TECHNICAL GUIDANCE TO IMPLEMENT BIOAVAILABILITY-BASED ENVIRONMENTAL QUALITY STANDARDS FOR METALS

April 2015

FOREWORD

Guidance documents have been produced to support the implementation of various aspects of the Water Framework Directive (2000/60/EC). These documents are aimed at delivering practical advice and assistance on various technical issues associated with the implementation of the Directive. However, adherence to guidance is not legally binding.

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 hazard of 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).

The revised Daughter Directive (2013/39/EU) includes annual average Environmental Quality Standards (EQS) for nickel (Ni) and lead (Pb) that refer to *bioavailable* concentrations. This Guidance Document has been developed to support the implementation of bioavailability-based EQSs for metals. This also includes consideration of specific pollutants common to many Member States, such as copper and zinc.

It is important to acknowledge that any relatively novel regulatory approach will present the need for changes to an existing way of working and potential challenges to implementation. The benefits of the new approach must outweigh disadvantages and this balance must be clearly apparent to technical, non-experts. The change required should provide environmental benefit alongside the opportunity for maintaining at the same level, or reducing, 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 text.

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

This is not CIS Guidance.

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GLOSSARY OF TERMS AND DEFINITIONS

Term	Definitions
BioF	The bioavailability factor. The BioF is based on a comparison between the expected bioavailability at the reference site and that relating 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. It is calculated by dividing the Generic or Reference EC10 by the calculated site-specific EC10.
BLM	This is a predictive tool that can account for variations in metal toxicity due to water chemistry. The tool calculates a site-specific effect concentration using information on the chemistry of local water sources, i.e. pH, calcium concentrations, hardness, dissolved organic carbon, etc.
User friendly bioavailability tool	Effectively is a simplified version of the BLM. It performs the same calculations as the BLM, but is run in MS Excel, requires fewer data inputs, and gives outputs that are precautionary relative to the full BLM but that are readily interpretable in the context of basic risk management and EQS compliance assessment.
HC5	Hazardous concentration for 5% of the species (based on the SSD).
DOC	Dissolved organic carbon. The input to the screening tool for DOC should be site-specific median concentrations from at least eight sampling occasions. Default waterbody values of DOC are available for some waterbodies[1].
ESR	Existing Substances Regulation (EEC No 793/93)
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.
AA-EQS	Environmental Quality Standard. A term used for the annual average.
$EQS_{dissolved}$ or $EQS_{totaldissolved}$	Environmental Quality Standard derived and measured as a total dissolved concentration, this is generally considered operationally as that metal passing through 0.45 µm filter.
$EQS_{bioavailable}$	Environmental Quality Standard derived under conditions representing high or maximum bioavailability. Also termed $EQS_{generic}$ or $EQS_{reference}$ (Section 1.3 in this Guidance).
Generic EQS	Generic Predicted No Effect Concentration, sometimes also termed the reference or generic EQS. This is representative of conditions of high bioavailability and is expressed as "bioavailable" metal concentration.
PEC	Predicted Environmental Concentration. These are usually replaced in the screening tool with measured environmental concentrations of

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[1] ENVIRONMENT AGENCY. 2010. The importance of dissolved organic carbon in the assessment of environmental quality standard compliance for copper and zinc. Draft final report SC080021/SR7a. Environment Agency, Bristol, UK.

	dissolved metal in the waters of interest.
PNEC	Predicted No Effect Concentration. This concentration is derived from the ecotoxicological data and site-specific water quality data using the BLM.
RCR	Risk Characterisation Ratio, also sometimes called the risk quotient. This is calculated by dividing the PEC by the PNEC. Values equal to or greater than 1 present a potential risk.
EC10	Effect concentration for 10% of the individuals in a toxicity test.

This document was drafted by wca (www.wca-environment.com) , on behalf of Eurometaux with input from Member State and Stakeholder experts. A separate Response to Comments (RCOM) document (titled: FINAL RCOM for bioavailability guidance for metals (November 2014)) accompanies this guidance.

1 INTRODUCTION

Across much of the world regulatory limit values (e.g., Environmental Quality Standards in the EU, Water Quality Criteria in the US, Australia, Canada, etc.) for metals in freshwaters vary according to water hardness (often with reference to specific taxa, e.g. Mance et al. 1984). However, following the adoption of these, water hardness has been found to be a poor explanation for differences observed for chronic toxicity for metals such as zinc, cadmium, copper, lead and nickel. This is illustrated for zinc in Figure 1.1.

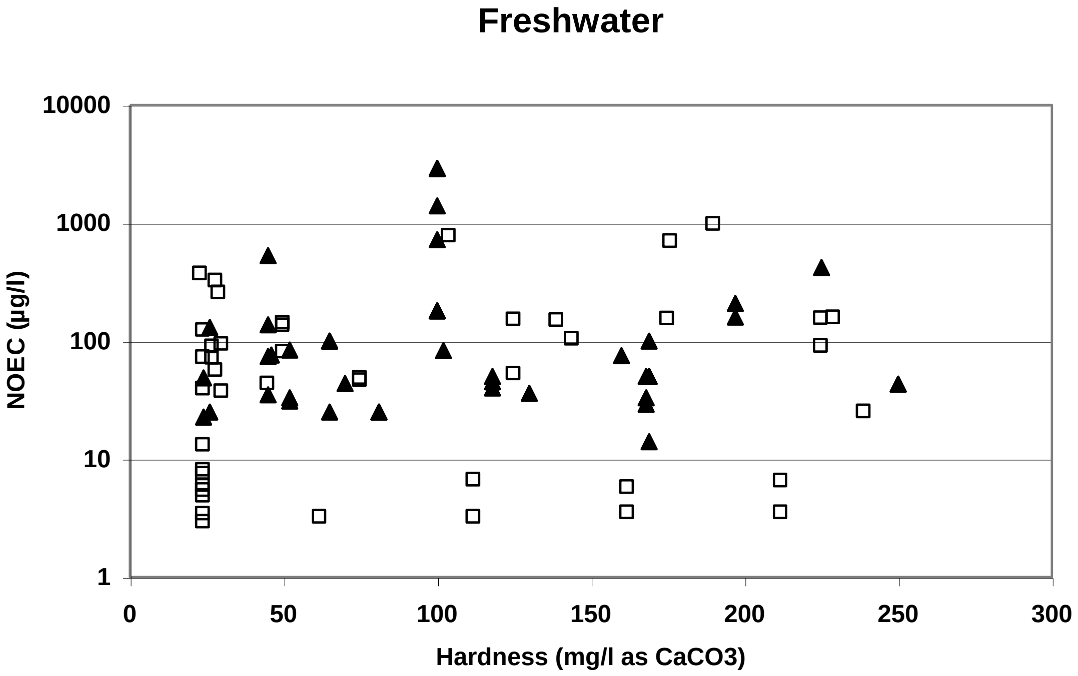

Figure 1.1 Relationship between hardness in freshwater and zinc toxicity, expressed as the NOEC (from: EU 2004).

The last 10-15 years have seen a major refinement in the scientific understanding of the behaviour, fate and toxicology of metals in the environment. As a consequence, the metrics conventionally used for the risk assessment of metals in soils, waters and sediments have been demonstrated to be prone to the incorrect estimation of likely ecological impacts (e.g. Zwolsman and De Schamphelaere 2007; Environment Agency 2008a; Environment Agency 2012b).

The revised Priority Substances Daughter Directive (2013/39/EU) includes annual average EQS for nickel (Ni) and lead (Pb) in the freshwater environment that refer to *bioavailable* concentrations. The concept of bioavailability influencing the hazard potential of metals in the environment is not new, but has received increased regulatory focus in Europe in recent years owing to a series of statutory and voluntary risk assessments performed under the Existing Substances Regulation (e.g. Nickel, EC 2008), which applied the approach. Efforts to incorporate the bioavailability concept into the risk assessment of industrial chemicals and the derivation of Environmental Quality Standards (EQS) under the Water Framework

Directive (WFD) have received scientific support more recently (e.g. SCHER 2010; EC 2011). However, accounting for the bioavailability of metals during the routine application of an EQS (i.e. as part of surface water classification under the WFD) represents a step change in the ways of working for most regulators and, indeed, stakeholders. Furthermore, there is very limited practical or regulatory-specific guidance available on the approaches that account for metal bioavailability, especially in relation to implementing an EQS (e.g. Brand et al., 2009).

Regulatory implementation of the bioavailability concept outside of Europe has focused on the use of acute ecotoxicity approaches to correct for long-term or chronic exposures (e.g. USEPA 2007). However, this approach is reliant on an assumption that the mode of action of a substance resulting in adverse effects after both acute and chronic exposure is similar, or that chronic effects can be accurately predicted from acute-to-chronic ratios, neither of which are supported scientifically. Therefore, approaches based on chronic data and validated under circumstances of long-term exposures are required to deliver robust long-term EQS and greater regulatory certainty.

1.1 Purpose and scope of guidance

This guidance needs to be both appropriate to, and useable by, practitioners in the regulatory and regulated communities. It must also reflect the full range of requirements and circumstances of those practitioners. This document provides the necessary guidance to facilitate the implementation of bioavailability-based standards for metals.

This guidance is concerned with the issue of checking compliance with EQS set with reference to dissolved bioavailable concentrations ($EQS_{bioavailable}$) which in Directive 2008/105/EU as amended by Directive 2013/39/EU currently are set for certain metals (Ni, Pb). It does not deal with the issue of setting an EQS or $EQS_{bioavailable}$.

It should be emphasized that in the cases where an $EQS_{bioavailable}$ has been set in EU this is the only legally binding EQS, here the $EQS_{bioavailable}$, for each substance which applies to all waters after bioavailability corrections of monitoring data. Thus when the concentration of bioavailable metal has been measured or calculated at a given site it can be compared directly to the $EQS_{bioavailable}$. In the case of permitting discharges to a given water body it could be a possibility to use bioavailability corrections to set a local compliance concentration. Such local compliance concentration shall not be exceeded as a consequence of the discharge.

This guidance document is based upon experiences and published regulatory reports (e.g. Vijver and De Koning 2007; Zwolsman and De Schamphelaere 2007; UBA 2008; Environment Agency 2009, Hommen and Rüdel 2012), open literature publications (e.g. Niyogi and Wood 2004; Peters et al. 2011a) and notes[2] from a 2011 workshop organised by Member States, where the implementation of bioavailability-based approaches were

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[2] http://bio-met.net/eu-member-state-workshop-on-metal-bioavailability-and-the-wfd/

discussed. This guidance also builds upon, and is in line with, CIS Guidance No. 27 (EC 2011).

The document has been drafted by different individuals, but has been steered and edited by a small group of Member State experts (The Netherlands, France and UK). This has involved the drafting and agreement of the guidance contents, commenting on complete drafts and final editorial control.

The primary focus of the guidance, at this current time, is *freshwaters* and in particular chronic or long-term exposures, i.e. the use of *annual average* EQS. Nevertheless, brief reference has been made to acute exposures and also corrections for availability in marine waters.

Links to European and other international reference documents are made where appropriate. The guidance has been written in a way that is generic and is not specific to a particular metal. This acknowledges the fact that the approaches described here, i.e. those based on the concept of the biotic ligand model, are currently only available for relatively few metals (copper, nickel, manganese, zinc). However, on-going research indicates that similar approaches are likely to be available soon for several additional metals (i.e. lead, cadmium, iron, cobalt).

The most scientifically robust $\mathrm{EQS}_{\mathrm{bioavailable}}$ are currently based on biotic ligand models (BLM), which are currently only available for relatively few metals (copper, nickel, manganese, zinc). At the time of the development of the latest draft of the Water Framework Directive there was no validated and accepted BLM for some metals, therefore complimentary availability-based approaches were adopted to define the $\mathrm{EQS}_{\mathrm{bioavailable}}$. The EQS for lead (an availability correction based on dissolved organic carbon) and cadmium (an availability correction based on water hardness) are examples of these complimentary approaches.

The scope of this guidance covers the practical steps required to implement an approach to account for bioavailability when using an $\mathrm{EQS}_{\mathrm{bioavailable}}$. The scientific basis of metal bioavailability and biotic ligand models are covered elsewhere (e.g. Heijerick, et al. 2002; Paquin et al. 2002; De Schamphelaere et al. 2005) and are only briefly described here in order to provide definitions and support the technical foundations of the approaches taken. The outline of the regulatory framework is described, as implemented by some Member States, as is the development of the user-friendly bioavailability calculation tools, including their required performance characteristics. The operational requirements of implementing $\mathrm{EQS}_{\mathrm{bioavailable}}$ are also illustrated by the experiences and preferences of several Member States. Calculations to support compliance and permitting are described step by step, as are the interpretation of results and 'trouble shooting'.

The answers to Frequently Asked Questions (FAQs), developed based on feedback received over the last five years, are addressed, together with a glossary, at the end of this guidance.

1.2 What is bioavailability?

There are many analytical and modelling techniques that purport to assess or measure metal bioavailability in freshwater. These include speciation-based modelling, ion selective electrodes, passive samplers such as diffusive gradients on thin films, kinetic ion exchange columns and ultrafiltration (e.g. Unsworth et al. 2006). However, these are effectively chemical measurements 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). These techniques can be considered to account for one half of what is bioavailability, i.e. the abiotic component.

Bioavailability can mean a number of different things depending on the particular area of science, but in relation to this guidance and the use of EQSs under the WFD, bioavailability is considered to be a combination of the physico-chemical factors governing metal behaviour (the abiotic part) and the biological receptor – i.e. its specific pathophysiological characteristics (such as route of entry, and duration and frequency of exposure).

Effectively this means that a measure of bioavailability will reflect what the organism in the water column actually "*experiences*" and so is of greatest regulatory relevance. This is important as it has long been established that measures of total metal in waters have limited relevance to potential environmental risk (e.g. Campbell 1995; Niyogi and Wood 2004).

1.3 What is the $\text{EQS}_{\text{bioavailable}}$ and how is it derived?

There are clearly several challenges associated with the derivation and implementation of EQS for metals that are not usually encountered when considering synthetic chemicals. These challenges include:

- Natural, or low level anthropogenic metal concentrations in surface waters;
- The form or speciation of a metal changes in response to water chemistry conditions;
- The form of the metal has a considerable influence upon bioavailability and subsequent ecotoxicity to aquatic organisms;
- Some metals are essential for the functioning of biological systems.

European Regulators have stressed the need to have only one numerical value for the EQS, if possible, for ease of implementation and communication. However, derivation of a single $\text{EQS}_{\text{dissolved}}$ for a metal for the whole of Europe will, in all likelihood, result in failures in waters where no adverse effects (i.e. reduction in ecological status) would be expected (because of low bioavailability) and conversely compliance where exposure would result in adverse ecological effects (because of high bioavailability).

By considering metal bioavailability, at both the EQS derivation and implementation stages, and by adopting a tiered, risk-based, approach (Section 2) it **is possible** to have a single value for an EU-wide metal EQS and not compromise its performance. This is possible where the EQS is expressed as an $\text{EQS}_{\text{bioavailable}}$. The $\text{EQS}_{\text{bioavailable}}$ corresponds to the bioavailable fraction (BioF) of dissolved metal in a sample, as determined by the physico-chemical characteristics of the water, and can be calculated using a biotic ligand model (BLM) or other calculation method (Section 3). To assess compliance, the bioavailable fraction of dissolved metal can be compared to the $\text{EQS}_{\text{bioavailable}}$. However, bioavailable metal is not the same metric as dissolved metal as only a fraction of the dissolved metal will usually be bioavailable. The exception to this will be where water chemistry conditions are particularly sensitive, the definition of which will vary with the metal. If a large part of the metal is bioavailable, the toxicological effects will mostly be high and the water will consequently be classified as "sensitive". This distinction is critically important as the application of an $EQS_{bioavailable}$ to dissolved metal monitoring data without appropriate correction will result in significant misclassification of the risk from the metal present in the water at a site. The only exception to this is where $EQS_{bioavailable}$ is compared to dissolved metal monitoring data in early tiers of a compliance assessment.

Alternatively, the bioavailable fraction of dissolved metal in a sample can be expressed as an $PNEC_{site\text{-}specific}$ or $PNEC_{local.}$ These metrics are expressed as dissolved metal and describe the concentration of dissolved metal at a site that, under the prevailing physico-chemical conditions, would correspond to a concentration of bioavailable metal equal to the $EQS_{bioavailable}$. Under conditions of low bioavailability the $PNEC_{local}$ could be appreciably greater than the $EQS_{bioavailable}$. Under conditions of high bioavailability the $PNEC_{local}$ would be similar to the $EQS_{bioavailable}$. Under conditions of maximum bioavailability (sensitive conditions), the $PNEC_{local}$ equals the $EQS_{bioavailable}$.

As discussed above, 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 initial assessments reduces the identification of false negatives, i.e. passing a water sample or site that should have failed (Type II errors).

It is therefore key to define the reference conditions that were used to derive the $EQS_{bioavailable}$. To do this it is necessary to have an understanding of the abiotic conditions that are likely to result in the greatest metal bioavailability (also known as the most sensitive conditions to metal exposures). Reference conditions vary between metals, however, an example is shown in Figure 1.2 for nickel (EC 2010a). The y-axis is the calculated HC5.

