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GUIDANCE ON THE INCORPORATION OF BIOAVAILABILITY CONCEPTS FOR ASSESSING THE CHEMICAL ECOLOGICAL RISK AND/OR ENVIRONMENTAL THRESHOLD VALUES OF METALS AND INORGANIC METAL COMPOUNDS

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GUIDANCE ON THE INCORPORATION OF BIOAVAILABILITY CONCEPTS FOR ASSESSING THE CHEMICAL ECOLOGICAL RISK AND/OR ENVIRONMENTAL THRESHOLD VALUES OF METALS AND INORGANIC METAL COMPOUNDS

Environment Directorate
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT
Paris, 2016
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FOREWORD

The OECD Workshop on Metals Specificities in Environmental Risk Assessment was held on 7-8 September 2011 in Paris. A number of conclusions and recommendations were agreed upon by workshop participants (ENV/JM/MONO(2012)2). Stemming from topics identified at this workshop, two guidance documents have been developed: Guidance on Selecting a Strategy for Assessing the Ecological risk of Organometallic and Organic Metal Salt Substances based on their Environmental Fate (ENV/JM/MONO(2015)2) and the document herein focusing on the incorporation of bioavailability concepts for assessing the chemical ecological risk and/or environment threshold values of metals and inorganic metal compounds.

Several models, tools and methods have been published in the past 20 years to include bioavailability in risk assessment and several OECD member countries already have developed frameworks and published guidance documents for taking metal specificities into account in environmental risk assessment (e.g. US EPA Framework for Metals Risk Assessment, US EPA 2007; Metal Environmental Risk Assessment Guidance (MERAG fact sheets), ICMM 2007; ECHA's Appendix on Environmental Risk Assessment for metals and metal compounds, ECHA, 2008; Common Implementation Strategy for the Water Framework Directive, EC 2011; Technical guidance to implement bioavailability based environmental quality standard for metals, WFD 2015). The aim of the current guidance is not to replace the aforementioned frameworks or guidance documents, but rather, to provide an overarching framework on how to apply these tools depending on which data are actually available/needed to assess the bioavailability of the metal under scrutiny. Further harmonisation of these approaches and methodology, where appropriate, over the different OECD countries is recommended and could facilitate a more worldwide application and the Mutual Acceptance of Data since using common assessment approaches may help comparing and exchanging data sets, which could result in significant resource savings.

The drafting of this document was initiated by Eurometaux and the International Council on Mining and Metals (ICMM) based on the MERAG fact sheets and subsequently reviewed, revised and endorsed by the member countries participating in the Task Force on Hazard Assessment. It is published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology who agreed to its declassification.

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1 INTRODUCTION

The degree to which metals are available and cause toxicity to aquatic, sediment burying and terrestrial organisms is determined by site-specific geochemical conditions controlling the speciation/precipitation and/or complexation of metals. In the aquatic environment these processes are generally controlled by pH and DOC (dissolved organic carbon). Furthermore, several cations (Ca, Mg, Na, K) are known to compete with metal ions for binding to the site of toxic action and hence reduce metal toxicity. In sediments sulfides, organic carbon and iron/manganese (oxy)hydroxides play a mitigating role as they provide important binding/absorption phases. For the soil compartment it has been demonstrated that clay minerals, organic carbon together with soil pH are the main drivers controlling bioavailability of metals. The wide variation of the physicochemical characteristics encountered in the environment is the main reason why no clear relationships have been observed between measured total concentrations of metals and their potential to cause toxic effects. Therefore, taking bioavailability into account is a refinement that improves the precision of environmental assessment approaches as it helps to increase the realism of the assessment and can help regulators to better understand the likelihood of the occurrence of adverse effects due to metal contamination.

With respect to the variability of abiotic and biotic conditions in the surface waters and sediments or the soil compartment, the limitations and uncertainties of these concepts need to be considered when applied in the context of an environmental risk assessment. When using the approach it needs to be considered whether the concept is appropriate and applicable within the respective assessment context.

The information presented in this document serves as a practical guidance for regulators, industry or other experts faced with assessing bioavailability for inorganic substances. This guidance provides a step-by-step explanation that can be used to implement bioavailability for the water, sediment and soil compartments and describes the key scientific principles. As ecotoxicity data are a key component of setting safe thresholds for metals the guidance focuses on how bioavailability corrections can be applied for the purpose of using the normalized ecotoxicity data in a risk assessment framework. The guidance is not intended to be used for classification purposes. Neither does this guidance cover organometallic compounds and organic metal salts for which a separate OECD guidance document has been developed (OECD, 2015). Also, the methods and frameworks presented in this guidance were not analysed for their applicability for manufactured nanomaterials. Areas that are currently also not explicitly covered are the influence of multi-metal mixtures or multiple metals in media (water, sediment, soil), and marine environments.

The structure of this guidance is the following. Chapter 2 gives a brief overview of the terminology and definitions used and introduce the pragmatic approaches that can be used in implementing bioavailability. Chapter 3 outlines for each environmental compartment (water, sediment and soil) in detail the specific steps that can be taken to take bioavailability into account. For the water compartment different approaches are presented going from the use of advanced tools such as the Biotic Ligand Model (BLM) to the simple measurement of dissolved metal concentrations. For sediments binding to different sediment phases (sulfides, organic carbon and Fe, Al Mn-oxy hydroxides), as key parameters controlling metal bioavailability, are being explored. For soil, next to the aspect of bioavailability time related aspects such as lab-to-field extrapolations are covered. More specific background information is presented in the associated appendices: selection of toxicity data (appendix 1), BLM (appendix 2), case studies (appendices 3, 4 and 5) and the relative importance of the dietary route (appendix 6).

2 CONCEPTS AND OVERVIEW

2.1 Terminology and Definitions

The "bioavailability" concept encompasses several operationally defined and interacting terms (Figure 1) (Me being the metal ion). Availability starts with physico-chemical considerations (environmental availability) but should be subsequently linked to different ecological receptors taking different uptake routes into account (ECHA, 2014). Within the different environmental compartments processes such as absorption to particles and organic carbon, metal sequestration and competition with major cations will reduce the free metal ion concentration that is available to bind with a biological membrane and would be bioaccessible. Once inside an organism the bioaccessible metal pool that is actually available to elicit a potential adverse effect will depend on the way the metal is internally distributed, metabolised, excreted, detoxified and bioaccumulated.

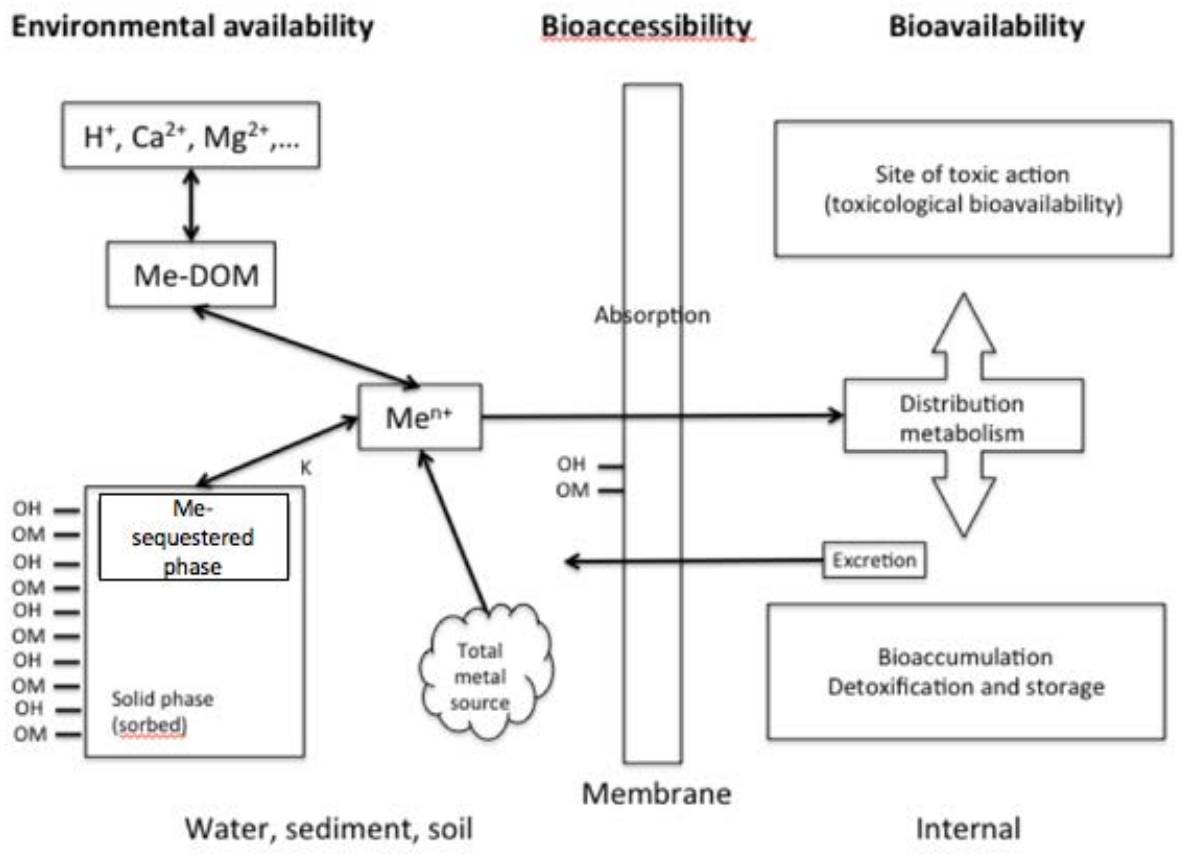

Figure 1 Simplified conceptual outline for metals bioavailability

The following definitions apply in this guidance:

- **Environmentally available fraction:** the portion of total metal in soil, sediment, water and air that is available for physical, chemical, and biological modifying influences (e.g. fate, transport, bioaccumulation). It represents the total pool of metal at a given time in a system that is potentially bioavailable (McGeer et al., 2004; US EPA 2007).

- **Bioaccessible fraction:** is the fraction of the environmental available metal that actually interacts at the organism's contact surface and is potentially available for absorption or adsorption by the organism.

- **Bioavailability:** (or biological availability) means the extent to which a substance is taken up by an organism, and distributed to an area within the organism. It is dependent upon physico-chemical properties of the substance, anatomy and physiology of the organism, pharmacokinetics, and route of exposure." (UN-GHS, 2013). Hence metal bioavailability refers to the fraction of the bioaccessible metal pool that is available to elicit a potential effect following internal distribution; metabolism, elimination and bioaccumulation. For the purpose of this guidance, the term "metal bioavailability" is used more as a conceptual term as initially proposed by Meyer (2002).

- **Biotic Ligand:** Metal toxicity is simulated as the accumulation of metal at a biologically sensitive receptor, the "Biotic Ligand", which represents the site of action for metal toxicity. It is hypothesized to be placed on the gill surface.

- **Critical biotic ligand accumulation:** This critical concentration is referred to as the "LAx", or the (sub)lethal accumulation of metal on the biotic ligand associated with x% effect.

- **Critical bioavailable dissolved concentration:** is the critical ligand concentration recalculated to the specific physico-chemistry conditions occurring at a site and expressed as dissolved concentration.

- **Environmental threshold value (ETV):** is an environmental effects concentration below which adverse effects on the environment are not expected to occur. Examples of ETVs are Predicted No Effect Concentrations (PNEC), Environmental Quality Standards (EQS), Water Quality Criteria (WQC), Water Quality Standards, etc.

- **Environmental Exposure Concentration (EEC):** is an exposure benchmark value, which is compared with an Environmental Threshold Value in a risk assessment framework or for compliance checking. This Environmental Exposure Concentration could be single exposure metric (e.g. a high end value such as the 90^{th} percentile of a suit of all individual measured or modelled metal concentrations for a predefined site/region or could encompass multiple metrics. For example the exposure concentration could consider the entire exposure distribution (in a probabilistic way) or the 10^{th}, 50^{th} and 90^{th} percentile could be used to present a more detailed picture.

- **Reasonable Worst Case conditions (RWC):** considered to be the environmental conditions that maximizes bioavailability

- **Biogeochemical region:** Fairbrother and McLaughlin (2002) initially referred to this concept as metallo-regions where on a regional scale separate sub-regions are being defined using suitable methods to aggregate spatially explicit environmental variables. Another term frequently used in this regard is "ecoregion". At the moment the biological/ecological-part has been a bit underrated as the current existing biogeochemical regions are based on abiotic factors rather than quantified ecological metrics. If ecology can be considered, the approach further suggests that instead of using 'generic' species, it is preferable to use 'endemic' test organisms representative for the natural environment under investigation to characterise the sensitivity of the ecosystem.

Several quantitative metrics have been put forward to assess bioaccessibility and bioavailability. None of them can really be singled out to capture all the different aspects in relation to bioavailability of chemicals in general (ECHA, 2014) and some have a broader and more relevant applicability domain than others (e.g. free ion vs. dissolved concentrations). For metals, the free metal ion, its potential to complex/compete with other organic and inorganic ligands for the available biological binding sites and its internal distribution within an organism is key to understand metal availability. Although it is acknowledged that the free ion is not necessarily the best predictor for all metals as other metal species such as neutral species (e.g. AgCl, HgS) and anionic species (e.g. SeO^{2-}, AsO_4^{2-}) may contribute to the observed toxicity (Campbell, 1995). Taking into account the different processes and metal forms that could occur in an ambient water and/or test medium the following operationally defined terms will also be used to make a distinction between "Total metal", "Total dissolved metal" and "Dissolved metal" species of concern:

- **Total metal concentration:** comprises particulate (adsorbed/absorbed + precipitated) + dissolved (inorganic complexes + organic complexes + free ionic forms)

- **Total dissolved metal concentration:** refers to the fraction that passes through a filter of 0.45 μm and comprises inorganic complexes + organic complexes + free ionic forms

- **Dissolved metal species of concern:** refers most often to the free metal ion but other relevant metal speciation forms that could contribute to the observed toxic effect are also covered

2.2 Practical approaches for using bioavailability

This chapter provides a concise overview of the overall strategy and associated decision logic and steps for the evaluation of bioavailability in the aquatic (water and sediment) and soil compartment (Figure 2). It should be noted that the approaches presented allow that one can stop anywhere in the process and that at each refinement step the respective outcome gained realism and reduced uncertainty and conservatism. It is also recognized that it is not always necessary and/or possible to perform all steps. For example, the exposure could be low, or countries do not have the monitoring tools in place to perform the advanced tools, or no advanced bioavailability models have been developed for the metal under scrutiny.

In general, for each environmental compartment a clear distinction can be made between those metals for which bioavailability models and/or user friendly bioavailability tools have been developed and validated and those for which only alternative approaches can be applied. It is clear that in case bioavailability models are available for the metal under scrutiny these models will provide the most realistic assessment with the lowest uncertainty. For example, for the water compartment metal species bound to a biological target could be calculated using toxicity-based models such as the Biotic Ligand

Model (BLM). Due to its complexity and multiple calculation steps, simplified screening models have been developed that that mimic the results of BLMs. Note that due to the simplification and the limited input parameters that are required by screening models, the predicted bioavailability is accompanied by a higher uncertainty than the outcomes of BLMs. If applicable, the use of hardness based regressions could be considered. In case no advanced or simplified models are available, an alternative approach can be used in which the assessment could be based on measuring or modelling dissolved metal and/or free metal ion concentrations (water compartment).

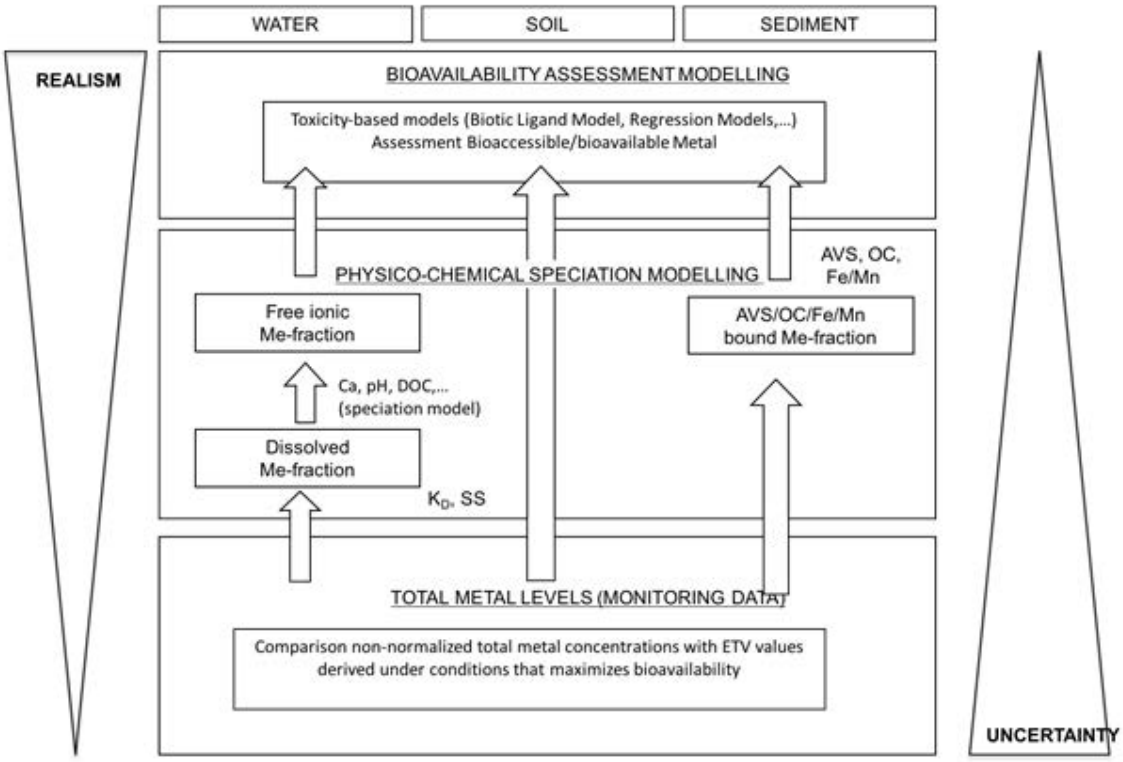

Figure 2: Overall strategy and associated decision logic and steps for the evaluation of bioavailability in the aquatic (water and sediment) and soil compartment.

The necessary abiotic factors needed for the application of bioavailability models can be obtained from existing monitoring databases for a specific region/area or from specific tailored monitoring campaigns (site specific). For a site specific assessment quite often median concentrations are used. For setting thresholds that could be used in a more cautious way, low or high concentrations (representative of realistic worst case conditions) of the abiotic factors should be selected.

Dissolved metal concentrations or free ion activities should be compared with ecotoxicity data expressed in the same way. In order to calculate the bioavailability of the metal in the test media, abiotic factors in the test medium should also be known. Furthermore, as metals are naturally occurring substances many organisms have evolved mechanisms to regulate the accumulation and storage of these metals which also influence their sensitivity towards these metals. This phenomenon should ideally also be considered in selecting adequate ecotoxicity data for risk assessment (see Appendix 1 section A.1.2). When test organisms have been cultured in conditions that are outside the natural background concentration ranges

such data should be considered with care and might even be discarded. It is, however, recognized that this may lead to a reduction in the number of useful ecotoxicity data which may even sometimes limit the possibility of using a Species Sensitivity Distribution (SSD). Another complicating factor is that quite often culture conditions are not reported and in that case expert judgment should be used to decide if the study can still be used or not.

The respective outcome of any type of bioavailability correction will result in a gain of realism and a reduction in uncertainty. In case metal concentrations are lower than generic ETVs it could be decided not to apply a bioavailability correction. Specific approaches for the different environmental compartments are described in Chapter 3.

3 PRACTICAL CONSIDERATIONS FOR IMPLEMENTING BIOAVAILABILITY

In a regulatory context metal exposure concentrations are typically compared to one single numerical value in order to screen out potential risks (i.e. in a risk assessment context) or to identify non-compliance (in case of the use of Environmental Quality Standards/guidelines, Water Quality Criteria etc.). Most often these values are expressed as total metal concentrations. In implementing bioavailability, the purpose is to recalculate both exposure and derived ecotoxicity thresholds to a metric that better reflects what an organism actually "experiences" under certain environmental conditions. Hence the application of the bioavailability concept to the water, sediment and soil compartment entails the normalization of conventional effect thresholds (for example toxicity thresholds such as EC50, EC10, EC20), expressed as total metal concentrations and exposure concentrations, using either soluble fractions, speciation or bioavailability algorithms. The next sections provide brief descriptions of the available approaches and tools available to implement bioavailability for the different compartments (water, sediment and soil).

3.1 Implementation of bioavailability for the water compartment

A schematic representation on how bioavailability can be implemented for assessing risks in the water compartment is presented in Figure 3.

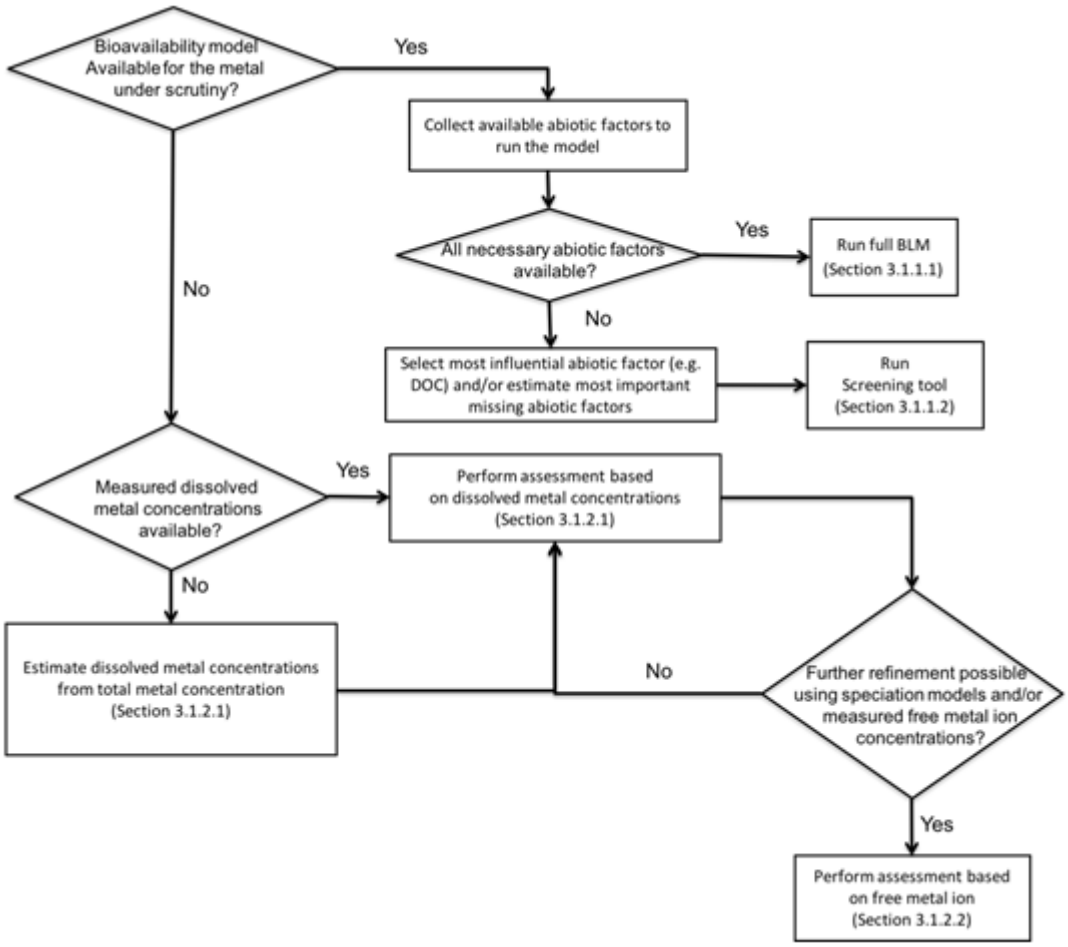

Figure 3: Simplified representation of the decision tree for the implementation of bioavailability correction for the water compartment

Preferentially, the assessment should be performed on a 'bioavailable' basis if appropriate toxicity related bioavailability models (e.g. Biotic Ligand Model and/or user friendly bioavailability tools) are available. The BLM provides a semi-mechanistic understanding of the interactions of metals with organic, inorganic and biotic ligands at the biological membrane, and allows for the quantification of the bioavailability under given abiotic conditions of the water. For several metals (Ag, Cd, Cu, Pb, Ni, Mn, Zn) most frequently used in industrial applications and found in contaminated areas, bioavailability assessment models such as Biotic Ligand Models (BLMs) are available which allows a semi-mechanistic understanding of metal toxicity. As the environmental risk assessments are predominantly driven by long-term effects (chronic toxicity), most of these BLMs have been developed for chronic endpoints. There are, however, a large number of publications on acute toxicity-based BLMs. More information on the validation of the BLMs and read across over different species is given in Appendix 2.

