearance of the tool if macOS is used though the functionality remains the same.

The front page of the tool provides a description of the tool, including the validated and prediction ranges, 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.
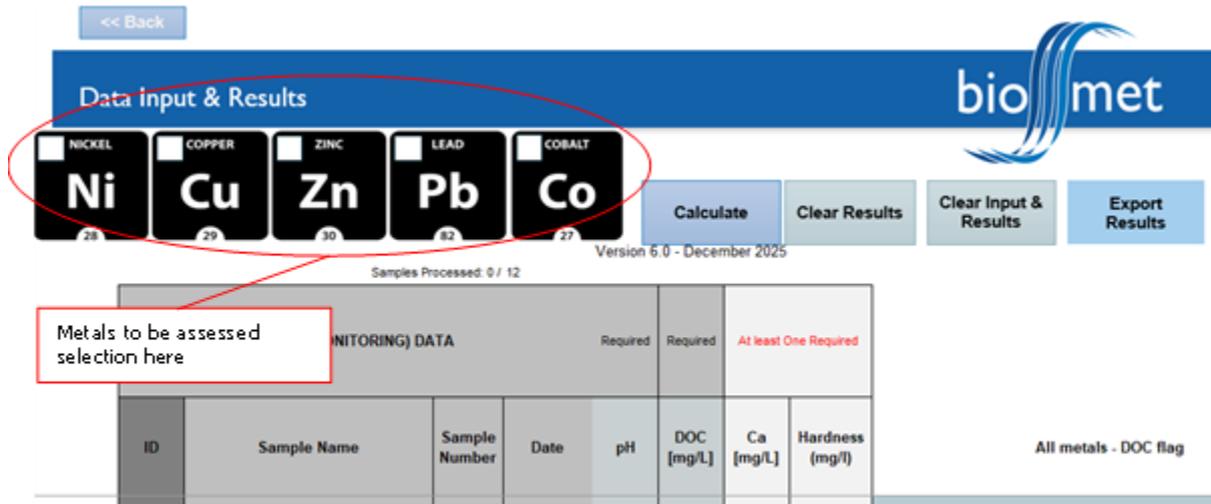
Figure 2.2   Metal selection prior to running the bio-met bioavailability tool

On clicking the 'Start' button the user is taken to the screen shown in Figure 2.2. Prior to running the tool the metals of interest to be assessed are required to be selected.
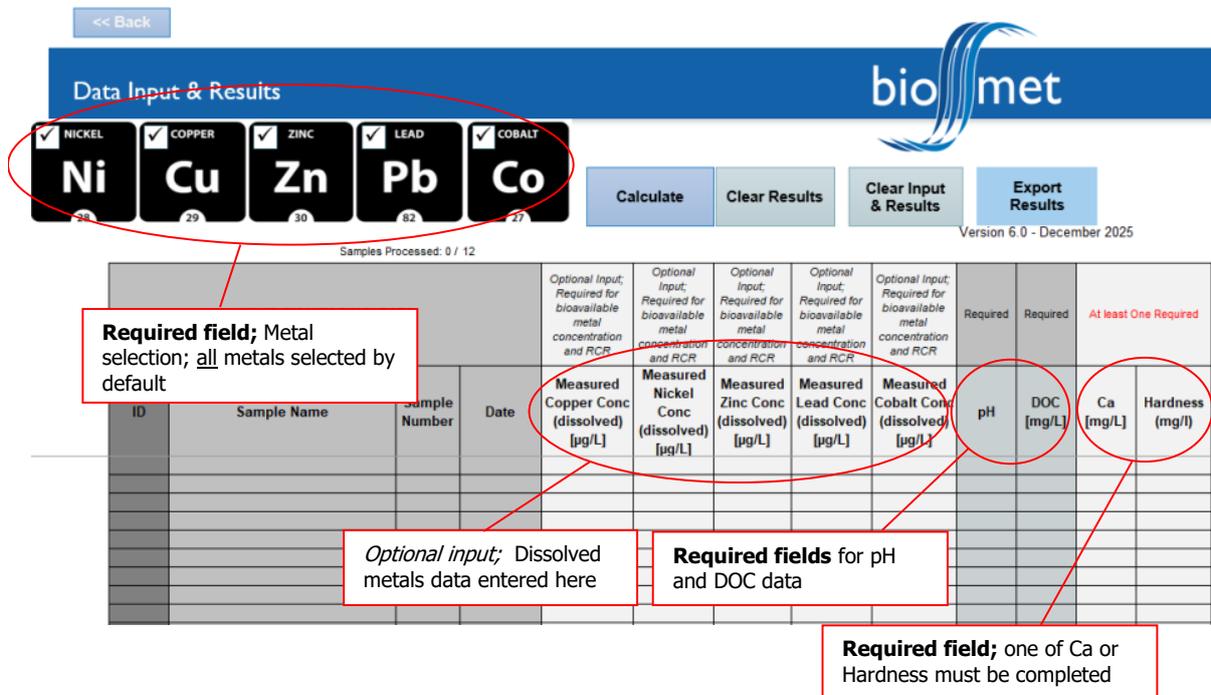
Figure 2.3   Required and optional input data to run the bio-met bioavailability tool

The required fields to run the model are highlighted in Figure 2.3. 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. A data input template file is also available on the bio-met website to assist in organising the input data into the correct format for running bio-met.

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) [µg l^{-1}], BioF, Bioavailable metal conc. [µg l^{-1}], RCR, Notes. When the calculations are complete a dialogue box (as shown in Figure 2.4 is shown).
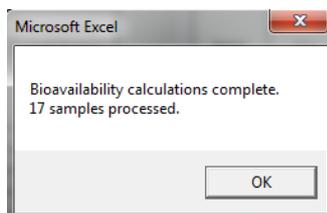
Figure 2.4   On-screen prompt once bioavailability calculations are complete

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

Figure 2.5 shows the screen that will be seen once bioavailability calculations for each row have been completed.

Figure 2.5   The bio-met bioavailability tool once calculations are complete

The warning columns 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) and a link to further information on the warning is available in the guidance column where appropriate.

When physicochemical input parameter data are outside of the validated range for a particular metal but within the prediction range of a metal, calculations are performed based on "best-fitting" data from within the validated ranges, and should be interpreted with caution (further information is detailed in Sections 2.5). When a physicochemical input parameter data are outside of the validated and prediction ranges for a particular metal, no calculation is performed. If two physiochemical input parameters are outside of the validated range for a metal, no prediction is provided and guidance is provided on potential next steps.

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 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 EQS$_{bioavailable}$ for Ni was established by the European Commission at 4 µg Ni L^{-1}. 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: 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 EQS$_{bioavailable}$ 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 EQS$_{bioavailable}$ was established. The technical basis for the increase in the pH boundary is described by Nys et al. (2016a). In addition, the lower Ca boundaries of the Ni bioavailability models have also been extended from 2 mg L^{-1} to 0.5 mg L^{-1} based on the experimental evidence published by Peters et al. (2018).

At the time of this writing (December 2025), 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 as well as the extension of the lower Ca boundary down to 0.5 mg L^{-1}, and any consequences this may have on the determination of the EQS bioavailable. As such, the update of the look-up table for Ni reflects these anticipated boundary extensions.

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 considered to have "sensitive conditions".

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

Bioavailable metal concentration [µg 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 Bioavailable Metal Concentration 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:

$$RCR = \frac{Bioavailable\ metal\ concentration}{EQS_{bioavailable}}$$

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 2.6.

Bio-met calculation steps
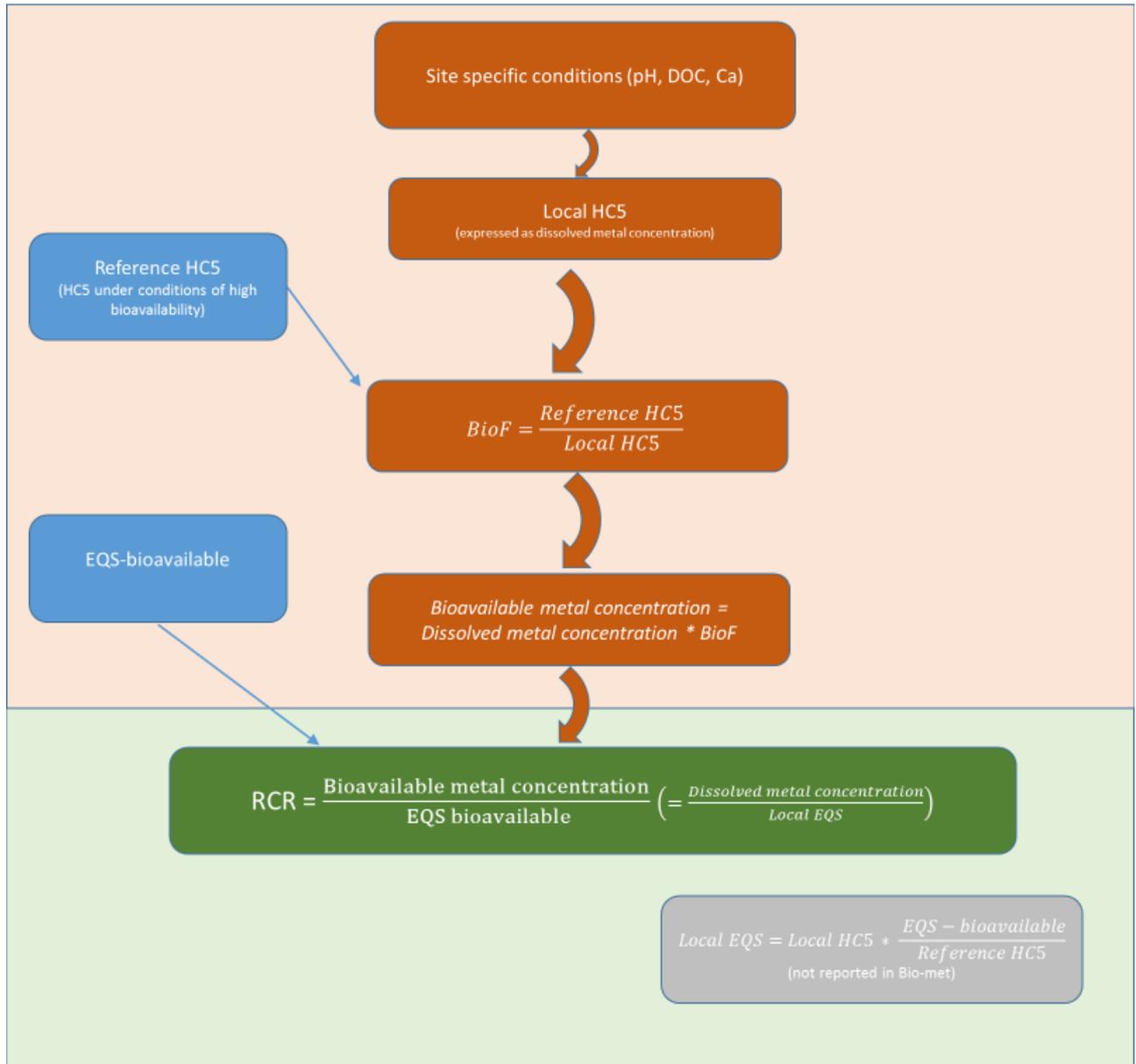
Figure 2.6   Calculation steps used in bio-met

2.2   Hardness Conversion Tool

bio-met bioavailability tool uses input data for Ca, rather than for water hardness. In bio-met version 6.0, if hardness data is available as mg L^{-1} of $CaCO_3$ and Ca data is unavailable, this can be entered directly into the tool (Figure 2.7) and the corresponding concentration of Ca from water hardness its calculated using the regression calculated by Peters et al. (2011a). 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. (2011a).

	Required	Required	At least one required	
c	pH	DOC [mg/L]	Ca [mg/L]	Hardness (mg/l)

Figure 2.7   Hardness Input Column

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 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 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 2.8 as detailed in Peters et al. (2011b).

Figure 2.8   Relationship between dissolved iron and dissolved organic carbon

DOC (mg L^{-1}) = 20.79 . Fe (dissolved, mg L^{-1}) + 2.32 r^2 = 0.738   Eq. 1

$\log_{10}$(DOC, mg L^{-1}) = 0.56 . $\log_{10}$(Fe, dissolved,mg L^{-1}) + 1.24 r^2 = 0.781   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, whist 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 outside the validated ranges of the full 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.

As part of the development of bio-met version 6.0, the Out-of-Boundary (OoB) behaviour of the full bioavailability normalisations was assessed. The OoB behaviour is defined as the behaviour of HC5-predictions when the bioavailability models are applied out of their application boundaries (based on the calibration and validation ranges) compared to the application boundaries. This information was used to inform the appropriate prediction ranges for each metal. For the OoB behaviour evaluation a limited structured physico-chemistry design was utilised, in which the direction in normalized HC5 when extrapolating outside the full bioavailability model boundaries was evaluated, i.e. whether predictions outside of the boundary were conservative compared to the boundary condition or not. A summary of the extent of the assessment for each physico-chemical input is presented in Table 2.1 and the metals specific conditions are presented in Appendix 4.

Table 2.1   Summary of the Out-of-Boundary (OoB) behaviour analysis conditions

Out of boundary parameter	Levels for OoB-behaviour analysis	pH conditions within boundaries considered	Ca conditions within boundaries considered
pH	0.25 and 0.5 pH units below lower pH boundary / above upper pH boundary	-	all Ca-levels considered in look-up table lay-out
Ca	0.5-fold below lowest Ca boundary / 1.5 and 2.0-fold above highest Ca boundary	pH levels within boundaries, with 0.5 pH unit interval	-
DOC	98th and >99th percentile of FOREGS DOC distribution		all Ca-levels considered in look-up table lay-out

The findings from this assessment resulted in the following prediction ranges (Table 2.2) being applied for each metal within bio-met (further information for each metal is included in Annex 3):

Table 2.2   Validated and Prediction Ranges in bio-met 6 for each metal

Metal	Validated Range		Prediction Range	
	pH	Ca (mg L^{-1})	pH	Ca (mg L^{-1})
Co	6.4 - 8.4	4.0 - 147	5.9 - 8.4	2.0 - 294
Cu	6.0 - 8.5	3.1 - 129	6.0 - 8.5	1.55 - 129
Ni	5.9 - 8.7	0.5 - 110	5.9 - 8.7	0.5 - 220
Pb	6.3 - 8.4	3.6 - 200	6.3 - 8.9	3.6 - 200
Zn	5.5 - 8.5	0.8 - 160	5.5 - 8.5	0.4 - 300

If a parameter is outside of the prediction range, or if both pH and Ca are outside the validated range for a metal, due to the uncertainty that would be associated with any calculations bio-met does not provide a prediction. In these cases, several options are available for treating conditions which are outside of the validated and predictions ranges of the BLMs are available.

1. For waters with extreme pH conditions, check the pH of the water

2. Apply the Full BLM model if appropriate

3. Consider 100% bioavailability of the metal (i.e. apply the EQS$_{bioavailable}$)

4. Extrapolation (e.g. consider complexation of metal by DOC only)

5. Bioassays (including ecotox. tests, WER, and ecological community monitoring)

6. Derive a site-specific Local EQS

If the pH of the water being assessed is the driving factor behind a prediction not being provided, the assessor may wish to confirm that the inputted value is correct and, if it is reliable, consider whether there are other issues associated with the pH of this water that may influence the ability to achieve 'Good Status'. Water pH can be an important factor affecting the health of an aquatic ecosystem and environments with extreme pH conditions may have other significant challenges in addition to metal contamination that will affect their ecological status.

Considering 100% bioavailability of the metal applies the $EQS_{bioavailable}$ outside the applicable conditions. Whilst this approach could be applied to many situations it can effectively result in a step change to the standard where the boundary conditions are met.

Extrapolation options could apply the BLMs outside their applicability ranges. This 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, Ca and DOC are limited to the allowable range, irrespective of the values entered into the tool, although actual measured values, which are outside these ranges, could be applied using the full BLMs. However, expert judgement is always needed to evaluate how far the full BLM can be extrapolated outside its validated ranges.

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 (WER) 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 $PNEC_{softwater}$ for Zn which was derived for the Existing Substances Regulations Risk Assessment Report on Zinc and Zinc compounds[20], although other approaches to developing specific localised standards may be more appropriate. A Water Effects Ratio approach could be used to correct the EQS under standard conditions in order to derive a site-specific Local EQS.

---

[20] https://dx.doi.org/10.2788/40041

REFERENCES

Boyd CE, CS Tucker, R Viriyatum. 2011. Interpretation of pH, acidity and alkalinity in aquaculture and fisheries. North American Journal of Aquaculture. 73:403-408.

Crommentuijn T, Polder MD, Van De Plassche EJ. 1997. Maximum permissible concentrations and negligible concentrations for metals, taking background concentrations into account. RIVM, Bilthoven, The Netherlands. Report no. 601501001.

De Schamphelaere KAC, Janssen CR. 2002. A biotic ligand model predicting acute copper toxicity for *Daphnia magna*: the effects of calcium, magnesium, sodium, potassium, and pH. Environmental Science and Technology 36: 48-54.

De Schamphelaere KAC, Vasconcelos FM, Heijerick DG, Tack FMG, Delbeke K, Allen HE, Janssen CR. 2003. Development and field validation of a predictive copper toxicity model for the green alga *Pseudokirchneriella subcapitata*. Environmental Toxicology and Chemistry 22: 2454-2465.

De Schamphelaere KAC, Janssen CR . 2004. Effects of chronic dietary copper exposure on growth and reproduction of *Daphnia magna*. Environmental Toxicology and Chemistry, 23(8), 2038-2047.

De Schamphelaere KAC, Janssen CR. 2004. Effects of dissolved organic carbon concentration and source, pH and water hardness on chronic toxicity of copper to *Daphnia magna*. Environmental Toxicology and Chemistry, 23(5), 1115-1122.

De Schamphelaere KAC, Janssen C. 2004c. Bioavailability of chronic toxicity of zinc to juvenile Rainbow Trout (*Oncorhynchus myskiss*): comparison with other fish species and development of a biotic ligand model. Environmental Science and Technology 38: 6201-6209.

De Schamphelaere KAC, Lofts S, Janssen CR. 2005. Bioavailability models for predicting acute and chronic toxicity of zinc to algae, daphnids and fish in natural surface waters. Environmental Toxicology and Chemistry 24: 1190-1197.

De Schamphelaere KAC, Van Laer L, Deleebeeck NME, Muyssen BTA, Degryse F, Smolders E, Janssen C. 2006. Nickel speciation and ecotoxicity in European natural surface waters: development, refinement and validation of bioavailability models. Report prepared for the Nickel Producers Environmental Research Association (NiPERA), Durham, NC, USA, Gent University, Laboratory of Environmental Toxicology and Aquatic Ecology, Gent, Belgium. Available online (Last accessed: 10/3/2015): https://biblio.ugent.be/input/download?func=downloadFile&recordOId=5785290&fileOId=5785373.