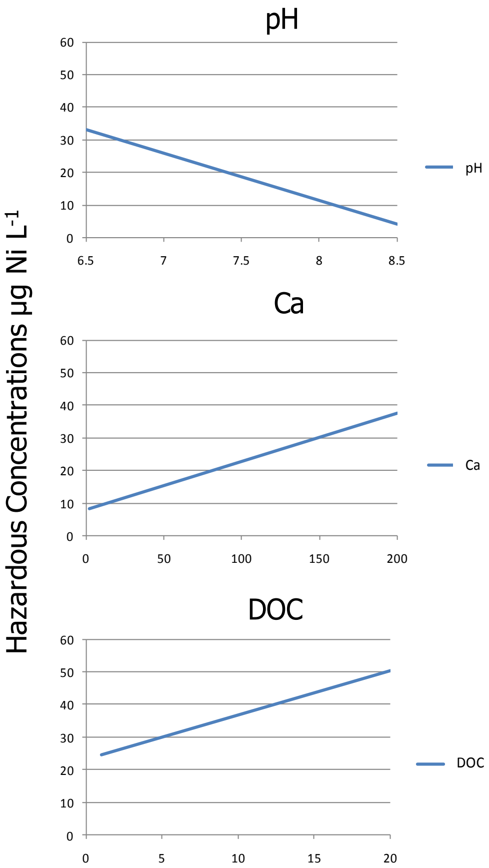

Figure 1.2 **Predicted 'stylised' changes in the ecotoxicity of dissolved nickel using the bio-met bioavailability tool. Results are expressed as an HC5, for pH, calcium (Ca mg l^{-1}) and dissolved organic carbon (DOC mg l^{-1}). Individual parameters were varied while the other two parameters remained constant (pH 7, Ca 120 mg l^{-1}, DOC 2 mg l^{-1}) (from: EC 2010a).**

In order to be able to identify conditions of high bioavailability, a biotic ligand model can be used to predict the dissolved nickel concentrations that may cause low level effects for particular water chemistry conditions (Section 3.1). So, a biotic ligand model (BLM) is a predictive tool that can account for variations in metal toxicity and calculates a site-specific Predicted No Effect Concentration ($PNEC_{site\text{-}specific}$) using information on the local water chemistry i.e. pH, calcium concentrations, hardness, dissolved organic carbon, etc.

Using the example of nickel and the NiBLM, a $PNEC_{site\text{-}specific}$ can be calculated across EU waters. The final reference condition selected must represent reasonable worst case bioavailability conditions in order that the $EQS_{bioavailable}$ is adequately protective of nearly all EU waterbodies when applied as a screening step within a tiered compliance assessment process (Figure 2.1)(EC 2010a). It is clear from Figure 1.2 that waters with low DOC and relatively high pH represent conditions of greatest sensitivity to nickel exposures.

Table 1.1 shows the 5th and 10th percentiles of $PNEC_{site\text{-}specific}$ derived using the NiBLM for EU datasets from England, Wales, Scotland, Sweden, Austria, Spain, The Elbe and Northern

France. This table is amended from the EQS summary sheet for nickel (EC 2010a). As shall be specified later in this Guidance Document (Section 4), the performance of this kind of exercise requires individual freshwater sample data for which all salient determinands (pH, DOC, etc) have been measured at the same time. From the table it can be seen that the Austrian waters are the most sensitive to nickel.

In a practical application of the EQS Technical Guidance (EC 2011) the reference condition for the $EQS_{bioavailable}$ is selected in order to ensure that 95% of EU waters from the most sensitive region are protected. In the case of nickel the most sensitive region of the investigated datasets for nickel 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 absolute lowest EQS derived from the underlying bioavailability relationship. The $EQS_{bioavailable}$ selected from the approximate 5th percentile of the Austrian dataset is 4.0 µg Ni L^{-1} (pH 8.2, DOC 2 mg L^{-1}, Ca 40 mg L^{-1}). Therefore, the $EQS_{bioavailable}$ is a dissolved metal concentration, but for water conditions (high pH and low DOC) that result in that dissolved metal concentration being highly bioavailable.

The derivation of a WFD EQS according to Technical Guidance (EC 2011) requires that QS in 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 sensitivity compared. The selection of compartments/receptors at risk is based on an understanding of the fate and bioaccumulation properties of the substance of interest. The selection of the 'overall' EQS for a substance from the various different compartment/receptor QS is based on the protection of the most vulnerable compartment/receptor (i.e. the most stringent standard). This concept is no different when considering $QS_{bioavailable}$ for the aquatic compartment. In order for a $QS_{bioavailable}$ to be selected as the 'overall' EQS it must be protective of all compartments across the likely conditions observed across the EU. Where an $EQS_{bioavailable}$ would not protect other receptors/compartments under certain water chemistry conditions the QS for the alternative receptor would become the overall EQS for those water conditions. This would have the effect of setting an upper limit to the range of possible $PNEC_{site-specific}$ derived from the bioavailability relationship.

Table 1.1 **The amended* 5th and 10th percentiles of Predicted No Effect Concentrations for nickel for EU Member States as calculated using the bio-met bioavailability tool (EC 2010a).**

Dataset and number of samples	10th Percentile	5th Percentile
England, Wales and Scotland (n = 184)	6.62	5.86
France (n = 249)	5.28	4.64
Austria (n = 1553)	4.34	3.7
Spain (n =48)	7.34	7.32
The Elbe (n =294)	8.22	7.46
Sweden (n = 3997)	11.2	10.08

Dataset and number of samples	10th Percentile	5th Percentile
Walloon (n = 559)	6.36	5.82
All data (n = 6885)	**6.58**	**5.2**

*The EQS for Nickel has an assessment factor of 1 in the amended Directive, although the EQS sheet for Nickel has not been updated to account for this.

In addition to $EQS_{bioavailable}$ being derived for nickel with the use of BLMs under the Amended Daughter Directive (2008/105/EC revised by 2013/39/EU) an $EQS_{bioavailable}$ is also given for lead. Although this is correctly detailed as an $EQS_{available}$ in the respective EQS dossier. The EQS for lead is an example of how a correction for water chemistry that can mitigate ecotoxicity may be accounted for in the compliance assessment of a metal. For lead there is a strong relationship between chronic ecotoxicity and DOC of the water. A precautionary relationship has been established between existing test data and DOC and this can be used to correct the measured Pb exposures in the sample from the waterbody into an "available" lead exposure.

This approach is used in other member states, for trace elements where the scientific evidence supports relationships or corrections based on mitigating water chemistry characteristics (e.g. for copper in marine waters, Environment Agency 2011). Implementing these approaches is discussed further in Section 3.3.

1.4 Using this guidance

This guidance establishes practical approaches for the implementation of $EQS_{bioavailable}$ for metals. For certain aspects of the implementation of $EQS_{bioavailble}$ there are currently several available options. The selection of an appropriate option will depend on existing regulatory frameworks in individual Member States. Figure 1.3 shows a schematic of the structure of this guidance and will assist in the identification of the most relevant sections for specific situations. This is an acknowledgement that some organisations and Member States have begun to establish the mechanisms required for the implementation of $EQS_{bioavailable}$, but for others this work has not yet started. The early sections of the guidance are descriptive, outlining principles and processes, with the latter sections detailing the practical and interpretative steps to be taken to implement the approach in a regulatory framework.

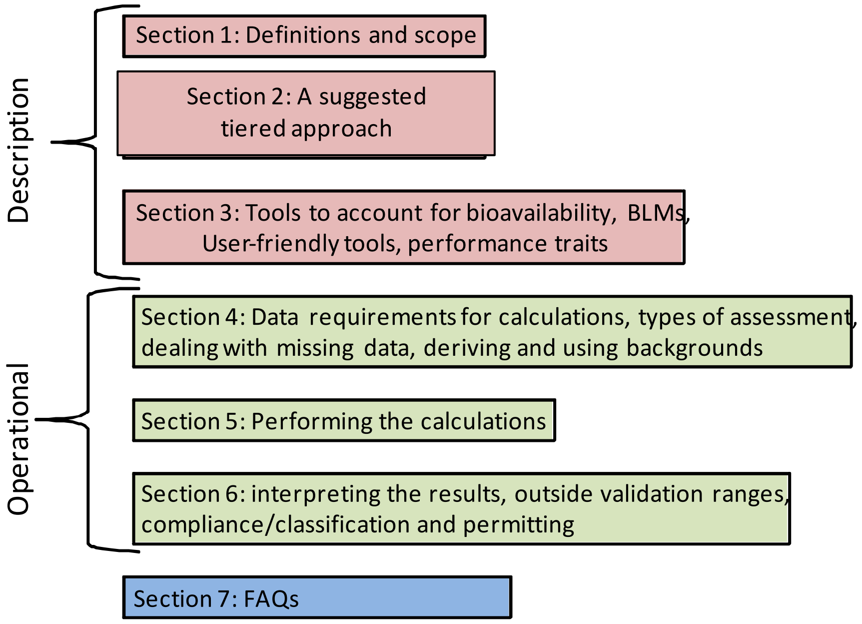

Figure 1.3 **Key sections of this guidance as split between descriptive and operational components.**

2 TIERED APPROACH FOR USING BIOAVAILABILITY

2.1 Why use a tiered approach to assess $EQS_{bioavailable}$?

The application of $EQS_{bioavailable}$ for metals within a tiered approach is consistent with classic risk assessment paradigms in that early tiers of assessment are precautionary, but simple to perform with large numbers of sites / waterbodies (as information requirements are low). The intention is to remove (screen out) low risk [of EQS failure] sites / waterbodies during early tiers of assessment. As progress is made through the assessment tiers the data and calculation requirements increase, but this effort is restricted to sites / waterbodies where metals potentially pose the greatest risk, thereby impeding the achievement of good ecological or chemical status. In applying this approach to the implementation of an $EQS_{bioavailable}$ for metals it is possible to have a single numerical value as the EQS, derived for reasonable worst case conditions (i.e. high bioavailability), but also be able to account for local water chemistry in a practical way (Comber et al. 2008; Environment Agency 2008).

A tiered approach to the implementation of $EQS_{bioavailable}$ also replaces the need for EQS banding, as conventionally used for EQS that vary with hardness. Banding can often result in dramatic changes in an EQS with relatively small changes in water hardness (e.g. moving from 200 to 201 mg $CaCO_3$ L^{-1} increases the cadmium EQS by greater than 60%). However, by using information on site-specific water chemistry factors that affect metal bioavailability in later tiers of compliance assessment, it is possible to provide greater realism to field conditions.

The tiered approach described below is also relatively easy to communicate to stakeholders as the yes/no decisions for progression are clear (at least through the first two tiers).

2.2 A suggested tiered approach, as applied in the UK

The tiered approach described here is one currently implemented in the UK. Although it is not prescribed, it suggests a logical process that might have value for other agencies. Each tier performs a function leading to a decision about classification (pass/fail), or the sample passes on to the next tier for further evaluation (Figure 2.1) so that a decision can be reached:

- **Tier 1.** The first tier in the scheme considers a direct comparison of the annual average concentration from monitoring data (dissolved metal – see Section 4.2) with the $EQS_{bioavailable}$ (so for nickel this would be 4 µg Ni L^{-1} and for lead, 1.2 µg L^{-1}). Although the $EQS_{bioavailable}$ is expressed as a "bioavailable" concentration, in the first tier of assessment it is compared to dissolved metal measurements. This means that the assessment is precautionary and false negatives are minimised. This tier is applicable to all freshwater waterbodies and the additional supporting physico-chemical parameters used for the calculation of the bioavailable fraction of metal (as discussed in Section 4) are not required. Only the dissolved metal concentrations (expressed as an annual average) are needed (Section 4.2). Sites, or samples, exceeding the $EQS_{bioavailable}$ at this tier progress to the second tier of the assessment.

- **Tier 2.** Ideally, this tier of assessment makes use of a user friendly tool[3] for the calculation of local metal bioavailability (either as bioavailability facto, concentration on bioavailable metal, BioF or $PNEC_{site-specific}$). User friendly tools perform bioavailability calculations to enable a comparison between the measured dissolved metal concentration at a site and the $EQS_{bioavailable}$. Matched water chemistry and metal data is preferred, but if these are not available, assumptions based on historic data or data from neighbouring locations can be used to identify if the collection of matched data is required for a robust assessment. Some Member States have automated these first two tiers in their Laboratory Information Management Systems (LIMS). For most metals, the effect of local backgrounds is most easily accounted for at tier 3. However, for some metals, where the EQS is expressed as an added risk approach (see Section 4.5), background concentrations should be accounted for as part of the EQS compliance assessment instead i.e. Tier 2. Currently, this would apply only to freshwater EQSs for zinc.

- **Tier 3**. Is not as specific as the first two tiers and is termed "local refinement". This tier would provide an opportunity to consider local issues that might affect the assessment of risk due to metals, e.g. local background concentrations of metals, or a more robust assessment of local water chemistry conditions (including possible running the full BLM). This tier can include several different options and alternatives that are aimed at confirmatory support for the identification of an exceedance at Tier 2 (Section 6). The form of this tier will depend upon local regulatory considerations.

- **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. Consideration of a programme of measures to mitigate the situation, within the appropriate cost/benefit framework, may be required.

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[3] User friendly tools are described in greater detail in Section 3 of this guidance.

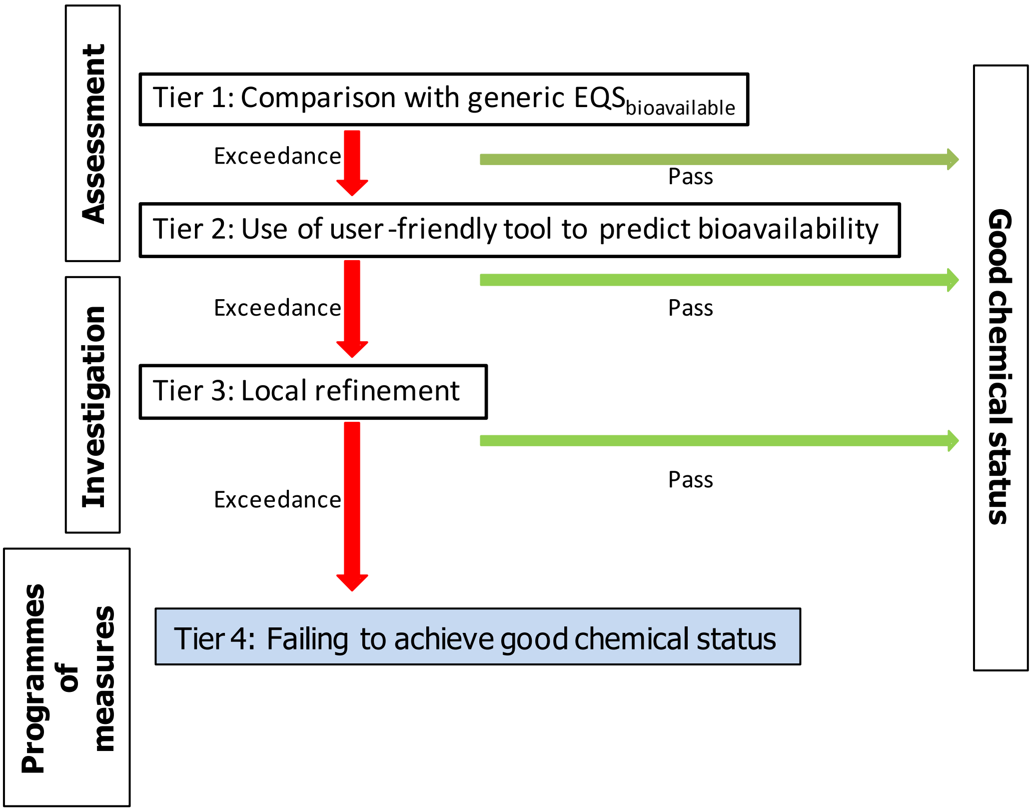

Figure 2.1 **Flow diagram of the possible stages of a tiered EQS compliance assessment under the Water Framework Directive (updated from Environment Agency 2009b).**

3 TOOLS TO ACCOUNT FOR (BIO)AVAILABILITY

There are a range of potential tools to estimate the influence of water chemistry on metal ecotoxicity, including speciation models and surrogate measures of availability, such as Diffusive Gradients in Thin films (Warnken et al. 2009). In this section, we outline two approaches for the implementation of two EQS in the current Amended Daughter Directive (2008/105/EC revised by 2013/39/EU), these methods are utilised widely for Specific Pollutants at Member State level. The first approach is the use of user-friendly tools based upon biotic ligand models, the second is accounting for availability through the development of relationships, based on scientific evidence and supporting ecotoxicity data, to account for the mitigating effects of water chemistry parameters upon chronic toxicity, such as for the current lead EQS (Section 3.3).

The tool that most effectively accounts for metal bioavailability, the only one with a direct link to toxicological endpoints, and hence that with the greatest potential to be applied in EQS derivation and compliance assessment, is arguably a BLM (Santore et al. 2001, 2004).

A BLM is a mathematical model that uses information on water chemistry, e.g. pH, calcium concentration, alkalinity, dissolved organic carbon (DOC) to predict metal toxicity. A BLM accounts for both the biological component (including ion competition at the biotic ligand) and chemical complexation interactions in the water column. A BLM is derived from a structured programme of ecotoxicity testing (numerous tests conducted under different physico-chemical conditions) using a single species of aquatic organism. Therefore several discrete BLMs are usually required to describe the bioavailability of a metal across several trophic levels i.e. fish, invertebrates and algae. Whilst BLMs are derived for a particular species, it is possible to perform cross species validation and extrapolation of a BLM model to allow all available ecotoxicity data for a metal (as long as sufficient information on water physico-chemistry parameters are reported alongside ecotoxicity endpoints) to be "normalised" to a specific water physico-chemistry (e.g. Van Sprang et al. 2009). When BLMs are applied to a species sensitivity distribution (through a process termed "full normalisation") site-specific PNEC or BioF values can be derived from the normalised HC5 value. Figure 2.2 shows various SSDs for nickel, normalised to different water physico-chemistry conditions and corresponding HC5 values (EC 2008).