Since BLMs have high data requirements and require multiple complex calculation steps, user friendly screening tools have been developed that in essence performs the same calculations as the BLM but requires fewer data inputs focusing on those parameters that influencing the bioavailability the most such as DOC, pH and Ca (section 3.1.1.2). Alternatively, methods can be used that estimate the parameters that are missing. These screening tools will also accommodate circumstances in which the required information on all abiotic parameters to run the BLMs are not available. Their outcome is however more uncertain relative to the BLM but are readily interpretable in the context of basic risk management and EQS compliance assessment.

In case no bioavailability models are available the preference is to use measured data (dissolved). If deemed more appropriate speciation could be taken into account at his stage. If no measured data are available total concentrations could be converted to dissolved concentrations. However, it should be recognized that the latter approach introduces quite a lot of uncertainty.

3.1.1 Use of bioavailability models

3.1.1.1 Overview of models and tools

For quite a lot of metals BLMs are available. Most BLMs have been developed for regulatory purposes. One of the barriers to the widespread use of BLMs in the regulatory arena is the amount of information which is required for making BLM calculations (Peters et al, 2011). Some BLM may require up to 10 measured input parameters, which might hamper the application in standard monitoring programs (Verschoor et al, 2012). Therefore, several user-friendly BLM-based bioavailability software tools for assessing the aquatic toxicity of relevant metals (mainly copper, nickel and zinc) have been developed (Rüdel et al, 2015):

- Bio-met: a "lookup table" based tool in both MS excel spreadsheet and online formats developed by ARCHE (Belgium)/WCA-environment (UK) (www.bio-met.net);
- M-BAT: an algorithm based tool in MS Excel developed by WCA-environment (UK);
- PNEC.Pro: an algorithm based tool in MS Excel developed by Deltares (The Netherlands) (www.PNEC-pro.com).

In Table 1 an overview is given of the most relevant parameters that influence the bioavailability of metal species in the water compartment. In order to normalise toxicity data towards physic-chemical conditions different datasets for abiotic factors (and environmental concentrations) should be considered depending on the goal of the assessment (i.e. threshold derivation, site-specific assessment etc.). Often,

DOC is the most influential parameter and therefore used in most tools. The necessity to consider hardness related parameters depends on the metal of concern. For silver, sulphide is an essential input parameter. The presence of inorganic ligands such as SO_4^{2-}, Cl^-, are generally less important, although they are required to run a BLM.

Table 1: Overview of the most relevant parameters that influence the bioavailability of metal species in the water compartment. Those indicated moderate and major should be measured. Minor is nice to have. Shaded area gives the magnitude of importance

Water compartment		Relative importance		
Metal	Physico-chemical parameter	Minor	Moderate	Major
Cu	DOC			
	Hardness (Ca^{2+}, Mg^{2+})			
	pH			
	Other inorganic ligands (SO_4^{2-}, Cl^-, Na^+, K^+, etc.)			
Zn	DOC			
	Hardness (Ca^{2+}, Mg^{2+})			
	pH			
	Other inorganic ligands (SO_4^{2-}, Cl^-, Na^+, K^+, etc.)			
Ni	DOC			
	Hardness (Ca^{2+}, Mg^{2+})			
	pH			
	Alkalinity			
	Other inorganic ligands (SO_4^{2-}, Cl^-, Na^+, K^+, etc.)			
Pb	DOC			
	Hardness (Ca^{2+}, Mg^{2+})			
	pH			
	Other inorganic ligands (SO_4^{2-}, Cl^-, Na^+, K^+, etc.)			
Mn	DOC			
	Hardness (Ca^{2+}, Mg^{2+})			
	pH			
	Other inorganic ligands (SO_4^{2-}, Cl^-, Na^+, K^+, etc.)			
Ag	DOC			
	Hardness (Ca^{2+}, Mg^{2+})			
	pH			
	Sulfides			
	Chlorides			
	Other inorganic ligands (SO_4^{2-}, Na^+, K^+, etc.)			
Cd	DOC			
	Hardness (Ca^{2+}, Mg^{2+})			
	pH			
	Chlorides			
	Other inorganic ligands (SO_4^{2-}, Cl^-, Na^+, K^+, etc.)			

As can be seen from Table 2, the screening tools only need a basic set of commonly determined water parameters as input. The other parameters are eliminated from the screening tools due to their limited influence on the normalized ecotoxicity result. For example for Bio-met the input parameters required for BL modelling has been simplified by estimation of certain water chemistry parameters (e.g. alkalinity, Mg, Na, K, SO4, Cl) from Ca concentrations. A simplified hardness calculator/convertor tool is available online to perform these transformations (www.bio-met.net). In addition, historical data have been used to allow estimation of DOC default values for many waterbodies and hydrometric areas in England and Wales (Environment Agency, 2009). Verschoor et al (2012) simplified the BLMs for Cu, Ni and Zn by developing 1-, 2- and 3- parameter transfer functions. Using these functions, requiring a maximum of 3 measured water chemistry parameters meet the requirements of monitoring efficiency, conceptual justification and reality best. The parameters DOC, pH and Mg or Ca appears to be the main descriptors. If only one parameter needs to be singled out for a bioavailability correction it would be DOC.

Data sets of abiotic factors as well as environmental concentrations should be representative of the area under investigation. The breadth of the data sets will usually be proportional to the scope of the assessment, i.e. broader data sets will be necessary for regional assessments with national to continental scales due to spatial variability, compared to local assessments which address site-specific operational scales. It is particularly important to take relevant abiotic factors into account for the metal under investigation

Table 2: Overview of required (red) and optional (orange) input requirements of bioavailability screening tools. Optional input parameters decrease the uncertainty of the normalized ecotoxicity data.

Metal	Tool	DOC	Hardness*	pH	Ca	Mg	Na
Cu	Biomet						
	M-BAT						
	PNEC-pro						
Zn	Biomet						
	M-BAT						
	PNEC-pro						
Ni	Bio-Met						
	M-BAT						
	PNEC-pro						
Pb	Biomet						
Mn	M-Bat						
Cd	Hardness equation						

*If only hardness is given this value can be used to derive Ca concentrations needed as input in the screening tools.

Table 3 gives an overview of several readily applicable screening tools and chronic BLMs that have been reported in the literature. It should be noted that it is unknown if all mentioned tools have been properly validated (see appendix 1). Acute BLMs that have been derived from acute ecotoxicity data that are less frequently used in some jurisdictions (e.g. US) are given in appendix 2.

Table 3: Overview Chronic BLMs and screening tools

Metal	(driving abiotic factor)	Available chronic bioavailability tools	Available screening tools
It should be noted that pH is not always specifically included as driving abiotic factor, but is only mentioned when specific binding constants for H^+ have been derived. Impact of pH, however, is often indirectly included in the speciation calculation.			
Ag	Silver: bioavailability effects due to DOC and sulphide (expressed as Chromium Reducible Sulphide (CRS). Silver will be toxic under conditions where the molar concentration of Ag exceeds the molar concentration of CRS (cfr SEM/AVS approach for metals in sediment)	www.hydroqual.com/wr_blm.html	No
Cd	Predominantly competition with major (hardness) cations	www.hydroqual.com/wr_blm.html	Hardness correction equation
Cu	Predominantly DOC-effect, some competition with major cations	www.hydroqual.com/wr_blm.html	www.pnec-pro.com www.bio-met.net http://www.wfduk.org/resources/rivers-lakes-metal-bioavailability-assessment-tool-m-bat
Pb	Clear effect of DOC on toxicity, but speciation complicate by precipitation with phosphate	www.leadblm.com www.hydroqual.com/wr_blm.html http://vminteq.lwr.kth.se/	Under development
Ni	Effects from both DOC and competing ions	www.hydroqual.com/wr_blm.html	www.pnec-pro.com www.bio-met.net http://www.wfduk.org/resources/rivers-lakes-metal-bioavailability-assessment-tool-m-bat
Mn	Evidence of a protective effect from hardness – chronic BLM development Competition from Ca^{2+} for fish and invertebrates, and competition from H^+ for algae Limited effect of DOC (limited binding of Mn)		http://www.wfduk.org/resources/rivers-lakes-metal-bioavailability-assessment-tool-m-bat
Zn	Effects from both DOC and competing ions	www.hydroqual.com/wr_blm.html	www.pnec-pro.com bio-met.net http://www.wfduk.org/resources/rivers-lakes-metal-bioavailability-assessment-tool-m-bat

3.1.1.2 Practical implementation using the Biotic Ligand Models (BLMs)

Figure 4 presents the general stepwise procedure when using BLMs in the assessment of metals and the steps are described in further detail below. In comparing the environmental concentrations and the effect concentrations, care should always be taken that both are expressed in the same units (Figure 4). The sequence and rationale is similar in case BLM screening tools are used.

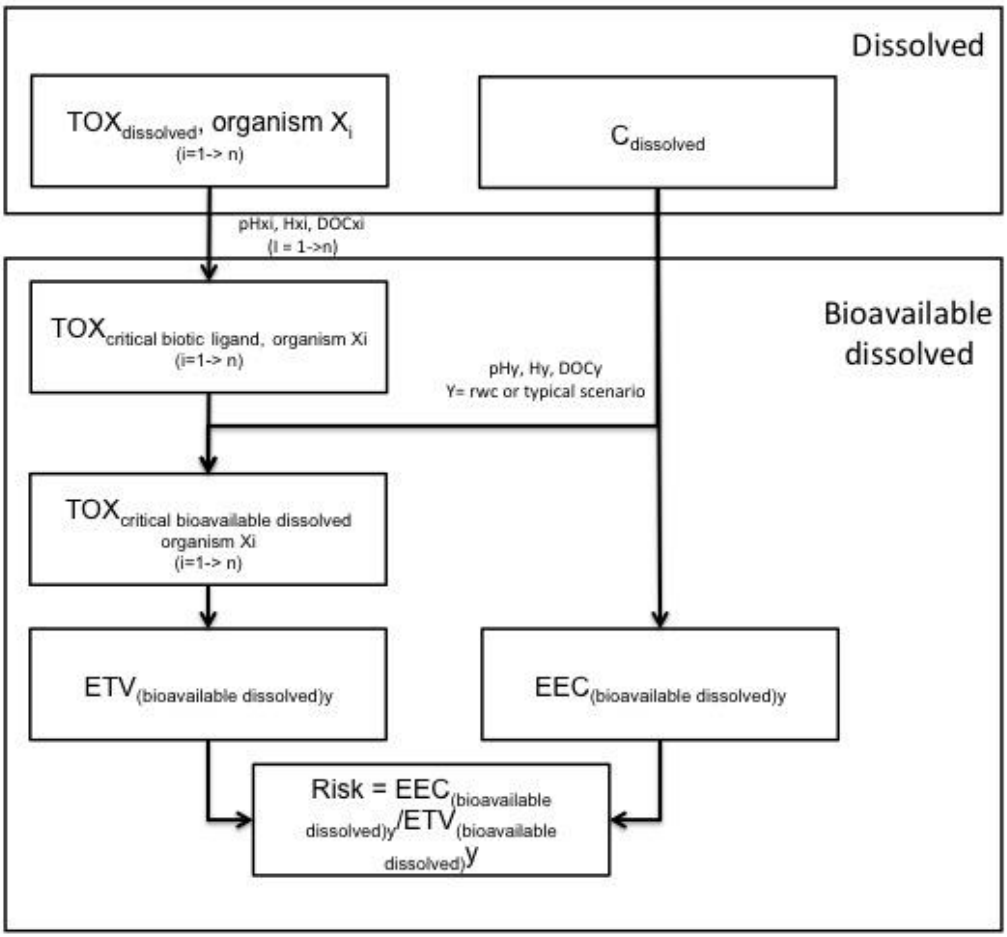

Figure 4: Framework for incorporation of bioavailability models for the water compartment. X = test conditions, Y = reasonable worst-case conditions or typical conditions (depending on the scenario), H = hardness, DOC: dissolved organic carbon.

STEP 1: Determine the critical biotic ligand accumulation ($TOX_{critical\ biotic\ ligand,\ organism\ xi}$) calculated from the organism specific toxicity values ($TOX_{dissolved,\ organism\ xi}$), expressed as dissolved concentration. Organism-specific bioavailability models should be used as much as possible for that purpose (see section on cross-species extrapolation in Appendix 2 for further guidance).

STEP 2: Recalculate each organisms specific critical biotic ligand binding ($TOX_{critical\ biotic\ ligand,\ organism\ xi}$) into a critical bioavailable dissolved concentration ($TOX_{(critical\ bioavailable\ dissolved)y,\ organism\ xi}$) for a specific area under investigation, characterized by a specific set of water-quality conditions (pHy, Hy, DOCy).

STEP 3: Use the critical bioavailable dissolved concentrations ($Tox_{(critical\ bioavailable\ dissolved)y,\ organism\ xi}$) to derive a cautious environmental threshold value (ETV) using assessment factor approaches (data poor metals) or statistical extrapolation methods (species sensitivity distributions) (EC, 2011). This value is subsequently compared to the selected modelled and/or measured environmental exposure concentration (EEC) equally expressed as dissolved concentrations. The EEC is calculated from all individual $C_{dissolved}$ values for a predefined environment.

Some considerations:

- In the context of a generic risk assessment the BLMs can be used to convert the effects data to well characterized specific local or regional conditions (i.e. establishing $ETV_{local,\ bioavailable}$ or $ETV_{regional,\ bioavailable}$) or reasonable worst case conditions (i.e. establishing $ETV_{reference,\ bioavailable}$)

- Conceptually, the BLM framework is a valid descriptor of metal bioavailability when toxicity is a result of exposure to the dissolved metal ion. It should, however, be noted that for some metals combined toxicity effects due to both the dissolved and precipitated metal forms have been demonstrated under specific environmental conditions (e.g. aluminium). For those cases the semi-mechanistic BLM framework needs to be extended to account for the additional physical effects due to the interaction between precipitated forms and the biotic ligand.

- BLMs are developed for a well-defined chemical (abiotic factors) and toxicological data set that define their validated application boundaries. In case the local water chemistry falls outside the BLMs application domain it does not immediately prohibits the use of the model. For example if for increasing pH values (6-8) the BLM predicts less toxicity than the upper pH limit of the BLM (i.e. 8) could be used as a conservative estimate for toxicity. The BLM should, however, not be used in the other direction when toxicity would increase. In that case it should just be flagged that the water chemistry falls outside the boundaries. Eventually spot checks can be used to verify if the model could be extended into that region.

- Validated BLMs exist only for a limited number of biological species that are representative for the typical trophic levels encountered in the aquatic environment (fish, algae, invertebrates). For these species, toxicity data generated under different abiotic conditions can be normalized to a common set of abiotic conditions (e.g. biogeochemical region) as long as these abiotic parameters fall within the geochemical boundaries of the developed bioavailability model (e.g. range of pHs, hardness, DOC). However, for those species for which no specific bioavailability model has been developed cross-species extrapolation is recommended (Appendix 1). While cross-species extrapolation between species belonging to similar taxonomic groups seems justified in most cases (i.e. BLMs developed for *O. mykiss* can be applied to ecotoxicity data for other fish species, BLMs for *D. magna/C. dubia* can be applied to ecotoxicity data for other invertebrates, and BLMs for *P. subcapitata* can be applied to ecotoxicity data for other unicellular alga), "spot check" validations are needed for species belonging to dissimilar taxonomic groups.

- However, even if a model has been validated it is clear that the applicability domain of the current BLMs do not cover all relevant metal species. For example the BLM /free ion concepts

are less suited to predict effects of metal particulates (e.g. physical effects ad clogging gills), transformation to organometallics (e.g. methylation), release of hydrophobic organometallic (OM) and organic metal salts (OMS) (OECD, 2015), bioavailable low molecular weight complexes (e.g. metals bound to natural dissolved organic matter), other inorganic metal species such as AgCl, HgS (Campbell, 1995), cationic inorganic complexes (e.g. $Cd(OH)^+$) etc.

- Screening models can be used in situations where limited data are available on the abiotic factors needed to run the BLM.

The complete bioavailability normalisation procedure using cross-species extrapolation is illustrated below in further details to a reference scenario (e.g. RWC) but could equally be adapted to a specific local and/or regional scenario, respectively.

Full and RWC cross-species extrapolation

Predict TOX values at reasonable worst case conditions (RWC) for those bioavailability influencing abiotic factors affecting the acute/chronic toxicity, i.e. using the bioavailability model of the trophic level (or the justified model) for the test organisms for which the bioavailability models were originally developed and for those species for which application within the same taxonomic group could be justified. See appendix 3 example A.3.3, A.3.4 and A.3.5 b. The latter is an example on how bioavailability corrections can also be applied at the exposure side. In that case a Bio-F is calculated on the ETV level (Equation-1)

$$Bio-F_{reference} = \frac{ETV_{bioavailable,reference}}{ETV_{dissolved,generic}} \qquad \text{(Eq-1)}$$

1) For those species for which the trophic level specific bioavailability model could not be justified using spot checks (Appendix 2), a bioavailability factor (Bio-F) should be applied to derive the $TOX_{bioavailable,\ reference}$. This Bio-F can be calculated by comparison of the $Tox_{bioavailable,\ reference}$ with the $TOX_{dissolved,generic}$ of those species for which the BLM was originally developed (Equation-2). The most conservative value (smallest correction for bioavailability, $Bio\text{-}F_{reference}$) should then be used[1]. See appendix 3 example A.3.5a).

$$Bio-F_{reference} = \frac{TOX_{bioavailable,reference}}{T0X_{dissolved,generic}} \qquad \text{(Eq-2)}$$

2) When more data are available for the same species, calculate the species geometric mean value[2]

[1] In worst case even if there is no justification to apply the most conservative bioavailability model, a correction based on speciation modelling only could be an alternative to at least account for differences in abiotic factors.

[2] The geomean calculation applies to data related to the same species and endpoint (in similar test conditions) since growth for example could be a more sensitive endpoint than reproduction. In that situation the most sensitive endpoint (lowest value) for that species would be chosen.

3) Derive a cautious environmental threshold value ($ETV_{bioavailable,\ reference}$) using assessment factor approaches (data poor metals) or statistical extrapolation methods from all normalized $TOX_{bioavailable,\ reference}$ values

4) This value is subsequently compared to the modelled and/or measured environmental exposure concentration (EEC) expressed as dissolved concentrations using the relevant environmental concentrations as follows:

$$RCR = \frac{EEC_{dissolved}}{ETV_{bioavailable,\ reference}} \qquad \text{(Eq-3)}$$

5) For the water compartment kinetics are faster than for the soil and sediment. Therefore, the use of the total risk approach in combination with bioavailability should be considered as the standard approach. However, in certain specific cases the added risk may still have its merits if it can be shown that the background observed is essentially from natural origin and not anthropogenically from past historical activities. If it cannot be demonstrated that the geochemical background has a negligible anthropogenic origin then a precautionary approach (total approach) is recommended. In case the use of the added risk approach[3] can be justified and no compliance is reached and bioavailability can be taken into account, similar to the total risk approach both the toxicity values and the background values should be corrected for bioavailability. The added risk approach assumes that only the anthropogenic added fraction of a natural element that contributes to the risk for the environment should be regulated/controlled. Although this approach acknowledges that negative effects from the bioavailable fraction of the background concentration on some organisms in the ecosystem may occur or that organisms may even have become acclimated/adapted to it, from an environmental policy point of view such effects may be ignored and may even be regarded as desirable, since these effects may in theory lead to an increase in ecosystem differentiation or biodiversity (Crommentuijn et al, 1997). Potential environmental risks (RCR) are characterised based on the following quotient (Equation 4):

$$RCR = \frac{EEC_{add,bioavailable}}{ETV_{add,\ bioavailable}} \qquad \text{(Eq-4)}$$

Where $EEC_{add,bioavailable} = (EEC_{total} - Cb_{site/region})_{bioavailable}$ and $ETV_{add,\ bioavailable} = (ETV_{total} - Cb_{\ culture\ medium})_{bioavailable}$

Cross-region extrapolation

If the BLMs need to be applied in other regions than those for which the BLM was originally developed (i.e. cross-region extrapolation), a feasibility assessment is needed. This analysis could entail a reality check if the range of abiotic factors encountered in the region of interest falls within the boundaries of abiotic factors defined by the BLM. Next to the chemical component, it has to be acknowledged that for other species adapted to a different eco-region a comparison in sensitivities between the different species in that region and those used in the BLMs could be warranted. If deemed appropriate the SSD could be reconstituted on the relevance of the culture conditions for the region under consideration or could include

[3] The concept was developed and published by: T. Crommentuijn et al. Maximum permissible concentrations and negligible concentrations for metals, taking backgrounds concentrations into account, Netherlands, Institute of Public Health and the Environment, RIVM, Bilthoven, RIVM report N° 601501001, 1997

species relevant for the region (temperate species vs tropical species). Recently, alongside the experience in European freshwater bodies (EU RARs for Ni, Cu, Zn; van Sprang et al. 2009), experience has been gained to assess freshwater quality using BLMs in different regions worldwide. For example in Japan (Hayashi 2013), South and Central America (Natale and Leis 2008; Villavicencio et al. 2011; Casares et al. 2012), and in North America (e.g. Khan et al. 2012). Several ongoing programmes are successfully demonstrating the applicability of the Biotic Ligand Model to sensitive fish and invertebrate species in important tropical/subtropical freshwater ecosystems such as the Everglades (USA), Amazon basin (Brazil) and the Mekong/Lancang basin (Southeast Asia). A larger programme is calibrating the copper BLM to another diverse tropical/subtropical river system – the Mekong/Lancang, stretching from mountainous Yunnan Province in China to its delta in southern Vietnam. Here BLM application studies on sensitive fish and invertebrates are being conducted (Wu et al., 2014; Wang et al., 2014a; Chen et al., 2014; Wang et al., 2014b.). Initial results again demonstrate that the copper BLM developed from temperate species and water types can successfully be calibrated to tropical species and environmental conditions. Most studies are based on acute toxicity testing over a range of physical/chemical conditions. Studies are underway on chronic toxicities.

