Guidance on information requirements and chemical safety assessment

Appendix R.7.13-2: Environmental risk assessment for metals and metal compounds

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Guidance for the implementation of REACH

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1. GENERAL INTRODUCTION

1.1 Aim of this guidance

The presence of metals in the environment due to natural processes (resulting in a natural background concentration of metals in all environmental compartments, including organisms) and the chemical processes that affect the speciation of metals in the environment have implications for both the environmental exposure and effects assessment of metals.

The following key issues require specific recognition when performing a chemical safety assessment (CSA) for metals and metal compounds:

- Metals are a class of chemicals of natural origin and have been in use for a long time. Subsequently natural background and historical emissions should be taken into account in a CSA.
- Metal data sets can be data-rich, requiring extensive data treatment (e.g. statistical, probabilistic tools);
- Speciation is of paramount importance, metals can occur in different valences, associated with different anions or cations, and can be associated to adsorptive agents, such as Dissolved Organic Matter (DOM) in water, or bound to minerals in sediment or soil. Speciation highly depends on environmental conditions and chemistry;
- The adsorption/desorption behaviour of a metal strongly depends on prevailing environmental conditions.
- Differences in (bio)availability

The aim of this guidance is to assist the REACH registrant in how to perform the chemical safety assessment for metals and metal compounds, taking into account the issues listed above.

The guidance provided therefore presents the general building blocks of a risk characterisation strategy for managing the potential risks presented by metal/metal compounds. To this end, tiered approaches are advocated since data availability will depend to a large extent on the type of metal/metal compound for which a CSA has to be developed.

Some of the refinement tools presented in this guidance document are only applicable for data-rich metals (e.g. Ni, Cu, Zn etc.). Since it can be anticipated that most metals and metal compounds that will go through the REACH process will be more data-limited, the guidance provided always starts out from the situation that no data or only limited data are available. The further necessity for performing a more detailed CSA and the incorporation of (bio)availability concepts pertains in fact both to the estimated environmental exposure and effect levels. If enough data are available the deterministic approach can be developed into a probabilistic approach.The guidance is supplemented by explanations and practical advice, this being illustrated with examples whenever possible.

Organo-metallic compounds are not explicitly covered by this annex unless they act, through their degradation products, as significant sources of the metal ion. It is considered that these organo-metallic compounds can generally be assessed as individual substances in accordance with the general procedures laid down in the guidance for information requirements and the chemical safety assessment.

Alloys can be assessed on the basis of this annex, particularly in relation to the rate and extent to which alloys can produce soluble (bio)available ionic and other metal-bearing species in the various environmental media.

1.2 General terminology

In this annex the following terminology will be used for some key terms:

- **total concentration of a metal**: for terrestrial and sediment systems, the concentration of a metal that is determined after destruction of the mineral matrix. For aqueous systems: the total amount of metal present, including the fraction sorbed to particles and to dissolved organic matter and the fraction in the mineral matrix;
- **dissolved concentration of a metal**: most often, the dissolved fraction in ecotoxicity tests refers to the fraction that passes through a filter of 0.45 µm. It should be noted, however, that this definition may not necessarily refer to the metals in solution. In the range of 0.01-0.45 µm colloid inert particles containing metal ions that remain suspended, may still exist;
- **available fraction of a metal**: the fraction of the total metal that is extractable from the substrate with chemical (e.g. neutral salt, water extraction) or physical means (shaking, pore water collection), and that is generally considered to be a better estimate for the fraction that is potentially available for organisms than the total concentration;
- **bioavailable fraction of a metal**: bioavailability is a combination of factors governing metal behaviour and the biological receptor (such as route of uptake, duration and frequency of exposure). As such the bioavailable fraction is dependent on the metal forms that prevail under specific environmental conditions and the biological receptors and can be defined as the metal fraction that can be taken up and that can interact with the organism’s specific metabolic machinery. Bioavailability is organism specific – what is bioavailable to a wheat plant is not necessarily bioavailable to an earthworm;
- **toxicological bioavailable fraction:** the fraction of the concentration that is adsorbed and / or absorbed by an organism, distributed by the systemic circulation and ultimately presented to the receptors or sites of toxic action;
- **natural background concentration**: the natural concentration of an element in the environment that reflects the situation before any human activity disturbed the natural equilibrium As a result of historical and current anthropogenic input from diffuse sources the direct measurement of natural background concentrations is challenging in the European environment;
- **ambient background concentration**: the sum of the natural background of an element with diffuse anthropogenic input in the past or present (i.e., influence of point sources not included);
- **baseline background concentration:** the concentration of an element in the present or past corresponding to very low anthropogenic pressure (i.e., close to the natural background).

2. EXPOSURE ASSESSMENT

Aim and structure of this section

The guidance for information requirements and the chemical safety assessment gives general guidance for ES (Exposure Scenario) development. The aim of this chapter is to explain the various metal-specific considerations that should be taken into account in the exposure assessment of a CSA. In the first part, guidance is given on modelling metal emissions (section 2.1 and 2.2). In the second part, the use of monitoring data for metals is explored, since measured data are available for many metals. Emphasis is put on the selection of adequate monitoring data (section 2.3.2), how to deal with the natural background (section 2.3.4) and historical contamination (section 2.3.4). Finally, guidance is given on which abiotic parameters drive metal bioavailability for the various environmental compartments (section 2.4) and the ecoregion concept is introduced (section 2.4.2), with an explanation of the way in which this can be implemented in the risk assessment process. The general outline of this chapter is given below:

- **2.1 General introduction**
    - ***2.1.1 Guidance for the local exposure assessment***
    - ***2.1.2 Guidance for the regional exposure assessment***

- **2.2 Guidance on metal-specific aspects in exposure modelling**
    - ***2.2.1 Adjusting multimedia fate models for metals***
    - ***2.2.2 Modelling adsorption/desorption processes***

- **2.3 Guidance on metal-specific aspects in selecting measured data**
    - ***2.3.1 Introduction***
    - ***2.3.2 Data selection and handling***
    - ***2.3.3 Determination of natural background concentrations and historical contamination***
    - ***2.3.4 How to deal with natural background concentrations and historical contamination***

- **2.4 Guidance on the incorporation of bioavailability in the exposure assessment**
    - ***2.4.1 Introduction***
    - ***2.4.2 Guidance on the use of the ecoregion driven approach***

2.1 General introduction

For data-limited metals, modelling is the only way to estimate emissions and PEC's. For data-rich metals, the combination of modelling and the use of monitoring data is often an appropriate way to identify the predominant intended or unintended sources. The major benefit of monitoring data is that they are integrative (natural and all anthropogenic sources), but they may be influenced by local point sources. Both approaches have their value and a weight of evidence approach should be used to derive conclusions on the adequate control of risk. This weight of evidence approach should include attention to: relative contributions of the sources, natural versus anthropogenic, and local source versus regional background. In practice, monitoring data may be of different nature, using different analytical techniques with different limits of detection, may have been performed at different times, which requires careful interpretation of the different monitoring data Guidance on how to address local and regional exposure calculations is given in the following sections.

2.1.1 Guidance for the local exposure assessment

For the life cycle stages of manufacture, formulation and industrial use the local releases of a single site have to be assessed taking into account the amount that the registrant is registering. If no emission data are available, a modelling approach using conservative default emissions (e.g. ERC (Environmental Release Classes tables) should be used to develop the appropriate exposure scenario. When the use of ECR tables turn out to be too conservative it could be worthwhile to refine the exposure scenario by developing generic scenario based on reliable and representative emission factors extracted from other site-specific information representative for the sector in which the registrant is working[1] (Example 2.1)

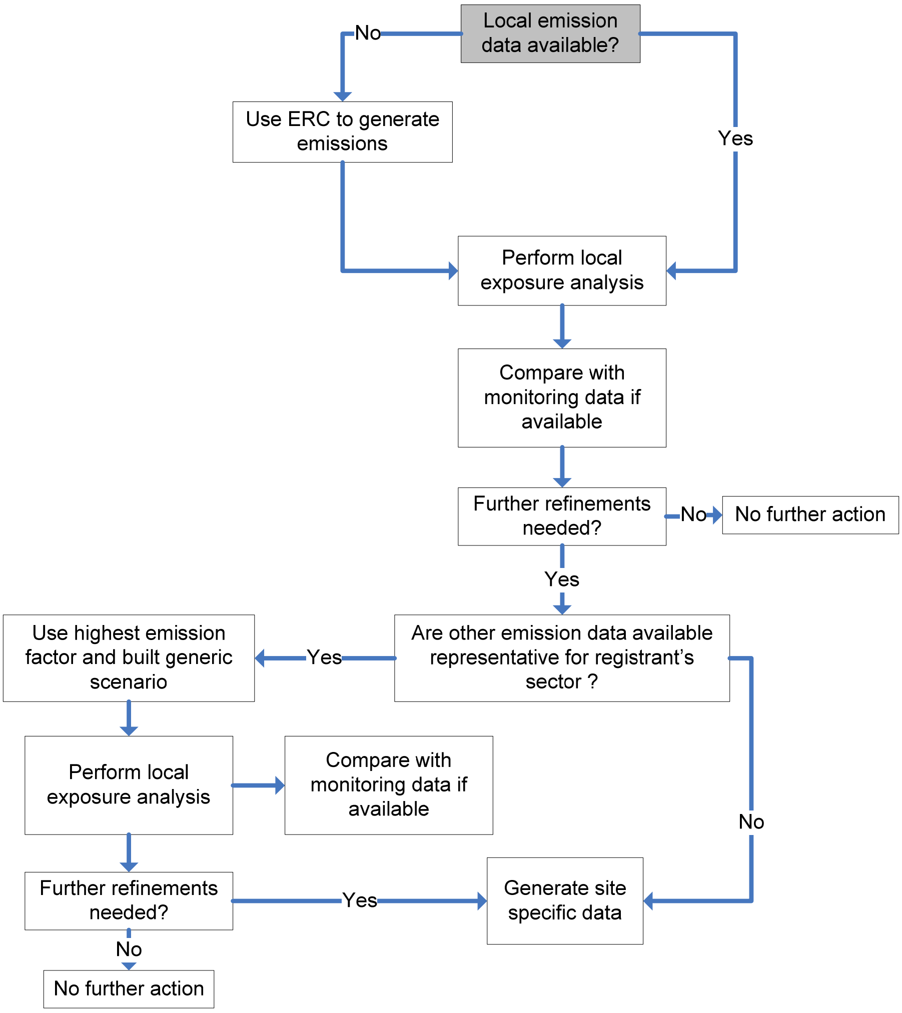

Figure 1: guidance on local exposure analysis

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[1] When the ERCs turn out to be too conservative, a tier 2 Exposure scenario can be developed. Next to sector-specific information made available by industry, regulatory sector documents -e.g. IPPC (Integrated Pollution Prevention and Control) Reference Documents for different industry sectors i.e. BREFs- can also be used in order to assess emission factors. Besides, these documents provide process information and information on typical emission reduction measures for the sector that can serve as a basis for the estimation of the potential for releases to air and water. Please note that the information reported in IPPC documents relates mainly to IPPC compliant companies; meaning, companies that follow BAT (Best Available Techniques) requirements. For non-compliant companies, industry information should be provided in order to estimate emission factors. Also relevant information can be found on the OECD website on Pollutant Release and Transfer Register (www.PRTR.net) that includes emission data in Europe as well.

The generic scenario should cover:

- A representative tonnage consumed/produced in the registrant's sector
- Sufficient numbers of sites involved per sector
- The commonly used production processes in the sector.
- The regional distribution of the activities (spread in the region of interest)

If local monitoring data are available these can be compared with the modelled data. This comparison could result in the identification of for example historical contamination (section 2.3.3) or could be used for a reality check. If no measured data are available, there is no need for the collection of monitoring data if no risk is identified under a conservative modelling approach using reasonable worst-case (RWC) default values. If potential risks are identified using the modelling approach, collection of site-specific monitoring data on the metal content and bio-availability parameters can further reduce uncertainties and improve the assessment.

Example 2-1: Development of generic exposure scenarios for the nickel plating industry (EU Ni RAR, 2007)

Although this example is not specific for metals, it illustrates that the metal surface treatment sector is a typical sector with a large number of Small and Medium Enterprises (SME) widely distributed over Europe. In order to adequately estimate the emissions from this sector, the following methodology was developed. .The plating industry uses both Ni metal and Ni compounds (Ni sulphate, Ni chloride) in its processes. The total EU-15 amount of Ni metal and compound used is estimated at 22,165 tonnes (expressed as Ni). The total number of Ni plating sites in the EU was estimated to be 808 (year 2000 information). Exposure data were available from 137 plating companies located in Finland, Sweden, Denmark, UK, Germany, France, Italy, Spain and the Netherlands (e.g. water emission factors, see Table 1).

The total amount of Ni metal and Ni compounds used by those plating companies is 4,160 tonnes (expressed as Ni). Based on the total number of plating sites in the EU, the collected information represents only 17% of these plants (137 sites in total). The tonnage-based coverage of 18.8% corresponds well with the number of site-based coverage. On the other hand, information for the major plating countries (France, Germany, Italy, UK) -representing 80-85% of the capacity- is fairly well covered. It was concluded that, for the Ni plating sector, a representative emission factor dataset had been established that could be used to set the reasonable worst-case emission factor for generic scenarios; i.e. 90P emission factor due to the large number of data points (>10dp).

Table 1: Overview of site-specific water and sewer emission factors for the Ni plating sector

Industry sector	No. of sites (report/EU)	No. of data points	WATER		
			Average	Min	Max
Plating (all countries)	137/ 808				
Electroplating	(131)	47	2.84×10^{-3}	1.21×10^{-6}	2.04×10^{-2} 90P:$7.47\text{x}10^{-3}$
Electroless plating	(6)	2	3.29×10^{-3}	4.75×10^{-4}	6.10×10^{-3}
Country-specific:					
UK	sewer	9	5.48×10^{-3}	2.29×10^{-6}	2.04×10^{-2}
Germany	sewer	10	1.05×10^{-3}	2.16×10^{-5}	6.25×10^{-3}
Italy	water	12	1.31×10^{-3}	1.01×10^{-4}	4.86×10^{-3}

Generic scenarios for non-covered Ni plating sites

Two scenarios were developed and taken forward in the risk assessment used:

- In the first generic exposure scenario, the 'average remaining tonnage' Ni used/produced per site is calculated from the total remaining tonnage used in the EU and the number of remaining companies in that sector. Emissions to water are estimated applying the 90P representative emission factors for the sector (large dataset>10dp). A default number of emission days and a pre-defined environment are assumed in the exposure calculations (EUSES 2.0). (See also guidance for information requirements and the chemical safety assessment).

- In the second generic exposure scenario, a 'realistic worst-case rest tonnage' Ni used/produced per site is calculated on the basis of the average remaining tonnage per site and the variance of the known sites (assuming log normal distribution). Water emissions are calculated applying the 90P representative emission factor for the sector. A default number of emission days and a pre- defined environment are assumed in the exposure calculations (EUSES 2.0) (See also guidance for information requirements and the chemical safety assessment).

From the collected exposure information, it could be concluded that the majority of the plating companies in the EU discharge their waste water to a municipal STP, where an additional Ni removal of 40% takes place. This has also been considered in the developed generic scenarios.

2.1.2 Guidance for the regional exposure assessment

The guidance given in this section is not always only relevant for metals but the issues raised have quite often being observed for metals. At regional scale (i.e., for the assessment of diffuse ambient concentrations) the use of both measured data and modelled data is recommended if data are available (data-rich metals). Measured data provide a quantification of the contribution of all possible metal compounds, processes and sources to the environment. Although modelled data have the possibility of missing releases from unintended uses/sources (see example 2-2) or excluding sources due to regulatory issues (e.g. biocides, mining medical use), their use in parallel with measured data can be of added value. The outcome of the modelling can be used to differentiate between both the natural background and the concentration added by past and recent anthropogenic activities that are both integrated in ambient measured monitoring data. At the end, a comparison between modelled and measured data has to be performed in order to select the most appropriate exposure estimate to take forward in the risk characterisation.

For data-limited metals, monitoring data may be lacking and in those cases a choice has to be made as to whether to initiate a monitoring programme or to only use modelling as a way forward for carrying out the exposure assessment. The decision as to whether or not to embark on a monitoring programme should be based on a detailed evaluation of the use pattern of the metal (dispersive use versus contained use), the intrinsic toxicity, and, more importantly, the potential for release and likelihood of exposure. In this regard, it should be noted that the potential for release and exposure is not merely determined by the volume in which the product is being produced. The use pattern (e.g. contained versus wide dispersive use) can influence to a larger degree the release of the metal. Before embarking into an extensive monitoring programme, an extended model exercise could be conducted, in which various use/dispersion scenarios are evaluated. If potential risks are identified using the modelling approach, collection of regional monitoring data on the metal content and bioavailability parameters can further reduce uncertainties and improve the assessment (section 2.4).

Example 2-2. Importance of releases of intended and unintended use of metal and metal compounds on a regional level

For a regional exposure assessment the releases of unintended uses should not be neglected since they may contribute in a significant way to the regional background concentration used in the exposure modelling. A release from an unintended use is defined as the release of a metal during an activity for which the presence of the metal is not needed for the actual use. A release of an intended use can be defined as the release during the actual use of the metal/metal compound during the whole metal product life cycle of a registrant.

Examples of releases of unintended uses are:

- Combustion of fossil fuels (Ni, Pb)

- Impurities in phosphate fertilizers (Ni, Pb)

Examples of releases of intended use:

- Corrosion/run-off from building structures (Cu, Ni, Pb)
- Brake pads (Cu)
- Ammunition (Pb)

The importance of the releases of unintended use for the various metals can be very different. For nickel, for example, the releases from unintended uses like combustion processes and the use of phosphate fertilisers seem to result in about 50% of total nickel emissions on a regional level.

In order to account for releases of unintended uses on a regional scale, information on metal emissions should be collected from National Emission Inventories from different European countries and the European Pollutant Emission Register (EPER). If this information is not available for the specific metal, the contribution of releases from unintended uses to the total releases should be estimated by quantifying the metal content in e.g. fuels, fertilisers and quantifying the applied tonnages of these products (mass balance).

2.2 Metal-specific aspects in exposure modelling

2.2.1 Adjusting multimedia fate models for metals

Most of the current guidance on the use of multimedia models for the purpose of chemical safety assessments has been developed mainly from the experience gained on individual organic substances. This means that the methodology used /assumptions made cannot always be applied directly to metals without modification.

Specific guidance and background on how to run the various models in order to derive the modelled local and regional PEC concentrations can be found in the relevant documents dealing with the subject The main metal-specific attention points that should be taken into account when conducting the modelling exercise are addressed in Table 2.

Table 2: Use of multimedia fate models for metals

Parameter	Value	Remark
Water solubility		• prediction of the environmental concentration should be based on the relevant soluble metal ion /or other metal species that is bioavailable or may become available through transformation processes. Speciation models may be used to determine the soluble fraction • metals are not soluble but can be transformed and subsequently release soluble and sparingly soluble metal compounds • in some cases, the metal compound will be only poorly soluble and sufficiently stable to not rapidly transform to a water soluble form. Under these circumstances, the substance itself should be assessed taking into account its specific partitioning characteristics. For the aquatic environment, it can be assumed as a first estimate that the substance will dissolve up to its water solubility limit, and that this fraction will be the bioavailable form. Refinement of the assessment can be done by taking into account kinetics of the dissolution
Vapour pressure	Set vapour pressure to	• most metals, except for mercury compounds and

	minimum value (in EUSES $1x10^{-06}$ Pa)	several organometallo compounds, have a very low vapour pressure and therefore adjusting the vapour pressure in models, such as EUSES, will ensure that modelled distribution to air is negligible
Henry coefficient	Set Henry value to minimum value (in EUSES $4x10^{-06}$ $Pa.m^3.mol^{-1}$	• Volatilisation can be ignored for metals, except for mercury compounds and several organometallo compounds. Consequently, the Henry-coefficient should be set to a very low value
Adsorption to aerosol particles		• Most of the metal present in the atmosphere will be bound to aerosols. Consequently,, an extremely low value should be used for the vapour pressure e.g. $10E^{-06}$ to ensure that the metal fraction associated to aerosols ($Fass_{aer}$) is almost equal to one. If a valid measured value is available, this value should be used.
Octanol-water partitioning coefficient	Use modelled/measured partition coefficients K_p water-soil, water-sediment and water-suspended matter	• The octanol-water partitioning coefficient is not appropriate for metals; modelled / measured partition coefficients K_p should be used instead, taking into account environmental conditions and chemistry
Biotic and abiotic degradation rates	0	• Biotic and abiotic degradation rates should be set to zero for metals
Elimination in STP	Use measured/modelled partition coefficient for water-sludge	• These values are difficult to find for metals and quite often it is more obvious to obtain removal efficiency rates (expressed in percent) than sludge-water partition coefficients (see example 2-3)
Time scale	20-100 years	• For metals, steady-state is typically only reached after several decades or even thousands of years. Steady-state concentrations are uncertain at such time scales and the time scale is no longer relevant for risk assessments. It is therefore necessary to calculate both the PEC values after a surveyable time period of 100 years and the PEC at steady-state. The time period at which PEC equals PNEC should also be calculated for risk management purposes
Adsorption-desorption	Use measured partitioning coefficients for the environmental compartments of concern	• The transport of metals between the aqueous phase and soil/sediment/suspended matter should be described on the basis of measured soil/water, sediment/water and suspended matter/water equilibrium distribution coefficients (Kd; also called partition coefficient, Kp) (see also section 2.2.2).

Example 2-3: Overview of removal rates for metals (%) in municipal Sewage Treatment Plants (STPs) in the Netherlands (CBS, 2007)

Metal	2000	2004	2005
Arsenic (As)	52	54	54
Cadmium (Cd)	54	73	81
Chromium (Cr)	78	83	80
Copper (Cu)	89	92	92
Mercury (Hg)	72	74	77
Lead (Pb)	86	87	86
Nickel (Ni)	53	57	55

Zinc (Zn)	77	81	82

Metal removal rates for Dutch STPs are weighted average removal rates calculated as the ratio of total metal input to Sewage Treatment Plants (STP) versus total metal output of 100 Dutch urban waste water treatment plants. The implementation of new techniques for the removal of phosphates and nitrates in the 90s also resulted in a better removal of metals. Longer residence times and low sludge loads result in an increase in adsorption of metals to activated sludge particles, and higher removal rates are thus observed (CBS, 2007).