De Schamphelaere KAC, Nys C, Janssen CR. 2014. Ecotoxicity of lead (Pb) to freshwater green algae: development and validation of a bioavailability model and inter-species sensitivity comparison. Aquatic Toxicology 155: 348-359.

Deleebeeck NME, De Schamphelaere KAC, Janssen C. 2008. A novel method for predicting chronic nickel bioavailability and toxicity to *Daphnia magna* in artificial and natural waters. Environmental Toxicology and Chemistry, 27(10), 2097-2107.

Deleebeeck NME, De Schamphelaere KAC, Janssen CR. 2007a. A bioavailability model predicting the toxicity of nickel to rainbow trout (*Oncorhynchus mykiss*) and fathead minnow (*Pimephales promelas*) in synthetic and natural waters. Ecotoxicology and Environmental Safety 67: 1-13.

Deleebeeck NME, Muyssen BTA, De Laender F, Janssen CR, De Schamphelaere KAC. 2007b. Comparison of nickel toxicity to cladocerans in soft versus hard surface waters. Aquatic Toxicology 84: 223-235.

Deleebeeck NME, De Schamphelaere KAC, Janssen CR. 2008. A novel method for predicting chronic nickel bioavailability and toxicity to *Daphnia magna* in artificial and natural waters. Environmental Toxicology and Chemistry 27: 2097–2107.

Deleebeeck NME, De Schamphelaere KAC, Janssen CR. 2009a. Effects of Mg2+ and H+ on the toxicity of Ni2+ to the unicellular green alga *Pseudokirchneriella subcapitata*: model development and validation with surface waters. Science of the Total Environment 407: 1901–1914.

Deleebeeck NME, De Laender F, Chepurnov VA, Vyverman W, Janssen CR, De Schamphelaere KAC. 2009b. A single bioavailability model can accurately predict Ni toxicity to green microalgae in soft and hard surface waters. Water Research 43: 1935-1947.

European Commission (EC). 2007. Proposal for a Directive of the European Parliament and of the Council on Environmental Quality Standards in the Field of Water Policy and Amending Directive 2000/60/EC. COM(2007) 397 final. Brussels (BE): EC.

Heijerick DG, De Schamphelaere KAC, Van Sprang PA, Janssen CR. 2005. Development of a chronic biotic ligand model for Daphnia magna. Ecotoxicology and Environmental Safety 62: 1-10.

Heijerick DG, KAC De Schamphelaere, CR Janssen. 2007. Draft Interim Report on the development and validation of chronic Cobalt Biotic Ligand Models for freshwater environment: an initial BLM for freshwater green alga *Pseudokirchneriella subcapitata*. Prepared for the Cobalt Development Institute.

International Organisation FOR Standardisation (ISO). 2005. Soil quality: Guidance on the determination of background values. ISO 19258: 2005. International Organization for Standardization.

Nys C, Janssen CR, Mager EM, Esbaugh AJ, Brix KV, Grosell M, Stubblefield WA, Holtze K, De Schamphelaere K. 2014. Development and Validation of a Biotic Ligand Model for Predicting Chronic Toxicity of Lead to *Ceriodaphnia Dubia*. Environmental Toxicology and Chemistry, 33(2): 394–403.

Nys C, Janssen CR, De Schamphelaere KAC. 2016b. Development and validation of a chronic Pb bioavailability model for the freshwater rotifer *Brachionus calyciflorus*. Environmental Toxicology and Chemistry, 35 (12): 2977-2986.

Nys C, Janssen CR, Van Sprang P, De Schamphelaere KAC. 2016a. The effect of pH on chronic aquatic nickel toxicity is dependent on the pH itself: Extending the chronic nickel bioavailability models. Environmental Toxicology and Chemistry, 35(5): 1097-1106.

Oregon State University Aquatic Toxicology Laboratory. 2013a. Chronic Toxicity of Cobalt to the Cladoceran, *Ceriodaphnia dubia*: Refinement and Validation of the Biotic Ligand Model. Prepared by Oregon State University

Oregon State University Aquatic Toxicology Laboratory. 2013b. Chronic Toxicity of Cobalt to the Cladoceran, *Ceriodaphnia dubia*: Validation of the Biotic Ligand Model using Natural Waters. Prepared by Oregon State University Aquatic Toxicology Laboratory, Albany, Oregon, USA. March 2013.

Oregon State University Aquatic Toxicology Laboratory. 2013c. Chronic Toxicity of Cobalt to the Fathead Minnow, *Pimephales promelas*: Refinement and Validation of the Biotic Ligand Model. Prepared by Oregon State University Aquatic Toxicology Laboratory, Albany, Oregon, USA. Feb 2013.

Oregon State University Aquatic Toxicology Laboratory. 2013d. Chronic Toxicity of Cobalt to the Fathead Minnow, *Pimephales promelas*: Validation of the Biotic Ligand Model using Natural Waters. Prepared by Oregon State University Aquatic Toxicology Laboratory, Albany, Oregon, USA. Feb 2013.

Oregon State University Aquatic Toxicology Laboratory. 2015. Chronic toxicity of cobalt, under varying pH conditions, to the green algae, *Pseudokirchneriella subcapitata*: Empirical data for the Biotic Ligand Model (BLM). Prepared by Oregon State University Aquatic Toxicology Laboratory, Albany, Oregon, USA. Mar 2015

Oregon State University Aquatic Toxicology Laboratory. 2017. Chronic Toxicity of Cobalt to the Cladoceran, *Ceriodaphnia dubia*, in Natural Waters: Validation of the Cobalt Biotic Ligand Model. Prepared for the Cobalt Development Institute, CDI Study 78. Final report. Oct 2017.

Oregon State University Aquatic Toxicology Laboratory. 2020a. Chronic toxicity of cobalt to the green algae, *Raphidocelis subcapitata*: Expansion of the empirical database for bioavailability modeling. Prepared for the Cobalt Institute, CI Study E01. Final Report: August 2020.

Oregon State University Aquatic Toxicology Laboratory. 2020b. Chronic Toxicity of Cobalt to the cladoceran, *Ceriodaphnia dubia*: Expansion of the empirical database for bioavailability modeling. Prepared for the Cobalt Development Institute, CDI Study E01. Final Report: August 2020.

Oregon State University Aquatic Toxicology Laboratory. 2022. Chronic toxicity of cobalt to the cladoceran, *Ceriodaphnia dubia*: Expansion of the empirical database for bioavailability modeling (2021). CI Study E08.

Parametrix. 2005. Short-term chronic toxicity of cobalt to *Ceriodaphnia dubia*.

Parametrix. 2009a. Early life-stage toxicity of cobalt to the fathead minnow (*P. promelas*) under flow-through conditions.

Parametrix. 2009b. Short-term chronic toxicity of cobalt to the fathead minnow (*P. promelas*) under flow-through conditions.

Parametrix. 2010a. Chronic Toxicity of Cobalt to the Cladoceran, *Ceriodaphnia dubia*: Framework for the Development of a Biotic Ligand Model (BLM). Prepared by Parametrix, Corvallis, Oregon. April 2010

Parametrix. 2010b. Chronic toxicity of cobalt to the cladoceran, *Ceriodaphnia dubia*, in natural waters. Prepared by Parametrix, Corvallis, Oregon. April 2010.

Parametrix. 2010c. Chronic Toxicity of Cobalt to the Fathead Minnow (*Pimephales promelas*): Framework for the Development of a Biotic Ligand Model (BLM). Prepared by Parametrix, Corvallis, Oregon. April 2010

Parametrix. 2010. Chronic toxicity of cobalt to the fathead minnow, *Pimephales promelas*, in natural waters. Prepared by Parametrix, Corvallis, Oregon. April 2010.Peters A, Merrington G, De Schamphelaere K, Delbeke K. 2011a. Regulatory consideration of bioavailability for metals: simplification of input parameters for the chronic copper biotic ligand model. Integrated Environment Assessment and Management, 7(3), 437-444.

Peters A, Crane M, Adams W. 2011b. Effects of iron on benthic macroinvertebrate communities in the field. Bulletin of Environmental Contamination and Toxicology 86:591-595.Peters A, Merrington G, Schlekat CE, De Schamphelaere K, Stauber J, Batley G, Harford A, van Dam R, Pease C, Mooney T et al. 2018. Validation of the nickel biotic ligand model for locally relevant species in Australian freshwaters. Environmental Toxicology and Chemistry, 37 (10): 2566-2574.

Salminen R. (Chief-editor), Batista MJ, Bidovec M, Demetriades A, De Vivo B, De Vos W, Duris M, Gilucis A, Gregorauskiene V, Halamic J, Heitzmann P, Lima A, Jordan G, Klaver G, Klein P, Lis J, Locutura J, Marsina K, Mazreku A, O'Connor PJ, Olsson S.Å, Ottesen RT, Petersell V, Plant J.A, Reeder S, Salpeteur I, Sandstrom H, Siewers U, Steenfelt A, Tarvainen T. 2005. Geochemical Atlas of Europe. Part 1: Background Information, Methodology and Maps. Geological Survey of Finland, Espoo, Finland. [cited 2019 January 10]. Available from: http://weppi.gtk.fi/publ/foregsatlas/

Schlekat CE, Van Genderen E, De Schamphelaere KAC, Antunes PMC, Rogevich E, Stubblefield WA. 2010. Cross-species extrapolation of chronic nickel biotic ligand models. Science of the Total Environment 408: 6148-6157.

Tipping E. Corbishley HT. Koprivnjak JF. Lapworth DJ. Miller MP. Vincent CD. Hamilton-Taylor J. 2009. Quantification of natural DOM from UV Absorption at Two Wavelengths. Environmental Chemistry. Rapid Communication. October.

Van Sprang P, Nys C, Blust R, Chowdhury J, Gustafsson JP, Janssen CR, De Schamphelaere KAC. The derivation of effects threshold concentrations of Pb for European freshwater ecosystems. Environmental Toxicology and Chemistry 35: 1310-1320.

Van Regenmortel T, Berteloot O, Janssen CR, De Schamphelaere KAC. 2017. Analyzing the capacity of the *Daphnia magna* and *Pseudokirchneriella subcapitata* bioavailability models to predict zinc toxicity at high pH and low calcium concentrations and formulation of a generalized bioavailability model for *D. magna*. Environmental Toxicology and Chemistry 36: 2781-2798.

Working Group Chemicals. 2021. GUIDANCE NO 38 - technical guidance for implementing Environmental Quality Standards (EQS) for metals. Available online: https://circabc.europa.eu/sd/a/a705289f-7001-4c7d-ac7c-1cf8140e2117/Guidance%20No%2038%20-%20Technical%20guidance%20for%20EQS%20for%20metals.pdf(last accessed 12-12-2025).

Worms IAM, Wilkinson KJ. 2007. Ni uptake by a green alga. 2. Validation of equilibrium models for competition effects. Environmental Science & Technology 41: 4264-4270.

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* (Co and 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.

Cobalt (Stubblefield et al., in prep)
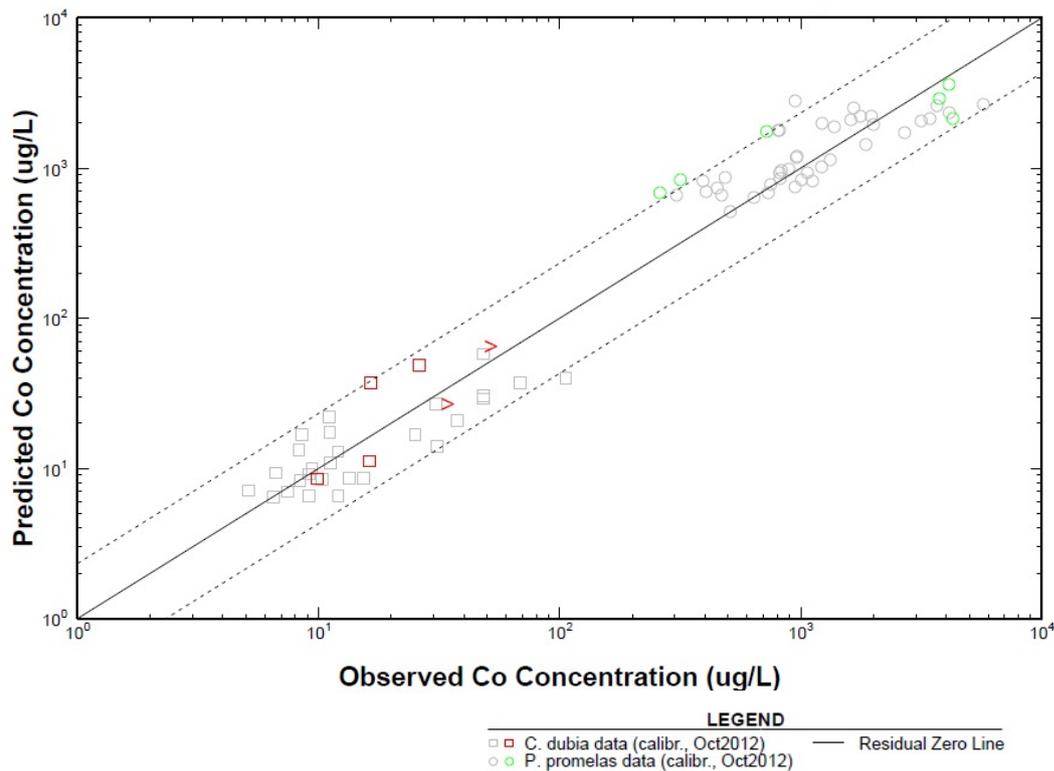
Figure A1.1. Predicted versus observed median effective concentration (EC50, expressed as mg filtered Co L^{-1}) of *Ceriodaphnia dubia* (squares) predicted with the chronic Co *Ceriodaphnia dubia* biotic ligand model (BLM) and of *Pimephales promelas* (circles) predicted with the chronic Co *Pimephales 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)

Copper (De Schamphelaere K. and C. Janssen. 2004)
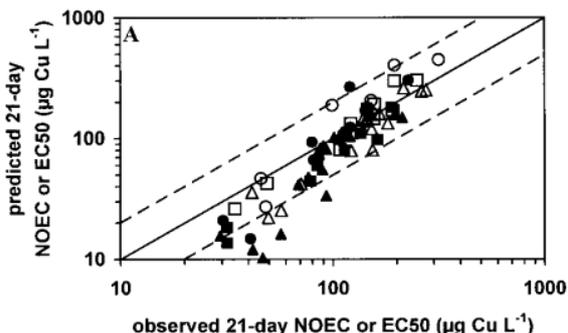
Figure A1.2. Predictive capacity of the chronic Cu biotic ligand model for *Daphnia magna* as shown by observed versus predicted 21-d no-observed-effect concentrations (NOECs; filled symbols) and 21-d 50% effective concentrations (EC50s; open symbols). Assumption for dissolved organic matter (DOM): 50% active fulvic acid Symbols: Bihain (●), Ossenkolk (Δ), 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)
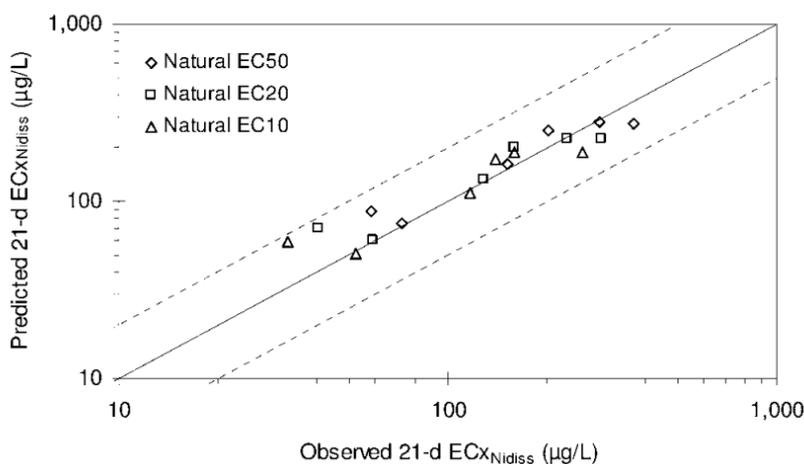
Figure A1.3. Predicted versus observed effect concentrations resulting in x% reduction of reproduction expressed as dissolved Ni concentration: predictions for all natural waters using optimized sensitivity parameters (Q50, Q20, and Q10, respectively). The solid line indicates a perfect match between predicted and observed ECx values; the dashed lines indicate ratios of 0.5 and 2 between observed and predicted ECx values.

Lead (Nys et al., 2014.)
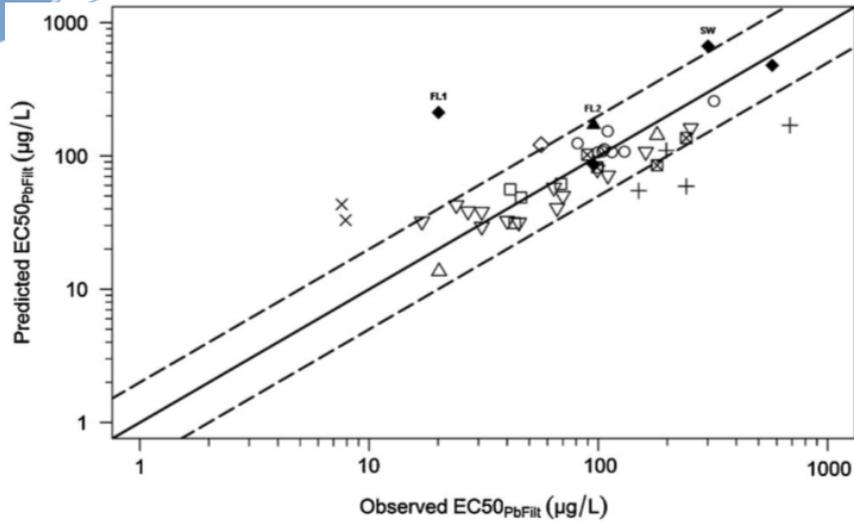
Figure A1.4. Predicted versus observed median effective concentration (EC50, expressed as mg filtered Pb L^{-1}) for the chronic Pb *Ceriodaphnia dubia* biotic ligand model (BLM) calibrated with the developed BLM calibrated with the clone-specific intrinsic sensitivities: predictions for the data used 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.

