Common Implementation Strategy
for the Water Framework Directive (2000/60/EC)

Guidance Document No. 38
Technical Guidance for implementing Environmental Quality Standards (EQS) for metals

Consideration of metal bioavailability and natural background concentrations in assessing compliance

Document endorsed by EU Water Directors at their meeting in Helsinki on 26-27 November 2019

Disclaimer:

This technical document has been developed through a collaborative framework (the Common Implementation Strategy (CIS)) involving the Member States, EFTA countries, and other stakeholders including the European Commission. The document is a working draft and does not necessarily represent the official, formal position of any of the partners. To the extent that the European Commission's services provided input to this technical document, such input does not necessarily reflect the views of the European Commission. Neither the European Commission nor any other CIS partners are responsible for the use that any third party might make of the information contained in this document. The technical document is intended to facilitate the implementation of Directive 2000/60/EC and is not legally binding. Any authoritative reading of the law should only be derived from Directive 2000/60/EC itself and other applicable legal texts or principles. Only the Court of Justice of the European Union is competent to authoritatively interpret Union legislation.

FOREWORD

Guidance documents have been produced to support the implementation of various aspects of the Water Framework Directive (2000/60/EC) (WFD) and its daughter directives. These documents aim to deliver practical advice and assistance on various technical issues associated with the implementation of the Directive. However, guidance is not itself legally binding and cannot be considered as a legal interpretation of the WFD or any other legal texts.

Whilst occurring naturally in the aquatic environment, certain metals are also considered to pose a hazard to the water environment of Europe of sufficient magnitude to be classified as Priority Substances under the Water Framework Directive. The ecotoxicological risk from certain metals is now understood to be associated with their “bioavailability”, which is controlled by site-specific water physico-chemistry (e.g. pH, dissolved ion concentrations, dissolved organic carbon).

Directive 2008/105/EC as amended by Directive 2013/39/EU includes annual average Environmental Quality Standards (EQS) for nickel (Ni) and lead (Pb) that refer to their *bioavailable* concentrations. This Guidance Document has been developed to support the implementation of bioavailability-based EQS for metals. It also includes consideration of river basin specific pollutants common to many Member States, such as copper (Cu) and zinc (Zn).

The EQSD also explicitly acknowledges the issue of natural background concentrations (NBCs) of metals. Recommendations for the derivation and consideration of NBCs in the context of assessing the chemical status of water bodies are also provided in this Guidance Document.

It is important to acknowledge that any relatively novel regulatory approach will present a need for changes to existing ways of working and potential challenges for implementation. Therefore, the benefits of the new approach must outweigh the disadvantages, and this balance must be clearly apparent to non-experts. The changes required should provide environmental benefits alongside the opportunity to maintain at the same level, or reduce, the regulatory burdens.

This Guidance Document is intended as a “living”, or “dynamic”, document that will be updated as application and experience of bioavailability-based approaches increases within the European Union and beyond. There are some remaining challenges to the implementation of bioavailability-based EQS, and these have been explicitly acknowledged in the different chapters.

This guidance is intended to be used by both the regulatory and regulated communities to promote common understanding of the best practices and challenges associated with implementing bioavailability-based EQS.

CONTENTS

TABLES

FIGURES

1. INTRODUCTION

1.1. Background, historical developments and level of regulatory acceptance

It has long been recognised that metal ecotoxicity in water varies with the chemical characteristics specific to each water body. Therefore, for inorganic cationic metals in freshwaters, many global regulatory jurisdictions set limit values that vary according to the water hardness. However, water hardness is just one of the several important parameters that explain the observed differences in metal ecotoxicity. For example, dissolved organic carbon (DOC) has also been shown to be a key parameter able to predict the behaviour, fate and toxicity of metals in aquatic systems (e.g. EA, 2012a).

Recent years have seen a major refinement in the scientific understanding of the behaviour, fate and toxicity of metals in the environment. Conventional metrics used for the risk assessment of metals in soils, waters and sediments have been demonstrated to be prone to incorrect estimation of the likely ecological impacts (e.g. Zwolsman and De Schamphelaere, 2007; EA, 2008a/b; EA, 2012b).

The Environmental Quality Standards Directive (EQSD) (Directive 2008/105/EC, EU, 2008b), which sets Environmental Quality Standards (EQS) for several substances in freshwaters at EU level, has since its adoption included a provision (Annex I Part B Para 3(b)) allowing Member States to take account of water quality parameters that affect the bioavailability of metals in their assessment of compliance with the EQS set under the Water Framework Directive (WFD). When the EQSD was amended (by Directive 2013/39/EU, EU, 2013), annual average EQS (AA-EQS) referring specifically to bioavailable concentrations were introduced for nickel (Ni) and lead (Pb) for freshwaters. Some Member States have also derived bioavailability-based AA-EQS for other metals that are river basin specific pollutants (RBSPs).

The concept of bioavailability influencing environmental risk from metals in the environment is thus not new, but it has received increased regulatory and scientific support in Europe in recent years, not only because of the developments under the EU water legislation but also as a result of a series of statutory and voluntary risk assessments performed under the Existing Substances Regulation (EEC) No. 793/93 (e.g. Ni, EU, 2008a). Although accounting for bioavailability of metals has been allowed under the EQSD, it has not been done routinely, and the application of bioavailable EQS represents a new way of working for most regulators.

1.2. Aims and scope of the guidance

The main aim of this guidance is to facilitate the consideration of bioavailability in the status assessment of freshwater bodies in relation to metals, including the application of EQS ($EQS_{bioavailable}$). It also aims to explain how natural background concentrations (NBCs) may be accounted for, including when no suitable bioavailability model exists.

Increasing amounts of practical regulatory-specific guidance on approaches to account for metal bioavailability are now available, especially in relation to implementing EQS (e.g. WCA, 2015; ISPRA, 2016; Swedish Marine and Water Authority, 2016; State of Oregon, 2016).

This implementation guidance intends to be generic and is thus not specific to any particular metal or region. While emphasising compliance assessment under the WFD, it attempts to show how bioavailability and NBC corrections can be used in different cases for risk assessment, prioritisation of measures or where available monitoring information may not be comprehensive.

The scope of this guidance covers the practical steps required to implement approaches that account for bioavailability by using a so-called $EQS_{bioavailable}$. It only briefly mentions the derivation of EQS or $EQS_{bioavailable}$ because existing guidance already details this subject (EC, 2018). The scientific basis of metal bioavailability and biotic ligand models (BLMs) is covered elsewhere in detail (e.g. Heijerick et al., 2002; Paquin et al., 2002; Niyogi and Wood, 2004; De Schamphelaere et al., 2005) and therefore only briefly described in this guidance (Sections 1.3 and 3.3) to provide definitions and support the technical foundations on which the approaches are based. The proposed tiered approach to include bioavailability correction and consider the NBCs is described in Chapter 2. The development of the simplified bioavailability calculation tools and their performance characteristics are described in Chapter 3; and the operational requirements for implementing $EQS_{bioavailable}$ are also illustrated by the experiences and preferences of several Member States. Chapter 4 outlines approaches to determining the NBCs of metals and provides case studies from several Member States. In Chapter 5, monitoring data requirements for status assessment are detailed, along with the role of incomplete historical data in performing a screening assessment/risk analysis. Finally, in Chapter 6, the calculations needed to support compliance assessment are described step-by-step, as are the interpretation of results and possible “trouble shooting”.

1.3. Bioavailability and natural background concentrations

1.3.1. Bioavailability

The term "bioavailability" can mean a number of different things depending on the particular area of science, but in relation to this guidance and under the WFD, bioavailability is considered to be a combination of the physico-chemical factors governing metal behaviour (the abiotic part) and the nature of the biological receptor – the combination resulting in specific physiological effects influenced by the route of entry, the duration and the frequency of exposure etc.. There are many analytical and modelling techniques that purport to assess or measure metal bioavailability in fresh water. These methods include speciation-based modelling, ion-selective electrodes, passive samplers such as diffusive gradients on thin films (DGTs), kinetic ion exchange columns and ultrafiltration (e.g. Unsworth et al., 2006). However, these approaches are effectively chemical measurements or modelling of the form of a metal in the water column, i.e. ‘availability’, with limited ability to take account of competitive effects at the ‘biotic ligand’ (e.g. a fish gill). Availability is only one of the two critical components that determine the bioavailability.

Figure 1 shows a conceptual diagram illustrating bioavailability, where BL is the biotic ligand and Me^{z+} represents the free metal ion activity in the water column. Using chemical equilibrium modelling, the BLM addresses the competition between the free metal ion and the other cations for complexation with a biotic ligand, which is the site where biological uptake may occur. Typically, these are the gill structures of fish and invertebrates, and the cell surfaces of algae. As illustrated below, the most important factors influencing bioavailability include acidity/basicity (pH), dissolved organic carbon (DOC), and hardness cations (calcium (Ca^{2+}), magnesium (Mg^{2+}), and sodium (Na^{+})).

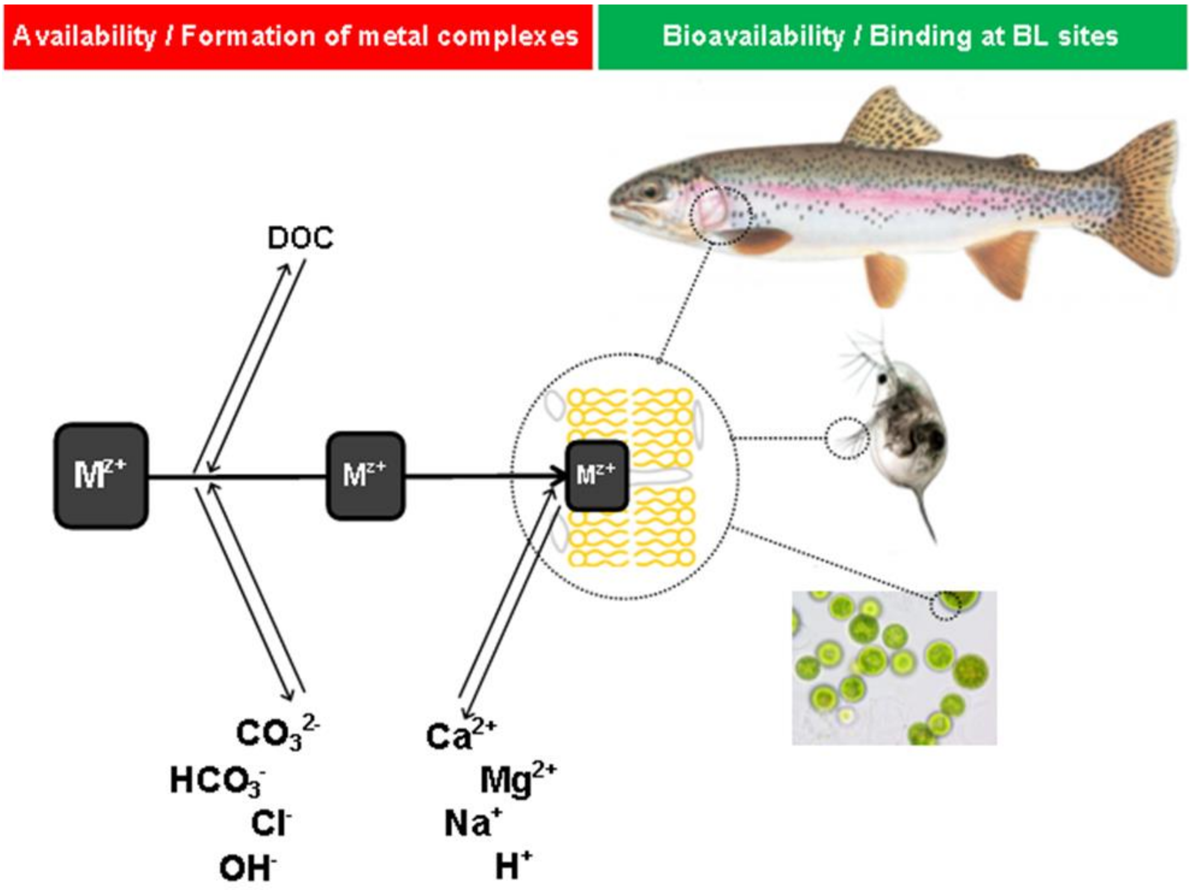


Figure 1: The Biotic Ligand Model (BLM) conceptual framework.

The Biotic Ligand Model (BLM) conceptual framework illustrating the abiotic speciation component and the biotic interactions (modified from www.windwardenv.com)

A measure of bioavailability reflects what the organism in the water column "*experiences*" regarding metal exposure and so what should be regarded as of regulatory relevance. It has long been established that measures of total metal in waters are of limited relevance to explaining the observed toxicity of waterborne metals to aquatic biota (e.g. Campbell, 1995; Niyogi and Wood, 2004).

Besides bioavailability, one of the challenges of implementing ecologically relevant metrics to assess risks from metals in the environment is how to deal with NBCs. It is important to state that consideration of NBCs does not equate to considering bioavailability. Both NBCs and bioavailability normalisations can be considered in the same integrated compliance checking process (Chapter 2).

1.3.2. Background concentrations

Member States, when assessing the monitoring results against the relevant EQS, may consider the NBCs for dissolved metals where such concentrations prevent compliance with the relevant EQS. The long history of metal mining and processing in Europe, along with numerous diffuse sources (essentially atmospheric deposition), means that the concentration of a metal in surface waters often consists of both a natural and an anthropogenic fraction (e.g. ISO, 2005; ECHA, 2008). In this context, the anthropogenic contribution (referring here to moderate diffuse inputs into the water, and not inputs from local point sources) to the environmental metal concentrations may be difficult to distinguish from the NBCs, which are considered to be the concentrations sourced only from natural geogenic cycling and processes. However, Member States should strive to reach an estimate of NBCs close to undisturbed conditions as outlined in Chapter 4.

1.4. How are bioavailability-based standards derived?

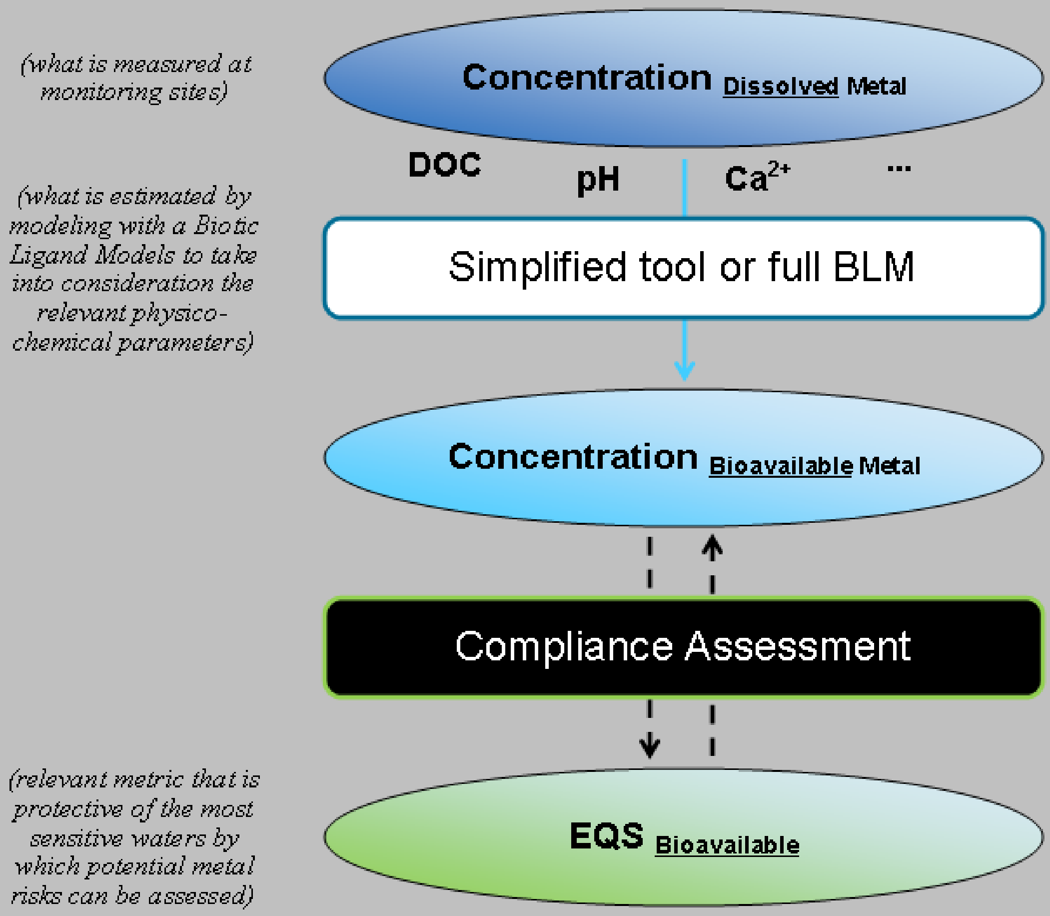
The derivation of EQS, including those based on bioavailability, has been addressed in a separate technical guidance document (EC, 2018). When deriving and implementing EQS for metals, there are clearly several challenges that are not usually encountered when considering synthetic chemicals. These challenges may notably include:

- The presence of low concentrations of metals from natural and/or anthropogenic diffuse sources;
- Changes in the form or speciation of a metal in response to water chemistry conditions;
- The considerable influence the form of the metal has upon bioavailability and its consequent ecotoxicity to aquatic organisms;
- The importance of some metals as essential elements for the functioning of biological systems.

European Regulators have stressed the need to have only one legal value for the EQS for individual metals, as for other substances, to ensure a level playing field. However, using a single EQS based on total dissolved concentrations for all European waters can result in quality failures in places where the bioavailability is low and, as a result, no adverse effects (e.g. reduction in ecological status) are necessarily seen. This is why the EQSD allows Member States to take bioavailability into account in assessing compliance.

By considering metal bioavailability already when deriving the EQS (expressed as an $EQS_{bioavailable}$), and by adopting a tiered, risk-based, approach (Chapter 2) to its application, it is possible to achieve a more consistent level of ecological protection despite different bioavailability conditions at different locations. To assess compliance, the bioavailable concentration of a dissolved metal, as calculated using a BLM or another equivalent tool from the measured total dissolved concentration, can be compared to the $EQS_{bioavailable}$. The bioavailable concentration is generally lower than the total dissolved concentration because only a fraction of it will usually be bioavailable unless water chemistry physicochemical conditions result in high levels of bioavailability. It should be noted that the influencing conditions vary with the considered metal. Under conditions of high bioavailability, the water will be considered as “sensitive”. This distinction is important because the application of an $EQS_{bioavailable}$ to dissolved metal monitoring data without appropriate correction for bioavailability may result in an overestimation of the risk from the metal present in the water at a given site. Therefore, although $EQS_{bioavailable}$ can be compared with total dissolved metal concentrations in the first tier of a compliance assessment, it will probably be necessary to consider bioavailable concentrations in a subsequent tier (Chapter 2).

Background understanding - Compliance assessment is based on the comparison of the bioavailable concentration of the metal in the water (calculated with Biotic Ligand) Models with the $EQS_{bioavailable}$.



There is only one value for the EQS

An $EQS_{bioavailable}$ has been set up under the EU WFD for nickel and lead. The EQSbioavailable is the only legally binding EQS for these substances and applies to the freshwater environment.

The $EQS_{bioavailable}$ is derived for a reference water chemistry condition that is representative of high (reasonable worst-case) metal bioavailability, termed “sensitive conditions”. When applied in a tiered approach, the use of a reasonable worst-case $EQS_{bioavailable}$ during the initial assessment reduces the identification of false negatives, i.e. approving a water sample or site that should have failed (Type II errors). It is important to define the reference conditions that were used to derive the $EQS_{bioavailable}$. To achieve this, it is necessary to understand the abiotic conditions that are likely to result in the greatest metal bioavailability (also known as the "most sensitive" conditions to metals exposure). The most sensitive conditions vary among metals and between taxonomic groups for a given metal, and therefore what may be considered sensitive for one metal may not necessarily be sensitive for another one.

To define conditions of high bioavailability, a BLM can be used to predict the dissolved metal concentrations likely to cause no toxic effect under a relevant combination of water chemistry parameters. Using appropriate bioavailability normalisation procedures, bioavailability-based predicted no-effect concentrations (PNECs) for different waters across the EU were calculated using existing monitoring datasets, resulting in a range of bioavailability-based PNECs relevant to the EU. The final set of reference conditions that is selected must represent reasonable worst-case bioavailability conditions in order to make the $EQS_{bioavailable}$ adequately protective of all EU waterbodies when applied as a screening step within a tiered compliance assessment process (EC, 2010).

As an example of this approach, Table 1 shows the 5th and 10th percentiles of site-specific PNECs derived using the Ni-BLM for an EU dataset from England, Wales, Scotland, Sweden, Austria, Spain, the Elbe Basin (DE), the Walloon region (BE) and Northern France. This kind of exercise requires individual freshwater sample data for which all relevant determinants (pH, DOC, etc.) have been measured at the same time as the dissolved metal. It is important to note that the PNECs reported in Table 1 are the hazardous concentration values affecting 5% of the species (HC5) with an assessment factor (AF) of 1.

In a practical application of the EQS Technical Guidance (EC, 2018) the reference condition for the $EQS_{bioavailable}$ is selected to ensure that 95% of EU waters in the most sensitive region are protected. In the case of Ni, the most sensitive region in the investigated datasets was Austria. The selection of a reference condition based on a low percentile of the most sensitive region prevents the “moving target” nature of basing $EQS_{bioavailable}$ on the lowest EQS derived from the underlying bioavailability relationship. Hence, the current $EQS_{bioavailable}$ of 4.0 µg/l is based on the 5th percentile of the distribution of PNECs of the Austrian dataset (3.7 µg/l) corresponding to water conditions with a high pH (8.2) and a low DOC content (2 mg/l), conditions that result in high Ni bioavailability. If additional monitoring data for sensitive ecoregions are collected, the set of reference conditions can change, and these changes can be considered in subsequent updates of the EQS for those affected substances.

Table 1: The amended* 5th and 10th percentiles of Predicted No-Effect Concentrations (PNECs) (µg/l) for Ni for EU Member States as calculated using the bio-met bioavailability tool (EC, 2010)

Dataset and number of samples	10th Percentile	5th Percentile
England, Wales and Scotland (n = 184)	6.62	5.86

Dataset and number of samples	10th Percentile	5th Percentile
Northern-France (n = 249)	5.28	4.64
Austria (n = 1553)	4.34	3.7
Spain (n =48)	7.34	7.32
The Elbe Basin (n = 294)	8.22	7.46
Sweden (n = 3997)	11.2	10.08
Walloon region (BE) (n = 559)	6.36	5.82
All data (n = 6885)	**6.58**	**5.20**

*The EQS for Ni has an assessment factor of 1 in the amended EQSD (EU, 2008b), although the EQS sheet (EC, 2010) for Ni has not been updated to account for this change in the assessment factor.

The derivation of a WFD EQS requires that Quality Standards (QS) for all relevant compartments (e.g. water, sediment and biota) and potential receptors (i.e. humans, sediment-dwelling biota, pelagic biota and top predators) are derived and their relative sensitivities compared. The selection of compartments/receptors at risk is based on an understanding of the fate, bioaccumulation properties, and calculated relevant doses of the substance of interest. For a given substance, the selection of the "overall" EQS from the various compartment/receptor QS values is based on the objective to protect the most sensitive compartment/receptor (i.e. by selecting the most stringent standard). This concept is not different when considering $QS_{bioavailable}$ for the aquatic compartment. Indeed, to select a $QS_{bioavailable}$ as the "overall" EQS it must be identified as protective of all compartments across the various likely conditions observed across the EU. Where due to certain water chemistry conditions an $EQS_{bioavailable}$ would not protect other receptors/compartments, the QS of the alternative assessment (e.g. secondary poisoning of top predators ($QS_{fw,secpois}$) or human health via drinking water ($QS_{dw,hh}$) would become the overall EQS. Consequently, situations may arise where particularly insensitive conditions (e.g. high DOC and dissolved calcium concentrations) may make the compliance with the $EQS_{bioavailable}$ too easy. This is also the case with some specific compartment/receptor QS ($QS_{dw,hh}$; $QS_{fw,secpois}$). As a result, they should rather be approached in terms of total dissolved concentrations. In such situations, potential risks to these specific receptors need to be assessed more carefully.

While the $EQS_{bioavailable}$ derived for Ni used a Ni BLM, the EQS for Pb is an example illustrating how a correction for water chemistry that can mitigate ecotoxicity may be accounted for in the compliance assessment of a metal for which no specific BLM exists. For Pb there is a strong relationship between chronic ecotoxicity and DOC in water. In the absence of a Pb BLM at the time of the 2013 amendment of the EQSD, a precautionary relationship was developed using only the DOC concentrations to determine the amount of "available" Pb from the measured Pb concentration. This approach is used in some Member States for metals for which the scientific evidence supports relationships or corrections based on mitigating water chemistry characteristics (e.g. for copper (Cu) in marine waters, EA, 2012c).

1.5. What about Maximum Allowable Concentration EQS (MAC-EQS)?

For most Priority Substances in the WFD, not only is an AA-EQS derived, but also a “Maximum Allowable Concentration EQS" (MAC-EQS), which is based on acute toxicity data. Acute BLMs are available for several trace metals, including Cu, Pb, zinc (Zn), silver (Ag) and Ni. These have mostly been developed in North America, using ecotoxicity data that fulfil US-EPA Water Quality Guideline requirements (e.g. US-EPA, 1985). These water quality criteria tend therefore to differ from the requirements of an EQS under the European WFD, particularly in relation to the taxonomic breadth of data.

Thus far, no $MAC_{bioavailable}$ has been derived under the WFD, and therefore compliance assessment is based on the dissolved concentration of the metal (MAC or $MAC_{generic}$).

1.6. What about marine waters?

Approaches to account for bioavailability using BLMs in marine waters are currently under development but are still some way from implementation. It is also not yet possible to account for bioavailability in transitional waters. Even without considering bioavailability, EQS derived according to CIS Technical Guidance Document No 27 (EC, 2018) are either for freshwaters or salt waters. As a default, a salinity of 5‰ is recommended as the cut-off between freshwaters and salt waters, unless other evidence suggests that a different one is appropriate for a particular water body. The EQSD specifies EQS for inland surface waters (lakes and rivers) and other surface waters (essentially transitional and coastal).
As mentioned previously, corrections based upon relatively simple relationships between ecotoxicity and mitigating water chemistry characteristics, such as DOC, have been developed by some Member States (e.g. EA, 2012c).

1.7. How to navigate through this document

This guidance presents practical approaches to implementing $EQS_{bioavailable}$ for metals. For certain aspects of implementation, several options are currently available; and the selection of an appropriate one will depend on the existing regulatory framework in each Member State. This flexibility also acknowledges that some organisations and Member States have already established mechanisms for implementing $EQS_{bioavailable}$, whereas others have only just begun. The earlier chapters (1, 2 and 3) of the guidance are descriptive, outlining principles and processes, while the later chapters (4, 5 and 6) detail practical and interpretative steps to implement the approaches in the context of the water regulatory framework.

The schematic below (Figure 2) shows how to navigate through the different steps that must be considered to account for bioavailability in a compliance assessment.

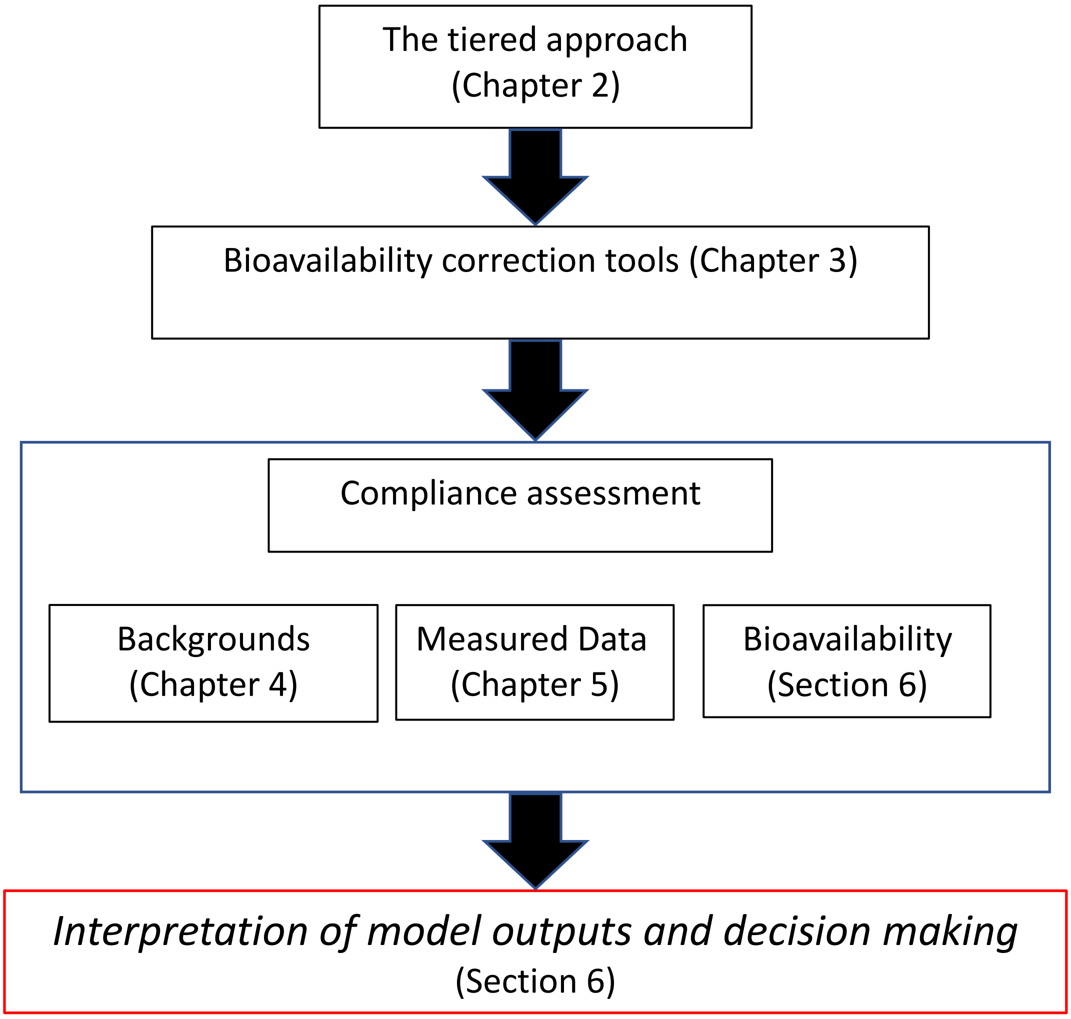


Figure 2: Steps that must be considered to account for bioavailability in a compliance assessment

2. TIERED APPROACH FOR USING BIOAVAILABILITY CORRECTION AND CONSIDERING NATURAL BACKGROUND CONCENTRATIONS (NBCS)

A tiered approach to compliance assessment under the WFD is presented for metals designated as priority substances (PS) and those designated by MS as RBSPs. At each tier, a decision about classification (pass/fail) can be taken, or a waterbody that “fails” can be further evaluated in a subsequent tier.

Two types of tiered assessment are described, one for metals with an EQS referring to a bioavailable concentration, and one for metals where the EQS refers to the total dissolved concentration. Both scenarios are outlined schematically.

In the first tier, the dissolved metal concentration is compared with the relevant EQS. In cases of non-compliance, the assessment can be further refined by integrating considerations of bioavailability (for those metals that have a bioavailability model), and the NBC (for all metals).

Consideration of bioavailability requires the use of bioavailability tools and specific information on physicochemical conditions of the water. These aspects are described in Chapters 3, 5 and 6. Guidance on determination of NBCs is outlined in Chapter 4.

2.1. Why use a tiered approach?

A tiered approach is consistent with classic risk assessment paradigms, in which the early assessment tiers are precautionary and simple to perform (as information requirements are low) on a large number of waterbodies. The intention of the early tiers is to remove from further assessment the waterbodies with a low risk of EQS failure; whereas, for the remaining cases, as progress is made through the tiers, the data and calculation requirements increase. In this way, additional effort is restricted to waterbodies where metals pose a risk to the achievement of good ecological or chemical status. In applying this approach to the implementation of an $EQS_{bioavailable}$ for a metal, it is possible to use a single numerical value as the EQS, derived for reasonable worst-case conditions (i.e. possible high bioavailability conditions) but to also account for local water chemistry in a practical way (Comber et al., 2008; EA, 2008b).

2.2. The tiered approaches

The tiered approach is a logical and flexible approach. Depending on whether the metal EQS is initially bioavailability-based or not, one of two different implementation scenarios will apply (Figures 3 and 4). Each tier leads to a pass or fail classification, i.e. if below the EQS or above/equal to the EQS, respectively, and in the latter case the waterbody can be assessed in the next tier. The first two tiers are primarily intended to inform the status assessment and the subsequent tiers for use as part of investigative action or to develop the programme of measures. At each tier, the relevant authority decides whether to stop the evaluation or progress to the next tier.

Total and added risk approaches and their use in metals risk assessment and/or EQS derivation

In metals risk assessment, in order to deal with the presence of NBCs, various methods have been developed, such as the Added Risk Approach (ARA) or the Total Risk Approach (TRA) concepts. The TRA assumes that exposure and effects should be compared for both the fraction comprising the NBC and the added anthropogenic component (i.e. organisms are not able to distinguish between the natural and the anthropogenic bioavailable part of the metal present in the environment). The ARA, on the other hand, assumes that species are fully adapted to the NBC and that only the anthropogenic added fraction of the metal contributes to the risk. The incorporation of the bioavailability concept into the risk assessment is the preferred way forward **for metals that have a bioavailability model**. In the approach for deriving an $EQS_{bioavailable}$, a TRA is adopted that integrates NBCs (EC, 2018).

In areas where NBCs prevent compliance with the relevant EQS, they can be considered when assessing compliance. This acknowledges that at some locations there is a significant natural background to which the indigenous organisms are tolerant, and which has little or no toxicological impact. To use this approach, the local or regional NBCs need to be established.

2.2.1. <u>Scenario 1:</u> Metals for which an $EQS_{bioavailable}$ has been explicitly derived

(See Figure 3)

- **Tier 1.** The first tier in the scheme considers a direct comparison of the annual average concentration from monitoring data (dissolved metal) with the $EQS_{bioavailable}$ (e.g.: for Ni 4 µg/l and for Pb 1.2 µg/l). Although the $EQS_{bioavailable}$ is expressed as a "bioavailable" concentration, in the first tier of assessment it is compared to the annual average dissolved metal concentration even though not all of it will be bioavailable. This means that the assessment is precautionary and false negatives are minimised. This tier is applicable to all freshwater water bodies, and the additional supporting physico-chemical parameters used for the calculation of the bioavailable metal concentration (as discussed in Chapter 5) are not required. Waterbodies exceeding the $EQS_{bioavailable}$ at this tier can progress to the second tier of the assessment.

- **Tier 2.** Ideally, this tier makes use of a simplified tool[1] to calculate local bioavailable metal concentrations at a given site from the measured total dissolved concentrations. The calculation requires information on local physico-chemical conditions from additional sampling parameters, i.e. pH, DOC and dissolved calcium (see Chapters 5 and 6). The annual average bioavailable metal concentrations can then be compared with the $EQS_{bioavailable}$ to assess compliance. Matched water

---

[1] User-friendly tools are described in greater detail in Chapter 3 of this guidance.

chemistry and metal concentration data are preferred, but if these are not available, assumptions based on historic data or data from neighbouring locations can be used to determine whether the collection of matched data is required for a more robust assessment (see Chapter 5). For Pb, a simple DOC correction is applied at this tier (see section 3.5.2)[2]. For cadmium (Cd), bioavailability can be considered in Tier 2 by applying the specific hardness-dependent EQS values as defined in the EQSD (EU, 2008b); in this approach, the dissolved Cd concentration is compared with the EQS corresponding to the hardness of the water. Sites or samples exceeding the $EQS_{bioavailable}$ can progress to Tier 3.

- **Tier 3**. At this tier, local conditions ("local refinement") can be investigated on a case-by-case basis to assess whether the metal concentration is expected to pose a risk to or via the aquatic environment. This tier can include several options that are aimed at confirmatory support for the identification of an exceedance at Tier 2. This may include:

    - The use of a more robust assessment of local water chemistry conditions affecting bioavailability, including the possibility to run the full BLM (notably for Pb; https://www.ila-lead.org/responsibility/lead-blm-tool) and/or further sampling and analysis, particularly where default values or proxies have been used as input parameters for the simplified tools.

    - Taking account of NBCs. The NBC of the metal (expressed as a dissolved concentration) has to be determined locally as described in Chapter 4. The value of the NBC is then compared to the annual average dissolved metal concentration in order to evaluate the contribution of the anthropogenic added fraction of the metal to the total metal to which aquatic organisms are exposed. If the NBC is in the range of the annual average dissolved metal concentrations and the anthropogenic added fraction is therefore almost negligible, it can be expected that there is little or no risk to local aquatic communities. This could be verified, for instance, in the ecological status assessment.

- **Tier 4.** At this tier, the failure of a site to achieve the $EQS_{bioavailable}$ has been clearly determined and so good status has also not been achieved. As required by the WFD, Member States should then design and implement measures to reach good status. Articles 4(4) and 4(5) of the WFD recognise that in some cases, natural conditions may affect the time taken by a water body to achieve good status or the possibility of achieving it at all. This can be the case for sites where, e.g., the sources of pollution have been reduced through the cessation of activities (e.g. closure of mines) but where contaminants persist, especially in the sediments, and may continue to affect the status of water bodies (EU Water Directors, 2017).

---

[2] The $EQS_{bioavailable}$ for lead was derived before the lead BLM was available and it was considered to represent the most reasonable approach at that time (SCHER, 2011).