In the absence of measured removal rates a default removal rate for cationic metals of 50 % seems reasonable.

2.2.2 Modelling adsorption/desorption processes

For organic non-ionic chemicals, adsorption/desorption processes are often based on octanol-water partition coefficients (K_{ow}) and the assumption that all adsorption is related to the organic matter (see Section 7.1.15 RIP 3.3 for general guidance). This approach can not be used to describe the partitioning of metal compounds in the various environmental compartments for the following reasons:

- The K_{ow} and K_{oc} concept is not applicable for inorganic compounds.
- Sorption is not controlled only by organic matter, but also by other solid phase constituents like clay minerals and oxides.
- The distribution of metals over the solid and liquid phase is not only controlled by pure adsorption/desorption mechanisms. Other processes like precipitation or encapsulation in the mineral fraction also play a role.
- Environmental conditions (pH, redox conditions, temperature, ionic strength) and the composition of the liquid and solid phase have a strong effect on the Kd of inorganic substances. As a result a wide range of Kd values have been reported.

Consequently, the distribution of metals between the aqueous phase and soil/sediment/suspended matter should be preferentially described on the basis of measured soil/water, sediment/water and suspended matter/water equilibrium distribution coefficients:

$$Kd = Cs / Caq \qquad \text{(Equation 1)}$$

Cs = total concentration of test substance in the solid phase (mg kg^{-1})
Caq = concentration of test substance in aqueous phase (mg L^{-1})

Kd values for metal and metal compounds are not true constants and can vary as a function of the metal loading and of environmental characteristics such as pH, ionic strength, redox conditions, or the composition of the liquid phase (Dissolved Organic carbon content i.e. DOC, concentration other complexing ions) and solid phase (organic matter, clay, oxides, sulphides (only for sediment)). Consequently, Kd's may differ from site to site and can change over time. This explains the wide range in Kd values observed for metals (see Example 2-4).

The relative importance of all these factors varies from metal to metal and depends on environmental conditions. However, pH is generally regarded as the most important factor in soils and in aquatic systems. The choice of the Kd values has important consequences for the outcome of the exposure assessment. Small Kd values will predict a larger PEC and higher risk in water, and large Kd values will lead to a large PEC in soils and sediments.

Example 2-4. Overview of metal partition coefficients for suspended matter, sediment (freshwater environment) and soil (median, 10P, 90P) (l/kg) (EU RAR)

Metal	Kp suspended matter (l/kg)				Kp sediment (l/kg)			
	N° of data	50P	10P	90P	N° of data	50P	10P	90P
Cadmium (Cd)	6	130,000 Log Kp=5.11	Min: 17,000 Log Kp=4.23	Max: 224,000 Log Kp=5.35	-	Cfr. Kpsusp. matter	Cfr. Kpsusp. matter	Cfr. Kpsusp. matter
Copper (Cu)	24	30,246 Log Kp=4.48	5,752 Log Kp=3.76	194,228 Log Kp=5.29	11	24,409 Log Kp=4.39	8,934 Log Kp=3.95	99,961 Log Kp=5.0
Lead (Pb)	19	295,121 Log Kp=5.47	50,119 Log Kp=4.70	1,698,244 Log Kp=6.23	5	154,882 Log Kp=5.19	35,481 Log Kp=4.55	707,946 Log Kp=5.85
Nickel (Ni)	39	26,303 Log Kp=4.42	5,754 Log Kp=3.76	117,490 Log Kp=5.07	17	7,079 Log Kp=3.85	2,138 Log Kp=3.33	16,982 Log Kp=4.23
Zinc (Zn)	14	110,000 Log Kp=5.04	Min: 64,000 Log Kp=4.81	Max: 176,000 Log Kp=5.25	-	73,000* Log Kp=4.86	42,667* Log Kp=4.63	117,333* Log Kp=5.07

* Kp sediment derived as Kp suspended matter / 1.5

Metal		Kpsoil l/kg		
	N° of data	50P	10P	90P
Cadmium (Cd)		280 Log Kp=2.45	ND	ND
Copper (Cu)	70 studies	2,120 Log Kp=3.33	Min: 6.8 Log Kp=0.83	Max: 82,850 Log Kp=4.92
Lead (Pb)	60	6,400 Log Kp=3.81	600 Log Kp=2.78	43,000 Log Kp=4.63
Nickel (Ni)	46	631 Log Kp=2.86	Min: 9 Log Kp=0.95	Max: 3,547 Log Kp=3.55
Zinc (Zn)	11	158 Log Kp=2.2	ND	ND

A number of reviews on appropriate Kd values have also recently been published. For example, the publications of Sauvé et al. 2000 and Degryse et al., 2006 contain Kd values for different metals in soils with min, max, mean and median values reported. Regression equations between Kd and soil constituents from the literature can also be used for predictive purposes provided that they have been developed based on data from soil types similar to those under consideration[2].

Guidance on Kd selection

Figure 2 gives an overview of the various steps that can be distinguished for selecting the most appropriate Kd value to be used in the CSA for metals and metal compounds.

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[2] It should be noted that the predictive validity of these equations is usually tested (and limited) to a limited domain of soil pedology)

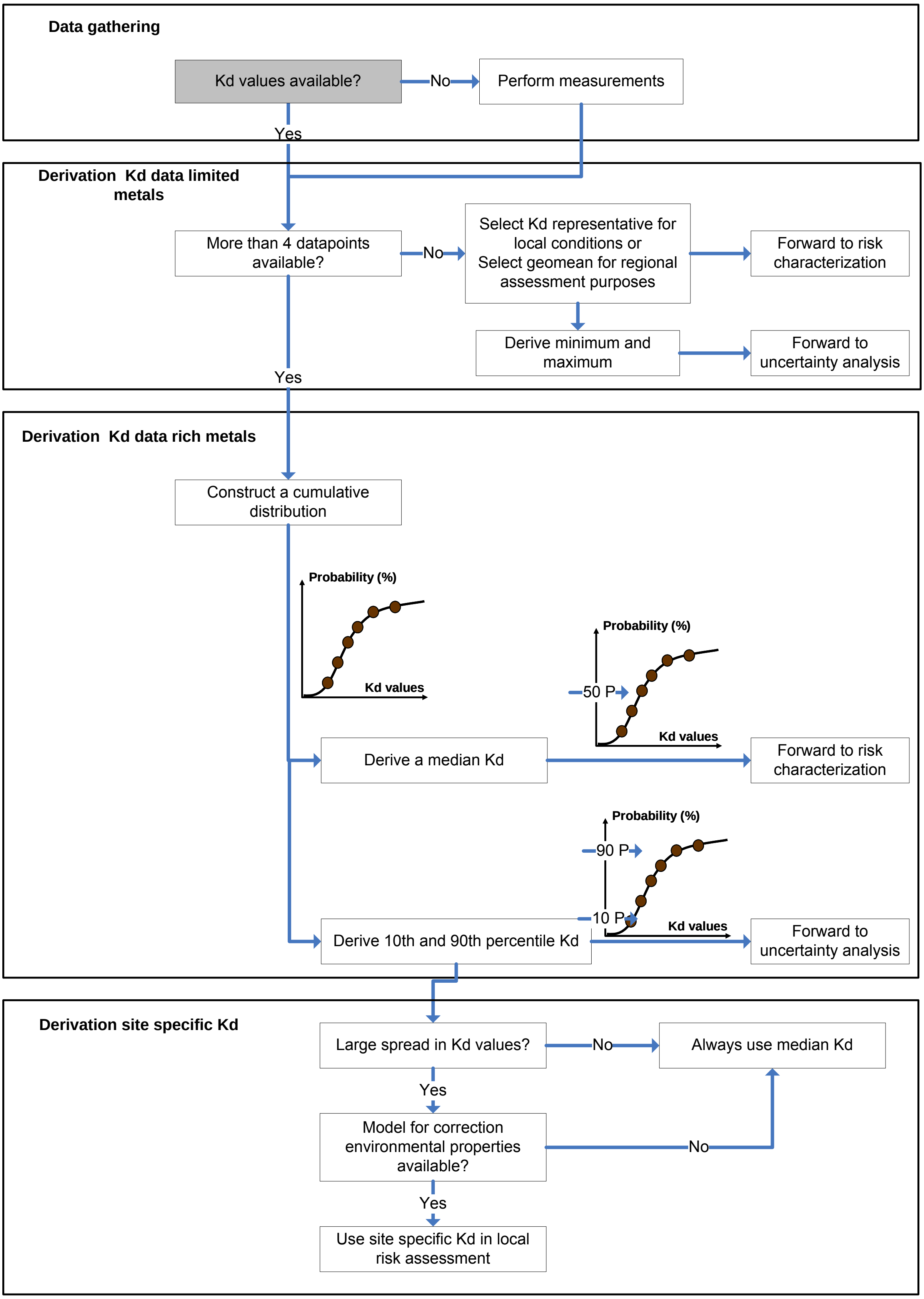

Figure 2: General overview guidance Kd selection for metals and metal compounds

The different steps are briefly described below.

Step 1: Data gathering and relevance check

If no reliable Kd data are available, Kd values must be experimentally measured. In RIP 3.3-2, a batch equilibrium method (OECD TG 106) is recommended for adsorption/desorption measurements for ionisable substances. At this moment, there is no specific standard method available for Kd determination of metals. Care should be taken that soil, sediments and suspended solids characteristics are selected that are representative of the environmental conditions encountered in the region of interest (Example 2-5).

Example 2-5: Indicative range of soil properties for the determination of a soil Kd

According to the guidelines for measurement of adsorption in soil (RIP 3.3-2), Kd values for ionisable substances, like metals, should be measured in a range of actual soils, covering a representative range in pH. Next to pH also clay, organic matter, oxides affect the solid/liquid distribution of metals in soils and a representative range in these properties is preferably also covered by the test soils. An indicative range of soil properties to be covered is as follows:

pH (0.01 M $CaCl_2$):	4.5-7.5
Organic matter:	2-20%
Clay	5-30%

In order to judge quality and usefulness, further information must be available on:

Analytics:

- extraction of the metal content of the solid phase (e.g. with aqua regia)
- sampling techniques of the solution phase (extraction of pore water for soil and sediment, filtration, etc.)
- analytical techniques

Key drivers determining the Kd value:

- composition of the solid phase (organic matter, clay, AVS (sediments))
- pH
- equilibration period after addition of metals

Preference should always be given to Kd values based on paired measured data in the solid and solution phase (e.g. measurements water and sediment concentrations should relate to the same sampling event). If no coupled data on metal concentrations in corresponding solid and solution phases are available, an alternative approach is proposed as a screening method for distribution coefficients. This approach is based on derived environmental concentration distributions for ambient or background dissolved metal concentrations in surface waters/soil pore water on the one hand and sediment/Suspended Particulate Matter (SPM)/soil metal concentrations on the other hand. Based on the median background or ambient concentrations respectively, water-sediment/suspended matter/soil Kd values can be derived. The combination of low-end and high-end values can be used to estimate a realistic range of variation between Kd-values. The disadvantage of this approach is that the values are not coupled.

Finally because Kd values are also concentration-dependent, they must be measured at environmentally relevant metal loadings.

Step 2: derivation of a representative Kd value for data-limited metals

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 judgement taking into account the representativity of the Kd value for the local scenario or, in the case of a regional assessment, a geometric mean is used. The minimum and the maximum values are taken forward to the uncertainty analysis.

Step 3: derivation of a representative Kd value for data-rich metals

When sufficient distribution coefficients are collected, it is possible to fit a normal, log-normal or other statistical distribution through the data points. Using "goodness-of-fit statistics", the distribution(s) that best fits the input data is selected for further assessment. When few distribution coefficients are available, only summary statistics (average, median, minimum and maximum) are calculated. The median Kd-value must be used in the exposure assessment and effect assessment of the CSA. In absence of site-specific Kd values, it is proposed to do an additional uncertainty analysis with a range of Kd values (10-90th percentiles).

Step 4: derivation of site- specific Kd values

For the risk assessment at local scale, the Kd values should, as far as possible, be representative of the environment of interest taking into account the major environmental characteristics influencing the Kd. For soils, the Kd can be derived per soil type of interest taking soil properties into account (pH, organic matter content, clay content, metal loading). For the aquatic compartment, Kd values should be derived under similar water quality parameters (pH, ionic strength, concentration of adsorbing phase) as those prevailing in the region of interest.

For sediments partition coefficients are redox dependent. This can be taken into account using different redox specific partition coefficients. These partition coefficients analysed in oxygen rich and anoxic (N2-atmosphere) sediments can be measured or can sometimes be found also in literature. The choice of a representative realistic worst case kd will have to be made case by case. Some metals form insoluble sulphide complexes in anoxic systems yielding higher Kd values. Other metals shift in redox state (Cr^{6+}:Cr^{3+}) with different sorption capacities.

The known/determined kinetic adsorption-desorption reactions may be fitted to several regression models:

- First order kinetics
- Second order kinetics
- Diffusion equation
- Modified Freundlich equation
- Elovich equation

Besides measuring site specific Kd values, those may also be indirectly estimated using field-validated models. For example:

- *Models based on an established empirical relationship between measured Kd values and soil properties.* Such regression models usually take the form of a linear relation between log Kd and soil properties or a Freundlich type equation. These models are simplifications of the complex soil system, and should not be extrapolated beyond the range of soil properties with which they were originally developed.. Regression models should be based on datasets of high quality that cover a large range of soil properties. From a practical point of view, it is preferable that only routinely measured soil properties (e.g. pH, % organic matter) are included in the model. An advantage of regression models is that these models may be calibrated on a large number of real soils
- *Field-validated surface complexation models.* Surface complexation models such as WHAM/SCAMP (Tipping, 1994, Lofts and Tipping , 1998) consider the soil or sediment as a set of independent reactive surfaces, and combine several models to describe sorption on (solid and dissolved) organic matter, oxides, and clay. These models are conceptually more attractive compared to the empirical regressions. However, extensive input information is needed, and assumptions about the relative reactivity of surfaces compared with model constituents (e.g. % active organic matter) are required. Because these models are derived for pure model constituents under laboratory conditions, an essential condition for their application is their validation for real soil/sediments systems. The use of modelled Kd values can therefore, at present, be used for estimation purposes only. Further research in this area may, however, allow appropriate use of modelled Kd values for future CSAs.

Uncertainty analysis

A Kd value for metals is not one single value and a wide range of Kd values is often observed. Using a range of Kd values in the CSA will help to highlight if the adsorption coefficient is an important factor for the environmental behaviour of the substance and to evaluate if the adsorption coefficient will not affect the outcome of the CSA to a large extent. Typically, uncertainty over the use of a particular value for a specific assessment is investigated by varying the value between high and low extremes – in a kind of sensitivity analysis. If a Kd distribution is available, a low-end value (e.g. 10th percentile) and a high-end value (e.g. 90th percentile) are selected for the sensitivity analysis. In the case of a limited data set, the minimum and maximum must be used as lower and upper bounds as worst-case scenarios. The representatitivity of the data available for the sites under assessment must also be discussed. The results of the uncertainty analysis can be used to check the robustness of the risk evaluation and could trigger further refinements when needed.

Example 2-6: sensitivity analysis PECregional derivation (Pb CSA, 2008).

Table 3: Added/total PEC values for the regional and continental environment

PEC values		**PEC$_{add}$ continental**	**PEC$_{total}$ continental**	**PEC$_{add}$ regional**	**PEC$_{total}$ regional**
K$_p$ sediment/suspended matter = 295,121 l/kg (median)					
PEC surface water (dissolved fraction)	µg/l	0.031	**0.12**	0.12	**0.22**
PEC sediment	mg/kg$_{dwt}$	4.8	**18.8**	19.2	**33.2**
K$_p$ suspended matter = 50,119 l/ kg (10P) ***Kp sediment = 35,481 l/kg (10P)***					
PEC surface water (dissolved fraction)	*µg/l*	0.17	0.26	0.62	0.71
PEC sediment	*mg/kg$_{dwt}$*	5.6	8.5	20.0	23.1
K$_p$ suspended matter = 1,698,244 l/ kg (90P) ***Kp sediment = 707,946 l/kg (90P)***					

PEC surface water (dissolved fraction)	*µg/l*	*0.0055*	0.099	0.022	0.12
PEC sediment	*mg/kg$_{dwt}$*	*3.9*	65.8	15.7	81.6

Increasing the K_p value of suspended matter and sediment by a factor 5.75, from 295,121 l/kg to 1,698,244 l/kg, decreases the added predicted regional Pb concentration in surface water by a factor 6.0, from 0.12 µg/l to 0.02 µg/L. Taking into account the natural background value in surface water, the $PECtotal_{regional}$ value is reduced from 0.22 µg/l to 0.12 µg/l. At lower K_p (50,119 l/kg), more Pb remains in solution (higher Pb $PECadd_{regional}$ concentration in dissolved fraction: 0.62 µg/l) and less Pb will be sorbed on particles (lower Pb concentration in sediment/suspended matter: 20 mg/kg dw).

2.3 Guidance on metal-specific aspects in selecting measured data

2.3.1 Introduction

When using monitoring data care should be taken in selecting only those data conducted according to adequate QA/QC procedures (section 2.3.2). Furthermore, the relevance of certain monitoring data on metals should be carefully considered (section 2.3.2), depending on the purpose of the assessment. For example elevated metal concentrations in the proximity of an identified point source can be used to describe the local scenario, but are less representative for deriving diffuse ambient metal concentrations (regional scenario).

Measured data sets represent the sum of three different fractions:

- the natural (background) concentration,
- the dispersive anthropogenic input due to human activities (historical and recent)
- the site-specific anthropogenic input due to human activities (historical and recent)

Natural background concentrations and baseline concentrations within an environmental compartment may vary markedly by several orders of magnitude. High ambient metal concentrations caused by natural processes (e.g., high background concentrations in samples of geological active areas, rivers flowing through metal-rich areas) must not be discarded from the data set, but they should be separated from the generic exposure dataset and should not be used for generic risk assessment.

With the exception of a few remote and unpopulated areas, true natural background concentrations can hardly be found in the aquatic and terrestrial compartment as a result of historical emissions and current dispersive anthropogenic inputs. Consequently, the term “baseline concentration” is often used to express the concentration corresponding to very low anthropogenic pressure, i.e. in areas where past and present anthropogenic influences are considered low. But in e.g. (old) industrialized or mining areas, the concentration of metal that has been introduced to (or removed from) the environment by man over the past few decades or even centuries can be significant. This added fraction is often referred to as “historical contamination”. In many cases this historical contamination cannot be distinguished from the natural background concentration. The concentrations of metals found at historically contaminated sites often still have a significant influence on the quality of the surface waters, and will also significantly influence the metal levels observed in sediments downstream (section 2.3.3).

Finally, only a fraction of the metal present in the environment may be available for biological uptake dependent on various biotic and abiotic parameters. Consequently, for metals risk assessment purposes it is recommended that, besides background and ambient site-specific/diffuse metal concentrations, the distribution of parameters that determine metal (bio)availability are also be described and integrated in the exposure assessment (section 2.4) when it is deemed appropriate to use (bio)availability models.

For the above reasons, monitoring data on metal concentrations in the environment need to be interpreted and used with caution. In particular, the spatial scale of influence from point sources, the effects of local geology (natural background, section 2.3.3), the effects of historical contamination (section 2.3.3) and bioavailability (section 2.4) should be dealt with in a proper way in the CSA and subsequent exposure scenarios. Such a distinction can be done by an outlier analysis and/or expert judgement from knowledge of the sites situated at the high end of the concentration distribution curve as described in RIP 3.2.

2.3.2 Data selection and handling

Only the most relevant and reliable monitoring data should be incorporated for the purpose of preparing the chemical safety report. The following generic and metal-specific issues require special attention:

Analytical considerations

Sample treatment and analysis of reported metal concentrations should be in line with internationally accepted Standard Guidelines (ISO, ASTM Standards, QA guidelines developed under the EU Water Framework Directive, etc.). Care should be taken that no data are used that are compromised by contamination. Consequently, depending on the level of metal present, the use of “clean” and “ultraclean” techniques for sampling and analysis may be critical in order to obtain accurate data (US-EPA, 1994). For example it could be that sample gets contaminated during the filtering process which could yield dissolved concentration higher than the total concentration. If such contamination is apparent the data should not be used.

- For the aquatic environment, measured dissolved metal concentrations are preferred[3]. In determining the dissolved metal concentration water samples should be filtered (0.45 µm) prior to analysis[4]. The handling of the samples should not affect the dissolved metal fraction in any way; contamination during sampling and filtration should be avoided by using ultra-pure equipment. All laboratory equipment such as glassware, plastics, etc. must be rinsed with a diluted acid solution (e.g. 1% HNO_3 solution) and demineralized water before use in order to remove all metals adsorbed.). Acidification should be done after filtration. Appropriate quality assurance measures (e.g. procedural blanks, assessment of the matrix effect) are recommended.

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[3] If no dissolved data are available, an estimate of this fraction can be made using the total metal concentrations, amount of particulate material in the water sample and relevant physicochemical parameters such as the Kd. Unless these parameters are identified specifically, the indirect estimation of the dissolved fraction has additional uncertainty due to the assumptions related to these parameters.