Zinc (Nys et al. in preparation)
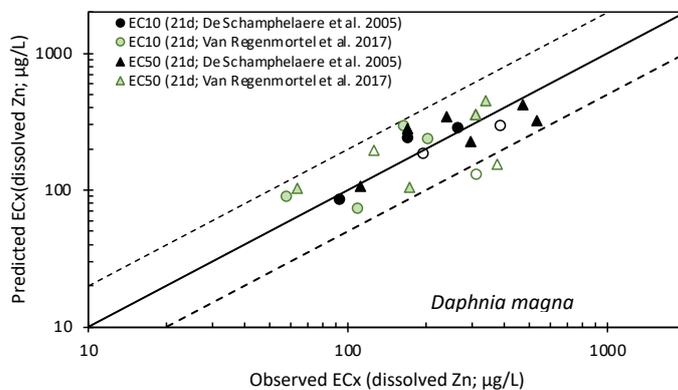
Figure A1.5. Predicted versus observed effect concentrations resulting in x% reduction of reproduction expressed as dissolved Zn concentration (21-d 10% effective concentrations (EC10s; circles) and 21-d 50% effective concentrations (EC50s; triangles)). The solid line indicates a perfect match between predicted and observed ECx values; the dashed lines indicate ratios of 0.5 and 2 between observed and predicted ECx values.

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 Co (Reference)	pH range for Cu (Reference)	pH range for Nickel (Reference)	pH range for Pb (Reference)	pH range for Zn (Reference)
Calibration range	Algae	6.1 - 8.4 (OSU 2015, OSU 2020a)	5.7 - 8.8 (De Schamphelaere et al. 2003b)	5.7 - 8.7 (Deleebeeck et al. 2009a)	6.0 - 8.0 (De Schamphelaere et al. 2014)	5.7 - 8.0 (De Schamphelaere et al. 2003a)
	Invert.	6.4-8.5 (Parametrix 2005; Parametrix 2010a; OSU 2013a; OSU 2020b)	5.6 - 8.7 (De Schamphelaere and Janssen, 2004a)	5.9 - 8.7[A] 6.5 - 8.7[B] (Deleebeeck et al. 2008; De Schamphelaere et al. 2006)	6.3 - 8.2 (Nys et al. 2014 ; Nys et al. 2016b)	5.5 - 8.0 (De Schamphelaere et al. 2003a)
	Fish	6.1 – 8.5 (Parametrix 2009a,b; Parametrix 2010c ; OSU 2013c)	6.6 - 8.7 (De Schamphelaere and Janssen, 2004c)	5.4 - 8.5 (Deleebeeck et al. 2007a)	6.3 - 8.2 (Van Sprang et al. 2016)	5.7 – 7.9 (De Schamphelaere et al. 2003a)
Validation range	Algae	7.0 – 7.8 (Heijerick et al. 2007)	5.5 - 8.7 (De Schamphelaere et al., 2003b, ECI 2008)	5.7 - 8.0 (Worms et al. 2007; Deleebeeck et al. 2009a)	6.0 - 8.4 (De Schamphelaere et al. 2014)	5.7 - 8.5 (De Schamphelaere et al. 2005; Van Regenmortel et al. 2017)
	Invert.	6.8 - 8.5 (Parametrix 2010b; OSU 2013b; OSU 2017)	5.5 - 8.5 (De Schamphelaere and Janssen 2004a, ECI 2008)	5.9 - 8.7[A] 6.5 - 8.7[B] (Nys et al. 2016a ; Peters et al. 2018)	6.0 - 8.6 (Nys et al. 2014 ; Nys et al. 2016b)	6.0 - 8.6 (U Gent 2015 unpublished report. De Schamphelaere et al. 2005; Van Regenmortel et al. 2017)
	Fish	6.6 - 8.1 (Parametrix 2010d ; OSU. 2013d)	6.0 - 8.6 (De Schamphelaere and Janssen 2004c, ECI 2008)	5.4 - 8.5 (Deleebeeck et al. 2007a; Peters et al. 2018)	6.4 - 8.0 (Van Sprang et al. 2016)	6.2 - 8.1 (De Schamphelaere et al. 2005)
Application range	"Full BLM"	**6.4-8.4**	**6.0 - 8.5**	**5.9 - 8.7**	**6.3 - 8.4**	**5.7 - 8.4**

[A] : *Daphnia magna* BLM
[B] : *Ceriodaphnia dubia* BLM

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 Co in mg L^{-1} (Reference)	DOC range for Co in mg L^{-1} (Reference)	DOC range for Ni in mg L^{-1} (Reference)	DOC range for Pb in mg L^{-1} (Reference)	DOC range for Zn in mg L^{-1} (Reference)
Calibration range	Algae	0.3 – 12.7 (OSU 2015)	1.3 - 20 (De Schamphelaere et al., 2003b)	0.1 * (Deleebeeck et al. 2009a)	2.1 - 10.6 (De Schamphelaere et al. 2014)	2.5 - 22.3 (De Schamphelaere et al. 2003a)
	Invert.	0.5 - 12.7 (Parametrix 2005; Parametrix 2010a; OSU 2013a; OSU 2022)	1.7 - 18 (De Schamphelaere and Janssen, 2004a)	0.1 * (Deleebeeck et al. 2008)	1.1 – 18.6 (Nys et al. 2014; Nys et al. 2016b)	0.3 – 5.0 (De Schamphelaere et al. 2003a)
	Fish	0.5 – 12.3 (Parametrix 2009a,b ; Parametrix 2010c; OSU 2013c)	1 ** (De Schamphelaere and Janssen, 2004c)	0.1 * (Deleebeeck et al. 2007a)	1.2 - 10.5 (Van Sprang et al. 2016)	0.3 - 22.9 (De Schamphelaere et al. 2003a)
Validation range	Algae	4.1 – 22.6 (Heijerick et al. 2007)	0 - 20 (De Schamphelaere et al. 2003b, ECI 2008)	2.5 - 25.8 (Worms et al. 2007; Deleebeeck et al. 2009b; Peters et al. 2018)	2.1 - 22.4 (De Schamphelaere et al. 2014)	0.3 - 12.0 (Van Regenmortel et al. 2017; De Schamphelaere et al., 2005)
	Invert.	0.7-19.8 (Parametrix 2010b; OSU 2013b; OSU 2017)	0 - 20 (De Schamphelaere and Janssen, 2004a, ECI 2008)	2.5 - 25.8 [A] 3.2 - 23.6 [B] (Deleebeeck et al. 2008 ; Peters et al. 2018)	0.4 - 31.5 (Nys et al. 2014 ; Nys et al. 2016b)	0.3 – 17.3 (Van Regenmortel et al. 2017; De Schamphelaere et al. 2005)
	Fish	0.1 – 19.8 (Parametrix 2010d ; OSU. 2013d)	0 - 18 (De Schamphelaere and Janssen. 2004c, ECI 2008)	3.8 - 18.4 (Deleebeeck et al. 2007a; Peters et al. 2018)	0.5 – 12 (Van Sprang et al. 2016)	2.8 - 22.9 (De Schamphelaere et al., 2005)
Application range	"Full BLM"	**0.5 - 19.8**	**0.1 - 30**	**0.1 - 30**	**0.4 - 27.3**	**0.3 - 22.9**

[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^{-1}. Since no natural water will include 0 mg L^{-1}, 0.1 mg L^{-1} 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 Co in mg L^{-1} (Reference)	Ca range for Cu in mg L^{-1} (Reference)	Ca range for Ni in mg L^{-1} (Reference)	Ca range for Pb in mg L^{-1} (Reference)	Ca range for Zn in mg L^{-1} (Reference)
Calibration range	Algae	4.0 -161 (OSU 2015))	5 - 160 (De Schamphelaere et al., 2003b)	2.4 - 144 (Deleebeeck et al. 2009a)	4.7 – 120 (De Schamphelaere et al. 2014)	1.5 - 65.4 (De Schamphelaere et al. 2003a)
	Invert.	4.2 - 147 (Parametrix 2005; Parametrix 2010a; OSU 2013a; OSU 2022)	7 - 179 (De Schamphelaere and Janssen, 2004a)	1.3 - 88 [A,B] (Deleebeeck et al. 2008 De Schamphealaere et al 2006)	9.6 - 98.8 (Nys et al. 2014 ; Nys et al. 2016b)	10.0 - 160.3 (De Schamphelaere et al. 2005; Heijerick et al. 2005)
	Fish	5.0 – 155 (Parametrix 2009a,b; Parametrix 2010c ; OSU 2013c)	14 - 94 (De Schamphelaere and Janssen, 2004c)	3.8 - 110 (Deleebeeck et al. 2007a)	5.7 – 83 (Van Sprang et al. 2016)	7.8 - 155.8 (De Schamphelaere et al. 2003a; De Schamphelaere and Janssen, 2004b)
Validation range	Algae	8.8 – 65.5 (Heijerick et al. 2007)	2.5 - 179 (De Schamphelaere et al. 2003b, ECI 2008)	1.0 - 144 (Worms et al. 2007; Deleebeeck et al. 2009b, Peters et al. 2018)	4.7 - 120 (De Schamphelaere et al. 2014)	0.8 – 159.1 (Van Regenmortel et al. 2017)
	Invert.	2.6 – 77.0 (Parametrix 2010b; OSU 2013b; OSU 2017)	2.5 - 179 (De Schamphelaere and Janssen 2004a, ECI 2008)	0.1-120 (Deleebeeck et al. 2007b ; Nys et al. 2016a ; Schlekat et al. 2010, Peters et al. 2018)	3.5 - 202 (Nys et al. 2014 ; Nys et al. 2016b)	3.6 - 155.9 (Van Regenmortel et al. 2017)
	Fish	2.6 – 77.0 (Parametrix 2010d ; OSU. 2013d)	3.1 - 129 (De Schamphelaere and Janssen 2004c, ECI 2008)	0.3 - 110 (Deleebeeck et al. 2007a; Peters et al. 2018)	3.6 – 84 (Van Sprang et al. 2016)	1.4 - 55.1 (De Schamphelaere et al. 2003a ; De Schamphelaere et al., 2005)
Application range	"Full BLM"	**4.0 - 147**	**3.1 - 129**	**0.5-110**	**3.6 - 204**	**2.0 - 160**

[A] : *Daphnia magna* BLM

[B] : *Ceriodaphnia dubia* BLM

Supportive Annex 3: Frequently Asked Questions accompanying the use of bio-met

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 <u>does mean</u> 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 if the findings from the OoB analysis (Section 2.4 and Annex 4) indicate that the prediction is likely to be conservative. <u>But</u> 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 in the tables below. Please note that an alert message may also be a combination of the common to all metals alert messages of Table A3.1 and the metal specific tables if more than one of the water chemistry parameters (pH, DOC and Ca) is outside the validated or prediction range or if the water represents high bioavailability conditions. The general guidance applicable to all metals in presented in Table A3.1, and the Co, Cu, Ni, Pb and Zn specific guidance is detailed in Tables A3.2, A3.3, A3.4, A3.5 and A3.6, respectively.

Table A3.1. Overview of flags and alerts for being outside the validated ranges and their implication common to all metals

Metal + parameter	Warning	Guidance	Advice
All metals + DOC >30 mg L^{-1}		*This water has a DOC concentration that is outside the range of bio-met. The bio-met prediction has been made using the highest applicable DOC concentration. The result provided is indicative*	The applicability range of the bio-met tool is set at 0.1 - 30 mg DOC L^{-1}. Therefore, the local HC5-calculations are held at an upper ceiling of 30 mg DOC L^{-1} when measured DOC concentrations entered are greater than 30 mg L^{-1}. Implication: 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. Several approaches are given in the bio-met Guidance Manual Section 2.5 to assist in these situations.
All metals + DOC <0.1 mg L^{-1}		*This water has a DOC concentration that is outside the range of bio-met. The bio-met prediction has been made using the highest applicable DOC concentration. The result provided is indicative.*	The applicability range of the bio-met tool is set a 0.1 - 30 mg DOC L^{-1}. Therefore, the local HC5-calculations are held at a lower ceiling of 0.1 mg DOC L^{-1} when measured DOC concentrations entered are lower than 0.1 mg L^{-1}. Implication: 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.
All metals	High bioavailability conditions resulting in Local HC5 ≤ Reference HC5. Under these sensitive conditions, as a further	*High bioavailability conditions resulting in Local HC5 ≤ Reference HC5. Under these sensitive conditions, as a further*	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.

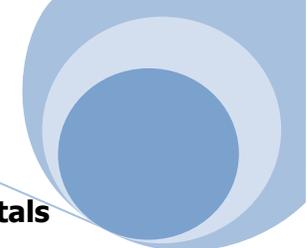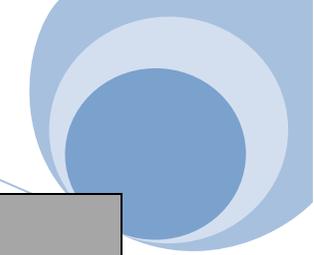| Metal + parameter	Warning	Guidance	Advice
	measure, the use of a full BLM may be required to determine the Local HC5.	*measure, the use of a full BLM may be required to determine the Local HC5.*	Under these sensitive conditions, as a further measure, the use of a full BLM may be required to determine the Local HC5.

Table A3.2 Overview of flags and alerts for being outside the validated ranges and their implication for Co

Metal + parameter	Warning	Guidance	Advice
Co + pH <5.9	pH is below the lower end of the validated and prediction range for Co	*This water has a pH that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in low pH conditions.*	The pH of the sample(s) under investigation is below the lower limit of the validated range for the CoBLM (pH 6.4-8.4), and therefore also for bio-met. Implication: The bio-met-local HC5 has not been calculated as the OoB assessment was performed to a lower pH of 5.9. For pH levels <5.9 it is not known whether predictions are likely to be conservative compared to calculations at a pH value of 6.4, the lower limit of the validated range, and therefore no prediction is provided. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.
Co + pH ≥5.9 but <6.4	pH is below the lower end of the validated range but within the prediction range for Co	*This water has a pH that is outside the range of bio-met. The bio-met prediction has been made using the lowest applicable pH.* *The result provided is indicative and is based on extrapolated full BLM calculations that show that the given prediction is likely conservative for decreasing pH down to pH 5.9.*	The pH of the sample(s) under investigation is below the lower limit of the validated range for the CoBLM (pH 6.4-8.4), and therefore also for bio-met. Implication: The bio-met-local HC5 has been calculated using a pH level of 6.4, the lower level of the validated range, as based on extrapolated full BLM calculations the given prediction is likely conservative for decreasing pH down to pH 5.9. The actual HC5 could be

Metal + parameter	Warning	Guidance	Advice
			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.
Co + pH >8.4	pH is above the higher end of the validated and prediction range for Co	*This water has a pH that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in high pH conditions.*	The pH of the sample(s) under investigation is above the upper limit of the validated range for the CoBLM (pH 6.4-8.4), and therefore also for bio-met. Implication: The bio-met-local HC5 has not been calculated as the OoB assessment indicated that predictions at pH levels >8.4 are unlikely to be conservative compared to calculations at a pH value of 8.4, the upper limit of the validated range. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.
Co + Ca <2 mg L^{-1}	Ca is below the lower end of the validated and prediction range for Co	*This water has a Ca concentration that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in low Ca conditions.*	The calcium concentration in the sample(s) under investigation is below the lower limit of the validated range for the CoBLM (Ca 4-147 mg L^{-1}), and therefore also for bio-met. Implication: The bio-met-local HC5 has not been calculated as the OoB assessment was performed to a lower Ca limit of 2 mg L^{-1}. For Ca concentrations of <2 mg L^{-1} is it unknown if predictions are likely to be conservative compared to a Ca concentration of 4 mg L^{-1}, the lower limit of the validated range and therefore, no prediction is provided. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.

Metal + parameter	Warning	Guidance	Advice
Co + Ca ≥2 but <4 mg L^{-1}	Ca is below the lower end of the validated range but within the prediction range for Co	*This water has a Ca concentration that is outside the range of bio-met. The bio-met prediction has been made using the lowest applicable calcium concentration.* *The result provided is indicative and is based on extrapolated full BLM calculations that show that the given prediction is likely conservative for decreasing calcium concentrations down to 2 mg L^{-1}.*	The calcium concentration in the sample(s) under investigation is below the lower limit of the validated range for the CoBLM (Ca 4-147 mg L^{-1}), and therefore also for bio-met. Implication: The bio-met-local HC5 has been calculated using a Ca concentration of 4 mg L^{-1}, the lower limit of the validated range, as based on extrapolated full BLM calculations the given prediction is likely conservative for decreasing calcium concentrations down to 2 mg L^{-1}. 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
Co + Ca >147 but ≤294 mg L^{-1}	Ca is above the higher end of the validated range but within the prediction range for Co	*This water has a Ca concentration that is outside the range of bio-met. The bio-met prediction has been made using the highest applicable calcium concentration.* *The result provided is indicative and is based on extrapolated full BLM calculations that show that the given prediction is likely conservative for increasing calcium concentrations up to 294 mg L^{-1}.*	The calcium concentration in the sample(s) under investigation is above the upper limit of the validated range for the CoBLM (Ca 4-147 mg L^{-1}), and therefore also for bio-met. Implication: The bio-met-local HC5 has been calculated using a Ca concentration of 147 mg L^{-1}, the upper limit of the validated range, as based on extrapolated full BLM calculations the given prediction is likely conservative for increasing calcium concentrations up to 294 mg L^{-1}. 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.
Co + Ca >294 mg L^{-1}	Ca is above the higher end of the validated and prediction range for Co	*This water has a Ca concentration that is outside the range of bio-met. No bio-met prediction has been made due to*	The calcium concentration in the sample(s) under investigation is above the upper limit of

Metal + parameter	Warning	Guidance	Advice
		the uncertainty in providing a prediction in high Ca conditions.	the validated range for the CoBLM (Ca 4-147 mg L^{-1}), and therefore also for bio-met. Implication: The bio-met-local HC5 has not been calculated as the OoB assessment was performed to an upper limit of 294 mg L^{-1}.For Ca concentrations of >294 mg L^{-1} it is not known if predictions are likely to be conservative compared to using a Ca concentration of 147 mg L^{-1}, the upper limit of the validated range, therefore no predictions are provided. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.
Co + pH <6.4 and Ca <4 mg L^{-1}	pH is below the lower end of the validated range for Co and Ca is below the lower end of the validated range for Co	*This water has a pH that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in low pH conditions.* *This water has a Ca concentration that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in low Ca conditions.*	The pH and Ca concentration of the sample(s) under investigation is below the lower limit of the validated range for the CoBLM (pH 6.4-8.4; Ca 4-147 mg L^{-1}) and therefore also for bio-met. Implication: The bio-met-local HC5 has not been calculated as two parameters are outside of the validated range of bio-met therefore there would be significant uncertainty in any predictions provided. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.
Co + pH <6.4 and Ca >147 mg L^{-1}	pH is below the lower end of the validated range for Co and Ca is above the higher end of the validated range for Co	*This water has a pH that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in low pH conditions.* *This water has a Ca concentration that is outside the range of bio-met. No bio-met prediction has been made due to*	The pH of the sample(s) under investigation is below the lower limit of the validated range and the Ca concentration is above the upper limit of the validated range for the CoBLM (pH 6.4-8.4; Ca 4-147 mg L^{-1}) and therefore also for bio-met.