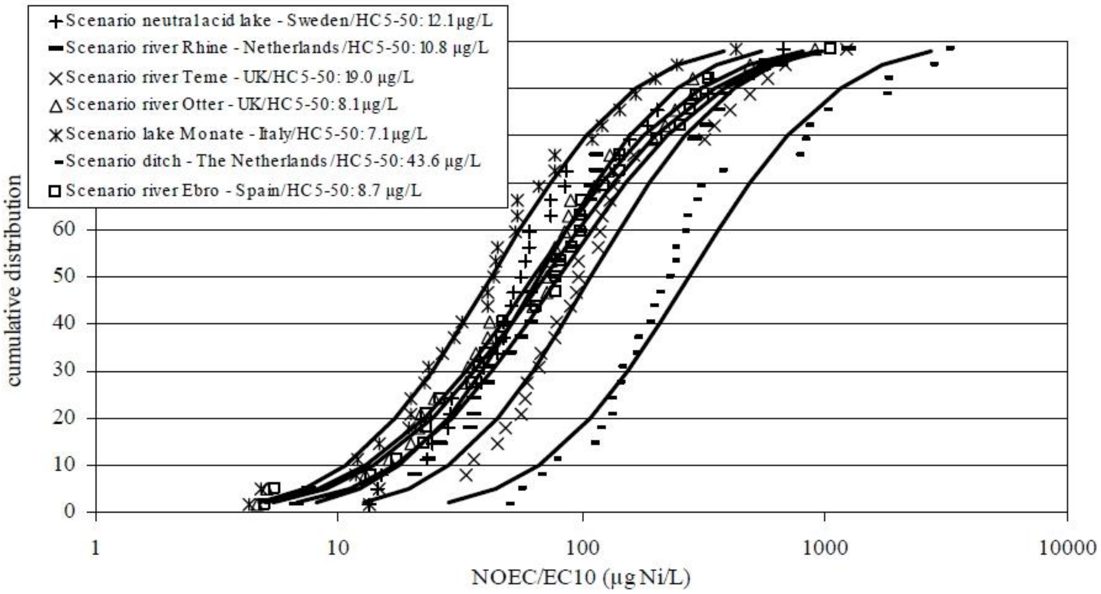

Figure 2.2 Examples of "full normalisation" of nickel SSD based on different water physico-chemistry (from EC 2008).

Some generalisations that can be made about BLMs (some of which are also described in the Section 8 FAQs) are:

- **Each model is developed from laboratory data for a single species tested under a range of different physico-chemical conditions using standard and field-collected waters.**
- **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 an alga.**
- **Discussions in previous guidance (e.g. EQS TGD, EC 2011), refer to <u>a</u> BLM for a metal. As described above this 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 readily applied to a different metal. Similarly, acute and chronic BLMs for the same metal may also not be interchangeable. There is a defined range of physico-chemical conditions (i.e. pH, DOC and calcium) over which a BLM has been validated (Section 6.1.1). These conditions are defined by the physico-chemical conditions 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.**
- **Acute and chronic BLMs have been developed, but until relatively recently all BLMs were not readily accessible to the user communities.**
- **In Europe, chronic BLMs have received more regulatory attention than acute models. This was driven by the ESR process which required a process with which to assess risks from long-term metal exposures.**

- **The BLMs for copper, nickel and zinc have been validated under field conditions to assess predictive capability.**
- **All of the current chronic BLMs, and those in development at the time of writing this guidance, have been funded by Industry with the testing and interpretation performed by universities, independent consultants and regulators.**
- **All of the BLMs currently fit for regulatory use are for freshwaters. Research is on-going at various universities considering specific models for marine waters. At present, only an 'availability' correction for copper using DOC in marine waters is currently embedded in regulatory frameworks (Environment Agency 2011).**

3.1 Biotic ligand models and user friendly tools

The discussions in this guidance are focussed upon chronic BLMs, and where reference is made to BLMs, unless explicitly stated, it refers to chronic models and specifically to the integrated versions of those models that can undertake full normalisation of an SSD. Despite the potential regulatory virtues of using BLMs, the reality is that they are relatively complex tools, comprised of a series of different elements e.g. metal speciation calculations, ecotoxicity database normalisation, species sensitivity distribution (SSD) fitting etc. (Environment Agency 2009b). Furthermore, the models are very data intensive in terms of the number of different physico-chemical input parameters required - although these can be simplified (e.g. Peters et al. 2011a) - and require considerable skill and expertise in terms of data processing (e.g. speciation calculations) and output interpretation (Environment Agency 2012a). All this may be reasonable in relation to performing an assessment for a small number of sites or waterbodies. However, use of BLMs is unlikely to be considered as fit for purpose for regulators, or indeed the regulated community, who have numerous regulatory duties to perform.

Therefore, in early 2009, the Environment Agency of England and Wales, in collaboration with Industry partners[4], assessed the potential of developing a simplified or "user-friendly" version of the existing BLM for copper, with the aim of being able to account for bioavailability for a large number of samples whilst requiring fewer inputs than the conventional model. The user friendly tool was also required to operate in a standard Microsoft Office 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 (Environment Agency 2009b) (Section 3.2). Effectively, a user friendly bioavailability tool mimics the BLM upon which it is based, but with a slightly reduced level of predictive performance (e.g. Environment Agency 2009c; 2010a; 2012; 2014a).

There are several user friendly bioavailability tools currently available for regulatory use (i.e. M-BAT, bio-met, PNECpro[5]). It is not the intention of this guidance to recommend any

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[4] European Copper Institute (ECI), International Zinc Association (IZA), Nickel Producers Environmental Research Association (NiPERA).

[5] www.pnec-pro.com

particular tool/s. However, in the next section the minimum performance characteristics that any user friendly bioavailability tool should have before application for regulatory purposes are discussed. A comparison between the performance of two of the currently available user friendly tools (that meet the performance characteristics given in Section 3.2), for three trace elements nickel, copper and zinc, using several European datasets, is given in Appendix 2 to this guidance.

In order to account for the numerous influences on metal speciation and toxicity, the available user friendly tools require data for a greater number of water chemistry input parameters than hardness-based metrics (Section 4). Other user friendly tools, not based on full bioavailability considerations or BLMs at present, exist for the application of EQS for lead, cadmium, copper-marine and likely several others (Section 3.3).

3.2 Performance characteristics of user friendly tools

To account for bioavailability using a user-friendly tool, it is important to be confident in the performance characteristics of that tool and also the scientific integrity of the datasets which are its foundation. The ecotoxicity datasets on which the BLMs for copper, nickel and zinc are based have received extensive regulatory peer-review (EU 2004; ECI 2007; EC 2008; EC 2010a; Environment Agency 2010b) and the details of the development of the models, cross-species extrapolation and validation in natural waters have been widely published in the open literature (e.g. Heijerick et al. 2002; 2005; De Schamphelaere et al. 2003; De Schamphelaere and Janssen 2004; Deleebeeck et al. 2007; Schlekat et al. 2010).

While there are few user-friendly bioavailability calculation tools currently available, this guidance should be applicable to these tools and may also be used to facilitate the development of further tools, for which the performance characteristics should be defined. Therefore, user-friendly tools for estimating bioavailability:

- **Should include the most contemporary, quality controlled ecotoxicity dataset for the respective metal;**
- **Should automatically default to the $EQS_{bioavailable}$ or generic EQS under "sensitive conditions" as defined by 2008/105/EC revised by 2013/39/EU;**
- **Need to be based only upon validated BLMs that have been assessed against ecotoxicity data generated in natural waters, mesocosms and in the field and shown to deliver predictions in line with those reviewed under Existing Substances Regulations;**
- **Where an EQS has been derived using a BLM, the user-friendly tool should be based upon that same full BLM. This should include the same ecotoxicity dataset, binding coefficients, normalisation process, speciation calculations, method to account for cross-species extrapolation and intrinsic sensitivity.**
- **Have validated physico-chemical boundary conditions that reflect the physico-chemical ranges of ecotoxicity data upon which the BLMs are based (Section 6.1.1), i.e. all the user friendly tools should have the same boundary conditions (unless policy decisions are made to depart from**

these). All relationships based upon ecotoxicity data and water chemistry have a validation range, i.e. the water chemistry conditions under which the tests were performed. Outside these ranges, user-friendly tools and the BLMs on which they are based, do not necessarily make incorrect predictions of bioavailability, but are less certain than predictions made within the validated range. Each metal has a different validation range, which reflects the different ecotoxicity datasets and water chemistry relationship that were used in their development;

- **Should give "flags" to indicate to the user when the water under consideration is outside those boundary conditions or applicability ranges (Section 6.1);**
- **Should give a within factor of two agreement to the respective BLM output;**
- **Have clear and transparent information on the derivation and validation of the tool;**
- **Have clear and sufficient documentation that describes how to undertake the calculations;**
- **Have a clear indication of the $EQS_{bioavailable}$ that is used in the calculations to facilitate regulatory interpretation.**

3.3 Compliance tools accounting for metal availability

Under the WFD, corrections for availability with varying water chemistry conditions can be made for lead and cadmium. These corrections are not as scientifically sophisticated as using BLMs as they may only take account of some, and not all, of the important factors influencing ecotoxicity. For the other metals and organo-metals; mercury and tributyl tin, no corrections are available.

For cadmium the correction is hardness-based, with the EQS varying over four water hardness bands. This is based upon a relationship developed by the USEPA for soft waters only with no data for harder and higher pH waters (e.g. Mebane 2010). There are no readily available compliance tools for cadmium.

The EQS for lead 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 lead toxicity. The EQS (EC2010b) assumes that there will not be any species in natural freshwater ecosystems for which the relationship between DOC concentration and EC10 would have a lower slope that that derived for *P.rapida* and thus is precautionary in nature. This relationship is given below in the equation (from EC 2010b):

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

Where:

$PNEC_{site}$ = Predicted No Effect Concentration at the site under consideration

$EQS_{available}$ = Generic or Reference EQS = EQS for a reference condition to ensure all water bodies are protected.

DOC = Dissolved Organic Carbon at the site under consideration

$DOC_{reference}$ = average Dissolved Organic Carbon (DOC) concentration in the ecotoxicity tests that the $EQS_{available}$ is based upon, 1.0 $mg.L^{-1}$.

An excel-based tool is available that facilitates the rapid undertaking of this calculation to assess compliance with the input of dissolved lead concentration and DOC. It is important to note that the $EQS_{available}$ for lead was derived before the lead BLM was available. Therefore for compliance purposes an availability correction based on dissolved organic carbon should be employed.

Member States have also developed similar water chemistry corrections for specific pollutants, i.e. national EQS (e.g. for copper marine, Environment Agency 2011b).

4 DATA REQUIREMENTS

This section provides guidance and further references on the data requirements for implementation of an $EQS_{bioavailable}$. A complete dataset (particularly for physico-chemical parameters) will not be available for every site / waterbody for which they are required. In these instances an assessment of bioavailability may still be possible using alternative data defaults or surrogates.

Equally, it is critically important to remember that the first tier in the risk assessment approach described in Section 2 (as shown in Figure 2.1) does not require information on water physico-chemistry as it assumes that metals in the water column are highly bioavailable. Compliance with the $EQS_{bioavailable}$ at the first tier means there is no further need to progress the site (or sample) through the subsequent tiers. This early comparison can be used to focus monitoring attention on particular waterbodies or river basin districts where water chemistry conditions may lead to ecosystems that are sensitive, or exposures to metals are expected. This may be an important consideration where regulators or stakeholders have limited data (or monitoring budget) available to adopt the bioavailability-based approach.

This section provides details on generic data requirements and some specific options for dealing with monitoring data, with a view to processing these data with a user-friendly tool to account for bioavailability.

4.1 Data handling considerations

To account for bioavailability using a user-friendly bioavailability tool requires that, ideally, the concentration of dissolved metal is accompanied by 'matched' data for supporting physico-chemical parameters. Those supporting parameters include, at the very least, dissolved organic carbon (DOC), pH and a measure of water hardness, preferably dissolved calcium (Section 4.3.1). However, for some user-friendly tools that are not based on BLM at present may not need all of the other three parameters. For example, the application of the $EQS_{bioavailable}$ for lead, which is based on a DOC correction, need only "matched" DOC data.

The term 'matched' here means that the supporting water chemistry parameters are sampled at the same site as where the metal concentration is taken and preferably also at the time, i.e. one sample is taken from a site from which the dissolved metal and supporting water chemistry parameters are all determined.

Matched data for all of the required inputs for the user-friendly tool to account for metal bioavailability is the preferred situation. However, it is recognised that this is not always a realistic situation, especially for those in the early phases of making the transition to consideration of an $EQS_{bioavailable}$.

Some options of how data may be used are provided below with some of the reasoning behind those options and the implications for selecting an option.

4.1.1 Calculating annual average concentrations for bioavailability

The way data from individual samples are treated can have an impact on predictions from the user-friendly tools. This is especially the case when considering comparison of monitoring data against an EQS that is derived as an annual average.

The annual average concentration of dissolved metal can be derived simply by taking the monthly sample concentrations for the year and dividing them by 12. However, in accounting for bioavailability using the user-friendly tools there is a need to provide similar summary statistics for the supporting water chemistry (unless all the data, including supporting determinands, are 'matched'). 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. ISO 2008; Environment Agency 2009b).

The preference for a bioavailability-based assessment is for the required supporting physico-chemical data to be matched to dissolved metal data on an individual sample basis (i.e. dissolved metal and supporting physico-chemical determinands 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) are calculated and the average computed and then compared to the $EQS_{bioavailable}$ using either a "face value" (direct comparison of the measured value against the EQS) or "confidence of failure" (accounting for variability in the sampling and measures) based compliance assessment. Decisions about or failure of bioavailable metal EQSs based on sampling are subject to uncertainty, like any other standard. Detailed guidance on assessment of compliance with standards is to be found in ISO Guidance (ISO 667-20:2008).

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. However, this approach may not be appropriate in 'flashy' or highly seasonal catchments as periods of high bioavailability may be obscured by the use of a summary statistic. Using a single mean 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 4.1.2).

Assessments of the influence of data aggregation have been undertaken to assess the influence of using individual matched data compared with averaging water quality data over a year and generating site-specific PNECs. 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 in regard to the appropriateness of aggregating data.

4.1.2 Dealing with variability within catchments

Variability within catchments is common rather than an exception and it is present when assessing compliance for all chemicals not just those for which bioavailability is to be accounted for. The magnitude of variability can be analysed from historic geographic 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. The influence of seasonality on water composition in some catchments is well understood (Verschoor et al. 2011). Parameters like DOC, Ca, Mg, and HCO_3, may vary up to a factor 2 easily in one year in some catchments. As a consequence, ratios between lowest and highest potential risk may occur with the same factor. 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 in fact 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 a catchment (e.g., downstream) may result in an EQS failure although 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 maybe 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 metal bioavailability. This is likely to be associated with factors that may influence DOC levels (Section 6.3) or pH.

4.2 Dissolved metal monitoring data

Dissolved metal concentrations (in µg L^{-1}), as noted in the amended WFD (EC 2013), refer to the concentrations of metals determined in a water sample obtained by filtration through a 0.45 µm filter or any equivalent pre-treatment.

Filtration can introduce numerous artifacts that, if unaccounted for, will lead to the collection of a highly heterogeneous and scarcely reliable data. At equal nominal pore size, different filter types may lead to different concentrations of trace elements in the filtrate. For the same filter material, membranes with a larger surface perform better than membranes with smaller surface (e.g., cellulose acetate filters with a diameter of 142 mm give higher concentrations than units with a diameter of 47 mm). Polycarbonate filters have a more accurate cut-off (better correspondence between nominal and actual), but tend to get quickly clogged and to yield lower concentrations of filterable elements. This feature and their higher costs do not make them the ideal choice for large monitoring programmes. There are some clear recommendations when undertaking a filtration of water samples in order to maintain an indication of factors potentially leading to filtration artifacts, including:

- Record the total volume of filtered water;
- Record the filter type (e.g. lot number, material, diameter, nominal pore size);
- Record the applied filtration technique (e.g. syringe, N_2 pressure, negative pressure – i.e., use of a hand pump);
- Record the details of the filtration procedure used to collect samples for metal and 'matched' ancillary analyses:
    - splitting of a sufficiently large filtered volume (collected using only one filter) into smaller aliquots for the various parameters
    - collection of independent aliquots for metals and 'matched' ancillary parameters during filtration of a single sample with a single filter
    - collection of independent samples for metal analysis and 'matched' ancillary parameters)
- Record the filtration rate (e.g., Time taken to filter 100 mL aliquots) or note any visible reduction in the filtration rate during sample collection.

Besides the 'reporting' about details of the filtration procedure listed above, a consistent way to collect filtered samples for analysis of trace elements is given below, but it is strongly recommended to refer to the USEPA document "Collecting water-quality samples for dissolved metals-in-water" (2000):

- Be sure to use only filters, syringes and sample containers that have passed quality control at the laboratory that will receive the samples for analysis
- Keep the filters and syringes in tight packaging until use. Follow clean procedures (USEPA 2000)
- Discard the first 4-5 ml, and filter 10-15 ml sample for analysis
- Filter the sample at the earliest convenience after collection (ideally, filter it in the field). If possible, filter into sample containers with preservation acid already added, so the samples are preserved immediately after filtration.
- If a second aliquot is needed for storage for further verification of the analytical results, collect it from the same primary bulk sample but filter it with a second filter following the procedure above;
- Perform a filtration blank, or ask the laboratory that will analyze the samples to do so. The filtration blank must be done in the same laboratory where samples are filtered or in the field if samples are filtered in the field. The water used for blank filtration shall have no measureable content of the metals of interest.

Analytical tools and techniques that can determine concentrations of trace elements in water samples are now readily available in commercial and regulatory laboratories. The primarily analytical methodology is Inductively Coupled Plasma Mass Spectrometry (ICP MS) as this offers the appropriate levels of sensitivity and quantitation needed to for the bioavailability-based approach. There is little point in undertaking an assessment of potential risks from metals accounting for bioavailability if the limit of quantitation or detection of the metal in question is greater than the $EQS_{bioavailable}$, unless there are values above the LoD. The QA/QC Directive clearly states the need to ensure that the LoD/LoQ is 30% of the EQS in order to assess compliance. This is often the case when considering historic (e.g. FOREGS[6]) or regulatory datasets[7] (Section 4.4.2).

As with all monitoring and assessment, the quality of the data are, in-part, dependent upon the skill, experience and understanding of the sampling and laboratory staff undertaking the assessment. Guidance is available elsewhere on these types of activities (e.g. USEPA 2000).