3.1.2 Use of alternative approaches to assess bioavailability.

In absence of specific bioavailability models alternative approaches can be used to account for bioavailability as outlined in detail hereunder. In case bioavailability models are lacking, the preferred way to account for bioavailability is to measure directly the dissolved metal concentrations, as total metal concentrations are a poor predictor of metal toxicity to aquatic organisms. Although measuring dissolved concentrations is becoming more and more common practice, sometimes only total concentrations are available. In that case, the dissolved concentration could be calculated from the total metal concentration. The dissolved concentration for soluble metals under biological relevant conditions is mainly driven by the adsorption (Kd) onto suspended solids. For less soluble metals (e.g. Pb, Al, Fe, Sn), the dissolved concentration is not only driven by adsorption phenomena; if the solubility limit is exceeded metal precipitation will also play an important role.

If deemed appropriate a further refinement step consists of estimating the free ionic metal concentration that is most likely to elicit a toxic response. This can be done by measurements or by using speciation programs often specifically designed for metals (e.g. WHAM, VISUAL MINTEQ, PHREEQC etc.), that take into account the presence of important binding ligands (e.g. Dissolved Organic Carbon (DOC), chlorides etc.) and the possible formation of precipitated metal forms. Free ion activities in local water types should be determined by the same speciation program or measurement technique that was applied to the toxicity tests, because outcomes of these model or measurement techniques can be variable.

3.1.2.1 Use of "Total Dissolved" Metal.

The use of ambient and/or total dissolved metal concentrations to report ecotoxicity data and the derivation of an environmental threshold value and risk ratio is done in the sequence as outlined in Figure 5 and detailed in steps 1-6.

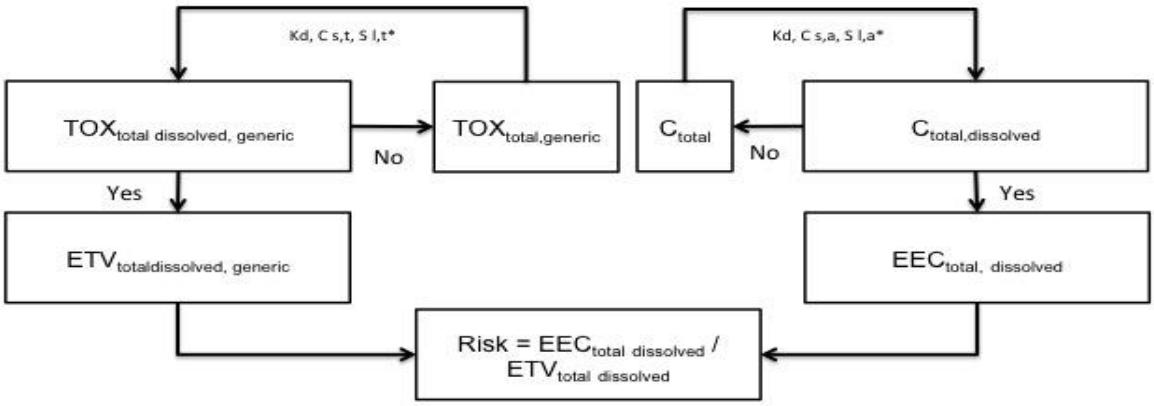

Figure 5: Framework for assessing risks of metals/metal compounds in water (sequence applies to both the local and regional environment) on a dissolved basis. (Tox = ecotox value), C = environmental concentration; PEC = predicted environmental concentration, SLa = solubility limit ambient, SLt = solubility limit toxicity test, *= applies for sparingly soluble metals only.

1. For soluble metals (i.e. metals that occur in a dissolved fraction under environmental relevant concentrations) the direct use of measured dissolved concentrations is preferred.

2. If dissolved measured concentrations are not available and exposure data are only expressed as total metal concentrations the individual C_{total} concentrations can be recalculated into $C_{total, dissolved}$ concentrations using Equation 5:

$$C_{total,\,dissolved} = \frac{C_{total}}{(1 + K_d \times C_{s,\,a} \times 10^{-6})} \qquad \text{(Eq-5)}$$

C_{total} = total environmental concentration (mg/L)
$C_{total, dissolved}$ = total dissolved environmental concentration (mg/L)
K_d = Partitioning distribution coefficient (L/kg)
$C_{s,a}$ = Suspended solids concentration in the ambient water (mg/L)

3. In a similar way the total concentrations in toxicity tests (TOX_{total}) concentrations are extrapolated into total dissolved concentrations in toxicity tests ($TOX_{total, dissolved}$) concentrations using Equation 6. Note that aquatic toxicity tests tend to maximize metal availability since most often DOC levels are low (< 2mg/L). Most toxicity tests are being conducted with reconstituted water and in those cases no additional conversion to a dissolved fraction has to be applied (i.e. the total concentration can be set equal to the dissolved concentration[4]). If natural waters are used, total concentrations should be recalculated using partition coefficients.

[4] It must be demonstrated that the organic particles (from e.g. faeces and food) that appears in the test system do not significantly affect the dissolved metal concentration in the test. Also surface adsorption could be the cause of decreased metal concentrations

$$TOX_{total,\,dissolved} = \frac{TOX_{total}}{(1 + K_d \times C_{s,\,t} \times 10^{-6})} \qquad \text{(Eq-6)}$$

TOX_{total} = total concentration in toxicity tests (mg/L)
$TOX_{total,\ dissolved}$ = total dissolved concentration in toxicity tests (mg/L)
K_d = Partitioning distribution coefficient (L/kg)
$C_{s,t}$ = Suspended solids concentration in toxicity tests (mg/L)

In case precipitation may occur under specific environmentally relevant conditions and/or for substances of very low solubility (<1mg/L), the toxicity data might need to be corrected taken the solubility limits of these metals into account. These solubility limits are mainly driven by abiotic environmental factors (e.g. pH, DOC) and could be estimated using specific speciation models[5] (e.g. Visual MINTEQ, PHREEQC) or experimentally derived. See example Appendix 3 case study A.3.1.

4. Calculate the Environmental Exposure Concentration $_{\text{total dissolved}}$ ($EEC_{\text{total, dissolved}}$) for a predefined local or regional environment (high end value e..g.. 90th percentile of the environmental concentration distribution)

5. From all available dissolved ecotoxicity threshold data a cautious environmental threshold value (ETV) is derived using assessment factor approaches (data poor metals) or statistical extrapolation methods[6]. The ETV is subsequently compared to the modelled and/or measured exposure data equally expressed as dissolved concentrations (Cfr step 2).

6. The risks for a regional or local environment are subsequently calculated from the comparison between the local/regional $EEC_{\text{dissolved}}$ and the $ETV_{\text{total,dissolved generic}}$ (Equation 7).

$$RCR = \frac{EEC_{dissolved}}{ETV_{total,\,dissolved,\,generic}} \qquad \text{(Eq-7)}$$

Some considerations:

- Kd values cannot be considered as true constants and will vary as a function of the metal loading and as a function of environmental characteristics such as pH (due to proton competition for binding sites) and ionic strength. Subsequently, as metal Kds will show a large degree of variability irrespective if equilibrium has been reached or not the assessment of the data quality and relevance of all collected measured Kd-values should be done with care. Ranges spanning different orders of magnitude have been reported in literature (Allison and Allison, 2005). Preference should always be given to coupled measured data for which information is available on both sampling and analytical measuring techniques. If only a limited data set of Kd values is available (less than 4 data points) the choice of the appropriate Kd value should be based on

[5] Some specific speciation models allow estimating precipitation of metals (e.g. Visual MINTEQ, PHREEQC) while others do not have that capacity (e.g. WHAM)

[6] For a large data set of toxicity data for one species the geomean is taken but in case of a small data set most often there is preference for selecting the most sensitive value rather than using a geomean value

expert judgment taking into account, the representativity of the Kd value for the site/region of interest. The minimum and maximum values can be used for the uncertainty analysis. When for data rich metals sufficient Kd values are available a log-normal distribution or other significant statistical distribution can be fitted through the data points. If the purpose of the assessment is to conduct an overall risk assessment covering all environmental compartments the median Kd value should be used in the exposure and effects assessment to avoid an underestimation or overestimation in the other compartments. If only the water compartment is being investigated, it could be useful to use Kd values representative for the area of concern or when a reasonable worst-case scenario is considered a low percentile value could be more appropriate. An additional uncertainty analysis with a range of Kd values (e.g. 10-90th percentiles) is recommended to indicate how risk ratios could be impacted.

- While for some metals the formation of solid precipitates will render the metal less toxic (e.g. lead) it has been observed for others that physical interactions between the precipitated metal forms and the active sites on an organism may occur and subsequently may also contribute to the observed toxicity. For example several authors have suggested that polymerization or precipitation of aluminium hydroxide at the gill may be responsible for observed respiratory effects in fish (Playle and Wood, 1989, 1990; Poleo, 1995). However, it should be noted that the latter effects could be transient in nature due to the use and disequilibrium of freshly prepared aluminium solutions in toxicity tests.

3.1.2.2 Use of "Dissolved metal" species of concern

In cases where appropriate (externally validated) speciation models (e.g. WHAM, visual MINTEQ, CHESS, PHREEQC etc.) and relevant input data on the main physico-chemical parameters driving the availability of a metal are available, the risk characterisation should be performed on basis of the dissolved metal species of concern. Most often the dissolved metal species of concern equals the free metal ion. However, the free metal ion is not necessarily the best predictor for all metals and other metal species such as neutral species (e.g. AgCl, HgS) and anionic species (e.g. SeO^{2-}, AsO_4^{2-}) may contribute to the observed toxicity (Campbell, 1995) and are captured in this term. Figure 6 gives an overview of the proposed tiered approach.

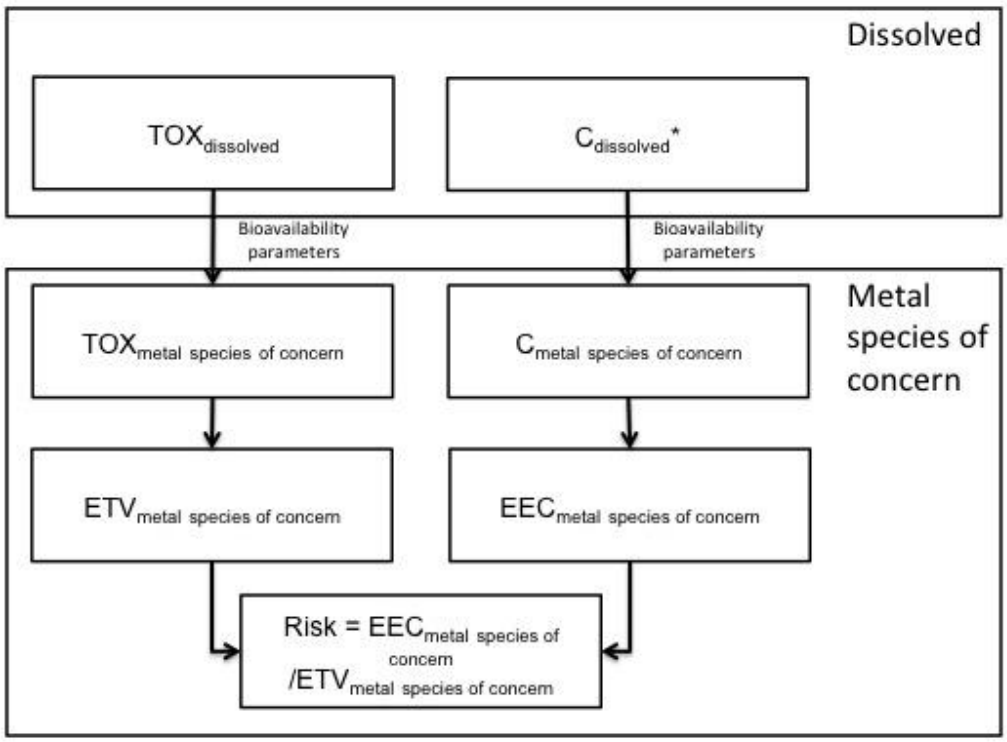

Figure 6: Framework for assessing risks of metals/metal compounds in water on a free metal ion basis (Tox = ecotox value, C = environmental concentration; *=sequence applies to both the local and regional environment)

- It is recommended to recalculate the reported total dissolved TOX concentrations ($TOX_{\text{total dissolved}}$) into Tox concentrations expressed as the metal species of concern ($TOX_{\text{dissolved metal species of concern}}$) using the appropriate speciation models (e.g. PHREEQC, WHAM, Visual MINTEQ,…) and taking into account the main physico-chemical conditions driving the bioavailability (e.g. pH, DOC,…) of the individual toxicity test result (i.e. for a specific test species and for the metal compound in question). If no specific information on relevant physico-chemical parameters is available, then the toxicity data should not be used unless the possibility of using default values instead for some of these parameters can be substantiated. For example for copper, Santore et al. (2001) used a default DOC value of 1 mg/L to calibrate the acute copper BLM.

- It is recommended to recalculate the total dissolved exposure concentrations ($C_{\text{total disssolved}}$) at the same level of bioavailability (expressed in the same units) as that used to recalculate the TOX concentrations, i.e. into metal species of concern exposure concentrations using the same speciation model (e.g. PHREEQC, WHAM, Visual MINTEQ, …). For that purpose, the physico-chemical parameters of the generic environment or site specific watershed driving the bioavailability (e.g. pH, DOC, …) should be gathered or estimated. Reference is given to either realistic worst case (e.g. $10^{th}/90^{th}$ percentile) or typical conditions (e.g. 50^{th} percentile), depending on the regulatory setting in which these values are used

- From all available $Tox_{\text{dissolved metal species of concern}}$ ecotoxicity threshold data a cautious environmental threshold value (ETV) is derived and compared to the_Environmental Exposure Concentration $_{\text{metal species of concern}}$ derived from all individual $C_{\text{dissolved metal species of concern}}$ values for a predefined

environment taking a high end value (e.g. the 90th percentile) of the environmental concentration distribution of the metal species of concern.

- The risks for a local or regional environment are subsequently calculated from the comparison between the $EEC_{\text{dissolved metal species of concern}}$ and the $ETV_{\text{dissolved metal species of concern}}$ (Equation 8):

$$RCR = \frac{EEC_{metalspeciesofconcern}}{ETV_{metalspeciesofconcern}} \qquad \text{(Eq-8)}$$

Some considerations:

- Next to measuring free ion activities chemical speciation models are more often used to try to accurately predict the distribution of an element amongst chemical species in an environmental system. It should be noted that depending on which speciation model is used and which parameter is the most influential, different speciation models may give different answers. Speciation modelling is often needed as direct measurement techniques predominantly focus on the quantification of the free metal ion concentrations, and even this approach is not always possible at environmentally low concentrations. The outcome of speciation models may vary and are sensitive to the selection of parameters that are included. Some of the reported uncertainties are (Van Briesen et al, 2010): decision rule uncertainty, model uncertainty, parameter uncertainty, and parameter variability (Finkel, 1990; Hertwich et al, 1999). Therefore, only validated and justified models for the metals under scrutiny should be used (e.g. used in Biotic Ligand Model development). Sensitivity analysis could be a valuable tool in this context to provide reassurance that any adopted approach is sufficiently precautionary. Further harmonization of speciation models is warranted in the future and existing speciation models are currently modified in an attempt to further improve their predictive capacity.

A non-exhaustive overview of some recent models or model versions that can be used to determine metal speciation are provided in Table 4.

Table 4: Overview of frequently used chemical speciation models (source: www.speciation.net)

Model	More information on model
CHEAQS Next - CHemical Equilibria in AQuatic Systems. This model is the successor of the models GECHEQ and CHEAQS Pro (developed by Wilko Verweij). - calculation of the concentration of complexes - complexation by natural organic matter (3 different models developed by Tipping and co-workers: Model V, Model VI and Model VII; - formation of solids due to oversaturation - Includes a surface complexation model to cover adsorption processes).	http://www.cheaqs.eu/
ChemEQL Determination of thermodynamic equilibrium concentrations of species in complex chemical systems. - Adsorption on up to five different particulate surfaces can be modelled - Simulations of kinetic reactions with one rate determining process - Calculation of two-dimensional logarithmic diagrams, (e.g. pe-pH) ChemEQL is an extended and user-friendly version of the original program MICROQL. It runs on MacOSX, Windows, Linux, and Solaris.	http://www.eawg.ch/research
MINTEQA2, version 4.03 A chemical equilibrium model for the calculation of metal speciation, solubility equilibria etc. for natural waters. - Ion speciation using equilibrium constants (based on the most recent NIST data) - Solubility calculation involving solid phases - Adsorption calculations with adsorption isotherms, based on five surface complexation models (Diffuse Layer, Constant Capacitance, Triple Layer, Basic Stern and Three Plane) - Ion-exchange processes covered (Gaines-Thomas formalism) - Metal-humic complexation can be simulated using the Gaussian DOM, the Stockholm Humic Model, or the NICA-Donnan model. - Visual MINTEQ is a Windows version of MINTEQA2 v.4.0	http://www2.epa.gov/exposure-assessment-models/minteqa2
PHREEQC (Version 2): A computer program for speciation, batch-reaction, one-dimensional transport, and inverse geochemical calculations - written in the C programming language that is designed to perform a wide variety of low-temperature aqueous geochemical calculations. - transport calculations involving reversible and irreversible reactions - Windows, Linux and MacOS versions are available	http://wwwbrr.cr.usgs.gov/projects/GWC_coupled/phreeqc/
WHAM7 – Windermere Humic Aqueous Model, version 7 Model for the calculation of equilibrium chemical speciation in surface and ground waters, sediments and soils (developed by E.Tipping). - Suitable for problems where the chemical speciation is dominated by organic matter - Model takes into account the precipitation of aluminium and iron oxides, cation-exchange on an idealized clay mineral, and adsorption-desorption reactions of fulvic acid - Ion accumulation in the diffuse layers surrounding the humic molecules is considered - Model calculations are performed with a BASIC computer code running on a Personal Computer.	https://www.ceh.ac.uk/services/software-models

- It is important to know, however, that some of the speciation models that were used for the development of BLMs – or that are even incorporated into their software, do not always represent the most current version. An overview of some of the models that have been used for BLM-

purposes are shown in Table 4 The choice for using a specific speciation model for a metal for modelling the dissolved species of concern and in particular the interaction between metals and (dissolved) organic carbon has been made for various reasons such as the state-of-the-art at the time of the Biotic Ligand Models development, or functionalities of the speciation model that meet the needs for a specific metal. Different speciation models are used for different metals: WHAM 5 (Cu, Zn, Co), WHAM 6 (Ni), Visual MINTEQ V3 (Pb) (Table 5).

Table 5: Rationale and overview speciation models used for translating total dissolved concentrations to total dissolved metal species of concern.

Speciation Model	Metals for which the speciation model is used	Rationale
Windermere Humic Aqueous Model, Version V (WHAM5)	Copper (Hydroqual, UGent Model) Zinc Cobalt	WHAM-5 model was originally used in the development of the first copper and silver BLM by Hydroqual, and was subsequently used in the development of BLMs for zinc and copper by Ghent University. The cobalt BLM (Hydroqual) was also developed with this version of WHAM
Windermere Humic Aqueous Model, Version VI (WHAM6)	Nickel	Initial modeling of nickel speciation with WHAM-5 resulted in poor predictions at low nickel concentrations. The use of the upgraded WHAM-6 model significantly improved predictive capacity of the BLM. The WHAM-6 model also allowed users to make adaptations to e.g. the binding and stability constants of metal (compounds) to organic carbon (fulvic acid, humic acid).
Visual MINTEQ version 3.0 (code built on MINTEQA2; optimized for metal complexing effects of DOM)	Lead	This model allowed a proper description of Pb-speciation with the NICA-Donnan model, and this was also the only speciation model at the time of development that took both Pb-precipitation as well as binding of Pb to organic material into account.

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- If there is a concern that the investigated metal binds strongly on colloids this should ideally be considered in calculating the speciation of dissolved metal because colloids can pass through filters and if ignored may have an impact on the overall outcome of the speciation exercise. However, at the moment, our understanding on colloids is limited and further research is needed in this field before this could be embedded in speciation calculations.

- For further guidance in selecting reliable and relevant ecotoxicity data for the purpose of bioavailability assessment the reader is referred to appendix 1.

3.2 Implementation of bioavailability for the sediment compartment

A schematic representation on how bioavailability can be implemented for assessing risks in the sediment compartment is presented in Figure 7.

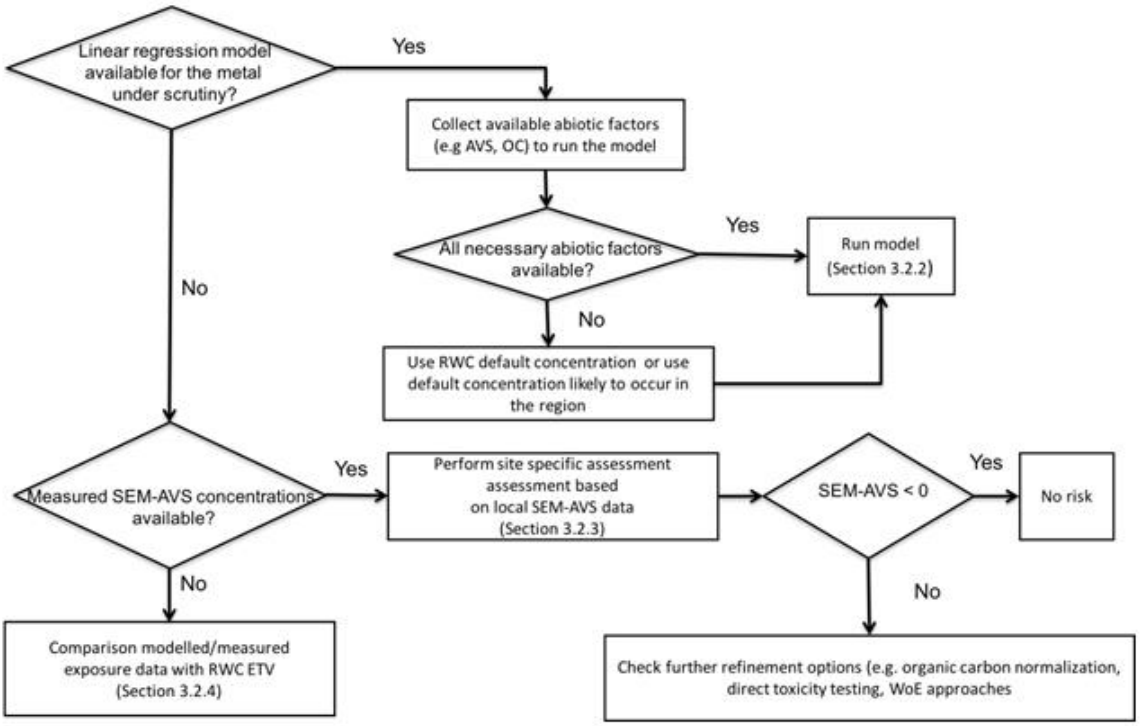

Figure 7: Simplified representation of the decision tree for the implementation of bioavailability correction for the sediment compartment.

Toxicity based models for predicting metal toxicity in sediments are scarce and/or under development. Linear regression models, that may be used to derive safe thresholds reflecting the conditions in sediments, have been developed for nickel (Vangheluwe et al, 2013). For copper a linear relationship with organic carbon have been established (EU-RAR Cu, 2008). For these metals the input parameters to run the regressions are limited to AVS and OC. If these parameters are unknown a default value can be chosen representing RWC conditions and/or the typical conditions derived from historical data for the site under evaluation. For most metals, however, no toxicity based model is available. In that case preference is to collect local SEM-AVS data. If this is not feasible the assessment should be based on a comparison from total metal concentrations with an ETV value derived under test conditions maximizing bioavailability (low AVS, low OC).