Metal + parameter	Warning	Guidance	Advice
		the uncertainty in providing a prediction in high Ca conditions.	Implication: The bio-met-local HC5 has not been calculated as two parameters are outside of the validated range of bio-met therefore there would be significant uncertainty in any predictions provided. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.
Co + pH >8.4 and Ca <4 mg L^{-1}	pH is above the higher end of the validated range Co and Ca is below the lower end of the validated range for Co	*This water has a pH that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in high pH conditions.* *This water has a Ca concentration that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in low Ca conditions.*	The pH of the sample(s) under investigation is above the upper limit of the validated range and the Ca concentration is below the lower limit of the validated range for the CoBLM (pH 6.4-8.4; Ca 4-147 mg L^{-1}) and therefore also for bio-met. Implication: The bio-met-local HC5 has not been calculated as two parameters are outside of the validated range of bio-met therefore there would be significant uncertainty in any predictions provided. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.
Co + pH >8.4 and Ca >147 mg L^{-1}	pH is above the higher end of the validated range for Co and Ca is above the higher end of the validated range for Co	*This water has a pH that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in high pH conditions.* *This water has a Ca concentration that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in high Ca conditions.*	The pH and Ca concentration of the sample(s) under investigation is above the upper limit of the validated range for the CoBLM (pH 6.4-8.4; Ca 4-147 mg L^{-1}) and therefore also for bio-met. Implication: The bio-met-local HC5 has not been calculated as two parameters are outside of the validated range of bio-met therefore there would be significant uncertainty in any predictions provided. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.

Table A3.3 Overview of flags and alerts for being outside the validated ranges and their implication for Cu

Metal + parameter	Warning	Guidance	Advice
Cu + pH < 6.0	pH is below the lower end of the validated and prediction range for Cu	*This water has a pH that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in low pH conditions.*	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. Implication: The bio-met-local HC5 has not been calculated as the OoB assessment indicated that predictions at pH levels <6 are unlikely to be conservative compared to calculations at a pH value of 6, the lower limit of the validated range. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.
Cu + pH >8.5	pH is above the higher end of the validated and prediction range for Cu	*This water has a pH that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in high pH conditions.*	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. Implication: The bio-met-local HC5 has not been calculated as the OoB assessment indicated that predictions at pH levels >8.5 are unlikely to be conservative compared to calculations at a pH value of 8.5, the upper limit of the validated range. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.
Cu + Ca <1.55 mg L^{-1}	Ca is below the lower end of the validated and prediction range for Cu	*This water has a Ca concentration that is outside the range of bio-met. No bio-met prediction has been made due to*	The calcium concentration in the sample(s) under investigation is below the lower limit of the validated range for the CuBLM (Ca 3.1-129

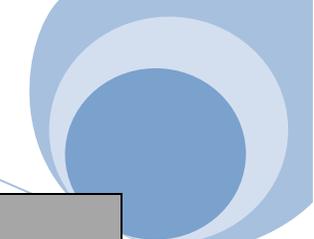| Metal + parameter	Warning	Guidance	Advice
		the uncertainty in providing a prediction in low Ca conditions.	mg L^{-1}) and the prediction range of bio-met based on the OoB analysis. Implication: The bio-met-local HC5 has not been calculated as the OoB assessment indicated that predictions at Ca concentrations of <1.55 mg L^{-1} are unlikely to be conservative compared to calculations at a Ca concentration of 3 mg L^{-1}, the lower limit of the validated range. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.
Cu + Ca ≥ 1.55 mg L^{-1} but < 3.1 mg L^{-1}	Ca is below the lower end of the validated range but within the prediction range for Cu	*This water has a Ca concentration that is outside the range of bio-met. The bio-met prediction has been made using the lowest applicable calcium concentration.* *The result provided is indicative and is based on extrapolated full BLM calculations that show that the given prediction is likely conservative for decreasing calcium concentrations to 1.55 mg L^{-1}.*	The calcium concentration in the sample(s) under investigation is below the lower limit of the validated range for the CuBLM (Ca 3.1-129 mg L^{-1}), and therefore also for bio-met. Implication: The bio-met-local HC5 has been calculated using a Ca concentration of 3.1 mg L^{-1}, the lower limit of the validated range, as based on extrapolated full BLM calculations the given prediction is likely conservative for decreasing calcium concentrations to 1.55 mg L^{-1}. 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.
Cu + Ca >129 mg L^{-1}		*! 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.*	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^{-1}), and therefore also for bio-met. Implication: The bio-met-local HC5 has not been calculated as the OoB assessment

Metal + parameter	Warning	Guidance	Advice
			indicated that predictions at Ca concentrations >129 mg L^{-1} are unlikely to be conservative compared to calculations at a Ca concentration of 129 mg L^{-1}. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.
Cu + pH <6.0 and Ca <3.1 mg L^{-1}	pH is below the lower end of the validated range for Cu and Ca is below the lower end of the validated range for Cu	*This water has a pH that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in low pH conditions.* *This water has a Ca concentration that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in low Ca conditions.*	The pH and Ca concentration of the sample(s) under investigation is below the lower limit of the validated range for the CuBLM (pH 6.0-8.5; Ca 3.1-129 mg L^{-1}) and therefore also for bio-met. Implication: The bio-met-local HC5 has not been calculated as two parameters are outside of the validated range of bio-met therefore there would be significant uncertainty in any predictions provided. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.
Cu + pH <6.0 and Ca >129 mg L^{-1}	pH is below the lower end of the validated range for Cu and Ca is above the higher end of the validated range for Cu	*This water has a pH that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in low pH conditions.* *This water has a Ca concentration that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in high Ca conditions.*	The pH of the sample(s) under investigation is below the lower limit of the validated range and the Ca concentration is above the upper limit of the validated range for the CuBLM (pH 6.0-8.5; Ca 3.1-129 mg L^{-1}) and therefore also for bio-met. Implication: The bio-met-local HC5 has not been calculated as two parameters are outside of the validated range of bio-met therefore there would be significant uncertainty in any predictions provided. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.

Metal + parameter	Warning	Guidance	Advice
Cu + pH >8.5 and Ca <3.1 mg L^{-1}	pH is above the higher end of the validated range Cu and Ca is below the lower end of the validated range for Cu	*This water has a pH that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in high pH conditions.* *This water has a Ca concentration that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in low Ca conditions.*	The pH of the sample(s) under investigation is above the upper limit of the validated range and the Ca concentration is below the lower limit of the validated range for the CuBLM (pH 6.0-8.5; Ca 3.1-129 mg L^{-1}) and therefore also for bio-met. Implication: The bio-met-local HC5 has not been calculated as two parameters are outside of the validated range of bio-met therefore there would be significant uncertainty in any predictions provided. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.
Cu + pH >8.5 and Ca >129 mg L^{-1}	pH is above the higher end of the validated range for Cu and Ca is above the higher end of the validated range for Cu	*This water has a pH that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in high pH conditions.* *This water has a Ca concentration that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in high Ca conditions.*	The pH and Ca concentration of the sample(s) under investigation is above the upper limit of the validated range for the CuBLM (pH 6.0-8.5; Ca 3.1-129 mg L^{-1}) and therefore also for bio-met. Implication: The bio-met-local HC5 has not been calculated as two parameters are outside of the validated range of bio-met therefore there would be significant uncertainty in any predictions provided. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.

Table A3.4. Overview of flags and alerts for being outside the validated ranges and their implication for Ni

Metal + parameter	Warning	Guidance	Advice
Ni + pH <5.9	pH is below the lower end of the validated and prediction range for Ni	*This water has a pH that is outside the range of bio-met. No bio-met prediction*	The pH of the sample(s) under investigation is below the lower limit of the validated range for

["

Metal + parameter	Warning	Guidance	Advice
			compared to calculations at a Ca concentration of 0.5 mg L^{-1}, the lower limit of the validated range. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.
Ni + Ca >110 mg L^{-1} but ≤220 mg L^{-1}	Ca is above the higher end of the validated range but within the prediction range for Ni	*This water has a Ca concentration that is outside the range of bio-met. The bio-met prediction has been made using the highest applicable calcium concentration.* *The result provided is indicative and is based on extrapolated full BLM calculations that show that the given prediction is likely conservative for with increasing calcium concentrations up to 220 mg L^{-1}.*	The calcium concentration in the sample(s) under investigation is above the upper limit of the validated range for the NiBLM (Ca 0.5-110 mg L^{-1}), and therefore also for bio-met. Implication: The bio-met-local HC5 has been calculated using a Ca concentration of 110 mg L^{-1}, the upper limit of the validated range, as based on extrapolated full BLM calculations the given prediction is likely conservative for increasing calcium concentrations up to 220 mg L^{-1}. 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 + Ca >220 mg L^{-1}	Ca is above the higher end of the validated and prediction range for Ni	*This water has a Ca concentration that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in high Ca conditions.*	The calcium concentration in the sample(s) under investigation is above the upper limit of the validated range for the NiBLM (Ca 0.5-110 mg L^{-1}), and therefore also for bio-met. Implication: The bio-met-local HC5 has not been calculated as the OoB assessment indicated that predictions at Ca concentrations of >220 mg L^{-1} are unlikely to be conservative compared to calculations at a Ca concentration of 110 mg L^{-1}, the upper limit of the validated range. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.

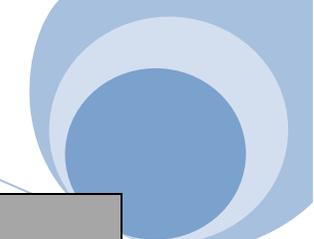| Metal + parameter	Warning	Guidance	Advice
Ni + pH <5.9 and Ca <0.5 mg L^{-1}	pH is below the lower end of the validated range for Ni and Ca is below the lower end of the validated range for Ni	*This water has a pH that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in low pH conditions.* *This water has a Ca concentration that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in low Ca conditions.*	The pH and Ca concentration of the sample(s) under investigation is below the lower limit of the validated range for the NiBLM (pH 5.9-8.7; Ca 0.5-110 mg L^{-1}) and therefore also for bio-met. Implication: The bio-met-local HC5 has not been calculated as two parameters are outside of the validated range of bio-met therefore there would be significant uncertainty in any predictions provided. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.
Ni + pH <5.9 and Ca >110 mg L^{-1}	pH is below the lower end of the validated range for Ni and Ca is above the higher end of the validated range for Ni	*This water has a pH that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in low pH conditions.* *This water has a Ca concentration that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in high Ca conditions.*	The pH of the sample(s) under investigation is below the lower limit of the validated range and the Ca concentration is above the upper limit of the validated range for the NiBLM (pH 5.9-8.7; Ca 0.5-110 mg L^{-1}) and therefore also for bio-met. Implication: The bio-met-local HC5 has not been calculated as two parameters are outside of the validated range of bio-met therefore there would be significant uncertainty in any predictions provided. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.
Ni + pH >8.7 and Ca <0.5 mg L^{-1}	pH is above the higher end of the validated range for Ni and Ca is below the lower end of the validated range for Ni	*This water has a pH that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in high pH conditions.* *This water has a Ca concentration that is outside the range of bio-met. No bio-met prediction has been made due to*	The pH of the sample(s) under investigation is above the upper limit of the validated range and the Ca concentration is below the lower limit of the validated range for the NiBLM (pH 5.9-8.7; Ca 0.5-110 mg L^{-1}) and therefore also for bio-met.

Metal + parameter	Warning	Guidance	Advice
		the uncertainty in providing a prediction in low Ca conditions.	Implication: The bio-met-local HC5 has not been calculated as two parameters are outside of the validated range of bio-met therefore there would be significant uncertainty in any predictions provided. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.
Ni + pH >8.7 and Ca >110 mg L^{-1}	pH is above the higher end of the validated range for Ni and Ca is above the higher end of the validated range for Ni	*This water has a pH that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in high pH conditions.* *This water has a Ca concentration that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in high Ca conditions.*	The pH and Ca concentration of the sample(s) under investigation is above the upper limit of the validated range for the NiBLM (pH 5.9-8.7; Ca 0.5-110 mg L^{-1}) and therefore also for bio-met. Implication: The bio-met-local HC5 has not been calculated as two parameters are outside of the validated range of bio-met therefore there would be significant uncertainty in any predictions provided. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.

Table A3.5. Overview of flags and alerts for being outside the validated ranges and their implication for Pb

Metal + parameter	Warning	Guidance	Advice
Pb + pH <6.3	pH is below the lower end of the validated and prediction range for Pb	*This water has a pH that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in low pH conditions.*	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. Implication: The bio-met-local HC5 has not been calculated as the OoB assessment indicated that predictions at pH levels <6.3 are unlikely to be conservative compared to

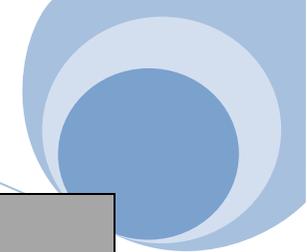| Metal + parameter	Warning	Guidance	Advice
			calculations at a pH value of 6.3, the lower limit of the validated range. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.
Pb + pH >8.4 but ≤8.9	pH is above the higher end of the validated range but within the prediction range for Pb	*This water has a pH that is outside the range of bio-met. The bio-met prediction has been made using the highest applicable pH.* *The result provided is indicative and is based on extrapolated full BLM calculations that show that the given prediction is likely conservative for increasing pH up to pH 8.9.*	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. Implication: The bio-met-local HC5 has been calculated using a pH value of 8.4, the upper limit of the validated range as based on extrapolated full BLM calculations the given prediction is likely conservative for increasing pH up to pH 8.9. 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 + pH >8.9	pH is above the higher end of the validated and prediction range for Pb	*This water has a pH that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in high pH conditions.*	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. Implication: The bio-met-local HC5 has not been calculated as the OoB assessment indicated that predictions at pH values >8.9 are unlikely to be conservative compared to calculations using a pH value of 8.4, the upper limit of the validated range. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.

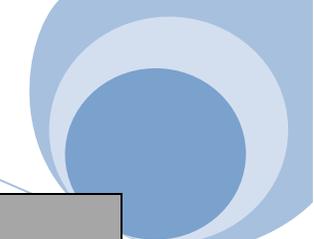| Metal + parameter	Warning	Guidance	Advice
Pb + Ca <3.6 mg L^{-1}	Ca is below the lower end of the validated and prediction range for Pb	*This water has a Ca concentration that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in low Ca conditions.*	The calcium concentration in the sample(s) under investigation is below the lower limit of the validated range for the Pb BLM (Ca 3.6-200 mg L^{-1}), and therefore also for bio-met. Implication: The bio-met-local HC5 has not been calculated as the OoB assessment indicated that predictions at Ca concentrations <3.6 mg L^{-1} are unlikely to be conservative compared to calculations at a Ca concentration of 3.6 mg L^{-1}, the lower limit of the validated range. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.
Pb + Ca > 200 mg L^{-1}	Ca is above the higher end of the validated and prediction range for Pb	*This water has a Ca concentration that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in high Ca conditions.*	The calcium concentration in the sample(s) under investigation is above the upper limit of the validated range for the PbBLM (Ca 3.6-200 mg L^{-1}), and therefore also for bio-met. Implication: The bio-met-local HC5 has not been calculated as the OoB assessment indicated that predictions at Ca concentrations >200 mg L^{-1} are unlikely to be conservative compared to calculations at a Ca concentration of 200 mg L^{-1}, the upper limit of the validated range. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.
Pb + pH <6.3 and Ca <3.6 mg L^{-1}	pH is below the lower end of the validated range for Pb and Ca is below the lower end of the validated range for Pb	*This water has a pH that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in low pH conditions.* *This water has a Ca concentration that is outside the range of bio-met. No bio-*	The pH and Ca concentration of the sample(s) under investigation is below the lower limit of the validated range for the PbBLM (pH 6.3-8.4; Ca 3.6-200 mg L^{-1}) and therefore also for bio-met.

Metal + parameter	Warning	Guidance	Advice
		met prediction has been made due to the uncertainty in providing a prediction in low Ca conditions.	Implication: The bio-met-local HC5 has not been calculated as two parameters are outside of the validated range of bio-met therefore there would be significant uncertainty in any predictions provided. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.
Pb + pH <6.3 and Ca >200 mg L^{-1}	pH is below the lower end of the validated range for Pb and Ca is above the higher end of the validated range for Pb	*This water has a pH that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in low pH conditions.* *This water has a Ca concentration that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in high Ca conditions.*	The pH of the sample(s) under investigation is below the lower limit of the validated range and the Ca concentration is above the upper limit of the validated range for the PbBLM (pH 6.3-8.4; Ca 3.6-200 mg L^{-1}) and therefore also for bio-met. Implication: The bio-met-local HC5 has not been calculated as two parameters are outside of the validated range of bio-met therefore there would be significant uncertainty in any predictions provided. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.
Pb + pH >8.4 and Ca <3.6 mg L^{-1}	pH is above the higher end of the validated range Pb and Ca is below the lower end of the validated range for Pb	*This water has a pH that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in high pH conditions.* *This water has a Ca concentration that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in low Ca conditions.*	The pH of the sample(s) under investigation is above the upper limit of the validated range and the Ca concentration is below the lower limit of the validated range for the PbBLM (pH 6.3-8.4; Ca 3.6-200 mg L^{-1}) and therefore also for bio-met. Implication: The bio-met-local HC5 has not been calculated as two parameters are outside of the validated range of bio-met therefore there would be significant uncertainty in any predictions provided. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.

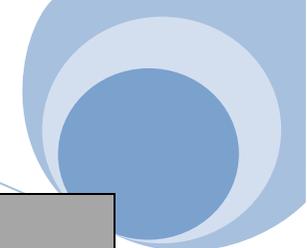| Metal + parameter	Warning	Guidance	Advice
Pb + pH >8.4 and Ca >200 mg L^{-1}	pH is above the higher end of the validated range for pb and Ca is above the higher end of the validated range for Pb	*This water has a pH that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in high pH conditions.* *This water has a Ca concentration that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in high Ca conditions.*	The pH and Ca concentration of the sample(s) under investigation is above the upper limit of the validated range for the PbBLM (pH 6.3-8.4; Ca 3.6-200 mg L^{-1}) and therefore also for bio-met. Implication: The bio-met-local HC5 has not been calculated as two parameters are outside of the validated range of bio-met therefore there would be significant uncertainty in any predictions provided. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.