Furthermore, the use of 'total' metal concentrations data in the bioavailability approach should best be avoided as the calculations are dependent upon the input of dissolved metal concentrations (as suggested to be measured by the WFD, EC 2013). Total metals data should only be used for the quantification of annual metal loads or for exercises related to assessment of the implementation of the biovailability-based approaches, and even then with an explicit acknowledgement that the results are at best **indicative**. For Tier 1, if total metal concentrations are below the $EQS_{bioavailable}$ then there is no need to proceed further. For Tier 2, total metals data may be used as a means of reducing the number of sites for which dissolved data may be required.

Historic regulatory metals data are often expressed as total concentrations, and attempts have been made, using the partitioning equations provided under REACH guidance[8], to estimate the dissolved concentration of zinc from a measured total concentration. These equations apply a suspended matter–water partition coefficient ($l \cdot kg^{-1}$) and the concentration of suspended solids ($kg \cdot l^{-1}$) to calculate the dissolved zinc concentration ($\mu g \cdot l^{-1}$). The Environment Agency of England (2009e) applied these equations to both the KP value from the Zinc Risk Assessment (EU 2004) of 110,000 l·kg-1 and using a fitted K_P value from measured data from 740 samples of Scottish surface waters. This dataset had matched sample information including pH, suspended solids, dissolved organic carbon (DOC), total organic carbon (TOC), dissolved Zn, and total Zn. A comparison of the observations and predictions of dissolved zinc concentrations using the fitted K_P value of 152,141 $l \cdot kg^{-1}$ is shown in Figure 4.1.

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

[7] http://water.europa.eu/

[8] http://echa.europa.eu/documents/10162/13632/information_requirements_r7a_en.pdf

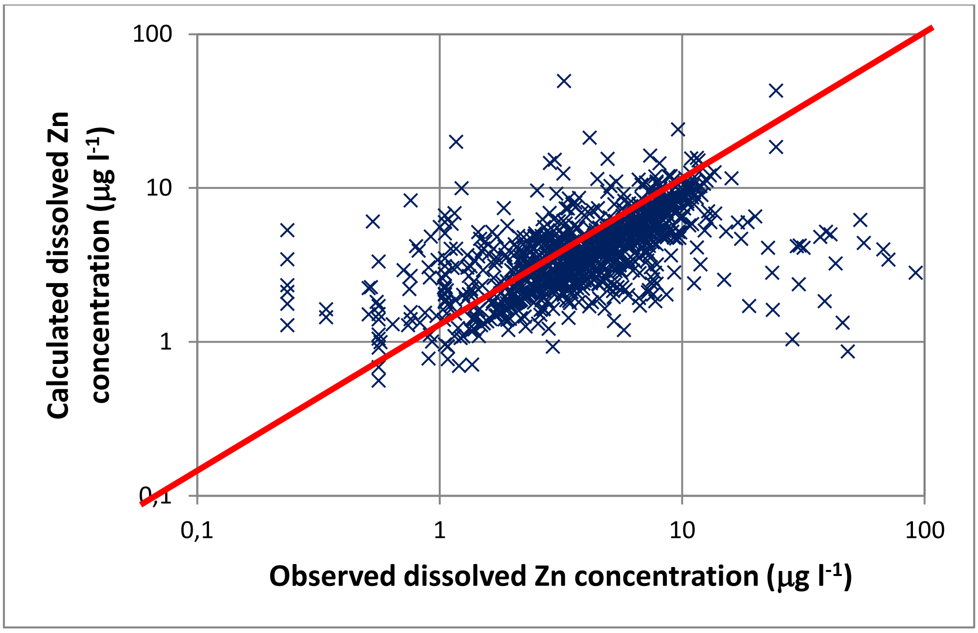

Figure 4.1 **Performance of predictions of dissolved Zn from total Zn freshwater data (from Environment Agency 2009e). The line represents the optimal 1:1 agreement.**

The standard deviation in the predictions of dissolved Zn concentrations from total Zn concentrations shown in Figure 4.1 is 7.7 µg L^{-1}. This means that approximately 95 percent of estimates of the dissolved Zn concentration will be accurate to within around 15 µg L^{-1} (the $EQS_{bioavailable}$ for zinc in the UK is 10.9 µg L^{-1}). The 95th percentile of dissolved zinc concentrations in the Scottish dataset that was used here for this testing is only 11.5 µg L^{-1}, indicating that in most cases the error will be greater than the result. The K_p-based approach is not recommended.

It is very important to enter dissolved metal data into the user friendly tools or availability calculations if undertaking compliance assessment. However, total metals data may be used for screening out sites and for feasibility studies. Conversions between total and dissolved metal concentrations are of limited reliability and will introduce a level of uncertainty into any regulatory decisions based on these types of data. Dissolved metal data are most appropriate in this approach.

4.3 Physico-chemical monitoring data

Almost all member states will have measures of pH and hardness in freshwaters (e.g. European Water Datasets[9]), although for the latter there is often limited consistency in methodologies between, and within, national datasets. However, it is for DOC where there are the fewest data. DOC is a required input to all user-friendly tools and it is a relatively cheap parameter to analyse. As has been indicated above, matched data are preferred.

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[9] http://water.europa.eu/

All assessments of potential risks are dependent upon the quality of the data used. In approaches accounting for metal bioavailability this is no less important and sampling, preservation of water and analysis should at all times follow the principles laid down in the CIS Guidance Note 19, Guidance on Surface Water Chemical Monitoring[10].

4.3.1 Measures of pH, Ca or hardness

Freshwater pH is routinely measured in samples in the field using a range of commercially available pH meters. Under some circumstances, for example when the transport time of the samples back to the laboratory is relatively short, measurement may be undertaken at the laboratory. Measures of pH are almost always included in the supporting suite of general water quality determinands for most samples.

Measures of water hardness in regulatory datasets can include for example; French, English, USA and German degrees, dissolved concentrations of calcium and magnesium, concentrations recorded as mg CaO L^{-1}, mmol L^{-1}, meq L^{-1}, etc. Therefore, the requirement, as a minimum, for the input of dissolved calcium into user friendly tools can represent a complex challenge.

However, it is possible to convert between these units and measures based on relationships developed for European freshwaters (and also predict missing inputs for some physico-chemical parameters if using the full BLMs, e.g. Peters et al. 2011a). A simplified hardness calculator/convertor tool is available online to perform these transformations[11]. For most of the metals for which BLMs are available, calcium/hardness has been shown to be the least influential parameter under most water chemistry conditions when compared to pH, and especially, DOC. For example, both Ca^{2+} and H^+ compete with nickel for occupancy of binding sites at the biotic ligand (and also similarly with DOC). High H^+ activities are found in acidic waters, and low H^+ activities in alkaline waters, whereas high Ca^{2+} activities are found in hard waters and low Ca^{2+} activities are found in soft waters. Softer waters, which have very little calcium competition for nickel binding to the biotic ligand, tend to have lower pH values, and therefore greater proton (H^+) competition for nickel binding to the biotic ligand. Consequently the overall effect of calcium, or hardness, on nickel toxicity is very limited. This relationship is reflected in the user-friendly tools with limited influence of changes in calcium concentrations upon the site-specific PNECs.

4.3.2 Dissolved organic carbon

Dissolved organic carbon (in mg L^{-1}) can be considered to be comprised of humic substances made up of heterogeneous polyfunctional polymers that are formed through the breakdown of plant and animal tissues by chemical and biological processes (Thurman, 1985). Importantly, DOC is not a single substance and not all DOC is the same. Yet all DOC contains functional groups (ligands) that bind free metal ions (the most toxic inorganic metal fraction) and reduce the interaction between free metal ions and aquatic organisms, and is consequently important for controlling adverse effects associated with metal exposures.

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[10] http://cliwat.eu/xpdf/Guidance%20no%2019%20-%20surface%20water.pdf

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

Dissolved organic carbon can come from natural and anthropogenic sources. Discharge derived DOC (such as sewage effluents) is likely to be of a different composition to natural DOC, and is comprised of proteins, amino acids, polysaccharides and synthetic chelating agents such as ethylenediamine tetraacetic acid (EDTA). The current evidence base suggests that this latter type of DOC has a much greater capacity to bind metals compared with similar concentrations of naturally sourced DOC (e.g. Sarathy and Allen 2005; Baken et al. 2011) (Section 6.3).

Nevertheless, the user friendly tools are based on the relationships established with the respective BLMs and the role of DOC binding was outlined in the European Risk Assessment Reports (e.g. ECI 2007). It was concluded that when considering metal binding to natural organic matter, typically 50 % of the DOC is to be considered as active fulvic acids (no binding to humic acids was considered). Despite the acknowledged variability of DOC binding properties, the variability in DOC characteristics is not large enough to substantially affect the predictive capacity of the different BLMs. This has been evaluated by performing toxicity tests with waters containing DOC from different sources (e.g., Deleebeeck et al. 2006). By assuming one 'average' binding property for the DOC in natural waters, most chronic ecotoxicity data for algae, daphnids and fish were predicted by an error of less than a factor of two. So the BLMs, without accounting for differences in DOC binding properties, perform equally well in estimating the chronic toxicity in a given water as performing an ecotoxicity test in that water.

Many Member States did not have routine DOC monitoring in place to perform bioavailability-based compliance assessments when $EQS_{bioavailable}$ where being discussed at EU level. However, many have since included DOC as a regular monitoring parameter (e.g. France, Ireland, UK, The Netherlands). DOC is needed for all the metals in the user friendly tools and also for lead. Furthermore, it is possible through screening-based feasibility assessments to prioritise those sites for which DOC measures are needed (this could be due to site sensitivity, potential metal exposures or highly variable hydrological regimes (Section 4.1.2).

4.4 How to deal with missing data?

Screening assessments or feasibility studies on implementing a bioavailability-based approach for metals have been undertaken by several Member States (Environment Agency 2009b; Geoffroy et al. 2010; Hommen and Rüdel 2012; Cousins et al. 2009; Hoppe et al. 2009). These assessments have been undertaken on relatively limited datasets compared to routine historic compliance assessments due to data limitations most often associated with a lack of DOC data or dissolved metal concentrations. However, these assessments are able to inform the decisions in relation to regulatory applicability of the BLMs and also the user friendly tools. This is particularly true in relation to the range of physico-chemical water chemistries and the validation boundaries of the user friendly tools (Section 6.1).

The user friendly tools require data inputs for monitoring data of at least pH, DOC and Ca (perhaps with the addition of magnesium and sodium for some tools). Without these, the user friendly tools will either not run or not run reliably. Dissolved organic carbon was a determinand that was not routinely monitored in freshwaters in many European Member States, including the UK. However, in the past a large amount of DOC data were collected across most Environment Agency regions. These historical data allow estimation of DOC default values for many waterbodies and most hydrometric areas in England and Wales (Environment Agency 2009b, Section 4.4.2). Importantly, as shown in Figure 2.1, only sites that progress through Tier 1 will require the collation of additional data, such as DOC.

Compromises from the ideal 'matched' data situation are possible in relation to reductions in spatial (less monitoring sites per water body or river basin district) or temporal (for example rather than determinands from one sample perhaps determinands from the same site but from samples taken during the same month or months) resolution.

Relationships, based on EU-wide water quality parameters have been derived to calculate the low to moderate impact physico-chemical inputs, if required, such as magnesium, sodium or alkalinity (e.g. Peters et al. 2011a).

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

4.4.1 Censored data – dealing with "less than" values

With all of the potential input data to the user friendly approaches, but probably most likely the metals concentration data, there are likely to be concentrations recorded as less than values (or < limit of detection – LoD). For the metals the change from total based measures, or dissolved measures linked to hardness, to $EQS_{bioavailable}$ can infer a considerable reduction in the numerical value of the standard. As set out in the QA/QC Directive (EC 2009), the limit of quantitation should be equal or below a value of 30 % of the relevant EQS. Therefore, going forward, the challenges of dealing with datasets in which many values are recorded as < LoD, so called censored data, should be greatly reduced. However, the situation for regulators and stakeholders is that current datasets to be used for regulatory assessments, or for testing the practicality of implementation of the bioavailability approach, can routinely have more than 30% of the data recorded as '< LoD'.

The Environment Agency of England (2009e; 2014b) has investigated approaches to the treatment of values reported as '< LoD' when analysing and processing monitoring data (e.g. Larson et al., 1997; Grunfeld, 2005; Mumford et al., 2006). However, these are often quite resource intensive approaches and are likely to be impractical when handling very large datasets. The required way forward in the QA/QC Directive is that all the values recorded as '< LoD' are halved and then treated as measured data (a substitution approach). Generally, this will produce a strong downward bias, but the approach can result in a dataset that bears little relevance to the actual metal concentrations in a waterbody, especially where there is a large proportion of censored data.

Several options exist for the manipulation of reporting limits and LoDs in highly censored monitoring datasets for the purpose of estimating metal concentrations (Table 4.1). These include substituting the values with zeros, excluding the data altogether (Zhao et al. 2007), substituting censored values with the detection limits (Larson et al. 1997) or substituting a value of half of the LoD (Paustenbach, 2001; EC 2009). Options for the treatment of LoDs have been trialed for monitored datasets and the implications are shown in Table 4.1, along with the influence upon the estimated value compared to the actual value (Environment Agency 2009e).

Table 4.1 Options for treating limits of detection for estimation of backgrounds for metals or averages of monitoring data (amended from Environment Agency 2009e).

Option	Basis of methodology	Possible influence on derived percentile?
1. LoD is halved	All the values recorded as <LoDs are halved and then treated as measured data.	Generally, this should produce a strong downward bias.
2. Extrapolation	Estimate true value of a percentiles by extrapolation from reported data.	Possibly higher than true values, but capable of deriving values that are below the LoD. Not precautionary.
3. LoD Removal	All the data listed as <LoD are excluded from estimation of the10th to 90th percentiles.	Reduces the dataset and produces percentiles that are likely to be high relative to the true values. Estimates cannot be lower than the LoD.
4. LoDs set to lowest	All the <LoDs are set to the lowest LoD in the dataset.	Precautionary approach where there are multiple LoDs, generally leading to relatively low and similar 5th and 10th percentiles
5. LoDs set to highest	All the <LoDs are set to the highest LoD in the dataset.	Not precautionary, but (as with Option 3) possibly leading to similar low percentiles values
6. LoD as reported value	All the <LoDs are treated as measured data at the recorded LoD.	Depends on how many measured data are available in the distribution tail and what proportion of the dataset is recorded as LoDs. May handle datasets with multiple reporting limits better than other methods.

Working with censored monitoring datasets is discussed further in Section 4.5, but it is clear that however the data are treated, there is likely to be a considerable affect upon any assessment of compliance in relation to both positive and negative bias. This bias needs to be considered in any assessment of potential risk.

Other approaches are available for the treatment of censored data within datasets. An alternative to substitution is to use a statistical technique to calculate a dataset’s descriptive statistics (i.e. mean, standard deviation and percentiles) incorporating censored data (Helsel 2005). In medical and industrial statistics the standard method for calculating descriptive statistics from censored data is the Kaplan-Meier method (Klein and Moeschberger 2003, Meeker and Escobar 1998). Kaplan-Meier (KM) is a non-parametric method designed to incorporate data with multiple censoring levels and does not require an assumed data distribution (e.g. log-normal), unlike similar maximum likelihood (MLE) techniques. KM estimates percentiles (including the median) and mean of the complete dataset, including the censored data, from a cumulative distribution function. This approach can provide information about the dataset as a whole, but not about any of the specific data points.

4.4.2 Using historic monitoring data

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

However, there are some key considerations that need to be borne in mind when using these types of data, and these include:

- The dataset is likely to have been collected for a different purpose than which it is currently being applied. This needs to be explicitly understood when drawing conclusions in relation to implementation of an $EQS_{bioavailable}$;
- Considerations need 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 LoDs. 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);
- Are the data spatially referenced? How representative are they of current sampling programs? Conclusions, in relation to compliance or potential risk, should not be drawn for a national scale if the historic data used in the assessment are not representative.

Nevertheless, historic monitoring data have been used to derive precautionary default inputs to the user friendly tools to undertake indicative compliance assessments (Environment Agency 2009a). In the UK, DOC data were collected routinely until about 10 years ago. DOC data therefore exists for many waterbodies in England and Wales, but these data are not current. An assessment was undertaken to see how these data could be used in order to provide a precautionary, indicative compliance assessment, accounting for bioavailability. 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 (e.g. Tack 2012). Indeed, at several locations the default DOCs estimated from historic data for hydrometric areas and waterbodies could be compared with a small subset of recent DOC monitoring data (Figure 4.2).

For the other water chemistry inputs, such as pH and dissolved calcium, these are generally measured at most sites, but where historic data are used mean or median values have been used (Environment Agency 2009a). The selection of the level at which a default value may be set requires a 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.