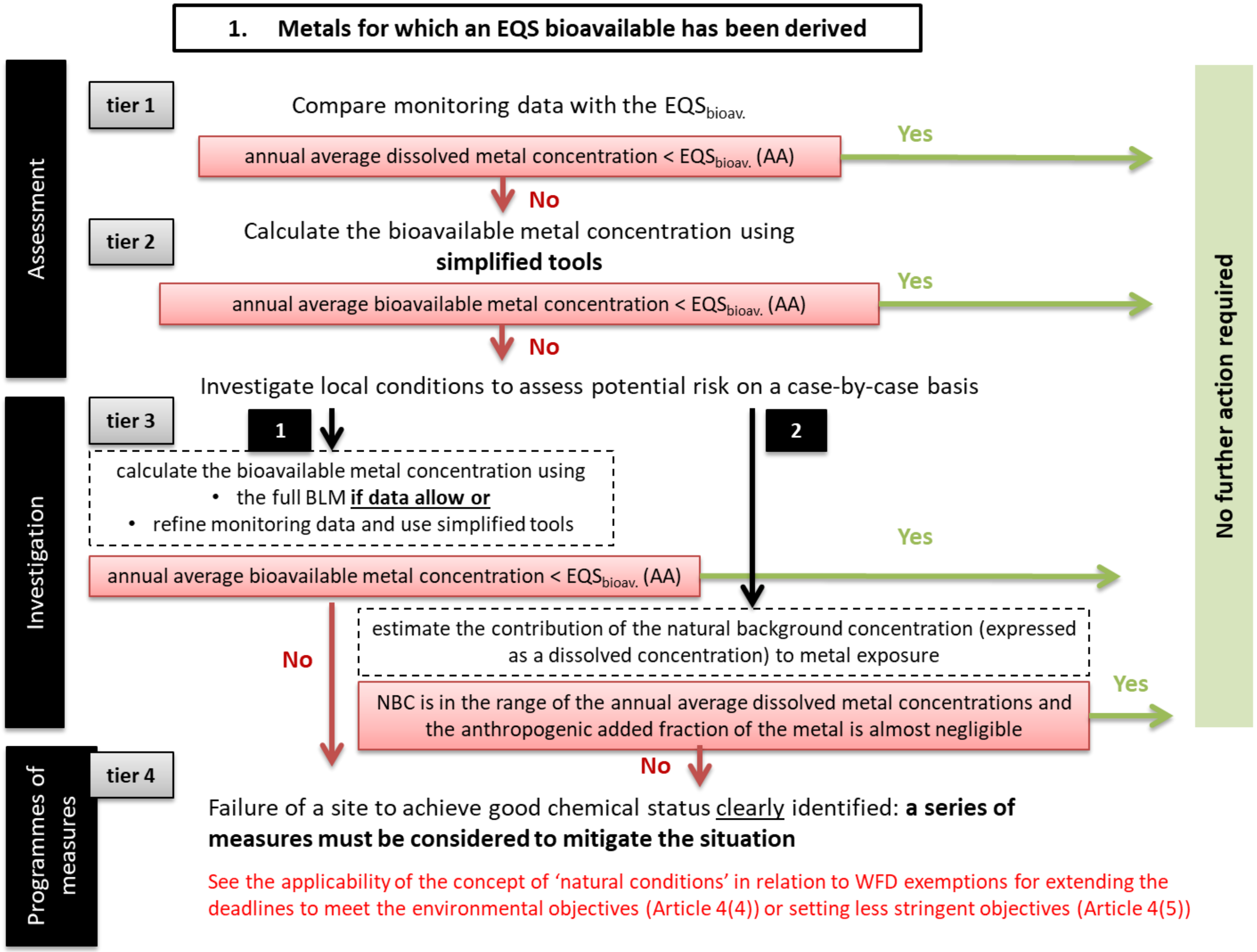


Figure 3: Flow diagram of the possible stages of a tiered EQS compliance assessment for a metal for which account of bioavailability can be taken (i.e. Scenario 1)

2.2.2. Scenario 2: Metals for which the EQS is derived generically (without explicit consideration of the bioavailability) and the NBC of the metal is considered

(See Figure 4)

- **Tier 1.** The first tier directly compares the annual average dissolved metal concentrations with the EQS. It is applicable to all freshwater waterbodies and only the dissolved metal concentrations are required. Sites, or samples, exceeding the EQS at this tier should progress to the second tier of the assessment.

- **Tier 2.** The second tier considers the NBC of the metal. The ARA is used in order to deal with the presence of a natural background. In the ARA, the toxicity data should first be re-evaluated: for each toxicity study that is used to derive the EQS, the metal background level measured in the culture or test media is subtracted from the effect value (i.e. NOEC and EC_{10}) in order to derive an “added EQS” – EQS_{add}. The media for toxicity test studies sometimes contain a background concentration, especially for essential metals and for tests using natural water as a diluent. In a second step, the NBC, expressed as a dissolved concentration, is subtracted from the monitoring data before comparing the net concentration with the EQS_{add} The NBC values applied at this tier need to be applicable to the relevant spatial scale (e.g. hydro-ecoregions or hydrogeological units), and consequently the chosen NBC needs to be sufficiently conservative to ensure that no sites are given the all-clear inappropriately (due to the application of an unrealistically high NBC for their local conditions). If the site or sample fails to fall below the EQS_{add}, the assessment should progress to Tier 3.

- **Tier 3**. At Tier 3, the region- or unit- NBC is replaced by a site-specific NBC (in practice, this is likely to be the first step of the assessment where NBC is accounted for). The wide variation in NBCs for metals makes it difficult to agree on a single NBC applicable over large geographic areas; to refine the assessment, it therefore seems more appropriate to assess the monitoring data in relation to background concentrations at a local scale.

- **Tier 4.** At this last tier, the failure of a site to achieve the EQS has been clearly determined and so good status has also not been achieved. Member States should implement the necessary measures to reach good status. As in Scenario 1, the applicability of Articles 4(4) or 4(5) of the WFD may also be considered.

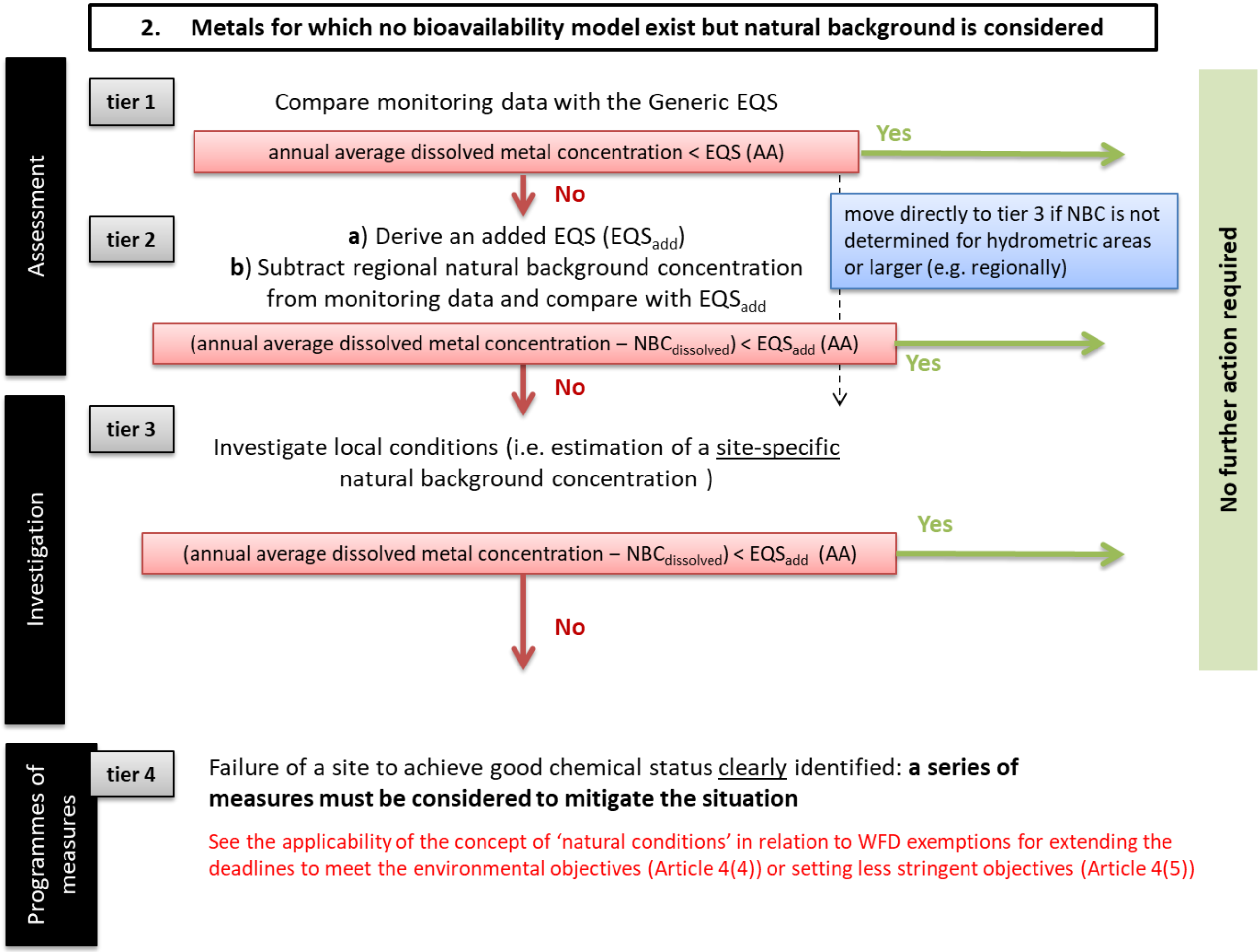


Figure 4: Flow diagram of the possible stages of a tiered EQS compliance assessment for a metal for which no bioavailability models exist but natural background is considered (i.e. Scenario 2)

3. BIOAVAILABILITY CORRECTION TOOLS

3.1. Overview

This Chapter presents simplified bioavailability correction tools, bio-met and PNEC-pro, which are derived from full BLMs. These tools allow consideration of metal bioavailability in Tier 2 of the approach presented in Scenario 1 in Chapter 2.

The following sections describe the characteristics of the simplified tools and their parent BLMs, including the approach used for the simplification, the underlying ecotoxicity dataset, speciation calculations, species-specific BLMs used by the simplified tools, and the bioavailability normalisation process.

Other relevant information is also provided, such as the water chemistry conditions for which the models have been calibrated (model development) and validated (testing the model's predictions against an independent dataset).

To check consistency, the outputs from the simplified tools and their respective parent BLMs were compared using the same monitoring dataset. Overall results indicate relatively good agreement between the models, with predictions falling within a factor of two. For Zn, the comparison showed a 2-2.5-fold systematic difference between the two models. These differences should be evaluated with respect to the uncertainty in assessment of field data collection (e.g. spot sampling) and chemical analysis (sensitivity of analytical methods) and compared to the variability typically observed between ecotoxicity test results for a given species. Despite such differences between the outputs from different tools, it is considered that including bioavailability in the compliance assessment will lead to a more scientifically robust outcome, whichever tool is used, and that a bioavailability assessment should therefore be conducted when appropriate scientific models are available.

Other bioavailability-based correction approaches are also presented for Cd and Pb in freshwaters, and Cu in marine waters (section **3.6**).

3.2. Preamble

There is a range of tools available to estimate the influence of water chemistry on metal ecotoxicity, including speciation models and surrogate measures of availability. In this section, two approaches are outlined for the implementation of the bioavailability EQS in the EQSD (EU, 2008b). These approaches are also widely utilised at Member State level for RBSPs. The first presented approach uses simplified tools derived from full BLMs (Section 3.4). The second presented approach accounts for availability through the development of empirical relationships - based on mechanistic principles, scientific evidence, and supporting ecotoxicity data - to account for the effects of water chemistry parameters on chronic ecotoxicity, as in the case of the current EQS for Pb (Section 3.6).

BLMs have already shown their usefulness as robust means to incorporate bioavailability in risk assessments (SCHER, 2010). Since the so-called "full-BLM procedures" require a high level of expertise (Fig. 5), generally inhibiting their implementation in the context of legal frameworks, “simplified tools” have been developed over recent years and made freely available for compliance assessment of monitoring data against bioavailability-based EQS. Regarding the simplified tools derived from full BLMs, this guidance explains the underlying key assumptions of the models, the methods used for simplification, and the limits and validation stage of each tool. An indication of the general performance of the tools is also presented for representative European surface waters and includes a comparison of the outputs from these models when using the same monitoring dataset.

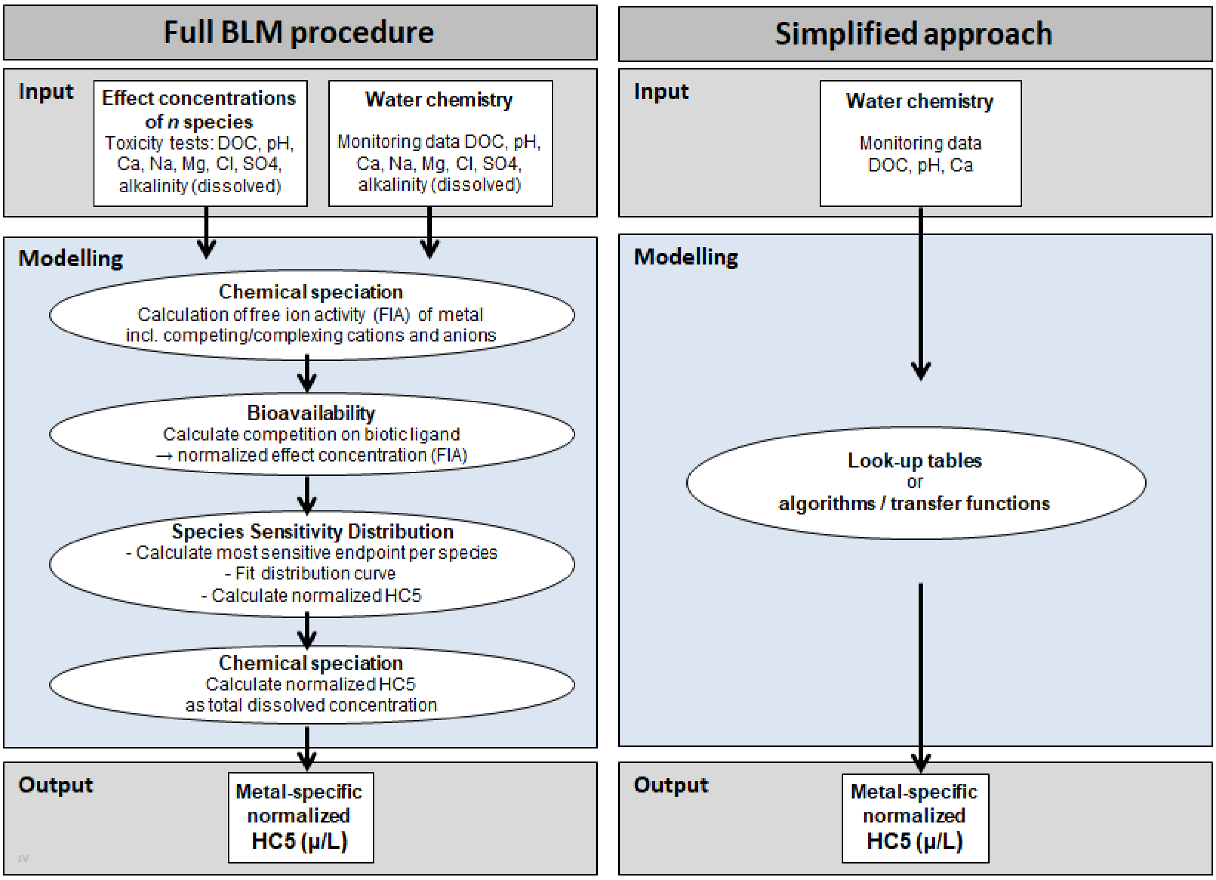


Figure 5: Overview of the full BLM procedure (left) and of the simplified approaches (right) to account for bioavailability in the calculation of the site-specific HC5

The Hazard Concentration for 5% of species (HC5) is the cut-off value of a species sensitivity distribution (SSD) for which 95% of the species are protected. The local HC5 is equivalent to the PNEC when an assessment factor (AF) of 1 is applied in the derivation of the site-specific PNEC.

3.3. Biotic ligand models (BLMs): Scientific underpinning

The modelling approach that most effectively accounts for metal bioavailability, with a direct link to toxicological endpoints, and thus with the greatest potential to be applied in EQS derivation and compliance assessment, is arguably the BLM (Merrington et *al*., 2016). A BLM is a mathematical model that uses information on water chemistry, such as pH, calcium concentration, alkalinity, dissolved organic carbon (DOC), to predict metal toxicity as a function of accumulation at a “biotic ligand” level in a (semi-)mechanistic way. BLMs include a speciation component and then account for both the influence of water chemistry on the free ion activity and the ion competition for the biotic ligand; the sensitivity of different species can then be defined according to a critical level of accumulation at the biotic ligand. Applying the full BLM to calculate a bioavailability-based HC5 requires distinctive sequential steps (Fig. 5):

- Calculate the metal speciation in individual ecotoxicity data records (EC_{10} values) using a chemical speciation model (e.g. the Windermere Humic Aqueous Model WHAM);
- Calculate the metal speciation in target site water, using relevant water chemistry parameters;
- Normalise individual ecotoxicity data records to target site water chemistry using the appropriate BLM;
- Transform normalised speciated ecotoxicity data to dissolved metal equivalent using WHAM software;
- Perform appropriate endpoint selection to determine species mean EC_{10};
- Populate Species Sensitivity Distribution based on normalised EC_{10} values expressed as dissolved metal concentrations;
- Calculate HC5 expressed as dissolved metal.

Databases containing ecotoxicity data were provided in earlier years in the EU Risk Assessment Reports (EU-RAR) for Cu, Ni and Zn, containing chronic dissolved EC_{10} values for a large number (i.e. > 30 in the case of Cu, Ni, and Zn) of freshwater species (European Copper Institute, 2008; EU, 2008a; EU, 2010). The number of species and trophic levels for which toxicity data are available however depend on the metal. The initial development of BLMs involved measuring accumulation of metals at the putative site of toxicity, which in the case of fish was the gill. The critical gill metal concentration can then be related to adverse effects (i.e. toxicological endpoints such as growth, reproduction, mortality) (Meyer et *al*., 1999; Di Toro et *al*., 2001), and the use of speciation models can be used to predict the influence of water chemistry on the accumulation of metal at the gill level. This provides the basis for calculating metal toxicity based on water chemistry conditions. The shortcoming of this approach is the need to quantify the metal concentrations directly present at the biotic ligand level. While this approach was demonstrated to work for acute exposures in fish, it was not possible to apply it to smaller organisms, e.g. invertebrates and algae. De Schamphelaere and Janssen (2002) identified an alternative approach, which involved obtaining the model parameters from toxicity tests performed in exposures where relevant water chemistry parameters (e.g., Ca, Mg, pH, etc.) were varied univariately. This approach has been used to develop chronic BLMs for a range of metals (Co, Cu, Ni, Pb, Zn) for standard algae, invertebrate, and fish species. To demonstrate that the models developed for standard species, e.g., *Daphnia magna*, can explain intraspecies sensitivity differences in a given ecotoxicity database, cross-species validation studies have been performed for a number of metals, including Ni (Schlekat et *al.*, 2010) and Pb (Van Sprang et *al.*, 2016).

In order to perform full BLM calculations, the composition of water chemistry parameters that influence metal speciation in test media should be known in detail (e.g. concentrations of DOC, H^+, Ca^{2+}, Mg^{2+}, Na^+, K^+, OH^-, Cl^-, SO_4^{2-} and HCO_3^-). To perform the normalisation procedure the matched data on water characteristics are necessary. The term "matched" here means that the supporting water chemistry parameters are sampled at the same site and preferably at the same time as where the metal concentration is measured. This means that monitoring programmes should dictate that a given sample is taken from the site where the dissolved metal and supporting water chemistry parameters are determined.

Whilst the BLM calculated concentrations are derived for particular species, it is possible to perform cross-species validation and so to extrapolate the BLM model to other, taxonomically distinct, species. Therefore, all available ecotoxicity data for a metal can (as long as sufficient information on water physico-chemistry parameters is reported alongside ecotoxicity endpoints) be “normalised” to a specific water physico-chemistry (e.g. Van Sprang et *al.*, 2009). When BLMs are applied to all species in a species sensitivity distribution (SSD), through a process termed “full normalisation”, local or site-specific HC5 values can then be derived from the bioavailability normalised SSD. Figure 6 shows various SSDs for Ni, normalised to different water physico-chemistry conditions and their corresponding HC5 values (EU, 2008a).

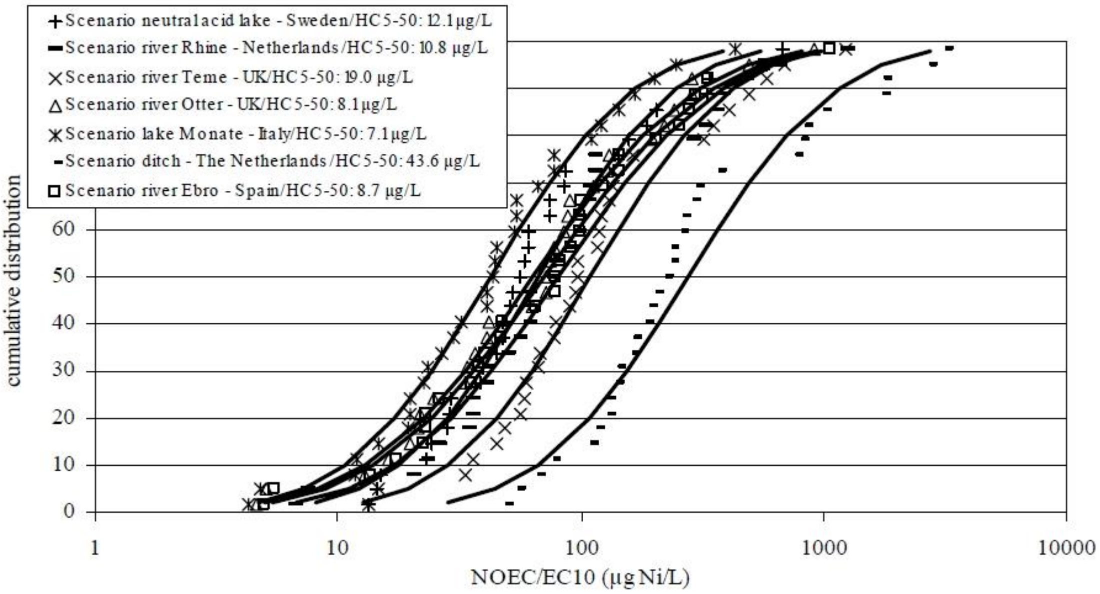


Figure 6: Examples of “full normalisation” of Ni Species Sensitivity Distribution (SSD) for various water types or ecoregions (from EU, 2008a)

The discussions in this guidance are focussed on chronic BLMs, and where reference is made to BLMs, unless explicitly stated, it therefore refers to chronic models and specifically to the integrated versions of those models that can undertake full normalisation of an entire SSD.

3.4. Validation of BLMs

As BLMs are developed on a species-specific basis, questions arise as to the ability of these species-specific models to normalise the ecotoxicity data of other organisms in the databases used to derive an EQS. There are different approaches for developing the quantitative information that can be used to address cross-species application of BLMs. In the case of Ni, information was already present in the literature to demonstrate that the BLM developed for rainbow trout could be applied to other fish species (Deleebeeck et *al*., 2007a), and that the BLM developed for the green alga *Pseudokirchneriella subcapitata* could be applied to other algal species (Worms et *al*., 2006). The normalisation of ecotoxicity endpoints includes the understanding of endpoint variability within each species in order to reduce the intra-species variability. Figure 7 shows the original (non-normalised) and BLM-normalised intra-species variability of Ni, expressed as the ratio between the highest and lowest endpoint from a specific species, i.e. max/min).

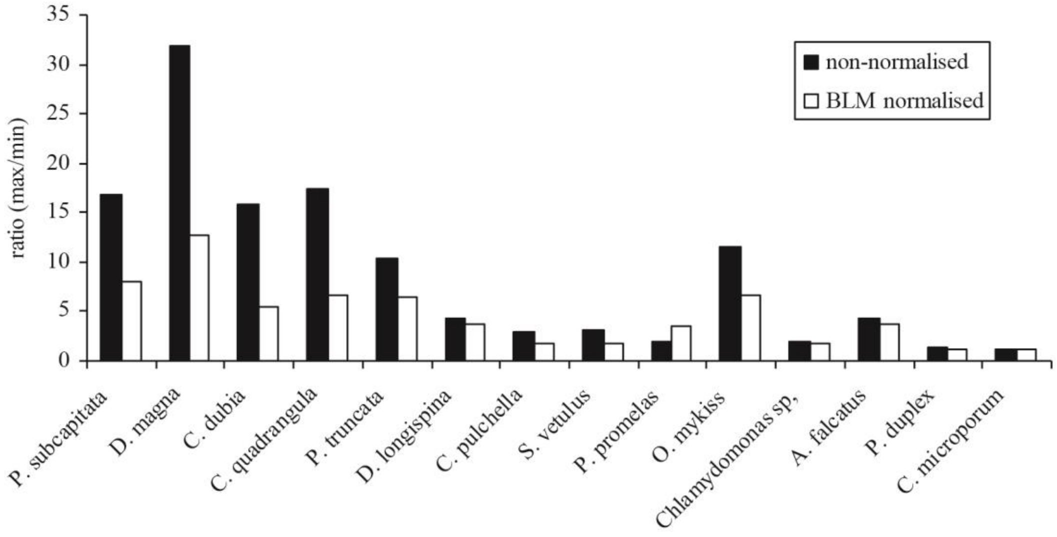


Figure 7: The intra-species variability expressed as max/min ratios, of the EC_{10}/NOECs, as µg Ni/L in the test medium and BLM-normalised to the River Rhine water chemistry conditions using the chronic Ni BLMs (from EU, 2008a)

This comparison shows that normalised-BLMs significantly reduced the observed intra-species variability among all invertebrate species. The use of the chronic Ni *D. magna / C. dubia* BLMs to do a “spot-check” of the applicability of the cladoceran models to other invertebrates, including a mollusc, a rotifer, and an insect, also support the use of chronic Ni BLMs from one species to another (Schlekat et *al*., 2010). Therefore, the BLMs reduce intraspecies variability to a level that is considered acceptable for regulatory use (Schlekat et *al*., 2010). Similar analyses have taken place for Pb (Van Sprang et al., 2016), for Zn (De Forest and Van Genderen, 2012) and for Cu as part of the EU voluntary risk assessment (European Copper Institute, 2008).

Considerations on regulatory application of BLMs in the European Union

Where BLMs have been developed under European regulatory scrutiny (e.g. ESR, REACH, WFD), there are generally at least three independent BLMs: for a fish, an invertebrate and algae.

Discussions in previous guidance (e.g. EQS Technical Guidance Document; EC, 2011a), refer to a single BLM for a metal. As described above, this in fact refers to an integrated version of all the respective BLMs for the different species, for that metal.

BLMs developed for one metal are specific to that metal and cannot be applied to another metal. Similarly, acute and chronic BLMs for the same metal may also not be interchangeable. There is a defined range of physico-chemical conditions over which a BLM has been validated. These conditions are defined by the water chemistry parameters (e.g. water hardness, pH, DOC) of the ecotoxicity testing used to develop the BLM. In some cases, the control performance of the test organisms used to develop the model (i.e. the ecology of the test species) will restrict this validated physico-chemical range.

The species-specific BLMs for Cu, Ni, Pb and Zn have been validated under field conditions to assess predictive capability.

All the BLMs currently fit for regulatory use in Europe are for fresh waters. Marine waters show little variability in pH or salinity, and so the main water chemistry parameter that can influence metal bioavailability is dissolved organic carbon. A bioavailability correction based on DOC in marine waters is currently embedded in some regulatory frameworks (EA, 2012c). Research is on-going to develop marine bioavailability models for other metals.

3.5. Simplified or “user-friendly” tools

The major barrier to widespread, practical use and implementation of BLMs is the conceptual complexity of the approach, combined with the practical complexity of performing the calculations, which requires chemical speciation calculations and normalisation of toxicity data (see also Fig. 5). Moreover, the speciation components of BLMs require measured data for a large amount of water chemistry parameters, some of which are not readily conducted in standard monitoring programmes, and some of which cannot be reliably estimated. Moreover, the influence of many of these parameters (e.g. SO_4^{2-}) has been shown to be negligible in terms of predicting toxicity. For these reasons, simplified and user-friendly bioavailability tools have been developed over the past years. These simplified modelling tools can be used as screening tools in lieu of complicated BLM procedures, and their use can facilitate the incorporation of bioavailability into the assessment of compliance to metal EQS.

A user-friendly bioavailability tool mimics the output of the full BLM normalisation process upon which it is based, but with a slightly reduced level of predictive performance (e.g. EA, 2009, 2012d, 2014; Peters et *al*., 2016; bio-met, 2017; Verschoor et *al*., 2017). A user-friendly tool should also operate in a standard software application (Excel in the case of all the existing user-friendly tools), have the potential to be automated (i.e. process large number of samples without user intervention), have readily interpretable outputs and deliver acceptable performance as measured against the BLM itself (EA, 2009). Simplified tools use a reduced set of water chemistry parameters as input (i.e., DOC, pH, hardness or dissolved calcium concentration, and dissolved metal concentration). Such tools seem appropriate to enable the implementation of routine site-specific water quality assessments.

Currently, there are three operational simplified tools, which are used by different European Member States in their EQS compliance practices. These are:

bio-met	www.bio-met.net
M-BAT	https://www.wfduk.org/resources/rivers-lakes-metal-bioavailability-assessment-tool-m-bat
PNEC-pro	www.pnec-pro.com

bio-met is a free online resource for determining compliance with EQS derived for metals under the WFD in the freshwater aquatic environment. bio-met is a "lookup-table" based tool, in which results are matched with nearby-results from full-BLM calculations. In early 2009, bio-met was developed as a collaborative initiative led by the European Copper Institute, the International Zinc Association and the Nickel Producers Environmental Research Association (NiPERA). The tools and resources on bio-met.net have been developed collaboratively by ARCHE Consulting and wca environment. This guidance addresses the latest version (4.0) launched in April 2017 (bio-met, 2017)[3].

M-BAT is a tool available online and is aimed at supporting compliance with bioavailability-based EQS for metals within the existing UK water monitoring system. The tool is based on algorithms developed from bioavailability normalised HC5 calculations using the respective BLMs. M-BAT is the first simplified tool that was developed in 2008 and updated in 2014. The development of M-BAT and the algorithms that are used in the bioavailability compliance determination can be found on the UK Water Framework Directive Technical Advisory Group website. This guidance addresses the last version as described in 2014 (M-BAT, 2014).

PNEC-pro V6 was developed after 2013 using data published after the metal RARs. PNEC-pro is an algorithm-based tool. It uses routines that select 1-, 2-, or 3-parameter functions, based on the input parameters that are entered by the user. PNEC-pro has been implemented in legal frameworks for EQS compliance testing and WFD reporting in The Netherlands in 2015. The tool was developed collaboratively by the research institute Deltares, the Dutch National Institute of Health and Environment (RIVM), and the Dutch Leiden University (CML). This guidance addresses the latest updated version (V6) launched in December 2016.

---

[3] A new version of bio-met (version 5.0) was released in 2019, although this review is still relevant to the updated version.

It is not the purpose of this guidance to recommend any particular tool. However, to account for bioavailability using a simplified tool, it is important to be confident in its general performance characteristics, in its performance with the specific water chemistries that will be investigated, and also in the scientific integrity of its datasets, which are its foundation (Section 3.5.1). While the eventual choice of a tool will be made by each Member State, based on its own particular circumstances and requirements, it is important that performance characteristics (e.g. precision and applicability domain relative to the BLMs they are supposed to mimic) of the simplified tools used for regulation purpose are understood, and that the possible differences in the outputs of the different bioavailability models are known (Section 3.5.4).

It is also important to note that other user-friendly tools, which are not based on full bioavailability considerations or BLMs at present, exist for the application of $EQS_{bioavailable}$ for Pb, Cd and Cu in marine waters, and are also likely to exist for several other metals (Section 3.6).

3.5.1. Characteristics of the simplified tools

M-BAT

The M-BAT tool is largely based on the bio-met ecotoxicity databases, and both M-BAT and bio-met tools generate relatively similar results (see Peters et *al*., 2016). Therefore, this section will focus only on the bio-met and PNEC-pro bioavailability tools.

bio-met

The basic approach behind the bio-met tool is based on a large database of more than 20,000 different combinations of key input parameters (pH, Dissolved Organic Carbon [DOC] and dissolved calcium [Ca] concentrations) and the corresponding HC5 calculations for various metals (Ni, Zn, Cu and Pb), using their respective validated BLM (see Table 2). 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 or site-specific HC5.

Briefly, the development of the bio-met tool included the following steps:

- Key input parameters driving the HC5 calculation are identified by means of a combination of sensitivity analysis and expert judgement;
    - DOC, pH and Ca (or hardness) have moderate to major impact on HC5 estimation;
    - Magnesium (Mg), sodium (Na), alkalinity, dissolved inorganic carbon (DIC), iron (Fe) and aluminium (Al) have low to moderate impact on HC5 estimation (depending on the metal of concern) but can be reasonably accurately calculated from Ca and pH (see Peters et *al*., 2011a);
    - A sensitivity analysis based on the STOWA (Foundation for Applied Water Research in the Netherlands) and FOREGS (Geological Survey of Finland) databases demonstrated that temperature, potassium (K), sulphate (SO_4) and

chloride (Cl) have negligible to low impact on HC5 estimation. Based on this sensitivity analysis, these parameters were each set at a reasonable worst-case value (i.e. Temp = 5°C, K = 25 mg/L, SO_4 = 100 mg/L, Cl = 160 mg/L);

- More than 20,000 different combinations of pH, DOC and Ca were simulated to calculate the HC5 for each metal using the full BLM models;
- The results provided by the bio-met tool are therefore full BLM calculations for a water chemistry that is similar (but not identical) to the water of interest.

The accuracy of the predictions of the bio-met bioavailability tool relative to the full BLM predictions was assessed using monitoring data from surface waters across the European Union (see WCA, 2015; Peters et *al*., 2016; Merrington et *al*., 2016; bio-met, 2017). These comparisons indicate that for all metals (Ni, Cu, Zn and Pb), predictions from the bio-met tool are within a factor of two (as used during the validation of the BLMs with observed toxicity in the field[4]) compared to the full BLMs.

PNEC-pro

The PNEC-pro bioavailability tool is a modelling framework that was recently developed to compute water-type specific chronic NOECs and HC5s for Cu-, Ni-, Zn- and Pb-BLM in a statistical software package R. It is derived from the full BLM normalisation procedure to facilitate routine evaluation of the bioavailability of metals in fresh waters. This was achieved by stepwise regression of full-BLM HC5 values of 371 water types, with their corresponding water chemistry parameters, and resulted in transfer functions with 1, 2 or 3 parameters (Verschoor et *al*., 2012). These transfer functions were included in PNEC-pro, which selects the most accurate function for a given set of water chemistry monitoring data.

The accuracy of the predictions of PNEC-pro relative to the full BLMs from which it is derived was assessed using monitoring data from 7 ecoregions in Europe representative of freshwater types with contrasting physico-chemical conditions (Verschoor *et al.* 2017). Results are available for all metals. For Ni, results indicate that HC5 values obtained with PNEC-pro 6 are well correlated with those computed with the Ni-RAR BLM: the HC5-values of PNEC-pro are well within the uncertainty range reported in the Ni-RAR with a correlation coefficient of 0.99 (n = 7) and the mean HC5-ratio between PNEC-pro BLMs and Ni-RAR is 0.90.

---

[4] The factor of two criterion for accepting a model's ability to accurately predict ecotoxicity is based on the test variability in ecotoxicity test results for a range of test organisms and metals (Garman et *al*., 2018; Meyer et *al*., 2018; Santore and Ryan, 2015; Erickson et *al*., 1987). The point here is that a model reducing intra-species variability within a factor of two is approaching the minimum variability that is expected for testing a given organism within the same laboratory.

3.5.2. Qualitative assessment of the BLMs that are used by the simplified tools

An overview of the BLMs that are used in the PNEC-pro and bio-met bioavailability tools is given in Table 2.

Several differences are observed:

- Ecotoxicity datasets differ substantially at least for some of the metals; for Ni and Zn PNEC-pro has more data but from fewer species. Bio-met reflects the ecotoxicity data that were agreed to for the determination of the Ni EQS. PNEC-pro incorporates more recent ecotoxicity data, which were not originally included in the Ni-RAR. A detailed description of these updates is given in Verschoor *et al.* (2017);
- For Zn and Cu, PNEC-pro and bio-met use different speciation modelling software (WHAM 6 vs Hydroqual WHAM 5, respectively). Their model parameters (i.e. values of binding constants for metal binding to dissolved organic matter, DOM) are also different but a direct comparison between the two versions of WHAM is not easy. In the calculation of the free Zn^{2+} activities the models used different % of DOM considered as reactive fulvic acid (FA);
- For Zn, a more recent invertebrate bioavailability model has been implemented in bio-met; equally, some biotic-ligand (BL) binding constants differ, notably those used in the algae models;
- HC5 calculations are performed on ecotoxicity data expressed either as a concentration in bio-met or as an activity in PNEC-pro (for this last, see Vink 2002).

Differences in both the BLMs and the simplification approaches are likely to lead to differences between the results of the simplified tools (see Section 3.5.4).

For Pb[5], the bio-met and PNEC-pro bioavailability tools both use the same Pb-BLM; bioavailability models, chronic toxicity database, and the normalisation procedure that are at the basis of the Pb BLM are described in De Schamphelaere et *al*. (2014), Nys et *al*. (2014) and Van Sprang et *al*. (2016). For both tools, chemical speciation calculations are performed with Visual Minteq 3.0, and complexation of Pb with dissolved organic matter (that is fulvic acid) is modelled following the NICA-Donnan model. As a consequence, comparisons of outputs for Pb indicate good agreement between the simplified tools (see Figure 15 and Table 6).

---

[5] Bio-met and PNEC-pro both include a full bioavailability normalisation approach for lead. Although it is not appropriate to use this full normalisation process for assessing compliance with the Pb EQS, it may be applied in Tier 3-1 to more robustly assess the influence of local water chemistry conditions on the bioavailability of lead (see Chapter 2, Figure 3).

Table 2: Key characteristics of the BLMs used by bio-met and PNEC-pro

Aspect	Metal	bio-met 4.0	PNEC-pro 6
Underlying ecotoxicity dataset	Ni	214 ecotoxicity data (EC_{10}s and NOECs) covering 31 species	233 ecotoxicity data covering 28 species
	Cu	139 ecotoxicity data covering 27 species	139 ecotoxicity data covering 27 species
	Zn	128 ecotoxicity data covering 22 species	132 ecotoxicity data covering 19 species
Speciation calculations	Ni	WHAM 6 is used to calculate metal speciation in solution	WHAM 6 is used to calculate metal speciation in solution
	Cu	Hydroqual/Windward BLM (WHAM 5)	WHAM 6
	Zn	Hydroqual WHAM 5	WHAM 6
Species-specific BLMs used by the simplified tools	Ni	**Algae:** *Pseudokirschneriella subcapitata* (Deleebeeck et *al*., 2009b) **Invertebrate:** *Daphnia magna* (Deleebeeck et *al*., 2008) and *Ceriodaphnia dubia* (De Schamphelaere et *al*., 2006) **Fish:** *Oncorhynchus mykiss* (Deleebeeck et *al*., 2007a)	**Algae:** *Pseudokirschneriella subcapitata* (Deleebeeck et *al*., 2009b) **Invertebrate:** *Daphnia magna* and *Ceriodaphnia dubia* (Deleebeeck et al. 2008; Nys et *al*., 2016a) **Fish:** *Oncorhynchus mykiss* (Deleebeeck et *al*., 2007a)
	Cu	**Algae:** *P. subcapitata* (De Schamphelaere et *al*., 2003b) **Invertebrate:** *D. magna* (De Schamphelaere and Janssen, 2004a) **Fish:** *Pimephales promelas* and *O. mykiss* (De Schamphelaere and Janssen, 2004c; ECI, 2008)	**Algae:** *P. subcapitata* (De Schamphelaere et *al*., 2003b) **Invertebrate:** *D. magna* (De Schamphelaere and Janssen, 2004a) **Fish:** *P. promelas* (Erickson et *al*., 1996), *O. mykiss* (Waiwood and Beamish, 1978)
	Zn	**Algae:** *P. subcapitata* (De Schamphelaere et *al*., 2003a) **Invertebrate:** *D. magna* (van Regenmortel et *al*., 2017) **Fish:** *O. mykiss* (De Schamphelaere et *al*., 2003a)	**Algae:** *P. subcapitata* (De Schamphelaere et *al*., 2005) **Invertebrate:** *D. magna* (Heijerick et *al*., 2005) **Fish:** *O. mykiss* (De Schamphelaere and Janssen, 2004b)
Bioavailability normalisation process	Ni	Normalisation is performed on all individual ecotoxicity data. Cross-species extrapolation is in accordance with the procedures outlined in the Ni RAR in which the most sensitive invertebrate BLM is used where no information is available on the most appropriate model for a particular species.	Normalisation is performed on all individual ecotoxicity data. Cross-species extrapolation is described in Verschoor et *al*. 2017 (SI). Algal BLM is used for toxicity normalisation of algae and higher plant species. *D. magna* BLM is used for the normalisation of *D. magna* ecotoxicity data. *C. dubia* BLM is used for the normalisation of *C. dubia* ecotoxicity data.