Table A3.6 Overview of flags and alerts for being outside the validated ranges and their implication for Zn

Metal + parameter	Warning	Guidance	Advice
Zn + pH <5.5	pH is below the lower end of the validated and prediction range for Zn	*This water has a pH that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in low pH conditions.*	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. Implication: The bio-met-local HC5 has not been calculated as the OoB assessment indicated that predictions at pH values <5.5 are unlikely to be conservative compared to calculations at a pH value of 5.5, the lower limit of the validated range. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.
Zn + pH >8.5	pH is above the higher end of the validated and prediction range for Zn	*This water has a pH that is outside the range of bio-met. No bio-met prediction*	The pH of the sample(s) under investigation is above the upper limit of the validated range

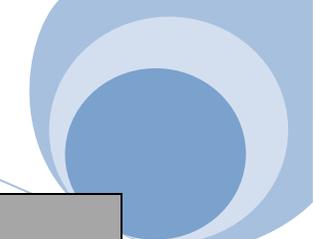| Metal + parameter	Warning	Guidance	Advice
		has been made due to the uncertainty in providing a prediction in high pH conditions.	for the ZnBLM (pH 5.5-8.5), and therefore also for bio-met. Implication: The bio-met-local HC5 has not been calculated as the OoB assessment indicated that predictions at pH values >8.5 are unlikely to be conservative compared to calculations at a pH value of 8.5, the upper limit of the validated range. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.
Zn + Ca <0.4 mg L^{-1}	Ca is below the lower end of the validated and prediction range for Zn	*This water has a Ca concentration that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in low Ca conditions.*	The calcium concentration in the sample(s) under investigation is below the lower limit of the validated range for the ZnBLM (Ca 0.8-160 mg L^{-1}), and the prediction range of bio-met based on the OoB analysis. Implication: The bio-met-local HC5 has not been calculated as the OoB assessment indicated that predictions at Ca concentrations of <0.4 mg L^{-1} are unlikely to be conservative compared to calculations at a Ca concentration 0.8 mg L^{-1}, the lower limit of the validated range. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.
Zn + Ca ≥ 0.4 mg L^{-1} but < 0.8 mg L^{-1}	Ca is below the lower end of the validated range but within the prediction range for Zn	*This water has a Ca concentration that is outside the range of bio-met. The bio-met prediction has been made using the lowest applicable calcium concentration.* *The result provided is indicative and is based on extrapolated full BLM calculations that show that the given*	The calcium concentration in the sample(s) under investigation is below the lower limit of the validated range for the CuBLM (Ca 0.8-160 mg L^{-1}), and therefore also for bio-met. Implication: The bio-met-local HC5 has been calculated using a Ca concentration of 0.8 mg L^{-1}, the lower limit of the validated range, as

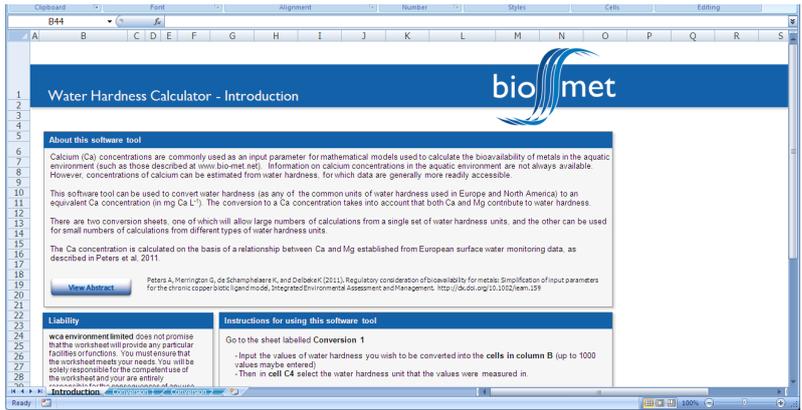| Metal + parameter	Warning	Guidance	Advice
		prediction is likely conservative for decreasing calcium concentrations down to 0.4 mg L^{-1}.	based on extrapolated full BLM calculations the given prediction is likely conservative for decreasing calcium concentrations to 0.4 mg L^{-1}. The actual HC5 could 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.
Zn + Ca >160 mg L^{-1} but ≤300 mg L^{-1}	Ca is above the higher end of the validated range but within the prediction range for Zn	*This water has a Ca concentration that is outside the range of bio-met. The bio-met prediction has been made using the highest applicable calcium concentration.* *The result provided is indicative and is based on extrapolated full BLM calculations that show that the given prediction is likely conservative for increasing calcium concentrations up to 300 mg L^{-1}.*	The calcium concentration in the sample(s) under investigation is above the upper limit of the validated range for the ZnBLM (Ca 0.8-160 mg L^{-1}), and therefore also for bio-met. Implication: The bio-met-local HC5 has been calculated using a Ca concentration of 160 mg L^{-1}, the upper limit of the validated range as based on extrapolated full BLM calculations the given prediction is likely conservative for increasing calcium concentrations up to 300 mg L^{-1}. 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 + Ca >300 mg L^{-1}	Ca is above the higher end of the validated and prediction range for Zn	*This water has a Ca concentration that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in high Ca conditions.*	The calcium concentration in the sample(s) under investigation is above the upper limit of the validated range for the ZnBLM (Ca 0.8-160 mg L^{-1}), and the prediction range of bio-met based on the OoB analysis. Implication: The bio-met-local HC5 has not been calculated as the OoB assessment indicated that predictions at Ca concentrations of >300 mg L^{-1} are unlikely to be conservative compared to calculations at a Ca concentration

Metal + parameter	Warning	Guidance	Advice
			of 160 mg L^{-1}, the upper limit of the validated range. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.
Zn + pH <5.5 and Ca <0.8 mg L^{-1}	pH is below the lower end of the validated range for Zn and Ca is below the lower end of the validated range for Zn	*This water has a pH that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in low pH conditions.* *This water has a Ca concentration that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in low Ca conditions.*	The pH and Ca concentration of the sample(s) under investigation is below the lower limit of the validated range for the ZnBLM (pH 5.5-8.5; Ca 0.8-160 mg L^{-1}) and therefore also for bio-met. Implication: The bio-met-local HC5 has not been calculated as two parameters are outside of the validated range of bio-met therefore there would be significant uncertainty in any predictions provided. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.
Zn + pH <5.5 and Ca >160 mg L^{-1}	pH is below the lower end of the validated range for Zn and Ca is above the higher end of the validated range for Zn	*This water has a pH that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in low pH conditions.* *This water has a Ca concentration that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in high Ca conditions.*	The pH of the sample(s) under investigation is below the lower limit of the validated range and the Ca concentration is above the upper limit of the validated range for the ZnBLM (pH 5.5-8.5; Ca 0.8-160 mg L^{-1}) and therefore also for bio-met. Implication: The bio-met-local HC5 has not been calculated as two parameters are outside of the validated range of bio-met therefore there would be significant uncertainty in any predictions provided. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.
Zn + pH >8.5 and Ca <0.8 mg L^{-1}	pH is above the higher end of the validated range Zn and Ca is below the lower end of the validated range for Zn	*This water has a pH that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty*	The pH of the sample(s) under investigation is above the upper limit of the validated range and the Ca concentration is below the lower limit of the validated range for the ZnBLM (pH

Metal + parameter	Warning	Guidance	Advice
		in providing a prediction in high pH conditions. *This water has a Ca concentration that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in low Ca conditions.*	5.5-8.5; Ca 0.8-160 mg L^{-1}) and therefore also for bio-met. Implication: The bio-met-local HC5 has not been calculated as two parameters are outside of the validated range of bio-met therefore there would be significant uncertainty in any predictions provided. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.
Zn + pH >8.5 and Ca >160 mg L^{-1}	pH is above the higher end of the validated range for Zn and Ca is above the higher end of the validated range for Zn	*This water has a pH that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in high pH conditions.* *This water has a Ca concentration that is outside the range of bio-met. No bio-met prediction has been made due to the uncertainty in providing a prediction in high Ca conditions.*	The pH and Ca concentration of the sample(s) under investigation is above the upper limit of the validated range for the ZnBLM (pH 5.5-8.5; Ca 0.8-160 mg L^{-1}) and therefore also for bio-met. Implication: The bio-met-local HC5 has not been calculated as two parameters are outside of the validated range of bio-met therefore there would be significant uncertainty in any predictions provided. Several approaches are given in the bio-met Guidance Manual Section 2.5 as to how to proceed in these cases.

2) Other questions

Other FAQs	
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.

Metal	pH	Ca (mg L^{-1})
Co	6.4-8.4	4.0-147
Cu	6.0-8.5	3.1-129
Ni	5.9-8.7	0.5-110
Pb	6.3-8.4	3.6-200
Zn	5.5-8.5	0.8-160

The full prediction ranges for each metal detailed in the table below and are based on predictions from the extrapolated BLM calculations (as detailed in Appendix 4 of this guidance):

Metal	pH	Ca (mg L^{-1})
Co	5.9-8.4	2.0-294
Cu	6.0-8.5	1.55-129
Ni	5.9-8.7	0.5-220
Pb	6.3-8.9	3.6-200
Zn	5.5-8.5	0.4-300

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. An analysis of the accuracy of predictions compared to the full BLM predictions where one parameter is outside of the application range has been performed, and only conservative predictions are provided within the tool. Further information on the analysis is detailed in Appendix 4.
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.
Can bio-met be used if Ca concentrations are not available but hardness is?	In bio-met version 6.0, if hardness data is available as mg L^{-1} of CaCO$_3$ and Ca data is unavailable, this can be entered directly into the tool and the equivalent concentration of Ca from water hardness its calculated using the regression calculated by Peters et al. (2011a). Additionally, a Hardness Conversion Tool has also been developed as a stand-alone tool and is available here: http://bio-met.net/wp-content/uploads/2016/10/110616_Hardness-conversion-tool_biomet.xlsm. 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. 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

<table>
<tr><td></td><td>

sheet, and the second conversion sheet can be used for small numbers of samples which are expressed in different hardness units.

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 the screenshot above) to mg L^{-1} $CaCO_3$, and also into an equivalent Ca concentration. This equivalent Ca concentration takes account of the fact that both Ca and Mg contribute to hardness.

</td></tr>
</table>

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 may represent an extrapolation for more than one parameter. If more than one parameters is outside of the validated range for the metal no bio-met prediction is provided due to the uncertainty associated with the prediction. Section 2.5 of this guidance provides more information on how to proceed in these situations.

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 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). 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.
How will toxicity be affected in high pH waters?	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.
How will toxicity be affected in high pH hard waters?	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.
How does DOC affect metal toxicity	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.
How will toxicity be affected in high DOC waters.	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^{-1}). Higher DOC concentrations will reduce toxicity, although the degree of any additional effect depends upon the metal in question.
What type of water chemistry conditions are most sensitive for copper toxicity?	Waters with low DOC concentrations are sensitive to copper toxicity. Extremes of pH can also increase toxicity.
What type of water chemistry conditions are most sensitive for nickel toxicity?	Waters with low DOC concentrations are sensitive to nickel toxicity. The combination of high pH and low hardness also increases toxicity.
What type of water chemistry conditions are most sensitive for zinc toxicity?	Waters with low DOC concentrations are sensitive to zinc toxicity. Extremes of pH, either high or low, and low hardness, also increase toxicity.
What type of water chemistry conditions are most sensitive for lead toxicity?	Waters with low DOC concentrations are sensitive to lead toxicity.
What type of water chemistry conditions are most sensitive for cobalt toxicity?	Waters with low DOC concentrations are sensitive to cobalt toxicity. High pH also increases toxicity.
What about lead?	For lead there is a full BLM, which has been implemented in the bio-met tool. However, the EQS for lead under 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:

|	
	$EQS_{site} = EQS_{bioavailable} + (1.2 \times (DOC_{site} - DOC_{reference}))$ Where: EQS_{site} = EQS at the site under consideration $EQS_{bioavailable}$ = EQS for a reference condition to ensure all water bodies are protected DOC_{site} = Dissolved Organic Carbon at the site under consideration $DOC_{reference}$ = average Dissolved Organic Carbon (DOC) concentration in the ecotoxicity tests that the $EQS_{bioavailable}$ is based upon = 1.0 mg L^{-1}.

Supportive Annex 4: Out of Boundary (OoB) Analysis

The Out-of-Boundary (OoB) behaviour of the full bioavailability normalisations is defined as the behaviour of HC5-predictions when the bioavailability models are applied out of their application boundaries (based on the calibration and validation ranges). This information is important as this will guide the out-of-boundary messages in the updated bio-met version. For the OoB behaviour a limited structured phys-chem design is proposed, in which the direction in normalized HC5 when extrapolating outside the full bioavailability model boundaries is evaluated. In practice, it is proposed to evaluate OoB behaviour for conditions where only 1 of the major phys-chem parameters (pH, DOC and Ca) is set to a value outside the applicability ranges at one time, as extrapolations with two or more parameters OoB are considered too uncertain.

The design of the OoB-behaviour analysis is dependent on the application ranges of the bioavailability normalization approach and, as such, is different for every metal. An overview of the levels considered for the OoB-behaviour analysis is given in Table A4.1. The OoB behaviour was evaluated against the closest phys-chem conditions that are within bioavailability model boundaries. This design allows us to evaluate in a systematic manner if out-of-boundary behaviour is dependent on conditions that are within the model boundaries (and are included in the bio-met look-up table entry).

Table A4.1 Overview of physico-chemistry condition considered for the Out-of-Boundary (OoB) behaviour analysis.

Out of boundary parameter	Levels for OoB-behaviour analysis	pH conditions within boundaries considered	Ca conditions within boundaries considered	DOC conditions within boundaries considered	Co	Cu	Ni	Pb	Zn
pH	0.25 and 0.5 pH units below lower pH boundary / above upper pH boundary	-	all Ca-levels considered in look-up table lay-out	<1st, 10th, 24th, 50th, 72nd, 90th percentile of FOREGS + upper limit:0.1, 1, 2, 5.1, 10.9, 17 and 30 mg L^{-1}	5.90, 6.15/ 8.65, 8.90	5.50, 5.75/ 8.75, 9.00	5.40, 5.65/ 8.95, 9.20	5.80, 6.05/ 8.65, 8.90	5.20, 5.45/ 8.65, 8.90
Ca	0.5-fold below lowest Ca boundary / 1.5 and 2.0-fold above highest Ca boundary	pH levels within boundaries, with 0.5 pH unit interval	-		2.0/ 220, 294 mg L^{-1}	1.55 / 194, 258 mg L^{-1}	0.25 / 165, 220 mg L^{-1}	1.8/ 306, 406 mg L^{-1}	0.4 / 200, 300 mg L^{-1}
DOC	98th and >99th percentile of FOREGS		all Ca-levels considered in look-up table lay-out	-	35, 50 mg L^{-1}				
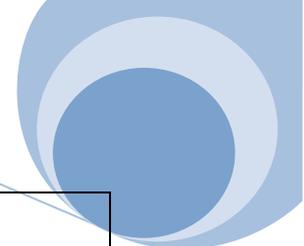
	DOC distribution				

Note that only one parameter was varied outside application boundaries of the full bioavailability normalization approach at one time, while all other parameters were kept within the application boundaries.

A4.1 Out of Boundary Analysis for Co

The full Co bioavailability normalization approach is applicable over the following physico-chemistry ranges: pH 6.4 - 8.4, Ca 4 - 147 mg L^{-1} and DOC 0.1 - 30 mg L^{-1}. Low pH (pH<6.4), high DOC (>30 mg L^{-1}), low Ca (<4 mg L^{-1}) and high Ca (Ca>147 mg L^{-1}) tend to be conditions that result either in higher or similar predicted HC5 with the full Co bioavailability normalization compared with the corresponding HC5 prediction at the border of the application range for Co (Table A4.2; Figure A4.1). As such, both the full bioavailability normalization predictions, as well as the bio-met predictions can be considered to be conservative relative to the expected HC5 outside the application boundary for Co. For high pH conditions (pH>8.4) a complex pattern was observed depending on Ca and/or DOC conditions.

A visualization of the out-of-boundary behaviour of the Co full bioavailability normalization approach and those predicted by bio-met is given in Figure A4.2. **bio-met predictions of local HC5 may be under conservative at high pH (pH>8.4).**

Table A4.2 Overview of out-of-boundary behaviour of HC5 predictions of the full bioavailability normalization approach (i.e. full BLM) for Co

	Description of out-of-boundary behaviour for Co
Low pH (pH<6.4)	Full BLM prediction out of boundary is less conservative compared with value within boundary → **Full BLM HC5 prediction at pH 6.4 is conservative for full BLM HC5 prediction at pH<6.4**
High pH (pH>8.4)	Complex pattern dependent on Ca- and/or DOC-level[b]
High DOC (>30 mg L^{-1})	Full BLM prediction out of boundary is less conservative[a] compared with value within boundary, although in specific conditions (i.e. pH 6.4 and 147 mg Ca L^{-1}) the prediction is similar compared with the value within boundary → **Full BLM HC5 prediction at DOC 30 mg L^{-1} is conservative for full BLM HC5 prediction at DOC>30 mg L^{-1}**
Low Ca (<4 mg L^{-1})	Full BLM prediction out of boundary is either less conservative or similar compared with value within boundary → **Full BLM HC5 prediction at Ca 4 mg L^{-1} is conservative for full BLM HC5 prediction at Ca<4 mg L^{-1}**
High Ca (>147 mg L^{-1})	Full BLM prediction out of boundary is either less conservative or similar compared with value within boundary → **Full BLM HC5 prediction at Ca 147 mg L^{-1} is conservative for full BLM HC5 prediction at Ca>147 mg L^{-1}**

[a] Less conservative means that full BLM HC5$_{OoB}$>full BLM HC5$_{IB}$. Similar means that full BLM HC5$_{OoB}$>full BLM HC5$_{IB}$ are within 5% of each other. More conservative means that the full BLM HC5$_{OoB}$<full BLM HC5$_{IB}$.

