FACT SHEET

05

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METALS ENVIRONMENTAL RISK ASSESSMENT GUIDANCE

MERAG

Bioavailability: water, soils and sediments

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CONTENTS

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 have the potential to 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 and soil pH are the main drivers controlling bioavailability of metals. The wide variation of the physico-chemical 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 will improve traditional 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.

The information presented in this document serves as guidance both for the national governmental institutions, industrial users and evaluating experts faced with implementing bioavailability for inorganic substances. This guidance provides the key scientific principles, tiered approaches and a step-by-step explanation that can be used to implement bioavailability for the water, sediment and soil compartments. As hazard data are a key component of setting safe ecotoxicity thresholds for metals, the guidance focuses on how bioavailability corrections can be applied for the purpose of using the normalised hazard data into a risk assessment framework. Two areas that this guidance does not cover are 1) the influences of multi-metal mixtures or multiple metals in media and 2) marine environments are not covered. Although Cu, Zn, and Ni have data on these areas, data for most other metals are scarce.

The structure of this guidance is the following: in section 2, a brief overview is given of the terminology and definitions used and the tiered approaches are introduced that can be used in implementing bioavailability. In section 3 each environmental compartment (water, sediment and soil) is outlined in detail with the specific steps needed to take bioavailability into account. For the water compartment, different approaches are presented going from measuring dissolved metal concentrations up to the use of more advanced tools such as the Biotic Ligand

Model (BLM). 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 features such as lab-to-field extrapolations are covered. Finally, specific examples on how to implement bioavailability are presented in every section.

2. Concepts and overview

2.1 Terminology and Definitions

The “bioavailability” concept encompasses several operationally defined and interacting terms (Figure 1) and the interpretation may differ depending on the context used (human health vs environmental research, type of regulation, etc). Availability starts with physico-chemical considerations (chemical availability) but should be subsequently linked to different ecological receptors taking different uptake routes into account (ECHA 2014).

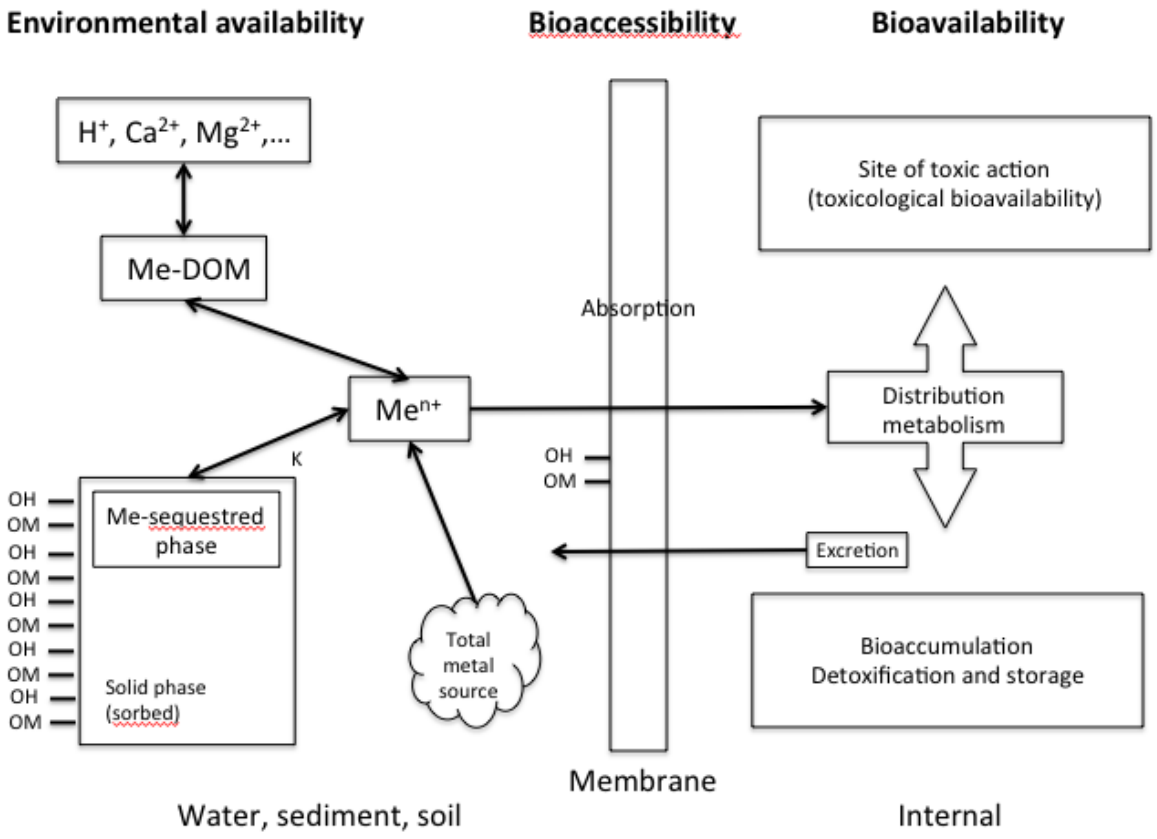


Figure 1: *Simplified conceptual outline for metals bioavailability (after M. McLaughlin personal communication). (“Me^{n+}” refers to the free metal ion.)*

The following definitions apply (to metals) in this fact sheet:

- **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).
- **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, 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.
- **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 hypothesised to be placed on the gill surface.
- **Critical biotic ligand accumulation:** is the critical concentration 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 ETV in a risk assessment framework or for compliance checking. The EEC is typically calculated from all individual measured or modelled metal concentrations for a predefined environment taking a high end value (eg the 90^{th} percentile) of the environmental concentration distribution at a site/region.
- **Environmentally available fraction: is** the portion of total metal in soil, sediment, water and air that is available for physical, chemical, and biological modifying influences (eg 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).
- **Reasonable worst case conditions (RWC):** are considered to be the environmental conditions that maximize bioavailability.

Several 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 then others (eg free ion vs dissolved concentrations). For metals, the free metal ion and 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 understanding metal availability. It is acknowledged that the free ion is not necessarily the best predictor for all metals because other metal species such as neutral species (eg AgCl, HgS) and anionic species (eg 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.

Over the last decade, significant efforts have been conducted to embed bioavailability concepts in predictive models such as the Biotic Ligand Model (BLM), SEM-AVS concept (SEM =

Simultaneously Extracted Metals; AVS = Acid Volatile Sulfides) and soil/sediment regression models[1]. Bioavailability models have become recognised and discussed within the regulatory community (US-EPA 2007; ECHA 2008; Ahlf et al 2009; Ruedel et al 2015). However, their implementation in the past has been lagging behind due to the lack of regular fit/science development and the availability of suitable and/or properly validated tools. Currently, the applicability and data needs of the different Biotic Ligand Models have been validated in some countries (UK, France) (David et al 2011) and more validated friendly-to-use models have been developed (Bio-met 2011).

Validation of the bioavailability models should both consider internal validation (ie, how well does a model predict based on the data on which the model was developed) and external validation (ie, how well can the model be used to give sound predictions of future settings). Similar OECD validation principles apply for the development and application of Quantitative Structure-activity Relationship (QSAR) models (OECD 2014a). As models get proper validation, it can be expected that these models may gain importance and be implemented more in the regulatory scene. However, even if a model has been validated, it is clear that the applicability domain of the current BLM models does not cover all relevant metal species. For example the BLM model/free ion concepts are less suited to predict effects of metal particulates (eg physical effects as clogging gills), transformation to organometallics (eg methylation), release of hydrophobic organometallic and organic metal salts (OMS) (OECD 2014b), bioavailable low molecular weight complexes (eg metals bound to natural dissolved organic matter), other inorganic metal species such as AgCl, HgS (Campbell 1995), cationic inorganic complexes (eg $Cd(OH)^{+}$) etc.

2.2 Tiered approaches for using bioavailability

Depending on the knowledge level for the metal/metal compound under investigation, bioavailability for the aquatic compartment (water and sediment) and soil can be introduced in a tiered approach as depicted in Figure 2.

[1] Regression models are empirical models that allow prediction of toxicity by using a regression equation derived from bivariate experiments.

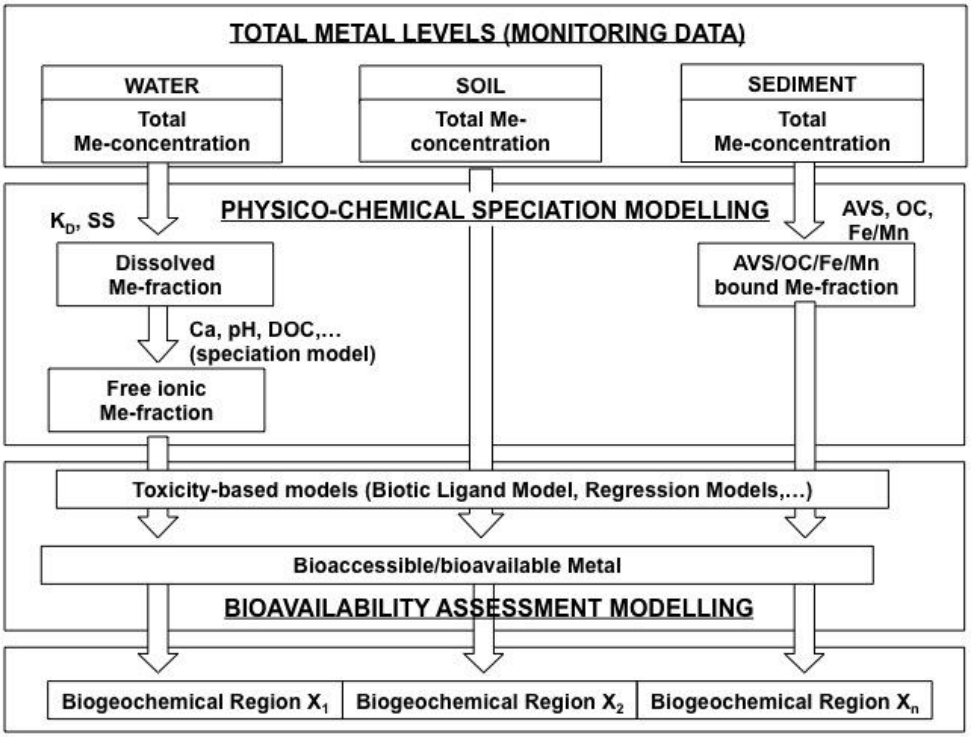


Figure 2: *Refinement levels for the incorporation of bioavailability concept for the water, sediment and soil compartment. Where SS = suspended solids; AVS = Acid Volatile Sulfides; OC = organic carbon; Fe/Mn = iron/manganese hydro-oxides.)*

A summary of the rationale for each refinement step for the different environmental compartments is given below. The sections where the methodological steps are covered in more detail are indicated between brackets.

Water compartment

Tier 1 (see section 3.2): For water, measuring dissolved metal concentrations is preferred because total metal concentrations are a poor predictor of metal toxicity to aquatic organisms. Although measuring dissolved concentrations is becoming more and more common, sometimes only total concentrations are available. In that case, if information is available on the amount of suspended solids (SS) present in the aquatic compartment, and the partitioning behaviour of the metal under scrutiny is known, then the dissolved concentration can be calculated from the total metal concentration. The dissolved concentration for soluble metals (eg, Cu, Ni, Zn) under biological relevant conditions is mainly driven by the adsorption coefficient (Kd) onto suspended solids. For less soluble metals (eg, Pb, Al, Fe, Sn), the dissolved concentration is not only

driven by the adsorption phenomena; if the solubility limit is exceeded, metal precipitation will also play an important role.

Tier 2 (section 3.3): A second refinement step consists of estimating the free ionic metal concentration that is most likely to elicit a toxic response. This can be done by using speciation programs often specifically designed for metals (eg, WHAM, VISUAL MINTEQ, PHREEQC etc), that take into account the presence of important binding ligands (eg, Dissolved Organic Carbon (DOC), chlorides etc) and the possible formation of precipitated metal forms. Because the outcome of these models can vary only validated and justified models for the metals under scrutiny should be used (eg, used in Biotic Ligand Model development). Free ion activities of some metals in solutions can also be measured, instead of estimated by speciation models.

Tier 3 (section 3.4): At this level, for several metals most frequently used in industrial applications / found in contaminated areas, bioavailability assessment models such as Biotic Ligand Models (BLM) are available which allows a semi-mechanistic understanding of metal toxicity. If local abiotic factors are known or where on a regional scale separate sub-regions are being defined using suitable methods to aggregate spatially explicit environmental variables (biogeochemical regions), the bioavailability assessment models can be used to calculate ecotoxicity values towards the conditions prevailing at the site/region.

Sediment compartment

***Tier 1* (section 4.3)** If no specific information is available to take bioavailability into account,, toxicity data expressed on a total metal concentration should be derived from experiments where test conditions reflect worst-case conditions.

Tier 2 (section 4.3): Multiple sediment properties determine the bioavailability of metals in sediments (eg, organic carbon, sulfides, iron/manganese oxy hydroxides etc). The relative importance of these properties differs depending on binding capacity of the metal 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 can be more important. In such cases normalisation towards the prevailing organic carbon/Fe content of the sediment can be considered.

Tier 3 (section 4.4): 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 has been established. If local abiotic factors (eg, AVS, total organic carbon (TOC)) are known or where on a regional scale separate sub-regions are being defined using suitable methods to aggregate spatially explicit environmental variables (biogeochemical regions), the current bioavailability assessment models (SEM-AVS, regression models) can be used to calculate the ecotoxicity threshold towards conditions prevailing at the site/region.

Soil compartment

Tier 1 (section 5.4.1): In the absence of specific information on bioavailability, toxicity data expressed on a total metal concentration should have been derived from experiments where test conditions reflect worst-case conditions.

Tier 2 (section 5.4.3): 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 (eg, 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 because 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, eg, because translocation of metals from plant root to shoot is restricted.

Tier 3 (section 5.4.3): Similar to the sediment compartment, multiple sorption phases present in the soil compartment could influence the bioavailability of metals (eg, organic carbon, Al, Fe or Mn oxides, clay minerals). The relative importance of these properties differs depending on the binding capacity of the metal and general chemical activity (eg, pH, concentration of competing ions). To correlate these soil/ properties to toxicity, for several metals (ie, Ag, Co, Cu, Pb, Mo,

Ni, Zn), toxicity based regression models are available. Empirical regression models have been derived covering a wide range of soil types and plants, invertebrates and micro-organisms. In addition, a clear discrepancy was observed between toxicity derived in laboratory-spiked soils and toxicity derived in field-contaminated soils and lab-field correction factors have been derived in order to correct for this. If local abiotic factors (eg,cation exchange capacity (CEC), pH; OC) are known or where on a regional scale separate sub-regions are being defined using suitable methods to aggregate spatially explicit environmental variables (biogeochemical regions), the available bioavailability assessment models can be used to calculate the ecotoxicity threshold towards conditions prevailing at the site/region.

2.3 Data availability for the bioavailability assessment/normalisation

In order to normalise toxicity data towards physico-chemical conditions, different datasets for abiotic factors (and environmental concentrations) should be considered depending on the goal of the assessment (ie, threshold derivation, site-specific assessment etc). More specifically, 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, ie, 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. In Tables 1 and 2, an overview is given of the relevant importance of the physico-chemical parameters for the different metal species that influence their bioavailability. For most metals, DOC, pH and hardness are key parameters in the water compartment.

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)			

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

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

* Mainly for divalent metals (Ag, Hg, Cu, Pb, Cd, Zn, Ni)

Table 2: *Overview of the most relevant parameters that influence the bioavailability of metal species in the sediment/soil compartment*

The abiotic factors 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.

Because the results of toxicity tests are also dependent on the bioavailability of the metal in the test media, especially those conducted in natural media, measurements of abiotic factors in the test medium should also be conducted. 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 Factsheet 3). 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. In that case, expert judgment should be used to decide if the study can still be used or not.

In a regulatory context, metal exposure concentrations are typically compared to one single numerical value in order to screen out potential risks (ie, 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 normalisation of

conventional effect thresholds (for example, toxicity thresholds such as EC50[2], EC10, EC20), expressed as total metal concentrations and exposure concentrations, using either soluble fractions, speciation or bioavailability algorithms. The next three sections provide brief descriptions of the tiered approaches and tools available to implement bioavailability for the different compartments.

3. IMPLEMENTATION OF BIOAVAILABILITY: WATER

3.1 General concept

A tiered approach for assessing risks is presented in MERAG Fact Sheet 1 by taking into account bioavailability in case a risk scenario was identified. If no risk is identified at a total metal level, the assessment may already stop there (assuming that the effect data were generated under realistic worst case scenarios).

The application of the bioavailability concepts to the water compartment consists in calculating the conventional estimated environmental effect threshold values (ETV, such as PNEC, EQS). Toxicity values (toxicity endpoint values such as $EC_{10,}$ EC_{20} etc) are put towards the conditions prevailing in a certain region or for a certain continent-wide percentage of surface waters using either total/dissolved fractions, speciation and bioavailability algorithms (eg, the free metal ion, Biotic Ligand Model) as visualized in Figure 3.

[2] Often the most robust value for developing bioavailability models to be used for normalisation purposes.