			Most sensitive invertebrate BLM is used for toxicity normalisation of other cladoceran, insect and amphipod species. *D. magna* BLM is used for toxicity normalisation of rotifers. *C. dubia* BLM is used for toxicity normalisation of molluscs and hydra. Fish BLM is used for toxicity normalisation of vertebrate species.
	Cu	Algal BLM is used for toxicity normalisation of primary producers. Invertebrate BLM is used for toxicity normalisation of all invertebrate species. Fish BLM is used for toxicity normalisation of all vertebrate species.	Algal BLM is used for toxicity normalisation of primary producers. Invertebrate BLM is used for toxicity normalisation of all invertebrate species. Fish BLM is used for toxicity normalisation of all vertebrate species.
	Zn	Algal BLM is used for toxicity normalisation of primary producers. Invertebrate BLM is used for toxicity normalisation of all invertebrate species. Fish BLM is used for toxicity normalisation of all vertebrate species.	Algal BLM is used for toxicity normalisation of primary producers. Invertebrate BLM is used for toxicity normalisation of all invertebrate species. Fish BLM is used for toxicity normalisation of all vertebrate species.
HC5 calculation	Ni	HC5 calculated from species sensitivity distribution on the basis of dissolved metal concentrations.	HC5 calculated from species sensitivity distribution on the basis of the chemical activity of the metal in solution, before converting the activity to a dissolved concentration through speciation modelling.
	Cu		
	Zn		

3.5.3. Calibration, validation and application ranges of the simplified tools

The calibration boundaries of the BLMs (and so the simplified tools) correspond to the extremes of the water chemistry parameters in the experiments used to develop the models. Many of the models (Cu, Ni, Pb, Zn) were developed by following the approach described in De Schamphelaere and Janssen (2002). This approach involves laboratory testing in synthetic or natural waters in which only one of the water chemistry parameters is univariately modified. As the calibration ranges are defined by the ecotoxicity datasets on which the BLMs are based, it is, without surprise, recognised that each metal has a different calibration range regarding the key water chemistry parameters. These ranges that primarily affect the interactions between metals and the water chemistry parameters of physiological relevance to the test organisms were identified in Table 3.

The validation range refers to the extremes of water chemistry parameters in experiments that are specifically designed to test the ability of a model developed using synthetic laboratory waters and a standard ecotoxicity test species, to predict metal toxicity against either natural waters (e.g., Deleebeeck et *al*., 2008) or other species within the ecotoxicity database (e.g., Schlekat et *al*., 2010).

The application range considers both the calibration and the validation ranges of the model, and so represents the aggregate range of water chemistry for which the model has been shown to accurately predict ecotoxicity tests. This terminology is consistent with the precedents set in the EU Risk Assessment Reports for Ni (EU, 2008a) and other metals.

This **does not** mean that the relationships developed and validated in the model do not hold where water conditions are outside of these ranges or that the derived $EQS_{bioavailable}$ is under-protective. It does mean that the certainty associated with calculations performed for water quality conditions outside of the calibrated boundaries has not been incorporated into the EQS. For example, data have been generated showing that Ni BLM predictions are still accurate above the pH application range (Nys et *al*., 2016a) and below the Ca application range (Peters et *al*., 2018a), as indicated in Table 3.

A common problem in BLM development, and arguably one of the reasons for the existence of the calibration ranges, is that the species typically used for BLM development (i.e. species commonly used in all laboratory ecotoxicity tests) are not tolerant to all the natural water chemistry conditions. For example, snails will not survive in control waters of very low hardness or pH; equally, they will not be present in ecosystems with naturally low hardness waters. Therefore, the calibration ranges of the BLMs will never be able to cover all EU water conditions. This is because there are fundamental difficulties in performing standard ecotoxicity tests in waters that are outside those conditions which are physiologically acceptable to the test organisms, i.e. it will not be possible to deliver acceptable control performances. In addition, these types of waters (at the extremes of pH or low hardness) often have very specific ecological compositions, which are rarely more sensitive to metal exposures than typical mid-range water conditions.

Table 3 indicates the calibration, validation and application ranges of the water chemistry parameters for the BLMs used in bio-met and PNEC-pro.

Table 3: Calibration, validation and application ranges of the water parameters for the BLMs used in bio-met and PNEC-pro

Supporting physico-chemical parameters		**BLM**	**Model**	**Nickel**	**Reference**	**Copper**	**Reference**	**Zinc**	**Reference**	**Lead**	**Reference**
pH	**Calibration range**	Algae	PNEC-pro	5.7-8.2		5.7-8.6		5.2-8.4		6.0-8.0	
			bio-met	5.7-8.7	Deleebeeck et *al*., 2009a	5.7-8.76	De Schamphelaere et *al*., 2003b	5.6-8.0	De Schamphelaere et *al*., 2003a	6.0-8.0	De Schamphelaere et *al*., 2014
		Invert.	PNEC-pro	5.9-8.2[A] 6.5-8.2[B]		5.6-8.4		5.2-8.4		6.3-8.2	
			bio-met	5.9-8.7[A] 6.5-8.7[B]	Deleebeeck et *al*. 2008; De Schamphelaere et *al*., 2006	5.6-8.74	De Schamphelaere and Janssen, 2004a	5.5-8.0	De Schamphelaere et *al*., 2003a	6.3-8.2	Nys et *al*., 2014; Nys et *al*., 2016b
		Fish	PNEC-pro	5.4-8.5		6.6-8.7		5.5-7.5		6.3-8.2	
			bio-met	5.4-8.5	Deleebeeck et *al*., 2007a	6.6-8.7	De Schamphelaere and Janssen, 2004c	5.7-8.1	De Schamphelaere et *al*., 2003a	6.3-8.2	Van Sprang et *al*., 2016
	Validation range	Algae	PNEC-pro	5.7-8.0		5.5-8.7		5.2-8.4		6.0-8.4	
			bio-met	5.7-8.0	Worms et *al*., 2006; Deleebeeck et *al*., 2009b	5.5-8.7	De Schamphelaere et *al*., 2003b; ECI, 2008	5.7-8.5	De Schamphelaere et *al*., 2005; Van Regenmortel et *al*., 2017	6.0-8.4	De Schamphelaere et *al*., 2014
		Invert.	PNEC-pro	5.9-8.7[A] 5.9-8.7[B]		5.5-8.5		5.2-8.4		6.0-8.6	
			bio-met	5.9-8.7[A] 6.5-8.7[B]	Nys et *al*., 2016a; Peters et *al*., 2018a	5.5-8.5	De Schamphelaere and Janssen 2004a; ECI, 2008	6.0-8.4	Gent U., 2015 unpublished report; De Schamphelaere et *al*., 2005; Van	6.0-8.6	Nys et *al*., 2014; Nys et *al*., 2016b

									Regenmortel et *al*., 2017		
		Fish	PNEC-pro	5.4-8.5		6.0-8.6		5.5-7.9		6.4-8.0	
			bio-met	5.4-8.5	Deleebeeck et *al*., 2007a; Peters et *al*., 2018a	6.0-8.6	De Schamphelaere and Janssen 2004c; ECI, 2008	6.2-8.1	De Schamphelaere et *al*., 2005	6.4-8.0	Van Sprang et *al*., 2016
	Application range	“Full BLM”	PNEC-pro	6.5-8.2		6.0-8.5		5.2-8.4		6.3-8.4	
			bio-met	**6.5- 8.2*****		**6.0-8.5**		**5.5-8.5**		**6.3-8.4**	
DOC (mg/L)	**Calibration range**	Algae	PNEC-pro	0.1		2-20		0.1-27.4		2.1-10.6	
			bio-met	0.1*	Deleebeeck et *al*., 2009a	1.3-20	De Schamphelaere et *al*., 2003b	0.3-22.3	De Schamphelaere et *al*., 2003a	2.1-10.6	
		Invert.	PNEC-pro	0.1		1.7-18		0.1-27.4		1.1-18.6	
			bio-met	0.1*	Deleebeeck et *al*., 2008	1.7-18	De Schamphelaere and Janssen, 2004a	0.3-17.3	De Schamphelaere et *al*., 2003a	1.1-18.6	
		Fish	PNEC-pro	0.1		1-20		0.3-23		1.2-10.5	
			bio-met	0.1*	Deleebeeck et *al*., 2007a	1**	De Schamphelaere and Janssen, 2004c	0.3-22.9	De Schamphelaere et *al*., 2003a	1.2-10.5	
	Validation range	Algae	PNEC-pro	2.5-25.8		0.1-20		1.7-25.0		2.1-22.4	
			bio-met	2.5-25.8	Worms et *al*., 2006; Deleebeeck et *al*. 2009b; Peters et *al*., 2018a	0-20	De Schamphelaere et *al*., 2003b; ECI, 2008	2.3-22.3	Van Regenmortel et *al*., 2017; De Schamphelaere et *al*., 2005	2.1-22.4	
		Invert.	PNEC-pro	2.5-25.8^{A} 3.2-23.6^{B}		0.1-20		0.1-22.1		0.4-31.5	
			bio-met	2.5-25.8 ^{A} 3.2-23.6 ^{B}	Peters et *al*., 2018a	0-20	De Schamphelaere and Janssen,	0.3–17.3	Van Regenmortel et *al*., 2017 De	0.4-31.5	

							2004a; ECI, 2008		Schamphelaere et *al*., 2005		
		Fish	PNEC-pro	3.8-18.4		0.1-18		2.5-22.9		0.5-12	
			bio-met	3.8-18.4	Deleebeeck et *al*., 2007a; Peters et *al*., 2018a	0-18	De Schamphelaere and Janssen. 2004c; ECI, 2008	2.8-22.9	De Schamphelaere et *al*., 2005	0.5-12	
	Application range	“Full BLM”	PNEC-pro	2.5-25.8		0.1-18		1.7-27.4		0.4-31.5	
			bio-met	**0.1-30**		**0.1- 30**		**0.3-22.9**		**0.4-27.3**	
Dissolved Ca (mg/L)	**Calibration range**	Algae	PNEC-pro	2.4-144		5-160		1.0-95.2		4.7-120	
			bio-met	2.4-144	Deleebeeck et *al*., 2009a	5-160	De Schamphelaere et *al*., 2003b	5.0-65.4	De Schamphelaere et *al*., 2003a	4.7-120	De Schamphelaere et *al*., 2014
		Invert.	PNEC-pro	1.3-88		7-179		1.0-95.2		9.6-99	
			bio-met	1.3-88^{A,B}	Deleebeeck et *al*., 2008; De Schamphealaere et *al*., 2006	7-179	De Schamphelaere and Janssen, 2004a	5.0-160.3	De Schamphelaere et *al*., 2005; Heijerick et *al*., 2005	9.6-98.8	Nys et *al*., 2014; Nys et *al*., 2016b
		Fish	PNEC-pro	3.8-110		14-94		7.9-158.2		5.7-83	
			bio-met	3.8-110	Deleebeeck et *al*., 2007a	14-94	De Schamphelaere and Janssen, 2004c	7.8-155.8	De Schamphelaere et *al*., 2003a; De Schamphelaere and Janssen, 2004b	5.7–83	
	Validation range	Algae	PNEC-pro	2.4-144		2.5-179		1.0-59.2		4.8-120	
			bio-met	2.4-144	Worms et *al*., 2006; Deleebeeck et *al*., 2009b	2.5-179	De Schamphelaere et *al*., 2003b; ECI, 2008	0.8–159.1	Van Regenmortel et *al*., 2017	4.7-120	De Schamphelaere et *al*., 2014
		Invert.	PNEC-pro	3.0-72.7		2.5-179		7.9-158.2		3.6-204	
			bio-met	3.0-72.7^{A} 1.3- 88^{B}	Deleebeeck et *al*., 2007b; Nys	2.5-179	De Schamphelaere and	4.8-155.9	Van Regenmortel et *al*., 2017	3.5-202	Nys et *al*., 2014; Nys et *al*., 2016b

					et *al*., 2016a; Schlekat et *al*., 2010		Janssen 2004a; ECI, 2008				
		Fish	PNEC-pro	3.8-83		3.1-129		7.9-158.2		3.6-84	
			bio-met	3.8-83.0	Deleebeeck et *al*., 2007a; Peters et *al*., 2018a	3.1-129	De Schamphelaere and Janssen, 2004c; ECI, 2008	1.4-55.1	De Schamphelaere et *al*., 2003a; De Schamphelaere et *al*., 2005	3.6-84	Van Sprang et *al*., 2016
	Application range	“Full BLM”	PNEC-pro	2.4-88.0		3.1-129		1.0-158.2		3.6-204	
			bio-met	**2- 88*****		**3.1-129**		**0.8-160.3**		**3.6-204**	

[A] : *Daphnia magna* BLM

[B] : *Ceriodphnia dubia* BLM

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

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

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

3.5.4. Comparison of the outputs from the simplified tools and the BLMs they are derived from

In this section, both the simplified tools and each of their respective BLMs are compared quantitatively using the approach described in Figure 8. For each metal, the predictions of the BLMs generated by PNEC-pro and bio-met are compared by using a limited monitoring dataset, which contains water chemistries related to 276 samples collected from different freshwater bodies in the Netherlands (see the description below). In contrast, predictions by the simplified tools were compared using an extensive pan-European water chemistry dataset (FOREGS). For each comparison, residual analysis, i.e. the ratio HC5 values from bio-met / HC5 values from PNEC-pro, is performed to determine if water chemistry is causing any bias. For each metal, the comparisons were made on samples/sites exhibiting pH values, DOC and Ca concentrations that are within the application range of both bioavailability tools (see 3.5.3). PNEC-pro includes an option to only enter a DOC concentration as input parameter (and no pH nor Ca concentration), but this option has not been evaluated in the present comparison.

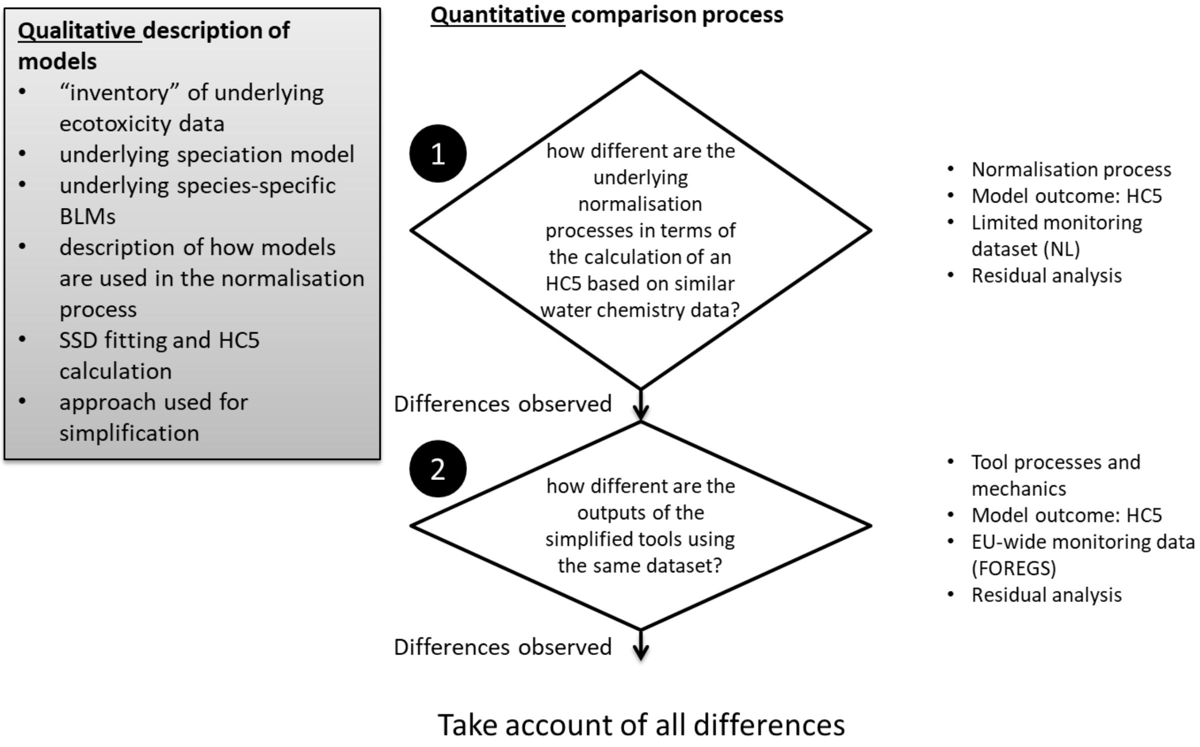


Figure 8: Flowchart of the main steps involved in the comparison of the bioavailability tools

3.5.5. Comparison of the predictions made by the full BLMs

The description of the physico-chemical properties of the sites that were used for the comparison of full BLMs is provided in Table 4.

Table 4: Description of water chemistry inputs to compare the BLMs used in PNEC-pro and bio-met

Percentile	pH	DOC (mg/L)	Ca (mg/L)	Na (mg/L)	Mg (mg/L)	Cl (mg/L)	SO_4 (mg/L)	HCO_3 (mg/L)
5th	6.62	2.9	32.9	15.1	5.7	23.5	26.2	41.3
10th	6.84	4.9	37.3	16.8	6.1	27.0	36.6	64.5
25th	7.16	7.4	41.1	22.4	7.0	33.8	49.2	96.4
50th	7.57	10.4	60.8	37.1	9.0	55.2	61.3	162.9
75th	7.87	13.6	81.3	63.1	14.1	100.4	82.6	220.4
90th	8.11	21.0	121.3	189.7	31.1	338.2	116.9	371.4
95th	8.26	26.8	149.1	370.4	49.3	709.0	159.8	429.8

Figure 9 shows for Ni the comparison between the PNEC-pro and the bio-met BLMs. Eighty percent (80%) of the 276 samples/sites were within the applicability domains of both BLMs and therefore processed for the comparison exercise. It can be observed that the data points are evenly distributed on either side of the 1 to 1 line; 12% of the predictions differ by a factor of more than two. This subset of predictions that are above a 2-fold difference generally corresponds to samples/sites with high pH and high DOC, high pH and low DOC, and high DOC and high Ca conditions. There is a high consistency in the predictions at low pH and the variations become considerable above pH 7.3.

Figure 10 shows the comparison for Cu. Seventy-eight percent (78%) of the 276 samples/sites were within the validation range of both BLMs and then further processed. It can be observed that HC5 predictions are relatively consistent and predominantly lie within a factor of two. Differences in the outputs are also pH-dependent, but to a lesser extent than that observed with Ni. In acidic waters, the bio-met BLM predicts higher toxicity than the PNEC-pro BLM. In contrast, in neutral and alkaline waters, the PNEC-pro BLM predicts slightly higher toxicity.

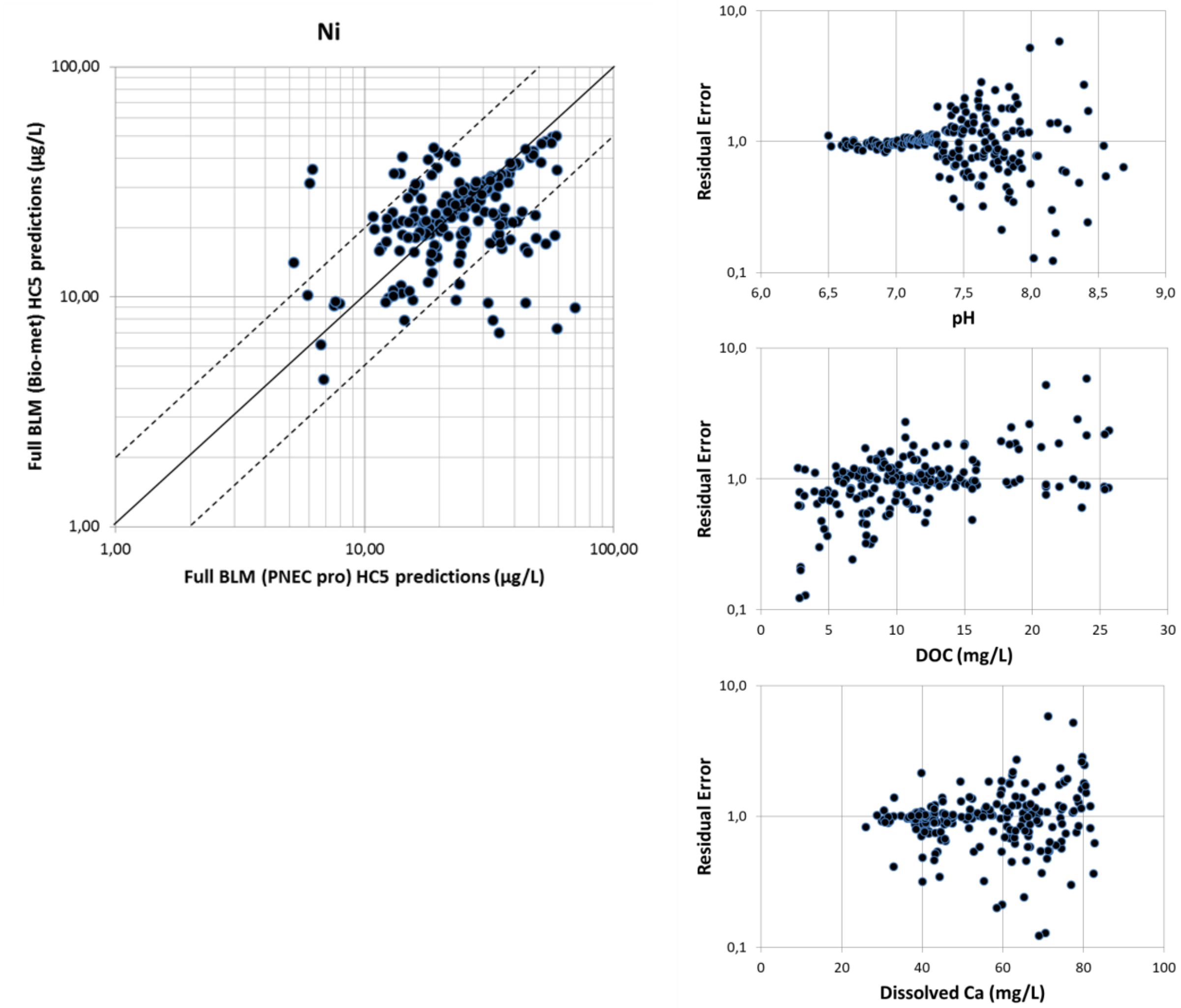


Figure 9: Comparison of HC5 values for Ni as calculated with the full BLMs

Comparison of HC5 values for Ni as calculated with the full BLMs of PNEC-pro or bio-met, and by using as inputs the monitoring data from Dutch fresh waters (*graph on the left*). The solid line is the 1:1 theoretical perfect agreement between the models' outputs, and the dashed lines show the predictions that are within a factor of 2 difference between the models' outputs.

The residual errors are indicative, for the different physico-chemical characteristics of the water bodies (pH, DOC, Ca), of the variations between both models' outputs; each is calculated as the ratio between the HC5 value generated with the full BLM of bio-met and the HC5 generated with full BLM of PNEC-pro (*three graphs on the right*).

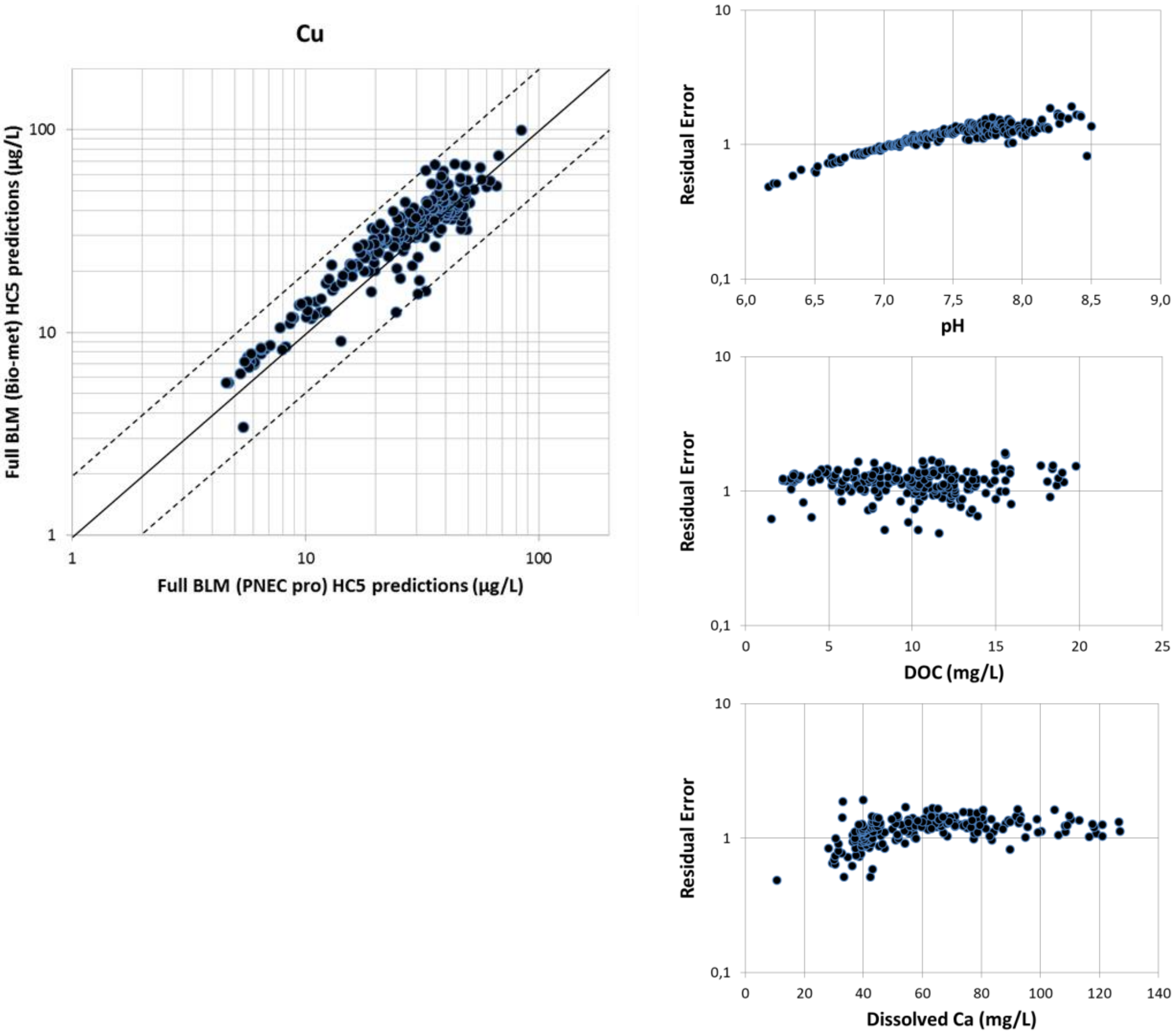


Figure 10: Comparison of HC5 values for Cu as calculated with the full BLMs of PNEC-pro or bio-met

Comparison of HC5 values for Cu as calculated with the full BLMs of PNEC-pro or bio-met, and by using as inputs the monitoring data from Dutch fresh waters (*graph on the left*). The solid line is the 1:1 theoretical perfect agreement between the models' outputs, and the dashed lines show the predictions that are within a factor of 2 difference between the models' outputs.

The residual errors are indicative, for the different physico-chemical characteristics of the water bodies (pH, DOC, Ca), of the variations between both models' outputs; each is calculated as the ratio between the HC5 value generated with the full BLM of bio-met and the HC5 value generated with the full BLM of PNEC-pro (*three graphs on the right*).

Figure 11 shows the comparison of BLM outputs for Zn. Eighty-one percent (81%) of the 276 samples/sites were within the applicability domains of the BLMs and were further processed. The outputs of the models are well correlated, but a consistent bias is observed, i.e. HC5 predictions with the PNEC-pro BLM are systematically 2.5-fold lower. A slight pH effect on the correlation is also observed when pH increases above 7.5.

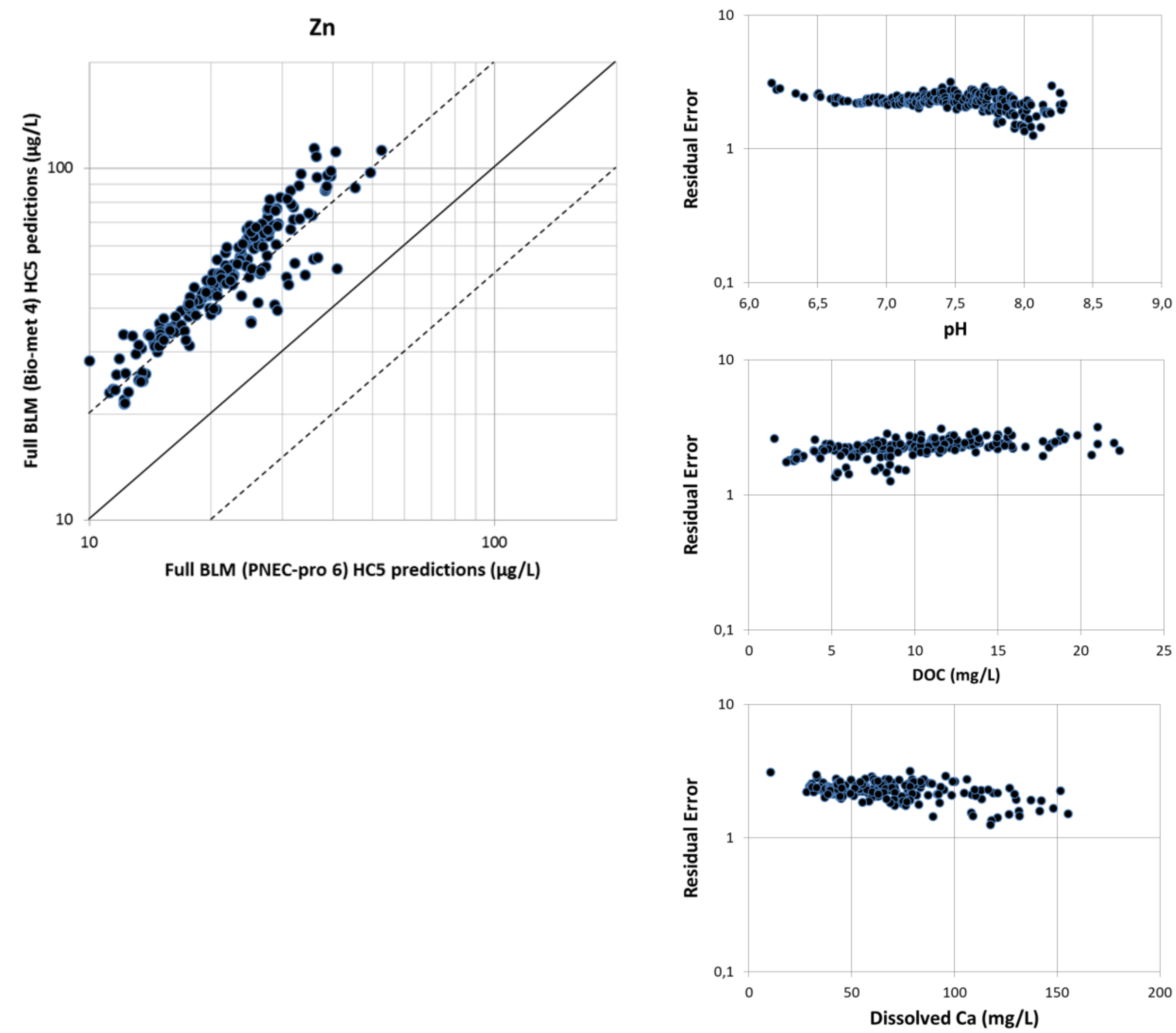


Figure 11: Comparison of HC5 values for Zn as calculated with the full BLMs of PNEC-pro or bio-met

Comparison of HC5 values for Zn as calculated with the full BLMs of PNEC-pro or bio-met, and by using the monitoring data from Dutch fresh waters (*graph on the left*). The solid line is the 1:1 theoretical perfect agreement between the models' outputs, and the dashed lines show the predictions that are within a factor of 2 difference between the models' outputs.

The residual errors are indicative, for the physico-chemical characteristics of the water bodies (pH, DOC, Ca), of the variations between both models' outputs; each is calculated as the ratio between the HC5 value generated with the full BLM of bio-met and the HC5 value generated with the full BLM of PNEC-pro (*three graphs on the right*).

3.5.6. Comparison of the simplified tools predictions

The FOREGS dataset has been used to compare the outputs (i.e. HC5) from the simplified tools. This database corresponds to 795 samples with measurements of pH, DOC and Ca in streams and rivers across Europe (covering 28 countries). The sample sites were selected to be representative of physico-chemistry conditions that are encountered in European fresh waters subjected to low anthropogenic pressures. Distributions of the key water chemistry variables from the FOREGS dataset are given in Table 5. In comparison to the limited dataset that was used for comparing the BLMs used by the simplified tools (Table 4), for the lower percentile (i.e. equal or below the 10th percentile) pH, DOC and dissolved Ca concentrations exhibit much lower values in the FOREGS dataset (e.g. for the 10th percentile: pH 6.40 vs 6.84, DOC 1.0 vs 4.9 mg/l, Ca 2.8 vs 37.3 mg/l, for the FOREGS dataset and the Dutch Water dataset, respectively).

Table 5: Distribution of the key water chemistry inputs used as inputs in the simplified tools for the comparison exercise

Percentile	pH	DOC (mg/L)	Ca (mg/L)
5th	6.10	0.6	1.8
10th	6.40	1.0	2.8
25th	7.00	2.0	7.5
50th	7.70	5.1	42.2
75th	8.10	11.1	90.6
90th	8.30	17.0	118.5
95th	8.50	23.4	146.1

Only the samples that fall within the application range of both simplified tools (see Table 3) were processed in this comparison exercise. For some sites/samples within the application ranges indicated in Table 3, PNEC-pro gives “OD” (out of domain) as output. The proportion of sites/samples in this situation depends on the metal (ranging from 1 to 20% of the dataset). These samples were excluded from the comparison since the output from bio-met could not numerically be compared to the PNEC-pro output.

Figure 12 presents the comparison of outputs (HC5) from the simplified models for Ni. A significant proportion of the samples in the FOREGS dataset are outside the application ranges of the tools (i.e. 51% and 60% for bio-met and PNEC-pro, respectively). It is important to note that not all the boundaries are equally important (see Section 6.4). Based on the relationships between Ni toxicity and water chemistry parameters, the most important boundary is the high pH because the toxicity of Ni is positively related to the pH. Hence, for waters with a pH greater than the upper pH boundary, Ni toxicity may be underestimated. When comparing the simplified models, a large proportion (i.e. 90%) of the predictions lies within a factor of two, and the median values of the predicted HC5 are comparable between the tools (i.e. 16.2 and 15.1 µg/l for PNEC-pro and bio-met, respectively; see Table 6). Similar to what was observed with their respective BLMs, residual analysis indicates that pH plays a major role in the observed differences, with a marked variation in the estimates beginning above pH 7.3, with PNEC-pro predicting higher Ni toxicity when pH increases.

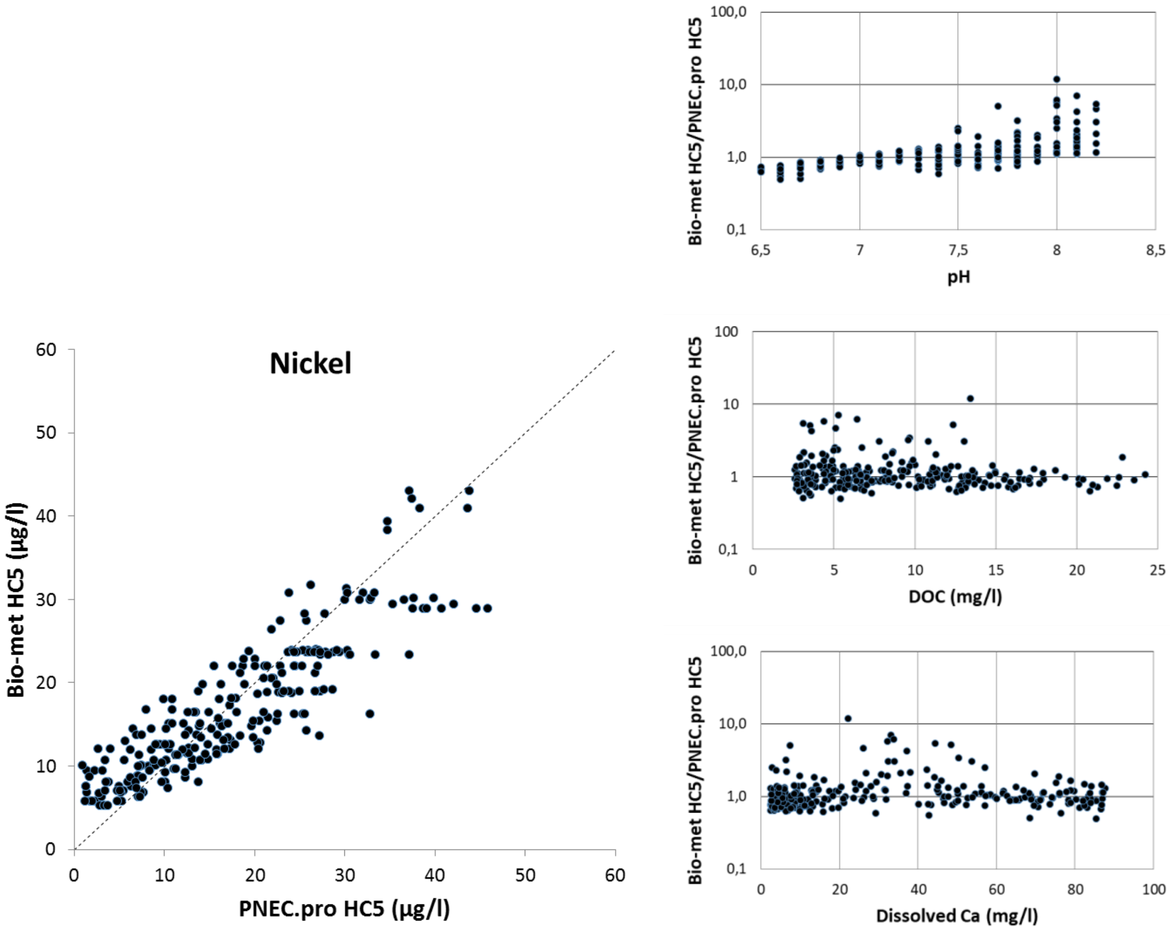


Figure 12: Comparison of HC5 values for Ni as calculated with the simplified BLMs of PNEC-pro or bio-met

Comparison of HC5 values for Ni as calculated with the simplified BLMs of PNEC-pro or bio-met, and by using the FOREGS dataset (graph on the left). The dashed line is the 1:1 theoretical perfect agreement between the models' outputs.