3.2.1 Key abiotic factors controlling metal toxicity in sediments

The main abiotic factors that drive metal toxicity in sediments are presented in Table 6. Those labelled required should be preferentially measured in test systems and collected for the site under investigation.

Table 6: Overview of the most relevant parameters that influence the bioavailability of metal species in the sediment

	Sediment
Required	Total Organic Carbon (TOC)
	Acid Volatile Sulfides (AVS)*
	Al/Fe/Mn oxides
Supportive information	Particle size (sand, silt and clay content)
	Pore water chemistry (total and dissolved metal concentrations, DOC, hardness, conductivity/salinity, ammonium etc.)

* mainly for divalent metals (Hg, Cu, Pb, Cd, Zn, Ni) but also for monovalent metals (e.g. Ag)

Sulfides binding

Acid Volatile Sulfides (AVS) has been demonstrated to be one of the most important factors controlling cationic metal activity (Hg, Ag, Cu, Pb, Cd, Zn, Ni) and metal induced toxicity in the sediment interstitial water system in both anoxic and suboxic sediments (Di Toro et al., 1990, Di Toro et al., 1992, US EPA, 2005). AVS is, however, in the first place an operational defined parameter indicating those sulfides that are readily extracted by the cold extraction of sediment in approximately 1 M HCl acid. Another term that is used in conjunction with AVS is SEM. SEM (Simultaneously Extracted Metal) can be defined as the metal, which is simultaneously extracted under the same conditions under which the AVS content is determined. If multiple metals are present it is necessary to use the term total SEM (Σ SEM). The equivalent extraction of sulfide (AVS) and metal, however, does not necessarily means that the metal is bound by sulfide alone. SEM refers to the metal associated with the sulfides and any other metal-bearing phase that is extracted in the cold HCl extraction used for AVS analysis (Allen et al., 1993). For example, metals adsorbed to iron oxides and particulate organic carbon will also be extracted.

The fraction of metals that may bind to sulfides in the sediment can be estimated using the SEM-AVS concept. In case the molar concentration of sulfides exceeds the molar concentration of metals than the metals will precipitate and pore water metal concentrations are expected to be low. If the molar concentration of AVS is lower than the amount of metals present in the sediment, the SEM-AVS difference gives the amount of SEM_{Me} that is not bound (excess SEM_{Me}) and consequently potentially bioavailable via the pore water. In the pore water other important ligands such as organic carbon and Fe/Mn oxides in the sediment or pH, DOC and hardness conditions in the pore water may further reduce bioavailability. The nomenclature of excess SEM_{Me} has to be interpreted as "bioavailable" for purposes of estimating the extent to which metal/metal compounds in sediments may cause toxicity (Equation-9).

$$SEM_{Me,\,bioavailable} = SEM_{Me} - \Delta\,\mathrm{AVS}_{\mathrm{Me}} \qquad \text{(Eq-9)}$$

Some considerations

- In applying the SEM-AVS model for a specific metal it has to be considered that metals are acting in a competitive manner when binding to AVS. This depends on the solubility limit of the metal-sulfide complex. The lower the solubility product the more stable the MeS complex. Ranked from the lowest to the highest solubility product the following sequence is observed: SEM_{Hg}, SEM_{Ag}, SEM_{Cu}, SEM_{Pb}, SEM_{Cd}, SEM_{Zn} and SEM_{Ni}, indicating mercury has the highest

affinity for AVS, followed by silver, copper, lead, cadmium, zinc etc. until the AVS is exhausted. The remaining SEM is that amount present in excess of the AVS. The SEM-AVS concept has proven to be successful to predict the lack of toxicity in spiked and field sediments. The concept is limited, however, in terms of predicting toxicity. Indeed the SEM-AVS concepts indicate how much metals are bound to sulfides and hence not bioavailable. But even if some metals are not bound to sulfides it does not mean that we need to see toxicity. That depends on the concentration of freely available metal and also on the real bioavailability of the metals in the pore water. That is why SEM-AVS < 0 is a good predictor of absence of toxicity but if SEM-AVS> 0 is not a good predictor of the start of toxicity. That will depend on the magnitude of exceedance and whether the bioavailable threshold is reached and does not always predict the absence of bioaccumulation (De Jong et al, 2009/2010, Lee et al, 2000 (See section 3.2.4).

- The SEM-AVS concept works mainly for metals occurring as cations (e.g. Cu^{2+}, Zn^{2+}, Pb^{2+}, …). Anionic forms such as oxyanions (e.g. MoO_4^{2-}, $Sb(OH)_6^-$, …) are not covered.

- Although metal sulfides can account for much of the non-toxic metal, it should be recognized that excess SEM can be bound to organic carbon (OC) and that sediment iron and manganese oxides may further reduce the fraction of bioavailable metal. The current SEM-AVS approach may therefore overestimate bioavailable metal and its toxicity towards sediment organisms in oxic surface layers with sufficient Fe and Mn (Costello et al., 2011).

Binding to other sediment phases (organic carbon, Fe/Mn-(oxy)hydroxides

Organic carbon has been identified as one of the major drivers for copper toxicity in sediments, but also other metals show a high affinity to bind with organic carbon sources. The affinity of metals to bind with organic carbon has been used by Di Toro et al. (2001, 2005) as the premise to build a model to predict not only the lack, but also the onset, of metal toxicity in spiked and field contaminated sediments (Di Toro et al.., 2001; Di Toro et al., 2005). In this context it is assumed that toxicity occurs if the excess SEM goes beyond the binding capacity of the organic carbon present in the sediment. Using this information it was shown that the organic carbon normalized excess SEM can be used to predict toxicity (Equation-10):

$$SEMx,oc = \frac{\Sigma SEM - AVS}{fOC} \qquad \text{(Eq-10)}$$

Where f_{OC} is the organic carbon fraction in the sediment.

But even in the absence of AVS (i.e. aerobic sediment), it is worthwhile exploring if a linear relationship can be established between the observed toxicity levels of the metal and the presence of organic carbon. If a relationship can be discerned the variability introduced by the presence of toxicity values generated at different organic carbon concentrations can be captured by normalizing each Tox value using the following formula:

$$TOX_{OC,\,normalized} = \frac{TOX_{total}}{fOC} \qquad \text{(Eq-11)}$$

TOX_{total} (mg Me/kgdw)
fOC = fraction organic carbon
$TOX_{OC,\,normalized}$ (mg/g OC)

Other important parameters

Fe and Mn oxides have been identified by different authors as important factors controlling bioavailability of certain metals such as nickel (Costello et al., 2011). These phases are particularly important because they have a large sorption capacity. Furthermore, they appear as coatings on the particles and occlude the other mineral components. As soon as reliable quantification models become available or equations have been established, the parameters could also be accounted for in the bioavailability correction in sediment. Even change in redox potential and hence speciation may govern metal toxicity as demonstrated with chromium where at a negative redox potential chromium is present in it less toxic trivalent form (Berry et al, 2004).

3.2.2 Use of bioavailability models

Empirically based regression models predicting the metal toxicity in spiked sediments based on sediment properties (e.g. AVS, TOC, Fe/Mn) are available for nickel for different benthic species. General information on how regression models are developed is included in section 3.3 dealing with the soil compartment. The models can be used to normalize the available toxicity data set to specific bioavailability conditions. For example, ETV RWC can be recalculated toward a reference situation such as 10^{th} percentile of the regional AVS/OC distribution in case no actual or historical AVS/OC data are available or ETV can be normalized towards the measured AVS/OC concentrations occurring at the site as presented here below.

1) Link the NOEC/EC_X values of the chronic ecotoxicity database (as total metal concentrations) with the relevant sediment parameters of the sediment (e.g. AVS, OC) in which the test was performed.

2) If regression models, taking the form *log(ECx) = intercept + slope * log(abioticfactor),* have been developed, the corresponding organisms specific slopes (from the regression analysis) can be used to normalize the NOEC/ECX values to "reasonable worst case" sediment properties (e.g. 10^{th} percentile AVS) or to specific local/regional conditions (actual or historical AVS concentrations prevailing on the site under investigation). The normalisation equation for RWC- and site-specific conditions is given here below (Equation-12and 13) is:

$$ECX_{RWC} = ECX_{test}\left[\frac{abioticfactor_{RWC}}{abioticfactor_{test}}\right]^{slope} \qquad \text{(Eq-12)}$$

test = scenario with typical local or regional conditions for which the Tox,test is derived
RWC = the realistic worst case scenario: is used as a reference scenario when correcting bioavailability with toxicity related models providing us with a $Tox_{,RWC}$ *corresponding to a maximized bioavailability*.

$$ECX_{Site-specific} = ECX_{test}\left[\frac{abioticfactor_{Site-specific}}{abioticfactor_{test}}\right]^{slope} \quad \text{(Eq-13)}$$

test = scenario with typical local or regional conditions for which the Tox,test is derived
site-specific = reflects the local AVS conditions

An example for nickel is given in appendix 3. Case study A3.2.

1) Derive a ETV_{rwc} or a $ETV_{site\ specific}$ using an SSD or AF approach

2) The risks for a local or regional environment are subsequently calculated from the comparison between the $EEC_{total,\ sediment}$ and the $ETV_{\ rwc\ /site\ specific}$ (Equation-14):

$$RCR = \frac{EEC_{totalmetal}}{ETV_{rwc\ /\ sitespecific}} \quad \text{(Eq-14)}$$

In case organic carbon has been identified as the major driver (e.g. copper), the ETVsediment can be calculated back to mg/kg dry wt. when a default OC value is assumed for the area/region under investigation. The latter value can be used as a generic ETV. In the EU, a standard sediment has a default OC value of 5 %. The risks for the local site can subsequently be calculated from the comparison between the EEC_{total} and the RWC $ETV_{normalized,\ OC\ (5\%)}$ taking into account site-specific information on the OC content (Equation-15)

$$RCR = \frac{EEC}{ETVnormalized_{,\ OCregion} \times \frac{fOC_{site}}{fOC_{region}}} \quad \text{(Eq-15)}$$

Some considerations:

- In case no bioavailability model is available for all biological species, care should be taken in the choice of the bioavailability model to be used in a cross-species extrapolation exercise for the sediment compartment. As various in faunal organisms disturb or alter (the sediment structure differently depending on their specific feeding type, mobility and life cycle (formation micro-habitats), the bioavailability model developed for another species that resembles a similar life strategy should be used.

3.2.2 Use of alternative approaches to assess bioavailability.

Application SEM-AVS concept

In absence of appropriate regression models linking directly the observed toxicity towards multiple sediment properties, that are determining the bioavailability of metals in sediments (e.g., organic carbon, sulfides, iron/manganese oxy hydroxides etc., the use of basic concepts such as the Acid Volatile Sulfide

concept and organic carbon normalization could be applied. The relative importance of these properties differs depending on the metal's binding capacity and general chemical activity. Estimating the amount of metals not bound to the sulfide pool can be very useful as it provides an estimate of the potentially bioavailable fraction available for uptake by benthic organisms. For other metals that exhibit a lower preference to bind to sulfides, binding to organic carbon and iron/manganese oxides could be more important. In such case normalisation towards the prevailing organic carbon/Fe content of the sediment could be considered.

The SEM-AVS approach has been proven to be quite accurate in demonstrating the absence of sediment-associated toxicity for a number of metals and can be applied to a specific region or a local site if extensive SEM-AVS data representative for that region or if site-specific local SEM-AVS measurements are available. Considering the observed co-variance between AVS and SEM_{Me}, it is recommended to take only measured coupled data into account to maintain the ecological relevance of the analysis (Vangheluwe et al., 2003, Vangheluwe et al., 2008). Low AVS-high SEM combinations are unlikely to be found. Knowledge with respect to spatial and seasonal variations of AVS and SEM levels is required for a proper application of the AVS concept in this context. SEM-AVS data should represent the seasonal worst case scenario (i.e. lowest AVS levels being measured, spring season). In addition, as there exist a redox gradient in sediments AVS levels tend to decrease with decreasing depth, as the redox potential in surficial sediments can be positive or less negative then deeper sediments. It is important to focus the analysis on the biological active layer of the sediment (0-20 cm). But even over this depth the AVS profile could differ dramatically and in top layers (0-2 cm) a significant lower amount of AVS can be present or can be even completely absent. Incorporation of measured SEM-AVS data in the risk characterisation should be performed as outlined here below.

1) From the compiled SEM and AVS data set the potential bioavailable SEM_{Me} fraction for each individual sampling point is derived by coupling the ΔAVS and SEM_{Me} data for that specific station.

$$EEC_{AVS\ normalized} = SEM_{Me,\ sampling\ point\ 1} - \Delta AVS_{sampling\ point\ 1} \qquad \text{(Eq-16)}$$

2) For the regional scenario, a distribution function of all individual (SEM_{Me} - ΔAVS) values across the region is elaborated. The $EEC_{AVS\ normalized,\ regional}$ is calculated as the higher value (e.g. 90th percentile) of the measured bioavailable $SEM_{Me.}$

3) For the local scenario the $EEC_{AVS\ normalized}$, local is taken as such.

4) If this value is < than 0, then 100 % of SEM_{Me} is bound to sulfide and no toxicity (risk) is expected to occur (Equations 15-16 example local scenario). In case this difference is > than 0 (meaning not enough AVS is available for binding with the SEM_{Me}), metals are potentially bioavailable and could -if present in high enough amounts- elicit a toxic response or present a risk to the sediment compartment.

$$SEM_{Me,\ sampling\ point\ 1} - \Delta AVS_{sampling\ point\ 1} < 0 \text{ (no risk scenario)} \qquad \text{(Eq-17)}$$
$$SEM_{Me,\ sampling\ point\ 1} - \Delta AVS_{sampling\ point\ 1} > 0 \text{ (potential risk scenario)} \qquad \text{(Eq-18)}$$

In case SEM-AVS > 0, a further weight of analysis can be conducted (e.g. direct toxicity testing, macro-invertebrate analysis etc.)

Comparison modelled/measured data with a reasonable worst-case (RWC) ETV

In the absence of measured SEM-AVS data a more generic approach using a generic reasonable worst-case environmental threshold value expressed as total metal concentration should be used. This generic ETV should be chosen to reflect a scenario which maximizes bioavailability and could serve as a conservative benchmark to ensure that results are protective for the majority of sediments (including those oxic/suboxic sediments). This goal is achieved by populating the effect database with toxicity data from tests conducted using spiked sediments with low AVS/ OC/Fe/Mn content that would probably represent high bioavailability conditions. Test results from sediments with high AVS/ OC/Fe/Mn content should be excluded from the database as outlined below.

1) The different generic toxicity values that are used in the sediments effects assessment are generally generated in sediments with varying physico-chemical characteristics known to alter metal bioavailability and toxicity (e.g. low/high AVS, low/high OC). In order to derive an ETV_{rwc}, only toxicity data from sediments exhibiting reasonable worst-case conditions for AVS, OC and Fe/Mn should be included in the calculations. Toxicity results from sediments with characteristics that mitigate metal toxicity (i.e. high AVS/ OC/Fe/Mn content) should be excluded.

2) Calculate the ETV_{rwc} from all toxicity $_{(low\ AVS,\ low\ TOC)}$ values. The ETV_{rwc} is expressed as total metal, which facilitates the comparison with available sediment monitoring databases that are also mostly expressed as total metal.

3) Calculate the $EEC_{total,sediment}$ from all individual C_{total} values for a predefined environmental site (local or regional) taking a high end value (e.g. the 90th percentile) of the concentrations of the metal of concern. As a natural sediment in lowland rivers will probably contain a certain amount of AVS, it is recommended to correct the EEC total by subtracting the amount of metal that could potentially bind with the sulfides present (note: the specific regional background of metals with a higher affinity is subtracted first). For example, for nearly oxidized sediments, it can be assumed that the AVS concentration in the sediment is low, i.e. an AVS concentration of < 1 µmol/g dry wt. can be used as default value available to bind with sediments. At this stage it is also assumed that the total metal concentration can be used as a conservative estimate of SEM_{Me}. $EEC_{AVS\ normalized}$ is then calculated using Equation-19.

$$EEC_{AVS\ normalized} = EEC_{total} - \Delta AVS_{default} \qquad \text{(Eq-19)}$$

$EEC_{Me,\ total}$ expressed as µmol/g.dry wt.

Δ {AVS} = {AVStotal} - {SEM_{Hg}}- {SEM_{Ag}}. …… (this computation is repeated until the next least soluble metal sulfides) .

4) The risks for a local or regional environment are subsequently calculated from the comparison between the $EEC_{total,\ sediment}$ and the ETV_{rwc} (Equation-20). Both parameters are expressed as mg total Me/kg dr wt.:

$$RCR = \frac{EEC_{totalmetal}}{ETV_{rwc}} \qquad \text{(Eq-20)}$$

3.3 Implementation of bioavailability for the soil compartment

The total (or aqua regia soluble) metal concentration in soil is often a poor predictor of metal toxicity to terrestrial organisms. The bioavailability and toxicity of metals or metalloids in soils is influenced by a number of abiotic factors such as:

- Variation in soil properties (e.g. pH, clay content, organic carbon content, (e)CEC) among soils
- Time since contamination/spiking in the tests (ageing processes)
- Form of metal added to the soil.

The relative importance of the soil properties differs depending on the metal's binding capacity and general chemical activity (e.g. pH, concentration of competing ions). For several metals (i.e. Ag, Co, Cu, Pb, Mo, Ni, Zn) toxicity based regression models are available correlating these soil/ properties to toxicity.

During the last decade, a lot of research has been performed in these fields, mainly triggered by the risk assessments for Zn, Cu and Ni under the European Existing Substances Regulation ((EEC) No 793/93) (see e.g. (V)RARs at http://echa.europa.eu/information-on-chemicals/information-from-existing-substances-regulation). Empirical regression models have been derived, covering a wide range of soil types, linking physico-chemical soil properties (e.g., pH, organic carbon and clay content, cation exchange capacity) with metal toxicity for plants, invertebrates and micro-organisms based on (pseudo-) total metal concentrations in soil (Smolders et al., 2009). Such regression models are useful to normalize effects data towards specific conditions of a site of interest and, hence, allow for the derivation of site-specific threshold concentrations, expressed as total metal concentration. Additionally, comparison of metal toxicity in laboratory and field conditions yielded toxicity-based empirical correction factors for a range of metals. The next sections provide brief descriptions of how these concepts have been developed and how they can be applied into a risk assessment context.

3.3.1 Incorporating long-term effects on metal bioavailability: derivation of a soil lab-to-field (L/F) factor.

Use of metal toxicity data derived from laboratory spiked soils included the risk of overestimating toxicity compared to field-contaminated soils. Short-term equilibration of the metal in soil, effects due to the added counter-ion, metal-induced acidification and higher ionic strength of the soil solution have large effects on metal chemistry in soil that are seldom representative of metal contamination occurring in the field. Examples of long-term equilibration reactions that may affect metal bioavailability and toxicity are inclusion of natural elements into the crystal lattices of soil minerals, the formation of insoluble precipitates, diffusion of metals into micro pores, occlusion by organic matter, etc.

Where the adverse toxicity effect of an elevated metal concentration is generally more pronounced in spiked soils than in historically contaminated field soils at the same total metal level, an additional lab-to-field translator should be incorporated This factor relates the differences in metal dose required between lab spiked and field contaminated soil to produce a same toxicity effect in a specific soil.

In order to correct for this discrepancy between freshly spiked and field contaminated soils, a lab-to-field (L/F) factor (also called leaching/ageing, or L/A factor) should be incorporated (Equation-21). This L/F factor relates the differences in metal dose required between lab-spiked and field-contaminated soil to produce a same toxicity effect in a specific soil.

$$Lab-to-Field(L/F)\,factor = \frac{EC_x / NOEC_{field\ /\ aged,add}}{EC_x / NOEC_{freshlyspiked,add}} \qquad \text{(Eq-21)}$$

This factor addresses the differences in toxicity between tests on soils spiked in the lab and tests on field contaminated soils using single species or micro-organisms functional tests due to differences in ionic strength, ageing of metals in soil.

Guidelines for L/F calculation:

- L/F factors should be calculated as a ratio between toxicity data generated from i) field-contaminated soils or laboratory-spiked, leached and aged soils (under equal conditions) and ii) freshly spiked soils.

- A minimum ageing period after spiking can be metal-specific. The experience for Zn, Pb, Cu and Ni indicated that 3 to 9 months is a good compromise between practical considerations, while still allowing a realistic amount of time for slow ageing/transformation reactions in soil. Longer ageing times may still result in a larger L/F factor. Because kinetics of ageing may be metal specific, the minimum ageing period required may be different for different metals.

- Soils should either be artificially leached before ageing or allow free drainage of percolating rainwater in order to remove the excess salts.

- As natural metal background concentrations are already "aged", the derivation of the L/F factors should be based on added concentrations.

- The L/F factors should be derived for a range of soils, ideally covering the relevant range in soil properties and for several species, representing the three trophic levels.

- The L/F factors are preferentially based on the ratio of EC_{50} because EC_{50} values are generally a more statistically robust estimate compared to e.g. EC_{10}. In cases where less than 50 % effect is observed at the largest dose tested, a ratio of lower effect levels should be selected. EC_{10}/EC_{20} values generally result in larger L/F factors due to larger relative differences. Only if no EC_x values are available is it acceptable to use NOEC values.

- In case no toxicity is observed at the largest dose tested in field conditions, in contrast with that in a corresponding freshly-amended soil, this information should not be ignored and an unbounded (lower) estimate for the L/F factor should be derived as the ratio of the largest dose tested under field conditions and the $EC_{10,add}$ (or NOEC) in the corresponding freshly amended soil.

Some considerations:

- The L/F factor does not address differences in effects between single species lab tests and multi-species tests (species interactions). The influence of the latter is addressed by comparing micro/mesocosm or field studies with the EQS based on single species/functional lab tests

- The selection of the most appropriate L/F factor is not straightforward and should be done in a pragmatic and cautious but realistic way, for example by selecting one generic value situated at the lower end of the spectrum. Preferentially, information on toxicity based L/F factors should be combined with information from changes in metal availability, e.g. based on changes in pore water concentration or isotopic exchangeability. When no relationship can be found between soil properties and the lab-to-field factors, and/or between organism and lab-to-field factor, all individual lab-to-field ratios should be aggregated into a frequency distribution in order to derive a generic lab-to-field factor on a statistical basis (e.g. Cu, Zn, Mo and Pb). The derivation of the L/F factor for Cu is described in paragraph A.4.3 of Appendix 4.

- In cases where a significant relationship between soil properties and the L/F factor is found, preference is given to derive soil-specific L/F factors for each EC_x to be corrected (e.g. Ni and Co). The derivation of the soil-specific L/F factor for Ni is described in paragraph A.4.4 of Appendix 4.

- It must be stressed that the L/F factor should not be applied on ecotoxicity data collected in field contaminated or in spiked and aged soils

An overview of the soil lab-to-field (L/F) factors for Cu, Ni, Zn, Co and Mo as incorporated in their European REACH dossiers is presented in Table A.4.2 of Appendix 4.

3.3.2 Use of bioavailability models

Empirical regression models can be used to correct the effects data for the differences in physico-chemical properties of the soils tested and to normalize results towards specific conditions of a site of interest. Figure 8 visualizes the general approach to derive the regression models.