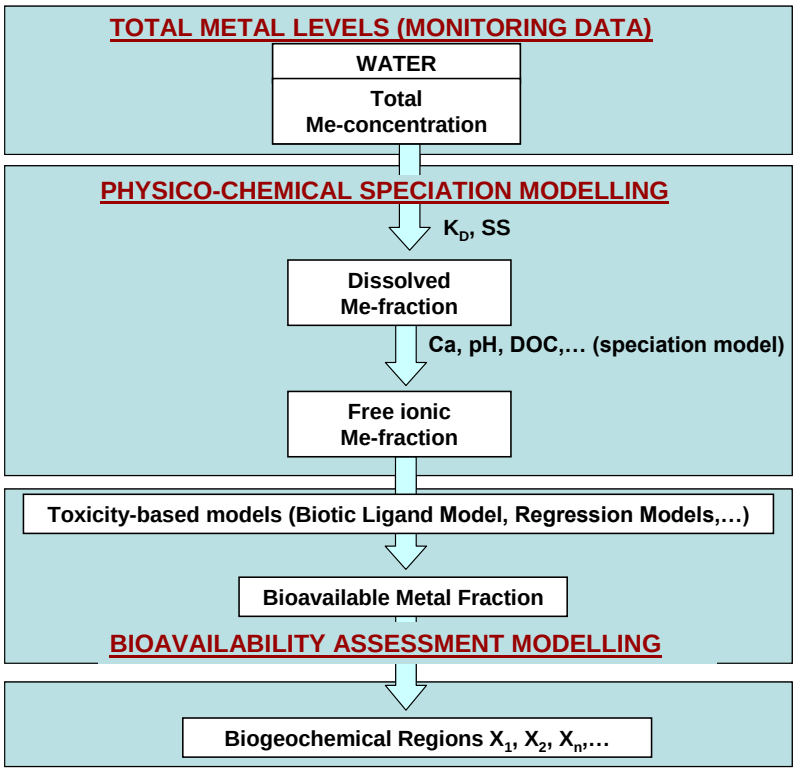


Figure 3: *Refinement levels for the incorporation of bioavailability concept for the water compartment. (Note total Me-concentration[3] is only needed if information on the "Total dissolved" fraction[4] is not available. The direct use of measured dissolved metal concentrations is preferred.)*

3.2 Use of "total dissolved" metal

A rudimentary but not preferred way of taking into account (bio)availability, is the use of dissolved concentrations. Translating a "total metal" concentration towards a "total dissolved metal" concentration takes several physico-chemical phenomena into account. The dissolved concentration of soluble metals (eg, Cu, Ni, Zn), under biologically relevant conditions, is mainly driven by the adsorption onto suspended solids. In case of less soluble metals, the solubility of the metals may be limited under specific environmentally relevant conditions (eg, Pb, Al, Fe, Sn). For these metals, the dissolved metal concentrations will not only be driven by the

[3] Total metal concentration and comprises particulate (sorbed + precipitated) + dissolved (inorganic complexes + organic complexes + free ionic forms).

[4] 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.

adsorption, If the solubility limit is exceeded, metal precipitation also plays an important role. While for some metals the formation of solid precipitates will render the metal less toxic (eg, lead), it has been observed for others that physical interaction 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 Al 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.

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 be subsequently used to make a distinction between "Total metal" and Total dissolved metal" :

- *Total metal concentration* comprises particulate (sorbed + 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.

It should be noted that the free ion is not necessarily the best predictor for the ecotoxicity of all metals because other metal species (eg, AgCl, HgS) may contribute to the observed toxicity (Campbell 1995).

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 4 and detailed in the text (steps 1-6 following the figure).

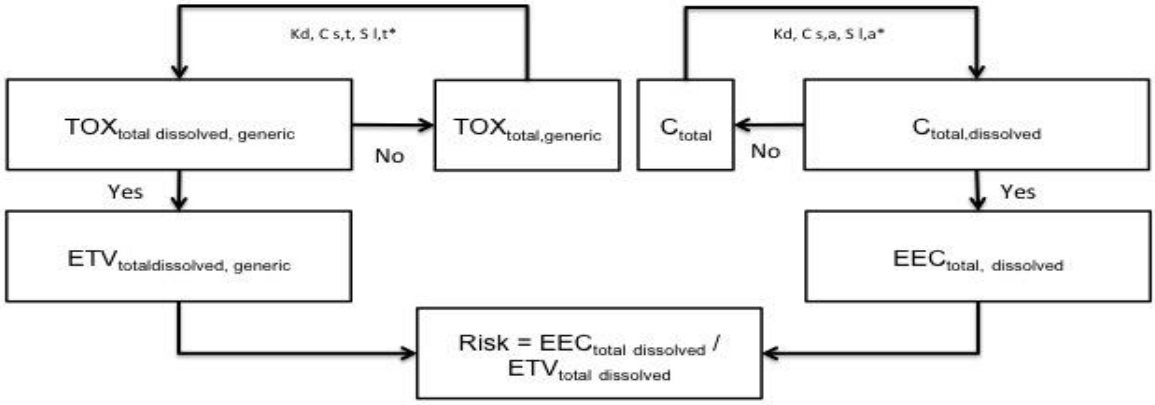


Figure 4: *Framework for assessing risks of metals/metal compounds in water (sequence applies to both the local and regional environment) on a dissolved basis. (Where Tox = ecotox value; C = environmental concentration; PEC = predicted environmental concentration; SLa = solubility limit ambient; SLt = solubility limit toxicity test; * = applies for sparingly soluble metals only.)*

1. For soluble metals (ie, 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 1:

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

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 (TOXtotal,) concentrations are extrapolated into total dissolved concentrations in toxicity tests ($TOX_{total, dissolved}$) concentrations using Equation 2. Note that aquatic toxicity

tests tend to maximise metal availability because 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 (ie, the total concentration can be set equal to the dissolved concentration[5]). If natural waters are used, total concentrations should be recalculated using partition coefficients.

$$TOX_{total,\,dissolved} = \frac{TOX_{total}}{(1 + K_d \cdot C_{s,t} \cdot 10^{-6})} \qquad \text{(Eq-2)}$$

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)

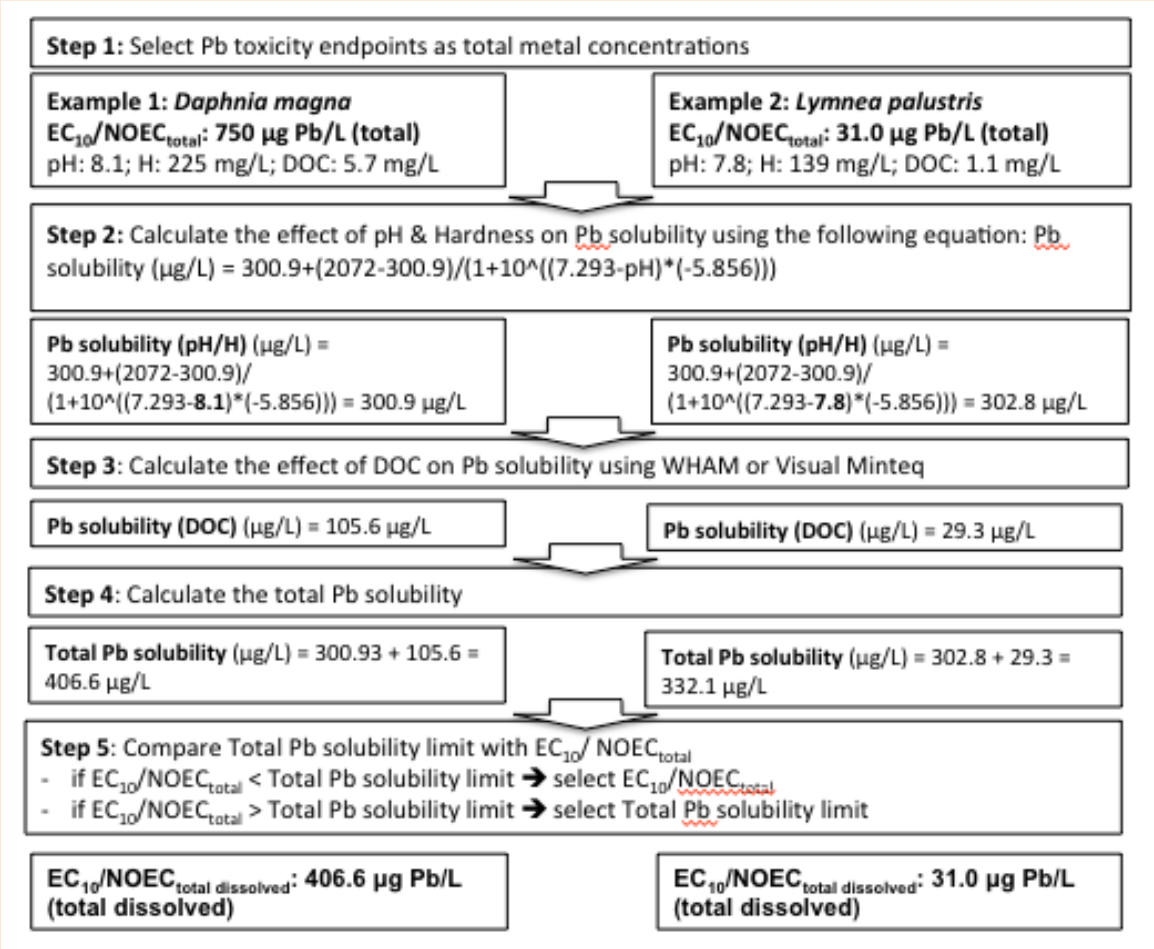
In case precipitation occurs under specific environmentally relevant conditions and/or for substances of very low solubility (<1mg/L), the toxicity data might need to be corrected by taking the solubility limits of these metals into account. These solubility limits are mainly driven by abiotic environmental factors (eg, pH, DOC) and could be estimated using specific speciation models[6] (eg, Visual MINTEQ, PHREEQC) or experimentally derived (Example 1).

[5] It must be demonstrated that the organic particles (from eg, 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.

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

Example 1: Derivation EC10 values for solubility-limited 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 (eg pH, hardness and DOC) and can be estimated using WHAM or Visual MINTEQ, Figure 1.1 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.



4. Calculate the Environmental Exposure Concentration $_{\text{total dissolved}}$ ($EEC_{totaldissolved}$) for a predefined local or regional environment (high end value eg, 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[7]. The ETV is subsequently compared to the modelled and/or measured exposure data equally expressed as dissolved concentrations (Cfr step 2).

6. The potential environmental risks (RCR) for a regional or local environment are subsequently calculated from the comparison between the local/regional $EEC_{dissolved}$ and the $ETV_{total,dissolved\ generic}$ (Equation 3).

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

In selecting an appropriate Kd value to be used in Eq-1 and Eq-2, it should be acknowledged that 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, Kd values 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 the literature (Allison and Allison 2005). (Allison ref is missing.) Preference should always be given to measured data for which synoptic 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 expert judgment taking into account the representativeness of the Kd value for the site/region of interest. The minimum and maximum values can be used for the uncertainty analysis. For data-rich metals, sufficient Kd values can be a log-normal distribution or another significant statistical distribution can be fitted through the data points. The median Kd value can subsequently be used in the exposure and effects assessment. An additional uncertainty analysis with a range of Kd values (eg, 10-90th percentiles) is recommended.

[7] 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.

3.3 Use of "dissolved metal species of concern (DMSC)"

In cases where appropriate (externally validated) speciation models (eg, 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 the basis of the dissolved metal species of concern. Most often the dissolved metal species of concern equals the free metal ion. Free ion activities can be directly measured. However, the free metal ion is not necessarily the best predictor for all metals and other metal species such as neutral species (eg, AgCl, HgS) and anionic species (eg, SeO^{2-}, AsO_4^{2-}) may contribute to the observed toxicity (Campbell 1995) and are captured in this term. Instead of 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 might 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 (eg, 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 harmonisation 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 is provided in Table 3.

Model	**More information on model**
CHEAQS Next - CHemical Equilibria in AQuatic Systems.	http://www.cheaqs.eu/

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).	
ChemEQL Determination of thermodynamic equilibrium concentrations of species in complex chemical systems. • Adsorption on upto five different particulate surfaces can be modelled • Simulations of kinetic reactions with one rate-determining process • Calcluation of two-dimensional logarithmic diagrams, (eg, 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	http://www2.epa.gov/exposure-assessment-models/minteqa2

using the Gaussian DOM, the Stockholm Humic Model, or the NICA-Donnan model • Visual MINTEQ is a Windows version of MINTEQA2 v.4.0	
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/
WHAM 7 – 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 and colleagues?). • 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

Table 3: *Overview of frequently used chemical speciation models (source: www.speciation.net)*

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 4).

Speciation Model	**Metals for which the speciation model is used**	**Rationale**
Windermere Humic Aqueous Model, Version V (WHAM 5)	Copper (Hydroqual, UGent Model) Zinc Cobalt	WHAM 5 model was originally used in the development of the first copper and silver BLM by HydroQual/HDR, and was subsequently used in the development of the BLMs for zinc and copper by Ghent University. The cobalt BLM (HydroQual/HDR) was also developed with this version of WHAM
Windermere Humic Aqueous Model, Version VI (WHAM 6)	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 eg, the binding and stability constants of metal (compounds) to organic carbon (fulvic acid and 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.

.

Table 4: *Rationale and overview speciation models used for translating total dissolved concentrations to total dissolved metal species of concern*

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 most 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.

Figure 5 gives an overview of the proposed tiered approach.

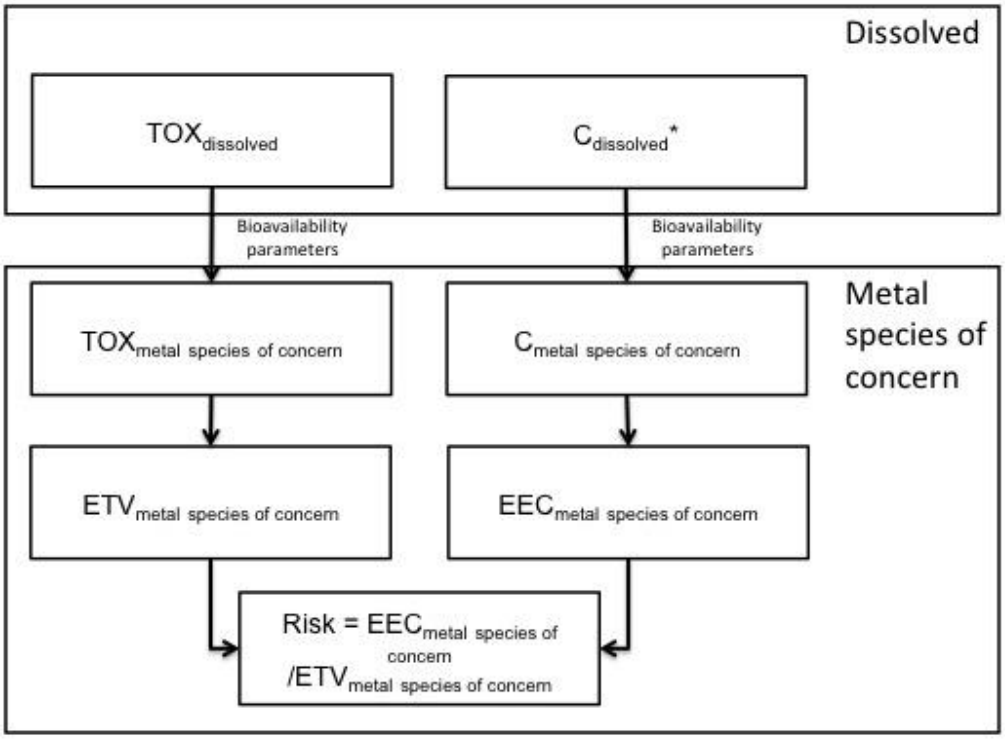


Figure 5 *Framework for assessing risks of metals/metal compounds in water on a free metal ion basis. (Where Tox = ecotox value; C = environmental concentration; * = sequence applies to both the local and regional environment.)*

7. It is recommended to recalculate the reported total dissolved TOX concentrations ($TOX_{total\ dissolved}$) into Tox concentrations expressed as the metal species of concern ($TOX_{dissolved\ metal\ species\ of\ concern}$) using the appropriate speciation models (eg, PHREEQC, WHAM, Visual MINTEQ,…) and taking into account the main physico-chemical conditions driving the bioavailability (eg, pH, DOC,…) of the individual toxicity test result (ie, 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 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.

8. It is recommended to recalculate the total dissolved exposure concentrations ($C_{total\ disssolved}$) at the same level of bioavailability (expressed in the same units) as that used to recalculate the TOX concentrations, ie, into metal species of concern exposure concentrations using the same speciation model (eg, PHREEQC, WHAM, Visual MINTEQ,…). For that purpose, the physico-chemical parameters of the generic environment or site-specific watershed driving the bioavailability (eg, pH, DOC,…) should be gathered or estimated. Reference is given to either realistic worst case (eg, 10^{th}/90^{th} percentile) or typical conditions (eg, 50^{th} percentile), depending on the regulatory setting in which these values are used.
9. From all available $Tox_{dissolved\ metal\ species\ of\ concern}$ ecotoxicity threshold data, a cautious environmental threshold value (ETV) is derived and compared to the Environmental Exposure Concentration $_{metal\ species\ of\ concern}$ derived from all individual $C_{dissolved\ metal\ species\ of\ concern}$ values for a predefined environment taking a high end value (eg, the 90^{th} percentile) of the environmental concentration distribution of the metal species of concern.
10. The risks for a local or regional environment are subsequently calculated from the comparison between the $EEC_{dissolved\ metal\ species\ of\ concern}$ and the $ETV_{dissolved\ metal\ species\ of\ concern}$ (Equation 4):

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

Example 2: Use of 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 lead, this difference can be substantial. Lead 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 (eg, 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 EU Water Framework Directive (EQS-TGD (2011). The process entailed the determination of significant relationships between DOC and chronic lead toxicity for different

freshwater species (*Cerodaphnia dubia*, *Pimephales promelas*, *Lemna minor*, *Pseudokirchienella subcapitata*, *Philodina rapida* and *Limnea stagnalis)*. The most conservative slope of the equation (ie, 1.2) was found for the rotifer *P. rapida* (Esbaugh et al 2012) and used to calculate the HC5-$50_{reference}$ based on the selection of chronic lead toxicity data with low DOC (ie, 1 mg/L on average). Subsequently the HC5-50 for a specific site can be calculated with the following equation:

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

3.4 Development and application of toxicity- related bioavailability models

3.4.1 General outline

Preferentially, the assessment should be performed on a 'bioavailable' basis. For this purpose, ambient dissolved metal concentrations and appropriate toxicity-related bioavailability models (eg, Biotic Ligand Model) could be used. 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 (ie, 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, for regulatory purposes, it could equally be applied at the exposure side using the Bio-F approach.

Figure 6 presents the stepwise procedure when using the BLMs in the assessment of metals and is described in further detail below. In comparing the environmental concentrations and the effect concentrations, care should be taken that both are expressed in the same units.