The residual errors are indicative, for the different physico-chemical characteristics of the water bodies (pH, DOC, Ca), of the variations between both models' outputs; each is calculated as the ratio between the HC5 value generated with the simplified BLM of

bio-met and the HC5 value generated with the simplified BLM of PNEC-pro (three graphs on the *right*).

Figure 13 shows the comparison of HC5 values obtained with the simplified tools for Cu. Twenty-five and 32% of the samples in the FOREGS dataset lie outside the applicability range of the bio-met and the PNEC-pro bioavailability tools, respectively. A significant proportion of the predictions (i.e. 30%) differ by a factor of more than 2. For Cu, the difference between the full BLMs underlying both tools is small (see Figure 10), whereas the difference between the simplified tools is much greater (up to a factor of 10, see below). This suggests that for Cu, differences between the simplification methods that are used in the simplified tools are more influential that the differences between the underlying full BLMs. bio-met predicts higher Cu toxicity in acidic waters than PNEC-pro. This was also observed in the underlying full BLMs. In neutral and alkaline waters, there seems to be no consistent difference between both tools, although the residual errors are quite scattered.

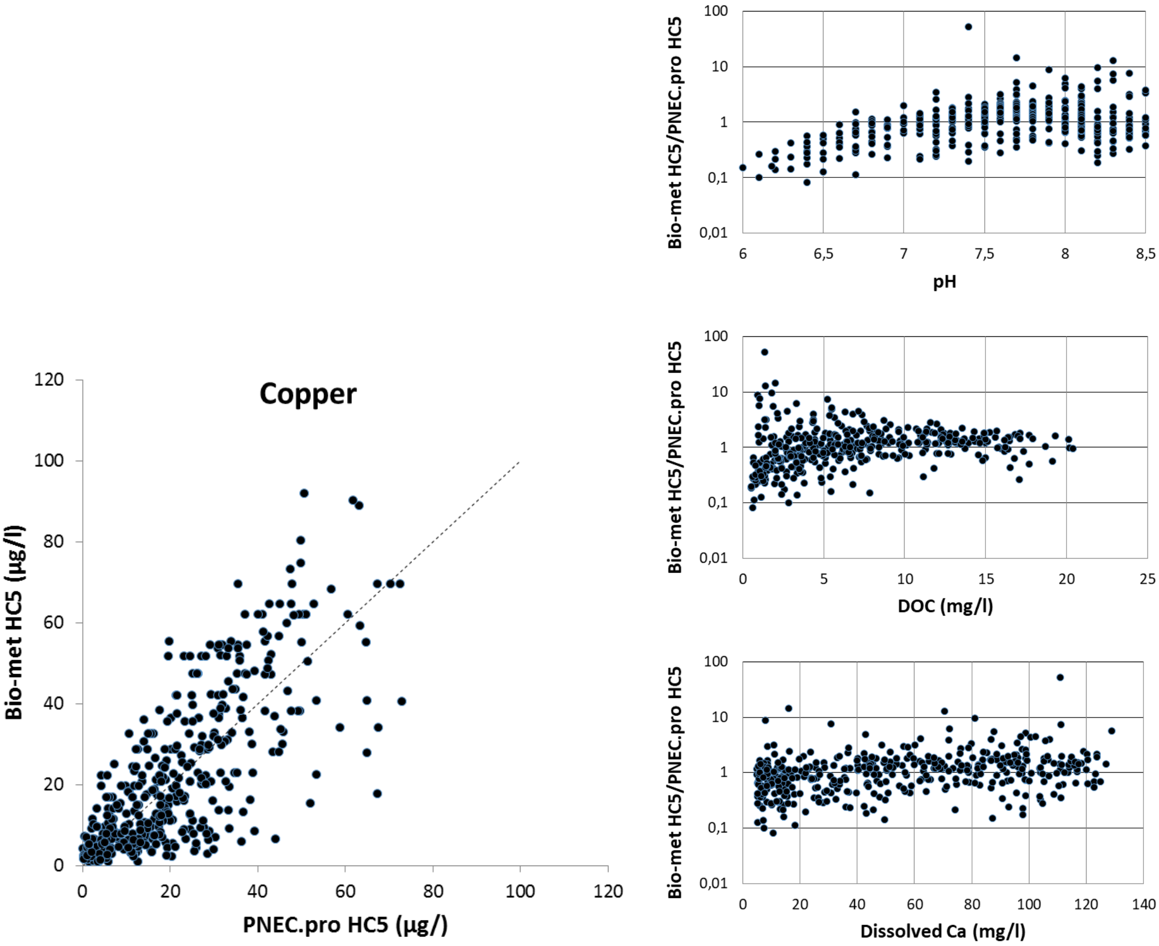


Figure 13: Comparison of HC5 values for Cu as calculated with the simplified BLMs of PNEC-pro or bio-met

Comparison of HC5 values for Cu as <u>calculated with the simplified BLMs of PNEC-pro or bio-met</u>, and by using the FOREGS dataset (*graph on the left*). The dashed line is the 1:1 theoretical perfect agreement between the models' outputs.

The residual errors are indicative, for the different physico-chemical characteristics of the water bodies (pH, DOC, Ca), of the variations between both models' outputs; each is calculated as the ratio between the HC5 value generated with the simplified BLM of bio-met and the HC5 value generated with the simplified BLM of PNEC-pro (*three graphs on the right*).

Figure 14 presents the comparison of HC5 values obtained with the simplified tools for Zn. Twenty-eight and 37% of the samples in the FOREGS dataset are outside the applicability range of the bio-met and the PNEC-pro bioavailability tools, respectively. As observed with their respective full BLMs, there is a systematic 2-2.5-fold difference in the predictions with bio-met predicting lower Zn toxicity in all water chemistry conditions (see also Table 6).

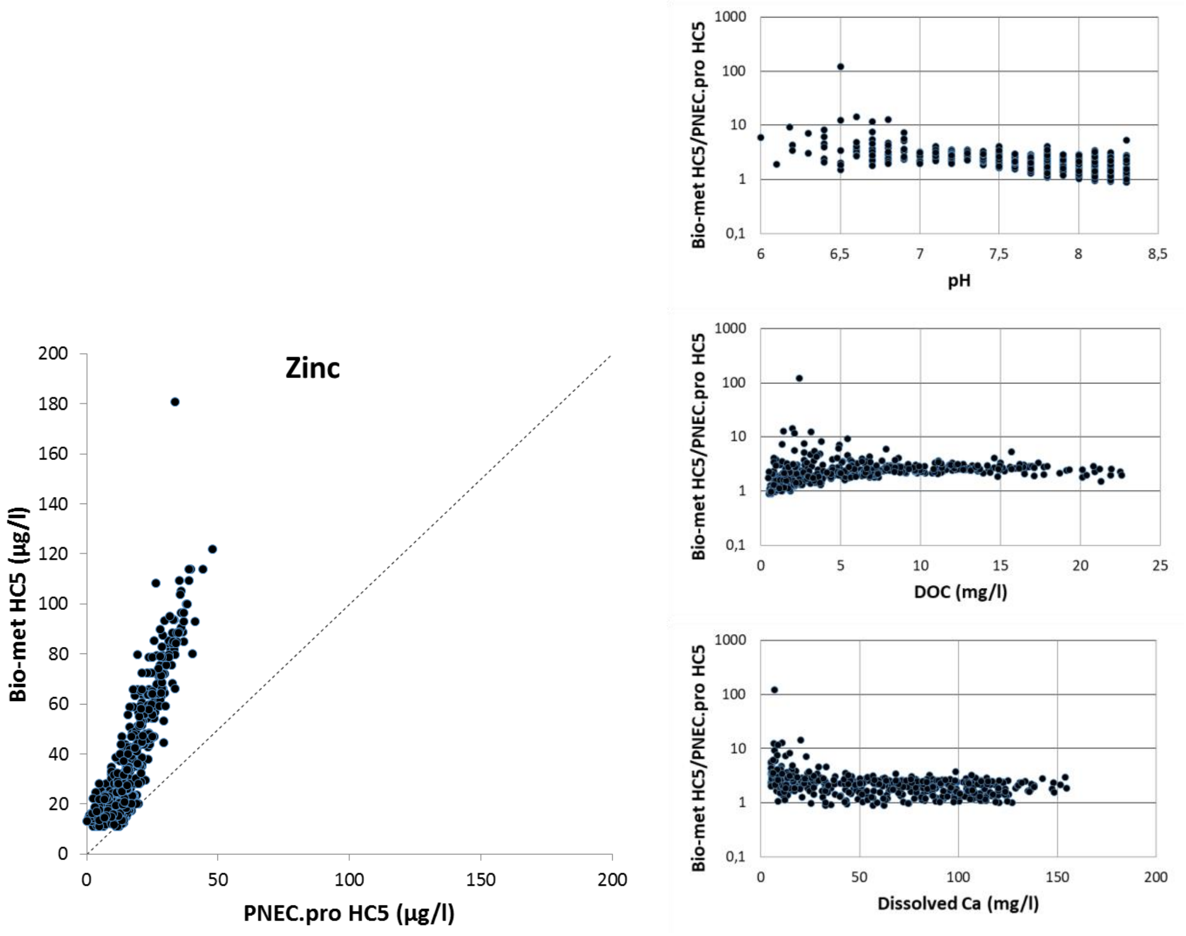


Figure 14: Comparison of HC5 values for Zn as calculated with the simplified BLMs of PNEC-pro and or bio-met

Comparison of HC5 values for Zn as calculated with the simplified BLMs of PNEC-pro or bio-met, and by using the FOREGS dataset (*graph on the left*). The dashed line is the 1:1 theoretical perfect agreement between the models' outputs.

The residual errors are indicative, for the different physico-chemical characteristics of the water bodies (pH, DOC, Ca), of the variations between both models' outputs; each

is calculated as the ratio between the HC5 value generated with the simplified BLM of bio-met and the HC5 value generated with the simplified BLM of PNEC-pro (*three graphs on the right*).

Figure 15 presents the comparison of the HC5 values for Pb as calculated with PNEC-pro and bio-met. Only 26% of the samples in the FOREGS dataset lie outside the applicability range of the bio-met tool. This proportion is larger for PNEC-pro (i.e. 47%). Overall, the HC5 values predicted by the tools are very similar (Table 6), and when comparing the outputs more than 95% of the predictions are within a factor of two. The data points of the relationship between PNEC-pro and bio-met are evenly distributed on either side of the one-to-one line. Residual analysis shows larger differences in the predictions for low DOC and high Ca conditions, and PNEC-pro predicts a slightly higher Pb toxicity above pH 7.5.

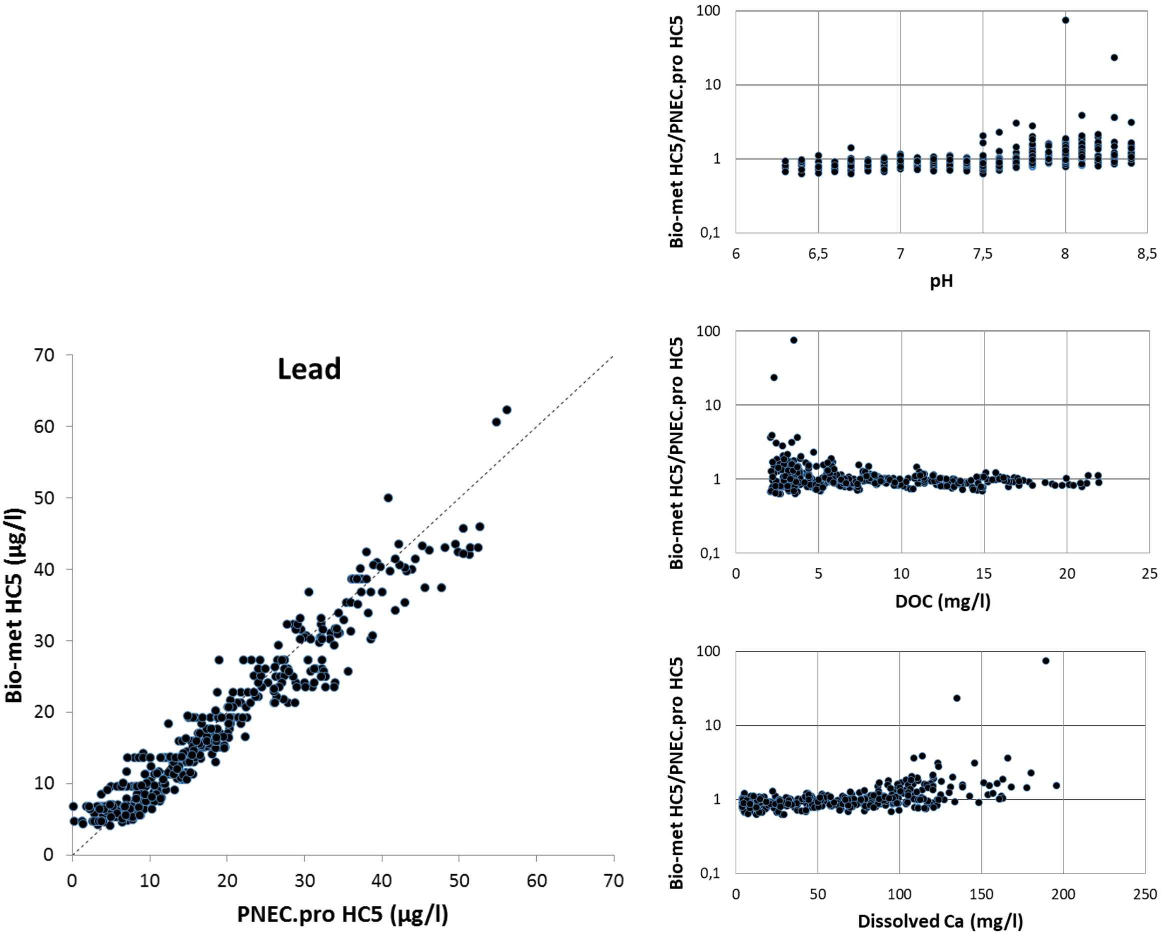


Figure 15: Comparison of HC5 values for Pb as calculated with the simplified BLMs of PNEC-pro or bio-met

Comparison of HC5 values for Pb as calculated with the simplified BLMs of PNEC-pro or bio-met, and by using the FOREGS dataset (*graph on the left*). The dashed line is the 1:1 theoretical perfect agreement between the models' outputs.

The residual errors are indicative, for the different physico-chemical characteristics of the water bodies (pH, DOC, Ca), of the variations between both models' outputs; each is calculated as the ratio between the HC5 value generated with the simplified BLM of bio-met and the HC5 value generated with the simplified BLM of PNEC-pro (*three graphs on the right*).

Table 6: Distribution of HC5 values obtained from the simplified tool of PNEC-pro and bio-met, and by using the FOREGS dataset

Percentile	Ni *N* = 256		Cu *N* = 412		Zn *N* = 489		Pb *N* = 416	
	PNEC-pro	bio-met	PNEC-pro	bio-met	PNEC-pro	bio-met	PNEC-pro	bio-met
5th	2.8	6.8	2.1	2.3	4.7	13.4	3.7	5.4
10th	5.0	7.6	3.7	3.5	6.9	14.9	5.2	6.4
25th	9.0	10.7	10.6	7.8	12.4	20.6	9.2	8.5
50th	16.2	15.1	19.2	17.1	17.1	32.4	16.0	15.1
75th	25.3	22.0	31.3	34.5	23.2	56.8	27.3	25.0
90th	31.1	28.9	44.8	53.8	29.5	78.8	36.7	35.4
95th	37.2	30.3	50.8	62.1	33.8	88.5	42.2	40.4

3.5.7. Concluding remarks

This chapter describes the relevance of integrating bioavailability into metal EQS compliance assessment and provides an overview of different tools that have been developed to facilitate bioavailability normalisation. As follows from the comparative analysis made on the tools (full BLMs and simplified versions), there are some differences observed in the results they produce. However, the group of experts that made the comparison unanimously agreed that including bioavailability in the assessment always leads to a more scientifically robust outcome, whichever tool is used. Therefore, despite some differences between these tools, it is recommended to always make a bioavailability assessment for those cases where scientific models are available.

By accounting for the influence of water chemistry, and without compromising environmental protection, incorporating bioavailability has the benefit of directing the assessors towards sites of genuine concern that should be addressed by risk management.

When choosing among simplified models, both qualitative and quantitative criteria should be considered, including the ease of use and the specific features (e.g. indication of the level of confidence associated with HC5 calculation), the level of validation of the results (with the full

BLMs and ecotoxicity data), the performance in high bioavailability conditions (high pH and low DOC), the consistency with the EQS derivation process, etc.

3.6. Other (bio)availability-based correction methods

Under the WFD, corrections for availability with varying water chemistry conditions are supported for some metals. These corrections are not as sophisticated and scientifically underpinned as BLMs, as they may only account for some of the important factors influencing ecotoxicity. This section briefly addresses some of these bioavailability correction methods.

3.6.1. Hardness correction (Cd)

For Cd, the bioavailability correction is related to water hardness, with the EQS varying over four water hardness bands. This is based upon a relationship developed by the US EPA for soft waters only, with no data for harder and higher pH waters (e.g. Mebane, 2010). The relationship between toxicity (log transformed) and the hardness (in mg $CaCO_3$/L, log transformed) is termed the “hardness slope” and has a value of 0.74 (EU, 2007). However, the Cd EU Risk Assessment Report (EU, 2007) concluded that further testing was required to assess the risks of Cd in a relatively soft water, i.e. water with hardness below 40 mg $CaCO_3$/L. The surveys indicated that the northern European countries have more than 10% of the sampled locations with hardness below about 10 mg $CaCO_3$/L, and also that waters of this character exist at some other locations in the Europe Union (EU, 2007). Currently, there is no operational tool to include a hardness correction for site specific assessment.

3.6.2. DOC-correction: Pb (freshwater) and Cu (marine)

The EQS for Pb is based upon data showing strong relationship between observed toxicity and DOC concentration for freshwater organisms and was derived using the “DOC slope” for *Philodina rapida*, a species of freshwater rotifer that displays limited influence of DOC on Pb toxicity. The Pb EQS Dossier (EC, 2011b) proposed a simple bioavailability correction for Pb, directly related to the DOC concentration, resulting in the equation:

$$PNEC_{site} = PNEC_{reference} + (1.2 \times (DOC - DOC_{reference}))$$

Where:

$PNEC_{site}$ = predicted no-effect concentration at the site under consideration;

$PNEC_{reference}$ = generic/reference EQS, i.e. 1.2 µg/L dissolved Pb;

DOC = dissolved organic carbon at the site under consideration;

$DOC_{reference}$ = average DOC concentration where $PNEC_{reference}$ is based upon, i.e. 1.0 mg/L.

The slope of 1.2 (µg/L Pb EC_{10} per mg/L DOC) was derived by using linear regression analysis of chronic toxicity data against DOC concentrations. The lowest slope was used as the most precautionary form of linear DOC correction, while acknowledging that the p-value for this slope was 0.067 (EC, 2011b). The $PNEC_{reference}$ value was derived by calculating the HC5-value for a “reasonable worst-case” scenario, corresponding to a mean DOC concentration of 1.0 mg/L, a mean pH of 7.56 and a mean hardness of 53.6 mg/L. This resulted in a HC5-value of 2.35 µg/L Pb. Because of an assessment factor of 2, the $\text{PNEC}_{\text{reference}}$ (i.e. $\text{EQS}_{\text{bioavailable}}$) was set at 1.2 µg/L dissolved Pb.

It is important to note that the $\text{EQS}_{\text{bioavailable}}$ for Pb was derived before the Pb BLM was available and at this time it was considered to represent the most reasonable approach (SCHER, 2011). Vink and Broers (2017) compared the performances of the DOC correction function and the Pb BLM of PNEC-Pro, using the water quality data of seven European ecoregions described in the RARs. It was concluded that the slope of the DOC correction function is approximately two times smaller than the slopes predicted by the full-BLM. Generally, the simple linear DOC-correction yields PNEC values which are a factor 2 lower than PNECs calculated with the Pb-BLM (in large part due to assessment factor of 2 used to DOC corrected PNECs). Similarly, in a compliance assessment of the Europe-wide bioavailable Pb EQS against freshwater monitoring data from 6 Member states and the FOREGS database, Peters et *al*. (2018b) showed that the DOC-correction tool is the most precautionary approach (regarding compliance assessment) followed by the simplified tool bio-met and then the Pb BLM.

Equally, increasing DOC concentrations has been shown to significantly reduce the ecotoxicity of Cu in marine waters. In the UK, an EQS adjusted to ambient conditions using a bioavailability correction based on the concentration of DOC was proposed (EA, 2012c). A relationship was developed between Cu ecotoxicity to marine organisms and DOC to provide a biologically relevant metric of Cu exposure. Using the DOC correction based on the relationship between active DOC and measured Cu toxicity to *Mytilus galloprovincialis* (EC_{10}s), each individual marine toxicity data (NOEC/EC_{10}) collated in the Cu risk assessment report (ECI, 2008) was normalised to a predefined DOC concentration relating it to the reference conditions (high bioavailability conditions) and used to construct the species sensitivity distribution and so obtain the reference PNEC.

3.6.3. Multiple Linear Regression (MLR)

A common reaction to the development and implementation of bioavailability-based standards that use BLMs is that these approaches are too complex and demand too many input data for routine use by relevant stakeholders. As a possible solution to this complexity (aside from the development of simplified tools that are based on BLMs) is an approach based on the use of stepwise multiple linear regressions, or MLRs.

Recently, it has been shown (Brix et *al*., 2017) how MLRs could be used to derive Cu thresholds for the protection of aquatic life in freshwater systems. The approach begins by identifying the water chemistry factors shown to influence Cu toxicity to freshwater organisms; the variables identified as important in Cu BLM being chosen *a priori* as the primary source of this information. Both acute and chronic ecotoxicity data covering a range of reliability and relevant criteria were analysed to determine the minimum number of critical variables necessary to provide a statistically significant model. The initial variables included DOC, pH, and hardness, and their interactions were also taken into account. For the development of species-specific models, datasets needed to comply with the following minimum ranges of variability in water chemistry: 100 mg/L hardness, 5 mg/L DOC, and 1.5 pH units. Five species followed these criteria for acute Cu data, whereas only one was available for chronic data. Most of the species-specific models included pH, hardness, and

DOC. Performances of the models were similar to the BLM-based approaches. The applicability of the approach described by Brix et *al*. (2017) to the European situations is limited by the availability of suitable chronic ecotoxicity data with which to derive the model.

Even more recently, the US EPA has issued revised Aquatic Life Criteria for aluminium (US-EPA, 2018) that is based on an MLR published by DeForest et *al*. (2018). The US EPA also includes an Excel Aluminium Criteria Calculator that allows the users to calculate the appropriate criterion for a given combination of water chemistry parameters[6].

---

[6] https://www.epa.gov/sites/production/files/2018-12/aluminum-criteria-calculator-v20.xlsm

4. METHODS TO DETERMINE NATURAL BACKGROUND CONCENTRATIONS (NBCS) FOR METALS

4.1 Overview

The following sections provide a definition of NBCs (Section 4.2) at different geographical scales (Section 4.3), how NBCs can be estimated (Sections 4.4 and 4.5) and case studies from individual Member States (Annex 2) showing how NBCs were determined in practice. The structure of the chapter is presented in the schematic below. The use of NBCs is to be considered in the tiered compliance approaches as described in Chapter 2.

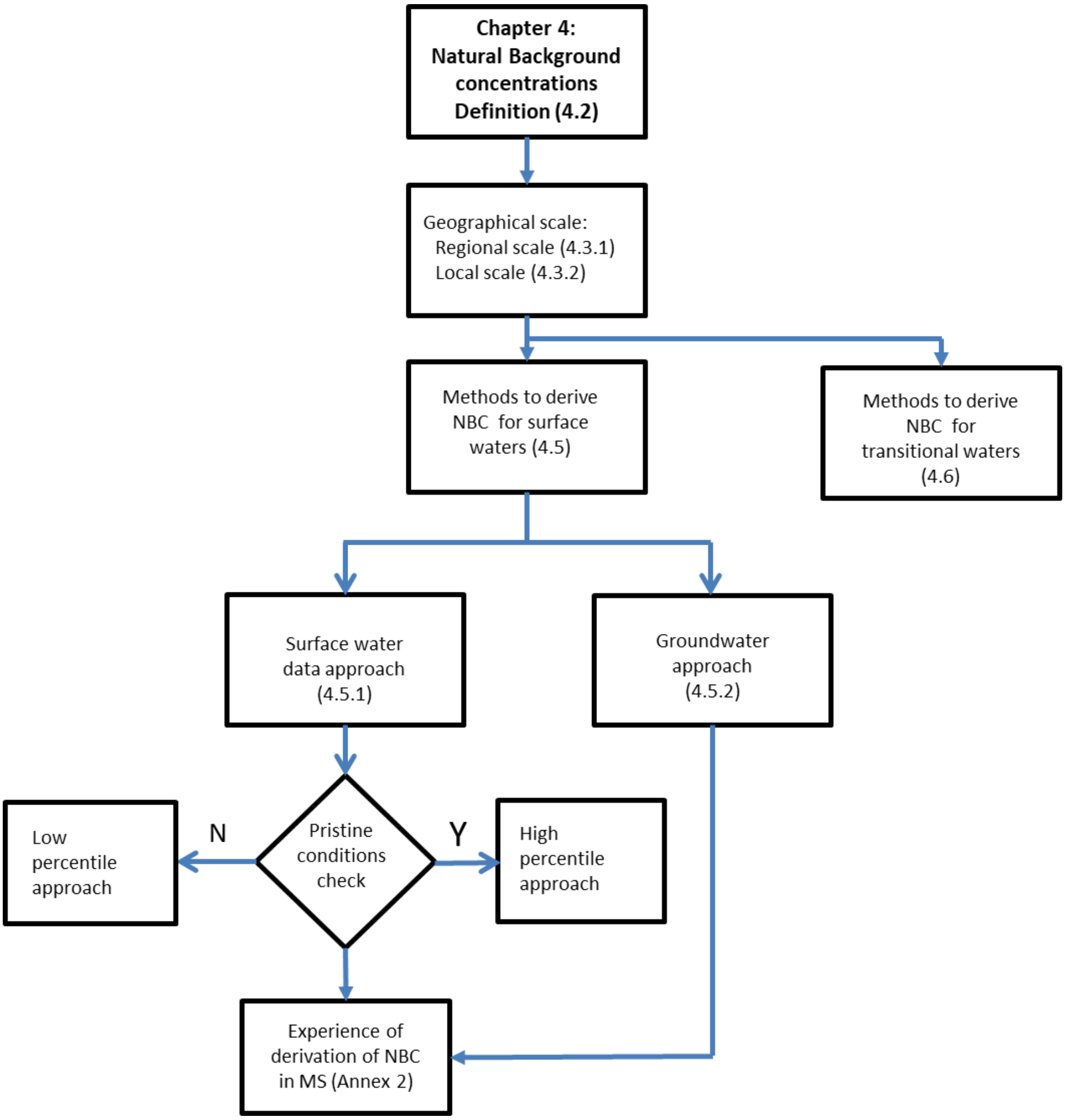


Figure 16: Flowchart of methods to determine the NBC

4.2 Definition of a natural background concentration (NBC)

According to the EQSD, Member States may, when assessing the monitoring results against the relevant EQS, take into account the NBC for dissolved metals and their compounds where such concentrations prevent compliance with the applied EQS.

NBC corresponds to the concentration of a metal present in the aquatic environment “within the range normally associated with undisturbed conditions”. The definition of undisturbed conditions requires that no anthropogenic contributions are present such as emissions, discharges, or losses. The NBC is therefore determined only by mineral and biogeochemical[7] factors. Nevertheless, even if the strict definition of undisturbed conditions requires that no anthropogenic contributions are present, ‘good status’ as defined in the Water Framework Directive (WFD) includes ‘*low levels of distortion*’ and ‘*slight deviations*’ resulting from human activity. In reality, true pristine areas are rare within Europe, and it must be considered on a case-by-case basis whether a given area represents undisturbed conditions for a specific metal.

Much of Europe’s landscape has been (and is still being) altered by human activities, e.g., mineral extraction and processing, agriculture or urbanisation. These activities can lead to anthropogenic emissions, discharges or losses of metal to the environment, and they can also create the conditions that increase releases to water bodies (e.g. crops such as conifers potentially creating acid soil conditions). These anthropogenic contributions to the measured environmental concentrations may be difficult to quantify and to distinguish from the NBCs, especially when they result from diffuse emissions (e.g. atmospheric deposition) instead of point source emissions.

However, Member States should strive to reach an estimate of NBCs that approximates undisturbed conditions, because the WFD and EQSD refer to “natural background” concentrations and they are necessary to assist in the appropriate interpretation of monitoring results. If NBCs are overestimated (because of significant anthropogenic contributions), an exceedance of the EQS can be falsely assumed to be natural. At the same time, if NBCs are underestimated for a particular area, the EQS will never be met in that area.

4.3 Geographical scale of NBCs

NBCs should be estimated in sites which have received none or only very limited influences from anthropogenic sources, and so are notably free of influence from point sources. At given locations the NBC may be strongly influenced by local geological and biological factors. A major contribution to the NBC will then be from the weathering of surface geology and any groundwater spring input.

---

[7] Microorganisms modify rates and mechanisms of chemical and physical weathering of rock, so they play a fundamental role in natural leaching and formation of suspended particles.

The derivation of NBCs should be done through a spatial/geographical approach by defining areas ("geological units") that are geochemically homogeneous for surface waters.

4.3.1 Regional scale

It is not possible to derive a European NBC for metals that would be applicable at large scale because of the substantial variation in NBCs from one catchment to another. NBCs can thus only be more robustly defined at a catchment or even sub-catchment scale.

Following this sub-scale logic, it is possible to use the hydro-ecoregions that have been defined on the basis of relief, climate and geology (Omernik, 1987; Moog et *al*., 2001; Wasson et *al*., 2002); (cf. Chandesris et *al*. 2013, see an example of application by France in Annex 2); or to use the hydrogeological units (cf. Schuster & Ullmann, 2017, see an example of application by Germany in Annex 2).

An example of a useful spatial data set is the European Geological Survey's (FOREGS) Geochemical Atlas of Europe[8], which provides environmental geochemical baseline data of high quality and consistency in Europe. The sample types collected were stream waters, stream sediments (< 0.15 mm), floodplain sediments (< 2 mm), top soils (0-25 cm), subsoils (25 cm layer within 50-200 cm depth) and humus (Salminen et *al*., 1998). The stream water data have been visualised as GIS-maps and plotted as cumulative distribution curves that can be used to estimate NBCs. It is based on Global Terrestrial Network cells (160 x 160 km^2) and sample locations within the cells have been randomised. However, the scale of the FOREGS' sampling grid and the consequent sampling resolution (e.g. 80 sites for the whole UK and 9 for the Netherlands) usually do not allow for the detection of elevated local NBC due to the occurrence of site-specific geological conditions at smaller spatial scales. Even though the FOREGS database is focused on sites with low anthropogenic input, the locations of the samplings do not often map onto WFD-specific sites. Therefore, when interpreting the upper ranges of the current distribution curve to evaluate NBCs for a specific river basin or a local area, care is required in order to exclude potential anthropogenic contributions. At the same time, however, it is important not to exclude elevated concentration resulting from local geological anomalies. All in all, the 90th percentile in the distribution of measured concentrations of the FOREGS dataset could be considered as general European upper limits for NBCs for metals in stream waters (Annex 1).

Most Member States have geological survey data or even national monitoring data that are obtained through sampling/analysing strategies similar to the ones used under the FOREGS project, but with a better spatial resolution. If available in a statistically reliable quantity and quality, these data can be used to derive regional NBCs. Member States should check

---

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

whether the estimated regional NBCs exceed the 90th percentile of FOREGS-EU data; and wherever this occurs, they should attempt to understand the source of these differences.

For marine sites, the European Environment Information and Observation Network[9] (EIONET) and the International Council for the Exploration of the Sea[10] (ICES) provide useful data for all countries bordering seas (i.e. the North Sea).

4.3.2 Local or site-specific scale

Site-specific NBCs may be developed for smaller areas of a “geological unit”, to reflect differences in local geology. Such areas may be characterised by elevated local NBC, which should be considered in compliance checking and, notably, possible risk management. This is the case, for example, of areas characterised by a particular geology associated with natural metal containing minerals. Areas with metalliferous geology are present throughout Europe. In such geological areas naturally enriched with metal-bearing minerals, the NBC may be significantly elevated due to the natural sources, up to levels where for a given metal the NBC could be higher than the EQS.

Metalliferous geological areas are generally well documented by national and local authorities. Their occurrence can be checked by e.g.:

- Local or national knowledge;
- Elevated metal levels in deeper groundwater;
- Awareness of past and present mining activity in the area;
- Geo-surveys, e.g. the GEMAS project (Geochemical Mapping of Agricultural and Grazing land Soil[11]; GEMAS 2014a,b) and the ProMine mineral deposit database (Cassard et *al*., 2015).

A number of distinct situations are possible, including naturally enriched areas where mining has not yet occurred, or naturally enriched areas where mining has historically occurred or is underway (see these distinct cases in Annex 3). As mining exploits natural geological anomalies, the presence of mining can be taken as a trigger to consider the likelihood of naturally elevated background concentrations, but mining should not cause increases in NBCs. Present-day consequences of historical mining activities may include elevated anthropogenic background concentrations that are sometimes extremely difficult for Member States to mitigate rapidly. Even in such cases, there will be a natural component that co-exists with the anthropogenic component of the prevailing concentrations. The ideal long-term goal must therefore be to reduce concentrations down to NBCs, which may still be anomalously high due to the local geology that hosted the old mine(s) and remains in place.

With this in mind, it is crucially important that Member States correctly account for local differences in natural/undisturbed conditions up front – in the process of determining the relevant objectives for the achievement of good status in each specific water body. In

---

[9] https://www.eionet.europa.eu/
[10] http://dome.ices.dk/views/ContaminantsSeawater.aspx
[11] http://gemas.geolba.ac.at/

practical terms, this will sometimes involve the implementation of a locally derived NBC that reflects unusually high natural background, and that is therefore higher than the regional, catchment or sub-catchment NBCs (see Chapter 2).

4.4 Methodologies to derive NBCs

There are several different possible approaches for estimating NBCs in fresh and marine waters. The ideal method would be one that allows users to:

- Provide a robust value, minimising methodological uncertainties;
- Be applicable over a defined spatial scale;
- Derive a single value as the NBC from a defined number of data, and for a defined homogeneous area; it is widely recognised that NBCs vary over a wide area and a single value may fail to reflect that range if the spatial coverage is too great (e.g. Reimann and Garrett, 2005);
- Be considered practical for the derivation of NBCs for all trace elements in all types of fresh or marine waters, and as far as possible, be evidence-based;
- Be straightforward and transparent to use by technical (but probably not expert) staff;
- Not incur excessive or prohibitive costs in time or financial resources to perform the derivation of NBCs.

Two main methods are discussed in the current guidance:

- **Surface water data approach:** the use of spatially based monitoring data in surface water after choosing an appropriate percentile dependent on the selected dataset, and on the evaluation of pristine conditions, anthropogenic sources and supporting information like flow characteristics.
- **Groundwater approach:** measurement of concentrations in groundwater and reading across to surface waters.

Another possibility is to derive NBCs in surface waters from local geological characteristics by applying **geological modelling**, such as the Watershed Mass Balance Models based on the use of erosion or buried sediments modelling. **Exposure modelling,** based on predicted discharges and using knowledge of the local geology, can also be used to derive NBCs. The data used should be appropriate to the required precision of the estimate. This modelling approach is relevant at a relatively local scale or under specific circumstances. It is difficult to validate, but it may provide a useful line of evidence in support of other approaches. The simplest way is to apply the so-called "**sediment approach**", i.e. the conversion of metal concentrations in suspended particulate matter (SPM) or sediment in undisturbed or pristine sites or in pre-industrial sediment by using partition equations (e.g. EqP approach). Since this method has an intrinsically high uncertainty, guidance will not be provided here for the determinations of NBCs.

4.4.1 Deriving NBC from surface water data approach

NBC information can be extracted from long-term temporal and spatial datasets from geographical surveys that address the determination of baseline concentrations of the metals under investigation and that cover the area of interest.

The data set should be of sufficient quality i.e. acquired with adequate sampling protocols and analytical methods with sensitivity limiting the number of measures below the limit of quantification (LOQ), so to ensure that the NBCs can be confidently estimated for trace metals. Data requirements are discussed in Chapter 5.

A pre-screening step is advised to exclude stations with known anthropogenic point sources. Data exclusion can also be performed by analysing the data distribution and detecting statistically significant outliers. But, especially in case of limited data availability, the *a priori* exclusion of data/sites based on the analysis of pressures may limit the number of data available for subsequent analysis and NBC derivation. In that case, a spatial analysis of data becomes essential, in order to reinforce the statistical quality of final results. A useful statistical tool to analyse the dataset is the use of probability plots graphical procedure to determine the distribution of a random variable (Wagner et *al*., 2014). If a dataset of a variable is normally (or lognormally) distributed, it is displayed as a straight line in the probability plot. Assuming the data originates solely from a homogeneous area, free of known anthropogenic influence, this straight line represents the background metal concentration. Examples of application of probability plots are given in Annex 2: Case studies of Germany and The Netherlands.

In essence, two types of surface waters can be distinguished as a basis for NBC estimation from surface water data:

- pristine waters under undisturbed conditions, and
- waters with ‘low levels of distortion’ and/or ‘slight deviations’ resulting from human activity”.

NBC values derived from these monitoring data sets on dissolved metals will be set as a specific percentile (cumulative frequency in %) of a distribution. The choice of a given percentile, or any other measure of the statistical properties of a dataset - e.g. the 5th or 10th percentile in the United Kingdom (Peters et *al*., 2012), the 10th percentile in The Netherlands (Osté et *al*., 2012; Osté, 2013), and the 90th percentile in Germany (LAWA-AO, 2015) - is determined by the degree to which the waters are influenced by anthropogenic activities.