[4] Different definitions for the dissolved fraction exist. Most often the dissolved fraction refers to the fraction that passes through a filter of 0.45 µm. It should be noted, however, that this definition may not necessarily refer to the metals in solution. In the range of 0.01-0.45 µm colloid inert particles that remain suspended may exist and these could account for 50 % or more of the “dissolved” 0.45 µm fraction

- With regard to the soil and sediment compartment, the digestion procedure of the samples is to be reported and the appropriate data selected. It is recommended that the metal fraction, which is released after *aqua regia* digestion, be used in exposure assessment. The aqua regia ($HCl + HNO_3$) digestion method releases all metal fractions except the fraction built into the crystal structure of the soil. The latter fraction can be determined using other methods (HF, X-Ray Fluorescence). The mineral fraction is not expected to be released over a reasonable time span under conditions normally encountered in nature. Other acids, like NaOAc or $NH_2OH.HCl$, are less strong than aqua regia and will not release all relevant metal fractions. Secondly, the *aqua regia* digestion method is harmonized as an International Standard (EN-ISO 11466 (1995) and is applied in most EU countries. Some countries used standard methods based on nitric acid for sediments or 6 N HCl for soils and hence numerous metal data are also available with this method. These data could be considered on a case by case basis. Potential deviations from the results of Aqua Regia digestion should be documented. For most metals this will not make a difference. In some cases, for example for the water compartment (EN-ISO 15587-2) a possible lower recovery compared to the *aqua regia* digestion method specified in EN-ISO 15587-1 has been observed for Cr, V and Mg.

- Proper analysis of metal monitoring data is quite often hampered by the presence of data where metal concentrations are non-detectable with multiple detection limits. For example the sensitivity of analytical techniques for metals has improved considerably over the last few years. As a result, older monitoring data typically have higher detection limits. For mixed data sets, recent data should be preferred and the detection limits are to be reported.

Data treatment

How to deal with detection limits

Although non-detects (concentrations below the detection limits) are not metal-specific, a lot of monitoring data is available for metals including non-detects. Non-detects may remain included in the exposure analysis. For non-detects in homogeneous data sets, taking the half of the value of the detection limit is commonly used in practice. This value represents the median of all values below the detection limit when an uniform distribution between zero and the detection limit is assumed.

2.3.3 Determination of natural background concentrations and historical contamination

Definitions of metal background concentrations

Natural metal background concentrations can contribute in a significant way to the total metal concentration measured in the environment. Due to the observed spatial and temporal variability, it is almost impossible to attribute single values to natural background concentrations of specific metals within a certain compartment. In certain regions, clearly elevated natural background concentrations can be encountered due to local geological conditions (mineralization).

Furthermore, as a result of historical and current anthropogenic input from diffuse sources the direct measurement of natural background concentrations is challenging in the European environment and most often it has to be evaluated case by case to which extent ambient background concentrations or baseline concentrations reported in some European monitoring databases can be retained as the natural background. The following definitions are used in this context:

- **natural background concentration**: the natural concentration of an element in the environment that reflects the situation before any human activity disturbed the natural equilibrium As a result of historical and current anthropogenic input from diffuse sources the direct measurement of natural background concentrations is challenging in the European environment;
- **ambient background concentration**: the sum of the natural background of an element with diffuse anthropogenic input in the past or present (i.e., influence of point sources not included);
- **baseline background concentration:** the concentration of an element in the present or past corresponding to very low anthropogenic pressure (i.e., close to the natural background).

Methods to determine natural background concentrations

An overview of methods that are available for determining natural/baseline background concentrations is provided in Table 4. Harmonised monitoring data collected in pristine (uncontaminated) areas are the preferred data for the assessment of the baseline concentration. For example the FOREGS Geochemical Baseline Programme (FGBP) database (Salminen et al, 2005) (http://www.gsf.fi/foregs/geochem/) contains recent, baseline concentrations for various environmental compartments (1st order stream water, stream sediment, floodplain sediment, soil, and humus). FOREGS was primarily designed as a standardization tool to derive metal concentrations in "pristine" areas, and thus derives the baseline concentrations in different environmental compartments. The concentrations are not representative of the true natural background concentrations, since they represent the concentration of an element in the present or past corresponding to assumingly very low anthropogenic pressure:.

- For the aquatic environment, the FOREGS database can be used in determining if a certain region has a high natural background or not. Measured baseline levels in surface waters in upstream areas (as measured in FOREGS) could be retained as an estimate for the natural background[5]. It has therefore been common practice to report/use the 10-90th percentiles of the baseline concentrations (from e.g. FOREGS stream waters) as regional background ranges.

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[5] Only relevant for metals that are not (or has not been) anthropogenically emitted to the atmosphere in significant amounts and that has been transported long range

- For soils, the organic layer is removed and only subsoil is sampled, which is likely to lead to an underestimation of the metal baseline concentrations and of the organic matter content. Due to this uncertainty, the FOREGS soil data can therefore not be considered as good quality baseline data to be retained as a representative value of natural background for the top soil compartment.

Further work for the determination of natural metal background concentrations is ongoing and could be used when those data become available.

Table 4: Overview of various methods that can be used to determine background metal concentrations. All presented methods have advantages and disadvantages. The final choice of a method should therefore be clearly substantiated in the CSA. If available preference should be given to direct measurements in uncontaminated (pristine) areas.

Water	**Soil**	**Sediment**
Direct measurement in pristine areas	Direct measurement in pristine areas	Direct measurement in pristine areas
Geochemical modelling: estimation methods on the basis of the contribution of weathering processes (erosion). This method is shown to be well applicable for assessing natural background concentration in aqueous systems (rivers).	The metal background concentration in soil depends on soil composition (sandy soil, clayey soils) and geochemical origin of the soil[6].Several countries (Belgium, The Netherlands, Denmark) have reported regression lines that predict metal concentrations as a function of soil texture: most often the clay content and the organic matter content determine the natural binding capacity of different soil parameters. As regressions were based on measured data, reported metal concentrations may be influenced by anthropogenic activities (e.g., atmospheric deposition) and may therefore be more representative of the baseline concentration than of the natural background. It should also be noted that the regression lines are quite often dedicated to the area for which they have been developed and may therefore not be representative for other soils. The applicability of such regression models to other areas can be validated using available background data from the ecotoxicity data set.	Assessment of metal concentrations in the deeper sediment layers, taking into account anthropogenic contributions and vertical distribution of metals towards these deeper layers. .
Calculation based on natural background concentration in sediment and the equilibrium partition coefficient. This may not be applicable if metal has been redistributed significantly in sediment column by diagenesis.	Information from deeper soil horizons (e.g. C-horizon), which are less affected by atmospheric deposition, can also be used for the estimation of background metal concentrations in pristine soils.	Calculation based on natural background concentration in surface water and the equilibrium coefficient
For surface water having ground water as its origin: assessment of the metal concentrations in the deeper ground water. It must be verified that the well-water or groundwater samples are free of current or historic pollution. Moreover, due to their contact with deeper, mineral rocks, metal background concentrations in these waters can be higher than those in surface waters where there is an additional dilution with rain water.		

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[6] For several metals, so-called “reference lines” were derived by correlating measured ambient background concentrations (total concentrations in the soil-matrix) at a series of remote rural sites in the Netherlands to the percentage lutum (%L) and the organic matter content (%H) of these soils (Ministry of VROM, 1994). The same approach has been followed in Flanders, Belgium (Ontwerp uitvoeringsbesluit, 1995). To this end the 90-percentiles of the ambient background concentrations measured were used. The metal-specific parameters of the regression equations represent the strength of binding of the various metals to soils of different clay and humus contents. The reference lines are not only used to calculate ambient background concentrations at given sites, but also to enable the extrapolation of laboratory toxicity data to standard-soil conditions. Some typical examples of reference lines derived in The Netherlands ([ ] = ambient background concentration in mg/kg soil, L = % lutum, H = % organic matter): $[Cu] = 15 + 0.6 \cdot (L + H)$; $[Zn] = 50 + 1.5 \cdot (2L + H)$ or $[Ni] = 10 + L$.

Determination of historical contamination

Next to elevated background concentrations, the presence of historical contamination may contribute significantly to measured total metal concentration. The contribution of historical contamination to the measured baseline concentration in surface waters can be important in historically contaminated areas (downstream) and in more enclosed water bodies with low turnover (e.g., lakes, reservoirs). Enclosed water bodies should therefore only be used for the determination of baseline levels when there is no indication that metal levels have been affected by anthropogenic contributions in the past. The largest influence of historical contamination is expected to occur in the soil and sediment compartments, which are natural sink compartments for metals and metal compounds.

Influence from historical contamination is not readily identifiable, since there is usually a mix of influences. The influence can be recognised by:

1) Using historical records that may provide information on former activities at sites, even local contamination levels of soils, waste disposal sites, etc.
2) Collecting detailed information about local water concentration profiles: when contamination by a historical site is expected, analysis up-and downstream of the area of influence can provide an idea of the importance of the local influence.
3) Evaluating information about local geology: small-scale ancient metal activity was often associated with local metal geology (natural mineralization can also influence water quality locally)
4) Evaluating the presence of elevated metal levels that cannot be explained by identified sources: these may suggest influence from historical contamination.
5) The use of ratios of stable lead isotopes [7].

2.3.4 Guidance on how to handle natural background concentrations and historical contamination

Historical contamination may contribute to a large extent to observed metal exposure levels. When historical contamination is due to past uses this should not be included for the assessment under REACH but assessed under other regulatory frameworks as necessary. When historical contamination is originating of a current use this should still be part of the evaluation process.

However, even for past uses knowledge on the influence of historical contamination can still be important for implementing risk management measures or developing risk reduction strategies. It is therefore important to compare the various contributing sources; if necessary, the use of approaches such as the added risk approach to have a better understanding of the relative importance of the *local versus regional risks and historical versus today's and future emissions and risks*. In areas influenced by high natural background values or for sites influenced with historical contamination, typically, differences between modelled and measured ambient metal concentrations can occur. Such areas should be assessed in a separate scenario.

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[7] a special case where there are good possibilities to define the anthropogenic influence is lead. The fact that the stable isotopes (^{204}Pb, ^{206}Pb, ^{207}Pb, ^{208}Pb) show different ratios in lead of different origin has successfully been used to separate the influence of historical and recent pollution of lead from the natural component in e.g. lake sediment profiles, peat, soil and teeth and bone tissues. The ratio mostly used in those cases is ^{206}Pb/^{207}Pb (e.g. Renberg et al., 1994, Brännvall et al., 2001). As lead is a common feature of complex sulphide ores, the isotopes may be used as a marker of historical mining activities, focused also on the processing of e.g. silver, copper and zinc.
Weiss et al. (2008) give an overview of the potential use of stable isotopes for a range of inorganic elements.

Guidance on the use of total versus added risk approach

In order to deal with the presence of a natural background, various concepts have been developed, such as the *Added Risk approach*[8] *(Added RA) and the Total Risk approach (Total RA)* concepts. In essence the Added RA assumes that species are fully adapted to the natural background concentration[9] and therefore that only the anthropogenic added fraction should be regulated or controlled[10]. The Total RA assumes that "exposure" and "effects" should be compared on the combination of the natural background and the added anthropogenic concentrations..

Both approaches can be used within a risk assessment context. The main driver for using either one of them is how the background level relates to the derived PNEC value which is in the initial phase derived using the total risk approach. In case it can be expected that the background is significant in comparison with the PNEC the Added RA may be employed as a pragmatic solution. In case the PNEC is close to the background but the use of the Added RA is chosen for regulatory purposes (e.g. Environmental Quality Standard setting) this should be done with care. In areas with high baseline levels it could be that environmental communities are already affected by the metal and every extra contribution of the metal could be detrimental.

It should also be mentioned that (especially for essential metals) also the media of toxicity studies will contain a background concentration. When this level is comparable to the natural background concentration, the outcome of the Added RA and Total RA should not deviate that much. When the background in the test media is remarkably lower, more preference should be given to the Total RA.

Specific guidance on the use of the Total RA and Added RA) is further outlined in Figure 3. The general idea behind the decision process is that the actual choice can either be driven by the question if the background is significant in comparison with the PNEC or by requirements set out in a regulatory context. The approach starts with approaches that require fewer resources and efforts, such as Tiers 1 and 1'. In any event, if the effects data are considerably above the background concentration, the difference between using the added or total risk approach could be negligible.

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

[9] For essential metals this seems most plausible. For non-essential metals this assumption is indeed less obvious.

[10] Although the added risk approach acknowledge that negative effects from the bioavailable fraction of the background concentration on some organisms in the ecosystem may occur, or that organisms may even have become acclimated/adapted to it, from an environmental policy point of view, such effects may be ignored and may even be regarded as desirable, since these effects may in theory lead to an increase in ecosystem differentiation or biodiversity (Crommentuijn et al, 1997).

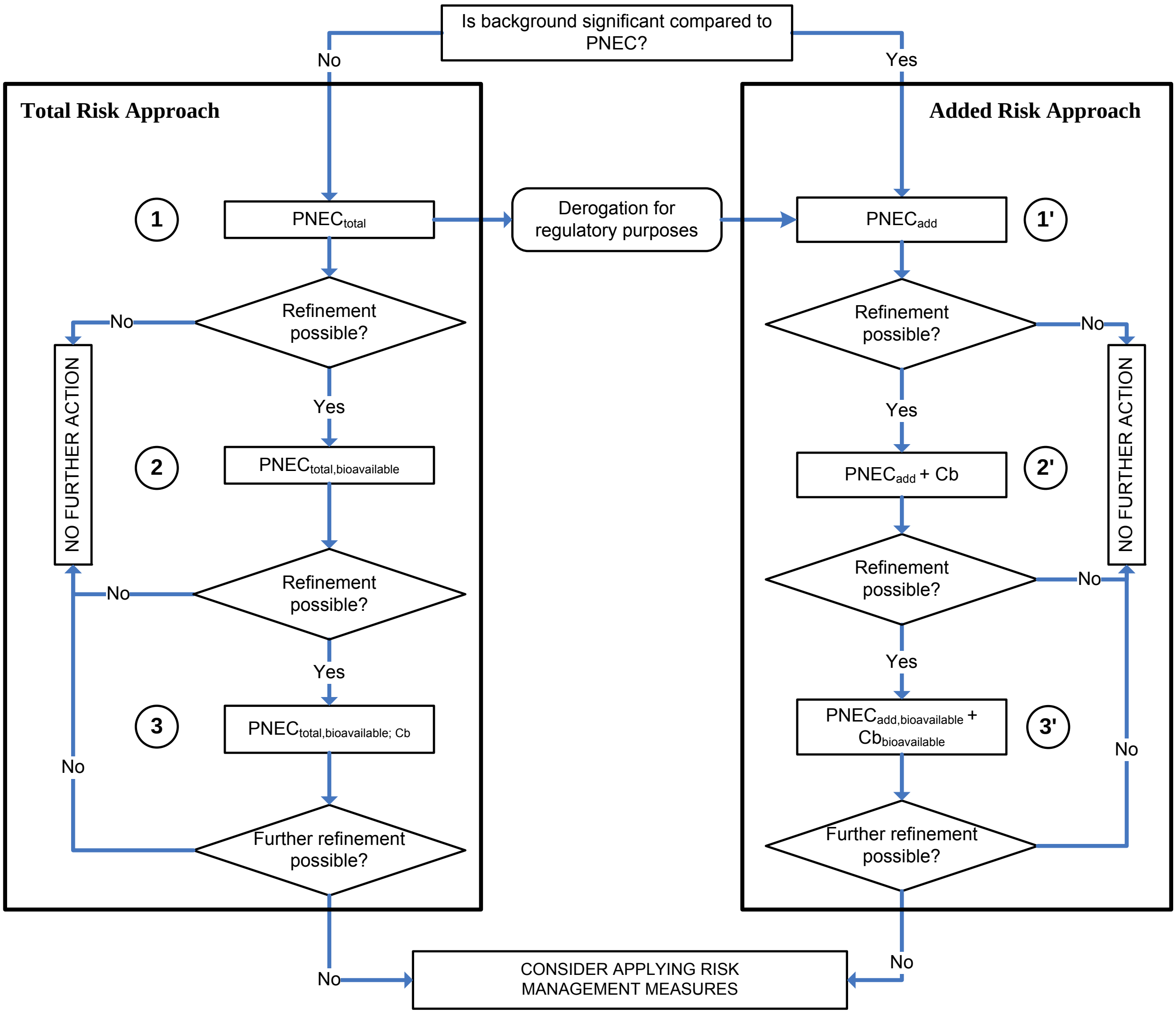

Figure 3: Tiered approach on potential application of total versus added risk concept

Legend:

Total Risk Approach

1. In cases where it can be expected that the background is not significant in comparison with the PNEC the total risk approach can be used. If differences in background need to be taken into account for regulatory purposes, the added risk approach could be chosen (see 1'.)

Potential environmental risks (RCR) are further characterised based on the following quotient:

$RCR = PEC_{total} / PNEC_{total}$

2. If RCR>1, a further refinement is possible when models are available to account for bioavailability Both the NOEC and the background values should be corrected for bioavailability.

Potential environmental risks (RCR) are further characterised based on the following quotient:

$RCR = PEC_{total, bioavailable} / PNEC_{total, bioavailable}$

3. The most accurate and ecologically relevant approach would be to account for both the effects of bioavailability and acclimation/adaptation (~Cb) on the effects/exposure data resulting in a $PNEC_{total, bioavailable + considering Cb.}$

Potential environmental risks (RCR) are characterised based on the following quotient:

RCR = $PEC_{total,\ bioavailable}$ / $PNEC_{total,\ bioavailable,\ +\ considering\ Cb.}$

Added Risk Approach

1'. In case there is a need for regulatory purposes (e.g. Water Framework Directive) or the natural background is significant compared to the PNEC, the added risk approach can be used. The first tier in compliance checking in a regulatory context when using the added risk could be to compare the PEC total with the PNECadd. If the PECtotal is below the PNECadd then consideration of the background (as in 2') will only make this difference bigger. This simple first step would ensure that only sites of concern are taken through the various tiers.

Potential environmental risks (RCR) are characterised based on the following quotient:

RCR = PEC_{total} / $PNEC_{add}$

Where $PNEC_{add}$ = calculated from the $NOEC_{total} - Cb_{culture\ medium}$

Most often Cb culture medium is similar to Cb test medium

2'. In the second tier the PEC total is corrected for the background value .
Potential environmental risks (RCR) are characterised based on the following quotient:

RCR = PEC_{add} / $PNEC_{add}$

Where $PEC_{add} = PEC_{total} - Cb_{site/region}$ and $PNEC_{add} = PNEC_{total} - Cb_{culture\ medium}$

3'. In cases where the added risk approach is used and there are still potential risks bioavailability can be taken into account in which both the NOEC and the background values should be corrected for bioavailability. However, care should be taken in seeing how the background correction is done (see below).

Potential environmental risks (RCR) are characterised based on the following quotient:

RCR = $PEC_{add,bioavailable}$ / $PNEC_{add,\ bioavailable}$

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

2.4. Guidance on the incorporation of bioavailability in the exposure assessment

2.4.1 Introduction

An important paradigm that controls the potential risks of metals and metal compounds due to exposure to metals and metal compounds is bioavailability. It is now clear that the dissolved free ionic metal species is far more bioavailable than most complexed metal species. It is also well established that many geochemical factors influence the metal speciation in water, sediment, and soil. Paradigms that explain the relationships between these geochemical factors and metal bioavailability and toxicity are explained in section 3. The information requirements in order to apply these concepts are fairly extensive. The choice of the concentrations of the physicochemical parameters that modify metal bioavailability needs to be representative for the environment under consideration.

Guidance on the measurement/selection of the main abiotic factors that drive bioavailability for the various compartments is given in Table 5. It is emphasized that, for the final risk characterisation, both exposure and effect concentrations should be expressed at the same level of (bio)availability.

Table 5: General recommendations for measuring some abiotic factors driving bioavailability for metals

Parameter	Relevance	Measurement recommendations
Aquatic compartment		
• Major cations (Ca, Mg, Na K, …),	Presence of cations like Ca and Mg may compete with the metal cations and thus reduce metal toxicity due to competition with metal ions for binding to the site(s) of toxic action.	Individual dissolved cation concentrations should be measured in filtered (0.45 µm) water samples
• pH • alkalinity	pH determines the metal speciation and, hence, the fraction of a metal that is present in a bioavailable (and toxic) form, e.g., the fraction of free metal ion will generally decrease with increasing pH. pH also determines the amount of protons which can compete with metal ions for binding to the site(s) of toxic action. Alkalinity is the water's capacity to resist changes in pH that would make the water more acidic, i.e., it is a measure for the capability of water to neutralize acid. In many natural water bodies the buffering system is carbonate-bicarbonate (H_2CO_3, HCO_3, and CO_3). These compounds can form complexes with dissolved metal ions and their presence can therefore affect metal speciation and bioavailability	Determination of the pH and alkalinity should be performed in the water body itself or in the sample immediately after it has been collected: pH of an enclosed water sample may change rapidly and is not always relevant for the sampled water body.
• DOC	Complexation of metal ions with dissolved organic carbon may affect metal bioavailability (and toxicity).	The dissolved organic carbon fraction should be determined in filtered (0.45 µm) water samples. As there is no clear relationship between total and dissolved organic carbon, determination of the total carbon content is not an acceptable alternative for the dissolved organic carbon fraction.
Sediment compartment		
• AVS	Sulphides form insoluble metal sulphide complexes with cationic metals, rendering metals unavailable	Spatial and temporal variability should be taken into account when sampled. It is recommended not to sample in summer time, when AVS (Acid Volatile sulphide) levels are expected to be high. Sampling depth should be 0-5cm.
• OC	Increasing organic matter content can result in decreasing bioavailability for both cations and anions	Organic matter = organic carbon*1.72 or directly determined by loss on ignition (only if organic matter >5%)

• pH	Increasing pH causes an increase in Kd value for cationic metals, and decreasing Kd values for anionic metal ions. However, sediment systems are better buffered then soils	Measured in 0.01 M $CaCl_2$
• Eh	Reduced conditions mitigate metal toxicity (presence of sulphides and change in redox state metals, e.g. Cr^{3+} versus Cr^{6+})	Redox potential should be measured in situ by preference
Soil compartment		
		General comment on soil sampling: representative sample of the top 10 (grassland) to 20 cm (arable land) should be taken, after removal of the litter layer (=organic matter still recognizable as leaves, twigs etc.) on top, but including the dark humus layer.
• pH	Increasing pH causes an increase in Kd value for cationic metals, and decreasing Kd values for anionic metal ions.	Measured in 0.01 M $CaCl_2$
• OC	Increasing organic matter content can result in decreasing bioavailability for both cations and anions	Organic matter = organic carbon*1.72 or directly determined by loss on ignition (only if organic matter >5%)
• eCEC	Increasing eCEC (effective Cation Exchange Capacity) causes may cause decreasing bioavailability for cationic metals in the soil	Measured at in situ soil pH (i.e. not at a buffered pH)
• Clay content	Increasing clay content (including oxides) can result in decreasing bioavailability for both cations and anions	Soil fraction smaller than 2 µm

In cases where a lot of monitoring data are available on the physico-chemical parameters influencing bioavailability, country-specific or region-specific distributions for these parameters should be elaborated. *Reasonable worst case conditions*, at regional scale, can then be defined as the lower (e.g. 10th %) or higher (e.g. 90th %) values of the obtained distribution of bioavailability modifying factors depending on the bioavailability models used. *Typical conditions* generally refer to average values of these parameters. A simple combination of low/high values may not always result in a realistic scenario in cases where influencing parameters co-vary. Consequently, where different physico-chemical parameters influence the bioavailable fraction of the metal, the reference scenario should, as far as possible, be a realistic combination of the relevant parameters. In local risk assessment scenarios, site-specific environmental data can also be used.