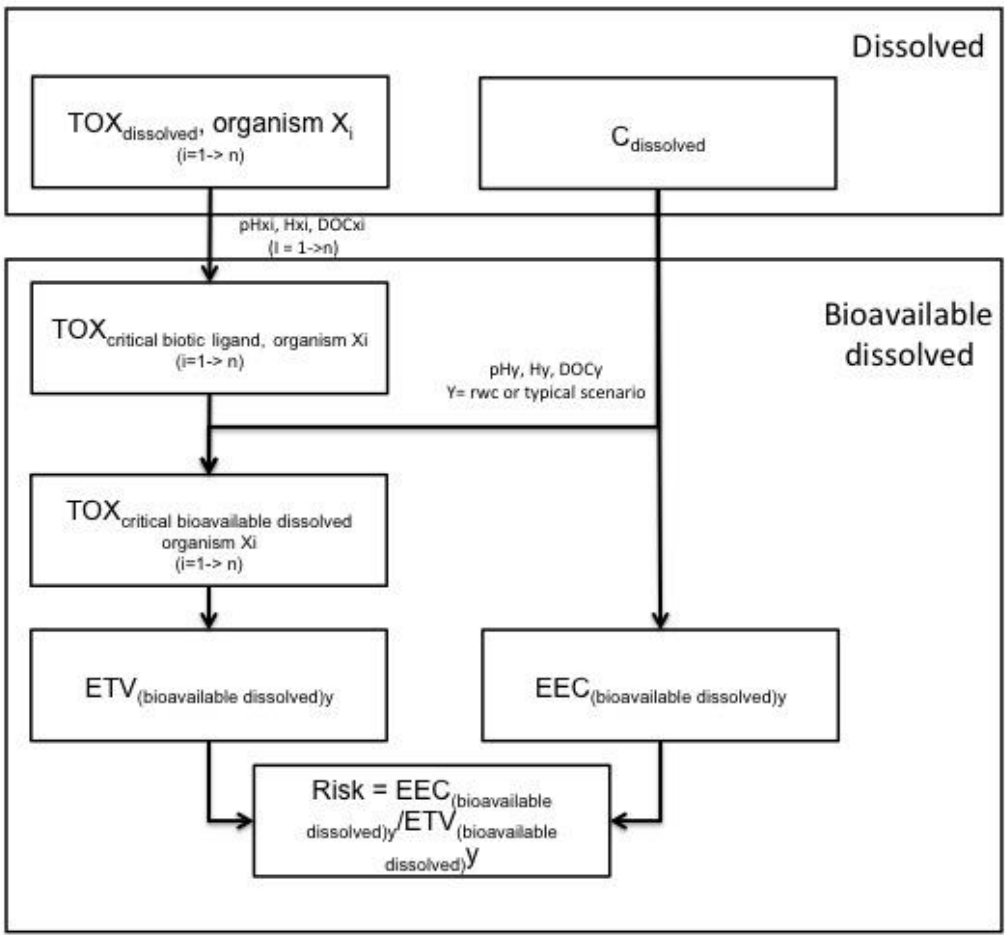


Figure 6: *Framework for incorporation of bioavailability models for the water compartment. Where X = test conditions; Y = reasonable worst-case conditions or typical conditions (depending on the scenario); H = hardness; DOC = dissolved organic carbon.*

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 3.4.3 on cross-species extrapolation 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, characterised 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. 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 taking a high end value (eg, the 90^{th} percentile) of the environmental concentration distribution of the metal species of concern.
In the context of a generic risk assessment and/or compliance checking, the BLM models can be used to convert the effects data to well-characterised specific local or regional conditions (ie, establishing $ETV_{local,\ bioavailable}$ or $ETV_{regional,\ bioavailable}$) or reasonable worst case conditions (ie, 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 (eg, aluminium). For those cases the 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. Furthermore, as BLM models are developed for a well-defined chemical (abiotic factors) and toxicological data set that define their validated application boundaries, it should be evaluated if the local water chemistry falls within the BLM application domain. If not, this does not immediately prohibit 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 (ie, 8), it could be used as a conservative estimate for toxicity. The BLM model 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.

Table 5 gives a non-exhaustive overview of several acute and chronic aquatic BLMs/screening tools that are reported in the literature. It should be noted that it is unknown if all BLMs have been properly validated (see section 3.4.2). The relevance for developing a BLM could be compartment specific (eg, a BLM for Mo was not developed for water because of its very low toxicity for the aquatic compartment, but bioavailability models were developed and deemed relevant for soil).

Metal	Available acute BLMs - main relevant publications
Most BLMs have been developed for regulatory purposes. Environmental risk assessments are predominantly driven by long-term effects (chronic toxicity), and therefore most BLM tools focussed on chronic endpoints. There are, 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 (ie,binding constants). Additional references (eg, 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; Janssen 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 al2011; 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 al2001/2002; Schwartz et al 2004; Todd et al 2009; Van Ginneken et al 1999.
Metal	**Available chronic BLMs (driving** **Reference/link**

	abiotic factor)	
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
Cd	Predominantly competition with major (hardness) cations	www.hydroqual.com/wr_blm.html
Cu	Predominantly DOC-effect, some competition with major cations	www.pnec-pro.com bio-met.net www.hydroqual.com/wr_blm.html http://www.wfduk.org/resources/rivers-lakes-metal-bioavailability-assessment-tool-m-bat
Pb	Clear effect of DOC on toxicity, but speciation complicated by precipitation with phosphate	www.leadblm.com www.hydroqual.com/wr_blm.html http://vminteq.lwr.kth.se/
Ni	Effects from both DOC and competing ions	www.pnec-pro.com bio-met.net www.hydroqual.com/wr_blm.html 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	http://www.wfduk.org/resources/rivers-lakes-metal-bioavailability-assessment-tool-m-bat

	H^+ for algae Limited effect of DOC (limited binding of Mn)	
Zn	Effects from both DOC and competing ions	www.pnec-pro.com bio-met.net www.hydroqual.com/wr_blm.html http://www.wfduk.org/resources/rivers-lakes-metal-bioavailability-assessment-tool-m-bat

Table 5: *Overview acute and chronic BLMs*

3.4.2 Validation of BLM models

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 model (Figure 7).

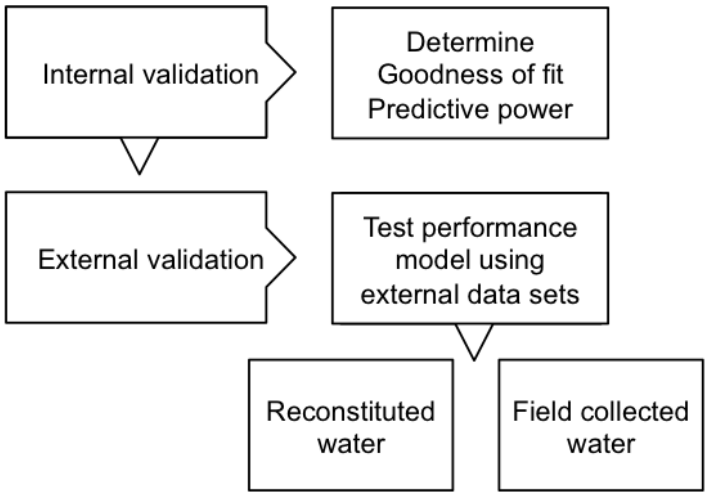


Figure 7: *Validation steps BLM.*

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 (ie, 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.
If a BLM is validated, it can be used to normalise single ecotoxicity data or the different data points of a species sensitivity distributions towards the condition prevailing in a region and/or local site (example 3).

Example 3: Application of Cu BLM normalisations towards different biogeochemical regions

The chronic BLM models 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 $[Cu]_{biotic\ ligand\ critical}$ is translated into a critical bioavailable dissolved concentration $[Cu]_{bioavailable,\ dissolved}$ for the area under investigation characterised 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 EQS 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 summarised in Table 3.1.

Table 3.1: Overview of the water chemistry and median HC5 values for the different EU eco-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, H 165 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	11.5	11.1

	mg/L		

The HC5 values for copper in common EU surface waters vary between 7.8 and 21.9 µg/L (Figure 3.1).

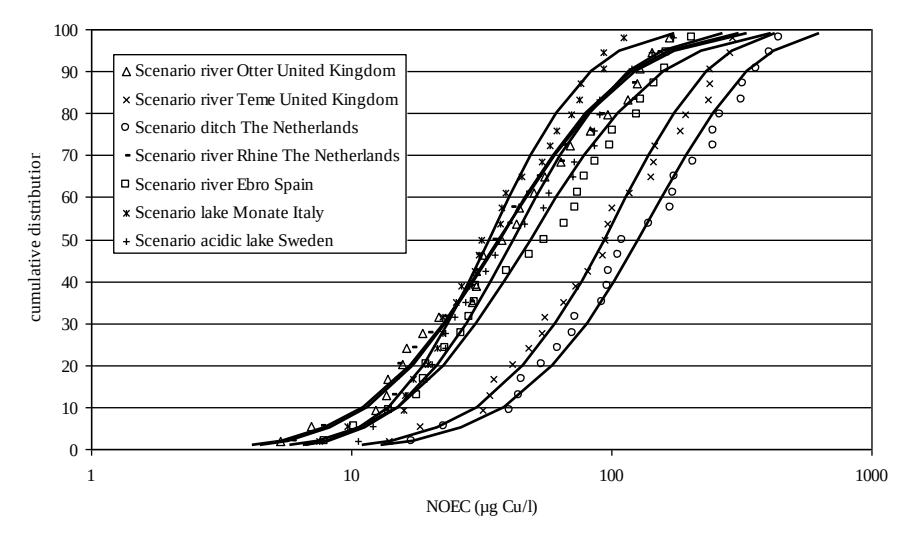


Figure 3.1: SSDs for different bioavailability scenarios

3.4.3 Cross-species extrapolation

The primary role of BLMs within chemicals management frameworks of metals is to remove the influence of test-specific abiotic conditions within ecotoxicity databases because this may introduce considerable variability and uncertainty. This could confound interspecies sensitivity comparisons, which are needed in the development and analysis of Species Sensitivity Distributions (SSDs) and, subsequently ETV determination. Validated BLMs exist only for a limited number of species to be 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 normalised to a common set of abiotic conditions (eg, biogeochemical region) as long as these abiotic parameters fall within the geochemical boundaries of the developed bioavailability model (eg, range of pHs, hardness, DOC). For those species for which no specific bioavailability model has been developed, cross-species extrapolation is recommended. The basis for a cross-species extrapolation is the assumption that the parameters which describe interactions between cations (notably Ca^{2+}, Mg^{2+}, and H^{+}) and the toxic free metal ion (eg, Ni^{2+}) are constant across organisms, and that only intrinsic sensitivity varies among species (Di Toro et al 2001; Van Sprang et al 2009). For example, one

possibility is to implicitly assume that BLMs can be extrapolated within taxonomically similar groups, ie, that BLMs developed for the rainbow trout *O.mykiss* can be applied to ecotoxicity data for other fish species, that BLMs for *D. magna/C. dubia* can be applied to ecotoxicity data for other invertebrates, and that BLMs for the green alga *P. subcapitata* can be applied to ecotoxicity data for other algae. This assumption can be supported by quantitative evidence showing that the available chronic BLMs can even predict toxicity for taxonomically dissimilar species with reasonable accuracy (Schlekat et al 2010). In any case, if cross-species extrapolation is used within dissimilar taxonomic groups, it should be verified on a case-by-case basis using spot checks if observed versus the predicted toxicity values fall within a factor of 2. It should be noted that normalisation using bioavailability models (eg, BLM) and cross-species extrapolation to other species for which no bioavailability model is available applies to any compartment where a bioavailability model is available.

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 8).

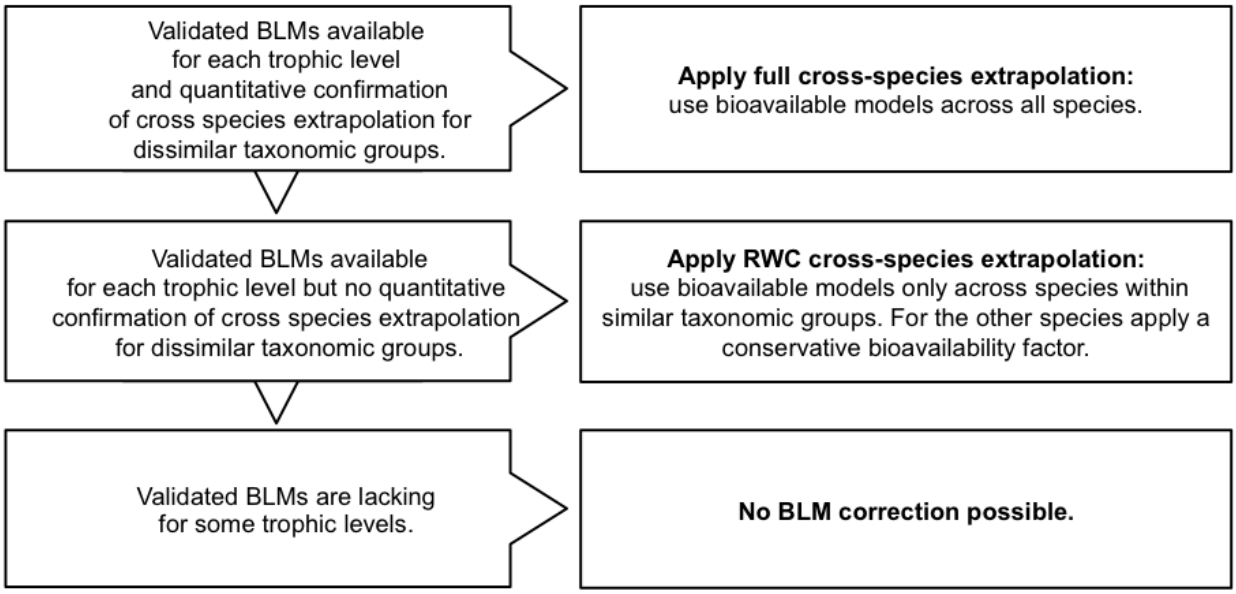


Figure 8: *Approach for cross-species extrapolation of bioavailability models.*

- In order to extrapolate across species in all typical trophic levels (algae/fish/invertebrates) a validated BLM should be available at least for each level.

- 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 BLM models can reduce the 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 significantly decreased (ie, min-max ratio is smaller after normalisation), 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 (eg, 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 (Example 4).
- In case cross-species extrapolation is only justified for some species and not for others (eg, 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 aquatic 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.

Example 4: 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 (ie, the invertebrates *Ceriodaphnia dubia* and *Daphnia*

magna, the fish Oncorhynchus *mykiss* and the alga *Pseudokirchneriella subcapitata*). Extrapolation of BLMs developed for one species (eg, the invertebrate *D. magna*) to other taxonomically similar (ie, crustaceans) or taxonomically dissimilar groups (ie, 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 were tested including the insect *Chironomus tentans*, the rotifer *Brachionus calyciflorus* and the snail *Lymnea stagnalis*. One plant species, the duckweed *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 4.1).

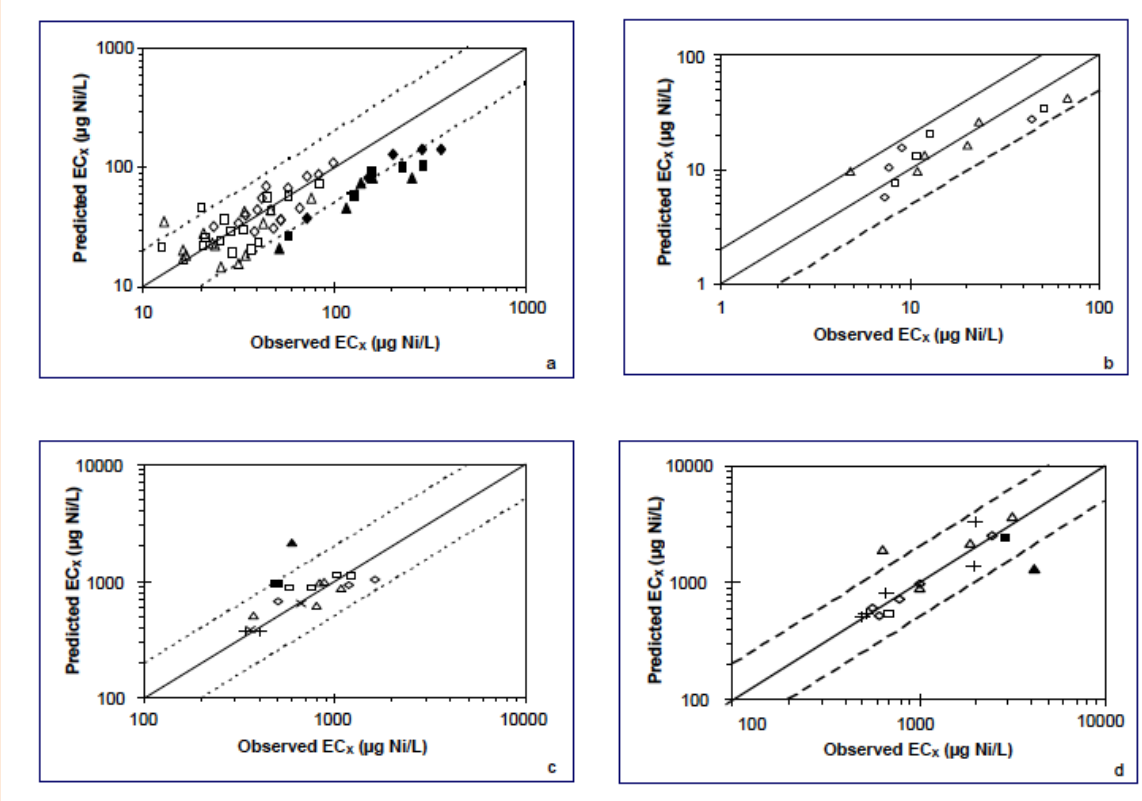


Figure 4.1: Overview of the relationship between observed and BLM predicted chronic toxicity values for the (a) *D. magna*, (b) *C. dubia*, (C) *P. subcapitata* and (d) *O. mykiss* . Note: logarithmic scales are used for the Y-and X-axis.