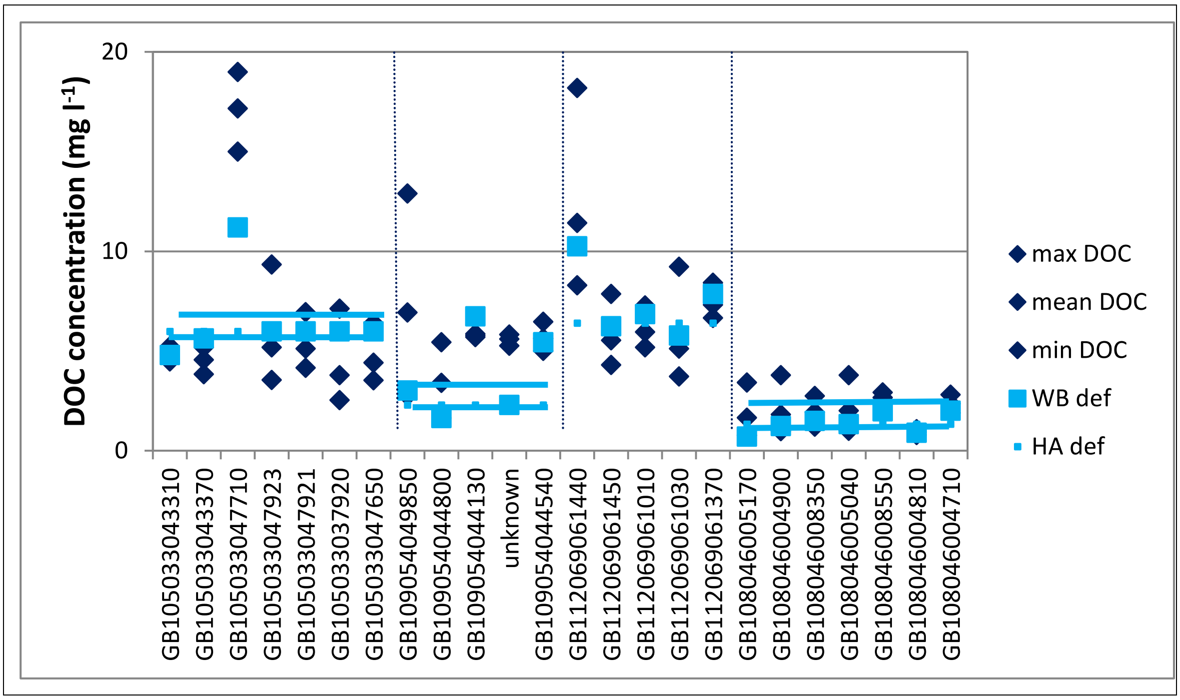


Figure 4.2 **Comparison of measured and default DOC concentrations. Dark blue diamonds indicate the minimum, mean and maximum measured concentrations from the recent subset of DOC monitoring. Large pale blue squares indicate waterbody default DOC concentrations and pale blue lines indicate hydrometric area default concentrations (from: Environment Agency 2009a).**

The DOC results from the monitoring program are shown in Figure 4.2 along with the default DOC concentrations, on both a waterbody and a hydrometric area basis (hydrometric areas are usually made up of several waterbodies and are of larger scale). In general the waterbody based default DOC concentrations, i.e. those derived on data representing a smaller spatial scale, appear to provide a reasonable estimate of the DOC concentrations which might be expected for a particular waterbody, although there are some cases where the default concentrations are towards the higher end of the range of observed DOC concentrations (from the monitoring). This latter issue may be due to relatively recent improvements in water treatment, reducing DOC levels, and so local information is important when assessing the use of historic data.

Historic 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.

4.4.3 Screening and hazard assessments involving partial datasets

Hazard assessments can be performed using the user-friendly tools to provide an indication of how sensitive waters are in relation to the individual metals. Effectively these assessments are undertaken without the input of dissolved metals data and can be used to derive site-specific PNECs to identify sensitive waters that may be targeted for additional monitoring or used to derive a generic $EQS_{bioavailable}$ that would be the protective proportion of all waters in a country of region (e.g. Table 1.1).

4.5 Use of background concentrations of metal

One of the challenges of implementing ecologically relevant metrics to assess risks from metals is dealing with background concentrations (Section 1.3). Very few areas in Europe can be considered to be free of anthropogenic influence, and therefore the measurable metal concentrations that we find today come from a combination of natural and anthropogenic sources. The long history of metal mining, working and use in Europe means that the usual or 'ambient' concentration of a metal in surface waters consists of both a natural geochemical fraction and an anthropogenic fraction (ISO 2005). In this context, the term 'anthropogenic fraction' refers to moderate diffuse inputs into the water, not the inputs from local point sources that generally result in a much elevated concentration. In this guidance, we have used the term 'ambient background concentration' or 'ABC' to mean the same as the ISO-defined term 'usual background concentration'.

Many regulatory organizations across the developed world make reference to the need or desire to estimate background concentrations of metals in waters (e.g. ANZECC 2000; CCME 2003; Alberta Environmental Protection 2012). From a recent review of regulatory methodologies for deriving ABCs, it is clear that there is little general acceptance of one particular methodology (Environment Agency 2014b). However, there tends to be a pragmatic acceptance that approaches for estimating ABCs are less related to scientific principles than regulatory expediency. The ecologically relevant ABCs are believed to be those that are derived over relatively small spatial scales because measured concentrations may change over larger scales and therefore to be relevant the background concentrations must be derived on a small scale (e.g. Roe and Hill 2011).

Two distinct approaches have been described in the EU TGD (Section 3.5.2.1; EC 2011) to account for background concentrations in the derivation and implementation of EQS for trace elements; total risk (TRA) and added risk (ARA).

The TRA makes no explicit account of ambient background levels in that no distinction is made between the fraction of a metal that is present in a waterbody for natural (or low anthropogenic pressure) reasons and the fraction added because of anthropogenic activities. The EQS derived for nickel and lead use the TRA.

The ARA makes a consideration of the ABC through the derivation of an EQS_{add} for which the background concentrations in the ecotoxicity tests have been subtracted. This EQS_{add} is the amount of metal that can be added to local ABC without adversely affecting the assessed ecosystem. The critical step in this process is the estimation of the ABC (see examples below). The EQS derived for zinc in the UK is an EQS_{add} (Environment Agency 2010b). The reasoning for this is that the considerable variability of ABCs for zinc across the UK (> 3 orders of magnitude) makes the TRA for zinc very challenging to implement.

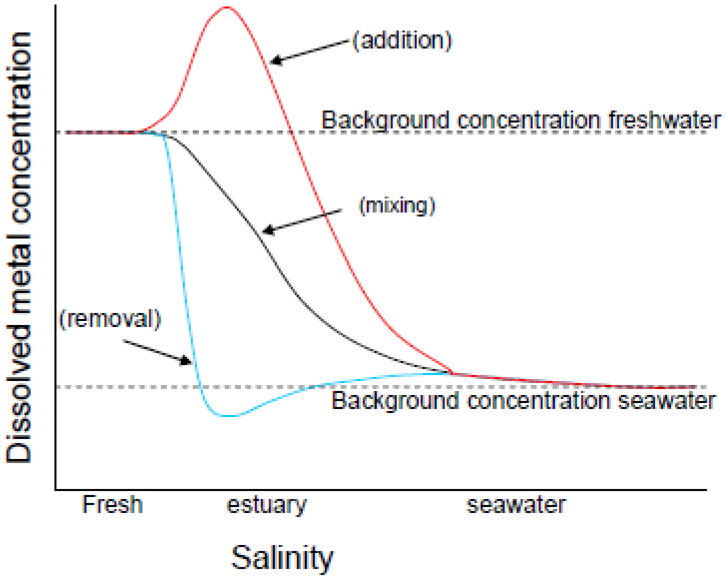
Where nationwide or regional approaches for deriving ABCs are required for use in compliance assessments historic monitoring data have been collated for waterbodies from which a low percentile is selected, as decided upon by policy makers, such the 5th or 10th percentile (e.g. Environment Agency 2009e; Osté et al. 2011, Osté 2013; Environment Agency 2014b). In CIS Guidance No. 27 (EC 2011) the recommendation is to use the 10th percentile.

How and where ABCs might be used, in combination with bioavailability approaches, is probably a local regulatory decision. We give some examples of how they might be derived and included in the tiered approach (Section 6.2.2). It is important to stress that while the concept of background estimation tends to be a simple one, the practicality and scientific rigor associated with the derivation is very limited, especially when compared to the bioavailability approach.

In the UK, ABCs have been derived specifically for zinc, as the $EQS_{bioavailable}$ for zinc is based on the 'added' approach as developed in the ESR for zinc (EU 2004). The ABCs have been developed for hydrometric areas, based on the 5th percentile of monitoring data of dissolved zinc concentrations where less than 30% of data are censored (i.e. where less than 30% of data are recorded as '< LoD'). For those hydrometric areas that have more than 30% censored data a national default has been derived, again based on dissolved zinc concentrations but all the data (45,567 data points) have been collated and a 5th percentile has been derived. For both the hydrometric areas and also the national 5th percentile the Kaplan-Meier method (Kaplan and Meier 1958) has been applied to estimate the lower tail of the distribution of dissolved zinc concentrations (Environment Agency 2014b). This method is currently recommended by both the USEPA and US Geological Survey for generating summary statistics from environmental datasets comprising censored data. In the UK, ABCs are considered for other metals, along with other lines of evidence, at Tier 3, in an effort to clarify the form and magnitude of an EQS exceedance, after consideration of bioavailability. The hydrometric area specific or default ABC for zinc can be subtracted from the monitoring zinc concentration before the bioavailability is considered (i.e. at Tier 2). Biomet has a facility to allow this consideration.

In The Netherlands a similar approach to that followed in the UK has been used to estimate background concentrations (Osté 2013). Specifically, the method is based on >100 monitoring data points using the 10th percentile for both freshwater and marine areas. The challenges are similar to those experienced in the UK, i.e. many measurements of metals in waters that below LoD, sometimes not enough measurements (<100 samples) or not measure of metals not as dissolved concentrations, but totals. There are several key differences in the programme to estimate ABCs in The Netherlands and the UK, including the coverage of many more metals, the greater scope of marine and transitional water databases for metals, the selection of the 10th percentile as the ABC and the data recorded as <LoD was used as half the LoD.

While not specifically related to implementation of bioavailability, in the report by Osté (2013) a methodology is provided to calculate dissolved background concentrations in Dutch transitional water. The method is based upon mixing behavior (figure below), as it is only mixing of seawater and river water that determines the background concentration of the metal in the transitional zone, neglecting the chemical processes.



Potential effects on dissolved metal concentrations in estuaries due to a changing salinity (from: Osté 2013).

The following equation can be used to perform the calculation which does require that requires the salinity at the location in transitional water, regularly measured, and both the background concentration in fresh water and in open sea.

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

Where:

Cbtransitional = dissolved background concentration at transitional water sampling station (µg l^{-1})
Cbsea = dissolved background concentration in seawater (µg l^{-1})
Cbfresh = dissolved background concentration in fresh (river) water (µg l^{-1})
salinity = salinity at the transitional water sampling station

In France, a methodology was recently developed for hydro-eco regions with different bedrock geology (crystalline basement, sedimentary or igneous rocks) to identify waterbodies at risk of having high ambient backgrounds for dissolved metals. Critical steps include the selection of sites where monitoring data for dissolved metal concentrations comply with the minimum performance criteria laid down by the QA/QC Directive (LoQ < 30% of the EQS values), and where direct anthropogenic metal inputs are negligible (based on land-use/land-cover geo-referenced data) (Chandesris et al. 2013).

In the end this methodology allows the classification and mapping of sites into three main categories:

- Areas with high, medium or low ABCs for dissolved metals.

●Areas with indeterminate ambient backgrounds because of lack of data or datasets with a large proportion of determinands whose LoQ are > 30% of the EQS value.
●Areas with indeterminate ambient backgrounds because the scale of investigation is not appropriate for areas with complex bedrock geology that are displaying high variability in dissolved metal concentrations.

Finally, this report proposes a methodology for the quantification of geochemical background concentrations in terms of sampling strategy and analytical protocols for trace metals, via a better use of existing knowledge while pursuing an operational objective for water managers.

In France, **no formal decision has yet been made** on how ABCs will be implemented within the EQS compliance assessment process. As in the UK, they will most likely be considered, along with other lines of evidence, at a later tier (i.e. local refinement), to better evaluate the impact of an EQS exceedance on local biological communities, after bioavailability being taken into account.

5 UNDERTAKING CALCULATIONS

Each user-friendly model has specific instructions and guidance associated with their use that can be found online. Each of the tools are based in excel and can be run on PCs using Microsoft Office. However, it is important to check on the respective websites to ensure the version of Microsoft Office used on the PCs is compatible (not too old). Bio-met can also be run online without the need to download software.

There are some general instructions that can be followed to make this process more straight-forward. These are:

- Ensure the data are arranged, by samples/site, in rows and the determinand 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, not 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 (Section 4.3.1);
- All of the tools will run data that is outside of the validated ranges, but provide 'flags' for when and why this has occurred (options for dealing with these data are given in Section 6.1.1);
- Run times for the tools are varying depending on the tools and also the size of the dataset being processed (and of course processing speed of the computer used). The Environment Agency have automated the calculation steps in their laboratory systems to avoid any data inputting errors (Paul Whitehouse pers comm.) and reducing the resource requirements. Bio-met has an online facility which means that it can be run remotely without the need to download the user friendly tool. The user-friendly tools can process more than 1000 rows of data at a time.

For some of the user-friendly tools it is possible to take account of the locally derived ABC in the calculation steps (e.g. bio-met), although this is only currently available for zinc (which has been derived as an $EQS_{bioavailable,\ add}$).

If an alternative generic, or reference, PNEC is required in some areas, as may be applicable for the specific pollutants, then the bioavailability correction can still be performed, provided that the conditions are within the applicability domain of the models, and the bioavailability coefficient for the water calculated. This bioavailability coefficient can then be applied to correct the site specific exposure for bioavailability in exactly the same manner as is performed in bio-met. The comparison between the site specific bioavailable metal concentration and the alternative generic PNEC can be according to the following equation.

$PEC_{Bioavailable(Site)} = PEC_{Dissolved(Site)} \times BioF$

Where:

$PEC_{Bioavailable(Site)}$ is the calculated bioavailable metal concentration at the site

$PEC_{Dissolved(Site)}$ is the measured dissolved metal concentration at the site

BioF is the bioavailability correction factor calculated by bio-met.

The calculated $PEC_{Bioavailable(Site)}$ can then be compared against the alternative generic PNEC in the usual manner.

6 INTERPRETING RESULTS ON BIOAVAILABILITY

All of the user friendly models have similar outputs that will 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 PNEC (dissolved) [µg L^{-1}] – this is the calculated concentration of dissolved metal that is equivalent to the $EQS_{bioavailable}$ based on local water conditions at the site. Under "sensitive conditions" (conditions of high bioavailability) the user friendly tool should return the $EQS_{bioavailable}$ as the compliance concentration, so for nickel this would be 4 µg l^{-1}.

BioF - is the ratio of the $EQS_{bioavailable}$ divided by the site-specific PNEC. This value is always 1 or less. When the value is 1 the metal, under the specific water conditions provided, is 100% bioavailable and the site is described as having "sensitive conditions".

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

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. The RCR column is not available when dissolved metal data for a row are not entered. **An RCR of 1 or greater indicates an exceedance of the $EQS_{bioavailable}$ and prompts a progression to Tier 3** in Figure 2.1.

The results of any compliance assessment will obviously be influenced by the value of the $EQS_{bioavailable}$. For nickel and lead these are applied Europe-wide. However, for specific pollutants these will be derived at a national level and could vary considerably between Member States.

6.1 Dealing with bioavailability estimates outside of the validation ranges of the ecotoxicity data

For EQSs that are derived on the basis of relationships between ecotoxicity data and water column physico-chemistry, such as for metals, there are likely to be some combination of those parameters in European freshwaters which will be outside the ranges of the test data on which the relationships are based (Section 3.2). These situations have been recognised in working with BLMs (Natale et al. 2007; Environment Agency 2009d) and there are options for dealing with these waters and sites.

6.1.1 What are validated ranges of the Biotic Ligand Models and what do they mean?

BLMs for predicting the chronic ecotoxicity of Zn, Cu and Ni under different freshwater chemistry conditions were originally developed as part of the EU risk assessments (for Nickel and Zinc and for a Voluntary Risk Assessment for Copper) performed under the Existing Substances Regulation 793/93/EC. 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 conditions for pH, hardness or calcium and DOC observed in different EU Member States. The validation boundaries of the BLMs (and so too the user friendly tools) represent the extremes of water quality conditions at which the validation chronic tests were undertaken (Table 6.1). 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.

A common problem in BLM development, and arguably one of the reasons for the existence of the validation ranges, is that the species typically used for BLM development (i.e. species commonly used in **all** laboratory ecotoxicity testing) are not tolerant of all natural water 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 validated ranges of the BLMs will never cover **all** EU waters. 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 performance. In addition, these types of waters (at the extremes of pH or low hardness) often have very specific ecological assemblages, which are not always more sensitive to metal exposures than typical mid-range waters.

Table 6.1 Validated water chemistry ranges of the BLMs for copper, nickel and zinc.

BLM	pH	Calcium, mg L^{-1}	DOC, mg L^{-1}
Cu	6-8.5	3.1- 129	30#
Ni	6.5-8.7	2.0-88*	30
Zn	6.0-8.5	5.0-160*	30

*These upper limits reflect only where the protective effect of Ca ceases, so above this value the site-specific PNEC is not going to be underprotective. Value of 30 mg L^{-1} for DOC equivalent to the 96th percentile of EU freshwaters, as given by FOREGS.

It is important to stress that *most* existing hardness-based limits have no validation and no evidence-based range of applicability, although these considerable uncertainties are rarely openly acknowledged. However, the ecotoxicity data from which hardness-based limits have been derived clearly *do not* cover the hardness ranges (i.e. from 0->200 mg $CaCO_3$ L^{-1}) over which they have been unquestioningly applied.

Nevertheless, explicit acknowledgment of validation ranges means that some waters will fall outside for some metals.

Two case studies, from the UK and Finland are provided in Appendix 1. The UK example shows a compliance assessment example where waters are above the upper range of the calcium concentrations of the nickel BLM for some waters. In a Finnish feasibility study of the nickel BLM, using the Bio-met model, it has been shown that typical values falling outside of the calcium and pH validation ranges are not likely to prevent the application of this model in Finnish surface waters. With decreasing pH there is an increasing amount of H^+ ions, which protects biota from Ni^{2+} ions. As the Bio-met model applies a default of pH 6.5 for all samples less than or equal to pH 6.5, it is most likely conservative in estimating local EQS values at low pH conditions. Calcium provides less of a modifying effect on nickel toxicity in the Bio-met BLM model and thus, water conditions falling below the validation range for calcium will not have a major impact on the model predictions.

6.1.2 What are the options for waters under investigation that fall outside the validation conditions of the model?

This subsection provides some considerations on how to deal with sites where the water chemistry conditions are outside the applicability range of the BLMs and so also the user friendly tools. The options provided here are not definitive and have been summarised from several studies (Environment Agency 2009c, 2009d), but may be considered to be policy, rather than science, related decisions.