2.4.2 Guidance on the use of the 'ecoregion driven approach"[11]

In cases where no site/region-specific measurements on abiotic factors are available, the (bio)availability concept as described above could still be applied using a set of default scenarios representing specific geographical characteristics. This approach is often called the "ecoregion approach". The ecoregion concept was originally developed to classify ecologically similar areas into **"**ecoregions," based on the recognition that ecosystems differ across large spatial scales resulting in ecologically distinct areas responding differently to environmental stressors, such as elevated levels of naturally occurring elements (e.g. metals) (ICMM, 2002).The original concept was built around the need to derive region-specific PNECs values, based on testing conducted with regionally relevant organisms that are acclimated to the elevated background levels of metals within each region However, our current understanding does not yet allow to take acclimation and adaptation issues properly into account. As such the ecoregions defined in this guidance document are not likely to be contiguous with the original definition of ecoregions. In this guidance document the eco region concept is used in a way to allow only to correct for differences in abiotic parameters (present in the different ecoregions) that are potentially affecting (bio)availability. As such these ecoregions should be merely considered as representative typical examples of specific EU conditions for which the critical parameters needed to run the (bio)availability model are readily available. This allows to parameterize the (bio)availability models without the effort of collecting an extensive database on site/region specific abiotic factors. This approach would in essence still allow the REACH registrant to set region-specific PNECs for a set of default example scenarios represented by various abiotic factors.

Potential environmental risks associated with the presence of a metal in a particular river/lake/soil can as such be assessed by comparison of the abiotic factors with those of a likely comparable typical scenario. I.e. in case it can be expected that the main abiotic factors mitigating the toxicity of the metal are comparable, potential risks in a particular river/lake is assessed through the comparison of the metal concentrations in that particular river/lake (PECriver/lake) with the normalised PNEC of the typical scenario with comparable values of abiotic factors. No further actions/refinement is required in case the PNEC typ. 1→x is not exceeded.

In case of potential risks identified or in case the values of some of the abiotic factors controlling the metal toxicity are significantly different (especially when a higher bioavailability of the metal is expected, e.g. in case of significantly lower DOC concentrations), it is recommended to calculate a normalised PNEC for the particular river/lake. In particular case more information on site specifc abiotic factors for the lake/river/soil of concern should be compiled and the PEC should be compared with the normalized PNEC for that particular river/lake/soil.

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[11] More specific information is also contained in the EU Risk Assessment Report on Nickel.

This concept is further elaborated for the aquatic and terrestrial compartments in Example 2-7.

Example 2-7: Scenario development for selected examples of typical EU ecoregions (Metal-CSA)

Aquatic environment

In the Ni-CSA, aquatic toxicity data were normalised towards typical physico-chemical conditions occurring in typical region specific EU surface waters. In order to achieve this, abiotic factors mitigating chronic Ni toxicity from both lakes and rivers were collected from different regions in the EU. The different scenarios were selected to provide *examples* of typical conditions covering a wide range of physico-chemical conditions (pH between 6.67 and 8.2; hardness between 27.8 and 260 mg/l $CaCO_3$, DOC between 2.5 and 27.5 mg/l) occurring in EU surface waters. Please note that for other metals maybe also other parameters could be important. The various scenarios considered are summarized in Table 6. In this exercise small (± 1,000 m³/d), medium-sized (± 200,000 m³/d) and large (± 1,000,000 m³/d) alluvial (eutrophic) rivers were considered. In addition, an example of a typical Mediterranean river was also included in this report. For the lakes, the focus was on the gathering of physico-chemical data for sensitive systems, i.e. oligotrophic and neutral-acidic lakes.

Table 6: Summary of the physico-chemical characteristics of the different selected examples of typical EU ecoregions in the Ni-CSA

	Water type	**Name**	**pH**	**H (mg/l $CaCO_3$)**	**DOC (mg/l)**
Rivers	Small (ditches with flow rate of ± 1,000 m³/d)	/	6.9	260	12.0
	Medium (rivers with flow rate of ± 200,000 m³/d)	River Otter River Teme	8.1 7.6	165 159	3.2 8.0
	Large (rivers with flow rate of ± 1,000,000 m³/d)	River Rhine	7.8	217	2.8
	Mediterranean river	River Ebro	8.2	273	3.7
Lakes	Oligotrophic systems	Lake Monate	7.7	48.3	2.5
	Neutral- acidic system	/	6.7	27.8	3.8
Boundaries	FOREGS database		6.4-8.3	/	0.9-17.0
	Swad database		6.6-8.1	16.4-253	2.6-12.4
	BLMs		5.5-8.5	6.3-480	0-18.4

It is emphasized that the abiotic factors of all selected scenarios are within the boundaries of the chronic BLMs for Ni.

Table 7 gives a more conceptual outline of the relative description of pH, hardness and DOC for the ecoregion scenarios, and the relative bioavailability for nickel that results from the combination of the abiotic parameters.

Table 7: Relative descriptions of pH, hardness, and DOC for the ecoregion scenarios, and the relative bioavailability that results from the combination of the abiotic parameters.

Scenario			Reference river(s)/lake(s)	Relative (bio)availability*
pH	Hardness	DOC		
L	H	H	Ditches	L
H	M	L	River Otter	H
M	M	H	River Teme	M
H	H	L	River Rhine	M
H	H	M	River Ebro	M/H
M	L	L	Lake Monate	H
L	L	M	Acidic lake	M

* the relative (bio)availability is metal specific. Here the relative bioavailability for nickel is given. L = low, M = medium and H = high.

The observed variability in abiotic parameters in these existing river/lake types results in major differences in bioavailability for nickel. It is hence relevant to define the regional PNEC on a "waterbasin-type" basis. This is consistent with the water basin-specific approach recommended for implementing bioavailability for the Water Framework Directive, as it ensures a commonprotection target for all surface waters in Europe.

Terrestrial environment

In the Cu CSA, eCEC, pH, OM and clay content were identified as the physico-chemical parameters influencing the toxicity of copper in soils. Different scenarios were selected to provide examples of conditions covering different land uses and covering a wide range of physico-chemical conditions in the EU that can affect Cu bioavailability and toxicity to soil organisms (pH between 3.0 and 7.5; CEC between 2.4 and 36 cmol/kg, clay between 7 and 46 %).

Table 8: Summary of the physico-chemical characteristics of the various examples of typical EU ecoregio**ns** in the Cu-CSA.

	Soil type	**Soil use**	**Country**	**pH**	**OM%**	**Clay%**	**CEC cmol/kg**
Agriculture	**1. Acid sandy soil**	Arable land	Sweden	4.8	2.8	7	2.4
	2. Loamy soil	Arable land	The Netherlands	7.5	2.2	26	20
	3. Peaty soil	Grassland	The Netherlands	4.7	40	24	35
Nature	**4. Acid sandy soil**	Forest	Germany	3.0	9	7	6
	5. Clay soil	Woodland	Greece	7.4	4.5	46	36
Agriculture + nature	**6. Loamy soil**	Arable + forest	Spain	6.2	2.7	17	12.8

3. EFFECTS ASSESSMENT

Aim and structure of this section

The aim of this chapter is to explain the various metal specific considerations that should be taken into account in the effects assessment part of a CSA. Generic guidance on selecting toxicity data and deriving a PNEC for the various environmental compartments is already addressed in the guidance documents on the information requirements for REACH.. However, similarly as for the exposure assessment, metal-specific aspects should be taken into account when selecting effects data for hazard assessment purposes for metals and metal compounds. In the first part a short summary is given of the metal-specific points that should be taken into account when selecting toxicity data for PNEC derivation Most of the guidance, however, is focused on how to account for (bio)availability in the aquatic, sediment and terrestrial compartment. The general outline of this chapter is given below.

3.1 Guidance on information requirements for toxicity data used for metals and metal compounds

Data selected for PNEC derivation for metal and metal compounds should adhere to the information requirements presented in the general guidance documents on information requirements for REACH This guidance document already contains the metal specific points of attention. In short, the following metal specific aspects were considered to be relevant in evaluating the appropriateness of ecotoxicity data for metal risk assessments:

- A proper description of the physico-chemical test conditions[12] that influence the speciation (i.e. availability) and bioavailability and toxicity, where these parameters are known.
- A strong preference for using measured data of the metal concentrations in the test media because of potential issues related to natural background, to analytical errors and to the limited solubility of some metals and inorganic metal compounds. In artificial media, where the metal background concentration is often very low compared to the effects levels, nominal concentrations can normally be used as long as the tests are based on soluble metal salts and the background concentrations in the test media are known[13]. When natural waters, sediments or soils are used instead of artificial test media, there could be a concern about the use of nominal values when the derived NOEC/EC_{10} values are close to the reported background values of the natural water, sediment or soil used, as these concentrations could potentially contribute to the observed toxicity in a significant way and, as a result, the use of a nominal value would overestimate toxicity.

- For sparingly soluble metals, measured data on the dissolved fraction are always required in order to obtain reliable toxicity test data.

- In the case of testing essential metals and metal components a proper description of the culture conditions, specifically related to the level of essential metals and inorganic metal compounds added or already present in the culture media could give valuable insight on issues such as acclimation.

- The possibility of hormesis[14] effects, observed for essential nutrients, needs to be considered when evaluating the calculation of EC_{10} values beyond the lowest tested concentration. In such cases, as positive effects should not be considered in the derivation of ECx other models than the conventional log-logistic dose-response model should be used to fit the toxicity data. For example the linear-logistic model of Brain and Cousens (1989) has been extended to allow EC_{50} and EC_{10} calculations (Van Ewijk and Hoekstra, 1993; Schabenberger et al., 1999, Cedergreen et al, 2005) in the case of hormesis.
- Test media containing chelators (e.g. EDTA) should be excluded from PNEC derivation.

- Artificial sediments used in studies should be characterised (e.g. particle size, organic matter (OM), cation exchange capacity (CEC)/anion exchange capacity (AEC)). If natural sediment is used in the test, it should be characterised preferably by origin, pH and ammonium content of pore water, total organic carbon content and nitrogen content, particle size distribution and percent water content. Also SEM (= Simultaneously Extracted Metals) and AVS (= Acid Volatile Sulphides), concentrations should preferably be measured as well as FeO.

- The kinetics of Me-DOC binding in aqueous test media demonstrated the need for an equilibration period between the metal and the test media prior to exposing the organisms in order to allow full Me-DOC binding. A pre-equilibration period of 12 hours has been

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[12] e.g. water: dissolved organic carbon concentration, water hardness, pH, alkalinity, presence of complexing agents such as humic acids and EDTA; e.g. soil: pH, CEC, organic carbon, metal background; e.g. sediments: organic carbon, Acid Volatile Sulphides, Fe-Mn oxyhydroxides etc): other cations and anions etc.

[13] For trace nutrients (Fe, Zn, Cu…), the addition of background concentrations to test media may be substantial and need to be considered

[14] Hormesis has been observed for both metals and organic substances and has been related to enhanced performance at low levels of induced stress (= at lower test concentrations).

recommended (e.g. Cu data). This may not be relevant when assessing acute effects at intermittent releases.

- Equilibration time water-sediment: after spiking the water-sediment system with the test substance, an equilibrium period is necessary to ensure partitioning of the substance between water-phase and solid-phase according to the substance-specific distribution characteristic. For metals and inorganic metal compounds, it is recommended that the concentration of the test substances be measured in the overlying water of semi static and static sediment toxicity tests, and that testing be initiated only when the overlying water concentration reaches stable concentrations (this can be more than 2 months for metals).

- Oxidation state. Many metals have more than one oxidation number, which poses several additional complications. Firstly, chemical characteristics, and thus toxicity can vary markedly between different oxidation states. Consequently, the oxidation number of the trace element(s) in a given substance must be known. This is not necessarily a trivial problem, as some materials could conceivably contain mixed oxidation states. Secondly, some oxidation states may be unstable in specific or all environmental compartments, meaning that distinct changes in bioavailability may occur during even a short-term toxicity assay (e.g. Cr(III)/Cr(VI)).

3.2 Read-across and QSAR

In case ecotoxicity data are lacking for a specific metal or metal compound, read-across ecotoxicity data from other inorganic compounds of the same metal could be considered. The basic assumption using this approach is that it is the bioavailable metal fraction that is causing the effects (e.g free metal ion or other specific metal species complexes (e.g. $CuOH^+$). Ecotoxicity data of simple soluble metal salts can be combined on condition that the metal ion is responsible for the effects observed for the metal salts considered (e.g. $CuSO_4$, $CuCl_2$). Appropriate standard ecotoxicity effects data (acute, chronic) measured for such soluble metal salt are thus combined by expressing the effects data (NOECs and PNEC) as dissolved (bioavailable) metal ion concentration (µg Me/L).

The development of QSAR methods for metals and inorganic metal compounds has not been as actively pursued as for organic substances. However, for some inorganic substances, predicting toxicity from chemical properties may be relevant. In this respect, Quantitative Ion Character-Activity Relationships (QICARs) and Quantitative Cationic-Activity Relationships (QCARs) have recently been developed (Ownby and Newman 2003, Walker et al. 2003). More research efforts are needed in this field, however, in order to develop and validate appropriate models.

3.3 Guidance on the derivation of the PNEC for metals and metal compounds

The number of available toxicity data for metals and metal compounds can vary widely, between no or a few data ($n < 3$, data limited metals) to more than 50 or 100 values (for data-rich metals). General rules for the derivation of a PNEC are provided in the guidance document on information requirements and chemical safety assessments. Depending on data availability, PNECs can be derived through the application of assessment factors or derived on the basis of statistical extrapolation. Calculation of a PNEC water/soil/sediment 1 using statistical extrapolation techniques can be considered when sufficient data are available (see section R.10.3.1.3 for minimum requirements).

For comparable data on the same end-point and species, the geometric mean should be used by default as the input value for the calculation of the species sensitivity distribution. When results are available from tests using different soils, sediments or water types and it is likely that the characteristics of these sediment, soil, or water types influence the results, the effect data should be normalised before further processing. If this is not possible, the lowest NOEC per end-point and species should be used.

If "(bio)availability" refinement is not possible following on from the analysis of the ecotoxicity data, a $\mathrm{PNEC}_{\mathrm{generic}}$, i.e. the PNEC not corrected for any "(bio)availability" should be derived. In cases where physico-chemical modelling and/or bioavailability models can be applied, this generic PNEC may be modified to:

1) a specific PNEC normalised to specific local or regional conditions (i.e. $\mathrm{PNEC}_{\mathrm{local,\ bioavailable}}$ or $\mathrm{PNEC}_{\mathrm{regional,\ bioavailable}}$). These specific conditions can be defined on a case-by-case basis, generally using typical values for the bioavailability modifying factors;
2) or a reference PNEC normalised to realistic worst-case conditions, i.e. $\mathrm{PNEC}_{\mathrm{reference,\ bioavailable}}$.
3) or a PNEC representative of a specific standard region (ecoregion approach)

The way a PNEC can be derived for metals and metal compounds for the various environmental compartments and how bio(availability) can be taken into account is explained in more detail in the subsequent sections. In section 4 guidance is given on the practical implementation of these concepts in the risk characterization phase.

3.4 Guidance on the incorporation of (bio)availability in the aquatic effects assessment

3.4.1 Use of dissolved concentrations

A rudimentary way of taking into account (bio)availability is the use of dissolved concentration. If dissolved concentrations are not given, the relation between the total and dissolved metal concentrations in ecotoxicity media has to be checked. For some metals (e.g. Cu/Zn), the data demonstrated that for these toxicity data no additional conversion to a dissolved fraction has to be applied (i.e. the total concentration can be set equal to the dissolved concentration[15]). For other metals, however (e.g. lead), evidence could be available to show that not all the metal is dissolved. Under these conditions, an additional conversion to a dissolved fraction has to be applied (Example 3-1). If natural waters are used, total concentrations can be recalculated to dissolved concentrations using partition coefficients.

Example 3-1 Correction for dissolved Pb concentration

For Pb, differences between total and dissolved metal concentrations might occur in toxicity tests and hardness is the main mitigating factor influencing the % dissolved Pb in the test media. Following that line of reasoning, the US EPA (1996) proposed a hardness dependent conversion factor (CF) for converting total recoverable Pb to dissolved Pb (Table 9) based on the following equation: CF=1.46203 - (ln(hardness)(0.145712)). Toxicity data

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[15] It must be demonstrated that organic particles (from e.q. faeces and food) that appears in the test systems throughout the test, do not significantly affect the dissolved metal concentration in the test.

expressed as total recoverable Pb could therefore be converted to dissolved concentration as follows: $EC_{10,dissolved} = EC_{10,total} * CF$.

Table 9: Conversion factor for Pb as a function of hardness.

Hardness (mg/l CaCO3)	Conversion factor (CF)
25	0.99
50	0.89
75	0.83
100	0.79
125	0.76
150	0.73
175	0.71
200	0.69
225	0.67
250	0.66
275	0.64
300	0.63
325	0.62
350	0.61
375	0.60
400	0.59

Case 1:
A chronic toxicity assay to *Oncorhynchus mykiss* performed in a well water with a hardness of 353 mg/l resulted in a total measured $EC10_{total} = 144$ µg total Pb l^{-1}

A conversion factor of 0.61 at a hardness of 353 mg/l was calculated. The calculated dissolved $EC10_{dissolved}$ was therefore: $EC10_{dissolved} = EC10_{total} \times CF = 144$ µg total Pb $l^{-1} \times 0.61 = 87.4$ µg dissolved Pb l^{-1}

Case 2:

A chronic toxicity assay to *Lepomis macrochirus* performed in a well water with a hardness of 44 mg/l resulted in a total measured $EC10_{total} = 70$ µg total Pb l^{-1}

A conversion factor of 0.91 at a hardness of 44 mg/l was calculated. The calculated dissolved $EC10_{dissolved}$ was therefore: $EC10_{dissolved} = EC10_{total} \times CF = 70$ µg total Pb $l^{-1} \times 0.91 = 63.7$ µg dissolved Pb l^{-1}

3.4.2 Use of speciation models

In cases where appropriate (externally validated), speciation models (e.g. WHAM, MINTEQA2, CHESS, etc (see Box 3.1)) and relevant input data (i.e. main physico-chemical parameters driving the availability of a metal such as pH, DOC,…) are available, NOEC and/or EC_{10} values should be expressed on the basis of the metal species of concern[16] in order to reduce uncertainty. If no specific information on relevant physico-chemical parameters is available, then speciation models should not be used unless the possibility of using default values instead for some of these parameters can be substantiated. If there is a concern that the investigated metal binds strongly on colloids, this should be considered when calculating the speciation of dissolved metal because colloids can pass through filters and if ignored may have an impact on the outcome of the speciation exercise. However, at the moment our understanding on colloids is limited and further research is needed in this field.

Box 3.1*:* Chemical speciation models and the importance of natural organic matter (NOM)

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[16] Most often this is the free metal ion but it should be noted that the free ion is not necessarily the best predictor for all metals, and other metal species such as neutral species (e.g. AgCl, HgS) and anionic species (e.g. SeO^{2-}, AsO_4^{2-}) may contribute to the observed toxicity (Campbell, 1995).

Currently there are a number of chemical speciation models or equivalent models that provide a good characterization of the metal chemical species in a solution containing inorganic ligands and well-characterized organic ligands. As binding of metals to organic matter are often one of the most dominating processes in natural water, it is essential that such speciation models include an accurate description of Natural organic Matter (NOM)-reactions with trace metals. NOM is not a homogeneous and well-defined substance and considerable variability can be observed in the structure and properties of NOM isolated from different sources. Given the complexity and variability of NOM and its importance in understanding metal bioavailability and as a critical input parameter to in speciation models and BLM (3.4.3) the question may arise if the variability in NOM need to be addressed when considering NOM effects on metal bioavailability? Comparative studies have shown that the NOM from different sources have very similar behaviour with respect to metal binding properties. Although some additional explanatory power may be attributable to variation in NOM quality, the effect overall is small especially relative to the primary effects of NOM quantity and NOM chemistry. These major effects are already well described by speciation models such as Windermere Humic Aqueous Model (WHAM, Model VI) and NICA-Donnan (Tipping, 1994/1998, Benedetti et al., 1995; Kinniburgh et al., 1996) and these model formulations can therefore be used to predict NOM effects. At most, the remaining differences that have been observed in chemical speciation measurements can be corrected by the use of a simple scaling parameter.

3.4.3 Use of Biotic Ligand Models (BLM)

In cases where ambient dissolved metal concentrations are reported and chronic BLMs[17] have been developed and validated for the metal/metal compounds of concern the NOEC and/or EC_{10} values should be expressed preferentially on a 'bioavailable' basis. In general, a given BLM will account for both the interactions of a metal ion with the media, which should be common to each model, and the interaction of the available forms of the metal with the organism, which is organism-/species-specific. This is because the competition for uptake between the free metal ion and other cations and protons at the site of toxicity is influenced by biological factors, e.g. the relative affinity for a metal ion versus a cation or proton at the uptake site can vary among organisms (see box 3.2). Since dietary exposure is not intrinsically incorporated in the BLM the relative importance of this exposure route should be evaluated on a case by case.