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 (ie, 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 standardization of Ni toxicity data.

1. The *P. subcapitata* BLM can be used to normalise the chronic toxicity to other algae,
2. The *O. mykiss* BLM can be used to normalise the chronic toxicity to fish and amphibians,
3. For cladocerans, insects and amphipods, the most stringent result of the *D. magna*, and *C. dubia* BLMs can be used, and

4. For rotifers, the *D. magna* BLM can be used and, for the invertebrates molluscs and hydra, the C. dubia (best fitting BLM) can be used.

Because it is expected that the mechanism of toxicity between short- and long-term exposures may differ, the use of acute bioavailability models to normalize chronic data should be considered with great care[8]. Such normalisation is only allowed in case the predictive capacity of these acute models for estimating chronic toxicity data is sufficient. In case of poor predictive power of the acute models towards chronic toxicity data, the acute model could only be used to normalise the acute toxicity data. The derivation of chronic effects levels could then be derived from the normalised acute toxicity data using an acute to chronic ratio.

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

[8] Both acute (eg Cu, Ni, Pb, Zn, Ag, Co etc Al according to Adams and Santore? …) and chronic (ie Zn, Cu, Ni, Pb) BLMs for metals have been developed/validated and proposed for regulatory purposes, both for environmental risk assessment exercises and for the development of site-specific water quality criteria. More details on the development, application and restrictions of using BLMs can be found in eg, Bossuyt et al (2004); Deleebeeck et al (2006, 2007a/b, 2008a/b/c); De Schamphelaere (2003); De Schamphelaere and Janssen (2002, 2004a, 2005, 2006); De Schamphelaere et al (2002, 2003a/b/c, 2004, 2005, 2006, 2014); Heijerick et al (2002a/b); Nijs et al (2013); Paquin et al (1999); Santore et al (2001); Van Assche (2008). In addition, comprehensive reviews with regard to the developed BLM models have been written by Paquin, Gorsuch et al (2002) and Niyogi and Wood (2004).

Full and RWC cross-species extrapolation

1. Predict TOX values at reasonable worst case conditions (RWC) for those bioavailability-influencing abiotic factors affecting the acute/chronic toxicity, ie, 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. The bioavailability corrections can also be applied at the exposure side. In that case a bioavailability factor (Bio-F) is calculated on the ETV level (Equation-5).

$$Bio\text{-}F_{reference} = \frac{ETV_{bioavailable,reference}}{ETV_{dissolved,generic}} \qquad \text{(Eq-5)}$$

2. For those species for which the trophic level specific bioavailability model could not be justified using spot checks, a 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-6). The most conservative value (smallest correction for bioavailability, Bio-$F_{reference}$) should then be used [9]. (See Example 5).

$$Bio\text{-}F_{reference} = \frac{TOX_{bioavailable,reference}}{T0X_{dissolved,generic}} \qquad \text{(Eq-6)}$$

3. When more data are available for the same species, calculate the species geometric mean value.
4. Derive a cautious environmental threshold value ($ETV_{bioavailable,\ reference}$) using assessment factor approaches (data-poor metals) or statistical extrapolation methods from all normalised $TOX_{bioavailable,\ reference}$ values.
5. This value is subsequently compared to the modelled and/or measured environmental exposure concentration (EEC) expressed as dissolved concentrations using the relevant environmental concentrations. (See Equation 7.)

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

In case the added risk approach[10] is used 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

[9] Under worst case conditions, 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.

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

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, because 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 8):

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

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

Example 5a: 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 where 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, daphnids 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.

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

Table 5.1: *Summary of reference NOEC values ($NOEC_{ref}$) for the three aquatic species for which BLMs have been developed under reference water chemistry conditions[11].*

[11] 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

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 algae, daphnid and fish. Furthermore, the BLM models provide sufficiently conservative outcomes that are in good accordance with the generic PNEC. Moreover, the BLM estimates generally overestimate toxicity, ie, the predicted NOEC values are lower than the experimental values, which provides further support that the BLMs result in sufficiently conservative outcomes. Therefore, the BLMs are regarded as being sufficiently validated and sufficiently conservative, thus protective of aquatic organisms.

The bioavailability factors (BioF) are then derived for each of the three (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, ie, the smallest correction for bioavailability.

For zinc, spot checks have been conducted and full cross-species normalisation 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 5b).

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

Example 5b: 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" (ie, 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. Therefore 23 sampling stations were potentially at risk.

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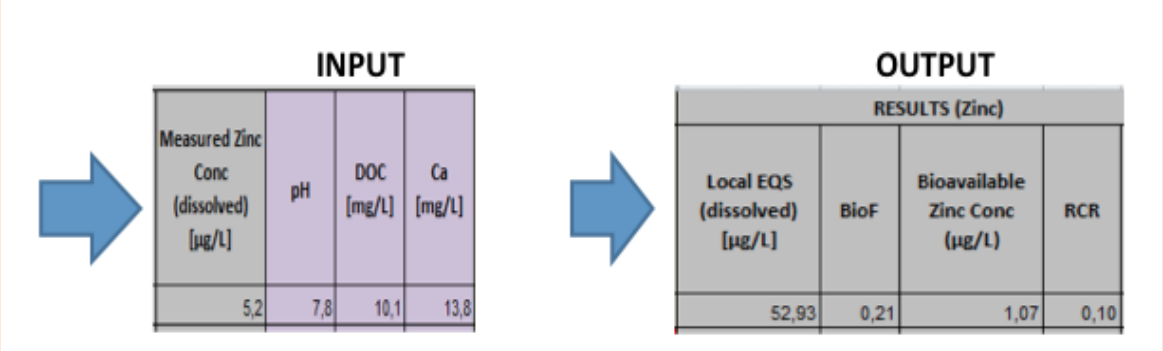
percentile of DOC. For *D. magna and O. mykiss*: 10th percentile of inorganic parameters (including pH and hardness). For *P. subcapitata*: 90th percentile of inorganic parameters (including pH and hardness).

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 (ie, 52.9 µg/L) was calculated using the Biomet tool. Comparing this with the EU generic EQS (ie, 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 (ie, 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 on the exposure side. As an example, the risk characterisation ratio (RCR) is calculated by applying the Bio-F both on the exposure side as on the effects side. In both scenarios the same RCR of 0.1 is calculated.

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

3.4.4 Cross-region extrapolation

If the BLMs need to be applied in other regions than those for which the BLM was originally developed (ie, 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 BLM models 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 species relevant for the region (temperate species vs tropical species). Recently, alongside the experience in European freshwater bodies (EU Regional Risk Assessments (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 (eg 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, 2014b; Chen et al 2014). 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.

3.5 Importance of dietary route of toxicity of metals

Information on the relevance of the dietary route for metal toxicity to pelagic organisms has received increasing attention that provide a number of studies utilising 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, 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 (eg, AWQC, EQS values, PNEC values) 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, ie, increased tissue concentrations have not been linked to adverse population/community effects. Although dietary exposure is not intrinsically incorporated in the BLM, the organisms in chronic toxicity tests are fed with algae that will absorb metals during the experiment, and hence the dietary component is covered to some extent and as such reflected in the derived-BLM parameters (stability constants).

4. IMPLEMENTATION OF BIOAVAILABILITY: SEDIMENT

4.1 General concept

Metal bioavailability in sediments is governed by different processes than in water (diffusion, re-mobilsation, burrowing, oxidation/reduction processes, etc), and in contradiction with nonionic hydrophobic substances, multiple sorption phases must be considered for metals (eg organic carbon, sulfides, iron and manganese oxy hydroxides). The relative importance of these binding phases differ depending on the binding capacity of the metal and general chemical activity. As for the water compartment, a specific tiered bioavailability normalisation approach is recommended for the sediment compartment (Figure 9).

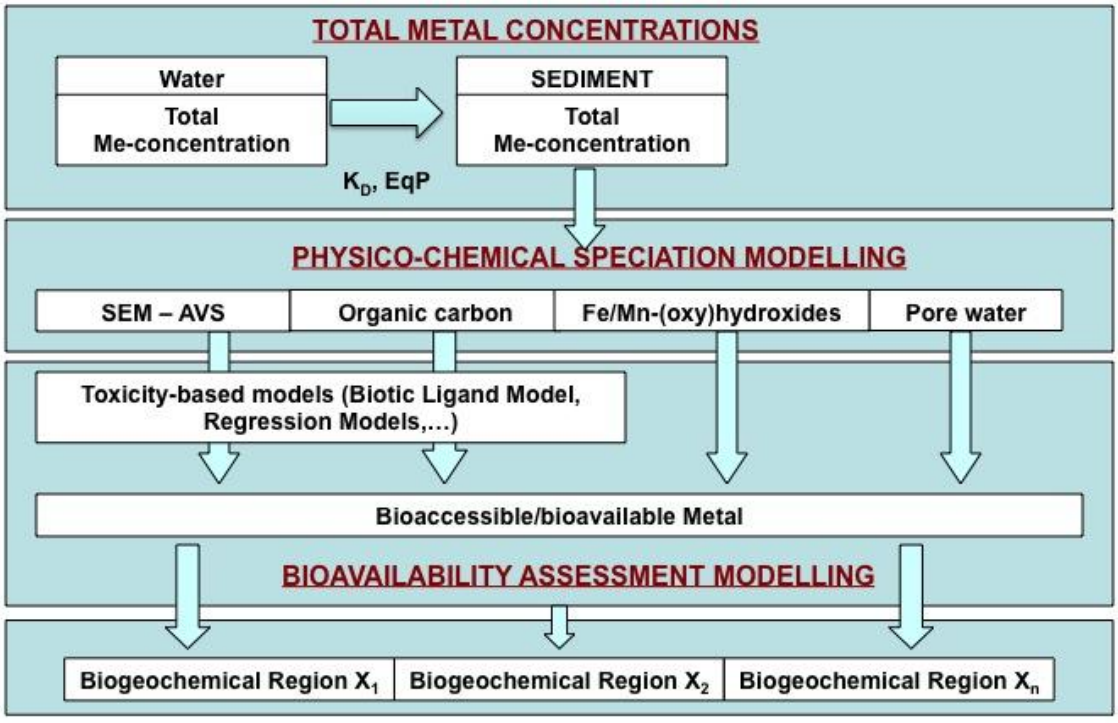


Figure 9 *Refinement levels for the incorporation of bioavailability concept for the sediment.*

In the absence of reliable whole sediment toxicity data (eg, data- poor metal substances), the equilibrium-partitioning approach (EP) is sometimes used as a lower-tier screening approach to generate surrogate whole sediment toxicity data. However, depending on the partitioning coefficient used, these data have the same intrinsic flaw as non-normalised whole sediment data that in most cases it is not clear if the data represent a realistic worst case with regard to

bioavailability of the metal under scrutiny. As a result, any EQS derived from sediment toxicity data solely expressed on a total metal concentration (dry or wet weight basis) has the potential to under or overestimate the risk. Therefore, procedures based on total concentrations have to be improved by taking into account those factors that governs bioavailability in sediments. Physico-chemical speciation and (bio)availability is taken into account, eg by estimating the amount of metal bound to organic carbon, Fe/Mn (oxy)hydroxides, and/or sulfides. The latter, Acid Volatile Sulfides (AVS), is a key partitioning phase controlling cationic metal activity and metal-induced toxicity in the sediment interstitial water system (also known as pore water) is (US EPA 2005). In addition, partitioning to Fe-Mn (oxy)hydroxides, speciation calculations (reduced forms under anoxic conditions – that is lacking oxygen) and interactions with dissolved and particulate organic carbon play a role. In case specific bioavailability models are available, attempts can be made to normalise the whole effect database towards the specific bioavailability conditions prevailing at a site.

4.2 Equilibrium partitioning approach (EqP)

The possible uncertainties introduced by the use of the EqP approach for metals need to be carefully weighed against the potential benefits of using the data (eg weight of evidence (WoF), screening method for data-poor metal substances). The EqP approach was originally developed for non-ionic organic compounds where it was shown that in absence of real sediment toxicity data the method could be used to predict sediment toxicity data from water only exposures taking into account the partitioning behavior of the compound and assuming that the pore water is the primary route of exposure. Because sediment toxicity data for some metals are also still lacking, this EP approach has already been applied to metals. However, validation studies of this concept to metals are scarce. Van Beelen et al (2003) studied the validity of the EP method for both organic compounds as metals to predict toxicity values for the soil compartment. The results showed that the EqP method can give significant over-or underestimations, due to inaccurate and widely variable partitioning coefficients or differences in species sensitivities (aquatic versus terrestrial species). The $HC_{5\text{-}50}$ values derived using the EP method were in 5% of the cases more than 20 times higher than the corresponding $HC_{5\text{-}50}$ values that were derived directly from soil toxicity tests (Van Beelen et al 2003). The Dutch Health Council stated that the EqP method was only suited for organic, apolar and not for very hydrophobic substances and not for metals (Gezondheidsraad 1995) because the variation in different partition coefficients for a single metal is large, introducing quite some uncertainty to the system. Because of this wide variability, the use of Kp values for the purpose of equilibrium partitioning was not

recommended for the derivation of ecotoxicological risk limits for metals in sediment (Verbruggen et al 2001). It is also very important to note that pore water geochemistry will vary substantially from that of the overlying water, eg, higher DOC, different pH, higher hardness etc. Overall, the results of whole sediments tests should be preferred because both the dietary and aqueous routes of exposure pathways are covered in these experimental designs. However, if the EqP approach is used in a screening approach, care should be taken to use relevant Kd values that reflect the bioavailability conditions occurring at the site. Because the EP approach is mainly focused on estimating exposure via pore water, it could be more worthwhile to look at pore water concentrations using peepers to collect these interstitial waters.

4.3 Physico-chemical speciation modeling

Similar to the water compartment, procedures based on total concentrations have to be improved by taking into account those factors that drive bioavailability of metals in sediments. Sulfides are a key partitioning phase controlling cationic metal activity (Hg, Ag, Cu, Pb, Cd, Zn, Ni) and metal-induced toxicity in the sediment interstitial water system (US EPA 2005). Additionally, organic carbon plays an important role in copper bioavailability and binding to Fe/Mn (oxy)hydroxides has been proven to be important for nickel (Costello et al 2011). 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 its less toxic trivalent form (Berry et al 2004).

4.3.1 Sulfides binding

Acid Volatile Sulfides (AVS) has been demonstrated to be one of the most important factors controlling metal toxicity in anoxic and suboxic sediments (Di Toro et al 1990, 1992; US EPA 2005). AVS is, however, in the first place an operationally 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 mean 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, then 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 metals 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).

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

- 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 will 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, that is not a good predictor of the start of toxicity. Toxicity will depend on the magnitude of metal exceedance of the sulfide 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 4.6).
- The SEM-AVS concept works mainly for metals occurring as cations (eg, Cu^{2+}, Zn^{2+}, Pb^{2+}, …). Anionic forms such a oxyanions (eg, 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 to bind other metals (Costello et al 2011).

4.3.2 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, 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-normalised 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 (ie, 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 normalising each Tox value using the following formula:

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

TOX_{total} (mg Me/kgdw); dw = dry weight
fOC = fraction organic carbon
$TOX_{OC,\,normalised}$ (mg/g OC)

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.

4.3.3 Use of metal concentrations measured in pore water

When dealing with bioaccessibility/bioavailability in sediments, it is suggested that the best metric for the moment is the freely dissolved pore water concentration, both for metals and organic chemicals (ECHA 2013). This is particularly true for selected cationic metals. Of course, if it can be expected that the dietary route could contribute significantly to the exposure (ie, conditions in the digestive track differ markedly from those in the sediment, then the assessment should not only focus on the pore water but should also take sediment ingestion into account (section 3.6).

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Dissolved metal pore water concentrations could be measured in pore water collected by different passive sampling methods (Peijnenburg et al 2014). Despite significant advances in the development and application of these devices, practical incorporation of these methods in contaminated sediment management decisions has been limited. Pore water concentrations are not often analysed in ecotoxicity tests and for many metals accurate pore water measurements can be difficult because the separation of pore water from sediments often results in experimental artifacts. To ensure consistency in the approaches to assess the bioavailable concentration in sediment pore water and overlying water, additional research is recommended before pore water normalisation can be routinely used.

4.4 Use of toxicity-related bioavailability models

Although the SEM-AVS approach has been proven to be quite accurate by demonstrating the absence of sediment-associated toxicity for metals, it is less useful in predicting the occurrence of toxic effects. Mechanistically based bioavailability models (sediment BLMs) or empirically based regression models predicting the metal toxicity in spiked sediments based on sediment properties (eg, AVS, TOC, Fe/Mn) are available or may be developed for establishment of environmental threshold values. The models can be used to normalise the available toxicity data set to specific bioavailability conditions (see tier 2 section 4.5). Further information on the

derivation of regression models in general is included in section 5.4.3 dealing with the soil compartment.
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 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.

4.5 Tiered implementation in a risk assessment context

The application of physico-chemical speciation modelling and/or the use of toxicity based bioavailability models are dependent on the knowledge and availability of information on relevant abiotic factors, and/or established relationships between toxicity and physico-chemistry of the sediments. Figure 10 represents the tiered approach that can be used to refine the risk assessment using the knowledge about sediment abiotic factors that drive toxicity (eg AVS or OC).