General criteria for the choice of percentile to derive NBC should be:

- **in pristine waters (not affected by humans) a high percentile (90th percentile, P90) should be used.**
- **in the case of waters affected by human activities, a low percentile (10th percentile, P10) should be used.**

The “**high percentile approach**” is especially suitable for deriving local NBCs in pristine areas. Its application is usually limited by data availability when national monitoring datasets are used. The quality of the dataset is critical. In fact, datasets used for deriving NBCs may not have been originally collected for this purpose; some parameters should then be checked, e.g. care should be taken to ensure that the magnitude of the LOQ is sufficiently below the EQS value, as required by QA/QC Directive. Monitoring efforts would need to be specific to these sites as few data are routinely collected in such “not at risk” sites because they are usually not included in operational monitoring where site selection has historically been influenced by likelihood of hazard. In the high-percentile approach the frequency of non-detects does not influence the P90 as long as the whole data population can be assumed to be homogeneous (i.e. the assumption of (log)normal distribution is not rejected).

For the “**low percentile approach**”, data pre-selection has a lower impact on NBC derivation, though it is recommended to apply it to discard areas/sites subject to direct anthropogenic inputs. Furthermore, the number of data that are above the LOQ needs to be sufficient to ensure an NBC can be estimated with a stated confidence.

At wider or regional scales, estimating NBCs based on a low percentile of existing surface water monitoring data is a practical and pragmatic solution. It is however emphasised that this approach may work primarily in areas with a rather homogeneous geology, where natural variability is limited. If the evaluation unit includes some local geological anomalies, the low percentile approach will inevitably exclude them. It is expected that a higher percentile better reflects the elevated local NBCs.

In **saltwater** (Practical Salinity Units, PSU > 25), the concentrations of metals (dissolved) in the open ocean should normally represent NBCs. Guidance is given in OSPAR (2005) on ambient metal concentrations measured in the waters of the North-East Atlantic area covered by this Convention. However, these data should be interpreted with care when deriving coastal NBCs. Indeed, the ranges presented for the different metals refer to open ocean ranges, which are usually lower in value than those from near and on the continental shelf areas (e.g. for Cd and Cu). In addition, it must be noted that total metal concentrations are typically measured at the open sea stations, whereas derivation of NBCs requires dissolved metal concentrations. If dissolved concentrations are not available in the open sea/ocean, monitoring data in coastal waters can be used, but then a P10 should be selected as a NBC.

Pristine condition check

A pristine area is an area that is unspoiled, in its original condition, i.e. not subject to anthropogenic pressures. As mentioned above, pristine areas can be hard to find in Europe, depending on the metal considered, and therefore, Member States should focus on areas with no point source anthropogenic emissions and endeavour to find areas with no or only very low anthropogenic pressure. The low anthropogenic pressure watersheds can be selected according to land use data (e.g. Corine Land Cover[12]), using the logic applied to inter-calibrate the results of the ecological status evaluation in accordance with the WFD

---

[12] https://land.copernicus.eu/pan-european/corine-land-cover

(Erba et *al*., 2009) and avoiding stations with diffuse and point sources of metals related to human activities.

Typically, headwaters or spring waters in resurgence zones/zones of groundwater outflows are considered as pristine, i.e. reflecting water status before any anthropogenic influence. They could be representative of upstream concentrations, but they are often not representative for the downstream concentrations due to the catchment contributions. Natural weathering of rock-forming minerals can increase concentrations downstream together with evaporation along the flow path (particularly if the catchment includes lakes). On the other hand, concentrations can decrease under undisturbed conditions because of changes in groundwater/interflow and runoff water shares to the surface water.

No site will be truly pristine, because even sites that are not directly impacted by local anthropogenic emissions may experience atmospheric deposition. Therefore, the ambient concentration may reflect the sum of the true NBC plus the input from atmospheric deposition. Atmospheric deposition may be hard to quantify, but the method below allows the identification of whether an area can be considered as pristine.

Because it is very difficult to *a priori* define a set of pristine sites, the pristine condition check is the essential step to determine the specific percentile used to derive the NBC. The pristine condition check can be done for each metallic element individually. This might lead to different percentiles for various elements in the same area. There are different possibilities to evaluate the pristine conditions:

- Use sediment profile by evaluating
  - the sediment profile (total metal contents),
  - the enrichment factors;
- Verify that there are no point-source metal inputs present in the area.

Pristine condition check-1 - Use of the sediment profile

Because the sediment column of a water body reflects the history of a basin, measuring of trace element concentrations in a sediment core is a way to check the pristine conditions of an area in a certain water body. The purpose of the sediment analysis would thus be to check if present-day metal levels are comparable with pre-industrial levels (Ferrand et *al*., 2012; Zglobicki et *al*., 2011). If deeper sediment samples are used to determine pre-industrial concentrations, the sediment core below the top layer should be relatively constant with depth. It has been shown (Swennen and Van der Sluis, 2002) that variation could be caused by natural processes (weathering, leaching, precipitation), leading to naturally enriched zones. The trace element concentration in a sediment core of a pristine area should be relatively constant with depth. Natural weathering of naturally metal-enriched zones might also give rise to some variation in the sediment profile in or adjacent to naturally mineralised zones. Note though that the chemistry of water within sediments has different drivers to those of the surface waters, so that aqueous concentrations of the two should not be compared directly.

An appropriate sediment profile can be observed at locations that have a significant sedimentation rate and that are not significantly affected by physical and biological mixing and diagenetic processes known to disturb the vertical distribution of pollutants (Kramer et *al*., 1991). Knowledge of sedimentation rates will help to estimate the depth needed for a proper sediment core and how to divide a sediment core into layers of certain periods. The particle size distribution is one of the most important parameters controlling metal concentrations in the sediment. Lake sediments and sediments accumulated under low hydrological energy and flow sections of rivers and estuaries are probably most appropriate for the analysis of (normalised) total metal content in sediment cores. Concentrations in the sediment could then be used directly to extrapolate to NBCs in water in pre-industrial areas, but this procedure, though presented, is not recommended because of its intrinsic high uncertainty (see Section 4.4).

In order to check for pristine conditions, sediment enrichment factors can be applied to sediment data. Sediment enrichment factors (see e.g. Norton et *al*., 1991) provide an indication of the ratio between the enrichment and the NBC in sediment.

The Enrichment Factor (EF) is an easy tool to assess the enrichment degree (Sinex and Helz, 1981). It is defined as follows:

$$\mathbf{EF = (Me/Al)_{sample} / (Me/Al)_{background}}$$

Where:

- $(Me/Al)_{sample}$ is the ratio of total metal and total aluminium (Al) concentrations in the (local) sample, and
- $(Me/Al)_{background}$ is the ratio of total metal and the total Al concentrations related to the (general) background.

The formula shows that the metal concentration needs to be normalised. In the equation Al is used, but Fe, total organic carbon (TOC), and grain size have also been used for normalisation (Nowrouzi and Pourkhabbaz, 2014). Another aspect of the formula is that not only the contents in the top sediment (sample) need to be measured, but that also a NBC is required. General earth crust data have been used as a background, but it is recommended searching for local background values by sampling deeper sediment layers that have been deposited in the pre-industrial period. In that case the equation can be modified by using the ratio of total metal and total Al concentrations in recent sediments and in pre-industrial sediments:

$$\mathbf{EF = (Me/Al)_{recent\ sediment} / (Me/Al)_{pre\text{-}industrial\ sediment}}$$

Total metal content in sediment versus metal concentration in pore and surface water

The text above mentioned metrics that provide insight in the sedimentation of metals in time. These approaches have been used frequently, but not to derive NBCs in surface water. Although the content of sediment is a good indicator of anthropogenic contributions, the ratios in pre-industrial and recent sediment may be unrelated to the background and recent concentrations in surface water for the following reasons:

- Due to anoxic conditions in sediments the partitioning between the solid (particulate) and the dissolved phase differs significantly in sediment compared to water.

- The concentration of metals in surface water is dependent also on other sources, like rainwater, upstream water, and groundwater.

Pristine condition check-2 - Pressures assessment

To prove that relevant metal sources are absent, the water manager has to make an inventory of the potential sources that can cause inputs of the substance into the water. Consequently, the presence of these sources in the area and in the area upstream needs to be thoroughly evaluated. If it appears (with a large certainty) that there are no anthropogenic sources present in the area, including no significant atmospheric deposition, the water may be considered as pristine for the specific element, and the value of the 90th percentile of the data (P90) can then be chosen as the NBC.

4.4.2 Deriving NBC from Groundwater

NBCs of metals in surface water can be estimated from their concentrations in connected groundwater bodies from the same river basin or sub-basin. Groundwater concentrations of trace elements may reflect local geological conditions. Large differences in metal concentrations have been observed in source waters taken all over Europe (Reimann and Birke, 2010). Notably, elevated concentrations in groundwater may indicate the presence of metalliferous geology in the area.

In general groundwater contamination from anthropogenic sources, in particular from aerial deposition, but also from other diffuse sources, is likely to be limited. This certainly will be the case when groundwater underlies catchments or sub-catchments in which soils are well buffered and display significant sorption properties with relatively high cation exchange capacity (CEC), high organic matter content, and a low output of dissolved organic material. In such cases, metals deposited on the soil surface are likely to be retained within the soil profile and thereby subject to normal bio-geochemical cycling.

There are potentially significant amounts of groundwater monitoring data available in many Member States that may be used for the derivation of NBCs. The use of groundwater data for the derivation of NBCs offers several advantages over other methods. These include:

- The wide availability of data on drinking water produced from groundwater sources;
- The likely close relationship between the underlying geology and the concentrations of trace metals in the water (depending on hydraulic conductivity); and
- Groundwater is less affected by anthropogenic inputs than the surface water in the same watershed.

However, several potential concerns arise from the use of groundwater data in regard to the following issues:

- Generally, groundwater concentrations of elements and ions are not related to those in surface water unless the groundwater constitutes a significant component of the surface water.

- There is often uncertainty about the relationship between surface and ground waters, particularly if redox conditions in groundwater or at the sediment-surface water interface change, resulting in large changes in the mobility of metals.
- Seepage water and interflow (drainage water), i.e., water in the unsaturated zone, also can have higher concentrations than groundwater and overlying surface waters because of the enrichment of metals in the soils. The use of element or isotopic ratios (such as Pb isotopes) may help to discriminate the anthropogenic impact from the natural ones.
- Shallow groundwaters can contain higher trace metal concentrations through the leaching of metals, also of anthropogenic origin, through soils which have limited buffering, low pH, poor sorption capacity and high rates of DOC outflow. (Note, however, that one also must account for the possibility of natural soil releases such as the ones from ‘acid-sulphate-soils’ or from the natural release by metalliferous rock).
- Some groundwater bodies are seasonally/regularly affected by contaminated surface drainage waters.

4.5 Baseline recommendations in deriving NBCs from water monitoring data

- Derive NBCs, with associated estimation of uncertainty, at the scale of homogeneous geographical and geological units. Geology and geological alterations are the main drivers of the dissolved metals background spatial variability in surface waters.
- The minimum size of the dataset is related to the data availability and the ability to estimate a reliable percentile value. Data should be sufficiently representative of the study area unit.
- The number of sites and the number of replicates for each site should be defined according to the statistical model in order to provide a reliable estimation of the uncertainty.
- High percentile approach (P90 as a default value) should be applied in pristine areas.
- In this case the database should be evaluated in a critical way to avoid sampling points impacted by known human activities. In the process of the statistical evaluation, outlier values in the distribution should be critically evaluated and, if necessary, be removed.
- If the dataset contains sites influenced by anthropogenic inputs, low percentile approach (P10 as a default value) should be used.
- The P10 value is less affected by high concentration data. Data preselection may be performed, but only in the case of confirmed outliers or if the sampling points are directly downstream of known point source discharges.
- In order to highlight local anomalies (whether they are geogenic or anthropogenic) spatial concentration distribution (such as probability plots) can help provide an insight into the sources of different statistical populations, enabling the identification of the background population from which NBC can be derived.

- This method shouldn't be used for metals for which atmospheric deposition is significant, as this would lead to an estimate of background concentrations influenced by this atmospheric deposition.
- The period of the data collection should be five to ten years. A shorter time period may be used if clear trends are observed, but it should at least cover a 3-year period.
- In the case of groundwater monitoring data, the period of the data collection should be up to 15 years.
- The monitoring data used should be distributed evenly over the year.
- Only measured dissolved metal concentrations (filtered through a 0.45 µm pore size) should be used, which is a standard procedure for WFD monitoring.
- The database is checked for whether it contains only fresh water or salt water data. The chloride concentration of fresh water should be less than 500 mg/l. The salinity of salt water should be at least 25 PSU.
- Data below the limit of quantification (LOQ) should be set to 0.5 x LOQ according to QA/QC Directive (European Union, 2009).
- A P10 can be derived if the percentage of data < LOQ is less than 30%. No P10 should be derived if more than 70% of the data are below the LOQ. In the range between 30 and 70% a decision must be taken based on detailed analysis of the data.
- A P90 can be derived if the percentage of the data < LOQ is less than 70%. No P90 should be derived if the percentage of data < LOQ is higher than 85%.

4.6 Estimating NBCs for transitional surface waters

In principle, the same approach used to estimate NBCs in freshwaters may be adopted for estimating NBCs in transitional waters. However, data are usually sparser, and the regional specificity mentioned above may be less appropriate because of the “smoothing” of the geological influences and the greater mixing of marine waters compared to freshwaters. Furthermore, it is difficult to derive a specific NBC for transitional waters because water composition in this transitional area varies from almost fresh water to almost sea water.

For transitional waters, a simple method has been developed based on the relationships among the various salinity gradients from fresh to brackish to salt waters. The generic method to derive NBCs in transitional waters is based on mixing behaviour (Osté et *al.*, 2013). Only the mixing of seawater and river water determines the background concentration in the transitional zone, neglecting chemical processes (e.g., flocculation and precipitation). Under these assumptions, the resulting dissolved background concentration in transitional water can be described by:

$$Cb_{transitional} = \frac{[salinity]}{35} * Cb_{sea} + \left(1 - \frac{[salinity]}{35}\right) * Cb_{fresh}$$

In which:

- $Cb_{transitional}$ = dissolved background concentration at transitional water sampling station (µg/l);

- $Cb_{sea.}$ = dissolved background concentration in seawater (µg/l);
- $Cb_{fresh.}$ = dissolved background concentration in fresh (river) water (µg/l);
- Salinity = salinity at the transitional water sampling station (PSU).

4.7 Take-home message

NBCs should be derived through a spatial/geographical approach by defining areas ("geological units") that are geochemically homogeneous for surface waters.

General criteria for the choice of percentile to derive NBCs from water monitoring data should be as follows:

- **in pristine waters (not affected by humans) a high percentile (90th percentile, P90) should be used;**
- **in the case of waters affected by human activities, a low percentile (10th percentile, P10) should be used.**

Sediment profiles can be useful to check the pristine conditions but are not recommended to derive background concentrations in surface waters.

Datasets for deriving NBCs should be critically evaluated using statistical tools (such as probability plots) in order to confirm the homogeneity of the population.

Statistical outliers should be checked and discarded if correlated with point-source discharges.

Local geological anomalies can be highlighted as a different population in probability plots, and then further defined by geological and mining maps.

5. MONITORING DATA / DATA REQUIREMENTS

5.1 Overview

This chapter provides information on monitoring covering: general principles (section 5.2); insights regarding the analysis of dissolved metals (section 5.3.1), pH and dissolved Ca (section .5.3.2), and DOC (section 5.3.3); how to deal with missing inputs by estimating the required parameters (sections 5.4.1) and by using the existing monitoring data (section 5.4.2); and ways to handle temporal (section 5.5.1) or spatial (section 5.5.2) variability.

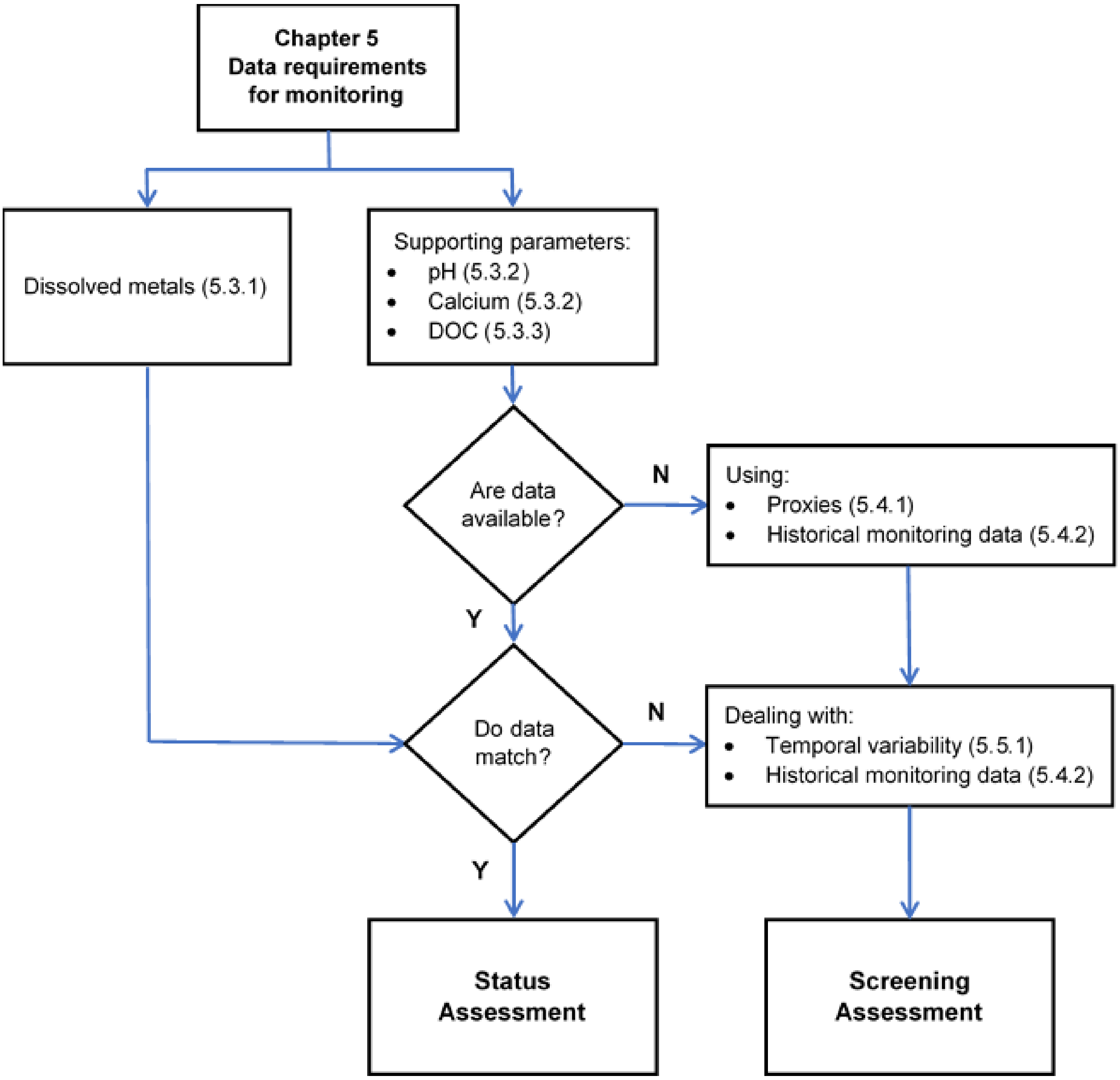


Figure 17: Flowchart of the different aspects related to the monitoring data

5.2 General principles

Accounting for bioavailability in compliance assessment using a simplified bioavailability tool requires, ideally, that the concentration of dissolved metal is accompanied by "matched" data on supporting physico-chemical parameters. These supporting parameters include, at the very least, dissolved organic carbon (DOC), pH and dissolved calcium. However, the application of the $EQS_{bioavailable}$ for Pb, which, at present, is based on a DOC correction, needs only "matched" DOC data.

In this guidance document, the term "matched" means that the supporting water chemistry parameters are sampled at the same site as the metal and preferably also at the same time, i.e. one sample is taken from a site from which the dissolved metal and supporting water chemistry parameters are all determined.

Dissolved metal concentrations and measurements of DOC, pH, and Ca vary temporally and spatially. Therefore, using matched data for all of the required input parameters for the simplified bioavailability tool increases the reliability of the results and a matched data monitoring scheme should therefore be used preferentially.

However, it is recognised that matched data may not always be available, especially for those Member States that are in the early phases of the transition towards the use of bioavailability-based EQS. Feasibility studies on implementing a bioavailability-based approach for metals have been undertaken by several Member States (e.g. EA, 2012a; Cousins et *al*. 2009; Hoppe et *al*. 2009; Geoffroy et *al*. 2010; Tack, 2012; Hommen and Rüdel, 2012). Some options on how to deal with the lack of matched data or missing data are provided in the following sections with some of the reasoning behind those options and the implications for selecting an option. Wherever alternative approaches are considered to address missing data, especially for DOC, the implications, in terms of uncertainty, are understood in the assessment.

High-quality data are a general prerequisite for WFD monitoring, EQS compliance assessment and subsequent decision-making. Any assessment of potential risks, including estimation of metal bioavailability, is highly dependent upon the quality of the used data. Protocols for water sampling, preservation and analysis should, at all times, follow the principles laid down in CIS Guidance Document No. 19 on surface water chemical monitoring under the WFD (EC, 2009b). Analytical methods for every variable should comply with the minimum performance requirements as stated in the QA/QC Directive (EC, 2009a).

5.3 Analyses

5.3.1 Dissolved metal analysis

Dissolved metal concentrations (in µg L^{-1}) as noted in the EQSD (EU, 2008b), refer to the concentrations of metals determined in a water sample obtained by filtration through a 0.45 µm membrane filter or any equivalent pre-treatment. The filter membranes used for metal analysis are usually made of nylon or cellulose nitrate. Many international standard methods are available for the determination of the concentration of metals in water, based on analytical techniques such as inductively coupled plasma mass spectrometry (ICP-MS), as described in the CIS Guidance n.19 (EC, 2009b).

5.3.2 Analysis of pH, dissolved Ca

An ISO standard for determination of pH in water is available (ISO, 2008a). This International Standard specifies a method for determining the pH value in rain, drinking and mineral waters, bathing waters, surface and ground waters, as well as municipal and industrial wastewaters, and liquid sludge. The ISO standard applies within the range pH 2 to 12 with an ionic strength below $I = 0.3$ mol kg^{-1} (conductivity at 25 °C, $\gamma_{25} < 2000$ mS m^{-1}) and in the temperature range 0 to 50 °C. In view of its great practical importance, accuracy and precision, only measuring using the pH glass electrode is described in this International Standard.

Relatively small errors in pH readings can result in significant misinterpretations of the chemical processes taking place. To ensure accuracy, pH must be measured in the field. Actions that help to improve data quality include the use of a good meter/electrode (accurate to 2 decimal places), the careful preparation before field activities, the careful transport of instruments, the frequent calibration, the measurement in a beaker (not the water body), and the allowance of time for equilibration. Instruments that allow measurement of very small samples, samples in wells, or continuous monitoring are available, but are more expensive and usually not as accurate. Under some circumstances, for example, when the transport time of the samples back to the laboratory is relatively short, the measurement may be undertaken at the laboratory.

Many international standards are available for the determination of calcium in waters, based on different analytical techniques such as ion chromatography (IC), atomic emission spectroscopy (AES), inductively-coupled plasma atomic emission spectroscopy (ICP-AES) and inductively-coupled plasma mass spectrometry (ICP-MS).

5.3.3 Dissolved organic carbon (DOC)

DOC analysis should be carried out according to the International Standard (ISO, 1999).

Total organic carbon (TOC) is a measure of the carbon content corresponding to the dissolved and the undissolved organic matter present in water. The definition of DOC in the ISO method is the *“sum of organically bound carbon present in water originating from compounds passing through a membrane filter of 0.45 µm pore size, including inorganic anions such as cyanate and thiocyanate”*.

Possible interferences from membrane filters in DOC determination have been discussed by Karanfil et *al*. (2003). Hydrophilic polyethersulfone (PES) filters and a hydrophilic polypropylene filter (both 0.45 µm absolute pore size and 47-mm disc size) were found to be the best options among those tested in the study.

Glass fibre depth filters are more suited for DOC analysis as they can be treated at high temperatures to remove organic matter, although the pore size discrimination is less precise than membrane filters used for metals’ analysis. This aspect could induce some mismatch between dissolved metal and DOC concentrations.

The method described in this International Standard applies to water samples containing organic carbon ranging from 0.3 mg/l to 1000 mg/l. The lower limit concentration is only applicable in special cases, for example, drinking water measured with highly sensitive instruments.

5.4 How to deal with missing input parameters?

Compliance assessment using the simplified tools requires data for at least pH, DOC and dissolved Ca. Without these, the simplified tools will either not run or not run reliably. Some of the required data, however, may not be available, particularly when Member States are just starting to consider the implementation of bioavailability based EQS. This section considers options for dealing with missing input parameters.

Historically, DOC has not been routinely monitored in freshwaters by many European Member States. Initially, therefore, many Member States did not have routine DOC monitoring in place to perform bioavailability-based compliance assessments with EQS. However, many have since included DOC as a regular monitoring parameter. DOC is needed for all the metals included in the user-friendly tools, including Pb. It is possible through screening-based feasibility assessments to prioritise those sites for which DOC measures are particularly important (this could be due to site sensitivity, potential metal exposures or highly variable hydrological regimes).

Some compromises from the ideal “matched” data situation may be possible. If data are available for chemical parameters from a waterbody but have not been taken at the same time as other parameters or perhaps not from exactly the same sample location, these data may still be used if the spatial and temporal variability of the water chemistry of that waterbody is relatively low.

5.4.1 Approaches for estimating required input data

The possibility of using approaches to estimate the required input data are discussed. For example, relationships based on EU-wide water quality parameters have been derived to calculate physico-chemical inputs (e.g. Peters et *al*., 2011a).

As a guiding principle, it should be emphasised that the use of estimates instead of measured (matched) data is acceptable for initial risk analysis, but for status assessment/water body classification the quality and relevance of the proxies should be clearly assessed and documented.

If calcium concentrations have not been measured, they can nonetheless be estimated. Measures of water hardness in regulatory datasets may often be in a variety of units, including: French, English, US and German degrees, dissolved concentrations of Ca and Mg, concentrations recorded as mg $CaCO_3$ L^{-1}, mmol L^{-1} and meq L^{-1}. Importantly, it is possible to convert between these units and measures based on relationships developed for European freshwaters. A simplified hardness calculator/convertor tool is available online to perform these transformations[13].

DOC has an important influence on bioavailability, so it is important to use actual monitoring data. However, in the absence of DOC monitoring data it is possible to use precautionary default values based on read-across from data for similar catchment types or to estimate DOC values. Where such data are used, the estimation of bioavailability could be used in the first tiers of compliance schemes to highlight the most sensitive areas, and for an initial risk analysis.

Some potential approaches to estimate DOC have been proposed, including **UV absorbance** (Tipping et *al*., 2009), **dissolved iron** (Peters et *al*., 2011b), or **fluorescence measurements** (Mueller et *al*., 2012). As an example, it has been demonstrated that matched data for dissolved (0.45 µm filtered) iron and DOC concentrations (also 0.45 µm filtered) in a variety of surface waters throughout Great Britain were positively correlated ($r^2 = 0.74$, $p < 0.001$) (Peters et *al*., 2011b).

Approaches to estimate DOC should be based on locally derived empirical relationships. But these relationships require that DOC is measured at least in a certain period and in some areas.

The possibility of using TOC instead of DOC can also be considered. Establishing an empirical correlation is limited by the spatial and temporal variability in suspended solids. Sweden has traditionally measured TOC, as opposed to DOC. Recognising that DOC is required for the user-friendly bioavailability tools, a conversion factor (**DOC = 0.9*TOC**) has been used (Weyhenmeyer and Karlsson, 2009); also, for the correction of Finnish data a **DOC = 0.94*TOC** conversion has been suggested (Mattsson et *al*., 2005). During initial screening assessment and feasibility studies, in the absence of measured DOC or TOC, other measurements such as water **colour** can be used to estimate TOC (e.g., Kortelainen, 1993).

Wherever alternative approaches are considered due to missing data, especially for DOC, it is important that the implications, in terms of reduced certainty, are understood in the assessment that is being made.

5.4.2 Using historical monitoring data

Some organisations hold extensive historic datasets for metals and also for physico-chemical parameters such as DOC, pH, etc. These data can offer an opportunity to perform a screening assessment or at least a scoping exercise on the feasibility of implementing a bioavailability-based approach.

However, some key considerations need to be borne in mind when using these types of data, including:

---

[13] http://bio-met.net/

- The dataset is likely to have been collected for a different purpose than such an exercise. This needs to be explicitly understood when assessing compliance with the EQS;
- The data set should be of sufficient quality, that is to say acquired with adequate sampling protocols, processed by using analytical methods with sufficient sensitivity so to limit the number of “less than LOQ” data, and collected using clean working practices in order to avoid any contamination (Nriagu et *al*., 1993, Horowitz et *al*., 1996).
- Consideration needs to be made of changes in methodologies (e.g. shift from Atomic Absorption Spectroscopy to ICP MS, change in hardness measure, move from total to dissolved metals) that may influence the accuracy and precision of the data, especially in relation to LOQs. Equally, there needs to be an understanding of the quality of the data from the analytical perspective (e.g. reference to ISO 17025 and ISO 5667);
- Data should be spatially referenced and representative of current sampling programmes. The conclusions in relation to compliance or potential risk should not be drawn at a national scale if the historical data used in the assessment are not representative.

As an example, historic DOC data exist for many waterbodies in England and Wales. An assessment was undertaken to see how these data could be used in order to provide a precautionary, indicative compliance assessment, accounting for bioavailability (EA, 2012a). The 25th percentile of the waterbody DOC concentration was selected as the default concentration as this generally gave a conservative estimate of measured values. These findings were also noted in France (Tack, 2012).

The other water chemistry inputs, such as pH and dissolved calcium, are generally measured at most sites. Where historic data are available for a site, mean or median values are often applied (EA, 2012a). The selection of the level at which a default value may be set requires careful consideration of the influence of the water chemistry parameter upon the ecotoxicity of the metal. For example, for a precautionary assessment it may be reasonable to select a percentile that reflects relatively sensitive bioavailability conditions (in France the 75th percentile pH values and the 25th percentile dissolved Ca concentrations from the monitoring database were selected as default inputs where/when data are lacking, see Tack 2012).

Historical monitoring data can be used to inform feasibility and screening assessment. However, these data need to be assessed on a case-by-case basis and estimated defaults are used at the lowest spatial scale possible.

5.5 Sampling strategy for supporting parameters

As with any environmental sampling, spatial and temporal variability of the medium being sampled needs to be considered. However, in most Member States, frameworks are already in place under the WFD to facilitate when, where and how often water samples are to be taken.

Some general principles of the effect of temporal and spatial variability on metal toxicity to aquatic organisms should also be identified so that decisions can be taken (De Laender et *al*., 2005). A study of the spatial and temporal variability of the ecological risks of Cu, Ni, and Zn, accounting for chemical speciation has been carried out in a wide range of surface water characteristics (Verschoor et *al*., 2011). Parameters like DOC, Ca, Mg, and HCO_3^-, may vary by up to a factor 2 in one year in some catchments. It has been shown that it is possible to identify vulnerable conditions and time periods, based on changes in water chemistry.

5.5.1 Dealing with temporal variability within catchments

If a reduction in sampling frequency of supporting parameters is planned, a documented knowledge of temporal and seasonal variability is required.

Temporal variability in rivers can be related to the high variability of flow conditions and catchment run-off, which strongly influences concentrations of calcium, DOC and pH values. Concentrations are also very variable during high-flow events, because at the beginning of the event they are controlled by run-off contribution, while in the last part of the event diluting effects are prevailing.

For lakes, pH is the main fluctuating parameter, and this fluctuation is tied to photosynthesis and respiration. In general, during periods of high photosynthetic activity, the pH increases.

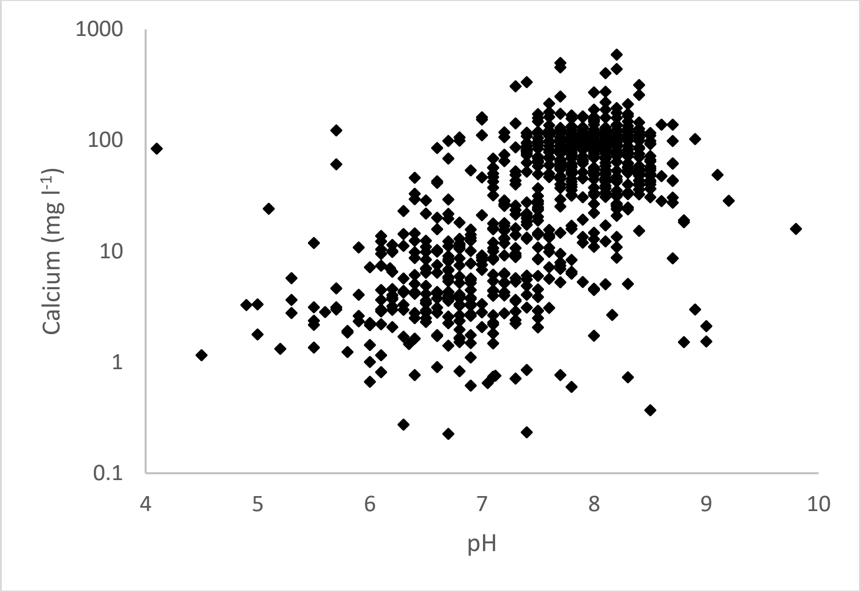
For parameters with a higher daily variability (e.g. pH) the choice of the time of the measuring should be considered to ensure that the value reflects the most sensitive condition.

The results of temporal variations have implications for a cost-effective setup of metal risk monitoring. It is a challenge to minimise the number of samples and parameters without losing information about risks.

5.5.2 Dealing with spatial variability within catchments

Variability within catchments is more common rather than an exception and it is relevant when assessing compliance for all chemicals, not just those for which bioavailability is to be accounted for.

The FOREGS dataset (Salminen, 2005) provides a basic indication of the range of physico-chemical parameters in European surface waters. These are one-off samples taken from streams and rivers across Europe. While the sample density is relatively limited, the coverage is on 28 countries. The sample sites were selected to be representative of conditions of low anthropogenic pressure. The 5th, 10th, 25th, 75th, 90th, and 95th percentiles, and medians, of key factors affecting metal bioavailability (pH, DOC, and Ca concentrations) in EU waters are listed in Table 5 (Chapter 3). Furthermore, it is important to realise that these are inter-correlated: for example, acidic waters tend to be soft and have high DOC concentrations, whereas hard waters often have lower DOC concentrations and higher pH (Figure 18).



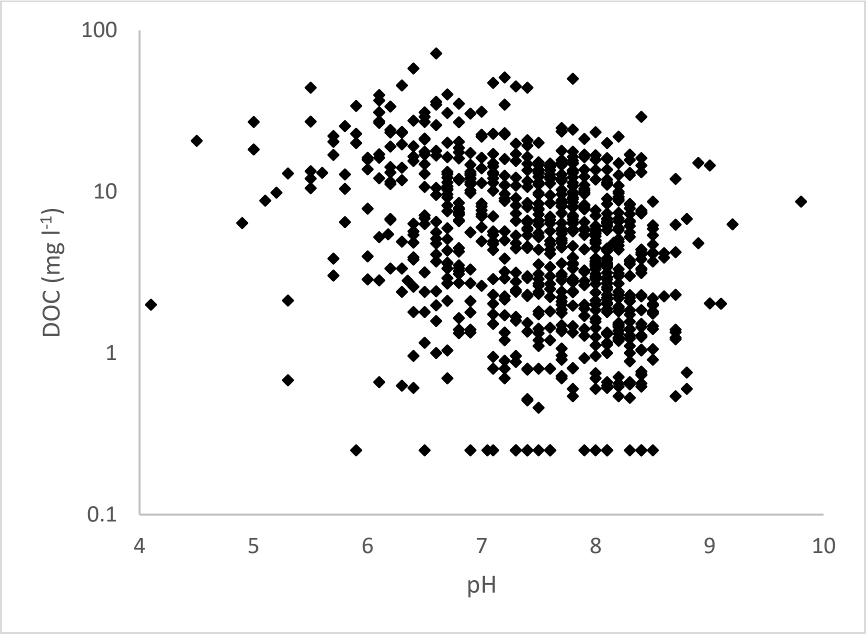


Figure 18: Spatial variation analysis of the relation between water physico-chemical characteristics

Two examples based on the FOREGS database: the Co-variation between Ca concentrations and pH values (top graph), and of the Co-variation between DOC concentrations and pH values (bottom graph).

The results of spatial variation analysis can help to select monitoring sites and monitoring parameters. The choice of monitoring sites will depend on the monitoring goal, but is usually more intense in areas where generic quality criteria are exceeded. For site selection, the origin of spatial variation in BLM parameters should also be considered.

Major BLM parameters such as pH and Ca are primarily determined by the geology of the area. DOC concentrations are likely to be controlled by land use. Trace metals like Cu, Ni, and Zn have natural origins, though their concentrations can be heavily elevated by industrial or historical point sources as well as diffuse sources related to population density and agriculture.

The magnitude of variability can be analysed from historical, geographical and/or temporal data and for some parameters is likely to be understood from previous typology and classification exercises under the WFD. The preferred situation is that dissolved metal concentrations and supporting data (such as DOC) are collected in the same sample at the same time, since these matched data enable the identification of cases where potential risks may occur.

Aquatic organisms may experience fluctuating exposures of metals due to variability in metal concentrations and/or alterations of the physico-chemical composition in the water body. This directly affects speciation and subsequent toxicity. As a consequence, ratios between lowest and highest potential risk may also vary. Similar observations were reported for pulse exposures of metals (e.g., Hoang et *al*., 2007), but attempts to couple pulse exposure models with acute BLMs generally fail due to delayed toxic effects (Meyer et *al*., 2007). To account for this matched water chemistry and dissolved metal data should be collected at the same time, in the same sample.

Under the WFD, water bodies are considered as homogeneous units of compliance. However, cases may occur where compliance at one site would result in an EQS pass, but physico-chemical changes along the catchment (e.g. downstream) may result in an EQS failure, even though metal concentrations are comparable. Cases like these may sometimes be related to permitting and discharge limits, as opposed to compliance. Zwolsman and De Schamphelaere (2007) discussed the changes in metal bioavailability on transition downstream through a catchment and how the measured data may be interpreted. This is obviously of great relevance to the longer rivers of Europe, where the water physico-chemical characteristics may be expected to be modified locally.

If the physico-chemical parameters affecting the metal bioavailability change rapidly within a waterbody, it is probably a practical step (i.e. a screening step) to select the conditions giving the reasonable worst-case (maximal) metal bioavailability. This is likely to be associated with factors that may influence DOC levels or pH.