[b] At a combination of low Ca concentrations (i.e. <40 mg Ca L^{-1}) and moderate to high DOC (≥2 mg DOC) the full BLM normalisation for high pH conditions (pH>8.4) may result in a lower HC5 compared to the full BLM normalized HC5 at pH 8.4.
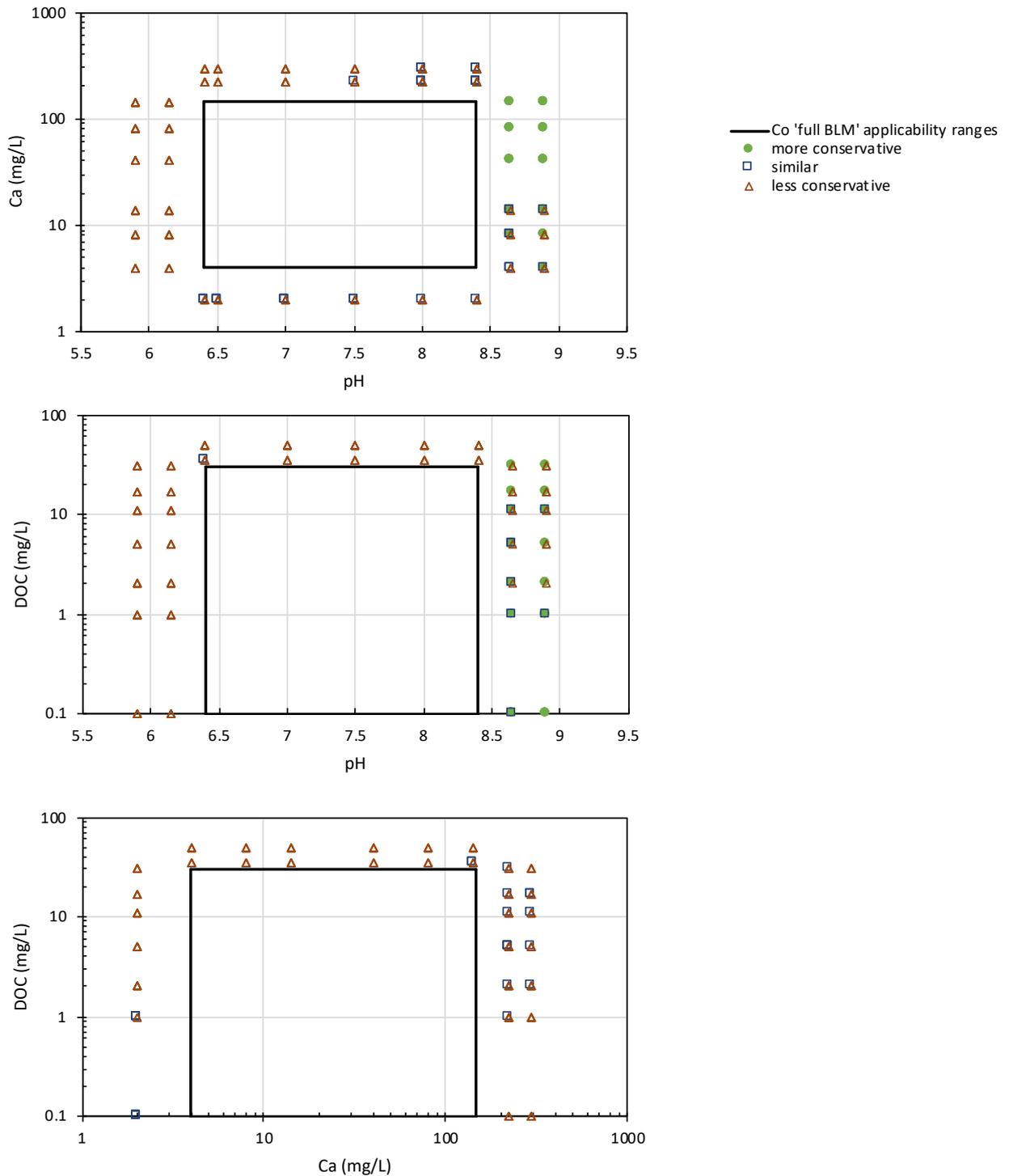
Figure A4.1 Overview of the outcome of the out-of-boundary (OoB) behaviour analysis for Co. Full lines indicate the application boundaries of the full bioavailability normalization approach for Co. Open triangles indicate that the OoB HC5 (HC5$_{OoB}$) is less conservative compared to the HC5 at the corresponding limit of the application range (HC5$_{IB}$) : i.e. full BLM HC5$_{OoB}$>full BLM HC5$_{IB}$). Open squares indicate that both values are relatively similar, i.e. within 5% of each other. Filled circles indicate that the OoB HC5 is more conservative compared to the HC5 at the corresponding limit of the application range (full BLM HC5$_{OoB}$<full BLM HC5$_{IB}$).
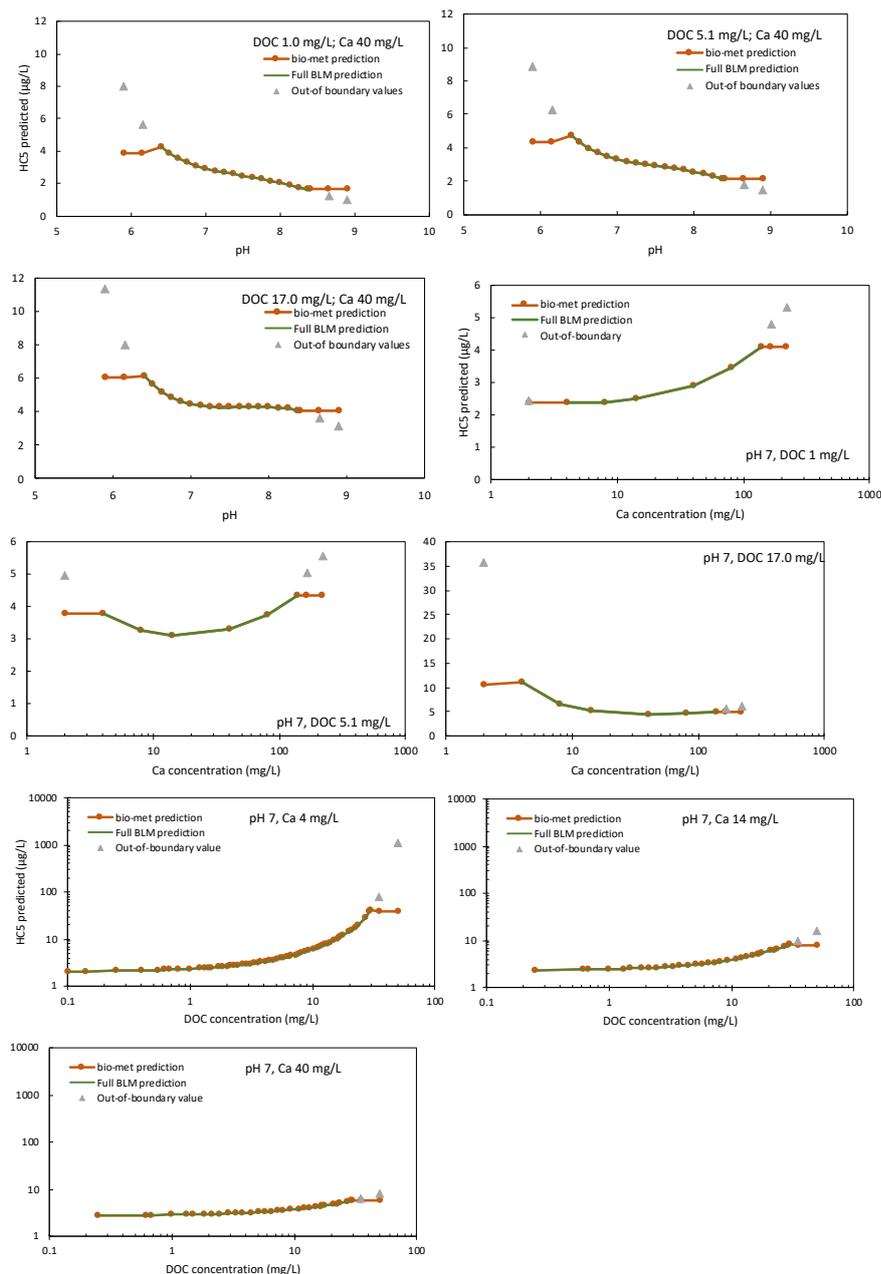
Figure A4.2 Overview of out-of-boundary behaviour of the full Co bioavailability normalization approach (green line) for selected physico-chemistry scenarios. Full bioavailability normalization HC5 predictions for conditions outside the application boundary of Co are visualized as grey triangles. For comparison, bio-met predictions are also shown.
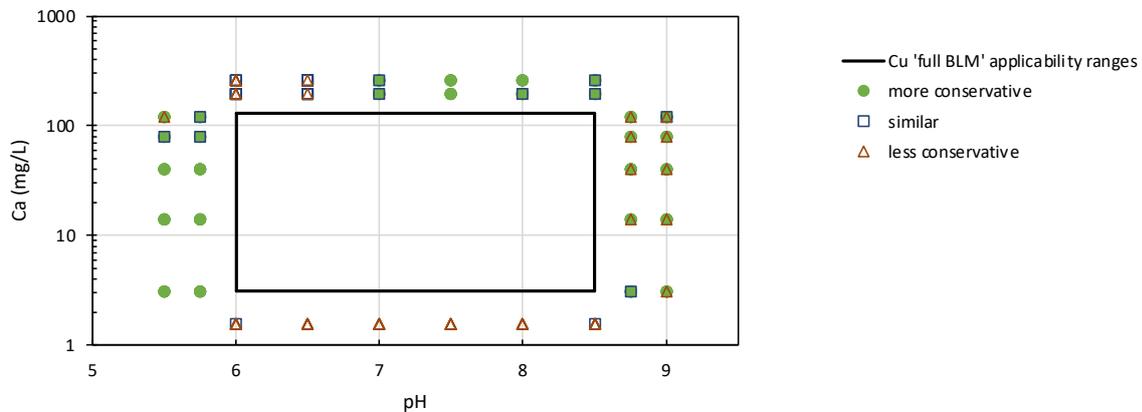
A4.2   Out of Boundary Analysis for Cu

The full Cu bioavailability normalization approach is applicable over the following physiochemistry ranges: pH 6.0 - 8.5, Ca 3.1 - 129 mg L^{-1} and DOC 0.1 - 30 mg L^{-1}. High DOC (>30 mg L^{-1}), and low Ca (<3.1 mg L^{-1}) tend to be conditions that result either in higher or similar predicted HC5 with the full Cu bioavailability normalization compared with the corresponding HC5 prediction at the border of the application range for Cu (Table A4.3, Figure A4.3). As such, both the full bioavailability normalization predictions, as well as the bio-met predictions can be considered to be conservative relative to he expected HC5 outside the

application boundary for Cu under these conditions. For conditions of low pH (pH<6.0), high pH (pH>8.4) and high Ca (Ca>129 mg L^{-1}) a complex pattern was observed depending on Ca and/or DOC conditions.

A visualization of the out-of-boundary behaviour of the Co full bioavailability normalization approach and those predicted by bio-met is given in Figure A4.. **bio-met predictions of local HC5 may be under conservative at low pH (pH<6.0), high pH (pH>8.5), and high Ca (Ca>129 mg L^{-1}).**

Especially for Ca, the relationship between bioavailability normalized HC5 and Ca is complex, and dependent on the considered DOC- and pH-level. The latter is because only one of the three original Cu bioavailability models include a Ca effect, i.e., the fish model (see Table 1 of Nys et al. 2024). As such, only in those bioavailability conditions where a fish species represents the most sensitive species in the SSD a positive relationship between Ca and HC5 is observed. This is the case for low pH (pH ~6) in combination with low DOC. Under high DOC conditions, due to competition between Ca and Cu for complexation at the NOM-binding sites such a positive relationship between Ca and Cu HC5 is not any longer observed. Note that in more recent versions of the Cu bioavailability models (Nys et al. 2024), also the invertebrate model includes a Ca effect. As such it is expected that a protective effect of Ca on Cu toxicity will be observed over a broader range of bioavailability conditions (i.e., also under some of the conditions where invertebrates become the most sensitive species in the SSD).
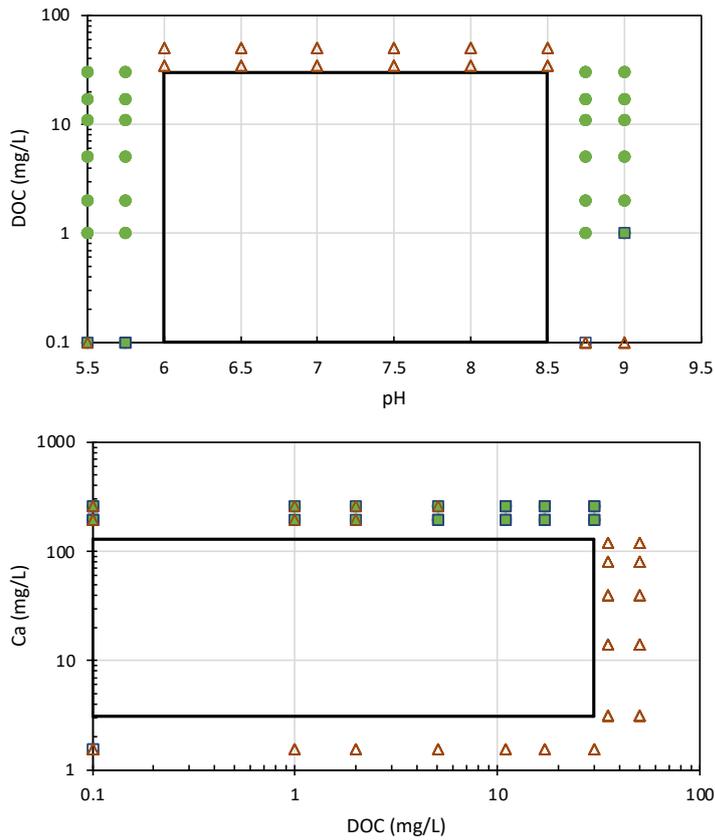
Figure A4.3 Overview of the outcome of the out-of-boundary (OoB) behaviour analysis for <u>Cu</u>. Full lines indicate the application boundaries of the full bioavailability normalization approach for Co. Open triangles indicate that the OoB HC5 (HC5$_{OoB)}$ is less conservative compared to the HC5 at the corresponding limit of the application range (HC5$_{IB}$) : i.e. full BLM HC5$_{OoB}$>full BLM HC5$_{IB}$). Open squares indicate that both values are relatively similar, i.e. within 5% of each other. Filled circles indicate that the OoB HC5 is more conservative compared to the HC5 at the corresponding limit of the application range (full BLM HC5$_{OoB}$<full BLM HC5$_{IB}$).

Table A4.3 Overview of out-of-boundary behaviour of HC5 predictions of the full bioavailability normalization approach (i.e. full BLM) for Cu
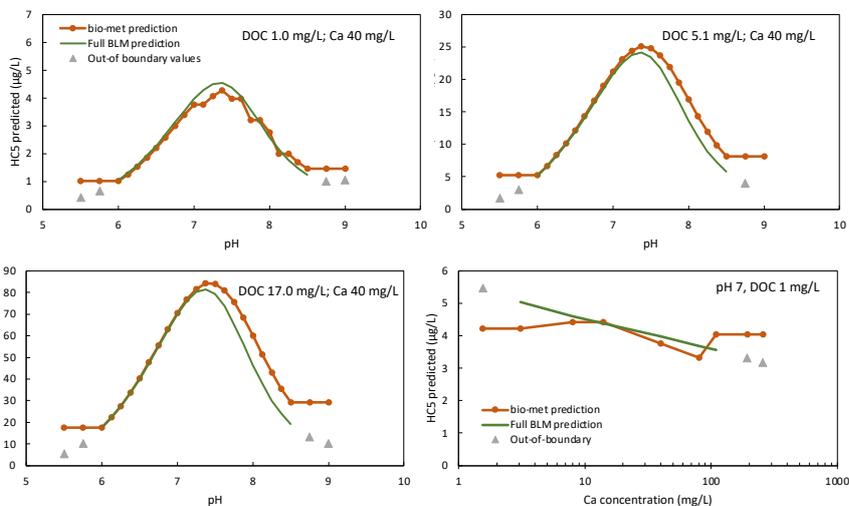
	Description of out-of-boundary behaviour for Cu
Low pH (pH<6.0)	Complex pattern dependent on Ca and/or DOC[b]
High pH (pH>8.5)	Complex pattern dependent on Ca and/or DOC[c]
High DOC (>30 mg L^{-1})	Full BLM prediction out of boundary is less conservative compared with value within boundary → **Full BLM HC5 prediction at DOC 30 mg L^{-1} is conservative for full BLM HC5 prediction at DOC>30 mg L^{-1}**
Low Ca (<3.1 mg L^{-1})	Full BLM prediction out of boundary is either conservative or similar compared with value within boundary → **Full BLM HC5 prediction at Ca 3.1 mg L^{-1} is conservative for full BLM HC5 prediction at Ca<3.1 mg L^{-1}**
High Ca (>129 mg L^{-1})	Complex pattern dependent on pH and/or DOC[d]

[a] Less conservative means that full BLM HC5$_{OoB}$>full BLM HC5$_{IB}$. Similar means that full BLM HC5$_{OoB}$>full BLM HC5$_{IB}$ are within 5% of each other. More conservative means that the full BLM HC5$_{OoB}$<full BLM HC5$_{IB}$.

[b] Full BLM prediction at low pH (pH<6.0) is generally more conservative compared with value within boundary, although in a few situations a less conservative or similar HC5 was observed. In general, the full BLM HC5 prediction at pH 6.0 is underconservative for full BLM HC5 prediction at pH<6.0.

[c] At low DOC concentrations (i.e. 0.1 mg DOC L^{-1}), the full BLM prediction at high pH (pH>8.5) is either similar or less conservative compared with the normalized HC5 within boundary. At all other evaluated DOC concentrations (≥1 mg DOC L^{-1}), full BLM predictions at high pH (pH>8.5) are generally more conservative compared with value within boundary. As such, at these DOC conditions the full BLM HC5 prediction at pH 8.5 is underconservative for full BLM HC5 prediction at pH>8.5

[d] At low DOC concentrations (i.e. 0.1 mg DOC L^{-1}), the full BLM prediction at high Ca (>129 mg Ca L^{-1}) is either similar or less conservative compared with the normalized HC5 within boundary. At all other evaluated DOC concentrations (≥1 mg DOC L^{-1}), full BLM predictions at high Ca (>129 mg L^{-1}) are generally more conservative compared with value within boundary. As such, at these DOC conditions the full BLM HC5 prediction at Ca 129 mg L^{-1} is underconservative for full BLM HC5 prediction at Ca>129 mg L^{-1}.
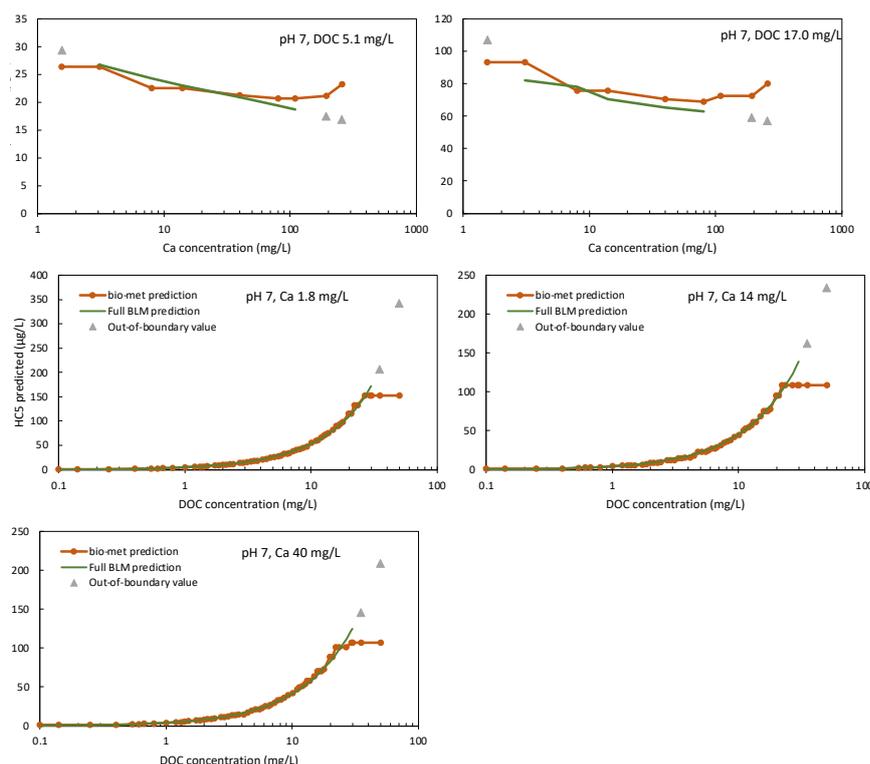
Figure A4.4 Overview of out-of-boundary behaviour of the full Cu bioavailability normalization approach (green line) for selected physico-chemistry scenarios. Full bioavailability normalization HC5 predictions for conditions outside the application boundary of Cu are visualized as grey triangles. For comparison, bio-met predictions are also shown.