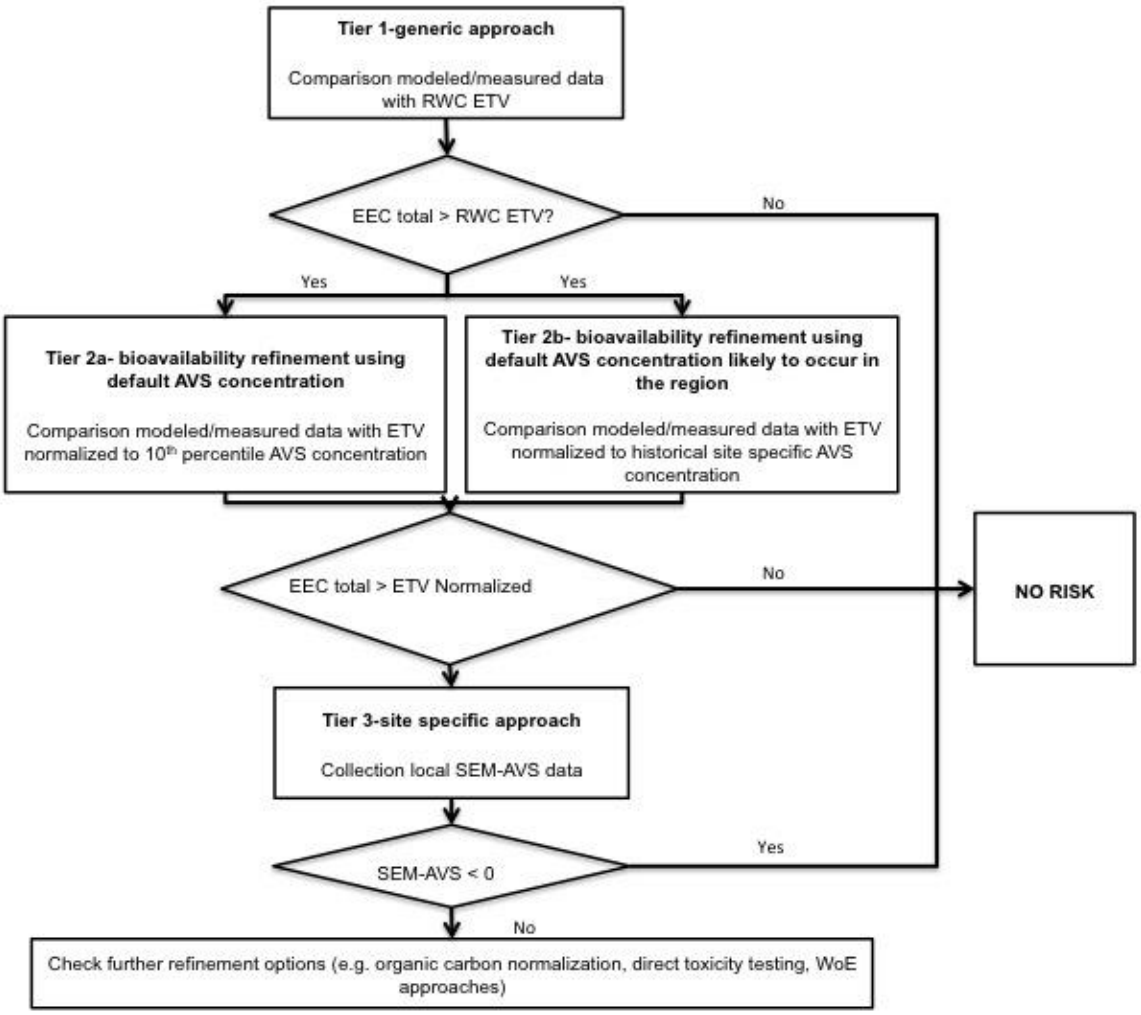

Figure 10 *Framework for assessing risks of metals/metal compounds in sediments using the SEM-AVS approach*

The scheme presented in Figure 10 gives the user the ability to perform a robust analysis of the potential risks depending on the data availability. In the highest tier (Tier 3) actual SEM-AVS analyses are conducted and a site-specific approach can directly be conducted for those divalent metals known to bind to AVS. However, in the absence of these data, a more generic approach using a generic reasonable worst-case environmental threshold value expressed as total metal concentration could be used (Tier 1) or in case adequate bioavailability models are available, these could be used to derive the ETV (Tier 2).

In the generic approach (Tier 1) an ETV is chosen to reflect a scenario which maximises 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. If a potential risk is identified in Tier 1 and an adequate regression model (eg AVS model for Ni, or OC for copper) is available, the ETV_{RWC} can be recalculated toward a reference situation such as 10^{th} percentile of the regional AVS/OC distribution (Tier 2a) in case no actual or historical AVS/OC data are available or ETV can be normalised towards the measured AVS/OC concentrations occurring at the site (Tier 2b). If no AVS-based bioavailability model is available, the only refinement step is to conduct paired measurements of AVS and SEM for the particular metal. In that case, the SEM-AVS difference is directly calculated. If SEM-AVS is < 0, no further action is required. If SEM -AVS is > 0, other lines of evidence can be explored (eg direct toxicity testing, macroinvertebrate analysis, etc). The different tiers are further elaborated in the subsequent sections.

TIER 1: comparison modelled/measured data with a reasonable worst-case (RWC) ETV

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 (eg, 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 (ie, high AVS/ OC/Fe/Mn content) should be excluded.
2) Calculate the ETV_{rwc} from all toxicity $_{(low\ AVS,\ low\ TOC)}$ values. The EQS_{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 (eg, the 90^{th} 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, ie 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-12.

$$EEC_{AVS\ normalised} = EEC_{total} - \triangle AVS_{default} \qquad \text{(Eq-12)}$$

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

$\triangle$ {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-13). Both parameters are expressed as mg total Me/kg dry wt.:

$$RCR = \frac{EEC_{totalmetal}}{ETV_{rwc}} \qquad \text{(Eq-13)}$$

TIER *2: Normalisation using toxicity-based models*

1) Link the NOEC/EC_X values of the chronic ecotoxicity database (as total metal concentrations) with the relevant sediment parameters of the sediment (eg 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 normalise the NOEC/ECX values to “reasonable worst case” sediment properties (eg 10th percentile AVS) or to specific local/regional conditions (actual or historical AVS concentrations prevailing on the site under investigation). The normalisation equations for RWC-and site-specific conditions is given here below (Equation-14 and Equation-15) are:

$$ECX_{RWC} = ECX_{test} \left[\frac{abioticfactor_{RWC}}{abioticfactor_{test}} \right]^{slope} \qquad \text{(Eq-14)}$$

Where test = scenario with typical local or regional conditions for which the $Tox_{,test}$ is derived, and 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 maximised bioavailability.

$$ECX_{Site\text{-}specific} = ECX_{test} \left[\frac{abioticfactor_{Site\text{-}specific}}{abioticfactor_{test}} \right]^{slope} \quad \text{(Eq-15)}$$

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

3) Derive a ETV_{rwc} or a $ETV_{site\text{-}specific}$ using an SSD or AF approach.
4) 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-16):

$$RCR = \frac{EEC_{totalmetal}}{ETV_{rwc\ /\ sitespecific}} \quad \text{(Eq-16)}$$

In case organic carbon has been identified as the major driver (eg copper), the ETV sediment 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 $ETV_{normalised,\ OC\ (5\%)}$ taking into account site-specific information on the OC content (Equation-17)

$$RCR = \frac{EEC}{ETVnormalized_{,OCregion} \cdot \frac{fOC_{site}}{fOC_{region}}} \quad \text{(Eq-17)}$$

TIER 3: Application of the SEM-AVS concept if actual measured regional/local SEM-AVS concentrations are available

The SEM-AVS concept 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, 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 (ie 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 than deeper sediments. It is important to focus the analysis on the biologically 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 $\triangle AVS$ and SEM_{Me} data for that specific station.

$$EEC_{AVS\ normalised} = SEM_{Me,\ sampling\ point\ 1} - \triangle AVS_{sampling\ point\ 1} \qquad \text{(Eq-18)}$$

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

$$SEM_{Me,\ sampling\ point\ 1} - \triangle AVS_{sampling\ point\ 1} < 0 \text{ (no risk scenario)} \qquad \text{(Eq-19)}$$

$$SEM_{Me,\ sampling\ point\ 1} - \triangle AVS_{sampling\ point\ 1} > 0 \text{ (potential risk scenario)} \qquad \text{(Eq-20)}$$

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

Example 6: 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 normalising the ecotoxicity data to RWC conditions reflected by the 10th percentile of the AVS concentrations present in European sediments.

TIER 2a: Bioavailability refinement using default AVS concentrations: comparison modelled/measured data with RWC EQS normalised 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 6.1.).

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

***Table 6.1**: Overview exposure data and risk characterisation-tier 2a*

TIER 2b: Bioavailability refinement using default AVS concentrations: comparison modelled/measured data with RWC PNEC normalised 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 normalised towards this AVS concentration yielding a EQS normalised of 225 mg/kg dry wt (Table 6.2).

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

***Table 6.2:** Overview exposure data and risk characterisation- Tier 2b*

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

TIER 3: 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 6.3), a site-specific approach can be followed.

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

Table 6.3: *Overview exposure data and risk characterisation- Tier 3*

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

Example 7: 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 (ie 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 characterise relationships for 7 test species between nickel toxicity and sediment characteristics (ie bioavailability regression models) (Table 7.1) (Vangheluwe and Nguyen 2015; Besser et al 2013).

Table 7.1: Overview slope and intercepts of the different bioavailability models.

Species	Life strategy	Intercept (S.E)	Slope (S.E.)
H. azteca	Swimmer, sprawler, surface deposit feeder	2.65 (0.11)	0.492 (0.11)
S. corneum	Burrower, surface deposit feeder	2.73 (0.01)	0.478 (0.011)

G. pseudolimnaeus	Swimmer, sprawler, surface deposit feeder	2.8 (0.13)	0.358 (0.13)
Hexagenia sp.	Burrower, surface and subsurface feeder	2.35 (0.06)	0.175 (0.07)
C. riparius	Burrower, surface and subsurface feeder	2.85 (0.017)	0.180 (0.017)
T. tubifex	Burrower, subsurface feeder	3.05 (0.006)	0.125 (0.006)

The normalisation procedure used the following equation:

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

Using the bioavailability models, data were normalised towards the realistic worst case (RWC) physico-chemical conditions prevailing in EU sediments (ie 0.8 µmol AVS/g dry wt). An overview of the normalised species mean L(E)C_{10} value for the most sensitive endpoint is provided in Table 7.2.

Table 7.2: Overview nickel sediment ecotoxicity database.

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

EU sediments (ie 0.8 µmol/g dry wt) for Ni is 109 mg. Ni/kg dry wt (Figure 7.1).

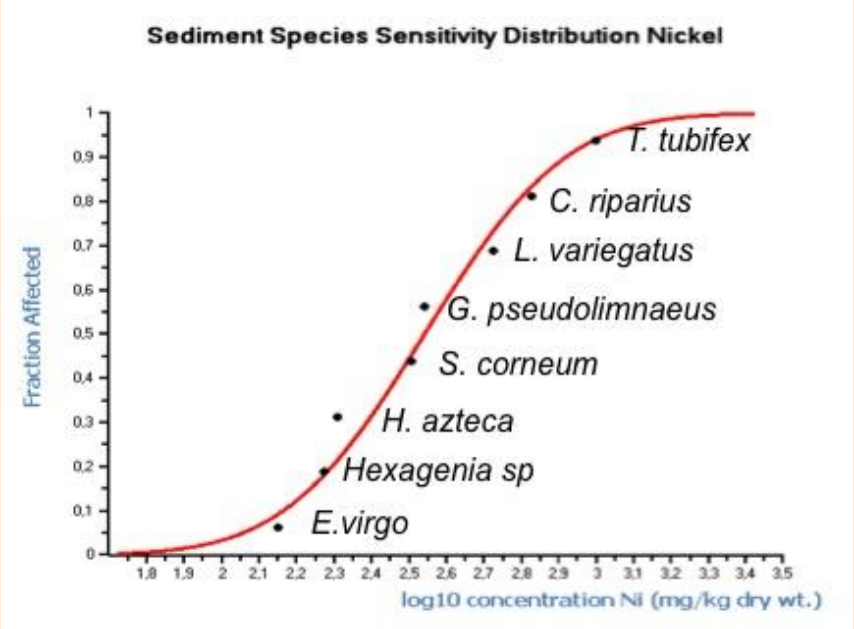

Figure 7.1: *SSD and median HC_5 derivation for Ni using normalised ecotoxicity data for a RWC sediment containing 0.8 µmol AVS/g dry wt.*

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 summarised in Table 7.3.

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)

Table 7.3: *Overview AVS concentrations and HC5-50 values for different selected biogeochemical regions. Where 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.

4.6 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 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. Further scientific research is, however, needed to assess the relative importance of the dietary route and its consequences for risk assessment purposes. In relation to this, it is essential to evaluate if the SEM-AVS model still holds for predicting that no toxicity should occur when excess AVS is present. 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 cases, bioaccumulation was best correlated with total metal content in the sediment irrespective of the AVS content (Lee at al 2000; De Jonge et al 2009, 2010). It has been found that the dietary route seems to play an important role in explaining these observations. It should, however, be 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 (eg *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, 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.

5. IMPLEMENTATION OF BIOAVAILABILITY: SOIL

5.1 Introduction

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:

i) Variation in soil properties (eg pH, clay content, organic carbon content, (e)CEC) among soils (Oorts et al 2006a; Song et al 2006; Criel et al 2008; Li et al 2010a, 2010b and 2013);

ii) Time since contamination (ageing processes, Lock et al 2006a; Oorts et al 2006b; Wendling et al 2009);

iii) Form of metal added to the soil (Degryse et al 2004; Ma et al 2013).

Soil properties, such as pH, organic carbon content and texture, determine the amount and type of metal species available for uptake by plants, invertebrates, and soil microorganisms, and the resulting toxic response or bioaccumulation of metal. Correction for the variation in soil properties among soils and hence normalisation to specific characteristics of a soil of interest requires an understanding of the relationship between soil physico-chemistry and metal toxicity on microbial function, plants and invertebrates. In order to perform such normalisation, speciation or bioavailability models must be available, allowing prediction of soil-specific metal toxicity.

Another aspect that needs to be taken into consideration for soils is that toxicity tests are typically based on soils that are freshly contaminated with soluble metal salts and results overestimate toxicity effects in historically contaminated soils at the same total metal level. The ecological relevance of toxicity tests after freshly spiking is questionable because metal spiking causes a sudden disturbance that is unrepresentative of a field where metals are added gradually and could equilibrate for several years.

In addition to the immediate, short-term effects of soil characteristics on metal bioavailability, it has also been shown that long-term equilibration (ageing) of metals in soil is a critical factor in determining bioavailability for most metals. (Italics for emphasis on important point) Long-term changes in metal bioavailability are due to phenomena such as inclusion of natural elements into the crystal lattices of soil minerals, the formation of insoluble precipitates, diffusion of metals into micro pores, etc. Spiking soils with soluble metal salts not only increases the metal content of a soil, but also increases the ionic strength of the soil solution and decreases the soil pH by the replacement of protons from the exchange complex with the metal cations. These changes in pH and ionic strength can also affect the biological response either directly or indirectly through their effect on metal bioavailability. The natural leaching of the excess cations and anions avoids these artifacts in field conditions. Both ageing and leaching processes significantly influence the bioavailable fraction of metals, thereby changing the soil toxicity profile over longer periods of time.

Most toxicity data for metals are based on tests with soluble metal salts, ensuring maximal solubility and bioavailability. However, contaminations are often caused by other, less soluble sources, eg oxides or metallic forms. The form of the metal added to a soil can have a major impact on its behaviour, bioavailability and toxicity. For example, the solubility and toxicity of the poorly soluble antimony trioxide in soil increases with time, and soil solution concentrations at equivalent total Sb concentrations only converge after 5 years with those of the more soluble $SbCl_3$ (Oorts et al 2008). Although less soluble metal sources in soil may slowly transform into more soluble forms, thermodynamic equilibrium may only be reached in the long term (years or decades). **At present, there is, however, no generally accepted approach for implementing considerations on the form and transformation rates of a metal into a risk assessment of metals in soil.**

Although it is generally accepted that the total metal concentration in soil is a poor predictor for toxicity to the environment, there is not yet a generally accepted method for measurement of the bioavailable fraction of metals in soil (Menzies et al 2007; Zhao et al 2006). As for the water compartment, the free metal ion in the pore water is considered to be the bioavailable metal species. However, 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 explain differences in bioavailability. Several soil extraction techniques have been used in order to predict metal bioavailability and toxicity in soils (eg pore water, 0.01M $CaCl_2$, 1 M NH_3NO_3, 0.43 M HNO_3, Diffusive Gradients in Thin-films (DGT),

cyclodextrin (HPCD), extraction, simulated earthworm gut (SEG) etc). However, no standard method applicable to most metals and soil types has been identified. If used for regulatory purposes, this should be done in a cautious way because 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, eg because translocation of metals from plant root to shoot is restricted.

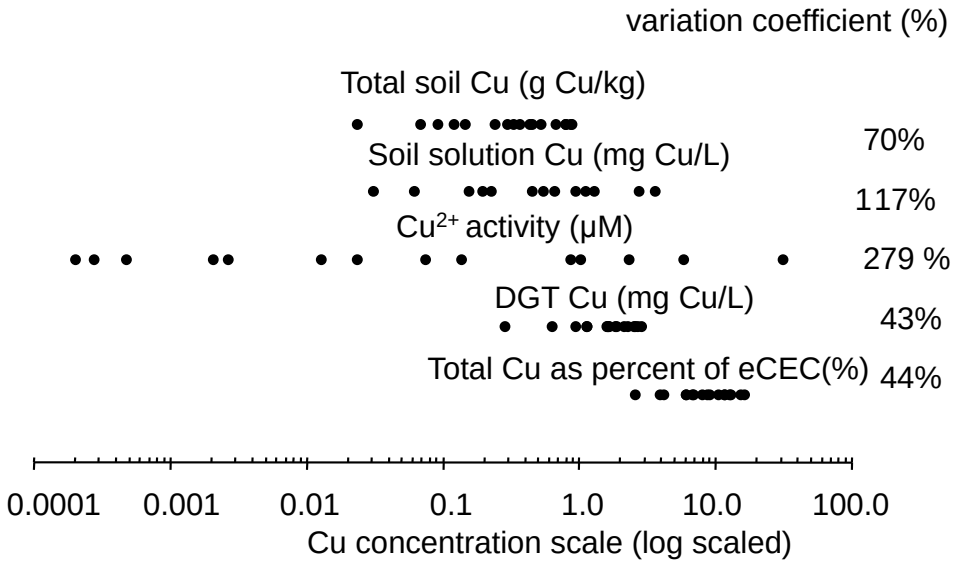

Figure 11: *Five different expressions of Cu toxicity (EC_{50}) to tomato seedling shoot yield in 17 freshly amended soils. Where the coefficients of variance is the standard deviation divided by mean of untransformed EC_{50} values. Where DGT = diffusive gradients in thin film technique; eCEC = effective cation exchange capacity, ie CEC at prevailing soil pH. Data adapted from Zhao et al 2006 and Smolders et al 2009.*

5.2 General concept

Data for (pseudo-) total metal concentrations, based on concentrated acid digestions, are most commonly available and used for risk assessment purposes. Toxicity data for soil organisms are preferentially expressed as total metal concentrations. As for water and sediment, a tiered approach is recommended for refinement of the effects assessment of metals in soil (Figure 12).