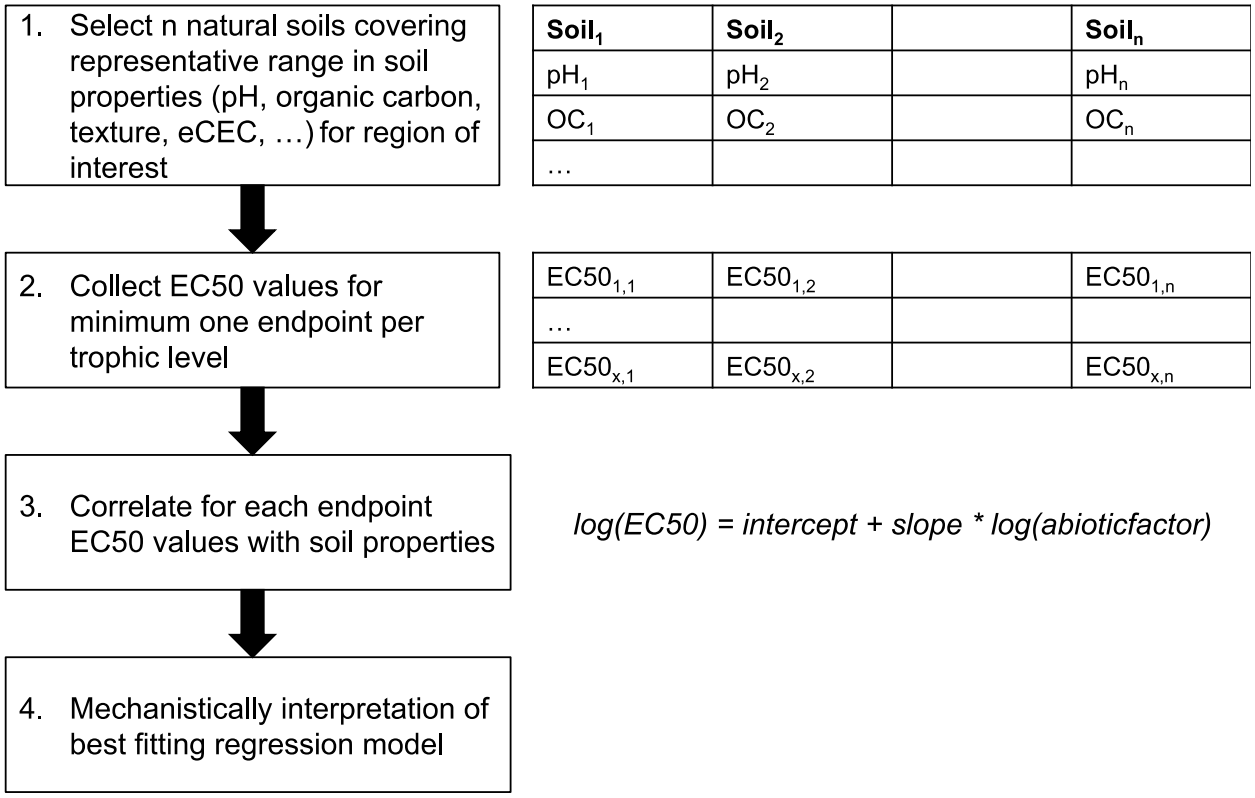

Figure 8: General approach for derivation of toxicity derived normalisation models for toxicity to terrestrial organisms.

Some considerations:

- The regression analysis is preferentially based on EC_{50} values[7] because of the larger robustness of this estimate compared to lower effect levels and the similar trends generally observed across the different effect levels (EC_{10}, EC_{20}, $EC_{50,}$, …).

- The EC_x values should be linked with the soil properties (pH, Organic carbon content, clay content, eCEC) of the soils in which the test was performed. The effective CEC (eCEC, i.e. CEC measured at prevailing soil pH) is preferred above the CEC measured at a buffered pH because the former is a better estimate for the in situ conditions.

- Regressions are preferably based on a log-log basis (except for pH as this is already a log-transformed parameter):

Regression models for accounting for the effect of soil properties on metal bioavailability and toxicity in soils have been derived for a wide range of European, Australian and Chinese soils in the framework of corresponding risk assessment processes in these regions (Table 8). Further information can be found in Appendix 4, section A.4.2). It must be noted that when several models are available for taking into account the effect of soil properties on metal bioavailability and toxicity for the same endpoint, they may have identified other soil properties as best predictor of metal toxicity for this endpoint. So far, not all major soil types globally occurring are covered yet and e.g. tropical scenarios are still missing. It is not yet known if

[7] For the Ni sediment regression models EC20 values were used.

the existing models are also applicable to such soils. A summary of the abiotic soil factors selected for the normalisation models developed for European soil is reported in Table 7. Table 8 gives an overview of the existing models for the different metals.

Table 7: Global availability of models to correct for the bioavailability of metals.

Geographical region	Metals	Endpoints
Europe	Cu, Ni, Pb, Zn, Co, Mo, Ag	Plants (monocotyledon and dicotyledon) Invertebrates (arthropod and annelid worm) Microbial processes (nitrification and C-respiration)
Australia	Cu, Zn	Plants (monocotyle) Microbial processes (nitrification and C-respiration)
China	Cu, Ni	Plants (monocotyle) Invertebrates (annelid worm) Microbial processes (nitrification)

Table 8: Overview of models to correct for the bioavailability of metals.

Metal	Organisms/Microbial processes	Abiotic factors	Reference
Ag	Plants	Organic C, pH and eCEC	Langdon et al., 2013
	Invertebrates	Organic C	Langdon et al., 2013
	Microbial processes	Organic C and eCEC	Langdon et al., 2014
Co	Plants	eCEC	Mico et al., 2008; Li et al., 2009
	Invertebrates	eCEC	De Schamphelaere et al., 2008
	Microbial processes	eCEC	Salpeteur et al., 2007
Cu	Plants	eCEC	Rooney et al., 2006
	Invertebrates	eCEC	Criel et al., 2008
	Microbial processes	eCEC, Organic C, clay and pH	Oorts et al., 2006
Pb	Plants	eCEC	Smolders et al., 2011
	Invertebrates	eCEC	Lanno, 2012
	Microbial processes	eCEC	Smolders et al., 2011
Mo	Plants	pH and clay	McGrath et al., 2010; Oorts et al., 2015
	Invertebrates	Clay	Van Gestel et al., 2011; Oorts et al., 2015
	Microbial processes	Clay	Oorts et al., 2015
Ni	Plants	eCEC	Rooney et al., 2007
	Invertebrates	eCEC	Van Eeckhout et al., 2005
	Microbial processes	eCEC	Oorts et al., 2006
Zn	Plants	pH and eCEC	Smolders et al., 2003
	Invertebrates	eCEC	Lock et al., 2003
	Microbial processes	Background Zn	Smolders et al., 2004

Next to these empirical models, some more semi-mechanistic models for the soil compartment were developed (e.g. Thakali et al., 2006a and 2006b, Lofts et al., 2013, Wang et al., 2013). These models are generally based on the biotic ligand model (BLM) concept as used for bioavailability corrections for the water compartment. The use of semi-mechanistic BLM-type models requires detailed and advanced soil solution modelling to provide the input data required which is still cumbersome. At present, the complexity of these semi-mechanistic approaches is still high and the predictive power is still not sufficient to be used

in a regulatory framework. Therefore, there is still a preference for empirical toxicity related bioavailability models. Semi-mechanistic models can however still be used as a validation of the correlations observed in the regression models

Derivation of soil threshold concentrations

The general framework for implementation of bioavailability corrections into derivation of ecological threshold concentrations for the soil compartment is presented schematically in Figure 9 (Smolders et al., 2009).

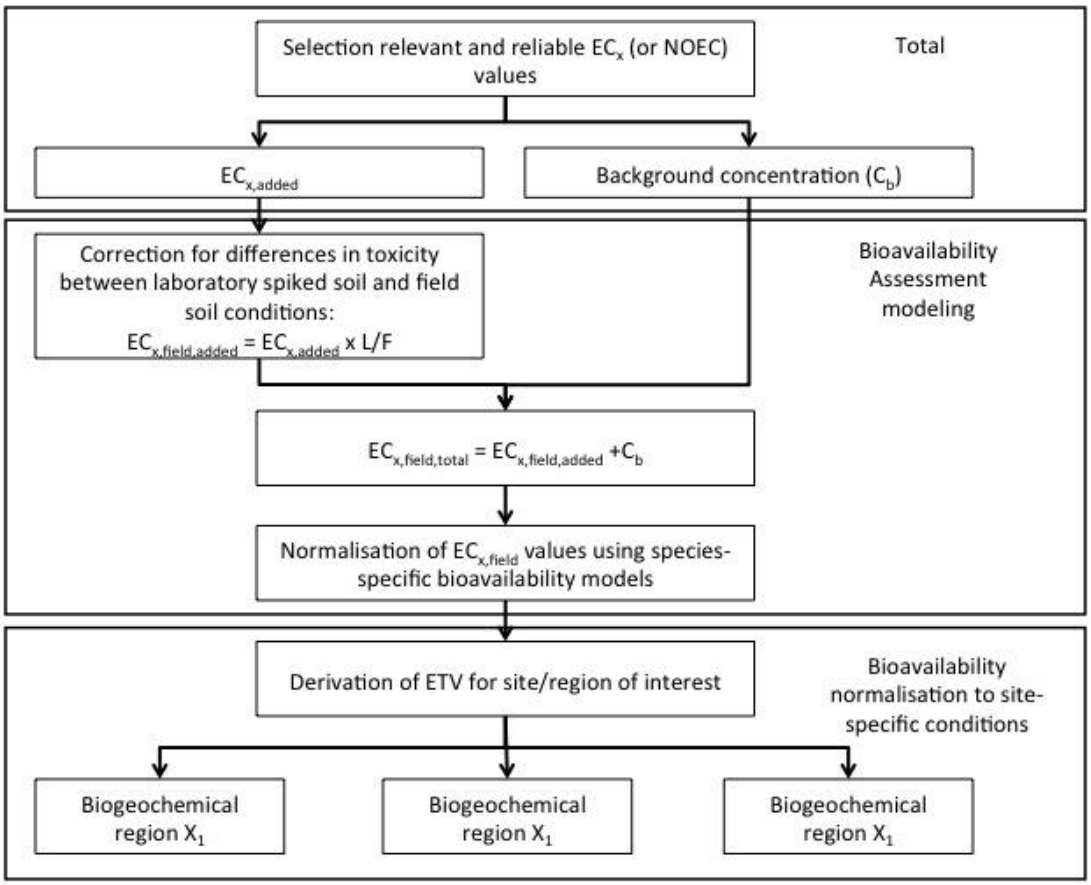

Figure 9: Framework for implementation of bioavailability factors into soil limits derivation.

Following steps can be distinguished:

- After selection of the reliable EC_x (or NOEC) values, the added EC_x values are derived by subtracting the metal background (C_b) of the tested control soils from the EC_x values based on total measured concentrations.

- Each individual $EC_{x,added}$ value is corrected for the discrepancy in toxicity between freshly-spiked soils in laboratory conditions and field-contaminated soils, by multiplying all individual added EC_x or NOEC values with the metal-specific lab-to-field (L/F) factor (see section 3.3.1). When no relationship is found between soil properties and the lab-to-field factor, one generic factor is

used for all soils (e.g. Cu, Zn, Pb). When the lab-to-field factor depends on the soil properties (e.g. pH for Ni and Co), a specific lab-to-field factor is calculated for the soil properties of the soil tested for each EC_x in the database. In a total approach, the metal background concentration from each individual test soil is then added again in order to calculate the total "field" EC_x or NOEC values. Previous steps should be omitted for toxicity data from tests in field contaminated or leached and long-term equilibrated soils.

- In the following step, the toxicity data are corrected for differences in metal availability among soils. Normalising for the effect of soil properties allows the calculation of a specific threshold concentration for the effect of metals to soil organisms in the soil under investigation. In case of the regression model approach (see section 3.3.2), each total or added "field" EC_x or NOEC value is normalized towards the soil properties of a specific target soil, using the slope of the metal- and organism-specific regression function (log-log based) and following equation (Equation- 22):

$$EC_{x,reference} = EC_{x,test}\left[\frac{abiotic\,factor_{reference}}{abiotic\,factor_{test}}\right]^{slope} \qquad \text{(Eq-22)}$$

reference is the soil for which the soil standard must be derived,

test is the tested soil,

abiotic factor is the soil property with which toxicity is correlated,

slope is the slope of the selected log-log based regression equation (in cases where the L/F factor is dependent on soil properties, the application of this factor will also affect the regressions between toxicity thresholds and soil properties and the slope from regressions based on L/F corrected EC_x values should be used. If the L/F factor is a constant value for all soils, regressions can be based on freshly spiked EC_x values).

- Finally, the environmental quality standard is derived based on the bioavailability-corrected toxicity data. If after normalisation multiple data are available for the same species or microbial process, a species/process mean value is calculated as the geometric mean from all data for the most sensitive endpoint for each species or process. This species/process mean approach is preferred for normalized data, where the remaining variation among data for a given species/process can be mainly attributed to intra-species variation in sensitivity. This is however not the case for non-normalized data, where variation between toxicity data is also caused by differences in bioavailability among soils). Depending on the data available, the EQS can be derived using the AF or SSD approach.

Where possible, the complete effects data set should be normalized. This assumes that reported effects data contain information on the abiotic soil bioavailability parameters of the test system. When such information is not available, these data cannot be normalized and used for the derivation of the bioavailability corrected soil threshold. The final definition of an environmental limit obviously requires several regulatory choices, e.g. on effect (choice of EC_x) and protection level (choice of HC_p) or on the organisms to be included (Checkai et al., 2014).

Cross species extrapolation

Where bioavailability models are available, they exist mostly for a limited number of species representing different trophic levels. Toxicity data generated for these species under different abiotic conditions can be normalized to a common set of abiotic conditions as long as these soil properties fall within the (geochemical) boundaries of the bioavailability model (e.g. range of eCEC, organic matter, pH). For those species for which no specific bioavailability model has been developed, it should be verified on a case-by-case basis whether the bioavailability model of another species could be applied. One exception is microbial activity where a function approach is preferably being used. Normalisation using bioavailability models and read-across to other species for which no bioavailability model is available applies to any compartment where a bioavailability model is available It must be noted that lab-to-field correction factors are derived based on the total weight of evidence and are not species or endpoint specific. Lab-to-field factors are therefore applied on all toxicity data derived in recently spiked soils.

The application of normalisation models across species (full normalisation) is based on several assumptions (e.g. similar binding and uptake mechanisms, similar stability constants between metals and the biotic ligands, similar site of action) and therefore the applicability across species needs to be investigated. The evaluation of full read-across of the available bioavailability models should include the following considerations:

- Mode of Action (MOA). A similar mode of action across species is a qualitative argument for read-across. In principle, it is very difficult to know the 'mode of action' of a metal ion for a particular species, and certainly one where only limited data are available. Even in circumstances where the same 'mode of action' is likely, there remains the uncertainty of whether the quantitative changes in physiological response to changes in metal ion availability will be identical between species.

- Physiological similarities of the species. Similarity of species can be used as justification for use of a particular model, e.g. use of model derived for one terrestrial arthropod species for normalisation of toxicity data of other arthropod species. Such extrapolation is widely used in environmental risk assessment for practical reasons, but is not without uncertainties and these need to be considered in drawing conclusions. Clearly there is a limit to how far such an extrapolation can be made before validity of the extrapolation should be confirmed. For read-across of bioavailability models among terrestrial organisms, the following rules should be taken into account:

    - Read-across is only accepted within the same trophic level (plants, invertebrates or micro-organisms). Extrapolation to a different trophic level is hence not allowed.

    - For terrestrial invertebrates, a distinction is made between hard-bodied (e.g. arthropods) and soft-bodied (e.g. annelid worms) organisms and read-across between these 2 groups is not recommended because of differences in the importance of dermal and oral exposure route between these groups of organisms.

    - Similarly, a distinction is generally made between microbial processes related to the nitrogen cycle (e.g. nitrification, N mineralization) and the carbon cycle (e.g. respiration) in soil and read-across between these 2 groups is also not recommended because of the larger functional redundancy for processed related to the C-cycle.

    - In case several potential models are available for read-across and there is no clear preference based on mode of action or physiognomy (e.g. for plants), the model with the smallest correction factors (i.e. the smallest slopes in case of regression models) should be selected as a conservative approach.

- Decrease in intra-species variability (e.g. max/min ratio) in EC_x values after normalisation. Normalisation of the individual EC_x data towards specific soil properties should significantly reduce the within species-variation in EC_x values. This is a quantitative illustration of the adequacy of the models and the significance of soil properties in controlling the bioavailability and toxicity of metals to soil organisms and further demonstrates the importance of normalisation of the toxicity data and separating the biological variation from the variation in metal availability because of varying soil properties.

- Similarity of the bioavailability models across species and endpoints tested. In case for one metal the same abiotic factors are selected for normalisation of toxicity values for all species and functions tested (e.g. eCEC for Co and Ni) and if all regression slopes are very similar, this points out that the relationships are consistent, and that models can be used across species. In such case, the choice of a particular relationship is not critical because the other relationships available will yield similar results. Therefore, even if a relationship would be incorrectly applied to a species in the database, this would not make a difference, as the "correct" relationship would be very similar to the one that was in fact used.

- In addition, validation with experimental data can also be considered, e.g. spot check validations for other organisms (i.e. few ecotoxicity data from tests performed under different geochemical conditions for a range of key soil parameters, e.g. eCEC, see also section 3.1.3 for the water compartment) or use of field or mesocosm data to validate the bioavailability corrections for organisms for which no specific bioavailability model has been developed. This is equally applicable to water and sediment.

If full read-across is justified the next step consists of applying the bioavailability model across species of similar trophic levels (e.g. applying the *Folsomia candida* bioavailability model for normalisation of the toxicity data from other arthropods …) towards a specific set of geochemical conditions. The bioavailability models normalize the effect concentrations (EC_x values) of the metal for each species' endpoint and therefore retain the intrinsic metal sensitivities of the different species and endpoints. The species-specific normalized EC_x values for the most sensitive endpoints are then used to derive the soil limit concentrations. For a large data set the geomean is taken but in case of a small data set most often there is preference for selecting the most sensitive value rather than using a geomean value.

In case read-across is only justified for some species and not for others (e.g. unexplained significant increase in variability after normalisation or different mode of action) an alternative cautious approach should be developed. In this approach the bioavailability models are only applied to those species within the trophic level for which the application can be justified. For those species for which application of a bioavailability model related to their trophic level cannot be justified, either all toxicity data (i.e. all EC_{10} values), the geometric mean or the worst-case (i.e. lowest) EC_x values can be selected for derivation of the soil limit concentrations. This decision should be made on a case-by-case basis and should consider e.g. the intra-species variation in toxicity data before and after normalisation.

Collection of abiotic factors

Information on the main abiotic factors controlling metal bioavailability needs to be collected in order to apply the toxicity models (Table 9). This type of data can be obtained from existing monitoring databases or from specifically tailored monitoring campaigns. The ETV can be normalized towards a standard set of soil properties for the most representative biogeochemical region (e.g. acid sandy soil); or information on the soil properties of the site of interest can be collected, allowing normalization of the ETV towards the site-specific conditions.

Table 9: Overview of the most relevant parameters that influence the bioavailability of metal species in the sediment/soil compartment

	Soil
Required	Total Organic Carbon (TOC)
	pH
	Effective Cation Exchange Capacity (eCEC, i.e. CEC at prevailing soil pH).
	Al/Fe/Mn Oxides
	Particle size (sand, silt and clay content)
Supportive information	Pore water chemistry (total and dissolved metal concentrations, pH, DOC, hardness, conductivity/salinity, etc.)

3.3.3 Use of alternative approaches to assess bioavailability.

Comparison modelled/measured data with a reasonable worst-case (RWC) ETV

If no regression models are available a realistic worst-case scenario can be used as a reference scenario, providing a soil limit value corresponding to a maximum bioavailability. Care should however be taken that the combination soil properties that maximize the bioavailability does not result in an unrealistic scenario ignoring the covariance among soil properties in natural soils. Therefore, where different parameters influence the bioavailability of the metal (e.g. Cu, Zn, Mo), the reference scenario should as far as possible be a realistic combination of the relevant soil parameters.

Use of soil extraction techniques

For soils, methods expressing metal toxicity in soil based on total soil solution metal concentration or free metal ion activity generally increases variability in toxicity thresholds among soils and hence does not really explain differences in bioavailability. Several soil extraction techniques have, however, been used in order to predict metal bioavailability and toxicity in soils (e.g. pore water, 0.01M $CaCl_2$, 1 M NH_4NO_3, 0.43 M HNO_3, Diffusive Gradients in Thin-films (DGT), cyclodextrin (HPCD), extraction. If used for regulatory purposes this should, however, be done in a cautious way as in their current stage of development extraction techniques are generally not validated with metal toxicity data to soil organisms. Most extractions and tests for bioavailability are indeed calibrated on uptake of metals by plants and invertebrates and not by their toxic effects to these organisms. Using bioaccumulation to calibrate soil extractions does not ensure that they predict toxicity, e.g. because translocation of metals from plant root to shoot is restricted.

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APPENDIX 1: PRACTICAL GUIDANCE IN SELECTING RELIABLE AND RELEVANT ECOTOXICITY DATA FOR THE PURPOSE OF BIOAVAILABILITY ASSESSMENT

A.1.1 Introduction

Several scoring systems are available to assess the reliability and relevancy of ecotoxicity data. The evaluation systems commonly used for studies assessment in different European regulations (ECHA, 2008a; EC, 2011) are usually based on the criteria established by Klimisch et al., 1997). However, some studies (Küster et al., 2009; Agerstrand et al., 2011a and b) indicated the need for an updated evaluation system as the Klimisch system is deemed not sufficiently detailed, tends to favour standard tests and/or tests conducted under Good Laboratory Practices (GLP) and was originally designed for evaluating mammalian toxicity data rather than ecotoxicity data. Recently, a new set of reliability and relevance Criteria for Reporting and Evaluating ecotoxicity Data (CRED-criteria) has been developed (Kase 2015a, 2015b; Moermond et al., 2015). The CRED-project aims at improving reproducibility, transparency and consistency of reliability and relevance evaluations of aquatic ecotoxicity studies (note that soil and sediment ecotoxicity data are not yet covered by the CREDS evaluation system). The CRED evaluation method provides more detailed guidance on how to evaluate study reliability and relevance, thus leading to greater consistency among individual reviewers (Kase, 2015b). However, many of these scoring systems have been developed with organic substances in mind. As metals are naturally occurring substances and the ecotoxicity of metals is strongly driven by the amount of metal that is bioavailable, it is imperative to take these aspects into account when evaluating the relevance and reliability of toxicity data generated with metals and metals compounds. For example, exposure conditions during the test could be different from those of the natural environment on which the risk assessment is done, e.g. the pH or hardness of the test medium may be outside the boundaries of the physico-chemical conditions encountered in a specific environment under investigation or the test organisms could be cultivated under very different conditions and as such be conditioned to a completely different environment.