An important factor in this issue is whether or not elevated exposures occur 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 user friendly tools likely to be important (i.e. when progressing from Tier 1 to Tier 2, Figure 2.1)(Appendix 1).

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 which commonly cause surface waters to be outside the applicability range of the BLMs (and so the user friendly tools) are hard waters (high calcium concentrations), where the calcium concentration may exceed the validation range, and soft waters where the calcium concentration is below the applicability range. Low pH may also cause waters to be outwith the applicable range of the BLMs, and in some instances both low pH and low calcium may be encountered in the same waters.

Hard waters, where the calcium concentration exceeds the BLM validation range, especially for the nickel and zinc BLM, can be treated 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 on the ”Biotic Ligand”, and increases in calcium concentrations do not result in further reductions in metal bioavailability (e.g. Heijerick et al. 2002; Deleebeeck et al. 2007b). This situation is automatically handled in the user friendly tools, M-BAT and bio-met by limiting the input data to the maximum allowable calcium concentration (i.e. can go no higher than the maxima for the BLM validation range), and predictions performed by doing so continue to be reliable.

In contrast, the boundaries established for soft, acid waters occur because the majority of test organisms used for BLM development are unable to survive and reproduce adequately under such extreme conditions. These water quality conditions may support different species than those found in harder water, higher pH environments. Protons (H^+) and Ca ions may both compete with metals for binding sites at the biotic ligand. As pH decreases the competition from protons will increase (resulting in lower metal bioavailability), whereas as hardness decreases the competition from Ca will decrease (resulting in higher metal bioavailability). The relative importance of competition from protons and Ca for each individual metal may affect organism responses under these conditions (Appendix 1). A reduction in pH may also result in a decrease in metal binding to DOC, due to increased competition from protons, resulting in increased metal bioavailability. Changes in pH can also result in changes to the inorganic speciation of a metal, and the fraction which exists as bioavailable species (e.g. Cu^{2+}), although significant changes in speciation around the lower pH limit for the BLMs are unlikely for Cu, Ni, and Zn, due to the dominance of the free ionic form under acidic conditions.

Several options for treating conditions which are outwith the applicability range of the BLMs and user friendly tools include[12]:

1. Apply the $EQS_{bioavailable}$ and assume and assume that there is no mitigating influence of water chemistry factors. This option applies the $EQS_{Bioavailable}$ outside the applicable conditions, and can effectively result in a step change to the standard where the validation conditions are exceeded (for example one water may be in the applicability range, but another just outside, effectively the first water would allow accounting for bioavailability whereas the second water would default to the $EQS_{bioavailable}$ and this may represent a considerable decrease).

2. Assume model predictions still apply outside validation conditions with the inherent uncertainties (Appendix 2). This applies the BLMs and assumes that no validation conditions apply and that the models can be extrapolated beyond their validated range.

3. Extrapolation of the physico-chemical boundaries of the validation range using speciation modelling to indicate likely influence upon exposures, acknowledging uncertainty (Environment Agency 2009c). At low pH and Ca concentrations there are likely to be physiological implications for interactions at the biotic ligand which 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) there is a possibility of deriving ecological assemblage specific EQS or site specific PNECs from field data (Appendix 1). Observations of organisms or communities in the field may provide a means of validating any predictions or assumptions that are made about the protection of aquatic ecosystems in relatively extreme environments, although it is likely that a combined weight of evidence approach including several complementary techniques would be required. Water Effect Ratios, which compare the results of toxicity tests in locally collected water and standardised water, have been widely used in the US. These tests use a Water Effects Ratio to correct the EQS under standard conditions to the local EQS. Such

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[12] http://bio-met.net/eu-member-state-workshop-on-metal-bioavailability-and-the-wfd/

tests would need to use test species which are appropriate to the water chemistry conditions of the local surface waters (many soft waters may be unsuitable for the culture of many crustacea). Ecological monitoring will be performed under the WFD and may provide a means to ensure that any deterioration in ecological quality can be identified, where there is uncertainty in the adequacy of an EQS 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 b. The use of additional biological monitoring may be valuable in supporting the application of the standards under potentially sensitive conditions. An example of a specific EQS would be the $\text{PNEC}_{\text{softwater}}$ for Zn which was derived for the Existing Substances Regulations Risk Assessment Report on Zinc and Zinc compounds (EU 2004), although other approaches to developing specific localised standards may be more appropriate (Peters et al. 2011b).

The copper BLM also includes a boundary condition for iron (307 µg L^{-1}) and aluminium (332 µg L^{-1}), although it is extremely unlikely that such conditions would be experienced without another of the validation conditions also having been breached. Extremely high iron and aluminium levels are most likely to occur in association with reduced pH, due to the tendency for both of these metals to form insoluble precipitates under circumneutral pH conditions. 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. At lower concentrations of iron and aluminium the competition for copper binding is lower and there is therefore a smaller reduction in the proportion of DOC complexed copper.

The nickel BLM 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 the two metals for DOC. Consequently, metals which bind strongly to DOC, such as copper and lead, will be less affected by this issue than more weakly bound metals such as nickel and zinc.

6.2 Compliance and classification

One of the key uses of an EQS under the WFD is to assess compliance with the measured monitoring data from surface waters. This result either drives (if a Priority or Priority Hazardous Substance, Annex IX or X of the WFD) or is a component of (Specific Pollutant, Annex VIII of the WFD) classification. Surface water bodies are classified according to their chemical and ecological status, which includes a consideration of the relevant EQS.

6.2.1 Exceedance and failure (what is a failure?)

Some Member States use the International Standard (ISO 2008) to take uncertainties associated with the use of monitoring data and the comparison with an EQS into account.

This is done through the calculation of the "confidence of failure"[13]. For the decision makers, who use the outcomes of a compliance assessment, a range of conclusions may be drawn from reporting a failure, such as taking legal action or undertaking costly programs of measures to rectify the situation. The consequences and ramifications of making the wrong decision are clearly variable, **but**, each decision requires its own degree of confidence, i.e. its own accepted risk of being wrong. This applies to all EQS, not just the $EQS_{bioavailable}$, and it is obvious that the more important the decision, the less the decision maker will want the influence of uncertainty and errors in sampling and measurement to lead to a wrong decision.

Most of the assessments that have been made related to the implementation of the $EQS_{bioavailable}$ have not considered this confidence of failure (e.g. Environment Agency 2014b), and so may be termed 'face value' assessments. Where confidence of failure has been considered when using an $EQS_{bioavailable}$ it can provide considerable assistance in interpreting data and prioritising actions and potential program of measures, especially when dealing with impacted sites, such as those impacted by metalliferous mining (Environment Agency 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 under very low bioavailability conditions, that drinking water standards are not breached at the point of abstraction or measured compliance of the drinking water standards (which is considered to be the tap, 98/83/EC).

6.2.2 What next if using user-friendly tools to confirm failure?

If an exceedance has been identified for a site (RCR = or > 1) through the use of the user friendly tools then the obvious question is; what next? From Figure 2.1, Tier 3, Local Refinement, is the next step. This 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 (an action which may potentially incur considerable cost). These might include:

- Consideration of local ABCs. For example a generic background value may have been used at Tier 1 for zinc, but at Tier 3 consideration may be of locally derived ABCs for all the metals for which an $EQS_{bioavailable}$ has been used (e.g. Environment Agency 2012b);
- If incomplete monitoring data have been entered in to the user-friendly tools then the correct data should be collected. The data may have been incomplete through the use of;
    - Total rather than dissolved metals data;
    - DOC defaults calculated from historic measurements (Section 4.4.2);

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[13] 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 2008).

    - Spatially or temporally mismatched measures (i.e. data not taken from exactly the same sites or at the same times as the metal measures);
    - Incomplete annual data that may represent only a limited subset of the year or exposures.
- Use of the full BLMs. The user friendly tools mimic, in a simplistic way, the full BLMs for each metal. As shown in Appendix 2 errors against the BLMs should be within a factor of 2, and so the use of the BLMs are likely to provide an assessment of greater accuracy. The collection and processing of additional input data to run the models requires considerable technical skill, as does the interpretation of the outputs. 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 program of measures.

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 with the total number entering at Tier 1 (e.g. EC 2011).

6.2.3 What about MACs?

There are acute BLMs available for several trace elements, including copper, lead, zinc, silver and nickel. Importantly, these have mostly been developed in North America, using ecotoxicity data that fulfill USEPA Water Quality Guideline requirements (e.g. USEPA 1985). These tend to differ from the requirements of an EQS under the WFD, particularly in relation to the taxonomic breadth of data.

However, of great importance is the fact that in order to implement an acute bioavailable approach there is a need to derive a $MAC_{bioavailable}$. Currently, no $MAC_{bioavailable}$ have been derived under the WFD.

Situations may arise when a MAC concentration for a metal is approached when bioavailability conditions are very insensitive. If this happens, for example the water has high DOC and high Ca for nickel, the insensitivity will mean that there are also few risks associated with acute exposures. In cases where a non-bioavailability corrected MAC is exceeded, but a bioavailability based AA-EQS is complied with, the MAC EQS is likely to be highly over-protective.

Nevertheless, it may be possible, under specific circumstances to consider acute bioavailablity, for example incident investigation. Although it is likely that this would require considerable expertise and the use of a weight of evidence based approach to reach a credible decision point.

6.2.4 What about marine waters?

Approaches to account for (bio)availability using BLMs in marine water are currently under development, but are still some way from implementation. Therefore, it is important to be able to establish at what point there is the transition between the use of the freshwater and transitional and coastal water EQS, this is likely to be the tidal limit.

As mentioned previously, corrections based upon relatively simple ecotoxicity relations with mitigating water chemistry characteristics, such as DOC have been developed by some Member States (e.g. Environment Agency 2011b).

6.3 Permitting discharges and accounting for metal bioavailability

Permitting of discharges using an $EQS_{bioavailable}$ is a considerable challenge and remains an area for which options are still in development. Unlike compliance assessment with an EQS, methods associated with permitting vary greatly between Member States. In some, there is an interest in extending the bioavailability concept to point source discharges so that permits are based on the risk posed by the bioavailable fraction. Others may give more weight to the total load of a metal emitted to a catchment, irrespective of its form.

The main challenge is adopting a bioavailability-based approach to permitting is that the water quality factors that affect metal bioavailability vary over time and from place to place. The properties of the effluent itself also affect the bioavailability of metals contained in the effluent (or inputs from further upstream).

6.3.1 Discharge derived DOC?

Dissolved organic carbon is clearly not all derived from natural sources and some dischargers may introduce considerable quantities of DOC into waterbodies from an effluent. This could give rise to the unintended effect of additional DOC inputs protecting against risks from metals. This is clearly not an intention of the bioavailability approach and certainly most wastewater treatment plants (WWTPs) have process controls that mean that DOC is unlikely to vary greatly and would be subject to limits in any case.

When permitting metal-containing discharges, regulators would need to decide whether to allow for the DOC contained in effluents, or only to consider the DOC in the receiving water. An allowance for discharge-derived DOC could lead to lower bioavailability (and hence more relaxed permits) than if this contribution was ignored.

Anthropogenic DOC is chemically distinct from the DOC derived from natural sources (like those arising from breakdown of leaf litter or arising from sediment and soil), but we are not yet clear how these differences would affect metal bioavailability. The limited evidence available suggests that metal binding tends to be stronger and complexation capacity greater for anthropogenic DOC (Section 4.3.2) than for ‘natural’ DOC. In this case, there would be a level of precaution built into assessments that make no distinction between anthropogenic and natural DOC.

6.3.2 Total vs dissolved metals concentrations

Some of the metal emitted in an effluent discharge will be in dissolved form (and therefore in a potentially bioavailable form) whilst some is likely to be in an undissolved form, perhaps as unavailable metal colloids. When deriving a permit, the regulator must consider whether emissions of undissolved metal in a discharge could give rise to dissolved metal in the receiving water some of which could, in turn, become bioavailable.

Preliminary modelling studies undertaken by the Environment Agency in the UK suggest that there is no significant net change in the proportion of dissolved and 'total' metal following release from a point source to a receiving water under a range of water quality conditions and dilutions. If this is a general pattern, this would simplify the introduction of bioavailability-based approaches to permitting because the undissolved fraction is unlikely to contribute to risk to aquatic.

6.3.3 Spatial and temporal variations in receiving water chemistry

The risk posed by a bioavailable metal is dependent on local water quality conditions so, when setting a permit for a particular location, it is necessary to define what the local receiving water conditions are (in terms of its DOC, pH and hardness). It is also important to understand how these vary in time. This would normally require a programme of monitoring for these parameters before a permit condition can be set. The receiving water conditions used to derive a permit would normally be reasonable worst case conditions that rarely give rise to higher bioavailability.

A further consideration is the possibility that the vulnerability of the receiving water changes further downstream. If the receiving water conditions lead to lower bioavailability, then conditions at the point of discharge would offer adequate protection. But if the combination of DOC, pH and hardness leads to increased bioavailability then this may need to be accounted for in the permitting decision, so that unacceptable risks further downstream do not occur.

6.4 Forward look

Bioavailability approaches and BLMs are becoming available for more metals (e.g. the chronic BLM for lead is expected to be available by the Autumn of 2014). The guidance described in this document will be suitable for these models too and the same supporting data (such as DOC), can be used for all metals.

In addition, approaches are also in development, and now nearing completion, for the implementation of bioavailability with sediment standards for trace elements. This includes advances in the understanding of the mitigating influence of acid volatile sulphide in metal bioavailability (SEM/AVS concept).

In addition revisions of the Metals Environmental Risk Assessment Guidance (MERAG) are currently under consideration that include outline concepts and guidance for the incorporation of bioavailability for the aquatic compartment (water and sediments, Factsheet 5)[14].

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[14] www.icmm.com

7 FREQUENTLY ASKED QUESTIONS

These questions have been compiled from queries received from Member State or Stakeholder experts.

Number	Question	Answer
		Complexity
1	The BLMs are too complicated to implement and interpret in a regulatory framework	Simplified BLM tools have been developed and are simple Excel spreadsheets that can either be used on any PC or alternatively the underpinning calculations can be embedded into laboratories or the regulatory compliance checking process.
2	The BLMs would require dedicated staff to run them and understand them	If used as stand-alone tools, many samples can be run through the Simplified BLM tools in a batch process that just requires data entry of the monitoring results. Simplified BLM tools can also be integrated into laboratory systems for automated production of outputs. The outputs are readily interpretable, including a simple risk characterization ratio.
3	The calculation of backgrounds is much easier to do	The use of natural background concentrations is not a replacement for bioavailability consideration. The calculation of backgrounds has much less scientific and technical pedigree than accounting for bioavailability using BLMs and so bioavailability should be given a greater influence than backgrounds when checking compliance, permitting etc. Backgrounds can be considered in the tiered approach following account being taken of bioavailability.
		Expense and resources
4	The BLMs or User-friendly BLM tools would be expensive to run and require more trained staff	In addition to being technically robust, the user-friendly BLM tools are freely available and simple to use, so minimal training is required to use them. In many cases, the help pages should provide sufficient information for users. In some cases it is possible for the estimation of bioavailability corrections to be automated within laboratory information management systems, thus reducing the need for resources.
5	The BLMs or user-friendly BLM tools require too many additional inputs that we do not measure	The user-friendly tools require a maximum of 4 inputs. These are the dissolved metal concentration, pH, calcium and dissolved organic carbon (DOC). Data for pH and calcium are usually more readily available than DOC. If site specific monitoring data for the key parameters are not available, default values based on historic data may be used. The full BLMs do require more inputs but these can be estimated from calcium using a freely available Excel calculation (from a peer-reviewed journal article) if there are no monitoring data.
6	We have no DOC data. Therefore we cannot implement the method.	DOC has an important influence on bioavailability so it is preferable 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 similar catchment types or to estimate DOC concentrations from other data that is available such as UV absorbance or dissolved iron (Peters 2011,).
		Scientific Rigor

7	The science on 'bioavailability' is not well developed	The science underpinning the understanding of bioavailability and BLMs is well studied. More than 500 papers have been published in the scientific literature on BLMs since 2000. SCHER Opinions over the last 4 years have supported the use of bioavailability-based approaches in ESR metals risk assessments and recent EQS guidance documents. The EU Technical Guidance for Deriving Environmental Quality Standards (TGD-EQS - EC 2011), supported by SCHER includes the use of BLMs for setting EQSs for metals. It is also notable that REACH guidance recognizes the use of BLMs in establishing Generic Exposure Scenarios for metals.
8	Where is the technical evidence to support this approach	There are a number of technical reviews of BLMs within the literature and the evidence is also presented and reviewed in the relevant ESR metals risk assessments. One key piece of evidence is that the predictions of toxicity from BLMs match what is observed in the field remarkably well and usually within a factor of 2.
9	These models don't cover all aquatic species, what about the species for which there are no BLMs?	Studies on different species have shown that the models used are broadly applicable between different species (the binding constants for both toxic metals and competing ions show remarkable consistency between different species) the BLMs are therefore applied to additional species by defining the sensitivity to the toxic metal (which is expressed as the fractional occupancy of the biotic ligand at the threshold level, e.g. EC10.)
10	The BLMs or user-friendly BLM tools do not account for dietary uptake of metals	For those metals where BLMs have been developed, the evidence is that direct toxic action of the metal on a receptor or 'biotic ligand' is the most sensitive endpoint. Therefore, the protection provided by using bioavailability and BLMs is more important and relevant than dietary uptake.
11	According to De Laender et al., (2005) & De Schamphelaere, (2003) toxicity for both Cu and Zn can be underestimated when applying BLMs on fresh waters with elevated levels of humic substances, Al, Fe and low pH. As these conditions are common here in Sweden what is the solution used in this model to prevent this?	It is not clear which specific papers are referred to here, although it is customary when performing BLM calculations for the purpose of EQS compliance assessment to assume that only 50% of the DOC is actually active. This is to ensure that DOC which may be inactive with respect to metal binding does not result in unprotective estimates. This approach was agreed under ESR some years ago.
12	Which geochemical model is used to calculate the chemical speciation in this version of chronic BLM?	The speciation codes are the same as those used in WHAM (Tipping 1994 Computers and Geosciences 20:973). However, for a chronic BLM, other type of speciation model (e.g., Visual Minteq) can also be used.
13	What about the influence of other metals present in the waters? Most important in Scandinavia Al & Fe?	Truly dissolved Fe and Al can compete for binding sites on DOC, although due to their tendency to precipitate any effects may be limited. The conservative assumption that only 50% of DOC is "active" is likely to result in the concentration of available binding sites being overestimated (rather than underestimated) even where there are appreciable levels of these metals in true solution. Boundary conditions for Al & Fe have been set in the chronic CuBLM.
14	These models are based on species that are not representative for our waters. Do we need to develop BLMs specifically for our waterbodies?	Studies show that the models are capable of predicting toxicity to species that are endemic to specific regions, e.g., Scandinavia (Deleebeeck et al 2007) Comparison of nickel toxicity to cladocerans in soft versus hard surface waters (Aquatic Toxicology 84:223.).
15	Not all DOC is created equal. We have special DOC in our waterbodies that is not considered in the BLM development.	Studies show that the models are capable of predicting metal toxicity in wide ranges of natural waters that exhibit ranges of DOC types.