5.6 Conclusions (or Take-Home Messages)

- **Status assessment** (which triggers starting measures) requires a decision at **a high certainty level**. **Matched data** for all of the required inputs for the user-friendly tool to account for metal bioavailability is the **preferred option**.
- **Screening assessments** or at least scoping exercises on the feasibility of implementing a bioavailability-based approach **can be performed using incomplete datasets**, with a risk analysis then based on:

.1 Historical monitoring data (from sites within the same ecoregion);

.2 DOC and Ca proxies from other measured variables;

.3 Dissolved metal fraction without any bioavailability correction (i.e. conservative approach).

- **If the physico-chemical parameters affecting the metal bioavailability change rapidly within a waterbody**, a practical step (i.e. a screening step) is to select the conditions giving the **reasonable worst-case (maximal) metal bioavailability**.

6 COMPUTING AND INTERPRETING THE OUTPUTS FROM SIMPLIFIED TOOLS

6.1 Overview

In this chapter guidance is provided on the characteristics and use of simplified tools for the assessment of compliance against a bioavailability-based EQS ($\text{EQS}_{\text{bioavailable}}$). The salient points from this chapter are as follows:

- The simplified tools are used in Tier 2 of the procedure, as described in Chapter 2 and summarised in Figure 3;
- Users need to understand the water chemistry boundaries of these simplified tools;
- The vast majority of European surface waters are expected to be within these boundaries;
- It is important to understand the water chemistry conditions in the waterbodies to which the tools are to be applied, to ensure these are appropriately covered by the calibration and validation ranges of the selected simplified tool;
- The tools can only be used with EQS that are derived as bioavailability-based EQS;
- A series of scenarios are identified at the end of this chapter with suggestions on how to proceed when a water sample is outside of the boundaries of the simplified tools.

Each simplified tool has specific instructions and guidance associated with its use and that can be found online. Each is based on an Excel sheet and can be run on any PC using Microsoft Office. However, it is important to check on the appropriate tool website to ensure the version of Microsoft Office used on the PC is compatible (i.e. not too old).

There are some general instructions that can be followed to make this process more straightforward:

- Ensure the data are arranged, by samples/site, in rows and the determinants in columns, and that these are in the same order as in the tool under consideration. This is readily done in Excel;
- Data must be formatted as numbers with decimal places; and without commas or other separators, such as hyphens;
- Data must not contain any symbols, such as ‘<’ or ‘>’, these will not be processed and will result in an error message;
- Ensure the units of the monitoring data entered are those required by the tool, this is very important for calcium;
- All of these tools will run with data that are outside of the calibration ranges, but will provide ‘flags’ or indications about when and why this has occurred (options for dealing with these data are given below);
- Run times for these different tools vary depending on both the tool and the size of the dataset being processed (and thus also depending on the computer processing speed). These simplified tools can process more than 1000 rows of data at a time.

For RBSPs such as Cu and Zn, the $\text{EQS}_{\text{bioavailable}}$ used as default values in the simplified tools reflects worst-case bioavailability conditions. However, some Member States might have derived alternative EQS for these metals. Nonetheless, bioavailability correction can still be performed, provided that 1) the water chemistry conditions in the water to which the tool is to be applied are within the applicability domain of the model, 2) the bioavailability of the metal in the water can be calculated (Chapter 5) and 3) the EQS has been derived as an $EQS_{bioavailable}$. If even only one of these conditions is not met, then the tools cannot be used.

Dealing with “less than” values

Potentially with any of the input data that can be entered into the simplified tools, and most likely with the metals concentration data, there are likely to be concentrations recorded as “less than” values (values below the limit of quantification, LOQ). $EQS_{bioavailable}$ for metals are generally considerably lower than EQS based on dissolved metal concentrations. As set out in the QA/QC Directive (EC, 2009a), the limit of quantification should be equal to or below 30 % of the relevant EQS. Therefore, going forward, the challenges of dealing with datasets in which many values are recorded as below the LOQ, the so-called “censored data”, are likely to be reduced. However, current datasets used for regulatory assessments, or for testing the practicality of implementation of the bioavailability approach, can routinely contain more than 30% of the recorded data as values “< LOQ”.

Under the QA/QC Directive, values recorded as “< LOQ” are to be replaced by half the value of the LOQ and then treated as measured data (substitution approach). Generally, this will produce a strong downward bias, which leads to an underestimation of the actual concentration; therefore this approach can result in a dataset that bears little relevance to the actual metal concentrations in a waterbody, especially where there is a high proportion of censored data. It is important to be aware that different LOQs can be present in the same dataset for the same determinant. To account for the values “<LOQ” in the dataset, one must know the numerical value of each LOQ.

Figure 19 below shows a schematic of the principal steps involved in the calculation of bioavailable metal concentrations to assist in the identification of the most relevant section of the guidance for specific situations/questions.

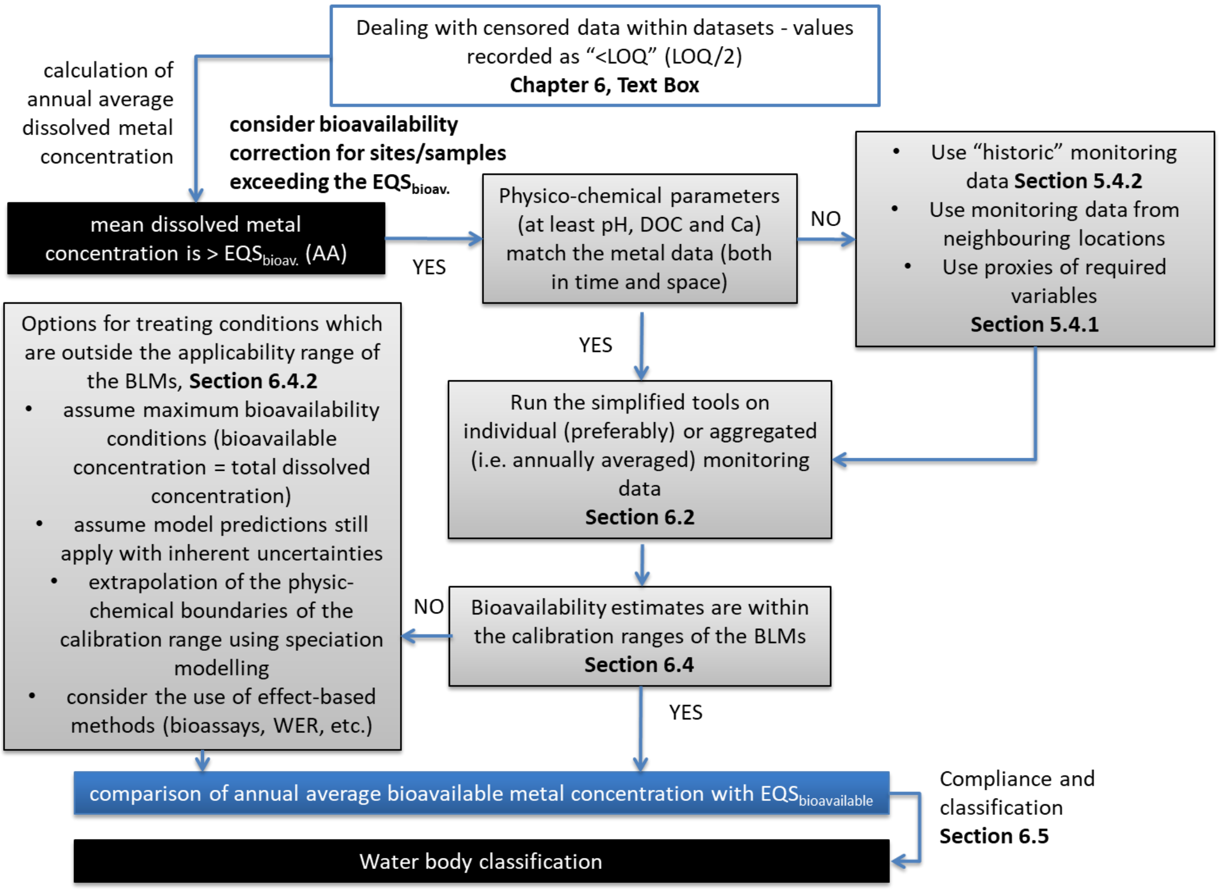


Figure 19: Flowchart describing the steps involved in the computation and interpretation of the results when processing the monitoring data with a simplified tool

6.2 Calculating annual average bioavailable concentrations

For a bioavailability-based assessment, the preference is that the required supporting physico-chemical data match with the dissolved metal data **on an individual sample basis** (i.e. dissolved metal and supporting physico-chemical determinants are quantified in the same sample). Using these data, the bioavailable metal concentration on each sampling occasion (usually over at least a 12-month period) is calculated, and the average value can be computed and compared to the $EQS_{bioavailable}$ using either a "face value" or a "confidence of failure" approach to compliance assessment. In the former, the measured average is compared directly with the $EQS_{bioavailable}$; in the latter, account is taken of the variability between samples. Compliance assessment based on sampling is subject to uncertainty with any standard. Detailed guidance on assessment of compliance with standards can be found in ISO Guidance (ISO, 2008b).

Where data for physico-chemical supporting parameters are only available as annual averages, these should only be applied to correct an annual average dissolved metal concentration. The annual average concentration of dissolved metal can be derived simply by summing the monthly sample concentrations for the year and dividing by 12. In accounting for bioavailability using the simplified tools, there is a need to provide similar summary statistics for the supporting water chemistry. For pH and hardness, these are probably best represented as averages. However, the log-normal distribution of DOC in the environment dictates that a median value is a more appropriate annual summary statistic (e.g. EA, 2012a).

It should be noted that the approach described above may not be appropriate in irregular-flow or highly seasonal catchments, as in these cases summary statistics may obscure periods of high bioavailability. Using one average dissolved metal concentration is then less preferable than correcting on a single-sample basis, as it may not be possible to assume that periods of high metal loads correspond with periods of low-bioavailability, and vice versa (Section 5.4).

Assessments of the influence of data aggregation have been evaluated to understand the influence of using individual matched data compared with averaging water quality data over a year. In the UK and France both methodologies led to very similar results, provided the datasets were of a reasonable size (100’s to 1000’s of points) and did not vary to a significant degree (e.g. Comber et *al*., 2008; Geoffroy et *al*., 2010; Ciffroy et *al*., 2013). However, this assessment needs to take place before conclusions can be drawn regarding the appropriateness of aggregating data.

6.3 Interpreting results on bioavailability

All of the simplified tools have similar outputs that include the calculation of some of the following, which will be filled in on the right-hand columns as the calculations are performed:

Local or site-specific 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 the simplified tools, the local HC5 of a metal (or the PNEC, as in the simplified tool PNEC.pro[14]) is an HC5 which reflects the bioavailability conditions at a specific site. It is based on the local water conditions at the site using a bioavailability model.

Under sensitive conditions, the local HC5 tends to approach the reference HC5, which is the lowest possible HC5 value reflecting the conditions of high bioavailability in European freshwaters[15]. For Ni, an assessment factor (AF) of 1 was used to derive the EQS so that the reference HC5 is equivalent to the $EQS_{bioavailable}$. In bio-met the bioavailability-based EQS for Ni is 4 µg L^{-1}, for Cu 1 µg L^{-1} and for Zn 10.9 µg L^{-1}. In PNEC-pro the bioavailability-based EQS for Ni is 4 µg L^{-1}.

---

[14] PNEC.pro also calculates a local HC5 but uses the term “PNEC” in the column header.

[15] The reference HC5 is derived in accordance with the procedure outlined in section 3.5.3.2, pages 64-65, of the CIS Guidance Document No. 27 (EC, 2018).

BioF (Bioavailability Factor) – is the ratio between the reference HC5 and the local HC5. This value is equal to 1 or is less. If the value is 1, the metal, under the provided specific water conditions, is 100% bioavailable and the site is targeted as having "sensitive conditions".

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

Bioavailable metal concentration [µg L^{-1}] – this is the estimated 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. If in a row the dissolved metal data are not entered, the BioF-column is then not available.

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 column may be highlighted red or flagged. If in a row the dissolved metal data are not entered, the RCR column is not available. The RCR is calculated as follows:

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

An RCR of 1 or greater indicates an exceedance of the EQS$_{bioavailable}$ and may thus prompt a progression to Tier 3 as indicated in Figure 3 (Chapter 2).

The results of any compliance assessment will obviously be influenced by the value of the EQS$_{bioavailable}$. For Ni and Pb the values are applied Europe-wide. However, for RBSPs they will be derived at a national level and could (although they should not if the Technical Guidance on Deriving EQS is followed) vary considerably between the Member States.

6.4 Dealing with bioavailability estimates that are outside of the calibration ranges of the ecotoxicity data

For EQS that are derived on the basis of the relationships between ecotoxicity data and water column physico-chemistry conditions, such as for metals, there are likely to be in European freshwaters some combination of those parameters that will be outside the ranges of the test data on which the relationships are based on; this is inevitable. These situations have been recognised when working with the BLMs (Natale et *al*., 2007; EA, 2009) and there are options for dealing with these waters and sites.

6.4.1 What are calibration ranges of the Biotic Ligand Models (and so also of the simplified tools) and what do they mean?

BLMs for predicting the chronic ecotoxicity of metals under different freshwater chemistry conditions were originally developed as part of the EU risk assessments conducted under the Existing Substances Regulation (Risk Assessments by Member State rapporteurs for, e.g. Ni and Zn, and Voluntary Risk Assessments by industry for, e.g. Cu). For that reason, they originally focused on the range of water conditions observed in Europe on a regional scale, i.e. they referred to the 10th - 90th percentile of the conditions for pH, hardness or calcium, and DOC observed in the different EU Member States. The calibration boundaries of some of the BLMs (and so also of the simplified tools that are based on those BLMs) represent the extremes of water quality conditions at which the chronic tests were undertaken and at which the relationships between physico-chemical conditions and toxicity were calibrated and validated (see Table 3). The datasets on which the simplified tools are based on are in the same situation. For example, PNEC-pro is based on 276 Dutch waters, which DOC 1st percentile is 2.38 mg L^{-1}, 5th percentile 3.27 mg L^{-1}, 10th percentile 4.85 mg L^{-1}. For bio-met, the DOC range is 0.1-30 mg L^{-1}. The 20 000 calculations in bio-met v4 (54 000 in v5) are based on waters through this range, reflecting EU-wide water chemistries, including waters with 0.1 mg L^{-1} DOC. The 1st DOC percentile is 0.108 mg L^{-1}, the 5th percentile 0.139 mg L^{-1}, the 10th percentile 0.194 mg L^{-1}.

6.4.2 What are the options for waters under investigation that fall outside the calibration conditions of the model?

This subsection provides some considerations on how to deal with sites where the water chemistry conditions are outside the calibration ranges of the BLMs, and so also their corresponding simplified tools. The options provided here are not definitive and have been summarised from several studies (EA, 2009).

An important factor in this issue is whether elevated exposures occur or not at the sites, because accounting for bioavailability will only be required where dissolved metal concentrations are at or above the $EQS_{bioavailable}$. Only in these cases is the question of the applicability of the simplified tools likely to be important (i.e. when progressing from Tier 1 to Tier 2, Figure 3).

Boundaries are set for DOC concentrations, although this only affects the exposure of the organisms to available metals. Boundaries are also set for both pH and calcium, which both affect uptake and binding at the biotic ligand and are physiologically important for aquatic organisms. The boundaries that commonly cause surface waters to be outside the applicability range of the BLMs (and so to the simplified tools) are very hard waters (with high calcium concentrations) in which the calcium concentration may exceed the calibration range, and very soft waters in which the calcium concentration may be below the applicability range. Low pH may also cause waters to be outside the applicable range of the BLMs, and in some instances both low pH and low calcium concentration may be encountered in the same waters.

Hard waters, where the calcium concentration exceeds the BLM calibration range, **for the Ni and Zn BLM**, can be computed relatively easily. The upper limit to the applicable range of calcium concentrations exists because there is a limit to the protective effect from calcium as a competitor for binding sites at the “Biotic-Ligand” level; and increases in calcium concentration do not result in further reduction of the metal bioavailability (e.g. Heijerick et *al*., 2002; Deleebeeck et *al*., 2007b). This situation is automatically handled in the simplified tools by limiting the input data to the maximum allowable calcium concentration (i.e. the input cannot go higher than the maxima of the BLM calibration range), and calculations performed by doing so continue to be reliable (at least for Ni and Zn for which a protective effect of calcium has been observed).

In contrast, the boundaries established for soft acid waters occur because the majority of test organisms are unable to survive and reproduce adequately under such extreme conditions. These water quality conditions may support different species than those found in harder waters and higher pH environments. Protons (H^+) and calcium (Ca^{2+}) ions may both compete with metals for binding sites at the biotic ligand level. As the pH decreases the competition from protons will increase resulting in a lower metal bioavailability; whereas as hardness decreases the competition from Ca will also decrease resulting in a higher metal bioavailability. The relative importance of competition coming from proton and calcium ions and directed towards each individual metal may affect organisms' responses under these conditions. Soft acid waters are also characterised by high DOC concentrations (> 20 mg/L), and the complexing capacity of these high DOC concentrations may compensate for processes like proton competition that may act to increase net bioavailability. A reduction in pH may also result in a decrease in metal binding to DOC, due to the increased competition from protons, resulting in increased metal bioavailability. Changes in pH can also result in changes to the inorganic speciation of a metal, and the fraction which exists as bioavailable species (e.g. Cu^{2+}), although significant changes in speciation around the lower pH limit for the BLMs are unlikely for Cu, Ni, and Zn, due to the dominance of the free ionic form under acidic conditions.

Options for treating conditions that are outside the applicability range of the BLMs and of the simplified tools include the following actions:

1. Applying the $EQS_{bioavailable}$ and assuming that there is no mitigating influence of water chemistry factors (all of the dissolved metal is bioavailable). This is generally likely to be precautionary and may therefore be the preferred option. This option applies the $EQS_{bioavailable}$ outside the applicable conditions, and it can effectively result in a step change to the standard where the calibration conditions are within the applicability domain of the BLM.

2. Assuming model calculations still apply outside calibration conditions with the inherent uncertainties. This means that the BLM is applied and that no calibration conditions are considered, and that the model can be extrapolated beyond its calibration range. Bio-met includes a default assumption in line with this option. It estimates the bioavailable metal concentration, but to do so, it uses a water chemistry that is just within the calibrated boundaries. A flag is included to caution the user that the water chemistry is outside the calibrated boundaries of the BLM, and that the result should be interpreted with care.

3. Extrapolating the physico-chemical boundaries of the calibration range by using speciation modelling to indicate likely influence upon exposures, but by also acknowledging the associated uncertainty (EA, 2012e). At low pH values and low Ca concentrations there are likely to be physiological implications regarding the interactions at the biotic-ligand level that should be understood when using this approach.

4. Considering using effect-based monitoring tools (including ecotoxicity tests, bioassays, certain biomarkers, Water Effect Ratios, and ecological community monitoring) to derive ecological assemblage specific EQS or site-specific PNECs from field data. The observations of organisms or communities in the field may provide a means of validating any predictions or assumptions made about the protection of aquatic ecosystems in relatively extreme environments; however, it is likely that a combined weight-of-evidence

approach including several complementary techniques would be needed. In water-effect ratio (WER) testing procedure, the toxicity of a metal is evaluated side-by-side in the site water and in laboratory reconstituted water, using a series of dilutions. Based on these tests, a site-specific PNEC can be determined by multiplying the $EQS_{bioavailable}$ by the value of the WER. Such tests would need to use test species, which are appropriate to the water chemistry conditions of the local surface waters; this may be challenging as, e.g. many soft waters may be unsuitable for the culture of many crustaceans. The choice of test species should take account of the water conditions to be tested, the bioavailability and the sensitivity of the species, and be fully justified. The choice of conditions for the laboratory (reference) water should also be carefully considered and justified. However, ecological monitoring that is performed under the WFD may provide a means to ensure that any deterioration in ecological quality can be identified, notably where there is uncertainty in the adequacy of an $EQS_{bioavailable}$ for relatively extreme environmental conditions. Some examples of the application of these types of assessments include Crane et *al*. 2007, Peters et *al*. 2011b, and Peters et *al*. 2014a and 2014b. The use of additional biological monitoring can be valuable in supporting the application of the standards under potentially sensitive conditions.

The Cu BLM validation notes an upper limit for iron (307 mg L^{-1}) and for aluminium (332 mg L^{-1}), although it is extremely unlikely that such conditions would be experienced without another of the calibration conditions having also been breached. Extremely high iron and aluminium levels are most likely to occur in association with reduced pH, due to the tendency for both metals to form insoluble precipitates under circumneutral pH conditions (e.g. pH 6.5-7.5). Iron and aluminium can affect the binding of other metals to DOC due to their very high affinity for complexation by DOC, which reduces the availability of binding sites for the less strongly bound metals, and so increases their bioavailability. At lower concentrations of iron and aluminium the competition for Cu binding is lower and there is then a higher proportion of DOC complexed Cu, which means that the Cu is less bioavailable.

The speciation methodology within the Ni BLMs (Schlekat et *al*., 2010, Nys et *al*., 2016a) includes consideration of competition for DOC binding from both iron and aluminium by assuming that their activity in solution is controlled by the precipitation of a solid phase (i.e. amorphous iron(oxy)hydroxides and amorphous aluminium(oxy)hydroxides) within the chemical speciation component of the model.

The importance of competition for DOC binding from other ions depends on the relative affinities of both metals for DOC. Consequently, metals that bind strongly to DOC, such as Cu and Pb, will be less affected by this issue than metals that bind more weakly such as Ni and Zn.

In Annex 4, some written advice, produced by the UK for users of their simplified tool M-BAT, is given, notably about what to do when cases are outside the calibration ranges and what the implications might be regarding making an assessment.

6.5 Compliance and classification

The key use of EQS under the WFD is to serve as benchmarks for assessing the status of surface waters. Member States assess compliance of the monitoring data from surface waters with the EQS for Priority Substances (in the assessment of chemical status) and RBSPs (in the assessment of ecological status).

6.5.1 Exceedance and failure: what is a failure?

[The following text applies to all EQS, not just the $EQS_{bioavailable}$]. Some Member States apply International Standard Organization guidance (ISO, 2008b) to take into consideration the uncertainties associated with assessing compliance based on a comparison of monitoring data and EQS values. This is done by calculating the "confidence of failure"[16]. For the decision makers who use the outcomes of a compliance assessment, a range of conclusions may be drawn from reported failure of an EQS. Member States may need to undertake costly programmes of measures to rectify the situation and to avoid penalties. The consequences of making the wrong decision are clearly variable. It is obvious that the more important the decision is, the less the decision maker will want uncertainty and errors in sampling and measurement to lead to a wrong decision, i.e. they will want a higher degree of confidence in the results on which the decision is based, and a higher degree of confidence in the correctness of the decision itself.

Most of the assessments that have been made on the implementation of the $EQS_{bioavailable}$ have not considered this confidence of failure (e.g. EA, 2014), and so may be termed "face value" assessments. Where confidence of failure is considered when using an $EQS_{bioavailable}$ it can provide considerable assistance in interpreting data and prioritising actions and potential programmes of measures, especially when dealing with impacted sites, such as those impacted by metalliferous mining (EA, 2012b).

For some trace elements, there may be a need, if the waterbody under consideration is used as a source of drinking water, to ensure that despite normally low bioavailability conditions the drinking water standards in Directive 98/83/EC (EU, 1998)[17] are not breached at the point of abstraction or at risk of being breached at the point of supply (the tap).

6.5.2 What comes next if the simplified tools have identified a failure?

If, through the use of the simplified tools an exceedance has been identified for a site (RCR = or > 1), the obvious question is then: what comes next? As presented in Figure 3, Tier 3 is the next logical step that corresponds to a "Local Refinement". The conduction of this step is dependent upon the type of assessment being undertaken, the type and quality of the data initially used, and the local regulatory context/policy in which the assessment is being made. Nevertheless, there are some options that may be considered at Tier 3 which could be viewed as a confirmatory step of the exceedance and provide an evidence base and a degree of certainty for assigning a failure to the site. These might include:

---

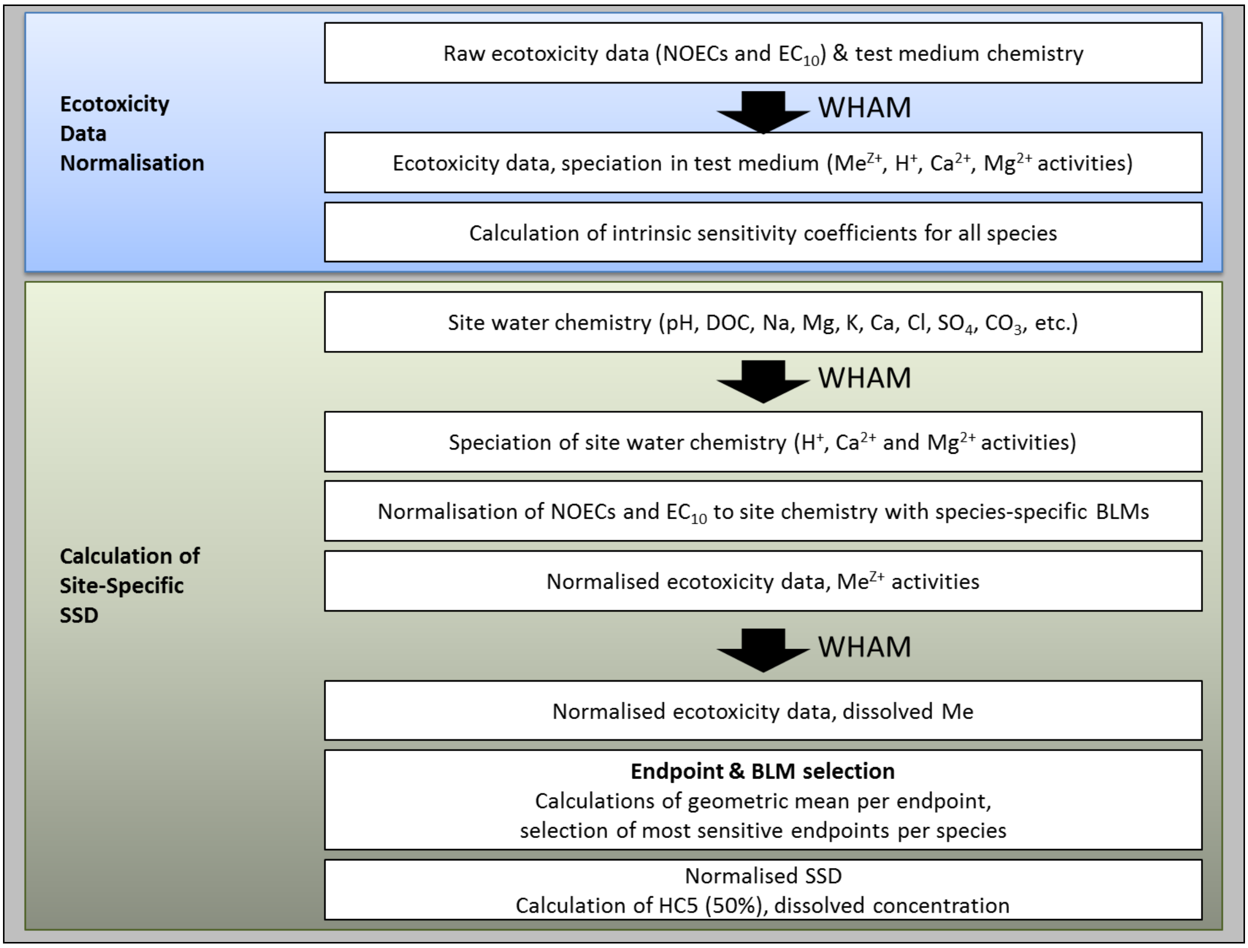
[16] The confidence of failure is a single statistic that replaces the need to compute different confidence limits for each type of decision. It varies on a scale from 0 % to 100 % (see ISO, 2008b).

[17] The revised Drinking Water Directive (Directive (EU) 2020/2184) enters into force on 12 January 2021, and Member States have two years to transpose it into national legislation.

- If incomplete monitoring data have been entered into the simplified tool, then the correct data should be collected. The data may have been incomplete because of the use of:
    - The total concentrations rather than the dissolved metals data;
    - The DOC defaults calculated from historical measurements (Chapter 5);
    - Spatially or temporally mismatched measurements (i.e. data not taken from the same sites or at the same times as the metal measurements);
    - Incomplete annual datasets that may represent only a limited subset of the year or of the range of exposure.
- Use of the full BLMs is likely to provide an assessment of greater accuracy. The collection and processing of additional input data to run the models require considerable technical skill, as does the interpretation of the outputs (see the flowchart in the textbox below). The costs of undertaking this exercise, would mean that this approach would really only be relevant for a relatively limited number of sites, but it would likely be considerably less than the costs associated with any programme of measures.
- Consideration of local NBCs (Figure 3 and 4). For example, a generic background value may have been used at Tier 1, but at Tier 3 locally derived NBCs may be considered.

Effectively these options represent the iteration in the risk assessment process. However, evidence suggests that these options would be carried out on relatively few sites/samples, when compared to the total number entered initially at Tier 1.

Bioavailability normalisation processes executed by the full BLM to calculate the local 5% hazard concentration (HC5). WHAM = Windermere Humic Aqueous Model; SSD = Species Sensitivity Distribution.


Ecotoxicity Data Normalisation
Raw ecotoxicity data (NOECs and EC_{10}) & test medium chemistry
WHAM
Ecotoxicity data, speciation in test medium (Me^{Z+}, H^+, Ca^{2+}, Mg^{2+} activities)
Calculation of intrinsic sensitivity coefficients for all species
Calculation of Site-Specific SSD
Site water chemistry (pH, DOC, Na, Mg, K, Ca, Cl, SO_4, CO_3, etc.)
WHAM
Speciation of site water chemistry (H^+, Ca^{2+} and Mg^{2+} activities)
Normalisation of NOECs and EC_{10} to site chemistry with species-specific BLMs
Normalised ecotoxicity data, Me^{Z+} activities
WHAM
Normalised ecotoxicity data, dissolved Me
Endpoint & BLM selection
Calculations of geometric mean per endpoint,
selection of most sensitive endpoints per species
Normalised SSD
Calculation of HC5 (50%), dissolved concentration

7. REFERENCES

Bio-met (2017). Bio-met bioavailability tool user guide (version 4.0). Guidance document on the use of the bio-met bioavailability tool. Available on www.bio-met.net.

Brix KV, DeForest DK, Tear L, Grosell M and Adams WJ (2017). Use of Multiple Linear Regression Models for Setting Water Quality Criteria for Copper: A Complementary Approach to the Biotic Ligand Model. Environ. Sci. Technol. 2;51: 5182-5192.

Campbell PGC (1995). Interactions between trace metals and aquatic organisms: a critique of the free-ion activity model. In: Tessier A, Turner DR. Editors. Metals speciation and bioavailability in aquatic systems. Chichester, UK. John Wiley and Sons, pp 45-102. 279.

Cassard D, Bertrand G, Billa M, Serrano JJ, Tourlière B, Angel JM and Gaál G (2015). ProMine Mineral Databases: New Tools to Assess Primary and Secondary Mineral Resources in Europe. Springer International Publishing Switzerland 2015. P. Weihed (ed.), 3D, 4D and Predictive Modelling of Major Mineral Belts in Europe, Mineral Resource Reviews, 51p.

Casiot C, Egal M, Elbaz-Poulichet F, Bruneel O, Bancon-Montigny C, Cordier MA, Gomez E and Aliaume C (2009). Hydrological and geochemical control of metals and arsenic in a Mediterranean river contaminated by acid mine drainage (the Amous River, France); preliminary assessment of impacts on fish (*Leuciscus cephalus*): Applied Geochem. 24: 787-799.

Chandesris A, Canal J, Bougon N and Coquery M (2013). Détermination du fond géochimique pour les métaux dissous dans les eaux continentales. Rapport final. Irstea. 65 p + Annexes (231 p.).

Ciffroy P, Charlatchka R, Ferreira D and Marang L (2013). Probabilistic approaches to accounting for data variability in the practical application of bioavailability in predicting aquatic risks from metals. Integr. Environ. Assess. Manag. 9: 508-516.

Comber SDW, Merrington G, Sturdy L, Delbeke K and van Assche F (2008). Copper and zinc water quality standards under the EU Water Framework Directive: The use of a tiered approach to estimate the levels of failure. Sci. Total Environ. 403: 12-22.

Cousins AP, Jönsson A and Iverfeldt A (2009). Testing the Biotic Ligand Model for Swedish surface water conditions – a pilot study to investigate the applicability of BLM in Sweden. IVL, B1858, Sweden. pp 61.

Crane M, Kwok K, Wells C, Whitehouse P and Lui GSC (2007). Use of field data to support European Water Framework Directive quality standards for dissolved metals. Environ. Sci. Technol. 41: 5014-5021.

De Laender F, De Schamphelaere KAC, Verdonck FAM, Heijerick DG, Van Sprang PA, Vanrolleghem, PA and Janssen CR (2005). Simulation of spatial and temporal variability of chronic copper toxicity to Daphnia magna and *Pseudokirchneriella subcapitata* in Swedish and British surface waters. J. Hum. Ecol. Risk Assess. 11: 1177-1191.

De Schamphelaere KAC and Janssen CR (2002). A biotic ligand model predicting acute copper toxicity for *Daphnia magna*: the effects of calcium, magnesium, sodium, potassium, and pH. Environ. Sci. Technol. 36(1): 48-54.

De Schamphelaere KAC, Heijerick DG and Janssen CR (2003a). Development and Validation of Biotic Ligand Models for Predicting Chronic Zinc Toxicity to Fish, Daphnids and Algae (Final report of ILZRO project ZEH-WA-1). Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University, Belgium. (Sponsor: International Lead and Zinc Research Organization, ILZRO, United States).

De Schamphelaere KAC., Vasconcelos FM, Heijerick DG, Tack FMG, Delbeke K, Allen HE and Janssen CR (2003b). Development and field validation of a predictive copper toxicity model for the green alga *Pseudokirchneriella subcapitata*. Environ. Toxicol. Chem. 22: 2454–2465.

De Schamphelaere KAC and Janssen CR (2004a). Effects of dissolved organic carbon concentration and source, pH, and water hardness on chronic toxicity of copper to *Daphnia magna*. Environ. Toxicol. Chem. 23: 1115–1122.

De Schamphelaere KAC and Janssen CR (2004b). Bioavailability and chronic toxicity of zinc to juvenile rainbow trout (*Oncorhynchus mykiss*): Comparison with other fish species and development of a biotic ligand model. Environ. Sci. Technol. 38: 6201–6209.

De Schamphelaere KAC and Janssen CR (2004c). Modelling copper bioavailability and toxicity in freshwater: uncertainty reduction for risk assessment (Chronic fish-BLM). Report to the European Copper Institute, included in the Copper Voluntary Risk Assessment as Appendix U.

De Schamphelaere KAC, Lofts S and Janssen CR (2005). Bioavailability models for predicting acute and chronic toxicity of zinc to algae, daphnids and fish in natural surface waters. Environmental Toxicol. Chem. 24(5): 1190-1197.

De Schamphelaere KAC, Van Laer L, Deleebeeck N, Muyssen B, Degryse F, Smolders E and Janssen CR (2006). Nickel Speciation and Ecotoxicity in European Natural Surface Waters: Development, Refinement and Validation of Bioavailability Models. Final Report. Ghent University (Laboratory of Environmental Toxicology and Chemistry) & Katholieke Universiteit Leuven (Division of Soil and Water Management), Belgium.[18]

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

DeForest DK and Van Genderen EJ (2012). Application of U.S. EPA guidelines in a bioavailability-based assessment of ambient water quality criteria for zinc in freshwater. Environ. Toxicol. Chem. 31(6): 1264-1272.

DeForest DK, Brix KV, Tear LM and Adams WJ (2018). Multiple linear regression models for predicting chronic aluminum toxicity to freshwater aquatic organisms and developing water quality guidelines. Environ. Toxicol. Chem. 37(1): 80-90.

---

[18] https://biblio.ugent.be/publication/5785290/file/5785373.pdf

Deleebeeck NME, De Schamphelaere KAC and 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. Ecotoxicol. Environ. Saf. 67(1): 1-13.

Deleebeeck NM, Muyssen BT, De Laender F, Janssen CR and De Schamphelaere KAC (2007b). Comparison of nickel toxicity to cladocerans in soft versus hard surface waters. Aquat. Toxicol. 84(2): 223-235.

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

Deleebeeck NME, De Laender F, Chepurnov V, Vyverman W, Janssen CR and De Schamphelaere KAC (2009a). A single bioavailability model can accurately predict Ni toxicity to green microalgae in soft and hard surface waters. Water Res. 43(7): 1935-1947.

Deleebeeck NME, De Schamphelaere KAC and Janssen CR (2009b). Effects of Mg2+ and H+ on the toxicity of Ni2+ to unicellular green alga *Pseudokirchneriella subcapitata*: Model development and validation with surface waters. Sci. Total Environ. 407(6): 1901-1914.

Di Toro DM, Allen HE, Bergman HL, Meyer JS, Paquin PR and Santore RC (2001). A Biotic Ligand Model of the Acute Toxicity of Metals. I. Technical Basis, Environ. Toxicol. Chem. 20(10): 2383-2396.

EA (Environment Agency) (2008a). Road Testing of „Trigger Values" for assessing Site Specific 286 Soil Quality. Phase 1- Metals, Science Report SC050054 SR1. Bristol, UK.

EA (Environment Agency) (2008b). Tiered approach to the assessment of metal compliance in surface waters. Science Report SC050054/SR1a, Bristol, UK.

EA (Environment Agency) (2009). Using biotic ligand models to help implement environmental quality standards for metals under the Water Framework Directive. Science Report SC080021/SR7b, Bristol, UK.

EA (Environment Agency) (2012a). The importance of dissolved organic carbon in the assessment of environmental quality standard compliance for copper and zinc. Released by the United Kingdom Technical Advisory Group (WFD-UKTAG) 2012. In collaboration with the European Copper Institute, the International Zinc Association, and the Nickel Producers Environmental Research Association. Bristol, UK.

EA (Environment Agency) (2012b). Ecological indicators for abandoned mines. SC090024/2/R. Bristol, UK.

EA (Environment Agency) (2012c). Proposed EQS for Water Framework Directive Annex VIII substances: copper (saltwater) (For consultation). Released by the United Kingdom Technical Advisory Group (WFD-UKTAG) Bristol, UK.

EA (Environment Agency) (2012d). Development and use of the manganese bioavailability assessment tool (Draft). Released by the United Kingdom Technical Advisory Group (WFD-UKTAG) 2012. Bristol, UK.

EA (Environment Agency) (2012e). Desk top study to evaluate options outside BLM thresholds (low pH and low calcium issues). Released by the United Kingdom Technical Advisory Group (WFD-UKTAG) 2012. Bristol, UK.

EA (Environment Agency) (2014). The development and use of the nickel bioavailability tool. Bristol, UK. Released by the United Kingdom Technical Advisory Group (WFD-UKTAG) 2014. Bristol, UK.