General data quality screening recommendations and further reading on those can be found in several guidance documents available on this topic (OECD, 1995; EC, 2000, EU-TGD, 2003; ECHA, 2011) and those referenced above. The subsequent paragraphs highlight some of the more metal specific issues and new concepts that could be taken into account when evaluating ecotoxicity data for metals and metals compounds. The main concepts that should be covered are:

- Metals are naturally occurring substances and hence natural backgrounds should be taken into account in selecting adequate ecotoxicity data
- The ecotoxicity of metals is strongly driven by the amount of metal that is bioavailable and this fraction is a function of the physicochemical conditions of the test media

A.1.2 Relevance criteria

Relevance of the test substance

- With the exception of tests conducted with complex materials (like substances of Unknown or Variable Composition, Complex reaction products or Biological Materials (UVCBs)), tests for metals should generally be conducted with high-purity soluble metal salts if used for the purpose of deriving environmental quality guidelines/standards. If tests are being conducted with sparingly soluble metals or metals known to complex rapidly with organic or inorganic species, preparation of exposure solutions should incorporate a proper equilibration time after spiking. It is important to note that for metals the free ion paradigm is overall the metric that is used for the interpretation

and expression of the effects data. This concept is important because it is widely used for read-across purposes. Indeed, the free metal ion and its potential to complex/compete with other organic and/or inorganic ligands for the available biological binding sites and its internal distribution within an organism is key to understanding metal availability. It is, however, acknowledged that the free ion may not necessarily be the best predictor of toxicity for all metals. For example, neutral metal species (e.g. AgCl, HgS) and anionic species (e.g. SeO^{2-}, AsO_4^{2-}) may contribute to the observed toxicity (Campbell, 1995). In addition, chemical interactions between metals and salts may lead to the formation of solid precipitates that may reduce bioavailability and toxicity or could lead to physical effects. For example, several authors have suggested that polymerization or precipitation of Al hydroxide at the gill may be responsible for observed respiratory effects in fish caused by Al exposure (Playle and Wood, 1989, 1990; Poleo 1995). Proper equilibration of freshly prepared test media could partly avoid this.

Relevance of the test medium

- Metals are naturally occurring substances and many organisms have evolved mechanisms to regulate their accumulation and storage. Moreover, some metals are essential nutrients (e.g., Co, Cu, Zn, and Ni for plants and algae), so insufficient concentrations can limit growth, survival and reproduction of the organisms. Such phenomena as acclimation/adaptation and essentiality should therefore be taken into account. Only data from ecotoxicity tests conducted at metal background concentrations (in the culture media) similar to the region under investigation and within the ecological niche of the organism should be deemed relevant. Species tested at very low background metal concentrations outside their ecological boundaries could be overly sensitive (due to deficiency issues or induced stress levels that would exert toxicity) and conversely, organisms cultured in media with elevated metal concentrations (both essential and non-essential metals) may become less sensitive. In particular, it is recommended that the essential metal concentration in the culture medium should be at least equal to the minimal concentration not causing deficiency for the test species used. Concentrations of non-essential metals should fall within the natural background variation of these metals. Defining minimal levels of metal background for selection of relevant culture media should only be performed in case there is scientific evidence that acclimation/adaptation phenomena are relevant for the metal under investigation. Ideally, culture media and test media used should be within the physico-chemical boundaries of the environment under study; this relates to pH, hardness, DOC and natural metal background, etc. If they are reported and test organisms have been cultured in conditions that are outside the natural background concentration ranges such data should be discarded. It is, however, recognized that this may lead to a reduction in the number of useful ecotoxicity data which may even sometimes limit the possibility of using a Species Sensitivity Distribution. Another complicating factor is that quite often culture conditions are not reported and in that case expert judgment should be used to decide if the study can still be used or not.

A.1.3 Reliability criteria

Exposure conditions

- For metals, the test design should be evaluated to ensure that exposure concentrations were well characterized and that proper equilibration time was provided to allow a stable bioavailable fraction. In some situations experimental methods must be modified to allow ample time for chemical reactions to occur that affect metal bioavailability. This is especially important for aquatic tests conducted under flow-through test conditions. For example, the kinetics of metal-DOC binding or sulfide precipitation (e.g. Ag) may require an equilibration period between the

metal and the test medium prior to exposing the organisms. For sparingly soluble metal salts freshly formed precipitates may obscure the toxicity test results. Test media containing chelators (e.g. EDTA or high DOC levels) should be avoided as they may decrease the metal bioavailability and toxicity. For sediment testing, semi-static and flow-through test designs are preferred since the sediment may act as a source of dissolved metals to the overlying water column (Wang et al., 2004). This could result in a build-up of metals in the overlying water causing toxicity via this route. If the latter is observed the tests should not be considered reliable. Static test designs are more prone to this phenomenon than test systems with sufficient water renewals.

- For sediment and soil testing, sediment testing and even sometimes aquatic testing, adequate time should elapse between mixing metal or metal compounds into the test medium and introducing biota (plants or soil/sediment species). Initial partitioning of metals, taking place within hours or days after addition of soluble metals to a moist soil, is often followed by much slower reactions, termed fixation or ageing, that further decrease the bioavailability of added metal with time (e.g. Buekers et al., 2008; Ma et al., 2006a and 2006b). However, equilibration in a water-only system will be reached within hours or days, while for sediments and soils full chemical equilibrium may only be obtained after several months or even years. Both short equilibration times and high spiked metal concentrations in sediments/soils will accentuate partitioning of metals disproportionately to the dissolved phase (Lee et al., 2004; Simpson et al., 2004). Results from standard tests in freshly spiked soil also generally overestimate toxicity in realistic field conditions (e.g., Lock et al., 2006; Oorts et al., 2006; van Gestel et al., 2012). Because it is practically not feasible to equilibrate all soils for several months after spiking with a soluble metal salt, correction factors are derived for the effect of this slow equilibration of metals in soil on their toxicity (see section 3.3.1.1 lab-to-field factor). It is however recommended that all soils are equilibrated for approximately one week after mixing the metal into the soil before introducing the test species in the soil and starting the ecotoxicity assays.
- Precautionary steps are needed to achieve more stable and environmentally realistic partitioning of metals in spiked sediments. Simpson et al. (2004) investigated the equilibration and bioavailability of metals in laboratory contaminated sediments in order to provide better guidance on acceptable procedures for spiking sediment with metals for use in the development of whole sediment toxicity testing. It was demonstrated that sediment spiked with Ni required a relatively long time for equilibration – as long as 70 days (d), compared to 15 d for copper, 40 d for zinc, and 45 d for cadmium. The addition of metals to the sediment causes major decreases in pH and increase in redox potential. Based on these and other findings new spiking methods have been developed and applied in sediment toxicity tests with the purpose of reducing the diffusional loss of nickel from the sediment phase into the overlying water in laboratory sediment toxicity tests (Brumbaugh et al., 2013; Besser et al., 2013). It is recommended that the overlying water of sediment toxicity tests is measured for the test substance, and that testing is initiated only when overlying water concentration reaches acceptable levels, e.g. EC10 levels derived from water only tests. Simulated aging and weathering processes may also be desirable but currently are not embedded in standard sediment test protocols.

Chemical analysis and description of physicochemical properties of the test media

- Considering the strong influence of water physico-chemistry on metal toxicity, the physico-chemical conditions (metal concentrations abiotic factors and biotic factors) should be adequately described and reported, especially if it is the intention to carry out corrections for bioavailability. The aquatic medium should be characterised by DOC concentration, hardness, pH, alkalinity and any other specific parameter of importance to the metal in question. With regard to metal concentrations, the current state-of-the-science dictates that only test results where metal concentrations have been measured should be used. Absence of measured exposure concentration

data is a clear reason to reject a data point. Measured data should reflect the dissolved fraction (< 0.45 μm) and/or total metal concentrations. Measurements of dissolved metal concentrations are critical to the assessment of sparingly soluble metals (particles and precipitation may occur) and in the use of natural waters as test media (adsorption to suspended solids may occur). A description of the filter methodology and its efficiency is therefore most relevant.

Concentration-effect relationship

- For essential elements, in theory, harmful effects may be observed due to metal deficiency caused by very low metal concentrations caused by particular bioavailability conditions used in the test. Therefore essential metals quite often exhibit biphasic dose-response curves visualized as U shaped or inverse U-shaped graphic forms (Alloway, 1995, Fairbrother and Kapustka, 1997, Van Assche et al, 1997). The concept that many metals are required for organisms health at one range of concentrations and are toxic in quantities that may be either more or less than the range is been referred to as the "window of essentiality" or the Optimal Concentration Range for Essential Elements (OCEE) (Van Assche et al., 1996). But also non-essential metals may elicit in low doses an increased performance that can be observed for example in growth or reproduction. This phenomenon is often referred to as the hormesis effect. If non-monotonic dose response curves are observed the conventional log-logistic dose response model is less useful to fit the toxicity data and adaptations need to be made.

APPENDIX 2: VALIDATION AND CROSS-SPECIES EXTRAPOLATION

OF BIOTIC LIGAND MODELS

A.2.1 Introduction

The conceptual part of the Biotic Ligand Model (BLM) can be considered in terms of three separate components. The first component involves the solution chemistry in the bulk water, which allows prediction of the concentration of the toxic metal species. These chemical speciation computations are standard and can be performed with any of the several speciation models that exist. A second component involves the binding of the toxic metal species to the biotic ligand. The final component is the relationship between the metal binding to the biotic ligand and the toxic response. The presence of the biological component (i.e. binding to the metal binding sites within an organism) suggests that the bioavailability correction should conceptually be applied on the effects' side of the equation. However, from a practical viewpoint for regulatory purposes it could equally be applied at the exposure side using the Bio-F approach (see section on Full and RWC cross-species extrapolation (section 3.1. and Appendix 3, case studies A.3.4 and A.3.5a and b).

A.2.2 Validation

Any BLM or equivalent bioavailability model used should be properly validated. Usually an internal/auto validation is performed as part of the development of a BLM (Figure A.2.1)

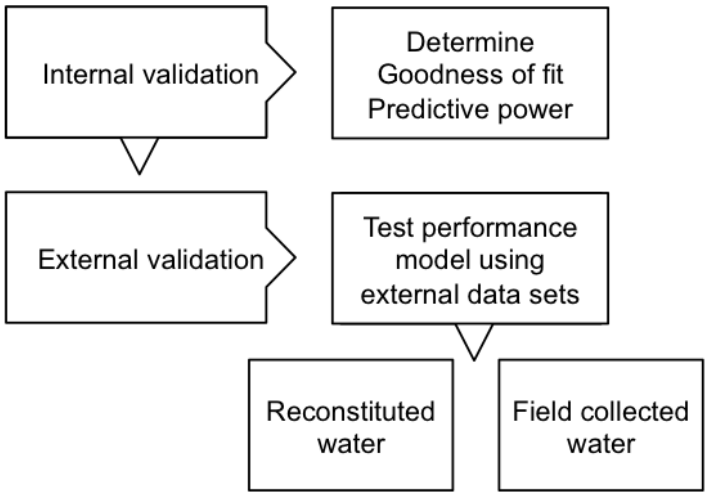


Figure A.2.1: Validation steps BLM

In this analysis, the goodness of fit of the model and its ability to predict the real toxicity values of the data used in the model for the species of concern is determined. More importantly however, is the external validation in which the predictive power of the model in estimating the results of toxicity data from external data sets (i.e. tests not used for the model development) is evaluated. These tests should be conducted in lab-reconstituted and/or field-collected natural waters with a range of abiotic parameters overarching the applicability domain of the BLM.

A.2.3 Cross-species extrapolation

Depending on the number of validated BLMs available per trophic level, a full-read cross-species extrapolation, a reasonable worst case (RWC) cross species extrapolation or no BLM correction can be applied (Figure A 2.2).

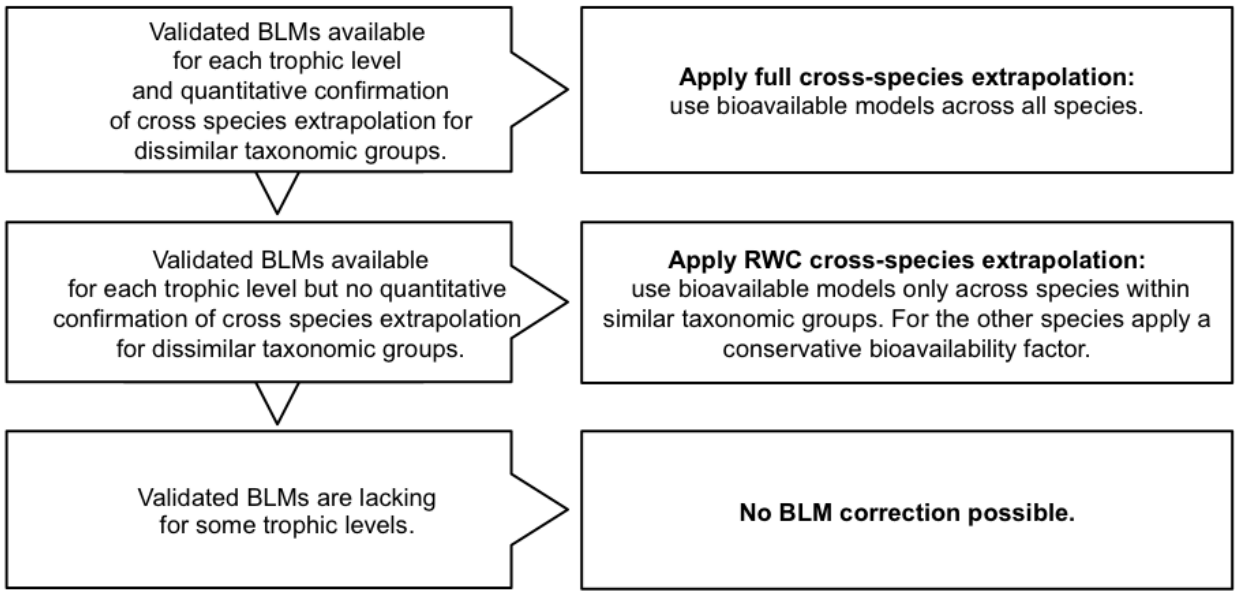


Figure A.2.2: Approach for cross-species extrapolation of bioavailability models.

Some considerations:

- In order to extrapolate across species in all typical trophic level (algae/fish/invertebrates) at least for each level a validated BLM should be available.
- the applicability of the bioavailability model across species can be assessed by comparing information on the mechanism of action (MOA) of the metal under consideration for the different species. If the MOA is different between two species the BLM should not be used. Another criterion for application is the extent in which the BLMs can reduce the in intra-species variability. This intra-species variability can be assessed by comparing the predicted vs. observed toxicity for the different species or by means of the max/min ratio between toxicity thresholds. If it can be demonstrated that the interspecies variability is significant decreased (i.e. min-max ratio is smaller after normalization) the bioavailability model can be used across species.
- These so-called spot checks consist typically of relatively few ecotoxicity tests, performed with species for which the BLM will be applied under different geochemical conditions for a range of key bioavailability parameters (e.g. pH & DOC)). Considering that sensitive species are driving the ETV derivation it should further be demonstrated that the developed/validated bioavailability models can be applied to the most sensitive species/taxonomic groups. For nickel in addition to the 3 trophic levels (algae, fish and daphnids) an additional species-specific BLM was developed for the daphnid *Ceriodaphnia dubia*. In case of a local assessment where endemic species may require specific protection the models should be equally protective for the typical endemic species of the database.
- In case cross-species extrapolation is only justified for some species and not for others (e.g. unexplained significant increase in variability after normalisation, different mode of action or no spot checks available to justify extrapolation to dissimilar taxonomic groups/trophic levels), an

alternative more precautionary cross-species extrapolation approach should be applied. In this RWC approach the bioavailability models are only applied to those species within the trophic level for which the application can be justified. For those species for which application of the bioavailability model cannot be justified a bioavailability factor based on the most conservative available bioavailability model should be applied.

- Conceptually, the BLM developed for animals are related to toxicity caused by uptake through gills, although the concept seems to work for gill-less organisms too. The relative importance of dietary exposure is discussed further in this document.

A.2.4 Use of acute BLMs

In some jurisdictions the use of BLMs developed on acute toxicity data are still used. Table A 2.1 gives an overall overview of available acute BLM's.

Table A 2.1: Overview Acute BLMs

Metal	**Available acute BLMs - main relevant publications**
Most BLMs have been developed for regulatory purpose. Environmental risk assessments are predominantly driven by long-term effects (chronic toxicity), and therefore most BLMs-tools focussed on chronic endpoints. There is, however, a large number of publications on acute toxicity-based BLMs. This work, however, was not always translated into the development of user-friendly and publicly accessible acute BLM tools/models. With regard to acute BLMs, this table only provides the most relevant references that report on key BLM parameters (i.e. binding constants). Additional references (e.g., publications that only focus on the metal-gill binding interaction) can be found in Ardestani et al (2014).	
Ag	Bury et al, 2002; Janes and Playle, 1995; McGeer et al, 2000; Morgan and Wood, 2004; Paquin et al, 1999.
As	Chen et al, 2009.
Cd	Clifford and McGeer, 2010; Francois L et al, 2007; Hatano and Shoji, 2008; Hollis et al, 2000; Jansen et al, 2002; Niyogi et al, 2004/2008; Playle et al, 1993; Rachou and Sauve, 2008; Schwartz et al, 2004; Van Ginneken et al, 1999.
Co	Richards and Playle, 1998.
Cu	Brooks et al, 2006; Constantino et al, 2011; De Schamphelaere and Janssen, 2002; De Schamphelaere et al, 2007; Di Toro et al, 2001; Ferreira et al, 2009; Gheorghiu et al, 2010; Hatano and Shoji, 2008/2010; MacRae et al, 1999; Meyer et al, 2007; Playle et al, 1993; Rachou and Sauve, 2008; Ryan et al, 2009; Santore et al, 2001; Taylor et al, 2003; Villavicencio et al, 2011; Welsh et al, 2008.
Hg	Klinck, et al, 2005.
Pb	MacDonald et al, 2002; Schwartz et al, 2004; Slaveykova and Wilkinson, 2003.
Mn	Francois et al, 2007; Peters et al, 2011.
Ni	Deleebeeck et al, 2007a,b/2008/2009a,b; De Schamphelaere et al, 2006; Keithly et al, 2004; Kozlova et al, 2009; Worms and Wilkinson, 2007; Wu et al, 2003.
U	Fortin et al, 2007.
Zn	Alsop and Wood, 2000; Clifford and McGeer, 2009; De Schamphelaere and Janssen, 2004; Heijerick et al, 2002a,b; Santore et al, 2001/2002; Schwartz et al, 2004; Todd et al, 2009; Van Ginneken et al, 1999.

APPENDIX 3: CASE STUDIES ON THE IMPLEMENTATION OF BIOAVAILABALITY FOR THE WATER COMPARTMENT

A.3.1 Case study: derivation EC10 values for less soluble metals: case study Pb

The solubility of lead is limited under specific environmentally relevant conditions. Its solubility limit is mainly driven by abiotic environmental factors (e.g. pH, hardness and DOC) and can be estimated using WHAM or Visual MINTEQ, Figure A3.1 shows an example of the translation of two ecotoxicity values expressed as total lead concentrations to dissolved Pb concentrations taking into account the actual solubility conditions occurring in the test.

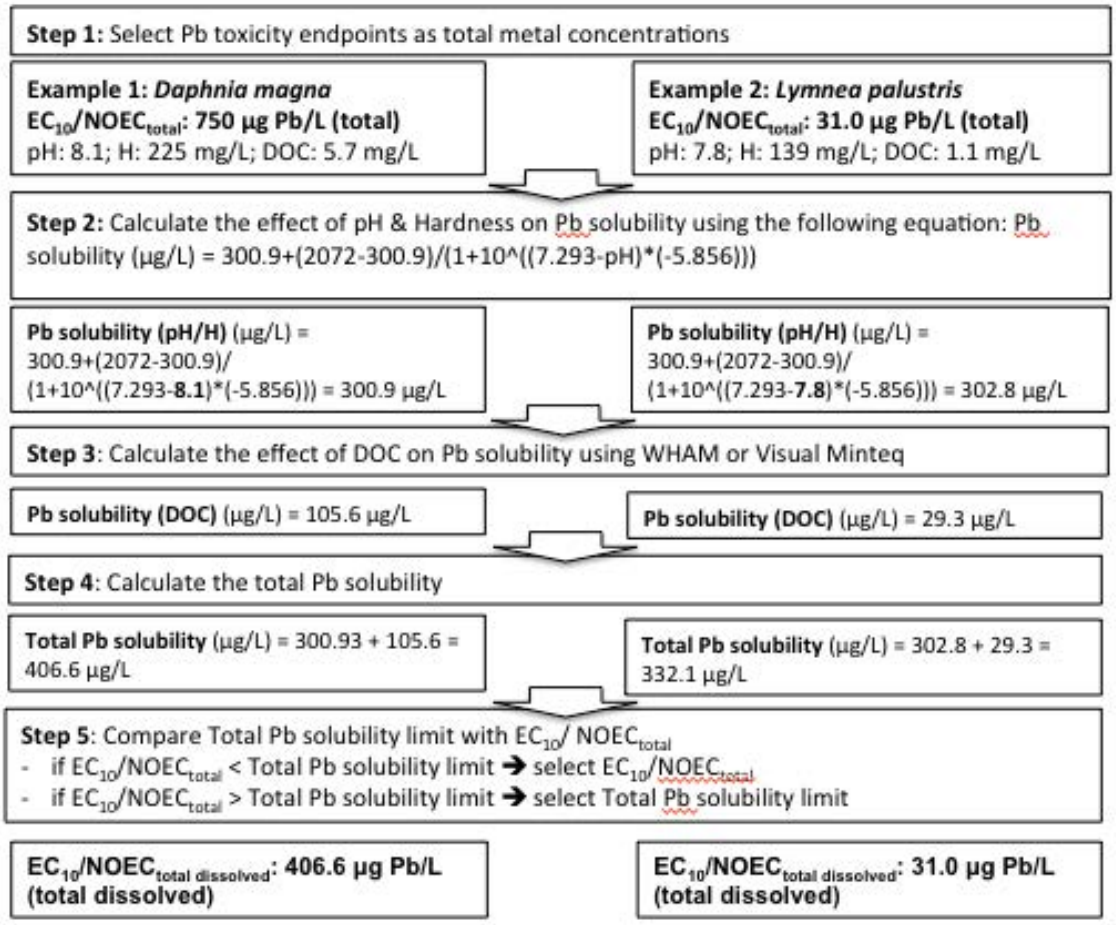


Figure A 3.1 Case study: translation from "total dissolved" to "dissolved metal" species of concern

For highly soluble metals such as Cu or Zn, the difference between total and dissolved (filtered) metal concentration is expected to be small in most surface waters and ecotoxicity test media. But in the case of a poorly soluble metal such as Pb, this difference can be substantial. Pb and its compounds will show indeed a low solubility under typical ecotoxicity testing, particularly at higher pH and alkalinity levels where lead carbonate and lead hydroxide minerals often tend to precipitate from exposure media (Kopittke et al., 2008). But also other parameters of the receiving water (e.g. DOC, hardness) may have an important

influence on the aquatic toxicity of lead. Recently, for lead a DOC based regression has been proposed for the aquatic EQS derivation in the context of the Water Framework Directive. (The process entailed the determination of significant relationships between DOC and chronic Pb toxicity for different aquatic species (*Cerodaphnia dubia*, *Pimephales promelas*, *Lemna minor*, *Pseudokirchienella subcapitata*, *Philodina rapida* and *Limnea stagnalis*. The most conservative slope of the equation (i.e. 1.2) was found for the rotifer *P. rapida* (Esbaugh et al., 2012) and used to calculate the $HC5\text{-}50_{reference}$ based on the selection of chronic lead toxicity data with low DOC (i.e. 1 mg/L on average). Subsequently the HC5-50 for a specific site can be calculated with the following equation:

$$HC5\text{-}50_{site} = HC5\text{-}50_{reference} + (1.2 \times (DOC_{site} - DOC_{reference})$$

A3.3 Case study: application of Cu BLM normalisations towards different biogeochemical regions

The chronic BLMs have been applied to a set of biogeochemical regions used as examples in the different EU metal risk assessments. For this exercise each organism specific critical biotic ligand accumulation $[Cu]_{\text{biotic ligand critical}}$ is recalculated into a critical bioavailable dissolved concentration $[Cu]_{\text{bioavailable, dissolved}}$ for the area under investigation characterized by a specific set of water-quality conditions (DOC, hardness and pH). Using the chronic BLMs will finally result in the derivation of different Species Sensitivity Distributions (SSDs) and ETV values for the different biogeochemical regions. The water chemistry and median HC5 values calculated for the different selected biogeochemical regions in EU-surface waters are summarized in Table A 3.1.