Box 3.2 Biotic Ligand Model concept

During recent years, the Biotic Ligand Model (BLM) has been proposed as a tool to evaluate quantitatively the manner in which water chemistry affects the speciation and biological availability of metals in aquatic systems. The BLM approach has gained widespread interest amongst the scientific, regulated and regulatory communities because of its potential for use in developing water quality criteria and in performing aquatic risk assessment for metals.

The conceptual part of the Biotic Ligand Model 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 BL. The final component is the relationship between metal binding to the biotic ligan (BL) and the toxic response.

Figure 4 shows the BLM-concept for zinc . Free zinc ions (Zn^{2+}) bind to the biotic ligand of organisms, which may be transport sites and / or toxic action sites. The concentration of Zn bound to the biotic ligand is directly proportional to the toxic effect, and independent of the physicochemical characteristics of the test medium.

The chemical activity of Zn^{2+} is, however, reduced by binding to organic (dissolved organic carbon, DOC) and

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[17] the Biotic Ligand Model (BLM) mathematically integrates the interaction of trace metal with solution phase ligands to predict its speciation and its subsequent interaction with receptor sites (the biotic ligand) on the organism (ICMM fact sheet N° 7).

inorganic ligands that reduce the bioavailability and thus reduce the toxicity. Inorganic ligands include OH^- and CO_3^{2-}. The concentrations of these ligands are increased at increased pH and increased alkalinity of the test medium, respectively. Cations in solution can compete with zinc for the biotic ligand, which also reduces bioavailability tot the biotic ligand and thus reduces toxicity. The speciation of Zn^{2+} is calculated by the WHAM V-model (Tipping, 1994), which is an integral part of the BLM software.

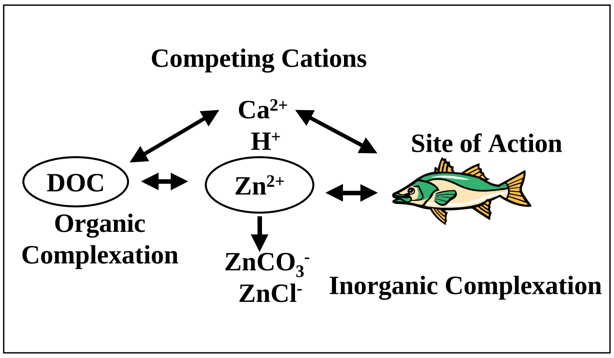

Figure 4: Summary of the BLM-concept

Chronic BLMs exist mostly for only a limited number of species representing various trophic levels (algae, fish, invertebrates). Toxicity data generated for these species under different abiotic conditions can be normalised to a common set of abiotic conditions (e.g. ecoregion) as long as these abiotic parameters fall within the geochemical boundaries of the model (e.g. range of pH, hardness, DOC). The use of organism-/species-specific models should be used as much as possible for that purpose. Guidance on how to develop a BLM is given in Example 3-2.

Example 3-2: general guidance on BLM development

The Biotic Ligand Model is the combination of a speciation module and a competition module, and both parts need to be developed in order to obtain a fully operational BLM for a specific metal. Background information on the principles of the BLM and BLM development can be found, for instance, in "Comparative biochemistry and Physiology, Volume 133C (2002); Special Issue: The Biotic Ligand Model for Metals – Current Research, Future Directions, Regulatory Implications".

1. Metal speciation module

An adequate and reliable prediction of the metal speciation in natural waters is essential for the development of a BLM, as the activity of the free metal ion (or other speciation fractions that may contribute to the overall toxicity) forms the base of BLM- predictions and normalizations. It may therefore be necessary to extend existing metal speciation models (e.g., WHAM 6.0) to enable speciation calculations to be carried out for the metal under investigation. The chemical constants that are required for this purpose can be found in open literature.

2. Development of the competition module

Step 1: Identification of data requirements
As a first step a data gap analysis on available, relevant information in literature should be conducted. Information should be collected on the following subjects:
1) Identification of physicochemical parameters that mitigate toxic effects

- Literature data
- Reduced test design: investigating the effect of a low/high concentration of a major cation on metal toxicity while all other concentrations of potential mitigating compounds are kept low and constant in the test medium [18]

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[18] for metals that only exist in an anionic form in the environment (e.g., molybdate, vanadate, arsenate), the effect of major anions instead of cations should be investigated. Until now no BLM for these type of metals have been developed

2) Collection of available binding constants of the metal under investigation (and mitigating compounds) to biological membranes (e.g., gills) and dissolved organic carbon (fulvic acids, humic acids).

Step 2: derivation of BLM constants

Derivation of binding constants for relevant free metal fraction(s) and competing ions to the biotic ligand. These constants can be determined on the basis of:

- Effects data generated by varying one abiotic factor while keeping all other mitigating factors as constant and low as possible (univariate test designs (cf. methodology described by De Schamphelaere and Janssen, 2002)

or

- Iterative fitting of a model to reliable existing effects data (if present), using available binding constants from literature as a starting point

3. Model validation

Natural surface water samples should be collected and should undergo a full physicochemical characterisation. This information is used as input parameter for the BLM which predicts the toxicity of the metal in the water sample (i.e., based on its speciation and occurring competing processes in the natural surface water samples). The predicted effects are then compared with observed effect levels that are generated in toxicity tests performed in the metal-spiked natural water sample. Model performance is evaluated by comparing observed and predicted effect levels in a 1:1 plot, and variation between both parameters should be equal or less than 2; a factor of two is considered to be a relevant value due to the natural variation in toxic response that is encountered in ecosystems under normal conditions.

3.5 Guidance on the incorporation of (bio)availability in the sediment effects assessment

Natural sediments used in ecotoxicological tests differ in characteristics such as organic matter, clay content and contents of sulphides. The (bio)availability of the test compound, and therefore the toxicity observed, is influenced by these sediment properties. If no data are available, toxicity has to be tested in a reasonable worst-case scenario, i.e. a sediment with high bioavailability of the metal substance tested. This ensures that results are protective for the majority of sediments. In cases where no SEM-AVS measurements are available the sediments t for which the bioavailability is limited due to the presence of substantial amounts of AVS should be excluded for the PNEC derivation. Only toxicity values originating from aerobic sediments with expected low AVS levels (e.g. artificial sediments or natural sediments with low OC and high sand fraction) should be used for deriving the $PNEC_{rwc}$.

3.5.1 Organic carbon normalisation

For metals that have a high affinity to bind with organic carbon, it is worthwhile exploring whether a linear relationship can be established between the observed toxicity levels and the presence of organic carbon. If a relationship can be discerned the variability introduced by the presence of toxicity values generated at different organic carbon concentrations can be captured by normalizing each $NOEC/EC_{10}$ value using the following formula:

$$NOEC / EC10_{OC,\,normalized} = \frac{NOEC / EC10_{total}}{fOC} \qquad \text{(Equation 2)}$$

$NOEC/EC10_{total}$ (mg Me/kgdw)
fOC = fraction organic carbon
$NOEC/EC10_{OC,\,normalised}$ (mg/g OC)

The PNEC sediment can be translated back to mg/kg dry wt. when a default OC value is assumed for the area/region under investigation. In the EU, a standard sediment has a default OC value of 5 % (example 3-3). In a similar way, the normalization could be performed with other sediment ligands such as Fe/Mn oxy hydroxides when it can be shown that a relationship exists between the observed toxicity and the ligand.

Example 3-3: organic carbon normalization (Cu)

The effect of organic carbon on the toxicity of copper was observed for all sediment species but explicitly proven by conducting tests with OECD sediments (containing no AVS) with high (9.8 % OC) and low (2.6% OC) organic carbon concentrations for the species *Tubifex*, *Hyalella* and *Chironomus* (Table 10).

Table 10: Means and ranges (across endpoints) of the ratio NOEC or EC_{50} in 9.8% OC to the NOEC or EC_{50} in 2.6% OC

	Total Cu (mg/kg dry weight)				OC-normalised Cu (µmol/g OC)			
	NOEC ratio		EC_{50} ratio		NOEC ratio		EC_{50} ratio	
	mean	range	mean	range	mean	range	mean	range
Tubifex	6.3	4.2-7.3	2.4	2.2-2.8	1.7	1.1-2.0	1.7	1.3-2.1
Hyalella	10	-	3.3	-	2.7	-	1.1	-
Chironomus	5.2	4.9-5.7	6.2	4.7-7.7	1.4	1.3-1.5	1.7	1.3-2.0
Overall	7.2	4.2-10	4.0	2.2-7.7	1.8	1.1-2.7	1.5	1.1-2.1

Overall, the results in Table 9 clearly illustrate that the uncertainty in toxicity due to a difference in organic carbon content is reduced from an average of factor 4.0-7.2 (cross-species) to an average of factor 1.5-1.8 (cross-species). Hence, an OC-normalization could reduce the uncertainty associated with differences in organic carbon concentration. The 4.0-7.2 reduction in toxicity is slightly higher than the 3.7-fold increase in %OC investigated. However, an analysis on the basis of EC_{50}s is statistically sounder, as EC_{50}s bear less uncertainty than NOEC values. The 4-fold reduction in toxicity observed based on EC50s is therefore very close to the 3.7-fold increase of the OC content. This finding therefore suggests that the data are more or less in line with a linear sorption isotherm on a log-log scale with a slope = 1, i.e. the K_{OC} concept (Mahony, 1996), $K_{Cu\text{-}OC} = Cu_T / Cu_{OC}$, which could imply that toxicity is caused by pore-water copper or overlying water copper in equilibrium with the sediment organic carbon.

Based on the evidence above, the calculation of the HC_5 for copper was based on organic carbon normalised data. A summary of the estimated HC_5 value (with the 90% confidence bounds) for the log normal function is provided in Table 11:

Table 11: Calculated $HC_{5\text{-}50}$ value (µmol/gOC) (with the 90% confidence bounds) for the organic carbon normalised data

HC_5 at 50% (& 90% confidence bounds) expressed as µmol/gOC	Type of fitting model	Parameters
27.4 (17.5-32.6) HC_5 at 50% (& 90% confidence bounds) expressed as µg/gOC 1,741 (1,112-2,071)	log normal	(1.71;0.199)

From Table 11, it can be deduced that the $HC5\text{-}50_{\text{sediment (benthic SSD)}}$ = 27.4 µmol/gOC = 1,741 µg Cu/gOC. According to the TGD (TGD, 1996) and the EUSES manual a standard sediment in the EU contains a weight fraction of 0.05 organic carbon (kg OC/kg solid). Hence the $HC5\text{-}50_{\text{sediment (benthic SSD)}}$ should be corrected for this organic content.

$HC5\text{-}50_{\text{normalised, 5 \% OC}}$ = $HC5\text{-}50_{\text{OC normalized}}$ x 0.05 = 1,741 µg Cu/gOC x 0.05 = **87.1 mg Cu/kg. dry weight**.

3.5.2 SEM-AVS normalization

The fraction of metals that may bind to sulphides in the sediment and thus be sequestered in the sediment can be estimated using the SEM-AVS concept. The basic concept behind the SEM-AVS approach is that the activity of most divalent metals (e.g. Hg, Zn, Ni, Cu, Pb, Cd, …) in sediments is controlled by the amount of acid-volatile sulphide (AVS) present in the sediment matrix. SEM and AVS are operationally defined parameters. AVS (Acid Volatile Sulphides) are those sulphides that are extracted by cold extraction (1 M HCL) of sediments. SEM (Simultaneously Extracted Metals), is the term used for those metals that are liberated under the conditions of the AVS analysis (ICMM fact sheet N° 10);. Incorporation of the AVS model is an improvement to sediment toxicity assessments, but the approach does have some limitations and should be considered more as one of the tools available to be used in a kind of weight approach. For example AVS concentrations have shown temporal and spatial (horizontal and vertical) variations depending on sediment type and hydrological conditions. Furthermore it should, be recognized that at the moment it is not possible to preclude unambiguously other routes of uptake, including exposure to metals via diet (Griscom et al, 2000, 2002.) which may become important during chronic exposure (e.g. a fraction of the AVS bound metals may still be available to organisms that ingest sediment particles, rather than just feeding from porewater). Another possible limitation of the model is that some sediment organisms create a micro-oxic environment by bioturbation.

The SEM-AVS difference gives the amount of SEM_{Me} for a specific metal Me that is not bound (excess SEM_{Me}) with sulphides, and consequently potentially available[19] (Equation 3).

$$SEM_{Me},_{(bio)available} = SEM_{Me} - \Delta\ \mathrm{AVS}_{\mathrm{Me}} \qquad \text{(Equation 3)}$$

In applying the SEM-AVS model for a specific metal it must be taken into consideration that metals act in a competitive manner when binding to AVS. Acknowledging the existence of competitive displacement kinetics the SEM-AVS model can be made metal-specific. The procedure that is used is to assign the AVS pool to the metals in the sequence of their solubility products. For example, ranked from the lowest to the highest solubility product the following sequence is observed for these six metals: SEM_{Hg} SEM_{Cu}, SEM_{Pb}, SEM_{Cd}, SEM_{Zn} and SEM_{Ni}. This means that copper has the highest affinity for AVS, followed by lead, cadmium etc until the AVS is exhausted. The remaining SEM is that amount present in excess of the AVS.

In cases where SEM-AVS values have been measured in a sediment toxicity test, the $NOEC/EC_{10}$ values should ideally be expressed as $SEM_{Me,\ (bio)available}$ and used to calculate the $PNEC_{\ AVS,\ normalised}$. If the SEM-AVS difference gives a negative value (i.e. no excess $SEM_{Me,\ (bio)available}$ present), then the use of LOEC values (giving positive SEM-AVS values) could be considered as the starting-point to derive NOEC values.

Example 3-4: Calculation SEM-AVS normalised PNEC (Cd)

In the risk assessment of Cd a bioavailable PNEC was calculated i.e. the excess Cd above the ‘available AVS’, in

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[19] Although it is recognized that 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 remainder of this section uses the nomenclature of excess SEM_{Me} as “available” for the purposes of estimating the extent to which metal/metal compounds in sediments may cause toxicity

formular form: total Cd minus available AVS, both values in µmol/g. The 'available' AVS is the total AVS corrected for the amount already used to precipitate Cu and Pb because these metal sulphides are less soluble than Cd (see below). This 'available Cd' concentration should be contrasted to the $PEC_{sediment}$ similarly expressed as 'available Cd' or, in a formular form:

$$Risk = PEC_{sediment,available}/PNEC_{sediment,available} = (PEC_{sediment}\text{-available AVS})/PNEC_{sediment,available}$$

The $PNEC_{sediment,available}$ is different from the generic PNEC of 2.3 mg Cd $kg^{-1}{}_{dw}$ because that PNEC was based on a sediment containing AVS, hence containing also a non-bioavailable fraction. There were only 2 sediment toxicity tests available within the data set that can be considered as chronic tests (test duration of other tests is 4-10 days and using mortality as endpoint). The statistical extrapolation technique will therefore not be used on the NOEC data. The $PNEC_{sediment,avaialable}$ is, therefore, derived by using the assessment factor (AF) method on the lowest NOEC value, expressed as 'available Cd', i.e. the total Cd NOEC minus available AVS, both expressed in molar units.

One NOEC is 115 mg/kg_{dw} or 1.02 $µmol/g_{dw}$, and the 'available' AVS in the sediments of that study is 0.37 $µmol/g_{dw}$ (AVS=0.5 $µmol/g_{dw}$ and Pb+Cu=0.13 $µmol/g_{dw}$). Hence, the 'available' NOEC is, 1.02-0.37= 0.67 µmol/g. Another NOEC fis 180 mg/kg_{dw} or 1.60 $µmol/g_{dw}$, and the 'available' AVS in the sediment of that study is estimated at about 0.87$µmol/g_{dw}$ (AVS=1.05 $µmol/g_{dw}$, ΣSEM=1.07 $µmol/g_{dw}$ in the control and SEM_{Zn}=0.89 $µmol/g_{dw}$; assuming a minor contribution of Ni, it is estimated that SEM Cu+Pb is 1.07-0.89=0.18; the 'available' AVS is therefore 1.05-0.18=0.87 $µmol/g_{dw}$). The 'available' NOEC is, then, 1.60-0.87= 0.73 $µmol/g_{dw}$.

The lowest NOEC, expressed as 'available Cd', is hence 0.67 µmol/g. This value is divided by an AF of 50. The choice of an AF of 50 instead of 100 is justified by the number of acute toxicity data showing no differences between species, yielding

$$PNEC_{sediment,available} = 0.67/50 = 0.013\ µmol\ Cd/g_{dw}\ (= 1.5\ mg\ Cd/kg_{dw}).$$

3.6 Guidance on the incorporation of (bio)availability in the terrestrial effects assessment

Natural soils used in ecotoxicological tests differ in characteristics such as organic matter and clay content, soil pH and soil moisture content. The bioavailability of the test compound, and therefore the toxicity observed, is influenced by these soil properties. If no data are available, toxicity has to be tested in a reasonable worst-case scenario, i.e. a soil with high bioavailability of the metal substance tested. This ensures that results are protective for the majority of soils. Guidelines for soil selection are presented in example 3-5.

Example 3-5: Guidelines for selection of reasonable worst-case scenario soil for toxicity testing:

General

Soils to be used for toxicity testing should not be deficient in elements essential for plant growth, nor should they have concentrations of essential elements that are in excess of those necessary for normal plant growth. Soils containing naturally high concentrations of metals should be avoided. Soils selected for toxicity tests should also not have had any recent application of biocides (Fairbrother et al., 2001).

Cations (e.g. Cu^{2+}, Ni^{2+}; Zn^{2+})

The bioavailability and toxicity of cationic metals in soil generally decreases with the increasing effective cation exchange capacity (eCEC) of the soil . Consequently, if no data are available, a soil with low eCEC and corresponding high bioavailability of the metals has to be selected, resulting in toxicity thresholds that are conservative for the majority of soils. Since eCEC is largely determined by the pH and organic matter and clay content of a soil, threshold values for these properties are also presented in case no information on eCEC is available.

eCEC	>5 and <15 $cmol_c\ kg^{-1}$
pH (as measured in 0.01 M $CaCl_2$)	>4.5 and <5 (corresponds to pH H_2O >5 and <5.5)
Organic matter	>1 and <3%
Clay	>5 and <10%

Minimum limits are defined in order to ensure viable populations of micro-organisms, plants and invertebrates in the control soil.

Anions (e.g. $Sb(OH)_6^-$, MoO_4^{2-})

Some metals form negatively charged complexes or oxyanions under the relevant environmental conditions. The sorption capacity for anions decreases with increasing pH, decreasing organic matter content and decreasing amount of oxides. The following threshold values for the standard soil properties (pH, organic matter and clay content) are proposed for the selection of a reasonable worst-case scenario soil for the testing of anions.

pH (as measured in 0.01 M $CaCl_2$)	>7 and <8 (corresponds to pH H_2O >7.5)
Organic matter	>1 and <3%
Clay	>5 and <10%

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 since metal spiking causes a sudden disturbance that is unrepresentative of a field where metals are added gradually and could equilibrate for several years. 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. Testing soils immediately after adding metals also ignores the effect of slow ageing reactions (inclusion of natural elements into the crystal lattices of soil minerals, the formation of insoluble precipitates, diffusion of metals into micro pores, etc.) on metal bioavailability. If possible these phenomena should be taken into account in the CSA (see section 4 risk characterization)

3.7 Guidance on bioaccumulation of metals and metal compounds

Most concepts and tools to assess the bioaccumulation/biomagnification potential of substances were originally developed on the basis of the observations made on a fairly limited number of neutral lipophilic organic substances that have shown that their potential to bioaccumulate and/or to biomagnify is directly related to the inherent properties of the substance. However, for naturally occurring substances such as metals, bioaccumulation is more complex, and many processes are available to modulate both accumulation and potential toxic impact. Many biota for example, tend to regulate internal concentrations of metals through (1) active regulation, (2) storage, or (3) a combination of active regulation and storage over a wide range of environmental exposure conditions. Although these homeostatic control mechanisms have evolved largely for essential metals, it should be noted that non-essential metals are also often regulated to varying degrees because the mechanisms for regulating essential metals are not entirely metal-specific. Some species (mostly plants) could also be adapted to a natural enriched environment and as such accumulate high levels of metals. Most often these phenomena are very local and not an overall concern for secondary poisoning and biomagnification.

From the above it is clear that it is not appropriate to apply classical concepts (e.g. use of bioconcentration factors; BCF -biomagnification factors; BMF) to metals as they are applied to organic substances.

As a result of these processes – and more specifically due to active regulation – at low metal concentrations, organisms accumulate essential metals (and often non-essential metals via the same uptake mechanisms) more actively in order to meet their metabolic requirements than when they are being exposed at higher metal concentrations. As a result, metal concentrations in tissue based on a range of exposure concentrations may be quite similar but the BCFs will be quite variable reflecting an inverse relationship (i.e., higher BCFs at lower exposure concentrations and lower BCFs at higher exposure concentrations) between metal concentrations and the corresponding BCF. In cases where the concentration dependency of BCFs can be demonstrated, it is therefore recommended to use regression models based on the observed inverse relationship (Figure 5) in order to derive the most appropriate BCF value for the region/site under investigation characterised by a specific soil/water metal exposure concentration (Brix et al, 2001).

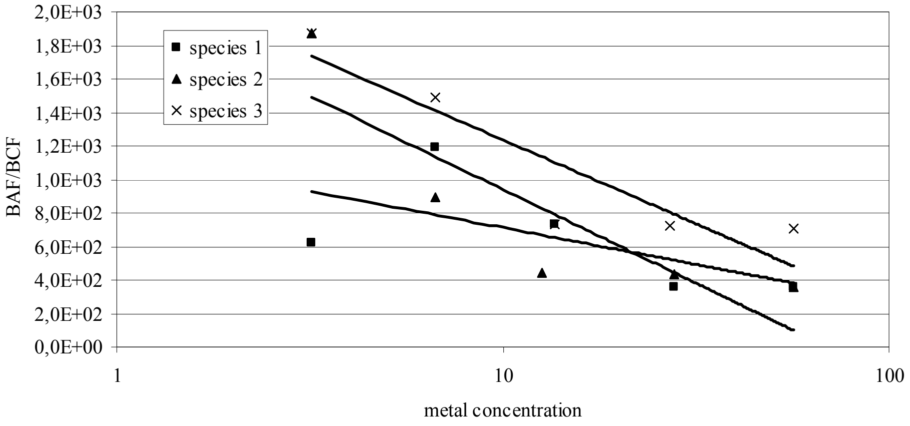

Figure 5: Inverse relationship between BCF/BAF and metal concentrations.