A4.3   Out of Boundary Analysis for Ni

The full Ni bioavailability normalization approach is applicable over the following physico-chemistry ranges: pH 5.9-8.7, Ca 0.5-110 mg L^{-1} and DOC 0.1-30 mg L^{-1}. The physico-chemistry input levels evaluated for the out-of-boundary analysis of Ni are visualized in **Error! Reference source not found.**. High DOC (>30 mg L^{-1}), and high Ca (Ca>110 mg L^{-1}) tend to be conditions that result either in higher or similar predicted HC5 with the full Ni bioavailability normalization compared with the corresponding HC5 prediction at the border of the application range for Ni (Table A4.4, Figure A4.5). As such, both the full bioavailability normalization predictions, as well as the bio-met predictions can be considered to be conservative relative to the expected HC5 outside the application boundary for Ni at these conditions. For low pH (pH<5.9) and high pH conditions (pH>8.7), as well as for low Ca (<0.5 mg L^{-1}) a complex pattern was observed depending on either Ca and/or DOC or DOC and/or pH conditions.

A visualization of the out-of-boundary behaviour of the Ni full bioavailability normalization approach and those predicted by bio-met is given Figure A4.6. **bio-met predictions of local HC5 may be under conservative at high pH (pH>8.7) and low Ca concentrations (Ca<0.5 mg L^{-1}).**

Table A4.4 Overview of out-of-boundary behaviour of HC5 predictions of the full bioavailability normalization approach (i.e. full BLM) for Ni

	Description of out-of-boundary behaviour for Ni
Low pH (pH<5.9)	Complex pattern dependent on Ca- and/or DOC-level[b]
High pH (pH>8.7)	Complex pattern dependent on Ca- and/or DOC-level[c]
High DOC (>30 mg L^{-1})	Full BLM prediction out of boundary is generally less conservative compared with value within boundary, although in specific conditions the prediction is similar compared with the value within boundary → **Full BLM HC5 prediction at DOC 30 mg L^{-1} is in general conservative for full BLM HC5 prediction at DOC>30 mg L^{-1}**
Low Ca (<0.5 mg L^{-1})	Complex pattern dependent on pH- and/or DOC-level[d]
High Ca (>110 mg L^{-1})	Full BLM prediction out of boundary is mostly less conservative compared with value within boundary[e] → **Full BLM HC5 prediction at Ca 110 mg L^{-1} is in general conservative for full BLM HC5 prediction at Ca>110 mg L^{-1}**

[a] Less conservative means that full BLM HC5$_{OoB}$>full BLM HC5$_{IB}$. Similar means that full BLM HC5$_{OoB}$>full BLM HC5$_{IB}$ are within 5% of each other. More conservative means that the full BLM HC5$_{OoB}$<full BLM HC5$_{IB}$.

[b] At low DOC to moderate DOC concentrations (i.e. 0.1-1.0 mg DOC L^{-1}), the full BLM prediction at low pH (pH<5.9) is either similar or less conservative compared with the normalized HC5 within boundary. At higher DOC concentrations (>5.1 mg DOC L^{-1}), full BLM predictions at low pH (pH<5.9) can result in more conservative HC5s compared with value within boundary. As such, at these DOC conditions the full BLM HC5 prediction at pH 5.9 may be underconservative for full BLM HC5 prediction at pH<5.9

[c] At Ca≥ 1.8 mg L^{-1}, full BLM predictions for pH>8.7 are generally more conservative HC5s compared with value within boundary. As such, at these conditions the full BLM HC5 prediction at pH 8.7 may be underconservative for full BLM HC5 prediction at pH>8.7. At Ca 0.5 mg L^{-1}, full BLM predictions for pH>8.7 are either similar or less conservative compared with value within boundary.

[d] Depending on the combination of mainly DOC, but also pH, the full BLM predictions for Ca<0.5 mg L^{-1} may result in a more conservative HC5s compared with value within boundary.

[e] One exception was noted at pH 8.7 and DOC 0.1 mg L^{-1}
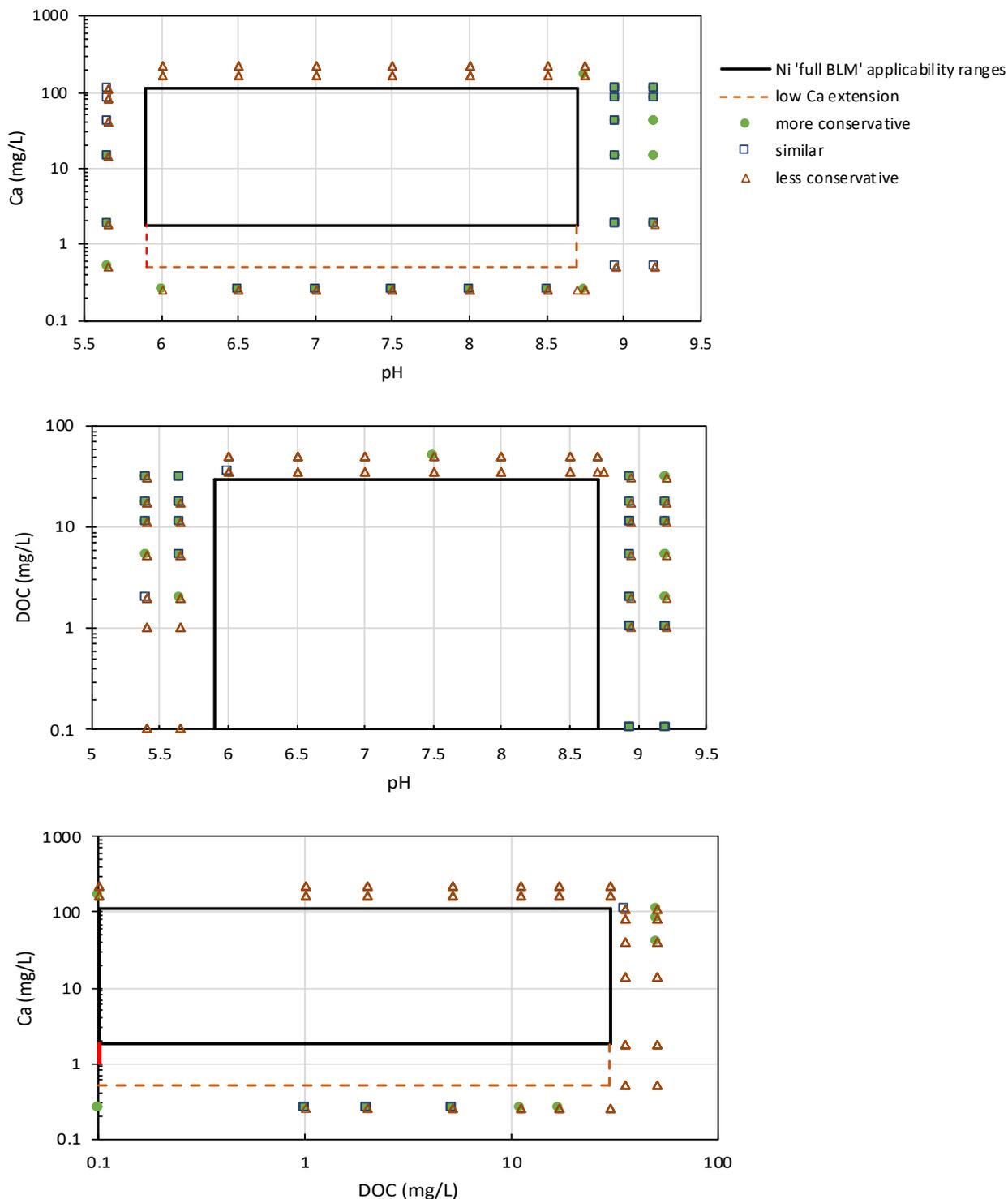
Figure A4.5 Overview of the outcome of the out-of-boundary (OoB) behaviour analysis for Ni. Full lines indicate the application boundaries of the full bioavailability normalization approach for Co. Open triangles indicate that the OoB HC5 (HC5$_{OoB}$) is less conservative compared to the HC5 at the corresponding limit of the application range (HC5$_{IB}$) : i.e. full BLM HC5$_{OoB}$>full BLM HC5$_{IB}$). Open squares indicate that both values are relatively similar, i.e. within 5% of each other. Filled circles indicate that the OoB HC5 is more conservative compared to the HC5 at the corresponding limit of the application range (full BLM HC5$_{OoB}$<full BLM HC5$_{IB}$).
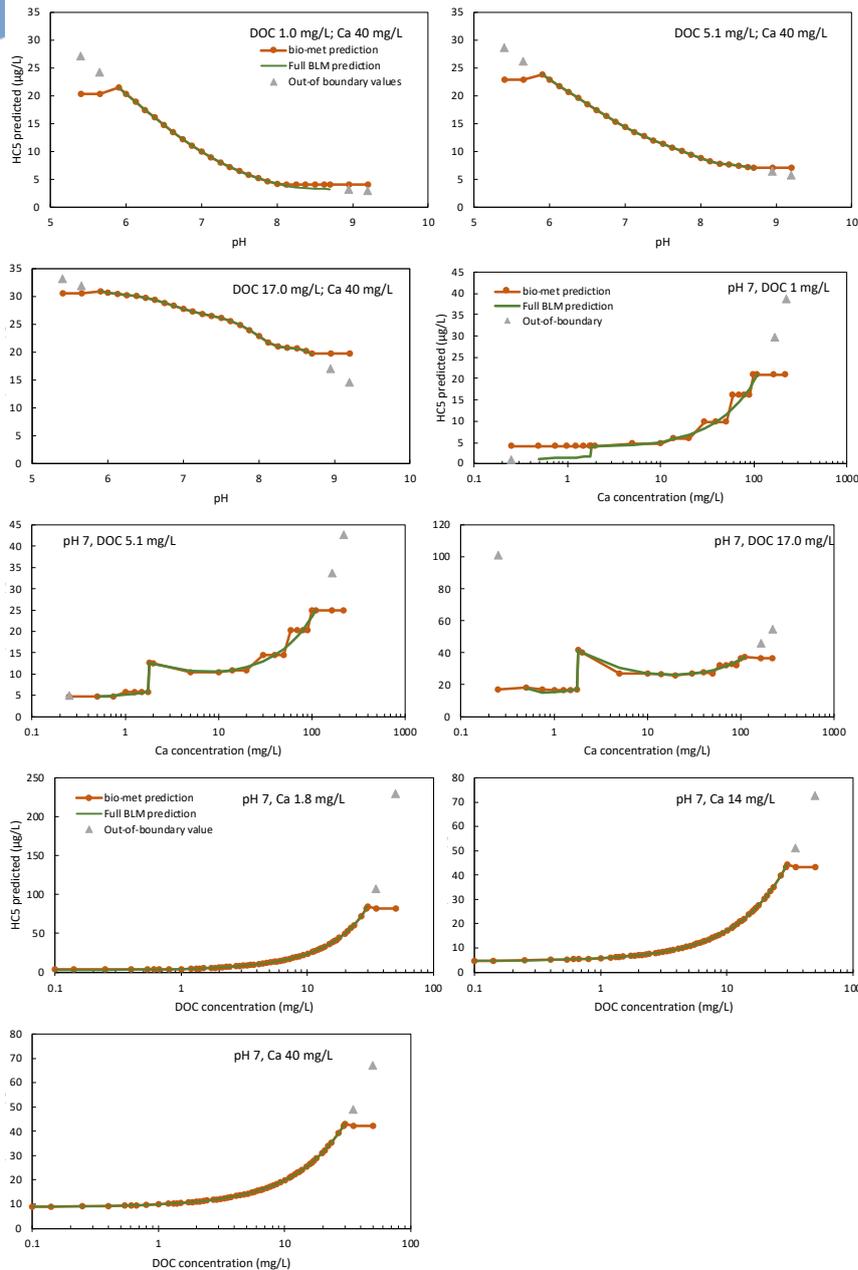
Figure A4.6 Overview of out-of-boundary behaviour of the full Ni bioavailability normalization approach (green line) for selected physico-chemistry scenarios. Full bioavailability normalization HC5 predictions for conditions outside the application boundary of Ni are visualized as grey triangles. For comparison, bio-met predictions are also shown. The 'jumpy behaviour' observed in the Ca-panels, represents the switch from the low Ca-extension to the normal Ni bioavailability models.

A4.4   Out of Boundary Analysis for Pb

The full Pb bioavailability normalization approach is applicable over the following physico-chemistry ranges: pH 6.3 - 8.4, Ca 3.6 - 140 mg L^{-1} and DOC 0.1 - 30 mg L^{-1}. High DOC (>30 mg L^{-1}), and high pH (pH>8.4) tend to be conditions that result either in higher or similar predicted HC5 with the full Pb bioavailability normalization compared with the corresponding HC5 prediction at the border of the application range for Pb (Table A4.5, Figure A4.7). As

such, both the full bioavailability normalization predictions, as well as the bio-met predictions can be considered to be conservative relative to the expected HC5 outside the application boundary for Pb under these conditions.

For low pH (pH<6.3), low Ca (<3.6 mg L^{-1}) and high Ca (>140 mg L^{-1}) a complex pattern was observed depending on either Ca and/or DOC or DOC and/or pH conditions. A visualization of the out-of-boundary behaviour of the Pb full bioavailability normalization approach and those predicted by bio-met is given Figure A4.8. **bio-met predictions of local HC5 may be under conservative at low pH (pH<6.3), low Ca (<3.6 mg L^{-1}) and high Ca (>140 mg L^{-1}).**

Table A4.5 Overview of out-of-boundary behaviour of HC5 predictions of the full bioavailability normalization approach (i.e. full BLM) for <u>Pb</u>

	Description of out-of-boundary behaviour for Pb
Low pH (pH<6.3)	Complex pattern dependent on Ca- and/or DOC-level[b]
High pH (pH>8.4)	Full BLM prediction out of boundary is either less conservative or similar compared with value within boundary. → **Full BLM HC5 prediction at pH 8.4 is in general conservative for full BLM HC5 prediction at pH>8.4**
High DOC (>30 mg L^{-1})	Full BLM prediction out of boundary less conservative compared with value within boundary. → **Full BLM HC5 prediction at DOC 30 mg L^{-1} is in general conservative for full BLM HC5 prediction at DOC>30 mg L^{-1}**
Low Ca (<3.6 mg L^{-1})	Complex pattern dependent on pH- and/or DOC-level[c]
High Ca (>110 mg L^{-1})	Complex pattern dependent on pH- and/or DOC-level[d]

[a] Less conservative means that full BLM $HC5_{OoB}$>full BLM $HC5_{IB}$. Similar means that full BLM $HC5_{OoB}$>full BLM $HC5_{IB}$ are within 5% of each other. More conservative means that the full BLM $HC5_{OoB}$<full BLM $HC5_{IB}$.

[b] Under most conditions, the full BLM predictions for pH<6.3 result in either similar or less conservative HC5-predictions compared to the within boundary HC5-predictions. However, at combinations of low Ca (<14 mg Ca L^{-1}) and certain DOC the full BLM prediction for pH<6.3 may result in a more conservative HC5-prediction compared to the within boundary HC5-prediction.

[c] Under most conditions, the full BLM predictions for Ca<3.6 mg L^{-1} result in either similar or less conservative HC5-predictions compared to the within boundary HC5-predictions, with the exception of combinations of low pH (pH<7.0) and low DOC (DOC<1 mg L^{-1}) where the full BLM predictions for Ca<3.6 mg L^{-1} may be more conservative compared to the within boundary value.