Table A.3.1: Overview of the water chemistry and median HC 5 values for some different EU biogeochemical regions

Biogeochemical region	Water chemistry	Median HC5 (best fit) (µg/L)	Median HC5 (log normal) (µg/L)
Ditch (The Netherlands)	pH 6.9, H 260 mg/L, DOC 12.0 mg/L	22.1	27.2
River Otter (UK)	pH 8.1, H165 mg/L, DOC 3.2 mg/L	7.8	7.8
River Teme (UK)	pH 7.6, H 159 mg/L, DOC 8.0 mg/L	17.6	21.9
River Rhine (The Netherlands)	pH 7.8, H 217 mg/L, DOC 2,8 mg/L	8.2	8.2
River Ebro (Spain)	pH 8.2, H 273 mg/L, DOC 3.7 mg/L	9.3	10.6
Oligotrophic Lake Monate (Italy)	pH 7.7, H 48.3 mg/L, DOC 2.5 mg/L	10.6	10.6
Acidic lake (Sweden)	pH 6.7, H 27.8 mg/L, DOC 3.8 mg/L	11.5	11.1

The HC5 values for copper in common EU surface waters vary between 7.8 and 21.9 µg/L.

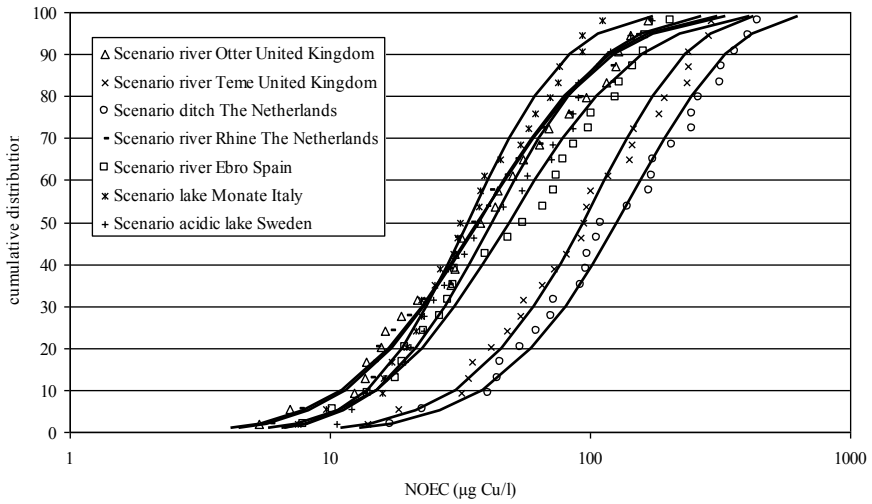


Figure A.3.2 Case study: justification cross-species extrapolation using spot checks

The chronic Ni aquatic toxicity database contains data for 31 species while fully validated chronic Ni BLMs are available for only 4 species (i.e., the invertebrates *Ceriodaphnia dubia* and *Daphnia magna*, the fish *oncorchynchus mykiss* and the algae *Pseudokirchneriella subcapitata*). Extrapolation of BLMs developed for one species (e.g. the invertebrate D. magna) to other taxonomically similar (i.e. crustaceans) or taxonomically dissimilar groups (i.e. insects, molluscs) should be justified. Sufficient information was available to convincingly demonstrate similarity in Ni toxicity mechanisms among different fish species and among different algae species, but not among different invertebrate or vascular plant species. Therefore a "spot-check" was undertaken to demonstrate the suitability of the developed BLMs to predict chronic Ni toxicity for organisms for which no BLM has been developed. In the spot-check study, four non-BLM organisms were tested. Three invertebrates including the insect *Chironomus tentans*, the rotifer *Brachionus calyciflorus* and the snail *Lymnea stagnalis* were tested. One plant species, *Lemna minor*, was also tested. Chronic toxicity tests were conducted with the "non-BLM" species with natural waters collected from sites selected to maximize variability in pH (6.9-8.0), hardness (16-256 mg/L Ca CO_3) and DOC (0.7-7.1 mg/L (ranges were kept within the boundaries of these parameters used in the development of the BLMs). The observed toxicity for each of the non-BLM species was compared with the predicted Ni toxicity from the BLMs (Figure A 3.3).

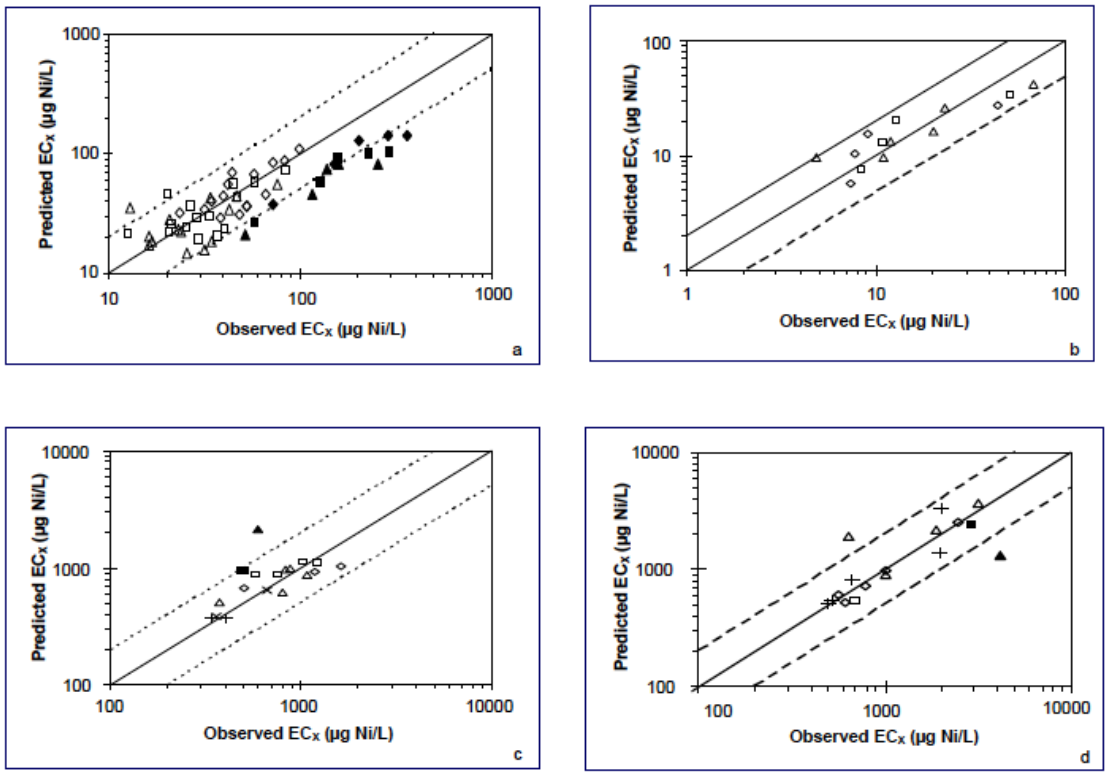


Figure A 3.3: Overview of the relationship between observed and BLM predicted chronic toxicity values for the (a) D. magna, (b) C. dubia, (C) P. subcapitata and (d) O. mykiss BLMs. Note: logarithmic scales are used for the Y-and X-axis.

Results showed that the BLMs were able to accurately predict Ni toxicity to the "spot check" species (Schlekat et al., 2010). Based on the results from the spot check exercise and other weight of evidence arguments (i.e. the ecological relevance of the BLMs, accuracy of the BLMs and the conservatisms of the proposed cross-species approach) the following final normalisation approach was determined to be appropriate for the normalisation of Ni toxicity data.

- the *P. subcapitata* BLM can be used to normalize the chronic toxicity to other algae,
- the *O. mykiss* BLM can be used to normalize the chronic toxicity to fish and amphibians,
- for cladocerans, insects and amphipods the most stringent result of the *D. magna*, and *C. dubia* BLMs can be used,
- for rotifers, the *D. magna* BLM can be used and, for molluscs and hydra, the *C. dubia* (best fitting BLM) can be used.

A.3.5 Case study: zinc compliance check using the Bio-F approach

A.3.5.a Example derivation conservative BIO-F value (RWC-cross species extrapolation)

As indicated in the guidance the most conservative Bio-F value should be used in order to correct for bioavailability if there is no justification for full cross species extrapolation. This was initially the case for zinc in where at the beginning three chronic BLMs are available for algae, fish and daphnids but no spot checks. Hence at the time for each the chronic NOEC values for algae, Daphnia and fish were predicted at

a site or a region X, using the BLMs under the site-specific conditions or water chemistry of that site or region. This will result in $NOEC_x$ values for that site or region. The chronic $NOEC_x$ values were then to be compared with a reference NOEC value ($NOEC_{ref}$). This $NOEC_{ref}$ value is calculated using the BLMs under reference water chemistry conditions.

Summary of reference NOEC values ($NOEC_{ref}$) for the three aquatic species for which BLMs have been developed under reference water chemistry conditions[8].

Species	**$NOEC_{ref}$**
O. mykiss	184
D. magna	86
P. subcapitata	21

This $NOEC_{ref}$ is a reasonable worst-case situation that mimics the situation where bioavailability of zinc is very high and thus can be regarded as a reference value for the bioavailability at the site or region X. The NOEC at the site or region X ($NOEC_X$) is then regarded as a surrogate for the actual bioavailable concentration of zinc at that site or region X, and is calculated with the BLM-models for the alga, Daphnid and fish.

The bioavailability factors (BioF) are then derived for each of the 3 BLM species as follows:

$$BioF_{water,X} = \frac{NOEC_{ref}}{NOEC_x}$$

The highest value of the three Bio-$F_{water,X}$ values for the three species is selected to ensure that a conservative approach and bioavailability factor (Bio-F) is taken, i.e. the smallest correction for bioavailability.

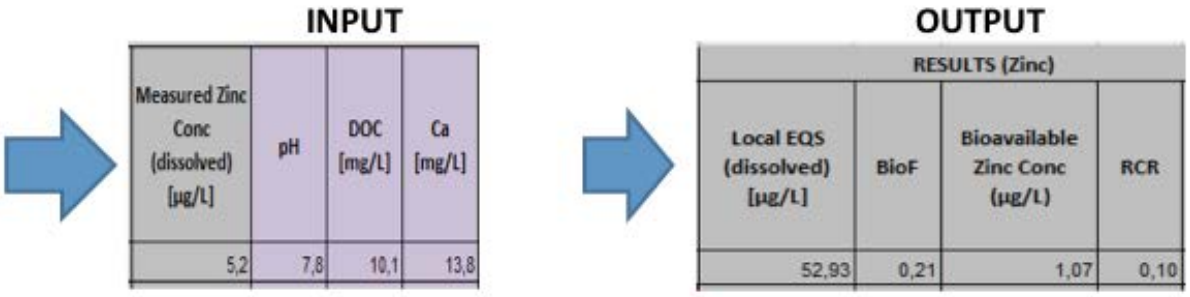
In the meantime for zinc spot checks have been conducted and full cross-species normalization is allowed. In that case if the bioavailability correction needs to be applied on the exposure side the Bio-F is calculated on the ETV level (see example A.3.5.b)

$$BioF_{water,X} = \frac{ETV_{ref}}{ETV_x}$$

[8] The reference water chemistry conditions (ref) are taken from the GEMS-B database (see Table 4.11 in Heijerick et al., 2003). The water chemistry conditions are selected as follows. For all organisms: 10th percentile of DOC. For *D. magna and O. mykiss*: 10th percentile of inorganic parameters (including pH and hardness). For *P. subcapitata*: 90th percentile of inorganic parameters (including pH and hardness).

A.3.5.b Bio-F using a full cross species extrapolation.

In this case study 29 stations at several Slovakian freshwater bodies were sampled to measure dissolved Zn concentrations. Ca, pH and DOC were documented by the Slovak Hydrometeorological institute (http://www.shmu.sk). As EU reference EQS for Zn the value of 10.9 µg/L derived by the UK authorities for the EU commission in 2010 has been used. This value is an EQS "added" (i.e. to be added to the natural background). According to the FOREGS database the natural background for Zn is 2.7 µg/L. As a first screen Zn dissolved concentrations were compared to the EU reference EQS. Only 6 stations did not exceed this threshold. 23 sampling stations were potentially at risk.
In order to take site-specific conditions into account the most conservative BioF was calculated. The bioavailability modelling was conducted using the Bio-met tool.



The bioavailable fraction of the Zn concentration measured (1 = 100 %) was calculated using the following equation:

$$BioF_{water,X} = \frac{EQS_{ref}}{EQS_x} = 10{,}9/52{,}9 \text{ ug Zn/L} = 0{,}21$$

The EQS site-specific (i.e. 52.9 µg/L) was calculated using the Biomet tool. Comparing this with the EU generic EQS (i.e. 10.9 µg/L) yields a BioF of 0,21.

If for example a local site has a measured dissolved zinc concentration of 5.2 µg/L the actual bioavailable Zn concentration can then be calculated to be 1.07 µg/L.

$$\text{Zn PEC}_{\text{bioavailable}} = \text{PEC} * \text{BioF}_{\text{water,X}} = 5{,}2 \text{ µg/L} * 0{,}21 = 1{,}07 \text{ µg/L}$$

Using the BioF value actually reduced the number of exceedances from 23 to 3 stations.

The presence of the biological component (i.e. binding to the metal binding sites within an organism) suggests that the bioavailability correction should conceptually be applied on the effects' side of the equation. However, from a practical viewpoint for regulatory purposes it could equally be applied at the exposure side. As an example the risk characterisation ratio (RCR) is calculated by applying the BioF both on the exposure side as on the effects side. In both scenarios the same RCR of 0.1 is calculated.

- BioF on effects side = Measured Zn concentration/EQS local = 5.2/52.9 (µg/L) = 0.1
- BioF on exposure side = Bioavailable Zn concentration/EU EQS Ref = 1.07/10.9 µg/L = 0.1

APPENDIX 4: CASE STUDIES ON THE IMPLEMENTATION OF BIOAVAILABALITY FOR THE SEDIMENT COMPARTMENT

A.4.1. Case study: Incorporation of bioavailability in the risk characterisation of Ni metal and chemical producer (Ni sulphate and Ni carbonate)

As a hypothetical example, a nickel metal and nickel chemical producing plant is located in Finland (site A). It is assumed that the site reported measured SEM-AVS data. This site, encoded ChP003, has been selected as an example to demonstrate the way bioavailability refinements can be incorporated in the overall sediment risk characterisation framework. For Ni a bioavailability model is available so RWC EQS value of 109 mg Ni/kg dry wt. can be calculated by normalizing the ecotoxicity data to RWC conditions reflected by the 10th percentile of the AVS concentrations present in European sediments.

Bioavailability refinement using default AVS concentrations: comparison modelled/measured data with RWC EQS normalized to a default AVS concentration.

Applying the bioavailability models for the different species for a 10th percentile AVS default concentration (0.8 µmol/g dry wt. Flemish database) yields a RWC PNEC of 109 mg/kg dry wt. With the default RWC AVS concentration, risks are still identified (Table A.4.1.)

Table A.4.1: overview exposure data and risk characterisation

Site	Clocal (mg Ni/kg dry wt)	PECregional (mg Ni/kg dry wt)	PECtotal (mg Ni/kg dry wt)	EQS normalized 10th percentile AVS	RCR
Site A	85.8	61.2	147	109	1.3

Bioavailability refinement using default AVS concentrations: comparison modelled/measured data with RWC PNEC normalized to the AVS concentration likely to occur in the region.

If AVS concentrations are available from earlier AVS measurements in the river sediments or from rivers in the region with similar characteristics an assessment can be made of the identified risks that are probable to occur. It is assumed that for the receiving Finnish river an AVS concentration of 8.0 µmol/g dry wt has been measured in the past. The RWC PNEC was subsequently normalized towards this AVS concentration yielding an EQS normalized of 225 mg/kg dry wt (Table A.4.2)

Table A.4.2: Overview exposure data and risk characterisation

Site	Clocal (mg Ni/kg dry wt)	PECregional (mg Ni/kg dry wt)	PECtotal (mg Ni/kg dry wt)	EQS normalized to historical AVS data	RCR
Site A	85.8	61.2	147	225	0.7

Using the available AVS concentration for the river it is unlikely that a risk will occur at the site (RCR < 1).

Bioavailability refinement using actual measured SEM-AVS data: site-specific approach to calculate the actual risks.

If actual SEM and AVS measurements were made upstream and downstream of the plant (Table A.4.3) a site-specific approach can be followed.

Table A.4.3: Overview exposure data and risk characterisation

Sediment (µmol/g dry wt.)	SEM Cu	SEM Pb	SEM Cd	SEM Zn	SEM Ni	Σ SEM	AVS	SEM-AVS
Downstream	0.083	0.044	0.002	0.439	1.118	1.686	6.18	< 0
Upstream	0.268	0.047	0.004	0.456	0.945	1.720	8.2	< 0

The AVS concentration measured downstream the plant, i.e. 6.2 µmol/g dry wt. is similar to the value measured in 2007 (i.e. 8.0 µmol/g dry wt.). The SEM-AVS calculation taking into account all metals present at the site is smaller than 0 predicting the absence of metal induced toxicity and hence no local risk.

A.4.2 Case study: Application of bioavailability models in the EQS derivation for nickel for freshwater sediments.

For nickel chronic sediment toxicity tests are available for 10 species of sediment dwelling organisms conducted in nickel-spiked sediments representing sediments with low and high nickel binding capacity (i.e. low AVS/Low TOC and high AVS/high TOC). In addition chronic toxicity tests were conducted with several additional nickel-spiked sediments with a wide range of AVS and TOC concentrations in order to characterize relationships for 7 test species between nickel toxicity and sediment characteristics (i.e. bioavailability regression models) (Vangheluwe & Nguyen, 2015; Besser et al., 2013).

Table A.4.4: Overview slope and intercepts of the different bioavailability models.

Species	Life strategy	Intercept	Slope
H. Azteca	Swimmer, sprawler, surface deposit feeder	2.65	0.492
S. corneum	Burrower, surface deposit feeder	2.73	0.478
G. pseudolimnaeus	Swimmer, sprawler, surface deposit feeder	2.8	0.358
Hexagenia sp.	Burrower, surface and subsurface feeder	2.35	0.175
C. riparius	Burrower, surface and subsurface feeder	2.85	0.180
T. tubifex	Burrower, subsurface feeder	3.05	0.125
E. virgo	Burrower, surface and subsurface feeder	2.21	0.218

The normalization procedure used the following equation:

$$ECX_{RWC} = ECX_{test}\left[\frac{abioticfactor_{RWC}}{abioticfactor_{test}}\right]^{slope}$$

Using the bioavailability models data were normalized towards the realistic worst case (RWC) physico-chemical conditions prevailing in EU sediments (i.e. 0.8 µmol AVS/g dry wt). The oligochaete *L. variegatus* is the only test organism without a specific bioavailability model, and hence this is the only data point in the SSD for which the bioavailability model for another species needs to be used. Because of the close similarity between tubificid/oligochaete worm behavior the *T.tubifex* model is used to normalize the *L. variegatus* data. This choice is considered precautionary as it has the smallest slope of all species tested (i.e. 0.125). An overview of the normalized species mean $L(E)C_{10}$ value for the most sensitive endpoint is provided in Table A.4.4.

Table A.4.4: Overview nickel sediment ecotoxicity database.

Taxonomic group	Species	Most sensitive endpoint	Normalized species mean (NOEC/$L(E)C_{10}$ value (mg Ni/kg dry wt.
Crustaceans	Hyalella azteca Gammarus pseudolimneaus	Biomas Biomass	203.5 348.4
Insects	Ephoron virgo Hexagenia sp. Chironomus riparius Chironomus dilutus	Biomass Biomass Development	141.1 188.7 673.5 > 762
Oligochaetes	Lumbriculus variegatus Tubifex tubifex	Abundance Biomass	529.8 1000.3
Molluscs	Sphaerium corneum Lampsilis siliquoidea	Biomass	322.1 > 762

Subsequently, a log-normal distribution was fitted through the ranked species mean toxicity data. From this SSD the median HC_5 was calculated using the ETX model. The SSD and the median HC_5 value for the normalized ecotoxicity data towards the RWC AVS conditions prevailing in the EU sediments (i.e. 0.8 µmol/g dry wt) for Ni is 109 mg Ni/kg dry wt (Figure A.4.1).

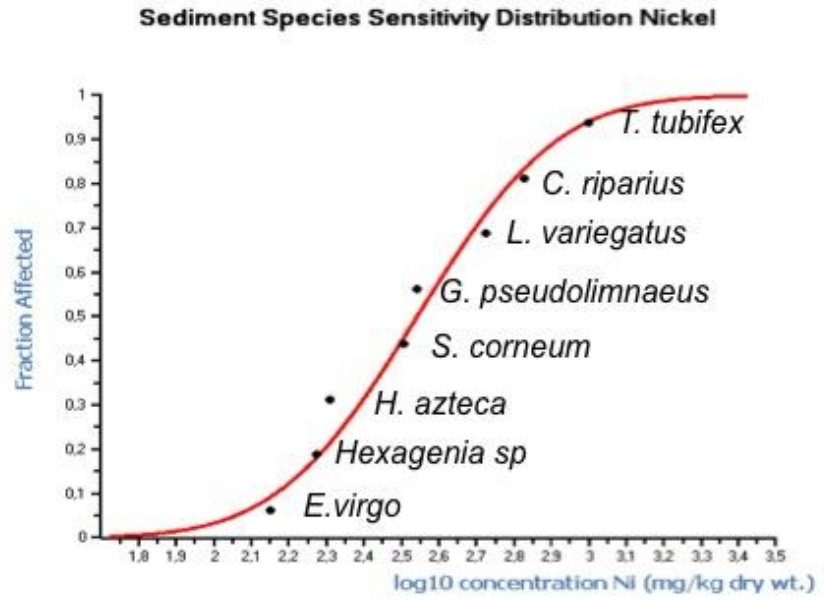


Figure A.4.1: SSD and median HC_5 derivation for Ni using normalized ecotoxicity data for a RWC sediment containing 0.8 µmol AVS/g dry wt

However, a range of AVS concentrations can be encountered in the EU (1-40 µmol/g dry wt.) resulting therefore in the setting of different EQS values for Ni. EQS values for typical eco-regions in EU sediments may vary, depending on the sediment chemistry, between 109 and 305 mg Ni/ kg dry wt). The AVS concentrations and $HC_{5\text{-}50}$/ values calculated for the different selected biogeochemical regions in EU freshwater sediments are summarized in Table A.4.5.