3.8 Guidance on secondary poisoning

Biomagnification of metals in aquatic organisms is rarely observed and if it does occur it frequently involves organic metallo species (e.g. methyl mercury) (Brix et al., 2000)). However, even for inorganic metal forms it is still recommended to examine their potential to biomagnify or cause secondary poisoning in specific food chains. In this regard it is especially important if organic metallo-species can be formed in some compartments (out of the scope of this guidance) or if the range over which homeostasis occurs is relatively small (e.g. selenium).

3.8.1 Identification of relevant food chains

The selection of appropriate food chains should be identified based on their relevance to exposure pathways and species in European environments, as well as reasonable conservatism in the exposure estimates (i.e., food chains based on food organisms that tend to bioaccumulate higher metal concentrations should be prioritized for this evaluation).

3.8.2 Derivation of $PNEC_{oral}$ values

The $PNEC_{oral}$ values represent dietary predicted no effect concentrations, below which food concentrations are not expected to pose a risk to birds or mammals. It is recommended to calculate $PNEC_{oral}$ values in two tiers. In Tier 1, derivation of $PNEC_{oral}$ values the lowest $NOEC_{oral}$ is divided by a default assessment factor and any species-specific differences in food ingestion rates and body weights is not taken into account. In needed in Tier 2, $PNEC_{oral}$ values could be derived based on species-specific food ingestion rate-to-body weight ratios for birds and mammals considered to be more relevant for the evaluation (example 3-6)

Example 3-6: Derivation of $PNEC_{oral}$ - Ni CSA, 2008

- Tier 1: tremors were observed for the toxicity studies with ducklings fed a dietary Ni concentration of 800 mg kg^{-1} or greater. At a dietary Ni concentration of 200 mg kg^{-1}, all ducklings survived and none developed tremors. Overall, therefore, 200 mg kg^{-1} appears to be an appropriate NOEC for the secondary poisoning analysis. No effects on growth, survival, or reproduction in chickens or mallards have been observed at this measured dietary concentration or lower.

In Tier 1 a generic bird $PNEC_{oral}$ value is calculated through division of the NOEC by an assessment factor (AF) of 30 to account for interspecies variation in sensitivity and lab-to-field extrapolation resulting in a Tier 1 Bird $PNEC_{oral}$ = 200 mg kg^{-1} / 30 = 6.7 mg kg^{-1}

In Tier 2 mallards are presumed to have different food ingestion rate-to-body weight ratios than other species considered in the exercise (e.g. the oystercatcher or European starling), the mallard-based dietary NOEC (see Tier 1) can be used to estimate oystercatcher- and starling-based dietary NOECs using the ingestion rate-to-body weight ratios for the two species. Oystercatchers or starlings are more relevant to the assessment of secondary poisoning for nickel since molluscs accumulate nickel to a greater degree so a bird with a largely mollusc-based diet would be more at risk and hence more relevant.

As a first step, a dose-based no observed adverse effect level (NOAEL) for the mallard can be estimated using the mallard duckling body weights and an allometric equation for estimating the food ingestion rate. The 28-day body weights of the mallards were used to determine the relevant body weight (457 g wet wt. based on the mean of males and females). Using the allometric equation for birds as described in literature results in an estimated food ingestion rate of 34.9 g d^{-1} on a dry weight basis (175 g d^{-1} on a wet weight basis assuming a water content of 80%). Thus, the estimated body weight (bw)-to-daily food intake (dfi) for mallard ducklings is estimated to be 2.6 (457 g body weight divided by 175 g d^{-1} wet weight.). The resulting estimated NOAEL for mallard ducklings is 77 mg Ni per kg body weight per day (mg kg^{-1} day^{-1}). Based on the mean body weight and food ingestion rate for the oystercatcher of 0.555 kg and 0.338 kg d^{-1} wet wt., respectively, this results in a bw/dfi of 1.6. Multiplying the duckling NOAEL of 77 mg kg^{-1} day^{-1} by the bw/dfi of 1.6 results in an estimated dietary NOEC for the oystercatcher of 123 mg kg^{-1}. In this Tier 2 the calculation of the $PNEC_{oral}$ can be made with much greater confidence, and thus a reduction of the assessment factor to 10 is possible resulting in a Tier 2 $PNEC_{oral}$ Oystercatcher = 123 mg kg^{-1} / 10 = 12.3 mg kg^{-1}.

3.8.3 Bioavailability of dietborne metal

The guidance given in this section is not always only relevant for metals but the issues raised have quite often being observed for metals. Currently additional guidance concerning these issues is under development for risk assessment of pesticides.

Often toxicity studies with mammals/birds used for the derivation of the oral PNECs are based on studies in which the animals are exposed to a highly soluble metal compound (e.g. metals salts). In such case, the oral PNECs are expected to overestimate the bioavailability of biologically incorporated metal in natural diets. In addition, in the terrestrial pathway, the bioavailability of soil-adsorbed metal in the earthworm gut is expected to have reduced bioavailability. Therefore, there is a need to derive a relative absorption factor [RAF] to refine the secondary poisoning analysis. RAFs can be determined for the ingestion of soil (soil RAF) and the ingestion of non-soil dietary items (dietary RAF). RAFs will be specific for the consumer organism in question, and may vary depending upon the dietary items under consideration.

Example 3-7: Incorporation of the bioavailability from the administration of metal salts (dietary RAF) – Ni CSA, 2007

Although limited bioavailability data are available for Ni, in one study human volunteers were provided nickel sulfate in drinking water in one experiment and nickel sulfate in food in a second experiment. The mass fraction of Ni dose absorbed from the gastrointestinal tract was 27% (±17%) for drinking water and 0.7% (±0.4%) for food. For example, in a 2-generation rat study, the NOAEL is based on a dose of 1.1 mg Ni kg^{-1} d^{-1} (gavage, dosing of Ni in water). Using a water-based absorption factor of 27% based on literature data, the NOAEL of the 2 generation rat study is associated with an absorbed Ni dose of 0.297 mg Ni kg^{-1} d^{-1} (1.1 mg Ni kg^{-1} d^{-1} × 0.27). Thus, if a mammal was consuming dietary Ni, the total dietary dose would need to be 42.4 mg Ni kg kg^{-1} d^{-1} to achieve the absorbed Ni dose of 0.297 mg Ni kg kg^{-1} d^{-1} (i.e., 0.297 mg Ni kg^{-1} d^{-1} / 0.007 = 42.4 mg Ni kg^{-1} d^{-1}).

3.8.4 Dietary composition

The guidance given in this section is not always only relevant for metals but the issues raised have quite often being observed for metals.

The conventional approach assumed that the proportion of different food types in the diet consists entirely of one realistic food type, e.g. worm in the terrestrial food chain and fish in the aquatic food chain. If concern is raised it may be possible to refine this too simplistic assumption in order to provide a more realistic indication of the risk. In order to refine this, data on food consumption of birds and mammals is essential. However these are rarely available, therefore it should be considered to use basic ecological knowledge on bird and/or mammal feeding behavior to model consumption appropriately. Data from stomach contents, faecal analysis, and pellet analysis can be used to determine likely food consumption.

Thus, a realistic mixed diet BAF value can be calculated using the following formula:

$$\mathrm{BAF}_{\mathrm{mixed\ diet}} = \sum_{\mathrm{i=1}}^{\mathrm{n}} f_i \times BAF_{\mathrm{i}}$$

with BAF_i the representative bioaccumulation factor for an individual prey species i; n: the number of prey species considered in the mixed diet of the predator; f_i: the proportion of the different food types in the mixed diet (value between 0 and 1).

Indirect exposure of man via the environment will be dealt with in HERAG.

4. RISK CHARACTERIZATION

Aim and structure of this section

The aim of this chapter is to explain how the metal specific principles set out in the previous chapters can be applied into a risk assessment context. As indicated it is imperative for metals and metal compounds that both PEC and PNEC are based on similar levels of (bio)availability. The general outline of this chapter is given below.

- **4.1 General guidance on information requirements needed to perform a risk characterization for metals and metal compounds**
- **4.2 Guidance on the risk characterization for the aquatic compartment**
- **4.3 Guidance on the risk characterization for the sediment compartment**
- **4.4 Guidance on the risk characterization for the soil compartment**
- **4.5 Guidance on the risk characterization for secondary poisoning**

4.1 General guidance on information requirements needed to perform a risk characterization for metals and metal compounds

The realism of the risk characterization for metals and metal compounds will depend to a large extent on how (bio)availability can be incorporated into the process. Depending on the availability of data on the abiotic factors, a tiered assessment approach is advocated (Figure 6).

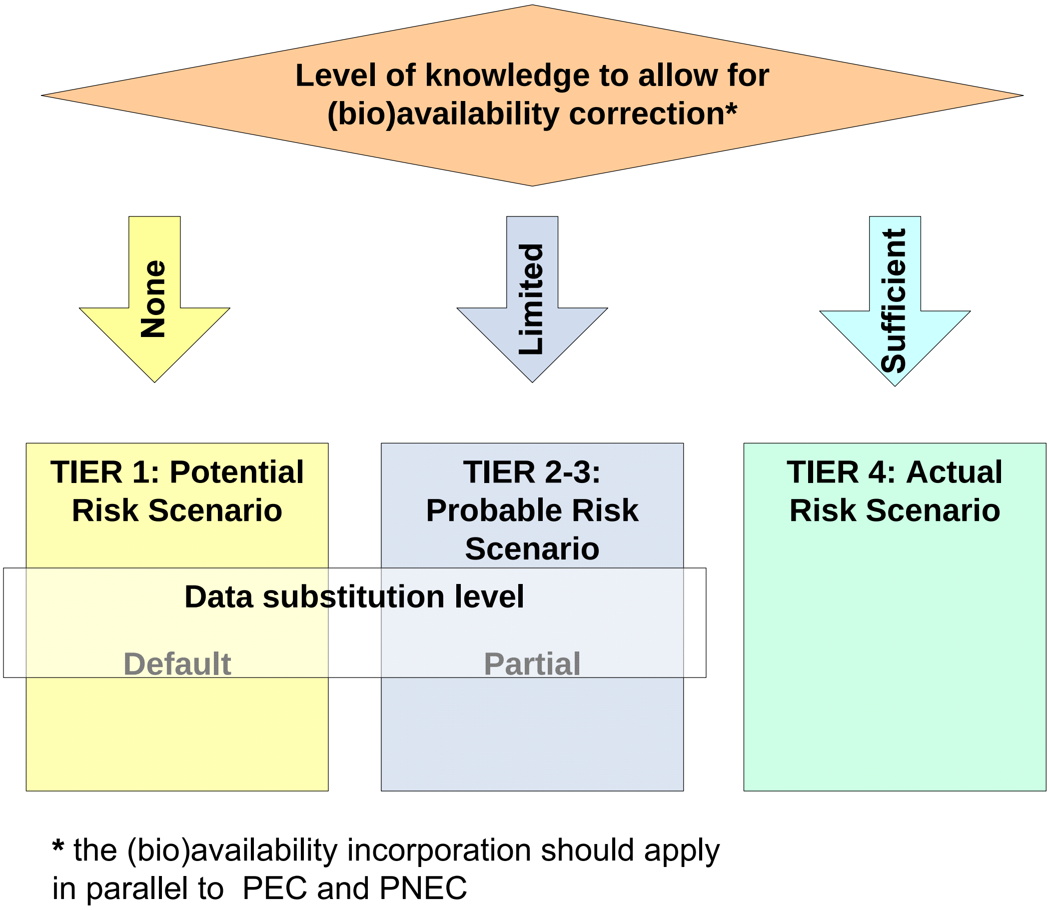

Figure 6: Tiered Approach for risk characterization (Tier 1, Tier 2, Tier 3 and Tier 4).

Tier 1 is the reasonable worst case default scenario. It represents the lowest degree of refinement of the tiered approach. When no data on abiotic parameters are available, a realistic worst case default PNEC is derived based on the precautionary principle. The PNEC derivation is based on a conservative combination of the abiotic parameters reflecting the EU relevant conditions. This tier provides a potential risk conclusion. Risk characterisation is performed by comparing the reference PNEC normalised to realistic worst-case conditions (i.e. $PNEC_{reference,\ bioavailable}$) with the site-specific PEC_{local} or with a specific region $PEC_{regional}$.

Tier 2 and 3 are substitute scenarios allowing for a partial degree of refinement. Tier 2 is based on the cautious allocation of a substitute ecoregion type to which surrounding physico-chemical conditions of sites (for the local assessment) or rivers/soils (for the regional assessment) belong, while Tier 3 relies on the cautious estimation of one of the relevant abiotic parameters (e.g. pH, hardness, DOC for the aquatic compartment or CEC, pH, organic matter and/and clay content for the terrestrial compartment.

Tier 3 therefore suggests that data on the other main abiotic factors are available. These tiers provide a probable risk conclusion, at the site-specific level.

Tier 4 allows for the highest level of refinement for risk characterization. Indeed, all relevant site specific abiotic parameters are present and therefore the actual risks could be estimated from the comparison of the $PNEC_{local,\ bioavailable}$ or $PNEC_{regional,\ bioavailable}$ with the site-specific PEC_{local} or with a specific region $PEC_{regional}$.

A further refinement of the risk characterization can be introduced in case several values of the abiotic factors controlling the metal toxicity are available for different sampling sites within a specific site or region. In that case, a distribution of normalised $PNEC_{local,\ bioavailable}$ or $PNEC_{regional,\ bioavailable}$ values for the particular site or region under consideration can be calculated and compared with the exposure concentrations of the same site or region. For each sampling point, the RCR will be calculated as the ratio between the $PEC_{local}/PEC_{regional}$ with the $PNEC_{local,\ bioavailable}$ or $PNEC_{regional,\ bioavailable}$ therefore resulting in a distribution of RCR values. Exceedance of the value of the RCR of 1 indicates potential risks for that particular site or region.

Specific guidance on how to conduct and apply the (bio)availability corrections in a risk assessment framework for the different environmental compartments (water, sediment and soil) is further outlined here below. It is again emphasized that for the final risk characterization both exposure and effect concentrations should be expressed at the same level of (bio)availability.

4.2 Guidance on the risk characterization for the aquatic compartment

A step-wise approach is proposed in Figure 7. Various situations for the calculation of a PNEC aquatic are defined, with an increasing level of refinement:

1) Derivation of a generic PNEC aquatic
2) Correction for differences in bioavailability:

- Use of dissolved concentrations
- Use of physico-chemical speciation models
- Use of Biotic Ligand Model

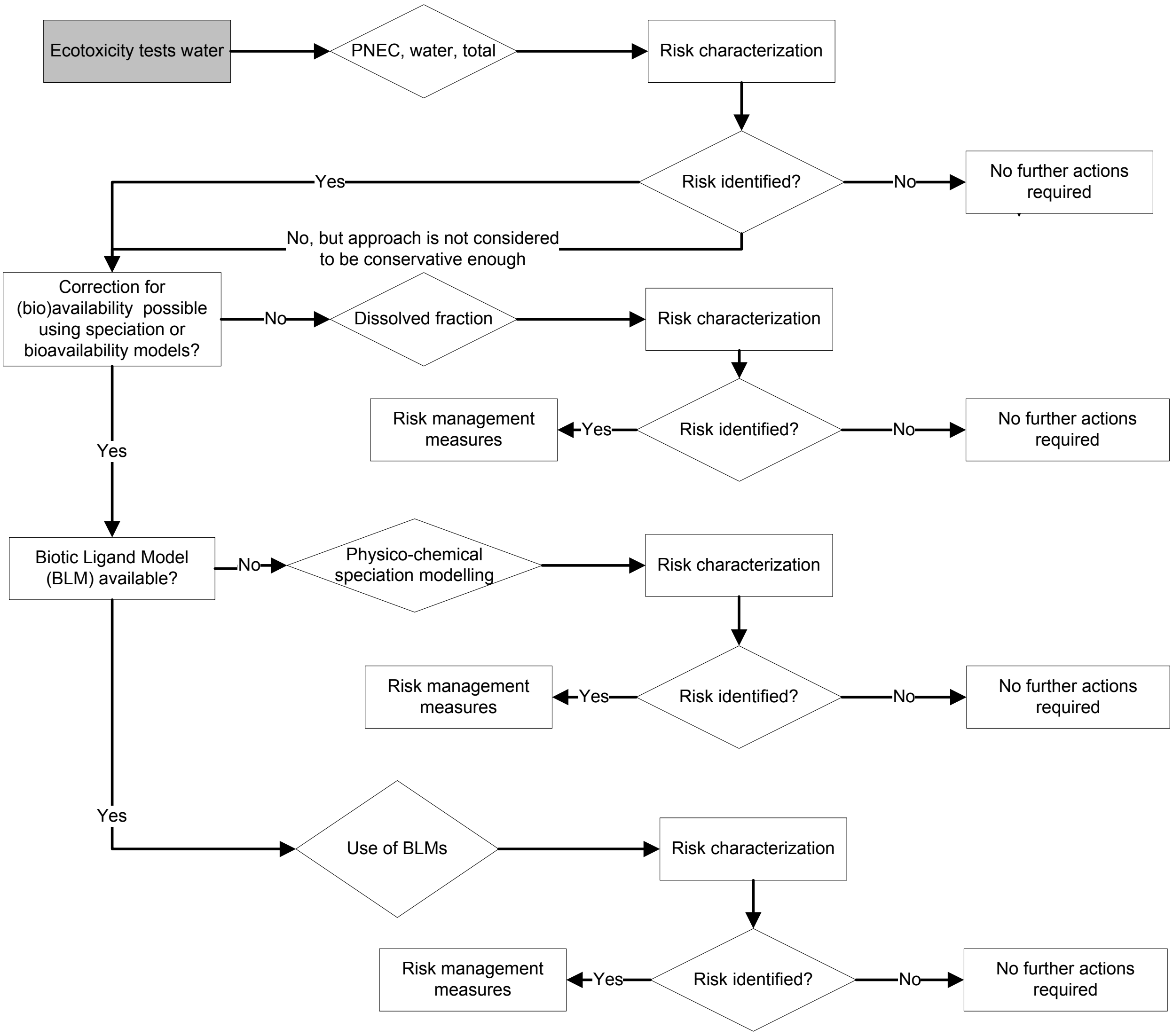

Figure 7: General framework for the aquatic risk characterisation.

Step 1: derivation of a generic PNEC aquatic

A generic PNEC water can be obtained on the basis of ecotoxicity data relevant to three trophic levels in the aquatic compartment:

- primary producers (algae)
- consumers (invertebrates)
- fish

The results from aquatic toxicity tests are usually expressed as total concentrations. Most aquatic toxicity tests conducted in artificial waters (low DOC, suspended solids) tend to maximize bioavailability and in those cases total concentrations can be considered equal to dissolved. If natural waters are used the potential influence of abiotic factors on the toxicity test results should be evaluated. If the toxicity data have been derived at a combination of abiotic factors which could have mitigated toxicity the approach is not considered to be conservative enough and a (bio)availability correction should be performed (step 2).

Step 2: (Bio)availability correction

Metal bioavailability and toxicity in the aquatic compartment does not solely depend on the total metal dose, but also on the physico chemical characteristics of the water and on biological characteristics. If possible, a correction for differences in (bio)availability of metals will further refine the risk assessment and allow the derivation of more field-relevant and site-specific PNEC values. This further refinement is not compulsory, but may avoid the identification of risk in some insensitive waters based on toxicity data derived in vulnerable waters.

Use of dissolved concentrations

In case ambient total metal concentrations are reported and no appropriate bioavailability models and/or relevant input data (i.e. physico-chemical parameters) are available, the risk characterization could be performed on a dissolved basis as outlined in Figure 8.

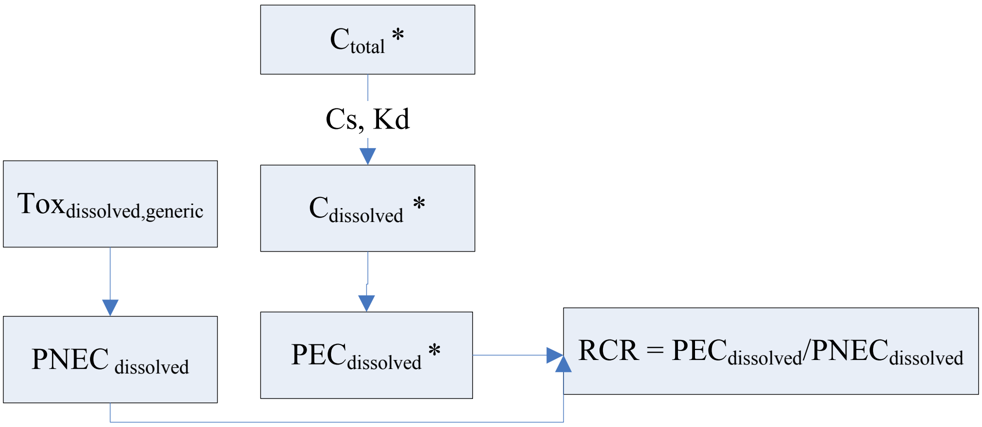

Figure 8: Framework for assessing risks of metals/metal compounds in water on a dissolved basis. (Tox = ecotox value = geometric mean in case of more that one value), C = environmental concentration; *=sequence applies to both the local and regional environment)

The translation of ambient total metal concentrations into the dissolved metal form is done using Equation 4:

$$C_{dissolved} = \frac{C_{total}}{(1 + K_d \times C_s \times 10^{-6})} \qquad \text{(Equation 4)}$$

K_d = Partitioning distribution coefficient (L/kg)
C_s = Suspended solids concentration (mg/L)

Use of physico-chemical speciation models

In case ambient total metal concentrations are reported and appropriate speciation models and relevant input data (i.e. physico-chemical parameters) are available, the risk characterization should be performed on basis of the metal species of concern[20] as outlined in Figure 9in order to reduce uncertainty. See also section 2.4 for guidance on selecting the physico-chemical parameters.