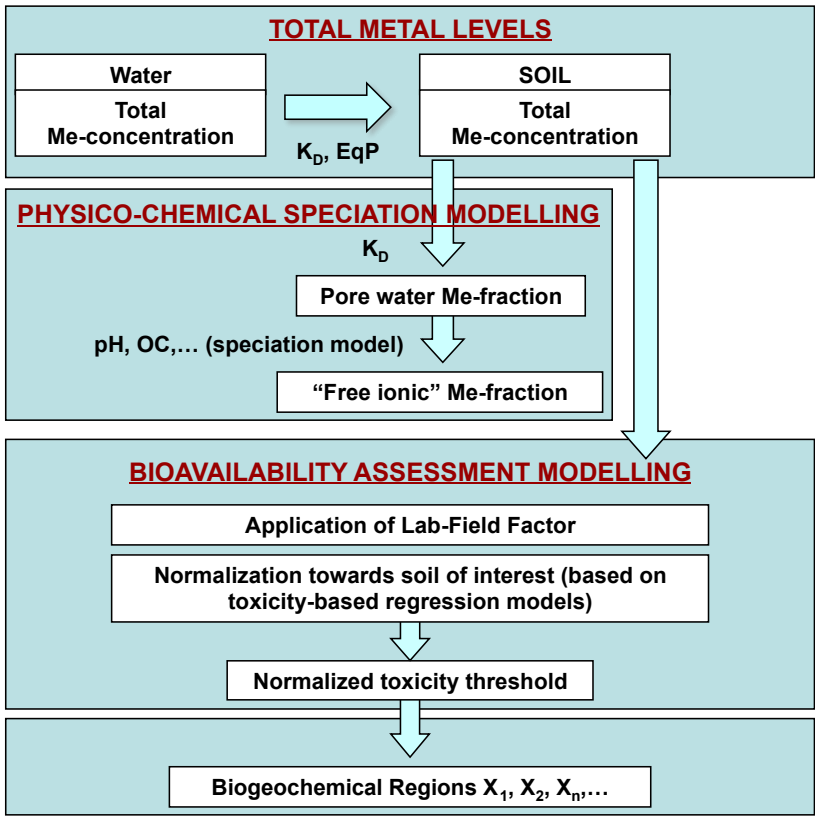

Figure 12: *Refinement levels for the incorporation of bioavailability concept for the soil compartment.*

In cases where no reliable toxicity data are available for the terrestrial environment, the equilibrium partitioning approach (EqP) is sometimes used as a lower tier screening approach to generate an EQS for soil based on toxicity data for the aquatic compartment and a soil:water distribution coefficient (Kd). The equilibrium partitioning approach is based on the assumption that soil toxicity expressed in terms of the freely dissolved metal concentration in the pore water (interstitial water) is the same as aquatic toxicity. It should be emphasised that substitution of terrestrial toxicity data by aquatic toxicity data should be used with caution. This is because the effects on aquatic species can only be considered as effects on soil organisms that are exposed exclusively to the soil pore water and may hence not be appropriate for organisms also exposed to soil particles and food via the dietary pathway. Furthermore, studies have shown that the equilibrium-partitioning method can give significant over- or underestimations, due to inaccurate partitioning coefficients or differences in species sensitivities between aquatic and terrestrial species (Van Beelen et al 2003). If the EqP approach is used in the screening, then care should be taken to use relevant Kd values that reflect the bioavailability conditions occurring at the site. Overall, the results of toxicity tests in whole soil should be preferred because both the dietary and aqueous exposure pathways are covered in these experimental designs. As reported above, natural soils used in ecotoxicological tests differ in physico-chemical characteristics,

which may significantly affect the bioavailability of the test compound, and hence the toxicity observed. Therefore, any EQS derived from toxicity data expressed on a total metal concentration, without any further information on bioavailability, has still the potential to under- or overestimate the risk. In the absence of specific information on the bioavailability of a metal, toxicity has to be tested in a reasonable worst-case scenario, ie, a soil with assumed high bioavailability of the metal substance tested as estimated based on physico-chemical speciation modeling and predicted partitioning. This ensures that results are protective for the majority of soils. Such reasonable worst-case soils have low organic carbon content (eg >0.5 and <2%) and low clay content (eg >5 and <10%). The pH would be low (eg <5.5) or high (eg >7) for metals occurring as cations (eg Cu^{2+}, Zn^{2+}, Pb^{2+}, …) or oxyanions (eg MoO_4^{2-}, $Sb(OH)_6^-$, …), respectively.

In case specific bioavailability models are available, toxicity data can be normalized towards the specific bioavailability conditions prevailing at a site. Bioavailability models were developed to correct for the differences in bioavailability due to varying soil properties or differences between lab and field conditions. First, a lab-to-field correction factor is used to translate results obtained from laboratory experiments to field conditions (see section 0). In a second step, a soil-dependent correction factor is applied in order to normalise the results towards the specific physico-chemical conditions of the site of interest (see section 0). Both correction factors are derived from an empirical approach. At present, no models are available to correct for the form of metal added (eg soluble salt versus poorly soluble metal compounds) and toxicity data for soluble metal salts are used as a worst-case reference for all metal forms.

5.3 Mechanistic versus empirical terrestrial bioavailability models

During the last decade, a lot of research has been performed on bioavailability and toxicity of metals in soil. Physico-chemical soil properties (eg pH, organic carbon and clay content, cation exchange capacity) are often significantly correlated with metal toxicity for plants, invertebrates and micro-organisms based on (pseudo-) total metal concentrations in soil. Empirical regression models are derived, covering a wide range of soil types (Smolders et al 2009). Such regression models can be used to normalise 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 differences between laboratory and field conditions were derived for a range of metals.

Next to these empirical models, some more mechanistic models were developed. These models are generally based on the biotic ligand model (BLM) concept as used for bioavailability corrections for the water compartment. Several levels of complexity are considered in this research (Figure 13). Most research so far has focused on derivation of biotic ligand models for terrestrial organisms (plants, invertebrates and micro-organisms) grown in nutrient solutions (Figure 13A; eg Lock et al 2006b; Antunes et al 2007, Mertens et al 2007). These studies show that the BLM concepts are also valid for terrestrial organisms and hence illustrate that the free metal ion in solution is the toxic metal fraction and that its bioavailability depends on competition with other ions (blue arrows in Figure 13).

In a higher level of complexity, the BLM concept was calibrated with results of toxicity studies in natural soils. The modeling is, however, based on the composition of the soil solution and at maximum only the critical free metal ion activity in the pore water was predicted from the total metal concentration in soil based on a speciation program (Figure 13B; eg, Thakali et al 2006a, 2006b; Lofts et al 2013). These terrestrial BLMs have also been calibrated using data from freshly amended soils in which the concentrations of the competing ions are higher than those in field-contaminated soils and in which no ageing reactions are taken into account. **In order to be directly applicable in a regulatory framework, a terrestrial BLM, or any mechanistic model, should preferentially start from total soil properties (total metal concentration, soil organic matter concentration, etc) and include ageing reactions (ie transition from labile to fixed metal).**

The use of mechanistic BLM-type models therefore requires detailed and advanced soil solution modeling to provide the input data required. Such mechanistic modeling, however, needs to consider many additional interactions on the solid-solution distribution from all critical components of the soil solutions (cations, protons, metals, dissolved organic matter, etc) as illustrated by the orange arrows in Figure 13C. At present, no full mechanistic model is available allowing accurate prediction of metal toxicity based on total soil characteristics.

Next to these biotic ligand type ion competition models, some alternative mechanistic models based on electrostatic and osmotic effects at the cell membrane have been developed recently (eg, Wang et al 2011, 2013). Although these models generally need more parameters to be fitted, and hence are rather data hungry, they provide a robust mechanistic framework to assess metal ecotoxicity to terrestrial organisms. At present, the complexity of these mechanistic approaches is still high and their 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. Mechanistic models can, however, still be used as a validation of the correlations observed in the regression models.

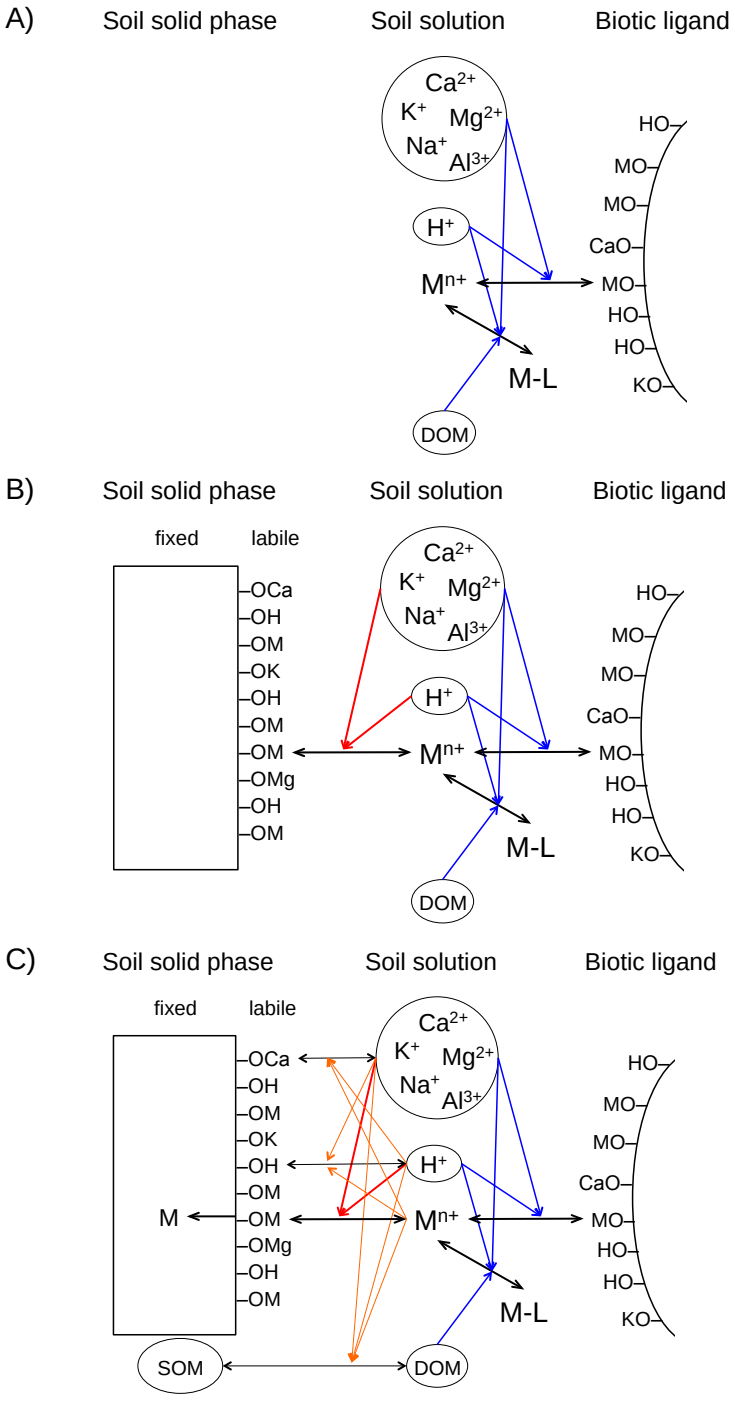

Figure 13: *Conceptual diagram explaining interactions of abiotic factors controlling toxicity of metal cations in soil. A) biotic ligand model only considering interactions in the solution phase. B) Terrestrial*

biotic ligand model based on interaction in the solution phase and including the solid-solution distribution of the metal. C) Hypothetical mechanistic model based on total soil properties and including ageing reactions concentrations. Where DOM = dissolved organic matter; SOM = soil organic matter; M-L = metal-ligand complex. See text for further explanation.

5.4 Toxicity-related bioavailability models

5.4.1 General outline

In considering the bioavailability of metals in soils, two phenomena on the ecotoxicity of metals to soil organisms should be considered:

1. The bioavailability and toxicity of metals to soil organisms is dependent on the equilibration time of metals in soil and the leaching of excess ions. Toxicity tests under typical laboratory conditions, ie no leaching and maximum a few days equilibration after spiking with a soluble metal source prior to start of the ecotoxicity assay, tend to overestimate bioavailability and toxicity of metals compared to field conditions (slow accumulation of metals, long-term equilibration, leaching through percolating rain water). Metal toxicity under field conditions is hardly detectable for some historically contaminated soils, or only observed at much higher doses than under laboratory conditions.
2. The toxicity response is dependent on the soil physico-chemistry (eg pH, cation exchange capacity, organic carbon content, clay content).

Therefore, soil-specific quality standards corrected for bioavailability should be calculated by applying bioavailability corrections that account for (i) differences in metal toxicity between freshly spiked soils and field-contaminated soil and (ii) effect of soil properties on the bioavailability and toxicity of metals. Both availability corrections should be integrated to derive toxicity data that are normalised to specific soil properties and field conditions.

5.4.2 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 (Figure 14). 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 soils 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.

In addition, the gradual accumulation of metals under field conditions and the different sources of the metal (different speciation and bioavailability) in the environment may further mitigate metal toxicity in the field.

Where the adverse 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 the same toxicity effect in a specific soil.

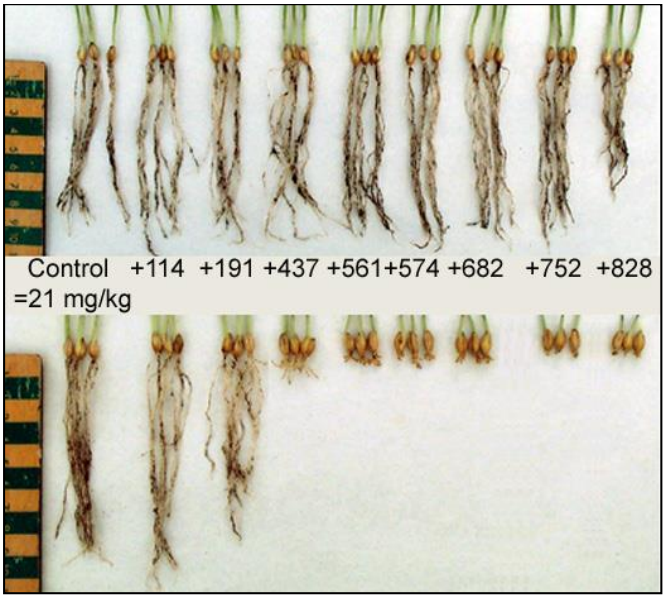

Figure 14: *Barley root elongation in a field-contaminated soil (top) and the corresponding control soil spiked with $CuCl_2$ to identical total Cu concentrations (bottom). Photo courtesy of Rothamsted Research.*

Correction for leaching and ageing: the lab-to-field (L/F) factor

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. 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.

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

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, and ageing of metals in soil. This factor does not address differences in effects between single-species lab test 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.

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 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 unique 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 (minerals?).
- 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 generally are a more statistically robust estimate compared to eg EC_{10}. In cases 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 highest 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 highest dose tested under field conditions and the $EC_{10,add}$ (or NOEC) in the corresponding freshly amended soil.

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, eg based on changes in pore water concentration or isotopic exchangeability (Ma et al 2006, 2013; Wendling et al 2009). 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 (eg Cu, Zn, Mo and Pb, Table 6 and Example 8a). 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 (Table 6 and Example 8b) (eg Ni and Co). 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.

Metal	L/F factor	Key soil properties for normalisation
Cu	2	eCEC*, Soil organic carbon content, clay content, pH*
Ni	1-3 (increasing as a function of pH)	eCEC
Pb	4	eCEC
Zn	3	eCEC, pH, background Co
Co	1-3.5 (increasing as a function of pH)	eCEC
Mo	2	Clay, pH

Table 6: *Lab-to-field factors and soil properties for normalisation for various metals as selected for European risk assessment dossiers*

*Where eCEC = effective cation exchange capacity, ie CEC at prevailing soil pH, as opposed to CEC measured at a buffered pH, pH measured in 0.01 M $CaCl_2$.

Example 8a: Derivation of L/F factor for Cu

Data availability:

1. 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);

2. Difference in Cu toxicity to plants, invertebrates and microbial processes between freshly spiked soils and corresponding leached soils (3);
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 8.1. The L/F factors range from 0.5-30, a 10th percentile of 1.5 and a median value of 2.8. These percentiles are still underestimates because many of the L/F factors are unbounded values, ie 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, ie toxicity is significantly lower in field-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, ie the suggested trend of increased toxicity upon ageing is statistically non-significant. *The overall evidence shows that Cu toxicity is almost consistently smaller (less) in aged soils than in freshly spiked soils. (Italic for emphasis)*

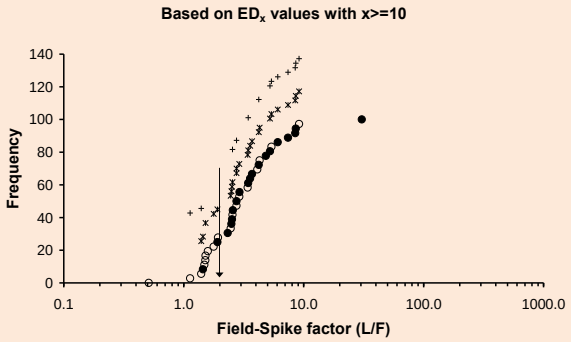

Figure 8.1: *Selection of a generic L/F factor for Cu based on the frequency distribution of all individual lab-to-field factors for Cu. Where open symbols refer to L/F factors derived from bounded toxicity thresholds, closed symbols refer to lower estimates of the L/F factors as they are derived from 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 years 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:

1. 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 similar overall for the soils tested.

2. 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 (less) 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 the absolute concentration should also be evaluated.