Table A.4.5: overview AVS concentrations and HC5-50 values for different selected biogeochemical regions.

Sediment	AVS concentration (µmol/g dry wt)	HC5-50 (mg Ni/kg dry wt)
RWC	0.8	109 (40-182)
SR	0.9	115 (43-191)
DOW	1.0	121 (46-201)
STJ	3.8	185 (75-296)
RR2	6.1	210 (85-337)
RR3	8.0	225 (91-336)
P30	12.4	249 (99-403)
STM	24.7	284 (108-470)
WB	38.4	305 (111-515)

RWC = reasonable worst case generic sediment; DOW = Dow Creek; P30 = US Geological Survey Pond 30; RR2 = Raisin River (site 2); RR3 = Raisin River (site 3); STJ = St. Joseph River; STM = south tributary Mill Creek; SR = Spring River; WB = West Bearskin Lake.

APPENDIX 5: IMPLEMENTATION OF BIOAVAILABALITY FOR THE SOIL COMPARTMENT EXPERIENCES EU-CHINA-AUSTRALIA

A.5.1. Bioavailability models derived for European regulatory frameworks

Risk assessments for metals in the framework of the European Existing Substances and REACH Regulations triggered research on the bioavailability and effects of metals and metalloids to terrestrial organisms (plants, invertebrates and microbial processes). Chronic toxicity tests were performed in a range of soils after various spiking treatments in order to develop soil bioavailability models (Table A4.1).
The toxicity tests covered the three trophic levels and major organism groups for terrestrial organisms: monocotyledonous and dicotyledonous plants, arthropods, earthworms and microbial N and C transformation processes. Soils tested were sampled from the top soils in agricultural and semi-natural areas across Europe to cover a representative range in soil properties (pH, organic carbon content, clay content and effective cation exchange capacity) and soil types in Europe. After air-drying and sieving, soils were amended in the laboratory with soluble metal salts and subjected to different toxicity tests one week after amendment. Additionally, some of the amended soils were leached with artificial rainwater or incubated outdoors with free drainage of percolating rainwater after metal salt amendment, and toxicity was compared before and after leaching or ageing. For some metals, field-contaminated soils were sampled as a gradient toward the point source, and an uncontaminated reference soil from the start of that gradient was amended to an identical total concentration to assess the combined effects of source of metal and of ageing.

Table A.5.1. Chronic toxicity data collected for the derivation of bioavailability models in Europe (number of different soils tested x number of different species and microbial processes tested).

Metal	Freshly amended soils	Spiked and leached soils	Spiked and experimentally aged (up to 18 months) soils	Field contaminated versus freshly amended control soils
Cu	19 x 7	3 x 7	3 x 7	4 x 7
Ni	16 x 7	3 x 7	3 x 7	No
Zn	15 x 6	No	No	4 x 7
Co	10 x 10	3 x 9	3 x 9	No
Pb	4 x 6	8 x 6	3 x 6	3 x 3
Mo	10 x 11	3 x 10	3 x 10	No

The effect of soil properties on metal bioavailability and toxicity in soils was tested for 6 to 11 different toxicity assays in 8 to 19 different soils covering a wide range in soil types and soil properties (Table A.5.1). All soils were sampled in Europe, except for Co, where 1 soil from Canada and 2 soils from the USA were included in the research project. Metal toxicity varied up to 2 orders of magnitude across soils. For most metals and species tested, metal toxicity (based on log EC50) was significantly related with (log transformed) soil properties (Table A.5.2 and Table A.5.3). The slopes of the selected linear regressions were used to normalise the data for the varying properties of the soils tested.

The changes in metal toxicity with leaching or ageing were typically studied for 6 to 10 endpoints in 3 to 7 different soils, while changes in metal chemistry were studied in up to 19 soils (Table A.5.1). The selection of the lab-to-field factor (or leaching-ageing factor) is based on a weight of evidence taking into account i) the changes in metal toxicity with leaching or ageing, ii) differences in toxicity between field contaminated

and corresponding laboratory-spiked control soils and iii) changes in metal availability, e.g. based on changes in pore water concentration or isotopic exchangeability in soil (Table A.5.2).

Table A.5.2. Bioavailability corrections for metal toxicity to soil organisms as incorporated in the European REACH dossiers.

Metal	Lab-to-field factor	Soil properties selected for normalisation toxicity data
Cu	2	eCEC, % organic carbon, % clay and pH*
Ni	1.0-4.0 (increasing as a function of pH)	eCEC
Zn	3	eCEC, pH and background Zn
Co	1.2-3.5 (increasing as a function of pH)	eCEC
Pb	4	eCEC
Mo	2	pH and % clay

* eCEC: effective cation exchange capacity = CEC at prevailing soil pH, pH measured in 0.01 M $CaCl_2$

A.5.2. Bioavailability models derived for China and Australia

Similar testing programmes were set up for implementation of metal bioavailability in regulatory frameworks in China and Australia (see e.g. Ma et al., 2012; NEPC, 2011). Copper and nickel toxicity to plants and microbial endpoints was tested in 17 Chinese soils, after different spiking treatments (with and without leaching) (Li et al., 2010, 2013). Likewise, copper and zinc toxicity to wheat and nitrification was tested in a range of Australian soils (Broos et al., 2007; Warne et al., 2008a and 2008b). Results from experiments in China and Australia also confirmed the strong variation in toxicity across soils and illustrate the need to implement the role of soil characteristics into the derivation of soil thresholds. Comparably as done for the European metal risk assessment dossiers, significant regressions between soil properties and metal toxicity were developed and it was proposed to use this information for normalisation of toxicity data in the derivation of ecological soil quality standards for these metals in soil (Table A.5.3). In some cases, different soil properties were identified as best related with metal toxicity to the same endpoint (Table A.5.3). These differences in the models can be due to either differences in soil types covered, potential differences in soil treatments (leaching and or equilibration), methodology (e.g. (e)CEC analysis), endpoints measured, etc. Next to models derived from toxicity data with Australian soils, also some 'European' models were used for the derivation of soil quality standards in Australia (Table A.5.3).

Apart from normalisation for variation in toxicity due to variation in soil properties, correction factors to account for the higher toxicity observed in freshly spiked soils compared to field contaminated soils were also proposed in both China and Australia. In Australia, the lab-to-field correction factors selected for Europe (Table 2) were used. In China, soil specific leaching factors were proposed based on comparative toxicity in freshly spiked and leached Chinese soils (Table A.5.4). Because there was no ageing model derived for Chinese soils, ageing models derived from European soils were used (Ma et al., 2006). In the models, an isotopic dilution technique was used to determine long-term changes in the lability of Cu added to soils leached under laboratory conditions, and also for soils incubated outdoors. The ratio in isotopically exchangeable fraction at 360 and 14 days after spiking a soil with a soluble metal salt was selected as the ageing factor (Ma et al., 2012).

Table A.5.3: Regression models selected for setting soil quality standards for copper in Europe, Australia and China

Endpoint	Geographical region	Regression equation*	Reference
Barley root elongation	Europe, Australia	Log EC50 = 1.56+0.69 log eCEC	Rooney et al., 2006
Tomato shoot yield	Europe, Australia	Log EC50 = 1.46+0.96 log eCEC	Rooney et al., 2006
Eisenia fetida (earthworm) reproduction	Europe, Australia	Log EC50 = 1.85+0.59 log eCEC	Criel et al., 2008
Folsomia candida (springtail) reproduction	Europe	Log EC50 = 1.63+0.96 log eCEC	Criel et al., 2008
Potential nitrification rate	Europe	Log EC50 = 1.41+1.07 log eCEC	Oorts et al., 2006
Substrate induced respiration	Europe	Log EC50 = 1.08+0.73 log OC+0.60 log clay	Oorts et al., 2006
Maize residue mineralisation	Europe	Log EC50 = 3.75-0.34 pH+0.74 log eCEC	Oorts et al., 2006
Wheat grain yield (field)	Australia	Log EC10 = 0.56+0.31 pH+1.05 log OC	Warne et al., 2008b
Folsomia candida (springtail) reproduction	Australia	Log EC10 = 1.499+0.8475 log eCEC	NEPC, 2011
Substrate induced nitrification	Australia	Log EC50 = 0.84+0.35 pH	Broos et al., 2007
Tomato shoot yield	China	Log EC10 = 0.635+0.092 pH+0.873 log CEC	Li et al., 2013
Bok choy shoot yield	China	Log EC10 = 1.554+0.706 log OC	Li et al., 2013
Barley root elongation	China	Log EC10 = 1.18+0.159 pH+0.597 log OC+0.702 log CEC	Li et al., 2010
Substrate induced respiration	China	Log EC10 = -2.247 + 0.565 pH + 0.283 OC	Wei, 2010
Bioluminescent bacteria	China	Log EC10 = -0.942 + 0.411 pH + 0.033 CEC	Ma et al., 2012

* eCEC: effective cation exchange capacity measured at soil pH; CEC: cation exchange capacity at pH 7; pH in European and Australian soils measured in 0.01M $CaCl_2$, pH in Chinese soils measured in H_2O; OC: % organic carbon, clay: % clay content

Table A.5.4: Leaching and ageing factors proposed for setting soil quality standards for copper in China.

Correction factor	Value
Leaching factor, pH≤7.0*	LF = 0.169pH-0.014CEC+0.012Clay+0.056
Leaching factor, pH=7~8.5	LF = 1.09pH+0.041CEC+0.003Clay-7.35
Leaching factor, pH≥8.5	LF = 6.92pH+0.264CEC-0.056Clay-60.3
Ageing factor	AF = 1.2 (pH 4.9) - 1.3 (pH 8.9) (based on difference in isotopic exchangeable fraction at 14 and 360 days after spiking)

*pH measured in H_2O

A.5.3 Case study: derivation of L/F factor for Cu

Data availability:

- Difference in Cu toxicity to plants, invertebrates and microbial processes between freshly spiked soils and corresponding experimentally aged soils (3) and field contaminated soils (4)
- Difference in Cu toxicity to plants, invertebrates and microbial processes between freshly spiked soils and corresponding leached soils (3)
- Changes in lability (isotopically exchangeable fraction) of Cu with increased equilibration time after spiking, tested in 19 European soils with contrasting soil properties and land use, spiked with $CuCl_2$ at the EC_{10} of a plant assay.

Derivation of L/F factor:

The frequency distribution of the 37 L/F factors available for Cu is shown in Figure A 5.1. The L/F factors range 0.5-30, a 10^{th} percentile of 1.5 and a median value of 2.8. These percentiles are still underestimates as many of the L/F factors are unbounded values, i.e. the true L/F factor is above the value indicated. In total 25 from the 37 ED_x ($x \geq 10$) based L/F factors are significantly larger than 1.0, i.e. toxicity is significantly lower in fields contaminated or artificially leached and aged soils compared to corresponding freshly spiked soils. None of the L/F factors smaller than 1.0 are significantly different from 1.0, i.e. the suggested trend of increased toxicity upon ageing is statistically non-significant. The overall evidence shows that Cu toxicity is almost consistently smaller in aged soils than in freshly spiked soils.

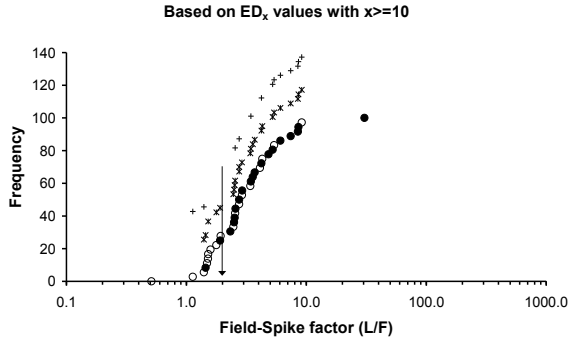


Figure A 5.1: Selection of a generic L/F factor for Cu based on the frequency distribution of all individual lab-to-field factors for Cu. Open symbols refer to L/F factors derived from bounded toxicity thresholds, closed symbols refer to lower estimates of the L/F factors as they are derived from unbounded toxicity data in the field contaminated soils. Values marked with an asterisk or cross on top are significantly different from 1.0 and 2.0, respectively. The selected L/F factor of 2.0 is indicated with the arrow.

L/F factors based on experimentally spiked and aged soils are generally smaller than those based on gradually contaminated and aged field soils. Also, more unbounded L/F factors (>x) are found in the field aged soils than in the experimentally aged soils. These differences could be explained by the shorter ageing time (up to 18 months) in the experimentally aged soils in comparison to the ageing time ranging from 8 to more than 70 years in the field contaminated soil. Further, differences in Cu availability between soils spiked once with a soluble form of Cu and soils in which Cu is added slowly over time may explain the discrepancy between laboratory and field aged data. The L/F factor will therefore be based on the field data. The experimentally aged data in the lab will be used as supporting evidence. There were no significant correlations between these factors and age of the Cu contamination, soil type or type of endpoint. This means that only a generic L/F factor can be used in the risk characterisation. A generic **L/F factor of 2.0** is proposed based on the following considerations.

- The L/F factor of 2.0 is about equal to the product of the median factor found for chemical fixation in several EU soils (factor 1.4) and the median factor for the effects of leaching on the Cu toxicity thresholds (factor 1.3). The ionic strength effect (leaching) is more important in soils with a low pH and CEC while the ageing effect is more important in high pH soils. The combination of both effects is overall similar for the soils tested.
- This factor is about the 10-15th percentile of the field contaminated soils and about the 25th percentile of all individual factors (field aged and experimentally aged). In the field contaminated soils only 1 L/F factor is significantly smaller than the proposed generic factor of 2.0. Similarly in the experimentally aged soils, only 1 L/F factor is found that is smaller than the proposed generic factor of 2.0. In other words, 5% of all generated L/F factors are significantly lower than the proposed generic factor of 2.0. However, besides the factor also the absolute concentration should be evaluated.

This generic leaching-ageing factor of 2.0 will be used on all individual $EC_{x,add}$ values of tests starting within 120 days after spiking to generate aged $EC_{x,add}$ values. For $EC_{x,add}$ values of tests in soils that have equilibrated for more than 120 days after spiking, the L/A factor is 1.0.

A.5.4 Case study: Derivation of L/F factor for Ni

Data availability:

- Difference in Ni toxicity to plants, invertebrates and microbial processes between freshly spiked soils and 3 corresponding leached and experimentally aged soils
- Changes in lability (isotopically exchangeable fraction) of Cu with increased equilibration time after spiking, tested in 16 European soils with contrasting soil properties and land use, spiked with $NiCl_2$ at the EC_{10} of a plant assay.

Derivation of L/F factor:

Clear differences in toxicity based L/F factors for Ni were observed among the 3 different soils tested Figure A 5.2). The fixation factor, calculated as the change in isotopically exchangeable fraction of Ni in soil between 1 day after spiking and 540 days equilibrated in outdoor conditions, ranged 0.7-4.0 with a median fixation factor of 1.0 and shows a clear increase with pH (Figure A.4.2). It is proposed to use the fixation factor, derived from an empirical chemical model as the L/F factor, i.e. $L/F=1+\exp^{(1.4(pH-7.0)}$ in which pH is the pH measured in $CaCl_2$ 0.01M. This equation is calibrated on soil aged maximally 1.5 year and soil pH ranged between pH 3.6 and 7.7. That empirical model predicts almost no ageing (L/F<1.2) up to pH 6 and L/F=2 at pH 7.0 and L/F=3 at pH 7.5. The L/F factor estimated from the fixation factor only accounts for the changes in the isotopically exchangeable pool, which is the fraction of the total that buffers the free metal ion activity in solution. This factor is a conservative estimate for the changes in toxicity for Ni, as shown in A.4.2. This factor will only be applied to $EC_{x,add}$ values of tests that are finished within 120 days after spiking whereas this factor is 1.0 for all tests performed on soils aged >120 days before the end of the test.

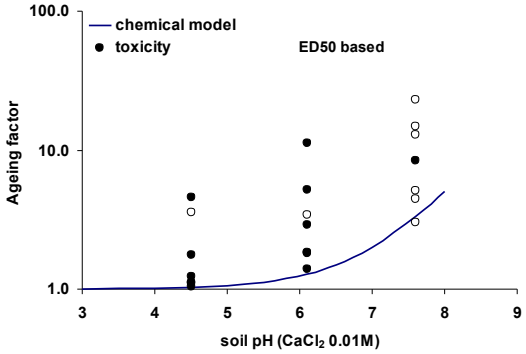


Figure A 5.2: The L/F factors for Ni based on toxicity (symbols) and the predicted factor changes in labile pool of Ni in soil (line). Open symbols are 'unbounded' values and are a lower estimate of the ageing factor. None of the ageing factors are significantly lower than those predicted by the chemical model.

A.5.5 Soil threshold calculator tools

All bioavailability correction models and the data selected for the EU REACH dossiers (Registration, Evaluation, Authorisation and Restriction of Chemicals; Regulation EC No 1907/2006) for a range of metals (Cu, Ni, Zn, Pb, Cd, Co and Mo) are compiled into a user-friendly tool, available at http://www.arche-consulting.be/metal-csa-toolbox/soil-pnec-calculator/. This spreadsheet calculates the predicted ecological risks of metals in soils, based on their Predicted No-Effect Concentrations (PNEC) to soil organisms, as derived in the EU REACH dossiers for these metals. PNEC values are calculated as the 5th percentile of the SSD divided by an additional assessment factor and are expressed as (aqua regia) total metal concentrations in soil. PNEC values are derived for different levels of refinement, depending on i) the availability of correction factors for the metal of interest and ii) the available data on soil properties for the site of interest. The input soil parameters required for calculation of site-specific PNEC values are dependent upon the metal under consideration (Table A.4.2), and are generally readily available soil parameters likely to be determined in routine soil analyses. In case no site-specific data are available, but the general soil type of the site is known, one of six standard soil types ('ecoregions') can be chosen in order to still derive a general PNEC value for this soil type. A similar interactive (Excel) calculation spreadsheet is available for calculation of Ecological Investigation Levels for metals under the National Environmental Protection (Assessment of Site Contamination) Measure in Australia (http://www.scew.gov.au/node/941).

APPENDIX 6: RELATIVE IMPORTANCE OF THE DIETARY ROUTE

A.6.1 Relative importance of dietary route for metals in the water compartment

Information on the relevance of the dietary route for metal toxicity to pelagic organisms has received increasing attention that provide a number of mainly laboratory studies utilizing systematic comparisons of toxicity of metals to an organism via water-only, diet-only, and combined water + diet exposures. The influence of dietary exposure on steady state tissue concentration has been well-established (Luoma and Rainbow, 2005). In the past studies investigating if dietary metal exposure could cause toxicity to aquatic invertebrates provided little insight (Meyer et al., 2005; De Schamphelaere et al. 2004; De Schamphelaere et al. 2007). Recently DeForest and Meyer (2014) reviewed the state of science about dietborne-metal toxicity to aquatic biota, with a focus on 13 metals: Ag, Al, As, B, Cd, Co, Cu, Cr, Mo, Ni, Pb, V, and Zn. Of those metals, Ag, As, Cd, Cu, Ni, and Zn have been demonstrated to cause dietborne toxicity to aquatic organisms in laboratory exposures at potentially environmentally relevant concentrations. That is, waterborne concentrations at or near existing waterborne criteria and guidelines (e.g., AWQC, EQSs, PNECs) sometimes result in dietborne concentrations that contribute to added toxicity to the most sensitive species (usually filter-feeding herbivores like freshwater daphnids and saltwater copepods) beyond the toxicity caused by waterborne exposure alone. However, up to now dietborne exposures have not yet shown adverse ecological impacts, i.e., increased tissue concentrations have not been linked to adverse population/community effects for the metals listed above. However, for metals such as selenium the dietary route has been identified as the primary pathway of exposure for both invertebrates as vertebrates (Chapman et al, 2009). Also for mercury dietary sources have been identified as important where it is primarily the methylated mercury forms that drive biomagnification and toxicity of this element in the foodchain (Scheuhammer et al, 2007). Organometallic compounds and organic metal salts are out of the scope of this guidance document and are covered in a separate OECD guidance document (OECD, 2015)

As BLM was developed for water exposures a dietary exposure is not intrinsically incorporated in the model. In the chronic toxicity tests are fed with algae and/or fish pellets that potentially absorb metals during the experiment. However, it is unclear to what extent the exposure duration is long enough to accumulate any significant metal quantities before being consumed and if this would really be reflected in the derived BLM parameters (stability constants) or not.

A.6.2 Relative importance of dietary route for metals in sediments

In assessing risks for the sediment compartment the dietary route could be of a relative higher importance than the aquatic compartment. Whole sediment toxicity tests are typically conducted using an array of test species with different life strategies. Several of these organisms (oligochaetes, chironomids etc.) are dependent on the ingestion and assimilation of sediment particles to survive. If those sediments have been contaminated with the metal of concern the dietary route is intrinsically included in the assessment. For those species getting additional uncontaminated food the dietary exposure could be underestimated. In relation to this it is essential to evaluate if the SEM-AVS model predicting that no toxicity should occur when excess AVS is present still holds. Studies examining the bioaccumulation of metals in anaerobic sediments showed in general that in most of the cases metal accumulation is reduced when SEM-AVS < 0 (Ankley et al, 1996). However, in some case bioaccumulation was best correlated with total metal content in the sediment irrespective of the AVS content (Lee et al, 2000, De Jonge et al, 2009, De Jonge et al, 2010). It has been found that the dietary route seems to play an important role in explaining these

observations. It should, however, kept in mind that bioaccumulation does not represent a toxicological effect and an unambiguous connection between observed levels of accumulation and effects is not frequently observed. For species with no important detoxification mechanisms (e.g. *Hyalella azteca*), however a relationship between internal body concentration and effects is well documented (Environment Canada, 2011). But in general, when detoxification systems are in place toxicity does not depend on total accumulated metal concentration but is related to a threshold concentration of internal metabolically available metal (Rainbow, 2007). Toxicity ensues when the rate of metal uptake from all sources exceeds the combined rates of detoxification and excretion of the metal concerned. Subsequently the biological significance of accumulated metal concentrations under SEM-AVS conditions < 0 will depend on the way organisms cope with the increased metal exposure. Metals extracted in the gut from the ingested metal sulfides are detoxified and stored in granules for the benthic oligochaete *Tubifex tubifex* while at an overload of the AVS system metals can be found in a more easily accessible pool (De Jonge et al, 2011). Overall the recent results support the tenet that AVS controls metal toxicity via the pore water in particular with relation to chronic effects and can therefore still be used in a risk assessment framework. Further scientific research is, however, needed to assess the relative importance of the dietary route and its consequences for risk assessment purposes.

A.6.3 Relative importance of dietary route for metals in soils

The dietary route is only relevant for terrestrial invertebrates that may take up contaminated soil or food via the oral route. Although earthworms ingest important amounts of soil, it is concluded that for metals the dermal route is the uptake route of importance (Vijver et al., 2003). Moreover, oral exposure to soils contaminated with the metal of concern is intrinsically included in the assessment. As for the sediment compartment, the dietary exposure could be underestimated for those species getting additional uncontaminated food during chronic toxicity testing and further scientific research is needed to assess the relative importance of the dietary route and its consequences for risk assessment purposes. However, the aqueous phase of the soil (soil pore water) is considered the main exposure route for most soil invertebrates (Ardestani et al., 2014). Because also plants and micro-organisms are exposed via the soil pore water, the dietary exposure route may be considered of lower importance in a risk assessment of metal toxicity to soil organisms.