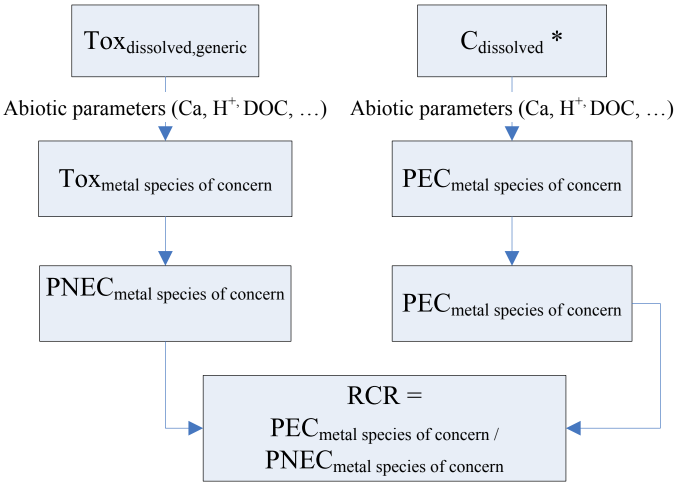

Figure 9: Framework for assessing risks of metals/metal compounds in water on a free metal ion basis (Tox = ecotox value = geometric mean in case of more that one value, C = environmental concentration; *=sequence applies to both the local and regional environment)

Guidance on bioavailability correction using BLM

The first step in using a toxicity related bioavailability model such as BLM consists in the determination of a critical biotic ligand accumulation ($\text{Tox}_{\text{critical biotic ligand, organism xi}}$) calculated from the experimentally generated organism specific toxicity values ($\text{Tox}_{\text{dissolved, organism xi}}$), expressed as dissolved concentration. In the second step of the approach each organism specific critical biotic ligand accumulation ($\text{Tox}_{\text{critical biotic ligand, organism xi}}$) is translated into a critical bioavailable dissolved concentrations ($\text{Tox}_{\text{(critical bioavailable dissolved)y, organism xi}}$) for a specific area under investigation characterized by a specific set of water-quality conditions (pHy, Hy, DOCy). Finally, these critical bioavailable dissolved concentrations ($\text{Tox}_{\text{(critical bioavailable dissolved)y, organism xi}}$)

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[20] Most often this is the free metal ion but it should be noted that the free ion is not necessarily the best predictor for all metals and other metal species such as neutral species (e.g. AgCl, HgS) and anionic species (e.g. SeO^{2-}, AsO_4^{2-}) may contribute to the observed toxicity (Campbell, 1995).

or the $PNEC_{(bioavailable\ dissolved)y}$) are compared with the dissolved environmental concentrations of the metal/metal compounds representative for the area under investigation. All individual for bioavailability corrected Tox and PNEC values are expressed as dissolved concentrations and are therefore at the same level of bioavailability as the environmental concentrations. The general outline of this approach is outlined in Figure 10.

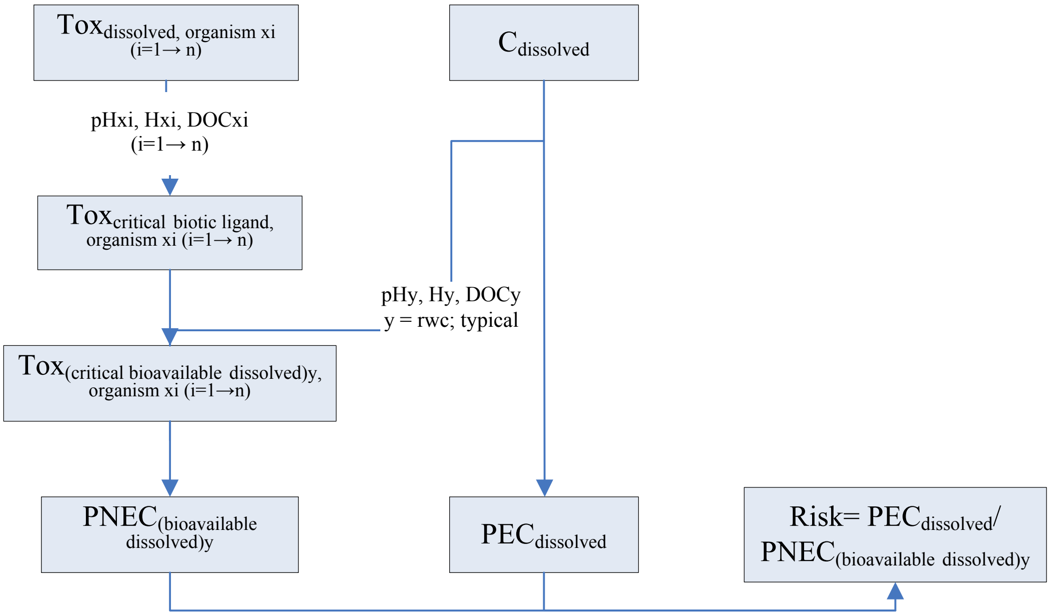

Figure 10: Framework for incorporation of bioavailability models in water

Organism-specific bioavailability models should be used as much as possible. Depending on the number of BLMs available two options are available to correct for bioavailability:

- *Baseline bioavailability correction* limited to those species for which an actual chronic BLM has been developed.
- *Full bioavailability correction* in cases where there is justification for using the originally developed chronic BLM for those species within the same trophic level for which no specific bioavailability model has been developed (e.g. insects, amphibians, molluscs).

Baseline bioavailability correction

A baseline bioavailability correction can only be conducted in cases where a BLM for algae, fish and invertebrates respectively is available. This correction is only performed for those species for which the BLM has originally been developed. For those species where no justification is available to apply the BLM across species a conservative bioavailability correction can be applied to normalize the other effects data for which no specific BLM model has been developed (Example 3-8).

Example 3-8: baseline bioavailability correction (Zn)

In the Zn EU RAR, it was considered that there was no sufficient scientific evidence to extrapolate the BLMs towards other species of the whole SSD. Consequently, the recommended way was to calculate the most conservative BioF for those organisms for which BLMs had been developed and validated.

Step 1: gather abiotic factors for particular site/region
Firstly, the chronic Zn-NOEC values for algae, invertebrates and fish (the 3 BLM species) were normalised for a particular site or region based on the site- or region-specific conditions or water chemistry, using the BLMs for the three aquatic species. An overview of the abiotic conditions for the river Meuse is outlined in Table 12.

Table 12: Overview of the abiotic conditions of the river Meuse

River	**DOC (mg/l)**		**pH**			**Hardness ($CaCO_3$ mg/l)**		
	10%	**50%**	**10%**	**50%**	**90%**	**10%**	**50%**	**90%**
River Meuse	1.9	2.6	7.4	7.6	7.9	143	205	244

Step 2: normalise the NOEC for the 3 BLM species towards site- specific/region-specific average and rwc conditions

According to the Zn EU RAR, both average (= 50% of pH, hardness and DOC for the 3 BLM species) and reasonable worst-case conditions RWC (= 10% of pH, hardness and DOC for fish & invertebrates; 10% of hardness and DOC, 90% of pH for algae) were used to normalize the NOEC values for the 3 different BLM organisms. This will result in different $NOEC_x$ values for the 3 BLM species normalised towards the conditions in the river, as shown in Table 13

Table 13: Overview of the normalised NOEC (µg/l) for the BLM species

River	**NOEC (µg/l) RWC conditions**			**NOEC (µg/l) Average conditions**		
	algae	**fish**	**invertebrate**	**algae**	**fish**	**invertebrate**
River Meuse	21	263	108	26	368	132

Step 3: normalise the NOEC for the 3 BLM species towards EU reference water chemistry conditions

The reference water chemistry conditions (ref) were taken from an EU wide database and used to calculate reference NOEC values for the 3 different BLM organisms. This was done For all BLM organisms, data were normalized towards reasonable worst case conditions., i.e. the 10% of DOC from the EU wide database is selected. For *D. magna* and *O. mykiss* the 10% percentile of pH and hardness are used for the estimation of the reference NOEC value, for *P. subcapitata* the 90% percentile of pH and 10% of hardness is used (for algae it was observed that toxicity of Zn was higher at higher pH which is in contrast with the two other BLM species). An overview of the reference NOEC values calculated for the 3 BLM species is provided in Table 14.

Table 14: Summary of reference NOEC values in µg/l ($NOEC_{ref}$) for 3 BLM species

Species	**$NOEC_{ref}$ (µg/l)**
O. mykiss	184
D. magna	86
P. subcapitata	21

Step 4: calculation of bioavailability factors BioF
The bioavailability factors (BioF) were then derived for each of the 3 BLM species as follows:

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

An overview of the BioF for the 3 BLM species for the RWC & average conditions in the river Meuse is provided in Table 15

Table 15: Summary of the BioF as calculated for the 3 BLM species

River	**BioF- RWC conditions**			**BioF - average conditions**		
	algae	**fish**	**invertebrate**	**algae**	**fish**	**invertebrate**
River Meuse	1.0	0.7	0.8	0.8	0.5	0.6

The highest value of the three BioF values for the three species is selected to ensure that a conservative approach and bioavailability factor (BioF) is taken, i.e. the smallest correction for bioavailability. The most conservative BioF for the RWC and average conditions are 1.0 and 0.8 for the river Meuse.

Step 5: calculation of the bioavailable PEC concentration

1) The bioavailable PEC value for Zn concentration in the river Meuse was calculated from:

$PEC_{bioavailable}$=PEC x $BioF_{,X}$

Monitoring data for the river Meuse were compiled from existing databases, and revealed a PEC value of 12.1 µg dissolved Zn/l for the river Meuse.
In the zinc RA, the added risk approach is applied, so the PECadd is calculated from the PEC monitored, which is equal to the PEC total. With a background zinc concentration for the Meuse river of 2-4 µg/l, the PECadd becomes 10,1-8,1 µg Zn/l.

An overview of the $PEC_{bioavailable}$ concentration based on RWC and average conditions is given in Table 16.

Table 16: Overview of the $PEC_{bioavailable}$ concentrations for the river Meuse

River	**$PEC_{bioavailable}$ (µg Zn/l)**	
	RWC	**average**
River Meuse	10.1 8.1	8.1 6.5

2) Similarly, the BioF values could also be used to derive bioavailable river-specific PNEC values that are protective for the river Meuse, using the following equation:

$PNEC_{bioavailable}$=PNEC/$BioF_{,X}$

The Zn EU RAR reports a PNEC of 7.8 µg dissolved Zn/l for the freshwater environment.

Table 17: Overview of the $PNEC_{bioavailable}$ concentrations for the river Meuse

River	**$PNEC_{bioavailable}$ (µg Zn/l)**	
	RWC	**average**
River Meuse	7.8	9.8

Step 6: Characterisation of the potential risks

1) Based on the $PEC_{bioavailable}$ (i.e. 10.1-8.1 µg Zn/l for the RWC; 8.1-6.5 µg Zn/l for average conditions) and the PNEC value of 7.8 µg dissolved Zn/l from the Zn EU RAR, the RCR could also be calculated as follows:

RCR = $PEC_{bioavailable}$ / PNEC

The RCR values for the river Meuse are 1.3-1.0 and 1.0-0.8 for the RWC and average conditions respectively.

Table 18: Overview of the RCRs for the river Meuse

River	**RCR**	
	RWC	**average**
River Meuse	1.3 1.0	1.0 0.8

2) Based on the $PNEC_{bioavailable}$ (i.e. 7.8 µg Zn/l for the RWC; 9.8 µg Zn/l for average conditions) and the PEC value of 10.1-8.1 µg dissolved Zn/l from the Zn EU RAR, the RCR could be calculated as follows:

RCR = PEC / $PNEC_{bioavailable}$

Similar RCR values for the river Meuse of 1.6 and 1.2 are calculated for the rwc and average conditions respectively.

Table 19: Overview of the RCRs for the river Meuse

River	**RCR**	
	RWC	**average**
River Meuse	1.3 1.0	1.0 0.8

Full bioavailability correction

The application of a BLM across species (full normalization) assumes similar mechanism of actions (e.g. similar stability constants between the cations (Ca, Mg, H) and the biotic ligands, similar site of action) and therefore the applicability across species, needs to be investigated on a case-by-case basis. Such analysis should consist of 'spot checking' of the BLMs for species for which no validation had been undertaken. The level of checking, e.g. testing of additional taxa to confirm applicability of the BLM 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. It is also needed to consider if certain keystone species or important groups of organisms/trophic levels are missing. The accuracy of such predictions should be within a acceptable range but could be altered depending on the robustness of the endpoint tested. Most importantly the variability should be reduced to a a significant extent. If the above information is not available, other evidence related to read-across of existing BLMs to other species can be used. Each of these bioavailability refinement criteria may bring some inherent uncertainties when used for full BLM normalisation:

- 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. Ideally, the same 'mode of' action would be demonstrated by the development of new species specific BLM. 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.
- Similarity of species can be used as justification for use of a particular BLM; this is plausible, and such extrapolation is widely used in environmental risk assessment for practical reasons. Such extrapolation 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.

However, at least 3 species are needed for 3 BLMs. If acute BLMs are available, acute tests could also be used in the "spot check" exercise. The reduction in uncertainty should be used as measure of the accuracy of the predictions. The acceptable accuracy will depend on the endpoint tested and the level of inherent uncertainty embedded in the extrapolation.

In case a model for a specific taxonomic group shows a better fit towards organisms belonging to a different taxonomic group (e.g. an invertebrate model fits better to the algae data than the algae model) there could be a need for further refinement of the latter. However, if both models are deemed valid after refinement the ecological relevance of the model should outweigh the reduction in uncertainty criterion.

An example of full bioavailability correction is given in Example 3-9

Example 3-9: Full bioavailability correction (nickel)

Because the Ni aquatic toxicity database contains several organisms for which no chronic BLMs currently exist, spot-checking validation testing for selected organisms within the database has been proposed for organisms belonging to different taxonomic groups than those for which BLMs have already been developed/validated. As such, the objective of this study was to conduct toxicity tests with a snail (*Lymnaea stagnalis*), an insect (*Chironomus tentans*), a higher plant (*Lemna minor*) and a rotifer (*Brachionus calyciflorus*) in five natural waters that represent those typically found within the major ecoregions of Europe (the selected waters have been chosen as being representative of a wide variety of ambient surface water types within the EU as regards the abiotic factors driving the BLMs).

The results for *Brachionus calyciflorus* show that good predictions for *B. calyciflorus* were obtained with the *D. magna* BLM. Predictions for all waters were accurate within a factor of two with the exception of the prediction for 1 site, which differed by a factor of 2.2. Similarly, good predictions (< factor 2) were obtained with the *D. magna* BLM model for the insect *Chironomus tentans and the* higher plant *Lemna minor.*

The results for the snail *Lymnaea stagnalis* indicate good predictions for *L. stagnalis* with the *C. dubia* BLM (Figure 11). All waters were predicted within a factor of 2 using the *C. dubia* BLM.

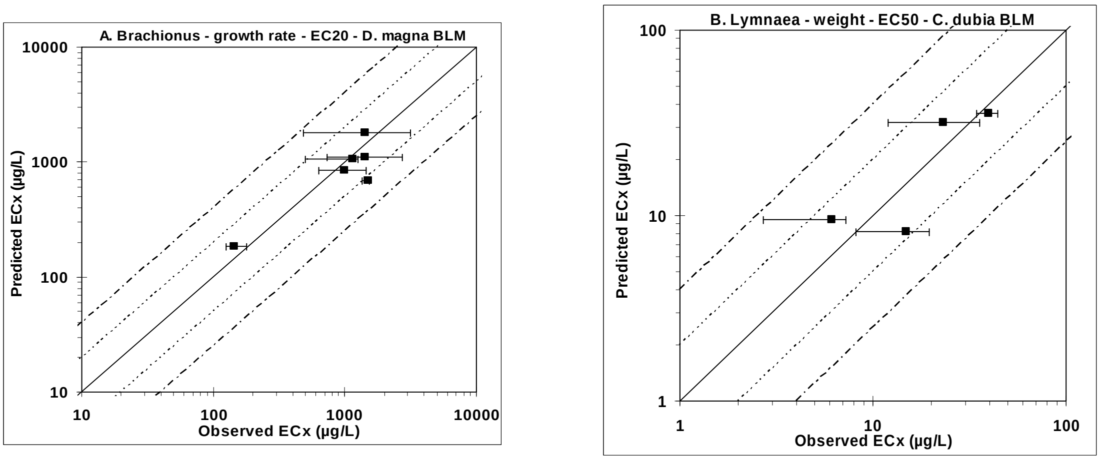

Figure 11: Observed nickel toxicity (EC20, in µg Ni/L) to the rotifer *Brachionus calyciflorus* compared with predicted toxicity using the Biotic Ligand Model developed for *Daphnia magna* and the snail *Lymnaea stagnalis,* with predicted toxicity using the Biotic Ligand Model developed for *Ceriodaphnia dubia*

Based on the results of the spot-checking study, the following full normalization approach was followed for the PNEC derivation of Ni:

- for algae, the *Pseudokirchneriella subcapitata* BLM was used;
- for higher aquatic plants, the *D. magna* (best fitting BLM) BLMs was used;
- for cladocerans, insects and amphipods, the most stringent BLM from the *D. magna* and *C. dubia* BLM is used;
- for rotifers, the *D. magna* BLM was used;
- for molluscs and hydra the *Ceriodaphnia dubia* (best fitting BLM) BLMs was used;
- for fish and amphibians, the *Oncorhynchus mykiss* BLM was used.

The main principles for normalization ecotoxicity data using bioavailability models (e.g. BLM) and read-across to other species for which no bioavailability model is available presented above applies also to the soil compartment. Similar to the water compartment bioavailability models are sometimes available and should be used in a similar way (e.g. spot checking concept, read across etc.)

4.3 Guidance on the risk characterization for sediments

A stepwise approach is proposed in Figure 12. Three different situations for the calculation of a PNEC sediment are defined, with increasing level of refinement:

1) Equilibrium partitioning (see also main guidance document-integrated testing strategy sediments)
2) Derivation of generic PNEC sediment
3) Correction for differences in bioavailability, allowing derivation of a site-specific PNEC sediment

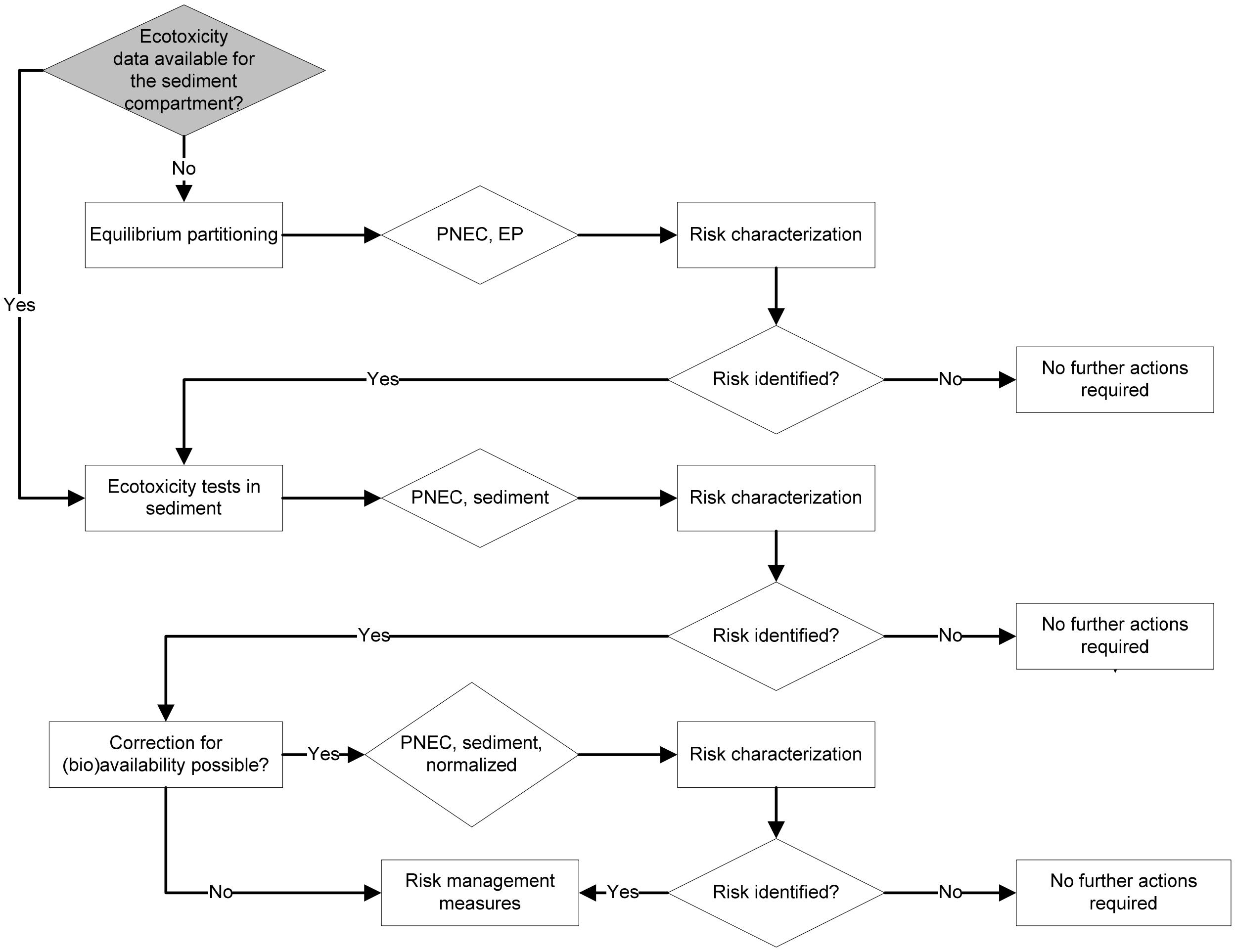

Figure 12: General framework for sediment risk characterisation.