3.

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.

Example 8b: Derivation of L/F factor for Ni

Data availability:

4. Difference in Ni toxicity to plants, invertebrates and microbial processes between freshly spiked soils and 3 corresponding leached and experimentally aged soils.
5. 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 7.1). 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 8.2). It is proposed to use the fixation factor, derived from an empirical chemical model as the L/F factor, ie $L/F=1+\exp^{(1.4(pH-7.0)}$ in which pH is the pH measured in $CaCl_2$ 0.01M. This equation is calibrated on soil aged maximally 1.5 years 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 Figure 7.1. 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.

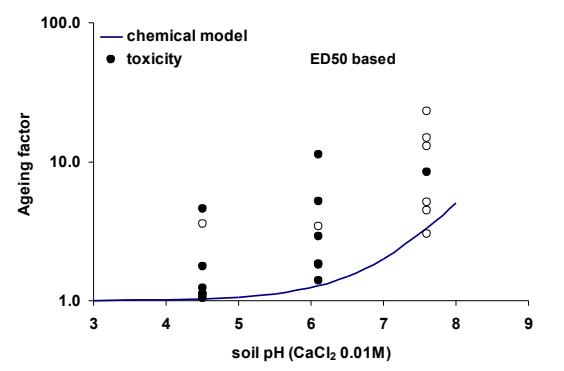

Figure 8.2: *The L/F factors for Ni based on toxicity (symbols) and the predicted factor changes in labile pool of Ni in soil (line). Where open symbols are 'unbounded' values and are a lower estimate of the ageing factor. None of the ageing factors are significantly lower than that predicted by the chemical model.*

5.4.3 Normalisation for variation in soil properties

Metal toxicity thresholds for the same endpoint can vary up to two orders of magnitude among different freshly spiked soil (Oorts et al 2006a; Rooney et al 2006; Criel et al 2008; Li et al 2009, 2010a; van Gestel et al 2011). Physico-chemical soil properties (eg pH, organic carbon content, clay content and cation exchange capacity) can explain a significant part of the variation in metal toxicity thresholds for plants, invertebrates and micro-organisms based on (aqua regia-) total metal concentrations in soil and empirical regression models are derived, covering a wide range of European soil types (Smolders et al 2009). Such regression models can be used to correct the effects data for the differences in physico-chemical properties of the

soils tested and to normalise results towards specific conditions of a site of interest. By doing so, these regressions allow for the derivation of site-specific environmental quality standards, expressed as total metal concentration.

The derivation of normalisation models according to the regression approach is shown in Figure 15 and should take into account the following considerations:

- The regression analysis is preferentially based on EC_{50} values because of the larger robustness of this estimate compared to lower-effect levels in 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, ie 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). Shown as:
  *log(ECx) = intercept + slope * log(abioticfactor)*

Although this is an empirical approach, it is strongly preferred that the models selected can be mechanistically explained. In this aspect, mechanistic approaches, such as biotic ligand-type or electrostatic models (Wang et al 2011, 2013), can be an effective validation of the correlations observed in the empirical regression approach. For example, regressions with eCEC for cationic metals can be explained by the higher sorption and hence lower bioavailability of cationic metals with increasing eCEC of the soil. Final selection of the models should therefore be based both on the statistical power (higher R^2_{adj}) of the regression model and the soil chemical considerations.

1. In order to ensure maximal cross-species extrapolation, models should be derived for a minimum of one species or endpoint for each relevant trophic level of soil organisms (see also section 0).

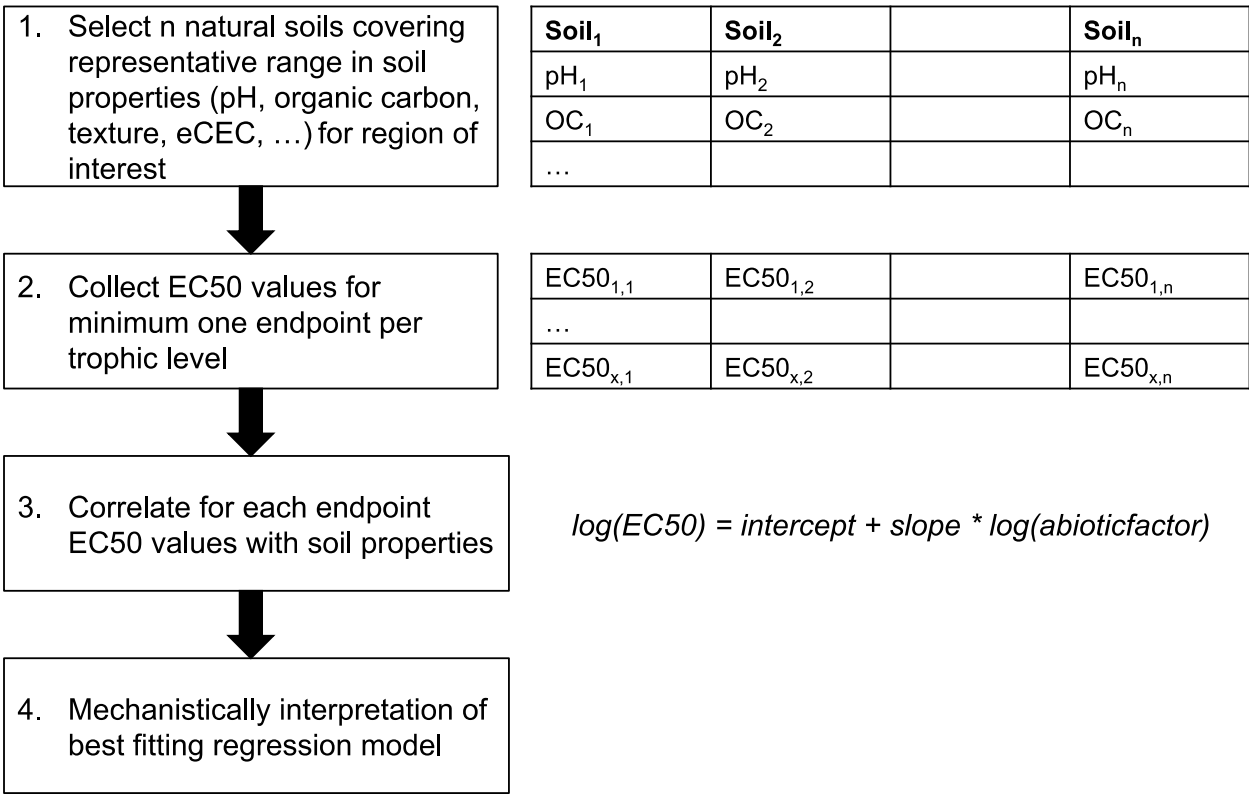

Figure 15: *General approach for derivation of toxicity derived normalisation models for toxicity to terrestrial organisms.*

Regression models used for accounting 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 7, Table 8, Example 9 and Figure 16). 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 (tropical scenarios are still missing). It is not yet known if 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 8.

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 (monocotyledon) Microbial processes (nitrification and C-respiration)
China	Cu, Ni	Plants (monocotyledon) Invertebrates (annelid worm) Microbial processes (nitrification)

***Table 7**: Global availability of normalisation models for metals.*

Metal	**Organisms/Micro-bial 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

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

Next to these empirical models, some more mechanistic models for the soil compartment were developed (eg Thakali et al 2006a, 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 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 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. Mechanistic models can, however, still be used as a validation of the correlations observed in the regression models.

A) B)

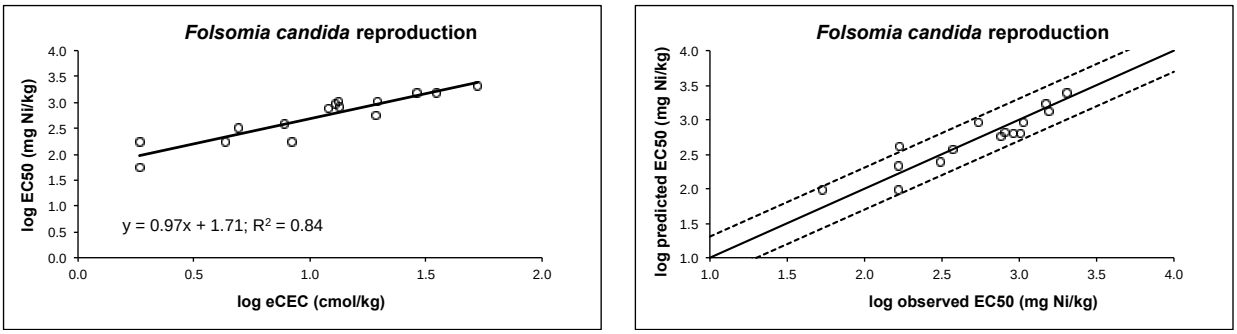

Figure 16: *Example of regression model for toxicity of Ni to the springtail Folsomia candida. A) Ni toxicity to Folsomia candida is best correlated with the eCEC of the soil. B) Observed versus predicted* EC_{50} *values based on eCEC of the soil and the regression equation in A). Where the full line is the 1:1 line and the dashed lines illustrate a factor of 2 deviation from the 1:1 line.*

Example 9: Normalisation models for Cu

Various regression models are available for normalisation of Cu toxicity data for soil organisms (Table 9.1). For example, three different regression models exist for effect of soil properties on toxicity of Cu to the microbial nitrification process in soil and all three models identified other soil properties as best related with Cu toxicity: (1) eCEC (European model); (2) pH (Australian model); or (3) total calcium concentration (Chinese model). These differences in the models can be due to either differences in soil types covered, or also to potential differences in soil treatments (leaching and/or equilibration), methodology (eg (e)CEC analysis), endpoints measured, etc.

Table 9.1 Soil properties selected for normalisation of Cu toxicity to terrestrial organisms.

Endpoint	**Geographical region**	**Regression**	**Reference**
Eisenia fetida (earthworm) reproduction	European soils	eCEC	Criel et al 2008
Folsomia candida (springtail) reproduction	European soils	eCEC	Criel et al 2008
Potential nitrification rate	European soils	eCEC	Oorts et al 2006a
Substrate-induced respiration	European soils	Organic carbon and clay content	Oorts et al 2006a
Maize residue mineralisation	European soils	pH and eCEC	Oorts et al 2006a
Barley root elongation	European soils	eCEC	Rooney et al 2006
Tomato shoot yield	European soils	eCEC	Rooney et al 2006
Substrate-induced nitrification	Australian soils	pH	Broos et al 2007
Substrate-induced respiration	Australian soils	clay	Broos et al 2007
Wheat shoot yield	Australian soils	CEC	Warne et al 2008
Substrate-induced nitrification	Chinese soils	Total calcium concentration	Li et al 2010b
Barley root elongation	Chinese soils	pH and organic carbon	Li et al 2010a

5.5 Implementation of bioavailability corrections into the derivation of soil threshold concentrations

5.5.1 General approach

The general framework for implementation of bioavailability corrections into derivation of ecological threshold concentrations is presented schematically in Figure 17.

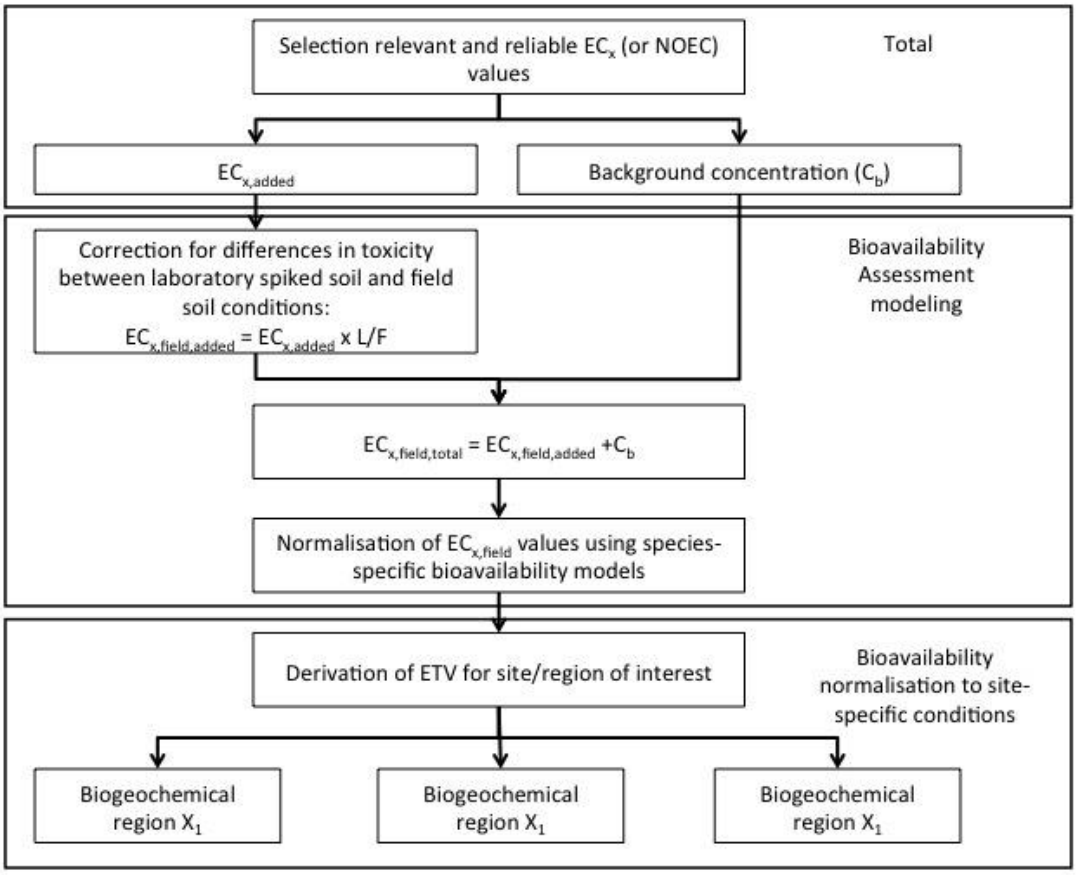

Figure 17: *Framework for implementation of bioavailability factors into soil limits derivation.*

The following steps can be distinguished (Smolders et al 2009):

1. 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.
2. 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. When no relationship is found between soil properties and the lab-to-field factor, one generic factor is used for all soils (eg, Cu, Zn, Pb). When the lab-to-field factor depends on the soil properties (eg, 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.

3. 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. Using the regression-model approach, each total or added "field" EC_x or NOEC value is normalised towards the soil properties of a specific target soil, using the slope of the metal- and organism-specific regression function (log-log based) and the following equation (Equation-21):

$$EC_{x,\text{reference}} = EC_{x,\text{test}} \left[\frac{\text{abiotic factor}_{\text{reference}}}{\text{abiotic factor}_{\text{test}}} \right]^{\text{slope}} \qquad \text{(Eq-21)}$$

Where "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 regression analysis 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.

4. 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

normalised 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-normalised data, where variation between toxicity data is also caused by differences in bioavailability among soils. Depending on the data available, the ETV can be derived using the AF or SSD approach.

5.

Where possible, the complete effects data set should be normalised. 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 normalised and used for the derivation of the bioavailability corrected soil threshold.

The final definition of an environmental limit obviously requires several regulatory choices, eg on effect (choice of EC_x) and protection level (choice of HC_p) or on the organisms to be included (Checkai et al 2014). The soil-specific limits in the European Union (EU) risk assessments were all set using the sensitivity distribution of all species tested and the hazardous concentration for 5% of the species (HC_{5-50}) estimated as the median fifth (5^{th}) percentile of the cumulative frequency distribution of the chronic NOEC or EC10 values from chronic toxicity tests for plants, invertebrates and micro-organisms (European Chemicals Agency 2008).

Example 10: Impact of normalisation on EQS for Ni

Reliable chronic terrestrial toxicity data are available for Ni toxicity to 11 plant species, 6 invertebrate species and 26 microbial endpoints, allowing the use of the SSD approach for derivation of an EQS. Nickel toxicity to all terrestrial endpoints is best correlated with the eCEC of the soil. Without normalisation, the toxicity values for varying soil properties, the HC_5 (ie 95% protection level) is 24.7 mg Ni/kg. Normalisation of toxicity values to a soil with low effective cation exchange capacity (eCEC = 5 $cmol_c$/kg) or to a soil with high effective cation exchange capacity (eCEC = 35 $cmol_c$/kg) results in a HC_5 of 10.3 and 94.4 mg Ni/kg soil, respectively.

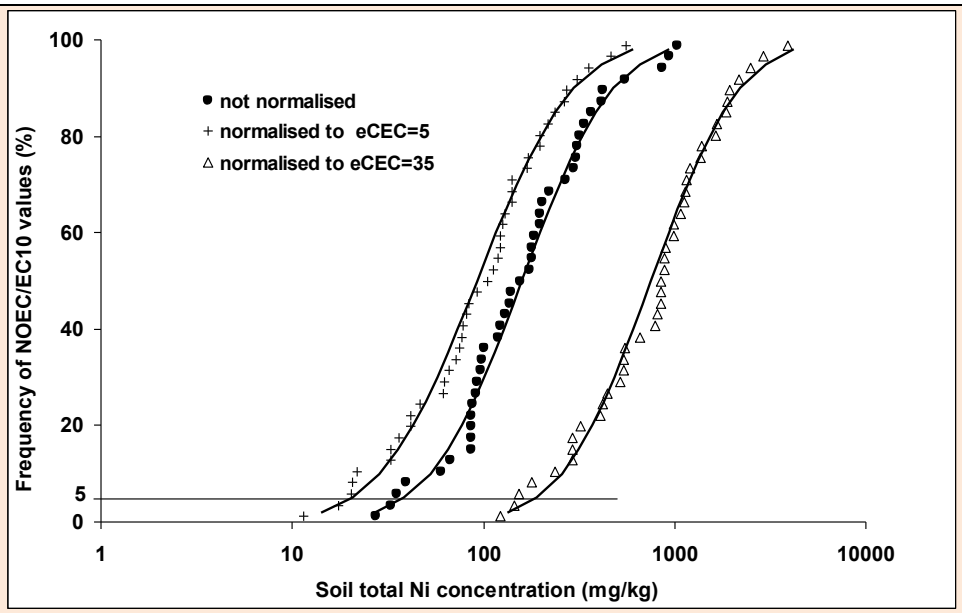

Figure 10.1. *The species sensitivity distribution for the effects of Ni on soil microbial processes, plants, and invertebrates as affected by Ni bioavailability. The raw EC_{10} and NOEC values are shown as well as the corresponding values corrected for bioavailability to a soil with low effective cation exchange capacity (eCEC = 5 $cmol_c/kg$) or to a soil with high effective cation exchange capacity (eCEC = 35 $cmol_c/kg$) (adapted from Smolders et al 2009).*

5.5.2 Normalisation and 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 normalised to a common set of abiotic conditions as long as these soil properties fall within the (geochemical) boundaries of the bioavailability model (eg 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. 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 (eg, 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 models, eg use of a 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 it 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 (eg, arthropods) and soft-bodied (eg, 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 (eg nitrification, N mineralisation) and the carbon cycle (eg respiration) in soil. Read-across between these 2 groups is also not recommended because of the larger functional redundancy for processes 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 (eg for plants), the model with the smallest correction factors (ie the smallest slopes in case of regression models) should be selected as a conservative approach.