16	Are the chronic and acute BLMs interchangeable?	We would strongly recommend that considerable caution is exercised when trying to make comparisons between different BLMs, even when they are for the same potentially toxic metal. This is especially important in the case of comparisons between acute and chronic BLMs, and particularly so in the case of copper due to the fact that whilst there is a protective effect of calcium on acute copper toxicity there is no protective effect of calcium on chronic copper toxicity, as your expert review of the copper BLM will no doubt have revealed. Your expert review will also no doubt have revealed that in addition to Cu^{2+}, $CuOH^+$ and $CuCO_3$ are also included as potentially toxic copper species in the chronic copper BLM, which is a further difference between the acute and chronic models.
17	According to Van Genderen et al. (2005) and Sciera (2004) using the acute copper BLM on waters with lower hardness then 50mg $CaCO_3$ may underestimate the toxicity of copper, in this case to larval fathead minnow. Is this addressed in the current version of the chronic BLM?	Calcium does not have a competitive effect on chronic copper toxicity, as can be readily observed by performing calculations in which Ca is varied but all other conditions remain constant with the chronic Cu BLM.
18	Studies have shown that BLM is systematically giving discrepancies in calculating reliable toxicity data for soft waters (a factor of 8) (Schamphelaere & Jansen,2004; Boeckman & Bidwell, 2006)	Boeckman & Bidwell 2006 - The effects of temperature, suspended solids and organic carbon on copper toxicity to 2 aquatic invertebrates - Water Air and Soil Pollution 171: 185. This study refers to acute tests, so uses a different BLM to the chronic Cu model, although the abstract states that LC50 values based on total copper concentrations were significantly greater than free ion LC50s for both species, suggesting that the BLM principles still apply. Schamphelaere & Jansen,2004 – neither ET&C 23:1115, nor ET&C 23:1365 indicate such an effect (both of which relate to the chronic Cu BLM).
19	The underpinning requirement of the BLMs is that the system is at equilibrium condition…but this never happens in nature!	Many natural systems exist in, or close to, a steady state pseudo-equilibrium, and in the vast majority of cases the assumption that the waterbody is close to equilibrium will be appropriate for bioavailability calculations.
Practical implementation and coverage		
20	What metals have BLMs and user-friendly tools?	There are BLMs and user-friendly tools for Cu, Ni, Mn and Zn. Under development are BLMs for, Co, Pb, Al and Fe. The BLM concept would not be appropriate for metalloids, such as Hg, because of the importance of volatility of inorganic forms and the key exposure route being an organic Hg form.
21	If a BLM is available for lead should I use this or the DOC correction to evaluate compliance with the WfD $EQS_{bioavailable}$	The latest WfD $EQS_{bioavailable}$ was derived before the lead BLM was available. Therefore for compliance purposes an availability correction based on dissolved organic carbon should be employed (see section 3.3 of this guidance)
22	The User-friendly tools are not the BLM.	The results of the user friendly models have been validated against the full BLMs and also use the same datasets.
23	The user-friendly tools show predictions that are mostly below the 1:1 line when plotted against the full-BLMs	The predictions are all within a factor of 2. For some metals, such as Cu, the exclusion of some parameters (especially Na) that may have a protective effect means that the predictions are overprotective. However, within a tiered risk based framework this is acceptable.
24	It is not possible to do a compliance assessment using these methods.	Compliance assessments using User-friendly BLMs have been undertaken in a number of countries including The Netherlands and the UK. If a generic bioavailability based EQS is in place then compliance assessments using User-friendly BLMs need not be any more complex than any other calculated parameter.

<table>
<tr><td>25</td><td>The pH/DOC/Ca do not cover the sensitive waters I’m interested in.</td><td>The applicable range of water chemistry conditions which the BLMs can be applied to is limited by the ability of standard test species to survive and reproduce under extreme conditions of pH and Ca. Typically low pH (,6) and low Ca concentrations (<5 mg l^{-1}) cannot be tolerated by standard test species (such as *Daphnia magna*), although the limited available testing on soft water species does not suggest that they are likely to be more sensitive than other sensitive species which have been tested. Furthermore, studies show that the models are able to predict toxicity to organisms from soft waters.</td></tr>
<tr><td>26</td><td>Is it possible to extrapolate beyond the boundaries of the models?</td><td>Following further testing, the datasets for Cu, Ni and Zn have validated boundaries for the BLMs that have been modified (see below). Options for what to do outside the boundaries are discussed further in Section 6.1.
<table>
<tr><th>Metal</th><th>pH</th><th>Calcium, mg L^{-1}</th><th>DOC$^{\alpha}$, mg L^{-1}</th></tr>
<tr><td>Cu</td><td>6-8.5</td><td>3.1- 160</td><td>30</td></tr>
<tr><td>Ni</td><td>6.5-8.7</td><td>2.0-88*</td><td>30</td></tr>
<tr><td>Zn</td><td>6.0-8.5</td><td>5.0-160*</td><td>30</td></tr>
</table></td></tr>
<tr><td>27</td><td>The upper Ca range on the model for Ni is only 88 mg L^{-1} we have waters that have much greater Ca. What is the implication of having a high Ca exceedance?</td><td>At Ca concentrations above this there are no additional protective effects (see above). So, while Ca concentrations may be higher in the waters the positive influence of ecotoxicity mitigation is limited.</td></tr>
<tr><td>28</td><td>How do I summarize the input data for the tool for the calculation of annual average compliance?</td><td>The ideal situation would be to have matched dissolved metal data and Ca, DOC and pH for every site for every sampling occasion. However, the difference between doing this and using annual average pH and Ca and an annual median for DOC is very limited. A median DOC value should be used as DOC may be more variable in waters and using the median is a more appropriate statistic.
It is probably possible in most cases to not need to measure values every time, for example hardness or Ca. But, for DOC it is difficult to tell immediately and generally needs a few years of monitoring to get default concentration for a waterbody, although some waterbodies this would not be appropriate. For very variable aquatic systems matched data should always be used in assessing annual average compliance.</td></tr>
</table>

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APPENDIX 1: Examples of issues – with worked solutions

Waters outside of the validated boundary conditions and compliance assessment

This appendix follows on from the information provided in Section 6.1 and provides two examples, from Finland and the UK, of how waters outside may be dealt with that outside of the validated boundary conditions of the BLMs (and so also user-friendly tools).

The UK has a relatively wide range of water chemistry conditions (Table A.1), reflecting a range of geological and land use conditions. The validated boundary ranges of the BLMs and so the user-friendly tools for nickel are 2 – 88 mg L^{-1} (Table 6.1). In an assessment of 916 sites with annual average data for dissolved nickel, calcium, dissolved organic carbon and pH, 71 sites had calcium concentrations greater than 88 mg L^{-1}.

Table A.1 Percentiles of measured data from 916 sites in the UK

Percentile of frequency distribution	pH	Ca (dissolved, mg L^{-1})	DOC (mg L^{-1})
10th	6.64	2.49	1.76
90th	8.12	120	9.40

These sites are therefore outside of the upper validation range and would be flagged in the user-friendly tools. The Environment Agency chose to assess which of these sites potentially represented a problem in the bioavailability assessment by initially looking at the potential metal exposure (i.e. measured nickel at the site). From Figure 2.1, consideration of bioavailability is only necessary if there is an exceedance of the generic EQS at Tier (e.g. 4 µg Ni L^{-1}). By undertaking this comparison of the measured nickel concentrations at the 71 sites with the generic EQS no sites give an RCR = or > 1 and so no further consideration is necessary.

If some of the sites had exceeded the generic EQS a further consideration, discussed in Section 6.1.2, could have been made and this is to determine the possible influence upon bioavailability of the parameter that is outwith the validation. In the case of calcium concentrations above the validated range for nickel there is a limit to the protective effect from calcium as a competitor for binding sites on the "Biotic Ligand", and increases in calcium concentrations do not result in further reductions in metal bioavailability (e.g. Deleebeeck et al. 2007b). Therefore, through a knowledge and understanding of the influence of the physico-chemical determinand upon the specific metal bioavailability it may be possible for the potential direction of ecological risk (i.e. more or less) may be understood. For example, increasing DOC above the limit will reduce bioavaiability of all metals, whereas an increase in pH above the upper validated range for nickel (Figure 1.2) may, other parameters remaining unchanged, mean an increase in bioavailability.

The BLM model validation ranges are likely to be challenged in some parts of Northern Europe were a cool Boreal climate and exposed Fennoscandian shield are primary conditions that would fall outside the validated ranges of the nickel BLM (e.g. low hardness and pH but high DOC). For example, in Finland among the 195,000 freshwater samples analysed for pH, 30% are lower than 6.5 and among the 35,600 samples analysed for calcium, 21% have concentrations below 2 mg L^{-1} according to the environmental administration data base search for the years 2004-2014. A feasibility study by the Finnish environmental administration was conducted concerning the nickel boundaries in the Bio-met BLM model. Chronic nickel algae tests (*P. subcapitata*, 72 h growth inhibition) were performed for samples covering typical water quality characteristics of Finnish waters. A line of evidence in support of the applicability of the Bio-met tool was the reduction in the variability of the EC50 concentrations of *P. subcapitata* when normalized to bioavailable nickel using the Bio-met tool.

Figure A.1 shows the results of algal toxicity tests performed in natural Finnish waters with the EC50 values expressed as both dissolved nickel (in black), and as bioavailable nickel (in red, as calculated by Bio-met). Samples where the difference between these two measures of exposure is relatively small, such as samples 5, 8, and 14, exhibit the greatest bioavailability of nickel, and approximately half of the dissolved nickel is present in bioavailable forms. Samples which show a very large difference between the EC50 expressed as a dissolved concentration and a bioavailable concentration, such as samples 10, and 11, have a very low nickel bioavailability. In all cases the bioavailable nickel concentrations are significantly lower than the dissolved nickel concentrations at the EC50.

Further support for the model applicability outside the boundary conditions was received after considering nickel behaviour in the waters of low pH and calcium conditions. Under lower pH conditions an increasing activity of H^+ ions protects biota from Ni^{2+} ions (by competing for binding sites on the biotic ligand). As the Bio-met model applies pH 6,5 conditions for all samples below this pH level, it is most likely conservative in estimating local EQS values. Calcium, on the other hand, has an insignificant effect on nickel behaviour in the Bio-met BLM model. Therefore, Finnish authorities have concluded that typical violations of the calcium and pH validation ranges in the Northern Europe are not a threat for the application of the nickel Bio-met BLM model.

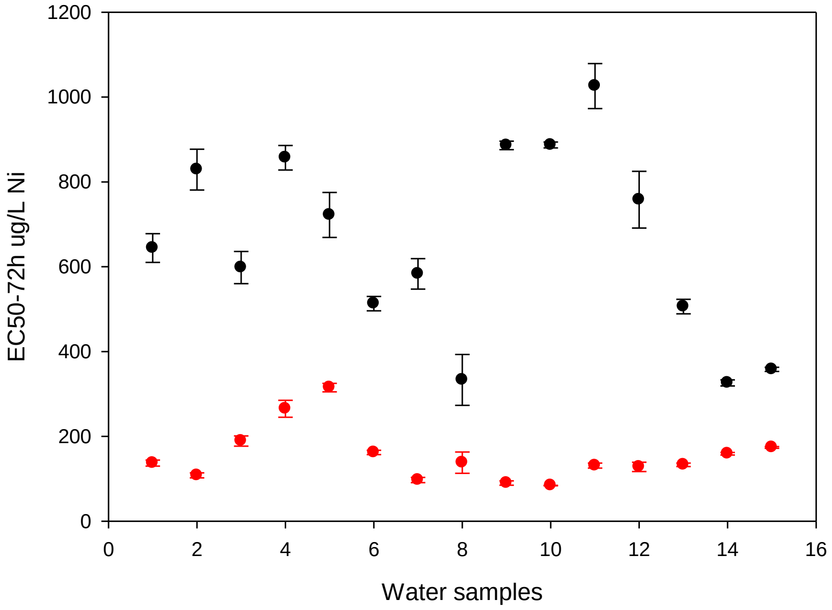

Figure A1.1 Algae EC50 values (mean +/- SD, n=3) before (black) and after (red) recalculation of the exposure concentrations.

APPENDIX 2: SYSTEMATIC COMPARISON OF USER – FRIENDLY TOOLS

Background

This comparison exercise has been performed between the two readily available user-friendly tools:

1. **bio-met bioavailability tool v. 2.3** – a "lookup table[15]" based tool in both ME Excel spreadsheet and online formats developed collaboratively by wca and ARCHE with funding from the metals industry (www.bio-met.net)
2. **M-BAT v.31** – an "algorithm" based tool in MS Excel developed by wca environment (based on bio-met database) with funding from the UK Environment Agency

The eventual choice of tool for regulatory purposes will be made by individual member states based on their own particular circumstances and requirements. However, it is important that the performance characteristics of bioavailability tools used for regulation (precision and applicability domain relative to the BLMs they are supposed to mimic), are understood in order that regulatory decisions based on the tools are scientifically defensible.

The aim of this appendix is to systematically and objectively evaluate the performance of the user-friendly tools listed above to assess the bioavailability of Zn, Ni and Cu in European freshwaters when compared to predictions using the "full" BLM. In any comparative assessment of this type it is critical that the performance of the tools are not simply compared to one another, but to an independent bioavailability estimate determined using a full BLM that provides the best estimate of risk.

Models and datasets

- For each dataset, HC5 predictions (site-specific PNECs) were made using the user-friendly tools and the "full" BLM. The user-friendly tools run swiftly and can efficiently manage large data sets (the calculations using the tools were only based upon pH, DOC and Ca inputs). The full BLM tools require greater data handling time:
- For Cu, despite the availability of the chronic hydroqual EU Risk Assessment tool, only batches of about 20 target waters can be run at a time and obtain reliable predictions. This model uses the ecotoxicity dataset originating from the Voluntary Risk Assessment produced under the Existing Substances Regulations.

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[15] A lookup table based tool has a database of many hundreds of site-specific LocalEQS/BioF values calculated from a metal BLM each of which is associated with accompanying information on the specific combination of water physico-chemistry used to make these predictions (the training dataset). Predictions of site-specific bioavailability at sites of interest outside of the training dataset are based on comparing the water chemistry of the site of interest to the permutations of site chemistry available in the site training dataset. Bioavailability estimates (i.e. LocalEQS/BioF) are obtained from the "best-fitting" entry in the training dataset. In the bio-met bioavailability tool a selection of potentially best fitting sites from the training dataset are identified and the most conservative (lowest Local EQS value) is returned.

- For Zn, there is no hydroqual EU Risk Assessment tool available, but BLM models can be used in combination with the Zn ecotoxicity data set to automatically run large data sets. This zinc ecotoxicity dataset was recently reviewed for relevancy and reliability by the UK when it derived its $EQS_{bioavailable}$ for zinc as a specific pollutant.

- For Ni, there is only a semi-automatic way to run normalisations with WHAM. Despite initial progress in automation only approximately 200 BLM predictions per day can be performed. This is the integrated BLM on which the Nickel EU RAR was based and on which the existing $EQS_{bioavailable}$ is also based.

In order to run the full BLMs it is important that the datasets used have the full complement of water chemistry inputs to run the models. These inputs need to include 14 to 15 water chemistry parameters measured for each site.

Datasets that fulfilled these criteria were obtained from EIONET or from direct contact with Member State regulators. Four sufficiently comprehensive datasets were identified from UK, NL, AT and FR, and represented a wide range of physico-chemical conditions. The French data was restricted to sites from the Rhone / Mediterranean and Corsica river basin districts. These data were then pre-processed, as follows, to avoid systematic bias in subsequent comparisons:

1. All water chemistries of these test data were within BLM applicability domains.

2. All BLM predictions less then $EQS_{bioavailable}$ were removed. For this exercise, the $EQS_{bioavailable}$ were 4.0 µg Ni L^{-1}, 1.0 µg Cu L^{-1} and 10.9 µg Zn L^{-1} (the latter two are UK Specific Pollutant $EQS_{bioavailable}$).

3. "Outlier" BLM predictions within each country dataset were also removed as these can have disproportionately influence the regression relationship used to compare performance of the user-friendly tool relative to the BLM. Outliers were identified as BLM predictions that were 3 times greater than the interquartile range of respective datasets as measured from the dataset's upper quartile.

In addition to analysis based on individual Member State data, composite datasets comprised of data pooled from several EU member states were also prepared to facilitate comparison of the performance of the tools. Composite datasets were prepared for each metal using equal numbers of datapoints from each contributing Member States to avoid introducing bias based on over-representation of certain types of water chemistry. The size of the individual Member State contribution to the composite dataset was limited to a number of datapoints consistent with 90% of the available data points for the Member State with the least data available i.e. where the member state with the smallest number of samples had 100 samples. The composite sample across member states would be comprised of 90 samples from each dataset. Samples corresponding to this size were then randomly sampled from each respective Member State dataset. This sampling was repeated three times (sample 1, sample 2 and sample 3) and analysis conducted independently to investigate if data selection was significantly influencing the response of each of the tools.