Step 1: Equilibrium partitioning

In cases where no reliable toxicity data are available for the sediment environment, a PNEC sediment can be calculated according to the equilibrium partitioning concept based on a $PNEC_{water}$ and a reasonable worst-case sediment: water distribution coefficient (Kd):

$$PNEC_{sediment}\ (mg\ kg^{-1}) = PNEC_{freshwater}\ (mg\ l^{-1}) * Kd\ (l\ kg^{-1}) \quad \text{(Equation 5)}$$

This method cannot replace toxicity data for sediment organisms, however, and should only be considered as a screen for identifying substances requiring further testing. As a reasonable worst case, the 10th percentile of Kd values for sediment is used (see section 2.2.2 in cases where no information on Kd values is available). If the adsorption is relevant[21], an additional assessment factor of 10 should be added to the RCR to take exposure via ingestion into account.

Step 2: derivation generic PNEC sediment

If the outcome of the equilibrium partitioning method results in a $PEC_{sediment}/PNEC_{sediment}$ ratio greater than 1, toxicity tests with sediment organisms are an essential requirement for a refined hazard assessment. See general guidance document on the information requirements for REACH.

Step 3: (bio)availability correction

Similarly to the hazard assessment for the aquatic compartment there is a need to take the metal (bio)availability of metals/metal compounds in sediments in to account. At the moment sediment BLMs have not yet been developed, and only a correction for chemical availability can be made. Metal availability in sediments is governed by various ligands/processes (e.g. organic carbon, sulphides, iron and manganese oxy-hydroxide and redox potential), and the relative importance of these binding phases may differ depending on the metals binding capacity and general behaviour). Various approaches can be used to take (bio)availability into account (see section 3). The use of partitioning to Fe-Mn (oxy)hydroxides, speciation calculations (reduced forms under anoxic conditions) and organic carbon normalization can be used if evidence is at hand that these factors do mitigate metal toxicity. For those metal/metal compounds that are susceptible to binding with sulphides or with organic carbon, the use of the SEM-AVS and/or organic carbon normalization could be appropriate (Figure 13).

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[21] For organic substances this has to be considered for substances with a log Kow > 5. For metals no specific Kd thresholds are available

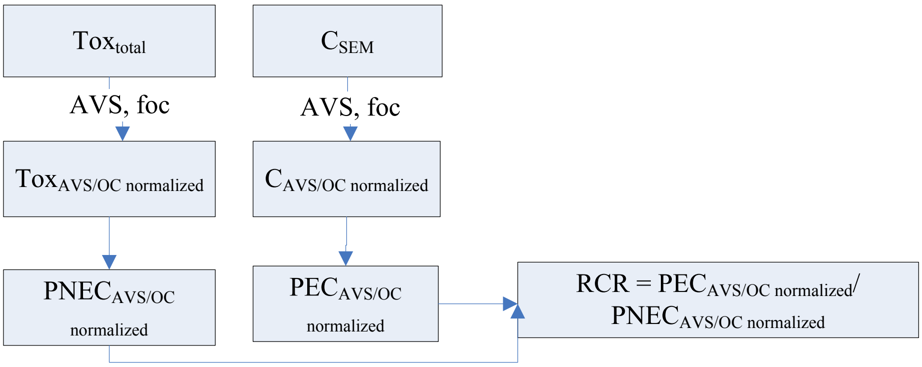

Figure 13: Framework for assessing risks of metals/metal compounds in sediments based on the SEM-AVS concept or using organic carbon normalization

4.4 Guidance on the risk characterization for the terrestrial compartment

A stepwise approach is proposed in Figure 14. Three different situations for the calculation of a PNEC soil are defined, with an increasing level of refinement:

1) equilibrium partitioning (see also main guidance document-integrated testing strategy soils)
2) derivation of a generic PNEC soil
3) correction for differences in bioavailability, allowing derivation of a site specific PNEC soil

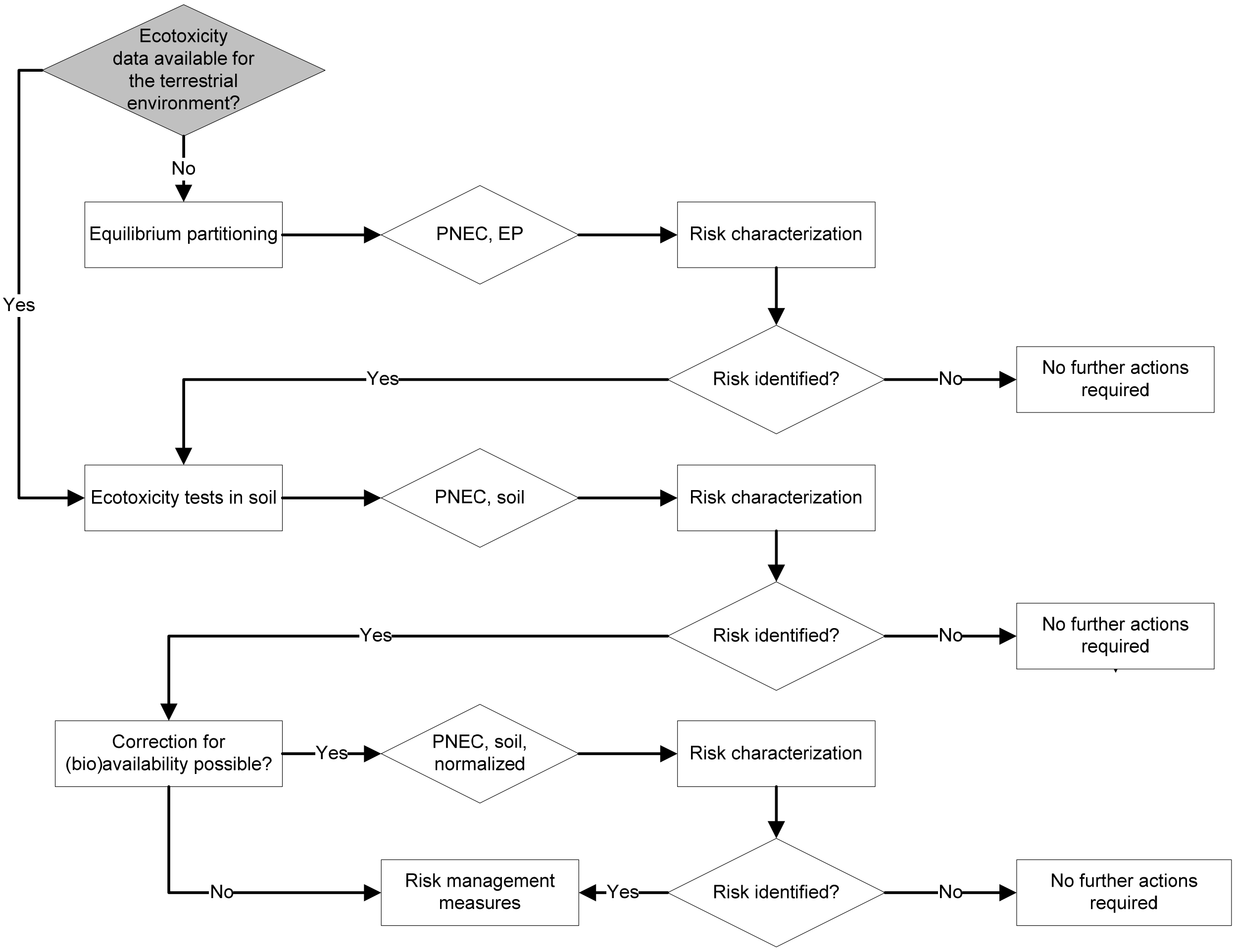

Figure 14: General framework for soil risk characterisation, applicable to both total and added risk approach.

Step 1: Equilibrium partitioning

In cases where no reliable toxicity data are available for the terrestrial environment or no terrestrial data are required depending on the tonnage band, a $PNEC_{soil}$ can be calculated according to the equilibrium partitioning concept based on a $PNEC_{water}$ and a reasonable worst-case soil: water distribution coefficient (Kd):

$$PNEC_{soil}\ (mg\ kg^{-1}) = PNEC_{freshwater}\ (mg\ l^{-1}) * Kd\ (l\ kg^{-1}) \qquad \text{(Equation 6)}$$

As a reasonable worst case, the 10th percentile of Kd values for soil is used (see section 2.2.2 if no information on Kd values is available). If the adsorption is expected to be high, an additional assessment factor of 10 should be added to the RCR to take exposure via ingestion into account.

Step 2: derivation generic PNEC soil

If the outcome of the equilibrium partitioning method results in a $PEC_{soil}/PNEC_{soil}$ ratio greater than 1, toxicity tests with soil organisms are an essential requirement for a refined hazard assessment. According to section 4.7.1 of the main TGD, a series of soil tests should ideally be designed to obtain data relevant to three trophic levels in soil (plants, invertebrates and micro organisms):

Step 3: (Bio)availability correction

Metal bioavailability and toxicity in soils does not solely depend on the total metal dose, but also on soil properties and time since contamination. Correction for differences in bioavailability of metals will further refine the risk assessment and allow for the derivation of more field relevant and site-specific PNEC values. This further refinement is not compulsory but may avoid identification of risk in some insensitive soils based on toxicity data derived in vulnerable soils.

Correction for leaching and ageing: the leaching-ageing (L/A) factor

In order to correct for this discrepancy between freshly spiked and field contaminated soils, a leaching-ageing (L/A) factor[22] should be incorporated. This L/A 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.

$$\text{Leaching-ageing (L/A) factor} = \frac{EC_x / NOEC_{Field\ /\ aged,\ add}}{EC_x / NOEC_{freshlyspiked,\ add}} \qquad \text{(Equation 7)}$$

Guidelines for L/A calculation:

- L/A factors should be calculated as a ratio between toxicity data generated from i) field or laboratory leached and aged soils and ii) freshly spiked soils.

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

- 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/A factor.
- Soils should either be artificially leached before ageing or allow free drainage of percolating rainwater in order to remove the excess salts.
- As natural metal background concentrations are already "aged", the derivation of the L/A factors should be based on added concentrations.
- The L/A 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/A factors could be based on either EC_{50} or EC_{10}/NOEC values. In cases of an appropriate test design robust EC_{10} (or EC_{20}) values can be estimated with low variability and these values should be used by preference[23].

The selection of the most appropriate L/A factor is not straightforward and should be done in a pragmatic and conservative but realistic way, for example by selecting one generic value situated at the lower end of the spectrum. In cases where there is a significant relationship between soil properties and the L/A factor, preference is given to derive soil-specific L/A factors. It must be stressed that the L/A factor should not be applied on ecotoxicity data collected in field contaminated or in spiked and aged soils.

Correction for variation in soil properties:

The bioavailability of metals and metal compounds in soils is largely controlled by soil properties (pH, Eh, organic matter, clay content, iron and manganese oxide content, mineralogy of the parent material). Correction for the variation in these properties among soils and normalization to soil specific characteristics requires an understanding of the relationship between soil physico-chemistry and metal toxicity on microbial function, plants and invertebrates. In order to perform this normalization, speciation or bioavailability models, mechanistically based bioavailability models or empirically based regression models predicting the metal toxicity in spiked soils based on soil properties (e.g. eCEC, pH, background metal, etc.) should be available or developed. These models/observed relationships allow the prediction of soil specific metal toxicity in laboratory spiking. Strong preference should be given to validated models.

This normalization procedure uses the following steps:

- Corrections should be based on toxicity data for a minimum of one species from all three trophic levels and for a range of soils that cover the natural variation in soil properties in the EU.
- Link the NOEC/EC_{10}/EC_{50} values of the chronic ecotoxicity database (as total metal concentrations) with the soil properties (CEC, pH and OM) of the soils in which the test was performed
- When the regression approach is used, the NOEC/EC_{10}/EC_{50} should be normalised using the corresponding organism-specific slopes (from the regression analysis) to 'reference' soil

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[23] EC10/EC20 values generally result in larger L/A factors due to larger relative differences. Only if no ECx values are available is it acceptable to use NOEC values.

properties or to specific local /regional conditions, i.e. to the driving abiotic factors of the soil for which the bioavailability corrections can be performed. Regressions are preferably based on a log-log basis:

$$\log(ECx/NOEC) = intercept + slope * \log(abioticfactor) \qquad \text{(Equation 8)}$$

In this case, the normalization equation is:

$$NOEC_{reference} = NOEC_{test}\left[\frac{abioticfactor_{reference}}{abioticfactor_{test}}\right]^{slope} \qquad \text{(Equation 9)}$$

reference = scenario for which PNEC must be derived
test = the abiotic factors of the soil in which the NOEC is derived

- In cases where the bioavailability model (e.g. terrestrial BLM approach is used, the NOEC/EC_{10} should be normalised using the corresponding organism-specific T-BLM. After normalization of all individual chronic toxicity data, species or process geometric mean values should be calculated and used for a normalised PNEC derivation.

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 (e.g. ecoregion) as long as these abiotic parameters fall within the geochemical boundaries of the bioavailability model (e.g. range of eCEC, organic matter, pH). For those species for which no specific bioavailability model has been developed, it should be verified on a case-by-case basis whether the bioavailability model of another species within the same trophic level can be applied. For guidance on this issue the reader is referred to the guidance given in the section hazard assessment of the aquatic environment.

Implementation of bioavailability correction

The correction for both differences in metal toxicity between freshly spiked soils and field contaminated soil and differences in metal toxicity among freshly spiked soils can be made as follows (Figure 15):

- Correct each individual generic added NOEC/EC_{10} value with the derived (organisms/soil specific) leaching-ageing factor (L/A-factor). This generates aged generic added NOEC/EC_{10} (L/A-F * NOEC/$EC_{10, \text{generic, added}}$) values.
- Add the individual background concentrations from the soil test media (C_b) to the corresponding L/A corrected generic added NOEC/$EC_{10,}$ values (C_b + L/A * (NOEC/$EC_{10, \text{generic, added}}$)). This step generates the aged generic total NOEC/EC_{10} values.
- Normalize the generated total aged NOEC/EC_{10} values to soil-specific aged NOEC/EC_{10} values, using equation 8, based on the total slopes from the organism-specific regression models or using relevant speciation/bioavailability models. In cases where the L/A factor is dependent on soil properties, the application of this factor will also affect the regressions between toxicity thresholds and soil properties and the slope from regressions on total aged NOEC/EC_{10} values should be used. If the L/A factor is constant for all soils, regressions can be based on total freshly spiked NOEC/EC_{10} values.

Calculate the soil-specific aged $PNEC_{total}$ based on the assessment factor or SSD approach

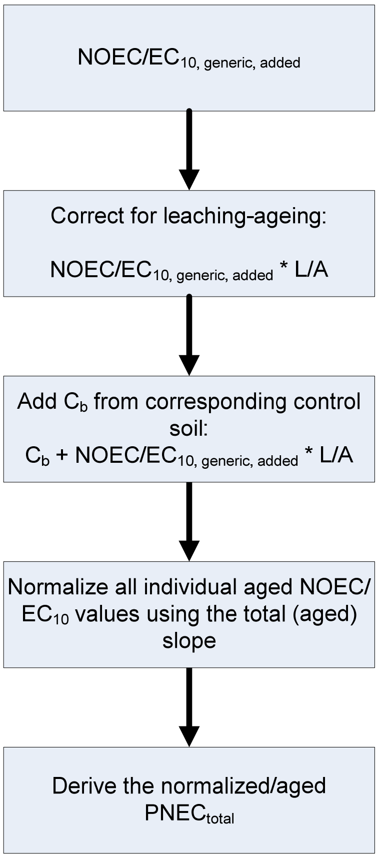

Figure 15: Framework for the calculation of a site specific PNEC soil.

Example 3-10 bioavailability correction for nickel in soil

eCEC is the driver for bioavailability correction across soils.
Reference situation: soil with eCEC of 15 $\text{cmol}_c\ \text{kg}^{-1}$

Case 1:
Chronic toxicity assay to *Lycopersicon esculentum* shoot yield in a sandy clay loam (pH 6.7, 1.9% organic matter, 9.6% clay, eCEC: 7.8 $\text{cmol}_c\ \text{kg}^{-1}$, C_b: 11 mg Ni kg^{-1})
equilibration period before start of test: 7 days

L/A factor is dependent on pH: (L/A=1 + exp(1.4*(pH-7.0))
Correction for soil properties: log $EC50_{\text{total, aged}}$ = 1.06 + 1.27*log eCEC (equation derived for *Lycopersicon esculentum*).

$EC10_{\text{added, generic}}$ = 118 mg Ni kg^{-1}
$EC10_{\text{added, aged, generic}}$ = 196 mg Ni kg^{-1}
$EC10_{\text{total aged, generic}}$ = 207 mg Ni kg^{-1}
$EC10_{\text{total, aged, corrected}}$ = 474 mg Ni kg^{-1}

Case 2:
Chronic toxicity assay to *Lolium perenne* yield in a sandy loam (pH 6.0, 2.9% organic matter, eCEC: 31 $\text{cmol}_c\ \text{kg}^{-1}$, C_b: 19 mg Ni kg^{-1}). Equilibration period before start of test: <120 days

L/A factor is dependent on pH: (L/A=1 + exp(1.4*(pH-7.0))
Correction for soil properties: log $EC50_{\text{total, aged}}$ = 1.57 + 1.12*log eCEC (i.e. equation for bioavailability correction for plants with the lowest slope (derived for *Hordeum vulgare*), because no specific equation available for *Lolium perenne*

$EC10_{\text{added, generic}}$ = 110 mg Ni kg^{-1}
$EC10_{\text{added, aged, generic}}$ = 137 mg Ni kg^{-1}
$EC10_{\text{total aged, generic}}$ = 156 mg Ni kg^{-1}
$EC10_{\text{total, aged, corrected}}$ = 69 mg Ni kg^{-1}

4.5 Guidance on the risk characterization for secondary poisoning

The guidance given in this section is not always only relevant for metals but the issues raised have quite often being observed for metals

The risk characterization component of this secondary poisoning evaluation is depicted in Figure 16.. The risk analysis is conducted in tiers, with the first tier being the most simplified and subsequent tiers being based on increasing levels of complexity. For each tier, a PEC_{oral}-to-$PNEC_{\text{oral}}$ ratio has to be calculated based on equation 10:

$$PEC_{\text{oral}}\text{-to-}PNEC_{\text{oral}}\ \text{Ratio} = (PEC_{\text{oral}} \times RAF) / PNEC_{\text{oral}} \qquad \text{(Equation 10)}$$

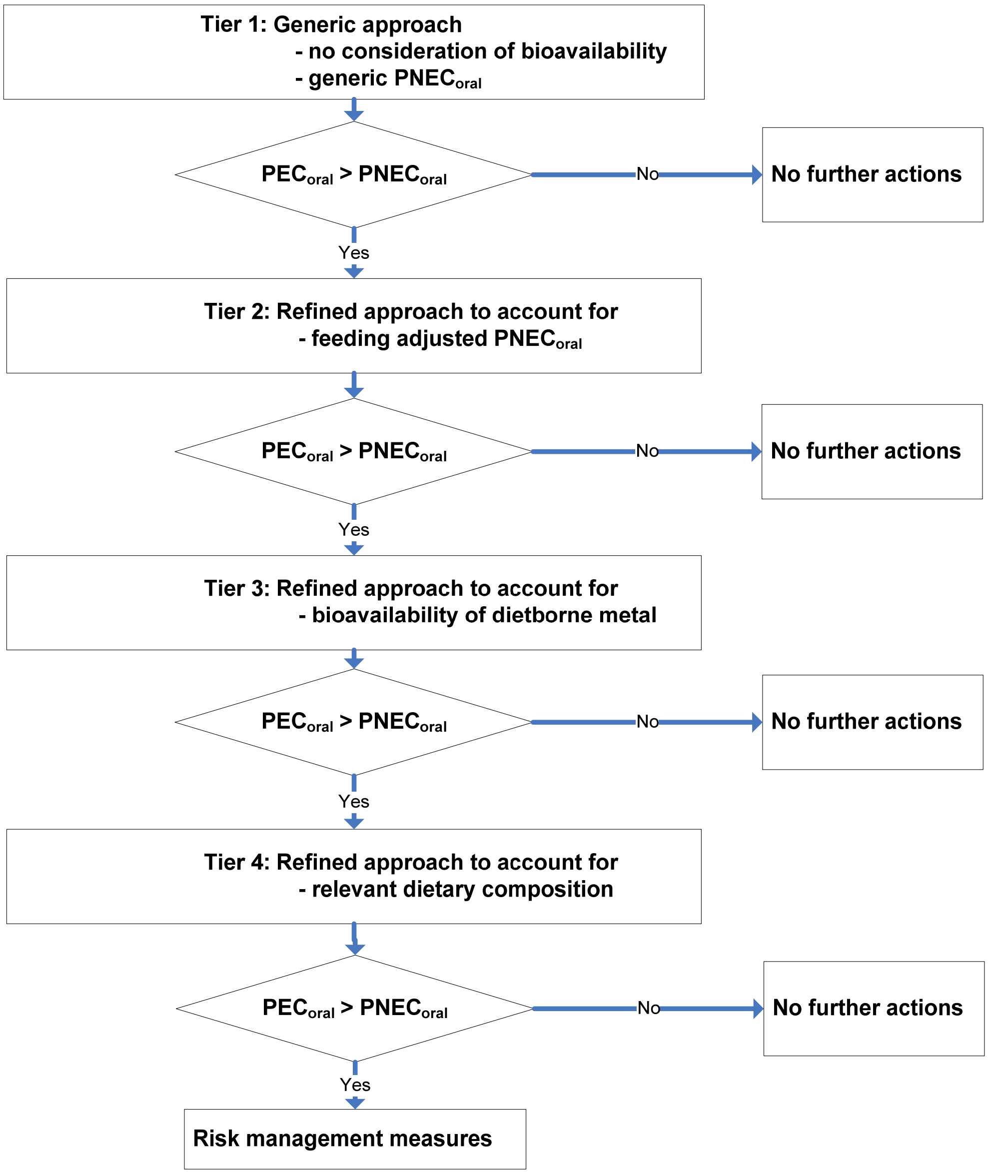

Figure 16: Framework for the calculation of a PNEC oral.

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