- **Decrease in intra-species variability (eg 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 where one metal has the same abiotic factors selected for normalisation of toxicity values for all species and functions tested (eg eCEC for Co and Ni) and if all regression slopes are very similar, these factors show that the relationships are consistent, and that the models can be used across species. In such cases, 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, because the "correct" relationship would be very similar to the one that was in fact used.
- **Spot-check validations for other organisms** (ie few ecotoxicity data from tests performed under different geochemical conditions for a range of key soil parameters (eg eCEC).
- **Field or mesocosm validation**.
- **Understanding of sensitive ecologically relevant species**.

The level of checking, eg testing of additional taxa to confirm applicability of the models, would be determined on a case by case basis taking into account the level of uncertainty in the extrapolations, and the extent to which it is necessary to reduce uncertainty. Considering that sensitive species are generally driving the assessment, it should be demonstrated that the developed/validated bioavailability models could be applied to the most sensitive species/taxonomic groups of the database. It is also needed to consider if certain keystone species or important groups of organisms/trophic levels are missing.

At present, no bioavailability models are available for acute toxicity endpoints and therefore it is not possible to use such acute bioavailability models to normalise chronic data. In case such models would be available, their use should be considered with great care. Such normalisation is only allowed in case the predictive capacity of these acute models is sufficient for estimating

chronic toxicity data. In the case of poor predictive power of the acute models towards chronic toxicity data, the acute model could only be used to normalise the acute toxicity data. The derivation of chronic effects levels could then be derived from the normalised acute toxicity data using an acute to chronic ratio.

If full read-across is justified, the next step consists of applying the bioavailability model across species of similar trophic levels (eg 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 normalise 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 normalised geomean EC_x values for the most sensitive endpoints are then used to derive the soil limit concentrations.

In case read-across is only justified for some species and not for others (eg unexplained significant increase in variability after normalisation or different mode of action), an alternative conservative 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 data, or the worst-case (ie lowest) EC_x values, are selected for derivation of the soil limit concentrations. This decision should be made on a case-by-case basis and should consider eg the intra-species variation in toxicity data before and after normalisation.

Example 11: Full cross-species normalisation for Cu

Seven chronic regression models are available for effect of Cu to different taxonomic groups: two different bioavailability models for higher plants belonging to different taxonomic groups, ie, *H. vulgare* (monocotyledon) and *L. esculentum* (dicotyledon); two different bioavailability models for invertebrates belonging to different taxonomic groups, ie, *E. fetida* (soft bodied) and *F. candida* (hard bodied); three different bioavailability models are available for different microbial processes, ie nitrification, substrate-induced respiration and plant residue mineralisation.
For those organisms or endpoints without a specific regression model, the most conservative model (ie, lowest slope) from similar species within the same trophic level is selected. The max/min ratios for the normalised toxicity data show in most cases to be smaller than a factor of 15, while originally a considerably higher intra-species variability (up to 73) was observed for the non-normalised NOEC data (Figure 10.1). This results in a reduction in intraspecies variability up to 78% for the microbial processes,

up to 66% for the invertebrates, and up to 60% for the higher plants. However, in some cases the max/min ratio is higher after normalisation. It must be stressed that in such cases the *absolute value* of the intra-species variability after normalisation is still very low, ie, 2.9 for *A. sativa*, 4.7 for *E. andrei*, 1.4 for *L. rubellus*, and 8.0 for glutamic acid. Application of the chronic regressions therefore seems to reduce the uncertainty associated with the effects assessment and can be applied for setting an ecologically more relevant EQS.

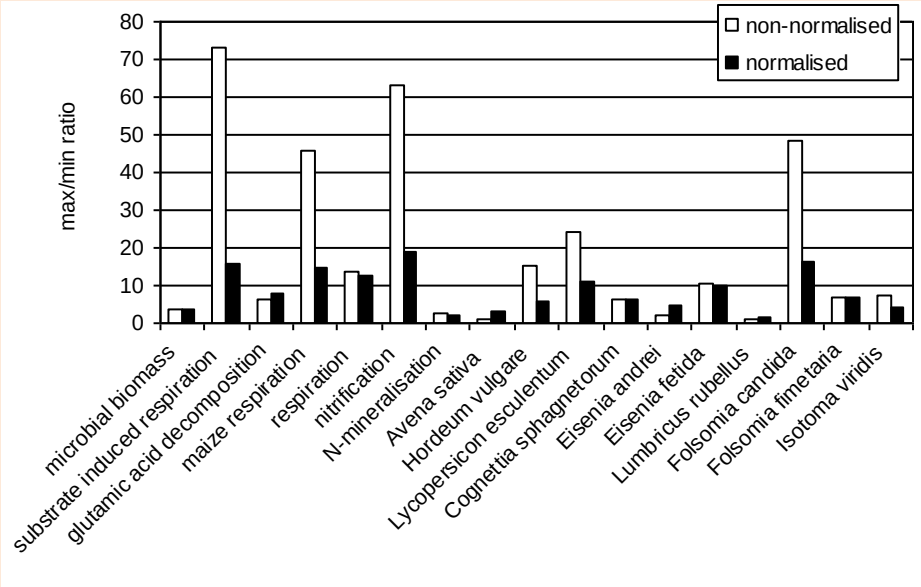

Figure 11.1. *The intra-species variability (expressed as max/min ratios) of the normalised and non-normalised EC10 and NOEC for Cu toxicity to terrestrial organisms, using the terrestrial chronic bioavailability regression models.*

Example 12: Partial cross-species normalisation for Pb

In the European risk assessment dossier for Pb, significant regression models are available for plants (shoot yield of tomato and barley), earthworm reproduction and microbial nitrification. Although the effect of soil properties on toxicity of Pb to arthropods (*Folsomia candida*) and microbial respiration processes were also studied, no significant correlation between Pb toxicity and soil properties was observed. Application of the regression models for plants, earthworms and nitrification to toxicity data for other species or endpoints within the same trophic level, did not show a consistent reduction in the intra-species variation of NOEC or EC10 values (as shown by the maximum/minimum ratio (Figure 12.1).

With the exception for *Zea mays*, the intra-species variation decreased for all plants after normalisation. Changes were, however, not large. Although the intra-species for *Zea mays* slightly increased after normalisation, its absolute value was still very small compared to all other species. The intra-species variation for *Eisenia fetida* and the nitrification process was strongly reduced after normalisation. However, the opposite trend was observed for toxicity data for *Folsomia candida* and respiration processes. Based

on these observations, normalisation models are not applicable for data for arthropods (*Folsomia*) and respiration processes (both basal respiration and substrate-induced respiration). Therefore, non-normalised toxicity data for these endpoints are used for the derivation of an EQS, while toxicity data for other endpoint (toxicity to plants, annelid worms and microbial nitrification process) were normalised towards specific soil conditions.

Because the intra-species variation in toxicity values for the effect of Pb to soil organisms was large compared to the inter-species variation in toxicity, it was assumed that overrepresentation of certain species in the cumulative distribution will not create a significant bias towards higher or lower toxicity thresholds. Therefore, all individual toxicity data (either normalised or not) were included in the SSD for direct effects of Pb to soil organisms.

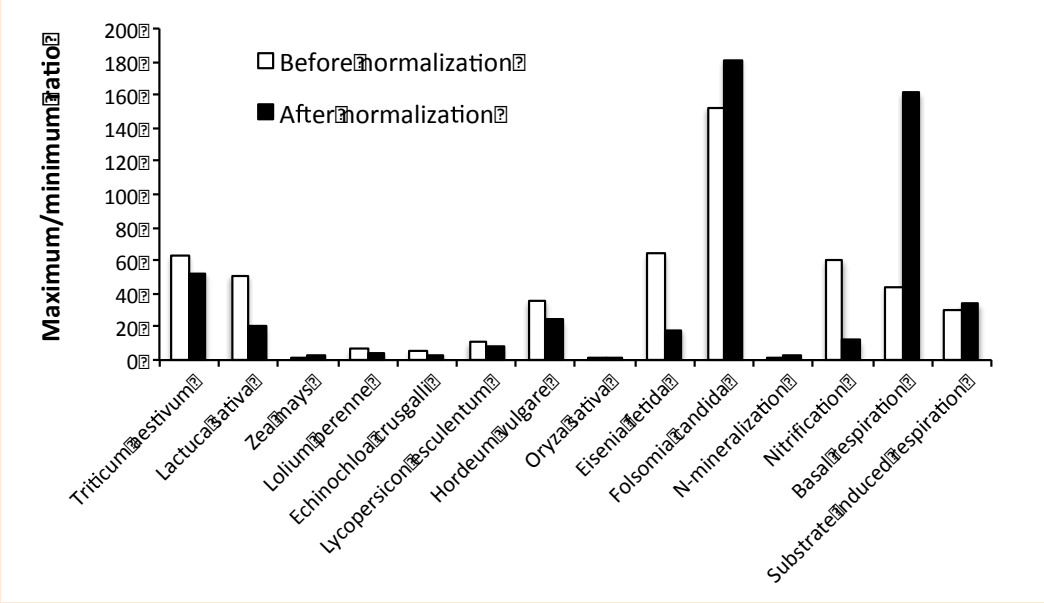

Figure 12.1 *The intra-species variability (expressed as max/min ratios) before and after normalization of the toxicity data for effects of Pb to terrestrial organisms with the terrestrial chronic bioavailability regression models. (Spelling of "normalisation" twice in Fig. 12.1 has to be done)*

5.5.3 Validation of bioavailability-corrected soil quality standards

Few soil limit values for metals used across the world for environmental and ecosystem protection have been validated. Validation in this sense means an assessment in the field in order to establish whether the protection goal for which the limit value was set is achieved. A study for the UK Environment Agency assessed several soil quality standards in terms of their effectiveness and practicality on a range of soils (Nicholson et al 2008). The 'effectiveness' was gauged in terms of whether the limit values were predictive of risk conditions for soils for which secondary data on soil biological process were also available.

In total, 95 field sites were identified with soil data on metal concentrations, soil physico-chemical properties and biological effects indicating ‘soil health’. These biological effects include a significant ($p<0.05$) reduction in crop yield, reduction in biomass, increase in respiration rate, or reduction in vertebrate numbers compared to a reference plot or exceeding Cd concentration in wheat grain.

Site-specific soil quality standards, based on lab-to-field correction factors and normalisation equations reported in the European Risk Assessment dossiers for Zn, Cu, Ni, Cd and Pb correctly assessed the absence or presence of risks in 72 of the 95 soils tested. These soil quality standards were under-protective of only six soils, ie the actual soil metal concentrations in these soils were below the quality standard but a significant negative biological effect was observed, while they were over-protective, ie effect predicted but not observed, for 17 soils. Soil quality standards considering bioavailability performed better than generic soil quality standards that do not take into account metal bioavailability in soil. These results therefore illustrate the validity of the lab-to-field correction factors and normalisation regression models applied.

5.5.4 Application in a risk assessment context

For the application of toxicity-related bioavailability models in a risk assessment context, a tiered approach for the incorporation of bioavailability in risk assessment procedures is proposed. At each tier, exposure concentrations should be compared to the environmental threshold value. In case this comparison leads to risks, more site-specific approaches should be developed based on site-specific information on soil properties (Figure 18).

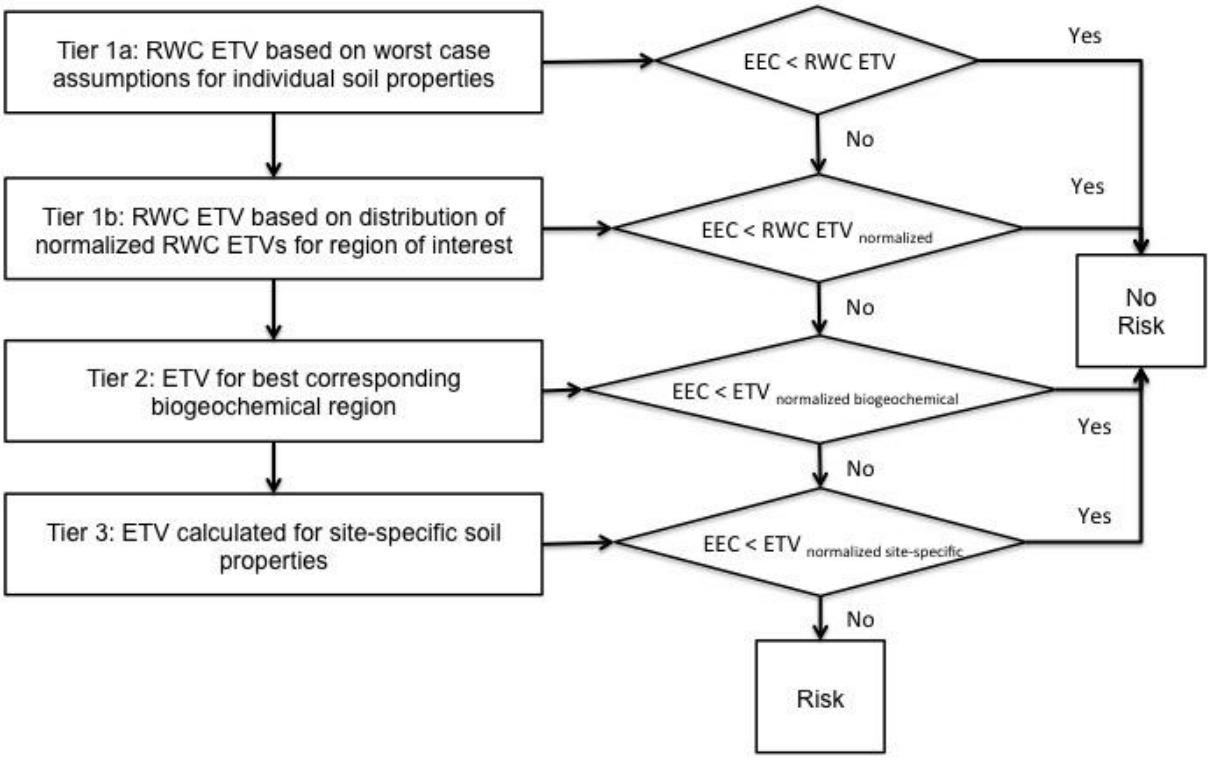

Figure 18: *Tiered approach for refinement of soil ETV values.*

Information on the main abiotic factors controlling metal bioavailability needs to be collected in order to apply the toxicity models. This type of data can be obtained from existing monitoring databases or from specifically tailored monitoring campaigns. As a first tier, the 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 maximise the bioavailability do 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 (eg Cu, Zn, Mo), the reference scenario should as far as possible be a realistic combination of the relevant soil parameters. It is therefore recommended that a reasonable worst-case ETV for a generic first tier local risk assessment is derived based on a distribution of normalised ETVs for a representative range of sites for the region/continent of interest. In case bioavailability models for a metal are all based on the same single soil property (eg eCEC for Ni and Co), worst-case estimates on the soil properties or the resulting ETV values result in the same reasonable worst-case ETV value.

Example 13: Selection of EQS for molybdenum

Mo toxicity in soil decreases, (= increase of the EQS) when clay content increases and pH of the soil decreases. The 5th percentile (HC_5) of EC_{10} and NOEC values normalised to a reasonable worst-case clay content (ie 10th percentile) and pH (ie 90th percentile) in arable soils across Europe is 1.0 mg Mo/kg dw (Table 13.1). This value is significantly different from the reasonable worst-case EQS as calculated based on the 10th percentile of the distribution of HC_5 values normalised to all individual clay-pH combinations in the same dataset for arable soil in Europe (10.5 mg Mo/kg dw). When sufficient actual site-specific EQS data are available, the latter approach is therefore strongly preferred for derivation of a reasonable worst-case EQS. The value of 1.0 mg Mo/kg corresponds to <1st percentile of all predicted site-specific HC_5 values across Europe, and can therefore be considered as overprotective. Soil pH and clay content are generally positively correlated and therefore soils with high pH (90th percentile) and low clay content (10th percentile) do not represent a realistic worst-case scenario.

Further information on the physico-chemical properties for the site of interest yields further refinement of the EQS. The EQS for the best corresponding biogeochemical region can be either over- or underprotective depending on the actual soil properties of the site.

Approach	**EQS (mg Mo/kg)**
Reasonable worst case EQS based on reasonable worst-case assumptions for clay and pH in Europe (data from GEMAS database, Reimann et al 2014)	1.0
Reasonable worst case EQS based on distribution of site-specific EQS values across Europe	10.5
EQS for standard loamy soil (biogeochemical region approach)	30.6
EQS calculated for soil-specific information (pH 7.0 and 25% clay)	22.4

Table 13.1: *Tiered approach for calculation of EQS for Mo in a loamy soil with pH 7.0 and 25% clay. EQS is calculated as the 5th percentile of EC_{10} and NOEC values. Reasonable worst-case estimates for PNEC for Mo in European arable soil.*

5.5.5 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 significant 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 plants and micro-organisms are also 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.

5.5.6 Soil threshold calculator tools

All bioavailability correction models and the data selected for the EU REACH dossiers (Registration, Evaluation, Authorisation 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 (Ta), 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 assigned 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).

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