EC (European Commission) (2009a). Directive 2009/90/EC of 31 July 2009 laying down, pursuant to Directive 2000/60/EC of the European Parliament and of the Council, technical specifications for chemical analysis and monitoring of water status. Official Journal of the European Union, L 201/36-38, 1.8.2009.

EC (European Commission) (2009b). Common Implementation Strategy for the Water Framework Directive (2000/60/EC). Guidance Document No. 19 Guidance on surface water chemical monitoring under the WFD. Technical Report – 2009 – 025.

EC (European Commission) (2010). Nickel and its Compounds (final revision Oct 12, 2010) EQS sheet. Prepared by Denmark, Danish Environmental Protection Agency on behalf of the European Union.

EC (European Commission) (2011a). Common Implementation Strategy for the Water Framework Directive (2000/60/EC) Guidance Document No. 27 Technical Guidance for Deriving Environmental Quality Standards. Technical Report-2011-055. Office for Official Publications in the European Communities, Luxembourg.

EC (European Commission) (2011b). Lead and its Compounds EQS sheet. Prepared by UK, Environment Agency on behalf of the European Union.

EC (European Commission) (2017). Common Implementation Strategy for the Water Framework Directive (2000/60/EC). Document WD2017-2-2 Natural Conditions in relation to WFD Exemptions. Document endorsed by EU Water Directors at their meeting in Tallinn on 4-5 December 2017.

EC (European Commission) (2018). Common Implementation Strategy for the Water Framework Directive (2000/60/EC) revised Guidance Document No. 27 – Technical Guidance for Deriving Environmental Quality Standards. European Communities.

ECHA (European Chemicals Agency) (2008). Guidance on information requirements and chemical safety assessment. Appendix R.7.13-2: Environmental risk assessment for metals and metal compounds. Helsinki, Finland.

ECI (European Copper Institute) (2008). European Union Voluntary Risk Assessment Report on copper, copper(II) sulphate pentahydrate, copper(I) oxide, copper(II) oxide, dicopper chloride trihydroxide. European Copper Institute, Brussels, Belgium.[19]

Erba S, Furse MT, Balestrini R, Christodoulides A, Ofenböck T, Van De Bund W, Wasson JG and Buffagni A (2009). The validation of common European class boundaries for river benthic macroinvertebrates to facilitate the intercalibration process of the Water Framework Directive. Hydrobiologia 633(1): 17-31.

---

[19] https://echa.europa.eu/fr/copper-voluntary-risk-assessment-reports

Erickson RJ, Benoit DA and Mattson VR (1987). A prototype toxicity factors model for site-specific copper water quality criteria. Revised September 5, 1996. EPA/600/X-87/290. U.S. Environmental Protection Agency, Environmental Research Laboratory–Duluth, Duluth, Minnesota, USA.

Erickson RJ, Benoit DA, Mattson VR, Leonard EN and Nelson HP (1996). The effects of water chemistry on the toxicity of copper to fathead minnows. Environ. Toxicol. Chem. 15(2): 181-193.

EU (European Union) (1998). Council Directive 98/83/EC on the quality of water intended for human consumption. Official Journal of the European Union, L 330/32-54, 5.12.2019.

EU (European Union) (2007) European Union Risk Assessment Report (RAR) on cadmium metal and cadmium oxide (EUR 22919 ENV (Part I: Environment) and EUR 22767 EN (Part II: Human health))[20], and 2008 Addenda to Part I[21].

EU (European Union) (2008a). European Union Risk Assessment Report (RAR) on nickel, nickel sulphate, nickel carbonate, nickel chloride, nickel dinitrate.

EU (European Union) (2008b). Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council. Official Journal of the European Union, L 348/84-96.

EU (European Union) (2010). European Union Risk Assessment Report on zinc metal[22]. Prepared by The Netherlands, RIVM, on behalf of the European Union. EUR 24587 EN.[23]

EU (European Union) (2013). Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. Official Journal of the European Union L 226/ 1-17, 24.8.2013.

EU Water Directors (2017). Natural Conditions in relation to WFD Exemptions. Water Directors Meeting, 4-5 December 2017, Tallinn.

Ferrand E, Eyrolle F, Radakovitch O, Provansal M, Dufour S, Vella C, Raccasi G and Gurriaran R (2012). Historical levels of heavy metals and artificial radionuclides reconstructed from overbank sediment records in lower Rhône River (South-East France). Geochimica et Cosmochimica Acta 82: 163-182.

---

[20] https://echa.europa.eu/documents/10162/4ea8883d-bd43-45fb-86a3-14fa6fa9e6f3

[21] https://echa.europa.eu/documents/10162/a3fdf036-c73f-47f0-a39f-bd5537e8ab23

[22] For zinc metal (CAS No. 7440-66-6), zinc distearate (CAS No. 557-05-1 / 91051-01-3), zinc oxide (CAS No.1314-13-2), zinc chloride (CAS No.7646-85-7), zinc sulphate (CAS No.7733-02-0) and trizinc bis(orthophosphate) (CAS No.7779-90-0) risk assessments were carried out within the framework of the EU Existing Substances Regulation (EEC) No. 793/93. For each compound a separate report was prepared. It should be noted, however, that this RAR on zinc metal contains specific sections (in the exposure and effect parts) that are also relevant to zinc compounds.

[23] https://publications.jrc.ec.europa.eu/repository/bitstream/111111111/15064/1/lbna24587enn.pdf

Garman ER, Meyer JS, Bergeron CM, Blewett TE, Clements WH, Elias MC, Farley KJ, Gissi F and Ryan AC (2018). Validation of bioavailability-based toxicity models for metals. In: Society of Environmental Toxicology and Chemistry, 4-8th November 2018, Sacramento, California, USA.

GEMAS (Groupement d'Études Méthodologiques pour l'Analyse des Sols) (2014a). Reimann C, Birke M, Demetriades A, Filzmoser P & O'Connor P (Editors). Chemistry of Europe's agricultural soils - Part A: Methodology and interpretation of the GEMAS data set. Geologisches Jahrbuch Reihe: Book B Band B 102, Schweizerbarth, Hannover, 523 pp. + DVD.

GEMAS (Groupement d'Études Méthodologiques pour l'Analyse des Sols) (2014b). Reimann C, Birke M, Demetriades A, Filzmoser P & O'Connor P (Editors). Chemistry of Europe's agricultural soils - Part B: General background information and further analysis of the GEMAS data set. Geologisches Jahrbuch: Book B Band B 103.

Geoffroy L, Tack K, and Andres S (2010). EQS for a metal: Is it possible to determine a lonely legal value? Interest of the Biotic Ligand Model (BLM)? Communication in the SETAC annual meeting, 23-27 May 2010, Seville, Spain.

Heijerick DG, DeSchamphelaere KAC and Janssen CR, 2002. Biotic ligand model development predicting Zn toxicity to the alga *Pseudokirchneriella subcapitata*: possibilities and limitations. Comp. Biochem. Physiol. C133(1-2): 207-218.

Heijerick DG, DeSchamphelaere KAC, Van Sprang P and Janssen C (2005). Development of a chronic zinc biotic ligand model for *Daphnia magna*. Ecotoxicol. Environ. Safety 62(1): 1-10.

Hoang TC, Gallagher JS, Tomasso JR and Klaine SJ (2007). Toxicity of two pulsed metal exposure to *Daphnia magna*: relative effects of pulsed duration-concentration and influence of interpulse period. Arch. Environ. Contam. Toxicol. 53(4): 579-589.

Hommen U and Rüdel H (2012). Sensitivity analysis of existing concepts for application of biotic ligand models (BLM) for the derivation and application of environmental quality standards for metals and evaluation of the approaches with appropriate monitoring data sets from German waters. Fraunhofer Institute for Molecular Biology and Applied Ecology, Schmallenberg. Report produced on behalf of the Umweltbundesamt (FKZ 363 01 352).

Hoppe S, Lithner G and Borg H (2009). Utvärdering av användbarheten av BLM i Svenska vatten. Report, ITM, SU. 186.

Horowitz AJ, Lum KR, Garbarino JR, Hall GEM, Lemieux C and Demas CR (1996). Problems Associated with Using Filtration To Define Dissolved Trace Element Concentrations in Natural Water Samples. Environ. Sci. Technol. 30(3): 954-963.

ISO (1984). Water quality - Determination of calcium content - EDTA titrimetric method. International Organisation for Standardization. ISO 6058:1984. Switzerland.

ISO (1999). Water quality - Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC). International Organization for Standardization. ISO 8245:1999(E). Switzerland.

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

ISO (2008a). Water quality – determination of pH. International Organization for Standardization. ISO 10523:2008(E). Switzerland.

ISO (2008b). Water quality - Sampling: Part 20: Guidance on the use of sampling data for decision making - Compliance with thresholds and classification. International Organization for Standardization. ISO 5667-20:2008(E). Switzerland.

ISPRA (Istituto Superiore per la Protezione e la Ricerca Ambientale) (2016). Linea guida per il monitoraggio delle sostanze prioritarie (secondo D.Lgs. 172/2015). Rome, Italy. Pp39.
Mance G, Brown VM, Yates J. 1984. Proposed environmental quality standards for List II substances in water – copper. Technical report TR210. Medmenham, UK: WRc. 11.

Karanfil T, Erdogan I and Schlautman RA (2003). Selecting filter membranes for measuring DOC and UV254. J. American Water Works Asso. 95(3): 86-100.

Kortelainen P (1993). Content of Total Organic Carbon in Finnish Lakes and Its Relationship to Catchment Characteristics. Can. J. Fisheries Aqua. Sci 50(7): 1477-1483.

Kramer KJM, Misdorp R, Berger G and Duijts R (1991). Maximum pollutant concentrations at the wrong depth: a misleading pollution history in a sediment core. Mar. Chem. 36(1–4): 183-198.

LAWA-AO (ED.) (2015). Berücksichtigung von natürlichen Hintergrundkonzentrationen bei der Beurteilung von Überschreitungen der Umweltqualitätsnormen nichtsynthetischer Schadstoffe. - Technische Anleitung zur OGewV, 1: 41 p.

Mattsson T, Kortelainen P and Räike A (2005). Export of DOM from Boreal Catchments: Impacts of Land Use Cover and Climate. Biogeochem. 76(2): 373-394.

M-BAT (2014). UKTAG River & Lake Assessment Method Specific Pollutants (Metals) - Metal Bioavailability Assessment Tool (M-BAT). Water Framework Directive – United Kingdom Advisory Group (WFD-UKTAG), July 2014. https://www.wfduk.org/sites/default/files/Media/Environmental%20standards/MBAT%20UKTAG%20Method%20Statement.pdf

Mebane CA (2010). Cadmium Risks to Freshwater Life: Derivation and Validation of Low-Effect Criteria Values using Laboratory and Field Studies. Scientific Investigations Report 2006–5245. Version 1.2, September 2010. U.S. Geological Survey, Virginia, U.S.A. pp 142.

Merrington G, Peters A and Schlekat CE (2016). Accounting for metal bioavailability in assessing water quality: A step change? Environ. Toxicol. Chem. 35(2): 257–265.

Meyer JS, Santore RC, Bobbitt JP, DeBrey LD, Boese CJ, Paquin PR, Allen HE, Bergman HL and Di Toro DM (1999). Binding of nickel and copper to fish gills predicts toxicity when water hardness varies, but free-ion activity does not. Environ. Sci. Technol. 33(6): 913-916.

Meyer JS, Boese CJ and Morris JM (2007). Use of the biotic ligand model to predict pulse-exposure toxicity of copper to fathead minnows (*Pimephales promelas*). Aquatic Toxicol. 84(2): 268-278.

Meyer JS, Traudt EM and Ranville JF (2018). Is the factor-of-2 rule broadly applicable for evaluating the prediction accuracy of metal-toxicity models? Bulletin of Environ. Contam. Toxicol. 100: 64-68.

Moog O, Schmidt-Kloiber A, Ofenböck T and Gerritsen J (2001). Aquatische Ökoregionen und Bioregionen Österreichs – eine Gliederung nach geoökologischen Milieufaktoren und Makrozoobenthos-Zönosen. Umwelt und Wasserwirtschaft, Wien. 106 p.

Mueller KK, Fortin C and Campbell PGC (2012). Spatial Variation in the Optical Properties of Dissolved Organic Matter (DOM) in Lakes on the Canadian Precambrian Shield and Links to Watershed Characteristics. Aquatic Geochem. 18(1): 21-44.

Natale OE, Gomez CE and Leis MV (2007). Application of the Biotic Ligand Model for regulatory purposes to selected rivers in Argentina with extreme water-quality characteristics. Integr. Environ. Assess. Manag. 3(4): 517-528.

Niyogi S and Wood CM (2004). Biotic ligand model, a flexible tool for developing site-specific water quality guidelines for metals. Environ. Sci. Technol. 38(23): 6177-6192.

Norton SA, Verta M and Kahl JS (1991). Relative contributions to lake sediment chemistry by atmospheric deposition. SIL Proceedings 1922-2010, 24(5): 2989-2993.

Nowrouzi M and Pourkhabbaz A (2014). Application of geoaccumulation index and enrichment factor for assessing metal contamination in the sediments of Hara Biosphere Reserve, Iran. Chemical Speciation & bioavailability 26(2): 99-105.

Nriagu JO, Lawson G, Wong HKT and Azcue JM (1993). A Protocol for Minimizing Contamination in the Analysis of Trace Metals in Great Lakes Waters. J. Great Lakes Res. 19(1): 175-182.

Nys C, Janssen CR, Mager EM, Esbaugh AJ, Brix KV, Grosell M, Stubblefield WA, Holtze K and De Schamphelaere KAC (2014). Development and validation of a biotic ligand model for predicting chronic toxicity of lead (Pb) to *Ceriodaphnia dubia*. Environ. Toxicol. Chem. 33(2): 394-403.

Nys C, Janssen CR, Van Sprang P and De Schamphelaere KA (2016a). The effect of pH on chronic aquatic Ni toxicity is dependent on the pH itself: extending the chronic Ni bioavailability models. Environ. Toxicol. Chem. 35(5): 1097-1106.

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

OGEWV (Oberflächengewässerverordnung) (2016). Verordnung zum Schutz von Oberflächengewässern vom 20. Juni 2016. - Bundesgesetzblatt, Jahrgang 2016, 28(I), Bonn.

Omernik JM (1987). Ecoregions of the conterminous United States. Annals of the Association of American Geographers 77: 118-125.

OSPAR (2005). OSPAR Convention for the Protection of the Marine Environment of the North-East Atlantic - Agreement on Background Concentrations for Contaminants in Seawater, Biota and Sediment. OSPAR Agreement 2005-6.

Osté LA (2013). Derivation of dissolved background concentrations in Dutch surface water based on a 10-percentile of monitoring data. Deltares-report 1206111.005-2.

Osté LA, Klein J and Zwolsman GJ (2012). Inventory of methods to derive natural background concentrations of metals in surface water. Deltares/KWR-report 1206111.005.

Paquin PR, Gorsuch JW, Apte S, Batley GE, Bowles KC, Campbell PGC, Delos CG, Di Toro DM, Dwyer RL, Galvez F, Gensemer RW, Goss GG, Hogstrand C, Janssen CR, Mc Geer JC, Naddy RB, Playle RC, Santore RC, Schneider U, Stubblefield WA, Wood CM and Wu KB (2002). The biotic ligand model: a historical overview. Comp. Biochem. Physiol. C – Toxicol. Pharmacol. 133(1-2): 3-35.

Peters A, Merrington G, Delbeke K and de Schamphelaere K (2011a). Regulatory consideration of bioavailability for metals: Simplification of input parameters for the chronic copper Biotic Ligand Model. Integr. Environ. Assess. Manag. 7(3): 437-444.

Peters A, Crane M and Adams W (2011b). Effects of iron on benthic macroinvertebrate communities in the field. Bull. Environ. Contam. Toxicol 86(6): 591-595.

Peters A, Merrington G and Crane M (2012). Estimation of background reference concentrations for metals in UK freshwaters. Environment Agency Science Project Number: SC080021/1a.

Peters A, Simpson P and Moccia A (2014a). Accounting for both local aquatic community composition and bioavailability in setting site specific quality standards for zinc. Environ. Sci. Pollu. Res. 21(1): 105-117.

Peters A, Simpson P, Merrington G, Schlekat C and Rogevich-Garman E (2014b). Assessment of the effects of nickel on benthic macroinvertebrate communities in the field. Environ. Sci. Pollu. Res. 21(1): 193-204.

Peters A, Schlekat CE and Merrington G (2016). Does the scientific underpinning of regulatory tools to estimate bioavailability of nickel in freshwaters matter? The European-wide environmental quality standard for nickel. Environ. Toxicol. Chem. 35(10): 2397-2404.

Peters A, Merrington G, Schlekat C, De Schamphelaere K, Stauber J, Batley G, Harford A, van Dam R, Pease C, Mooney T, Warne M, Hickey C, Glazebrook P, Chapman J, Smith R and Krassoi R (2018a). Validation of the nickel biotic ligand model for locally relevant species in Australian freshwaters. Environ. Toxicol.Chem. 37(10): 2566-2574.

Peters A, Wilson I, Merrington G and Chowdhury MJ (2018b). Are lead exposures a risk in European fresh waters? A regulatory assessment accounting for bioavailability. Bull. Environ. Contam. Toxicol. 100(1): 127-133.

Reimann C and Birke M (Eds.) (2010). Geochemistry of European Bottled Water. 268 p., 28 figs., 6 tab., 2 app., 67 maps, CD-ROM. Borntraeger Science Publishers, Stuttgart. ISBN 978-3-443-01067-6. (www.borntraeger-cramer.com/9783443010676).

Reimann C and Garrett RG (2005). Geochemical background - Concept and reality. Sci. Total Environ. 350(1-3): 12-27.

Salminen R, Tarvainen T, Demetriades A, Duris M, Fordyce FM, Gregorauskiene V, Kahelin H, Kivisilla J, Klaver G, Klein P, Larson JO, Lis J, Locutura J, Marsina K, Mjartanova H, Mouvet C, O'Connor P, Odor L, Ottonello G, Paukola T, Plant JA, Reimann C, Schermann O, Siewers U, Steenfelt A, Van der Sluys J, de Vivo B and Williams L (1998). FOREGS geochemical mapping field manual. Geological Survey of Finland, Guide 47. 36 p + 1 app.

Salminen R (2005). Forum of European Geological Surveys (FOREGS) Database EuroGeoSurveys. http://weppi.gtk.fi/publ/foregsatlas/.

Santore RC and Ryan AC (2015). Development and application of a multimetal multibiotic ligand model for assessing aquatic toxicity of metal mixtures. Environ. Toxicol. Chem. 34(4): 777-787.

SCHER (Scientific Committee on Health and Environmental Risks) (2010), Opinion on Chemicals and the Water Framework Directive: Technical Guidance for Deriving Environmental Quality Standards, October 2010.

SCHER (Scientific Committee on Health and Environmental Risks) (2011), Opinion on the environmental quality standards – lead, 25 May 2011.

Schlekat CE, Van Genderen E, DeSchamphelaere KA, Antunes PM, Rogevich EC and Stubblefield WA (2010). Cross-species extrapolation of chronic nickel Biotic Ligand Models. Sci.Total Environ. 408(24): 6148-6157.

Schuster H and Ullmann A – GD NRW – (2017). Natürliche Hintergrundkonzentrationen in Oberflächengewässern Nordrhein-Westfalens. – Zwischenbericht: 113 p., Ministerium für Umwelt, Landwirtschaft, Natur- und Verbraucherschutz des Landes Nordrhein-Westfalen (Ed.).

Sinex SA and Helz GR (1981). Regional geochemistry of trace elements in Chesapeake Bay. Environ. Geol. 3(6): 315–323.

State of Oregon (2016). Issue paper: Water quality standards revisions for freshwater copper. Oregon Department of Environmental Quality.

Swedish Marine and Water Authority (2016). Miljögifter i vatten – klassificering av ytvattenstatus. Vägledning för tillämpning av HVMFS 2013: 19. Swedish Marine and Water Authority, Gothenburg, Sweden.

Swennen R and Van der Sluys J (2002). Anthropogenic impact on sediment composition and geochemistry in vertical overbank profiles of river alluvium from Belgium and Luxembourg. J. Geochem. Explo. 75(1-3): 93–105.

Tack K (2012). Prise en compte de la biodisponibilité des métaux selon la DCE : guide méthodologique. INERIS report N°DRC-12-126834-07511A, Verneuil-en-Halatte, France.

Tipping E, Corbishley HT, Koprivnjak JF, Lapworth DJ, Miller MP, Vincent CD and Hamilton-Taylor J (2009). Quantification of natural DOM from UV Absorption at Two Wavelengths. Environ. Chem. 6: 472-476.

Unsworth ER, Warnken KW, Zhang H, Davison W, Black F, Buffle J, Cao J, Cleven R, Galceran J, Gunkel P, Kalis E, Kistler D, Van Leeuwen HP, Martin M, Noël S, Nur Y, Odzak

N, Puy J, Van Riemsdijk W, Sigg L, Temminghoff E, Tercier-Waeber ML, Toepperwien S, Town RM, Weng L and Xue H (2006). Model Predictions of Metal Speciation in Freshwaters Compared to Measurements by In Situ Techniques. Environ. Sci. Technol. 40(6): 1942-1949.

US-EPA (United States Environmental Protection Agency) (1985). Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses. Office of Research and Development, PB85-227049.

US-EPA (United States Environmental Protection Agency) (2018). Final Aquatic Life Criteria for Aluminum in Freshwater. Federal Register / Vol. 83, No. 245 / Friday, December 21, 2018 / Notices, pp 65663- 65665.[24]

Van Regenmortel T, Berteloot O, Janssen CR and De Schamphelaere KAC (2017). Analyzing the capacity of the *Daphnia magna* and *Pseudokirchneriella subcapitata* bioavailability models to predict chronic zinc toxicity at high pH and low calcium concentrations and formulation of a generalized bioavailability model for *D. magna*. Environ. Toxicol. Chem. 36(10): 2781-2798.

Van Sprang PA, Verdonck FAM, van Assche F, Regoli L, De Schamphelaere KAC (2009). Environmental risk assessment of zinc in European freshwaters: A critical appraisal. Sci. Tot. Environ. 407: 5373-5391.

Van Sprang PA, Nys C, Blust RJP, Chowdhury J, Gustafsson JP, Janssen CJ and De Schamphelaere KAC (2016). The derivation of effects threshold concentrations of lead for European freshwater ecosystems. Environ. Toxicol. Chem. 35(5): 1310–1320.

Verschoor AJ, Vink JPM, de Snoo GR and Vijver MG (2011). Spatial and Temporal Variation of Watertype-Specific No-Effect Concentrations and Risks of Cu, Ni, and Zn. Environ. Sci. Technol. 45(14): 6049–6056.

Verschoor A, Hendriks J, Vink JPM, De Snoo G and Vijver MG (2012). Multimetal accumulation in crustaceans in surface water related to body size and water chemistry. Environ. Toxicol. Chem. 31(10): 2269-2280.

Verschoor AJ, Vijver MG and Vink JPM (2017). Refinement and cross-validation of nickel bioavailability in PNEC-Pro, a regulatory tool for site-specific risk assessment of metals in surface water. Environ. Toxicol. Chem. 36(9): 2367-2376.

Vink JPM (2002). Measurement of heavy metal speciation over redox gradients in natural water – sediment interfaces and implications for uptake by benthic organisms. Environ. Sci. Technol. 36(23): 5130-5138.

Vink, JPM, Broers M. (2017). Risk assessment of lead (Pb) with biotic ligand models; Development of simplified functions in PNEC-pro V6. Deltares report 11200584-002, Utrecht, The Netherlands.

---

[24] https://www.epa.gov/wqc/aquatic-life-criteria-aluminum#2018

Wagner B, Beer A, Bitzer F, Brose D, Brückner L, Budziak D, Clos P, Fritsche HG, Hörmann U, Hübschmann M, Moosmann L, Nommensen B, Panteleit B, Peters A, Prestel R, Schuster H, Schwerdtfeger B, Walter T and Wolter R (2014). Explanations to the Web Map Service (WMS) "Groundwater background values": 22 p. Working group 'Groundwater Background Values' Grundwasser) of the Ad hoc Working Group Hydrogeology (Ad-hoc AG Hydrogeologie) of the Geological Surveys of the Federal States of Germany (SGD); (v3). [https://www.bgr.bund.de/EN/Themen/Wasser/Projekte/abgeschlossen/Beratung/Hintergrundwerte/wagner2014_en.html;jsessionid=D73F9A0ECB2990529E0637901B45816D.1_cid321?nn=1546392, cited 01.12.2017].

Waiwood KG and Beamish FWH (1978). The effect of copper, hardness and pH on the growth of rainbow trout. J. Fish. Biol. 13(5): 591-598.

Walter T (2008). Determining natural background values with probability plots. In: EU Groundwater Policy Developments Conference, 13-15 November 2008, UNESCO, Paris, France.

Walter T, Beer A, Brose D, Budziak D, Clos P, Dreher T, Fritsche HG, Hübschmann M, Marczinek S, Peters A, Poeser H, Schuster H, Wagner B, Wagner F and Wirsing Wolter R (2012). Determining natural background values with probability plots. – In: Groundwater Quality Sustainability: 331-341, CRC Press, DOI: 10.1201/b12715-32.

Wasson JG, Chandesris A, Pella H and Blanc L (2002). Les hydro-écorégions de France métropolitaine. Approche régionale de la typologie des eaux courantes et éléments pour la définition des peuplements de référence d'invertébrés. Ministère de l'Ecologie et du Développement Durable, Cemagref BEA/LHQ, Lyon. 190 p.

WCA (2015). Technical guidance to implement bioavailability-based environmental quality standards for metals. Eurometaux, Brussels.

Weyhenmeyer GA and Karlsson J (2009). Nonlinear response of dissolved organic carbon concentrations in boreal lakes to increasing temperatures. Limnol. Oceanogr. 54(6.2): 2513–2519.

Worms I, Simon DF, Hassler CS and Wilkinson KJ (2006). Bioavailability of trace metals to aquatic microorganisms: importance of chemical, biological and physical processes on biouptake. Biochimie 88(11): 1721-1731.

Zglobicki W, Ryzak M and Bieganowski A (2011). Changes in textural and geo-Chemical features of alluvia in the western part of the Lublin Upland over the past 1000 years. Quaestiones Geographicae, 30(1): 123-132.

Zwolsman J and De Schamphelaere K (2007). Biological availability and present risks of heavy metals in surface waters. Kiwa and Ghent University. By order of STOWA. STOWA report 2007-12. Belgium.

8. ANNEXES

ANNEX 1: General European upper limits for dissolved metal concentrations (μg/l) in stream waters calculated by P90 of the FOREGS dataset

	90th percentile	**95th percentile**
Element	**Europe**	**Europe**
Ag	0.005	0.010
As	2.4	4.0
B	92	175
Ba	76	104
Be	0.055	0.1
Bi	0.007	0.012
Cd	0.05	0.08
Ce	0.92	1.84
Co	0.58	0.95
Cr	1.38	1.89
Cs	0.050	0.091
Cu	2.34	2.96
Mo	1.06	1.70
Ni	4.7	6.3
Pb	0.41	0.63
Sb	0.21	0.31
Se	1.09	1.53
Tl	0.016	0.022
U	2.41	3.58
V	1.63	2.72
Zn	9.8	15.4

ANNEX 2: Indicative case studies from several MS to estimate NBCs in surface waters

Germany

According to the surface water ordinance (OGEWV, 2016), the NBC describes the concentration of a substance in a surface water body which is not (or hardly) influenced by anthropogenic contamination. The LAWA-AO (2015) established that the 90th percentile of the natural concentration distribution defines the NBC.

The presented method is a holistic approach for the evaluation of NBCs in surface waters. It is based on a technical guidance document developed and published by the Working Group on water issues of the Federal States and the Federal Government (LAWA-AO, 2015). These guidelines were devised and further developed within the framework of a project that aimed at the evaluation of NBCs in the North Rhine-Westphalia (Schuster & Ullmann, 2017). Based on these elaborations, different requirements need to be considered when determining NBCs:

To derive NBCs, monitoring datasets need to be collected. For the estimation of NBCs in streams, samples of stream waters and springs can be used. Measurements from bodies of standing water, however, should not be considered.

When working with monitoring data, it must be taken into account that not every measuring point is suitable for the evaluation of NBCs. Therefore, further data have to be compiled to assess the influence of anthropogenic emissions (like wastewater discharge, waste disposal sites, mining, agriculture, etc.) on each measurement point. Furthermore, the results of the Drivers-Pressures-State-Impacts-Responses (DPSIR) analysis, which helps to understand whether a surface water body (and thus the included measuring points) shows significant anthropogenic pressures, should be considered. While a pre-selection of the monitoring data with regard to anthropogenic inputs does not necessarily have to be carried out within the presented evaluation method, it is recommended to reduce the data that are anthropogenically influenced. The pre-selection, however, should be applied with care so as not to exclude too many (and/or wrong) data points.

To assess the plausibility of the background concentrations, the measurements should be compared with regard to additional information (as far as available) like the background values in rocks, soil or groundwater, mineral deposits and so on.

Evaluation units

In the derivation of NBCs, the scale of the evaluation unit has a significant influence on the results and their representativeness. A background concentration/value can only be considered reliable and representative if it is derived from a geochemically homogeneous unit. If a statistical analysis is performed using data which are spread over a whole country (or using evaluation units that are not geochemically homogeneous) the result can only impart a reference value and not an actual NBC, since local characteristics wouldn’t be captured.

In Germany, the so called ‘hydrogeochemical units’ (HGC) were derived from the hydrogeological units of the Hydrogeological Map of Germany 1:200.000 (HÜK200), taking into account geological-genetical and lithological criteria (Wagner et *al*., 2014). Based on these 186 HGC, it was possible to derive NBC in groundwaters (Walter et *al*., 2012). For the application to surface waters, it is recommended to include a hydrological criterion as well and so further differentiate the HGCs that depend on different catchment areas.

Evaluation method – probability plots

Probability plots are a graphical procedure to determine the distribution of a random variable (Wagner et *al*., 2014). On the y-axis of the graph the measured concentrations are plotted. In case of a Normally-distributed dataset, the grid is calculated linearly. Lognormal distributions require a logarithmically scaled y-axis. The grid upper x-axis is determined by an integrated normal distribution and shows the cumulative probability. The grid lower x-axis shows the standard deviation from the mean value (Wagner et *al*., 2014).

If a dataset of a variable is normally (or lognormally) distributed, it is represented as a straight line in the probability plot. Assuming the data originates solely from a homogeneous area, where there is no recording of anthropogenic influence, this straight line represents the background concentration/population.

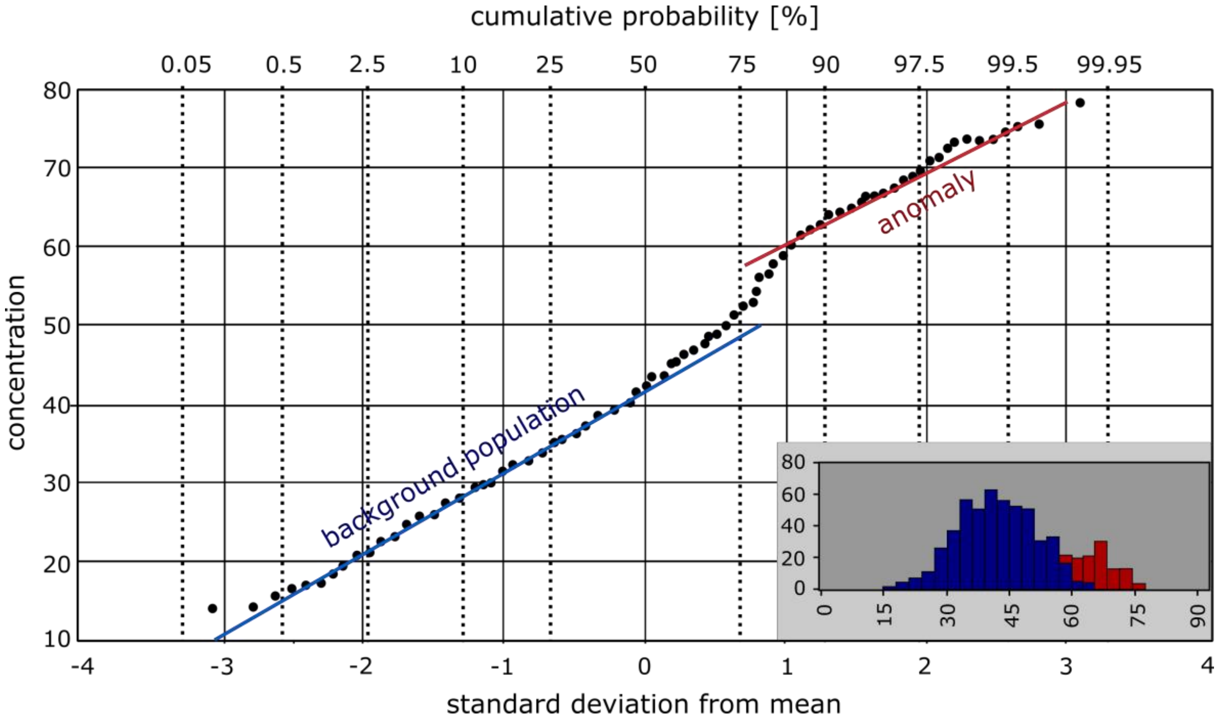


Figure Annex 2.1: Randomly generated mixed distribution (consisting of background population and an anomaly) (altered according to Walter et *al*., 2012)

Even though only datasets belonging to areas that are assumed to be geochemically homogeneous (evaluation unit) are to be evaluated collectively, some local anomalies are to be expected (whether they are geogenic, like mineral deposits or anthropogenic, like waste water discharges). In practice, the data of an evaluation unit thus does not necessarily consist of a single population (representing the background concentration), but can contain various, mixed populations. In the probability plot, these different populations will appear as several straight-line segments with different slopes. Therefore, the plot allows an immediate estimation of the heterogeneity of the dataset (Walter et *al*., 2012). To determine the background concentration (background population), the corresponding line segment has to be separated from the adjoining line segments (called anomalies) (Fig. 1). As a way to identify the background population and separate it from the anomalies, the data can be plotted in a GIS. By differentiating the measured concentration in terms of colour (or form) corresponding to the threshold concentration of the different line segments, one can control if certain populations cluster in specific areas (for example appear downstream of a wastewater discharge). Thus, the spatial concentration distribution can help to provide an insight into the sources of each population, enabling the identification of the background population. The background value is then assumed as the 90th percentile of the background population.

Concerning the statistical analysis, there are different requirements that need to be met. To avoid an imbalance between the different measuring points, only one result per measuring point should be imported into the probability plot. It was decided that the latest analysis is to be taken. If the latest measurement happens to be an outlier (doesn't comply with the criteria of mean ± 2.5·standard deviation) the preceding measurement is to be used. Values below the detection limit are imported into the probability plot as well (there is no need to use a substitute like half the detection limit). These values will be considered by extrapolation of the regression line. To guarantee a statistically reliable evaluation at least 10 measured values (which are above detection limit and part of the background population) are needed. The statistical evaluation can be performed semi-automatically using a Microsoft Excel software tool (Walter, 2008).

Step by step approach (Schuster & Ullmann, 2017)

1. Acquire data (it is important to differentiate between total content and dissolved content);
2. Pre-select the relevant data (no excessive pre-selection, rather exclude influenced data later on in the probability plot);
3. Assign data points to the evaluation units;
4. Check if each evaluation unit has enough data points (maybe define additional sampling sites);
5. Import data into probability plot:
    - check spatial distribution in GIS,
    - exclude data with significant anthropogenic input (anthropogenic anomaly),
    - in case of a geogenic anomaly (like a mineral deposit, etc.) it is possible to split the evaluation-unit and evaluate each region separately,
    - reduce data to background population;
6. Determine the background value (90th percentile);
7. Conduct a plausibility check.

Further and more detailed explanation concerning the approach to evaluate the background concentrations in surface waters as well as the functionality and application of probability plots can be found in Schuster & Ullmann 2017 and Walter et *al*. 2012.

The Netherlands

The Netherlands (Ministry of Traffic and Water Management) formalised NBCs in 1998 based on measurements in pristine areas (North West Europe) for a limited number of elements. New NBCs were derived in 2013, because NL need NBC for B and U in inland waters, and As, B, Ba, Mo, Ni, Se, Sn, U, and V in marine waters. After an evaluation of various methods (Osté et *al*., 2012) the 10-percentile of all monitoring data was chosen to derive new NBCs (Osté, 2013).

To estimate a low (10th) percentile of dissolved metal concentrations of all the monitoring data available for the water body or region, it is useful to first remove results with elevated concentrations sampled downstream of known point source discharges or pollution events. Moreover, there are often a large number of data reported as “less than” values, reflecting the analytical limit of quantification for the metal of interest. Some interpolation of the distribution of values is needed from the laboratory’s reporting limit (the “less than” value) and zero. Where datasets are small, or reporting limits are high, this can compromise the amount of data available to conduct this method.

If the distribution of the data has been checked (e.g. normal or log-normal), it is possible to derive the percentile based on the data measured above the LOQ. No background concentration is derived if more than 75% of the data are below the LOQ. In the range between 30 and 70% we check the distribution of the data. Based on extrapolation assessment it is decided whether a background concentration is determined.

The first example is the normal probability plot of Dutch zinc data (Figure Annex 2.2). If the data are normally distributed, the graph shows a straight line. For most metals, the log values are normally distributed. In the figure, the Log(zinc concentration) values show a straight line except for the low concentrations that are below the LOQ. About 40% of the data are below the LOQ. The assumption is that if these values below the LOQ had been measured properly, the line would be straight also in the left part of the graph. Therefore, the part of the graph without detection problems is extrapolated to the low concentration. The P10 is determined based on the P10 of the dashed line, which is 0.7 µg/l.

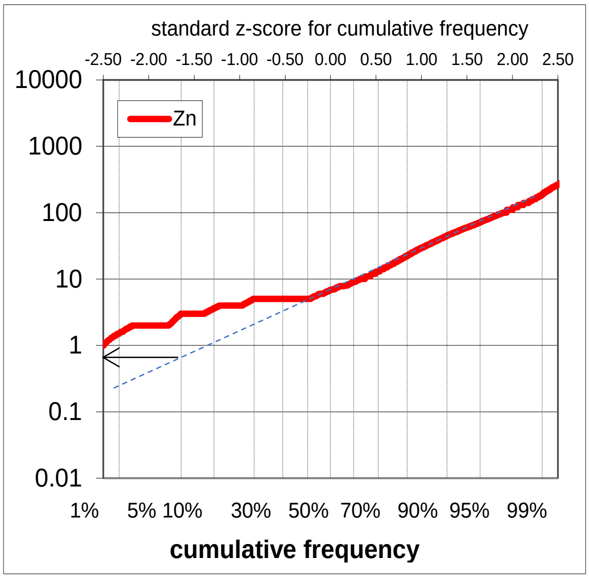


Figure Annex 2.2: Cumulative distributed concentration (log scale) of zinc in the Netherlands (Osté et *al*., 2012)

Two further examples are presented above. The light green (or grey) dashed line is the assumed distribution which is extrapolated to the lower range. The arrow represents the P10 value. Both Cd and Tl show a high number of values below the LOQ (flat part of the graphs), but extrapolation was only possible for Cd.