The figures in the results provide the number of data points that were processed in each dataset.

Performance criteria

In order to facilitate a judgement upon the performance of the user-friendly tools it is important to establish a set of criteria on which that judgement is to be made prior to performing the analysis. Therefore, we have considered the performance of a user-friendly tools to be based upon:

- Accuracy relative to BLM
    - statistical significance / slope / intercept of regression
- Precision of predictions (dispersion R^2/AIC)
    - Predictions within a factor of two (as used during BLM developments and assessment of ecotoxicity data)
- Extent of "false positives" and "false negatives"
- No predictions below generic $EQS_{bioavailable}$
- Ability to make predictions for all water chemistry conditions within applicability domain of BLM.

Figure A2.1 below provides a pictorial representation of the assessment criteria with six zones clearly delineated and described on the right hand portion of the figure. For the user-friendly tool there may be a regulatory desire to be precautionary in predictions and so zone 2 may be considered ideal, with zone 3 still within the factor of 2. Zone 4 is very precautionary and could reasonably result in the need to action when it is clearly not required. Zone 5 is under protective and is obviously a concern in a regulatory compliance assessment. Zone 6 gives values that are below the $EQS_{bioavailable}$, which again is a high undesirable and outwith the rationale behind the EQS development.

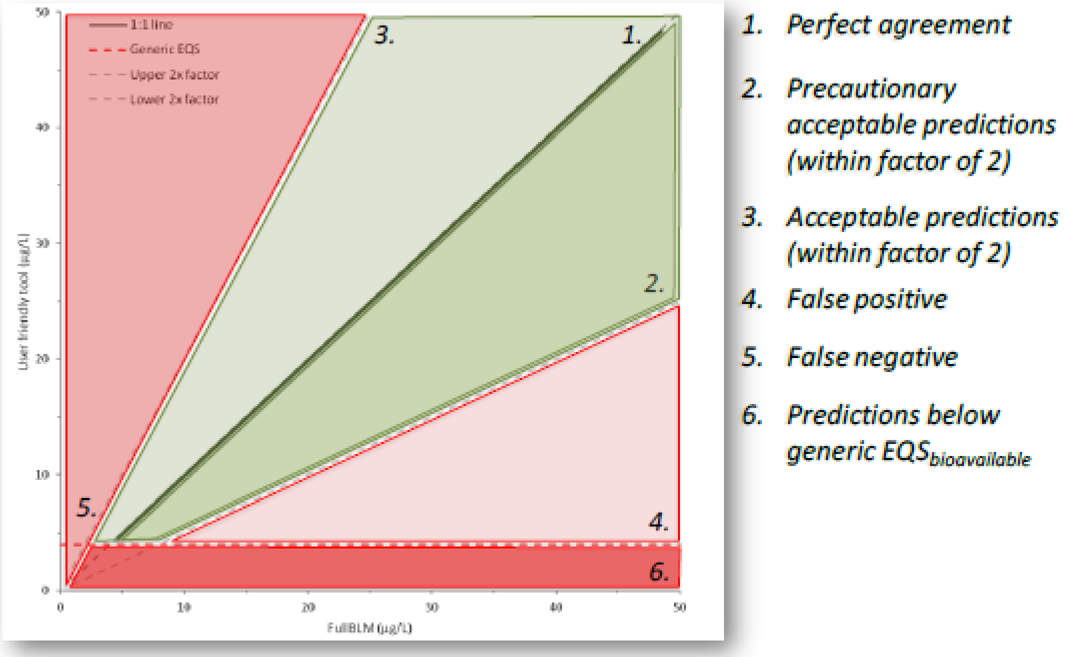


Figure A2.1 Pictorial representation of the criteria by which performance of the user-friendly tools is assessed.

Results

Figures A2.2 – A2.4 show the comparisons between the full BLMs for nickel, copper and zinc and the user-friendly tools. The sectors described in Figure A2.1 can be readily viewed in these comparisons.

Nickel

For nickel, bio-met provides relatively accurate and mostly precautionary predictions, with M-BAT showing slightly less accurate and less precautionary predictions, but all with the factor of two of the true predictions. Both of these tools utilise the original ecotoxicity dataset and BLMs used from the ESR process and the EQS derivation.

Copper

Figure A2.3 shows a comparison of the user-friendly tools with BLM outputs for copper. Bio-met is relatively accurate and precautionary under all conditions. M-BAT predictions are also precautionary and also within a factor of two of the 1:1 line. However, when the predicted PNEC is high (greater than 35 µg L^{-1}) there is a marked increase in false positives.

Zinc

Figure A2.4 shows the comparison for zinc and shows close alignment using bio-met, but with some predictions outside the upper factor of two. For M-BAT all of the values are within a factor two with the majority below the one to one line (i.e. a tendency to conservatism).

Figure A2.2 Comparison of full BLM predictions for nickel against user-friendly tool predictions for freshwater sites from the Netherlands, France and UK.

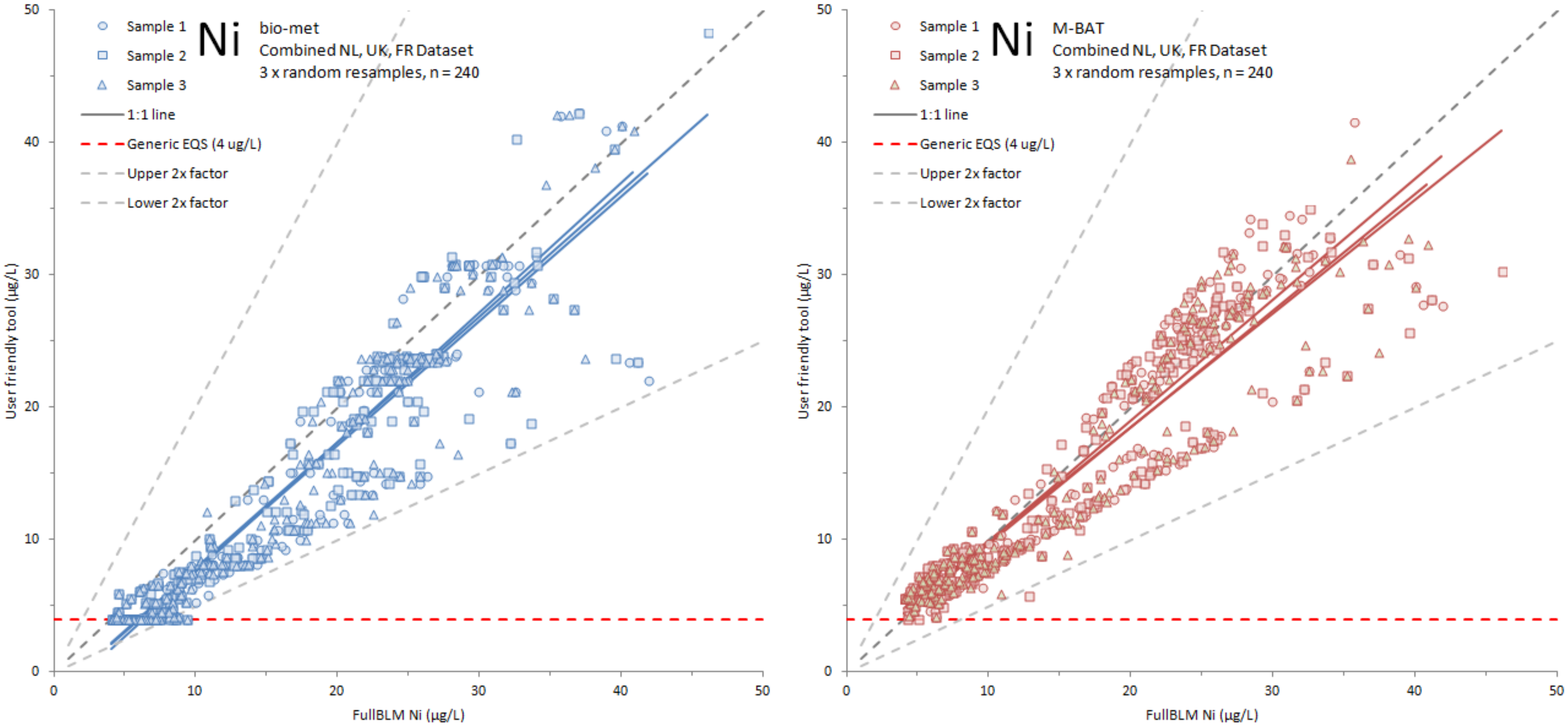


Figure A2.3 Comparison of full BLM predictions for copper against user-friendly tool predictions for freshwater sites from the Netherlands, France, Austria and UK.

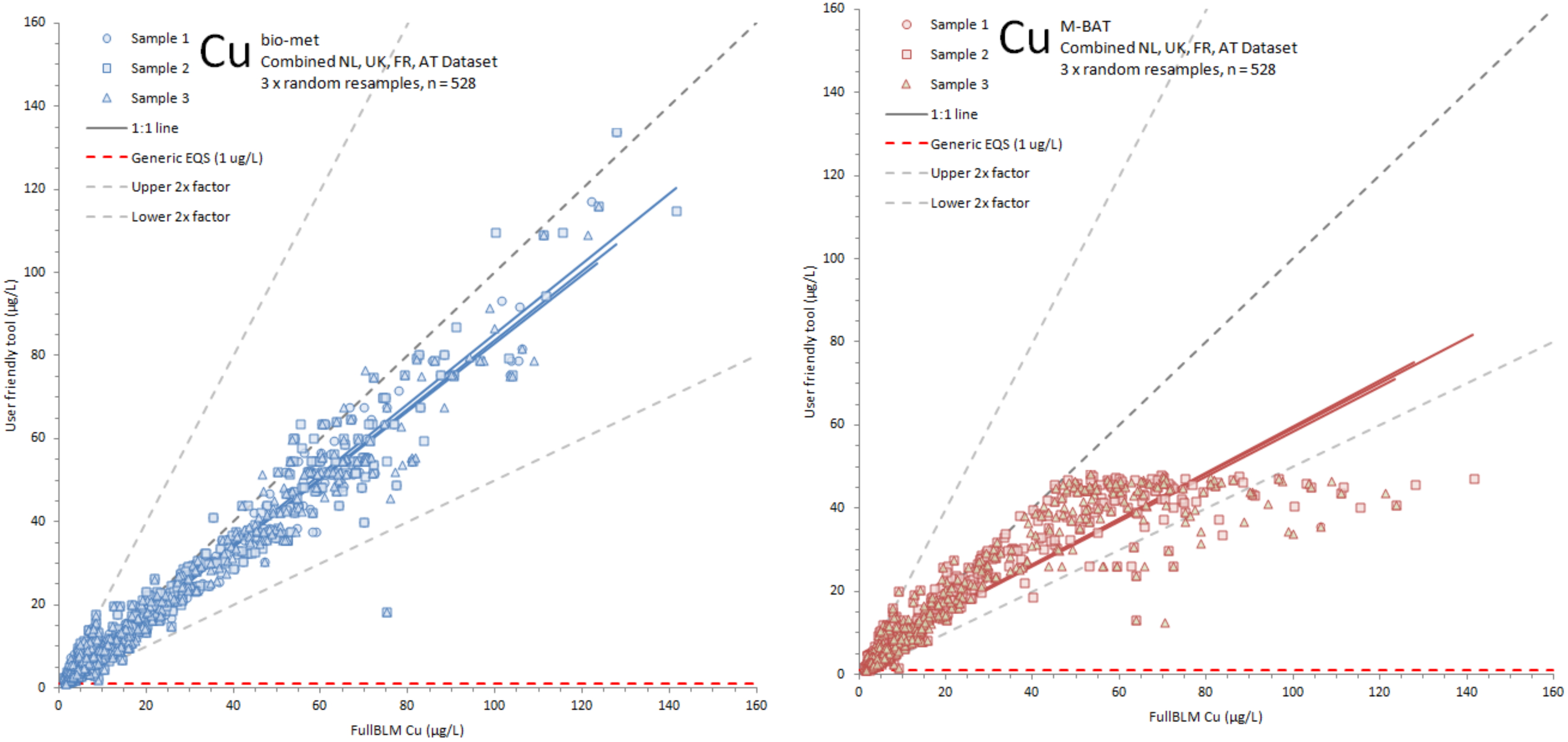
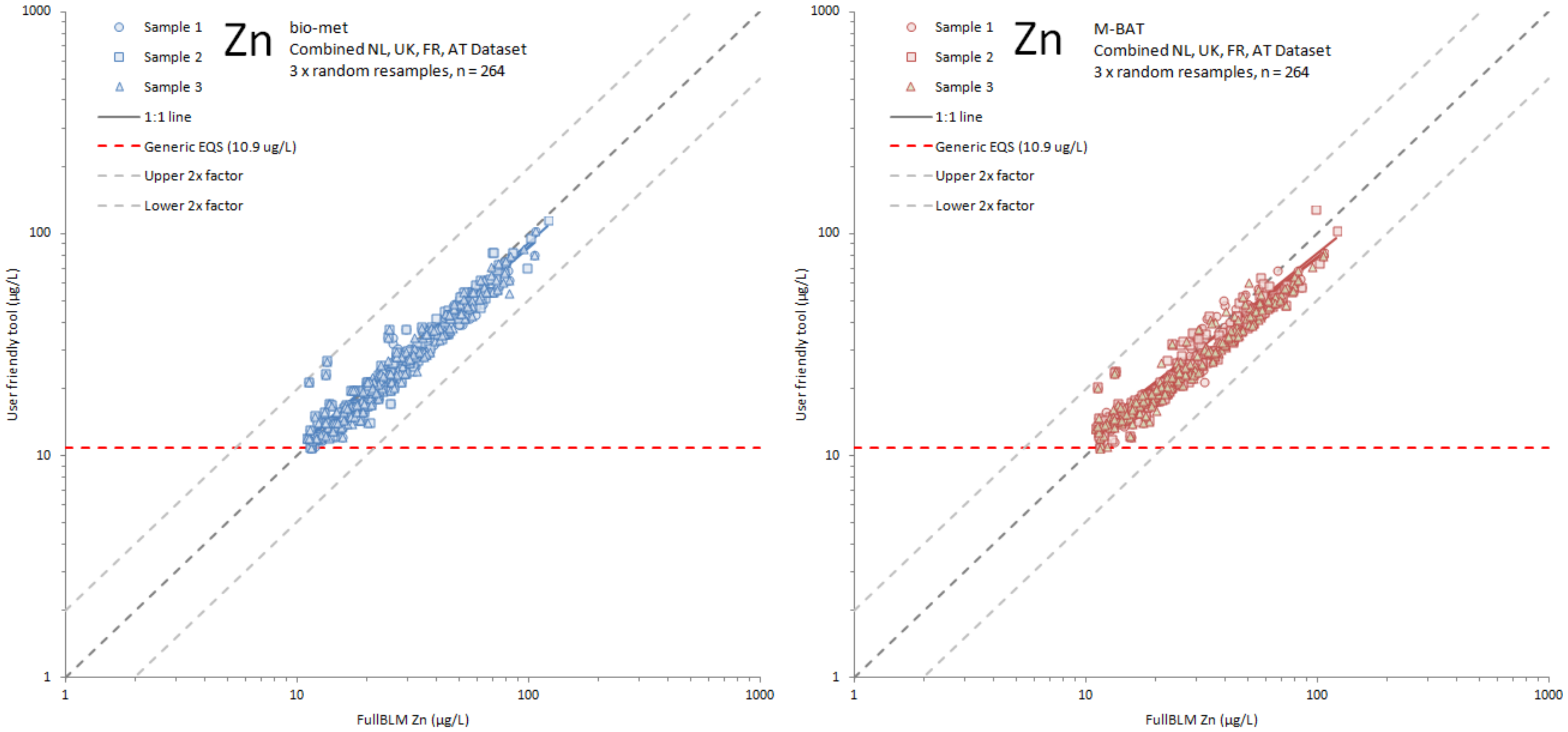


Figure A2.4 Comparison of full BLM predictions for zinc against user-friendly tool predictions for freshwater sites from the Netherlands, France, Austria and UK.



Interpretation

As observed for nickel, the relative difference between the predictions made by bio-met and M-BAT for zinc and copper are likely to be the consequence of different underlying processes to manage the ecotoxicity datasets (look-up tables vs algorithms) respective tools. As copper and zinc are not currently Priority Substances the harmonisation of underpinning BLM models is not crucial to their implementation. However, Member States should ensure that the BLM models underpinning user-friendly tools for Specific Pollutants, if adopted, are fit for purpose and, where possible, harmonised with models that have been subject to independent regulatory scrutiny, e.g. during the ESR technical review process or other form of peer-review and validation.

All of the tools provide precautionary predictions most of the time compared to the full BLMs. This is a desirable attribute when the tools are to be used within a tiered assessment approach. For both tools these are near the one to one line and mostly within a factor of two of the full BLM predictions. It follows that these screening tools might be expected to have a lower incidence of false positives. The main consequence of false positives is not so much about making mistakes about risk but, rather, about the effort that is needed to assess whether regulatory action is required or not.

M-BAT has been trained on UK freshwater compliance data, for which DOC concentrations are not typically above 10 mg L^{-1}. Samples in the test datasets in Figure A2.3 had DOC levels considerably greater than 10 mg L^{-1} and, as a result, predictions of risk from copper were highly precautionary. The Environment Agency are modifying M-BAT on the basis of the comparison presented above for copper.

For nickel, the tools are compared with the BLMs, methodology and datasets used for the Ni EQS. For nickel, a user-friendly tool should be based on these data. However, for copper and zinc, which are specific pollutants in most countries the models, datasets and methods that are used are down to the individual Member States. However, regulatory peer-reviewed ecotoxicity datasets and BLMs are readily available to use for these metals as well.