[d] The full BLM predictions for Ca>140 mg L^{-1} result mostly in similar or less conservative HC5-predictions compared to the within boundary HC5-predictions, with the exception of combinations of high Ca (>210 mg Ca L^{-1}), high pH (pH>7.5) and moderate to high DOC (>2 mg DOC L^{-1}) for which the full BLM predictions for Ca>210 mg L^{-1} may be more conservative compared to the within boundary value.
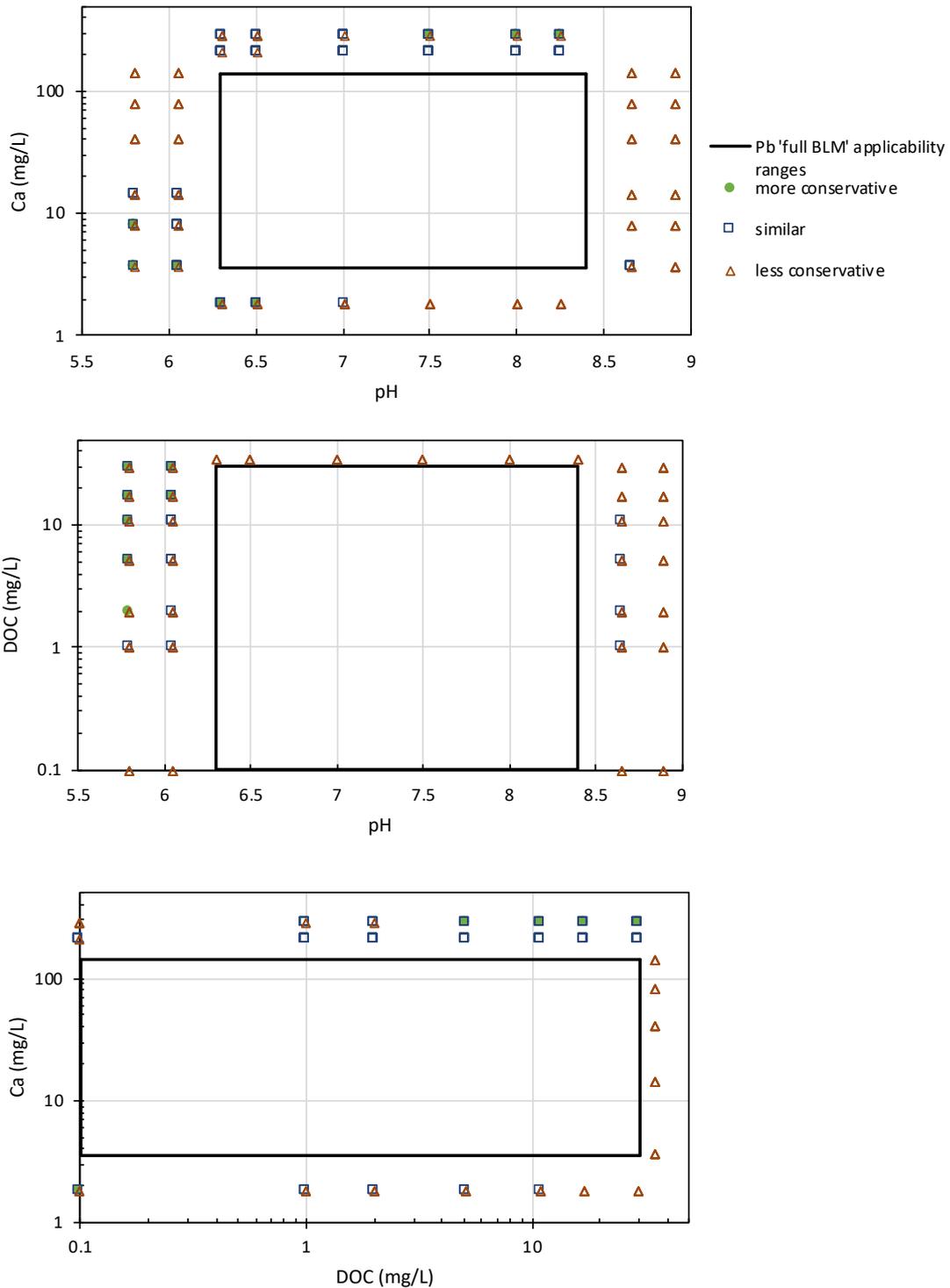
Figure A4.7 Overview of the outcome of the out-of-boundary (OoB) behaviour analysis for Ni. Full lines indicate the application boundaries of the full bioavailability normalization approach for Pb. Open triangles indicate that the OoB HC5 (HC5$_{OoB}$) is less conservative compared to the HC5 at the corresponding limit of the application range (HC5$_{IB}$) : i.e. full BLM HC5$_{OoB}$>full BLM HC5$_{IB}$). Open squares indicate that both values are relatively similar, i.e. within 5% of each other. Filled circles indicate that the OoB HC5 is more conservative compared to the HC5 at the corresponding limit of the application range (full BLM HC5$_{OoB}$<full BLM HC5$_{IB}$).
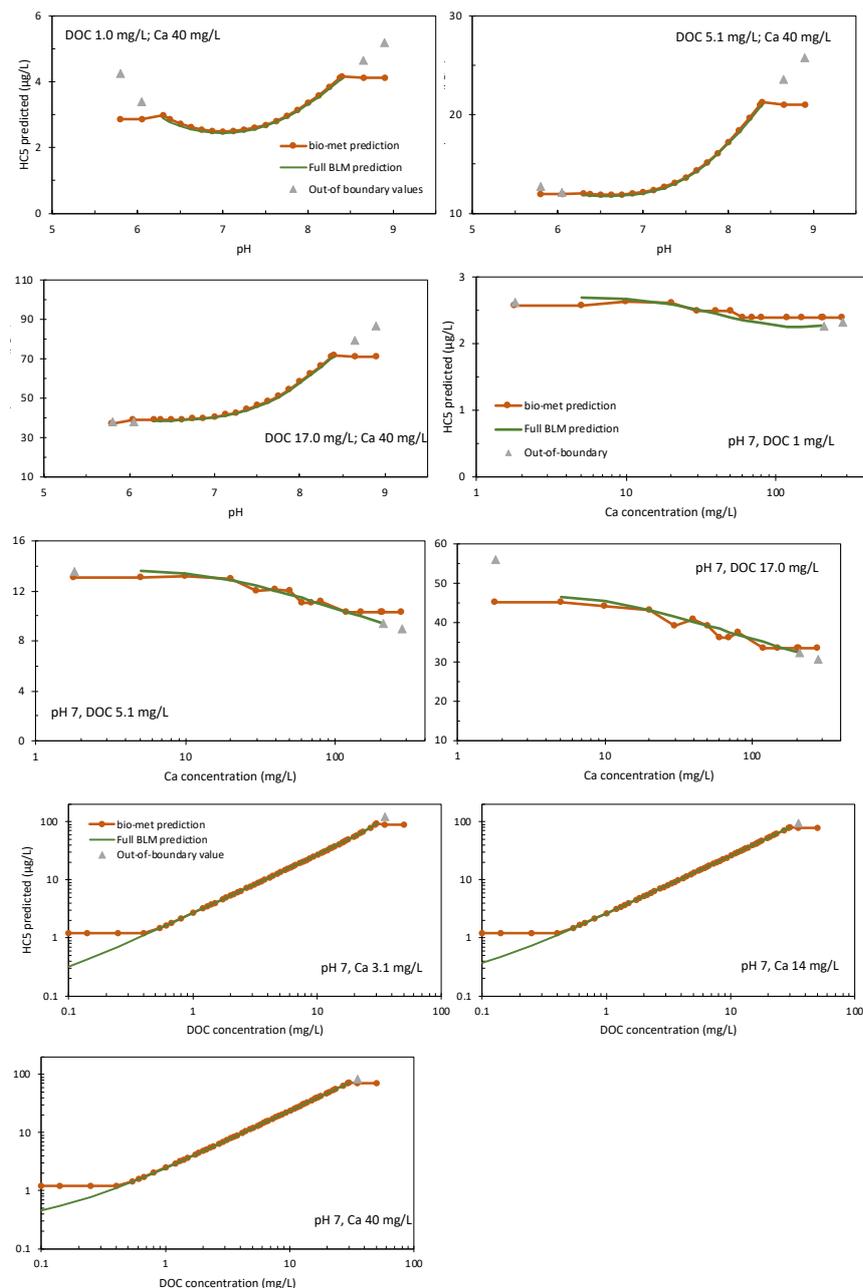
Figure A4.8 Overview of out-of-boundary behaviour of the full Pb bioavailability normalization approach (green line) for selected physico-chemistry scenarios. Full bioavailability normalization HC5 predictions for conditions outside the application boundary of Pb are visualized as grey triangles. For comparison, bio-met predictions are also shown.

A4.5 Out of Boundary Analysis for Zn

The full Zn bioavailability normalization approach is applicable over the following physico-chemistry ranges: pH 5.5 - 8.5, Ca 0.8 - 160 mg L^{-1} and DOC 0.1 - 30 mg L^{-1}. The physico-chemistry input levels evaluated for the out-of-boundary analysis of Zn are visualized in **Error! Reference source not found.**. High DOC (>30 mg L^{-1}), low Ca (<0.8 mg L^{-1}) and high Ca (Ca>160 mg L^{-1}) tend to be conditions that result either in higher or similar predicted HC5 with the full Zn bioavailability normalization compared with the corresponding HC5 prediction

at the border of the application range for Zn (Table A4.6). As such, both the full bioavailability normalization predictions, as well as the bio-met predictions can be considered to be conservative relative to he expected HC5 outside the application boundary for Zn. For both low pH (pH<5.5) and high pH conditions (pH>8.4) a more complex pattern was observed depending on Ca and/or DOC conditions.

A visualization of the out-of-boundary behaviour of the Zn full bioavailability normalization approach and those predicted by bio-met is given in Figure A4.4. **bio-met predictions of local HC5 may be under conservative at low pH (pH<5.5), and high pH (pH>8.4).**

Table A4.6 Overview of out-of-boundary behaviour of HC5 predictions of the full bioavailability normalization approach (i.e. full BLM) for Zn

	Description of out-of-boundary behaviour for Zn
Low pH (pH<5.5)	Complex pattern dependent on Ca- and/or DOC-level[b]
High pH (pH>8.5)	Complex pattern dependent on Ca- and/or DOC-level[c]
High DOC (>30 mg L^{-1})	Full BLM prediction out of boundary is either less conservative or similar compared with value within boundary, → **Full BLM HC5 prediction at DOC 30 mg L^{-1} is in general conservative for full BLM HC5 prediction at DOC>30 mg L^{-1}**
Low Ca (<0.8 mg L^{-1})	Full BLM prediction out of boundary is similar compared with value within boundary → **Full BLM HC5 prediction at 0.8 mg L^{-1} is conservative for full BLM HC5 prediction at Ca<0.8 mg L^{-1}**
High Ca (>160 mg L^{-1})	Full BLM prediction out of boundary is similar compared with value within boundary → **Full BLM HC5 prediction at 160 mg L^{-1} is conservative for full BLM HC5 prediction at Ca>160 mg L^{-1}**

[a] Less conservative means that full BLM HC5$_{OoB}$>full BLM HC5$_{IB}$. Similar means that full BLM HC5$_{OoB}$>full BLM HC5$_{IB}$ are within 5% of each other. More conservative means that the full BLM HC5$_{OoB}$<full BLM HC5$_{IB}$.

[b] At low DOC (at least upto 2 mg DOC L^{-1}): Full BLM out of boundary prediction is in general less conservative compared with value within boundary. At high DOC combined with low Ca (<40 mg Ca L^{-1}) Full BLM out of boundary prediction might become more conservative compared with value within boundary, under these conditions bio-met predicted HC5 may be under conservative.

[c] Full BLM out of boundary prediction is in most conditions either less conservative or similar compared with value within boundary. However, in some situation a more conservative prediction could be generated compared with the condition within the boundaries
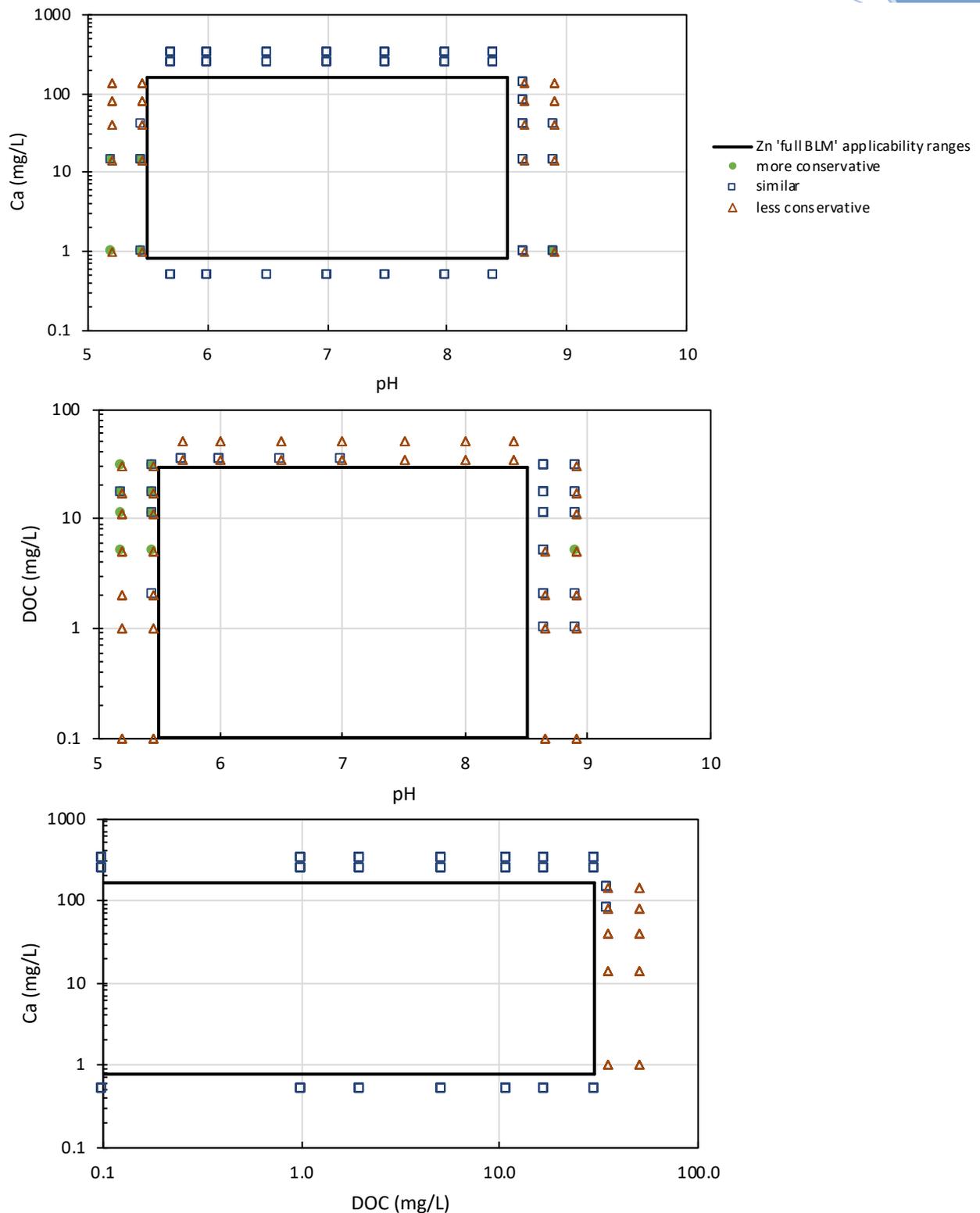
Figure A4.9 Overview of the outcome of the out-of-boundary (OoB) behaviour analysis for Zn. Full lines indicate the application boundaries of the full bioavailability normalization approach for Co. Open triangles indicate that the OoB HC5 (HC5$_{OoB}$) is less conservative compared to the HC5 at the corresponding limit of the application range (HC5$_{IB}$) : i.e. full BLM HC5$_{OoB}$>full BLM HC5$_{IB}$). Open squares indicate that both values are relatively similar, i.e. within 5% of each other. Filled circles indicate that the OoB HC5 is more conservative compared to

the HC5 at the corresponding limit of the application range (full BLM HC5$_{OoB}$<full BLM HC5$_{IB}$).
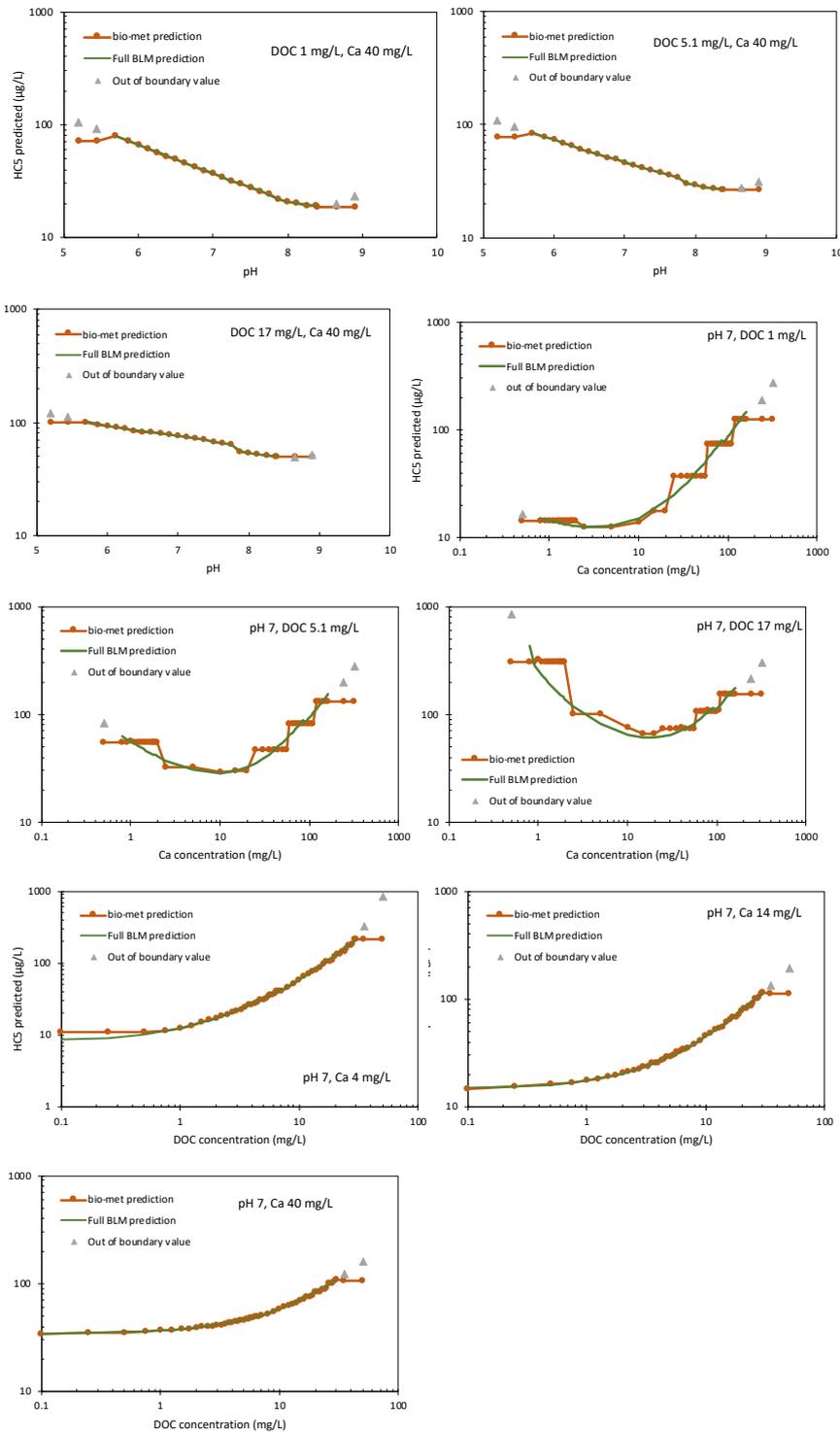

Figure A4.4 Overview of out-of-boundary behaviour of the full Zn bioavailability normalization approach (green line) for selected physico-chemistry scenarios. Full bioavailability normalization HC5 predictions for conditions outside the application boundary of Zn are visualized as grey triangles. For comparison, bio-met predictions are also shown.