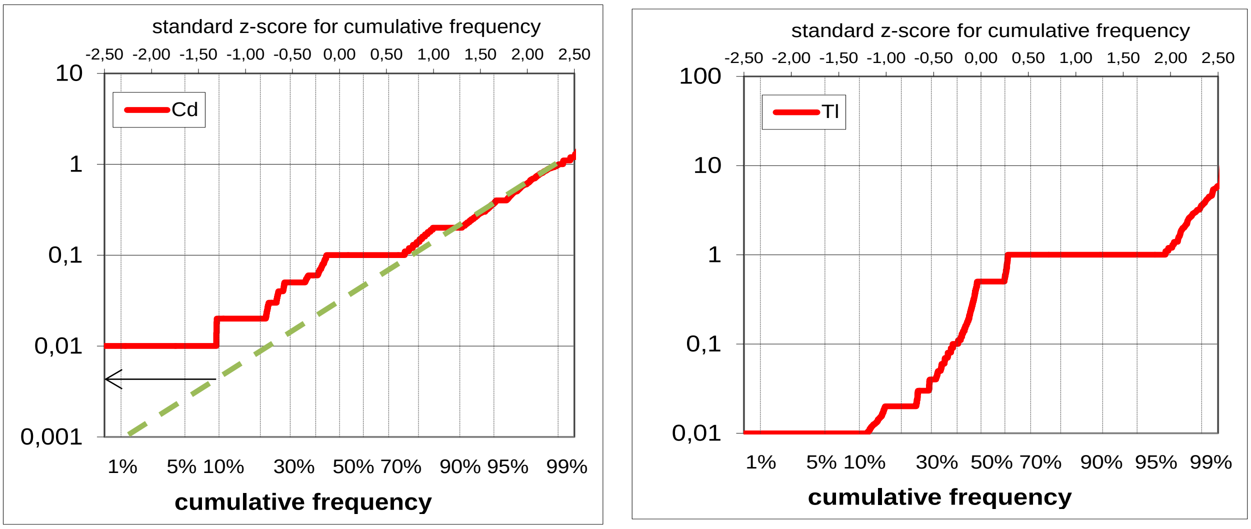


Figure Annex 2.3: Cumulative distributed concentration (log scale): Left: Cd extrapolation method = 0.005 µg/l; Right: Tl extrapolation is impossible.

The alternative **groundwater approach** has been tested in the province of Noord-Brabant, NL (Osté et *al*., 2012). The P50 or P90 values of the monitoring data in the groundwater were taken as the NBC in groundwater. High percentages were chosen, because the deep groundwater is considered to be undisturbed, so all values can be considered as ‘natural’.

However, the derived groundwater values were often much higher than the low percentile (P10) chosen in anthropogenically affected surface water as an NBC. It was concluded that the step from (anaerobic) groundwater to (aerobic) surface water is too complicated to directly “copy” the background groundwater concentrations into surface water concentrations. This method thus might be more valuable in areas holding aerobic conditions in the deep groundwater layers or where redox reactions can be successfully understood and modelled.

France

1. Principles

- Based on monitoring data: national or regional, dissolved metals concentrations in surface water, sampling 4 to 12 times per year.
- It requires a preliminary data examination and sorting (quality check): LOQ (vs. EQS), % of values above LOQ.
- Work within homogeneous geochemical (geological) units based on the hydro-ecoregions (Omernik,1987; Wasson et *al*., 2002; Moog et *al*., 2001) (USA, France, Austria).
- Selection of stations based on local or watershed land-cover (Corine Land Cover) and other geographical database: stations submitted to anthropogenic pressure are excluded for a first analysis.

Nonetheless, data from excluded stations could be reintegrated with the sub-set corresponding to a homogeneous geological unit; if they are close to the values observed for the non-impacted selected stations within the same area, this is a means to increasing the number of available data and their spatial coverage.

- Determination of geochemical background "levels" for each defined geological unit:
    - Low = close or < LOQ,
    - Medium (< EQS) and High (> EQS) if values are homogeneous within the geochemical unit.

If there is a high heterogeneity of the values within the geological unit, then it is necessary to adapt the scale of study for the background concentration (BC) determination, and perform a case study at local scale, as proposed by Casiot et *al*. (2009).

2. Procedure

2.1. **Data acquisition** and (pre)checking

LOQ (vs. 30% EQS), quantification frequency, more than one year of monitoring for each station. This preliminary step is necessary to scrutinize the quality of the monitoring dataset.

2.2. **Selection of non-impacted stations** (Corine Land Cover, soil and industrial database)

Existing databases in France are BASIAS (industrial sites, current or historical, with the potential to cause environmental pollution) and BASOL (polluted sites that, because of historical waste deposits or infiltration of pollutants, could prove a nuisance or pose a long-term risk to people or the environment). These databases are maintained by the BRGM (Bureau de Recherches Géologiques et Minières), the French geological survey.

2.3. Identification of geological homogeneous units

It is a compromise between limited size (geological homogeneity) and sufficient number of stations/data within each unit. Alternatively, it is possible to perform a spatial extrapolation of data using a kriging method (e.g. Seine Normandy watershed).

2.4. **Qualification/quantification** of geochemical background

- **Low / Medium / High background "level"** is determined if the available number of stations is not sufficient to determine a reliable mean/percentile (with associated confidence level) for the considered scale;
- **Mean or percentile background "value"** (BC) is calculated only if *n* is sufficient (statistical tests); P75 was used in our study; *n* should be higher for areas with higher heterogeneity (expert judgement necessary);
- **Alternatively, background "value"** can be calculated by kriging procedure (spatial extrapolation) with an associated confidence level.

If a high heterogeneity is observed at the scale of the geological unit, it is necessary to perform a specific analysis at the station' watershed scale, with additional data acquisition at this local scale.

3. Limitations and warnings

- The selection and *a priori* exclusion of impacted stations is reversible in some cases, as it is an approach by precautionary principle; in fact, anthropogenic pressure does not systematically have a direct effect on a given metal concentration in surface waters. So, depending upon the values observed for the excluded subset of stations in relation to the selected stations (non-impacted), they might be reintegrated in the final dataset for BC calculation;
- Non-quantified values, if replaced by LOQ/2, could induce a bias in the BC determination if actual metal concentrations are lower; an adapted statistical analysis might be useful, but better data quality might be required to calculate reliable BC values (ex. Cases of Hg and Cd);
- If data are heterogeneous within a given geological unit, there is no simple solution for BC determination. An additional data acquisition at local scale is most probably necessary.

4. Application to a case study

Example of application in the Seine River basin

Data compiled from monitoring programmes (surface and groundwater) along the Seine River basin, between 2005 and 2018, were compiled and scrutinized. The final selected dataset was only covering the period from March 2012 to February 2018. Then, as explained above, the catchments only slightly affected or unaffected by anthropogenic activities were identified ("reference" stations). Finally, the resulting representative averaged dissolved metal concentrations were interpolated by kriging to the whole basin at the local scale (water bodies catchment). This method allowed to establish background concentrations together with confidence levels for all water bodies catchments of the Seine-Normandy basin.

In the application of the method, instead of assigning a geochemical background per geological unit (as in Chandesris *et al.*, 2013), the average metal concentrations (from the Q0-Q75 quartiles interval) of "reference" data from monitoring stations were extrapolated by kriging across the basin district.

Data Analysis

The Seine-Normandy river basin districts have a large amount of dataset of quality measurements in surface and ground waters. For the eight metals defined in WFD, 893 710 measurements (dissolved fraction) were available for the period 2005-2018. First of all, monitoring stations were selected at the surface and near surface and underground water. Then, data was sorted by excluding measurements with a limit of quantification (LOQ) above 30% of EQS according to the so-called Quality Assurance/Quality Control Directive (EC, 2009).

	Cd	Hg*	Ni	Pb	As	Cr	Cu	Zn
EQS (µg/L) (WFD 2008, French decree of 27/7/15)	0.08 to 0.25	0.07	4	1.2	0.83	3.4	1.6	3.1 to 7.8
Required LOQ (EC 2009)	0.075	0.021	1.2	0.36	0.249	1.02	0.48	2.34

* MAC (EU, 2013)

Measurements above the LOQ were accepted; and when a measurement was equal or below the LOQ, we replaced data by the value of LOQ divided by two, according to the QA/QC directive (EC, 2009). Outliers have been removed before calculating, applying the proper statistical tests.

	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
Number of data with a conc. > LOQ	7339	855	4432	5190	2	5459	1915	4558
Number of data with a conc. ≤ LOQ	1359	1421	1752	870	18	2258	5136	3115
Proportion of data with a conc. ≤ LOQ	16%	62%	28%	14%	90%	29%	73%	41%

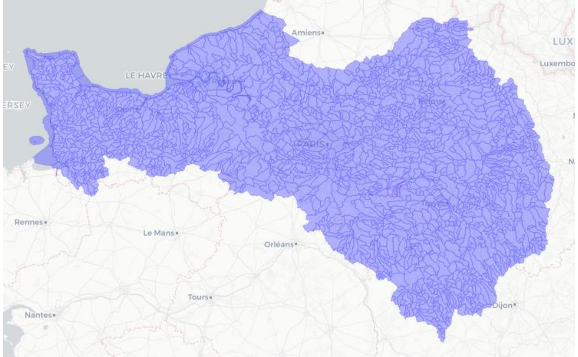
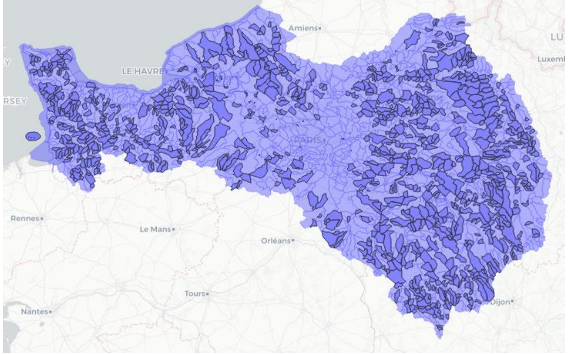
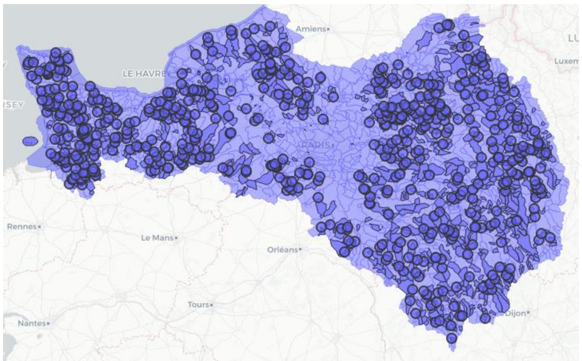
Then, for each "reference" station a representative average was calculated for the quantile 0 to 75 data subset.

At station scale, the criteria to accept data were the following: stations with at least four measurements (except for mercury), and stations with their standard deviation above the monthly average divided by 2.

List of poorly or unaffected water bodies catchment by anthropogenic activities

The list of poorly or unaffected water bodies catchment was defined by anthropogenic activities with the Corine Land Cover database of land uses. For each water body catchment, the percentage of artificialised land (such as urbanised, industrial, commercial, communication networks, mines, landfills and sites) was calculated. All water bodies of the catchment with less than 5% of artificialised land were accepted; as well as those that do not have any water bodies catchment with more than 5% artificialised land upstream themselves.

Example of arsenic (As)

	
1806 Seine-Normandy river basin districts water bodies catchment	936 water bodies catchment poorly or unaffected by anthropogenic activities
	
563 "reference" monitoring stations intersect with the 936 water bodies catchment	

Removal of local anomalies

Additional information was used to exclude other water bodies in the catchment, like the database of direct or indirect industrial discharges to surface waters related to metals activities; and the database on present and historical soil pollution. Finally, an automatic scan to look at local anomalies was performed in order to identify the local minimum concentration for each metal.

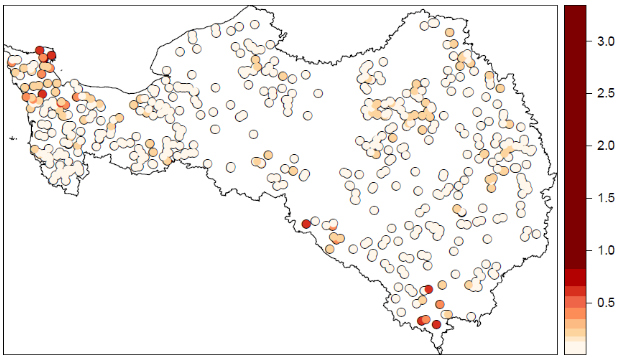
Metal	Number of excluded stations	Number of final “reference” stations	Number of measurements
Arsenic	39	524	6248
Cadmium	76	137	1407
Chromium	97	232	2236
Copper	327	352	3461
Nickel	387	261	3093
Mercury	113	5	10
Lead	180	249	3239
Zinc	204	148	1333

Kriging

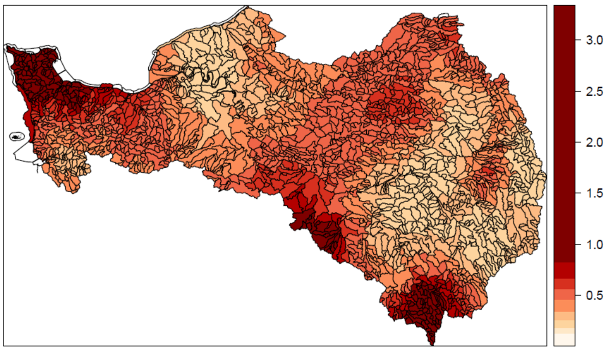
The interpolation was free in space at 360°. The best fitting variogram model was applied, between Circular, Spherical, Exponential, Matérn and Gaussian models, in view of the “SSErr“ (Sum of Squared errors), and by choosing a 1 km² grid.

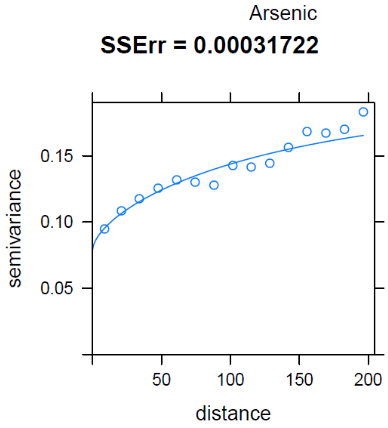
Results for arsenic

Map of monitoring “reference” stations and associated dissolved average As concentrations (in µg/L) used in the kriging calibration model



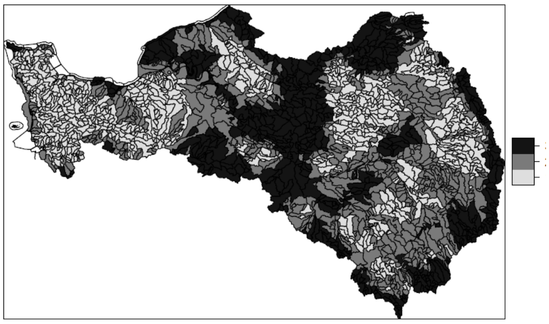
Map of kriging interpolated prediction average dissolved As metal concentration (in µg/L) by water bodies catchment





Variogram

Map of incertitude levels from kriging variance prediction



- Black: high incertitude
- Grey: medium incertitude
- White: low incertitude

In the end, a geochemical background and associated level of incertitude were proposed for each water body catchment. If a water body catchment has a high incertitude, its geochemical background concentration could be replaced by the minimal geochemical background determined in the basin.

Conclusion

Of the eight studied metals, arsenic showed the best results with a good calibration variogram model lead by high density and homogeneous distribution of stations over the basin. Furthermore, this density and distribution allowed an easy identification of pollution sources unlike other metals. The arsenic background concentration map is well correlated with the geological maps. In particular, adding a background level to the EQS (NBC added to it if needed and if uncertainty sufficiently lower) enabled shifting several stations from bad to good status for arsenic on the Triassic bedrock geology’s basin district areas like the Cotentin and the Morvan. For other metals, for instance Hg, the dataset quality was not sufficient to conclude on the BC.

UK

Derivation of ambient background concentrations for zinc

Ambient background concentrations (ABCs) have been derived for zinc as the $EQS_{bioavailable}$, which has been set for zinc in the UK by using the added risk approach (ARA) as developed in the ESR for zinc (EU, 2010). A summary of the derivation of the ABCs in fresh and salt waters is summarised below. The ABCs have been derived based on consideration of available surface water monitoring data.

Freshwater

ABCs for the freshwater environment have been estimated for zinc based on a low percentile of the available monitoring data. Where sufficient data were available the ABCs have been derived for a defined hydrometric area. In addition, a national value has also been derived based on consideration of all the available data. This default value can then be used in those hydrometric areas where insufficient data were available to enable a specific ABC to be derived.

The ABCs were based on a low percentile of the available monitoring data to ensure significant anthropogenic influences are excluded. The 5th percentile of the monitoring data for dissolved zinc concentrations has been used. For those hydrometric areas where there were very limited monitoring data or a high proportion (i.e. > 30%) of results were noted as less usable and then a specific ABC has not been derived. The Kaplan Meier method was used to estimate the lower tail of the distribution of the dissolved zinc concentrations.

Saltwater

Saltwater ABCs have been derived by assessing dissolved zinc data for around 43 000 samples taken from around the UK coast. A single ABC value has been derived for saltwater based on consideration of all the available data. As with the freshwater method, a low percentile of 5% has been used to exclude significant anthropogenic influences with the Kaplan Meier approach being used to estimate the lower tail of the distribution.

ANNEX 3: A number of distinct cases of geological areas with natural metal/bearing minerals

A number of distinct cases can be possible, all of which can be expected to display naturally occurring concentrations of metals above a generic EQS based on regional, national or EU datasets:		
Scenario		**Implications for NBC**
Naturally enriched areas where mining has not yet occurred at all.		Correct setting of objectives will require implementation of locally derived NBCs.
Naturally enriched areas where mining has historically occurred.	With elevated background concentrations that are entirely of natural origin.	Correct setting of objectives will require implementation of locally derived NBCs.
	With remaining anthropogenic contributions to the elevated background concentrations.	Best available measures for reducing the anthropogenic pollution may be rate-limited by the speed of natural processes, but also the measures should target compliance with a locally derived NBC that reflects the natural component of the background.
Naturally enriched areas where contemporary mining is underway.	With elevated background concentrations that are entirely of natural origin, ***or*** With authorised anthropogenic contributions to elevated background concentrations.	Correct setting of objectives will require implementation of a locally derived NBC that reflects the natural component of the background.
	With unauthorised anthropogenic contributions to elevated background concentrations.	Immediate means of redress should be sought.
Naturally enriched areas where mining has historically occurred, and contemporary mining is also underway.	With elevated background concentrations that are entirely of natural origin, ***or*** With authorised anthropogenic contributions from contemporary sites only	Correct setting of objectives will require implementation of a locally derived NBC that reflects the natural component of the background.
	With remaining anthropogenic contributions to the elevated background concentrations from historic workings only (authorised or not).	Best available measures for reducing the anthropogenic pollution may be rate-limited by the speed of natural processes, but also the measures should target compliance with a locally derived NBC that reflects the natural component of the background.
	With unauthorised anthropogenic contributions from contemporary sites only.	Immediate means of redress should be sought.
	With remaining anthropogenic contributions to the elevated background concentrations from historic workings that are reducing with time as a co-benefit of contemporary mining.	The reduction of historic pollution may be rate-limited by the speed of the contemporary mining method, but also the contemporary mine should be required to target compliance with a locally derived NBC that reflects the natural component of the background.

ANNEX 4: Advice accompanying the use of the M-BAT tool

Number	Question	Advice
	Flags/alerts for being outside validated ranges (see below)	The validation boundaries of the BLMs (and so too M-BAT) represent the extremes of water quality conditions at which the validation chronic tests were undertaken (shown on the introductory page of M-BAT). However, this does not mean that the relationships developed do not hold where water conditions are outside of these ranges or that the derived $EQS_{bioavailable}$ is underprotective. It does mean that the certainty associated with calculations performed for water quality conditions outside of the validated boundaries is not as high as if the waters were within the range of validation conditions. Hence, M-BAT does give calculations for waters that are outside of the validated ranges But the physico-chemical parameter(s) that is outside the range is 'held' at the limit of the validation. This introduces an additional level of uncertainty in the calculated result. How this might be dealt with is described below.
1	For Copper: *The measured DOC is above the upper limit of the validated range for Cu. The Site-specific PNEC has been calculated using a DOC value of 15mg /L. For further clarification please seek advice.*	The data outputs from the CuBLM on which M-BAT are based are only for water chemistry conditions with up to 15 mg DOC L^{-1}.[25] Therefore, the calculations are held at a ceiling 15 mg DOC L^{-1} when measured DOC concentrations entered are greater than 15 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.
2	For Copper: *The measured pH is below the lower limit of the validated range for Cu. The Site-specific PNEC has been calculated using a pH value of 6. For further clarification please seek advice.*	The pH of the sample(s) under investigation is below the validated range for the CuBLM, and so too M-BAT. Implication: The calculated PNEC could be higher, and so less precautionary than the real

---

[25] http://www.wfduk.org/sites/default/files/Media/Environmental%20standards/Copper%20M-BAT%20report%20-%20UKTAG.pdf

		situation. If the RCR shows a marginal pass then this requires further investigation. Several approaches are given in the guidance document given in the introduction.
3	For Copper: *The measured Ca is below the lower limit of the validated range for Cu. The Site-specific PNEC has been calculated using a Ca value of 3.1. For further clarification please seek advice.*	The calcium concentration in the sample(s) under investigation is below the validated range for the CuBLM, and so too M-BAT. Implication: The calculated PNEC could be higher, and so less precautionary than the real situation. If the RCR shows a marginal pass then this requires further investigation. Several approaches are given in the guidance document given in the introduction.
4	For Copper: *The measured pH is above the upper limit of the validated range for Cu. The Site-specific PNEC has been calculated using a pH value of 8.5. For further clarification please seek advice.*	The pH of the sample(s) under investigation is above the validated range for the CuBLM, and so too M-BAT. Implication: The calculated PNEC could be higher, and so less precautionary than the real situation. If the RCR shows a marginal pass then this requires further investigation. Several approaches are given in the guidance document given in the introduction.
5	*For Copper:* *The measured Ca is above the upper limit of the validated range for Cu. The Site-specific PNEC has been calculated using a Ca value of 93. For further clarification please seek advice.*	The calcium concentration in the sample(s) under investigation is above below the validated range for the CuBLM, and so too M-BAT. Implication: The calculated PNEC could be higher, and so less precautionary than the real situation. If the RCR shows a marginal pass then this requires further investigation. Several approaches are given in the guidance document given in the introduction.
6	For Zinc: *The measured Ca is below the lower limit of the validated range for Zn. The Site-specific PNEC has been calculated using a Ca value of 3. For further clarification please seek advice.*	The validated boundary condition for the ZnBLM for calcium, on which the zinc calculations in M-BAT are based[26], have a range of 3 – 160 mg Ca L^{-1}. Implication: The calculated PNEC could be higher, and so less precautionary than the real situation. If the RCR shows a marginal pass then this requires further investigation. Several approaches are given in the guidance

---

[26] http://www.wfduk.org/sites/default/files/Media/Zinc%20bioavailability%20assessment%20tool%20-%20UKTAG%20DRAFT_0.pdf

		document given in the introduction.
7	For Zinc: *The measured pH is below the lower limit of the validated range for Zn. The Site-specific PNEC has been calculated using a pH value of 6. For further clarification please seek advice.*	The pH of the sample(s) under investigation is below the validated range for the ZnBLM, and so too M-BAT. Implication: The calculated PNEC could be higher, and so less precautionary than the real situation. If the RCR shows a marginal pass then this requires further investigation. Several approaches are given in the guidance document given in the introduction.
8	For Zinc: *The measured DOC is above the upper limit of the validated range for Zn. The Site-specific PNEC has been calculated using a DOC value of 20mg /L. For further clarification please seek advice.*	The data outputs from the ZnBLM on which M-BAT are based are only for water chemistry conditions with up to 20 mg DOC L^{-1}. Therefore, the calculations are held at a ceiling 20 mg DOC L^{-1} when measured DOC concentrations entered are greater than 20 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.
9	For Zinc: *The measured Ca is above the upper limit of the validated range for Zn. The Site-specific PNEC has been calculated using a Ca value of 160. For further clarification please seek advice.*	The validated boundary condition for the ZnBLM for calcium, on which the zinc calculations in M-BAT are based[27], have a range of 3 – 160 mg Ca L^{-1}. Implication: The calculated PNEC is likely to be similar 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.
10	For Zinc *The measured pH is above the upper limit of the validated range for Zn. The Site-specific PNEC has been calculated using a pH value of 8. For further clarification please seek advice.*	The pH of the sample(s) under investigation is above the validated range for the ZnBLM, and so too M-BAT. Implication: The calculated PNEC could be higher, and so less precautionary than the real situation. If the RCR shows a marginal pass then this requires further investigation. Several approaches are given in the guidance document given in the introduction.

11	For Manganese: *The measured DOC is above the upper limit of the validated range for Mn. The Site-specific PNEC has been calculated using a DOC value of 20mg /L. For further clarification please seek advice*.	The data outputs from the MnBLM on which M-BAT are based are only for water chemistry conditions with up to 20 mg DOC L^{-1}. Therefore, the calculations are held at a ceiling 20 mg DOC L^{-1} when measured DOC concentrations entered are greater than 20 mg L^{-1}. Implication: When this flag is shown it is likely to mean that the calculated PNEC is slightly 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.
12	For Manganese: *The measured pH is above the upper limit of the validated range for Mn. The Site-specific PNEC has been calculated using a pH value of 8.5. For further clarification please seek advice.*	The pH of the sample(s) under investigation is above the validated range for the MnBLM, and so too M-BAT[28]. Implication: The calculated PNEC could be higher, and so less precautionary than the real situation. If the RCR shows a marginal pass then this require further investigation. Several approaches are given in the guidance document given in the introduction.
13	For Manganese: *The measured Ca is above the upper limit of the validated range for Mn. The Site-specific PNEC has been calculated using a Ca value of 200. For further clarification please seek advice.*	The validated boundary condition for the MnBLM for calcium, on which the manganese calculations in M-BAT are based, have a range of 1 – 200 mg Ca L^{-1}. Implication: The calculated PNEC is likely to be similar 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.
14	For Manganese: *The measured Ca is below the lower limit of the validated range for Mn. The Site-specific PNEC has been calculated using a Ca value of 1. For further clarification please seek advice*.	The validated boundary condition for the MnBLM for calcium, on which the manganese calculations in M-BAT are based, have a range of 1 – 200 mg Ca L^{-1}. Implication: The calculated PNEC is likely to be higher, and so less precautionary, compared to the real situation. If the RCR shows a marginal pass then this requires further investigation. Several approaches are given in the guidance document given in the introduction.

---

[28] http://www.wfduk.org/sites/default/files/Media/Environmental%20standards/Development%20and%20use%20of%20the%20manganese%20screening%20tool%20-%20UKTAG.pdf

15	For Manganese: *The measured pH is below the lower limit of the validated range for Mn. The Site-specific PNEC has been calculated using a pH value of 5.5. For further clarification please seek advice.*	The pH of the sample(s) under investigation are below the validated range for the MnBLM, and so M-BAT. Implication: The calculated PNEC could be higher, and so less precautionary than the real situation. If the RCR shows a marginal pass then this require further investigation. Several approaches are given in the guidance document given in the introduction.
16	For Nickel: *The measured pH is below the lower limit of the validated range for Ni. The Site-specific PNEC has been calculated using a pH value of 6.5. For further clarification please seek advice.*	The pH of the sample(s) under investigation is below the validated range for the NiBLM, and so too M-BAT. Implication: The calculated PNEC could be lower, and so less precautionary than the real situation. If the RCR shows a marginal pass then this require further investigation. Several approaches are given in the guidance document given in the introduction.
17	For Nickel: *The measured DOC is above the upper limit of the validated range for Ni. The Site-specific PNEC has been calculated using a DOC value of 20mg /L. For further clarification please seek advice.*	The data outputs from the NiBLM on which M-BAT are based are only for water chemistry conditions with up to 20 mg DOC L^{-1}. Therefore, the calculations are held at a ceiling 20 mg DOC L^{-1} when measured DOC concentrations entered are greater than 20 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.
18	For Nickel: *The measured Ca is above the upper limit of the validated range for Ni. The Site-specific PNEC has been calculated using a Ca value of 88. For further clarification please seek advice.*	The validated boundary condition for the NiBLM for calcium, on which the nickel calculations in M-BAT are based, have a range of 2 – 88 mg Ca L^{-1}. Implication: The calculated PNEC is likely to be lower, and so 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.
19	For Nickel: *The measured pH is above the upper limit of the validated range for Ni. The Site-specific PNEC has been calculated using a pH value of 8.7. For further clarification please seek advice.*	The pH of the sample(s) under investigation is above the validated range for the NiBLM, and so too M-BAT. Implication: The calculated PNEC could be higher, and so less precautionary than the real situation. If the RCR shows a marginal pass then this require further investigation. Several

		approaches are given in the guidance document given in the introduction.
20	For Nickel: *The measured Ca is below the lower limit of the validated range for Ni. The Site-specific PNEC has been calculated using a Ca value of 2. For further clarification please seek advice.*	The validated boundary condition for the NiBLM for calcium, on which the manganese calculations in M-BAT are based, have a range of 2 – 88 mg Ca L^{-1}. Implication: The calculated PNEC is likely to be higher, and so less precautionary, compared to the real situation. If the RCR shows a marginal pass then this requires further investigation. Several approaches are given in the guidance document given in the introduction.
Additional prompts and alerts		
21	For all metals (red shaded rows, white text): *Bioavailable metal concentration exceeds EQS based on site-specific conditions.*	This indicates an exceedance of the EQS. The RCR column characterises the potential risk.
Other queries		
21	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). The RCR is given in M-BAT
22	How important are the validation boundaries of the models?	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 (shown on the introductory page of M-BAT). Clearly, for waters which lie only slightly outside the BLM validation limit for a single parameter the results provided by M-BAT will be more reliable than those provided for a water for which two parameters lie significantly outside the validation range of the BLM.
23	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.
24	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, Mn, Zn) are anticipated. Sites with very low metal exposures (i.e. with dissolved concentrations below the reference $EQS_{bioavailable}$) are not expected to be impacted.
25	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. For metals which bind very strongly to DOC (e.g. Cu) they can also reduce DOC binding so softer waters may not be more sensitive for copper.
26	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.
27	How will toxicity be affected in soft, acid waters?	Soft acid waters can be very sensitive to metal toxicity, and any predictions made for such waters would represent an extrapolation for more than one parameter, consequently making the results more uncertain.
28	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 M-BAT) will generally be reliable, provided that the hardness is not extreme (i.e. more than twice as high as the upper validation threshold).
29	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. For some metals high pH can also increase the bioavailable forms of the metal in the solution, which could increase toxicity in some circumstances.
30	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.
31	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, smallest for manganese, and intermediate for nickel and zinc. Sites with low DOC concentrations will always be more sensitive than similar sites with higher DOC concentrations.
32	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. Higher DOC concentrations will reduce toxicity, although the degree of any additional effect depends upon the metal in question.
33	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.
34	What type of water chemistry conditions are most sensitive for manganese toxicity?	Waters with either low hardness, or high pH, are sensitive to manganese toxicity.
35	What type of water chemistry conditions are most sensitive for nickel toxicity?	Waters with low DOC concentrations are sensitive to nickel toxicity. The uncommon combination of high pH and low hardness also increases toxicity.
36	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.
37	What about lead?	For lead there is a BLM, but it has not been implemented. Further, the EQS for lead is based on a DOC correction. There is a separate spreadsheet tool available for assessing lead compliance.

ANNEX 5: Glossary of terms

Term	Definitions
AA-EQS	Annual Average Environmental Quality Standard. EQS expressed as an annual average value – to be protective against chronic exposure.
ARA	Added Risk Approach. This approach assumes that only the anthropogenic added fraction of the metal contributes to the risk for the environment.
BioF	The Bioavailability Factor. The BioF is based on a comparison between the expected bioavailability at the reference site and the one related to site-specific conditions. Through the use of a BioF, differences in (bio)availability are accounted for by adjustments to the monitoring data, but the EQS remains the same. The BioF is calculated by dividing the Generic or Reference EC_{10} by the calculated site-specific EC_{10}.
BLM	Biotic Ligand Model. This is a predictive tool that can account for variations in metal toxicity due to water chemistry. The tool calculates a site-specific bioavailable metal concentration using information on the chemistry of local water sources, i.e. pH, calcium concentrations, hardness, dissolved organic carbon, etc.
DOC	Dissolved organic carbon.
EC_{10}	Effect concentration for 10% of the individuals in a toxicity test.
EQS	Environmental Quality Standard. Concentration of a particular pollutant or group of pollutants in water, sediment or biota, which should not be exceeded in order to protect human health and the environment. Environmental quality standards are set as an annual average AA-EQS or a maximum allowable concentration MAC-EQS. Water EQS laid down in part A of annex I to Directive 2008/105/EU as amended by Directive 2013/39/EU are expressed as total concentrations in the whole water sample; except in the case of cadmium, lead, mercury and nickel where the water EQS refer to the dissolved concentration, i.e. the dissolved phase of a water sample obtained by filtration through a 0.45 µm filter or any equivalent pre-treatment, or, where specifically indicated, to the bioavailable concentration.
EQS_{add} or “added EQS”	Environmental Quality Standard derived using the added risk approach (ARA) In the added risk approach, an EQS_{add} is derived from toxicity

	data that are based on the added concentration of the metal in the ecotoxicity tests without the background concentration in the test media. The effect values (i.e. NOEC or EC10) are then calculated on the basis of these added concentrations defined as the total measured concentration in the test minus the background metal concentration present in the test medium or test water
$EQS_{bioavailable}$ or $EQS_{bioavail.}$	Bioavailable Environmental Quality Standard. This threshold value is derived under conditions representing high or maximum bioavailability.
$EQS_{generic}$ or Generic EQS	The Generic Predicted No-Effect Concentration (PNEC). This value is sometimes also termed the reference or generic EQS. The $EQS_{generic}$ is expressed as dissolved concentrations of metal and is derived without any consideration of bioavailability. The methodology for deriving a generic EQS for metals in water is similar to deriving a QS to protect pelagic species against any other type of substance.
ESR	Existing Substances Regulation (EEC) No. 793/93.
FOREGS	The European Geological Survey's Geochemical Baseline Database.
HC5	The hazardous concentration for 5% of the species in a Species Sensitivity Distribution (SSD). This value is used to derive the PNEC or EQS.
LOQ	Limit of quantification The LOQ can be defined as the lowest limit of measurand that can be determined with an acceptable level of repeatability precision and trueness
MAC-EQS	Maximum Allowable Concentration EQS. Set to be protective against acute exposure.
MLR	Multiple Linear Regression.
NBC	Natural Background Concentration.
NOEC	"No Observed Effect Concentration". This value is the highest concentration in a test with a mean response statistically not differing significantly from the mean response of the control if compared statistically. The NOEC

	value corresponds to about 10% t level of effect29.
P10	10th Percentile
P90	90th Percentile
PEC	Predicted Environmental Concentration. These values can be modelled but are usually replaced in the screening tool with measured environmental concentrations of dissolved metal in the waters of interest.
PNEC $\text{PNEC}_{\text{site}}$ or site-specific	Predicted No-Effect Concentration Predicted no-effect concentration at the site under consideration, derived from the ecotoxicity data and site-specific water quality data using the BLM.
RCR	Risk Characterisation Ratio. This ratio, sometimes also called the risk quotient, is calculated by dividing the measured environmental concentration (MEC) or the predicted environmental concentration (PEC) by the PNEC. RCR values equal to or greater than 1 are considered as presenting a potential risk.
Simplified tool (or user-friendly bioavailability tool)	These tools are simplified versions of the BLMs. They perform the same calculations, but are run in MS Excel, require fewer data inputs, and give outputs that are precautionary relative to the full BLMs but readily interpretable in the context of basic risk management and EQS compliance assessment.
SSD	Species Sensitivity Distribution. It is a cumulative probability distribution of toxicity values for a given substance in multiple species and is used to generate the HC5 value.
TRA	Total Risk Approach. This approach integrates the background concentrations when deriving an EQS.
WFD	Water Framework Directive. Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in

---

[29] See e.g. Crane and Newman (2000), Environ. Toxicol. Chem. 19: 516-519; Azimonti et *al*. 2015; EFSA supporting publication (2015): EN-906; and Warne & van Dam (2008), Australasian Journal of Ecotoxicology 14: 1-5.

	the field of water policy.
WHAM	Windermere Humic Aqueous Model. A chemical speciation model for metals in soil and water systems.

ANNEX 6: Contributors

Contributors to the CIS activity: Development of a guidance document on the implementation of bioavailability-based EQS for metals

Activity leads	
France	Olivier Perceval
The Netherlands	Dorien ten Hulscher

Guidance authors	
Country/stakeholder	**Author**
France	Marina Coquery
	Olivier Perceval
Ireland	Daragh Cunningham
Italy	Stefano Polsello
The Netherlands	Leonard Oste
	Jos Vink
Eurometaux	Frank van Assche
Euromines	Johannes Drielsma
Nipera	Chris Schlekat
WCA	Graham Merrington
	Adam Peters

Model comparison Activity	
Country/Stakeholder	**Participant**
France	Olivier Perceval (Activity lead)
ARCHE consulting	Frederik Verdonck
	Charlotte Nys
Danish EPA	Henning Clausen
Deltares	Jos Vink
	Bas van der Zaan
Ghent University	Karel De Schamphelaere
RIVM	Anja Verschoor
WCA	Adam Peters

Members of the subgroup	
Country/Stakeholder	**Participant**
Austria	Karin Deutsch
	Manfred Clara
Belgium	Jeroen Vanhooren
Czech Republic	Tomas Micanik
Denmark	Henning Clausen
France	Sandrine Andres
Germany	Anja Duffek
	Maren Hess
Hungary	Maldini Kresimir
Italy	Stefano Polsello
Ireland	Daragh Cunningham
Sweden	Ann Sofie Wernerson
The Netherlands	Jos Vink
	Eric Verbruggen
UK	Helen Wilkinson
EDF	Franck Marolleau
Euromines	Johannes Drielsma
	Jaap van Nes
IZA	Frank van Assche
Nipera	Chris Schlekat
Uniper	MarieJo Booth
WCA	Graham Merrington
	Adam Peters

External reviewers	
Affiliation	**Reviewer**
Fraunhofer Institute (Germany)	H. Rüdel
Geological Survey of North-Rhine Westphalia	H. Schuster
Geological Survey of North-Rhine Westphalia	A. Ullmann
INRS ETE, Canada	P.G.C. Campbell
RIVM, the Netherlands	W. Peijnenburg
Rijkswaterstaat, Netherlands	H. Maas