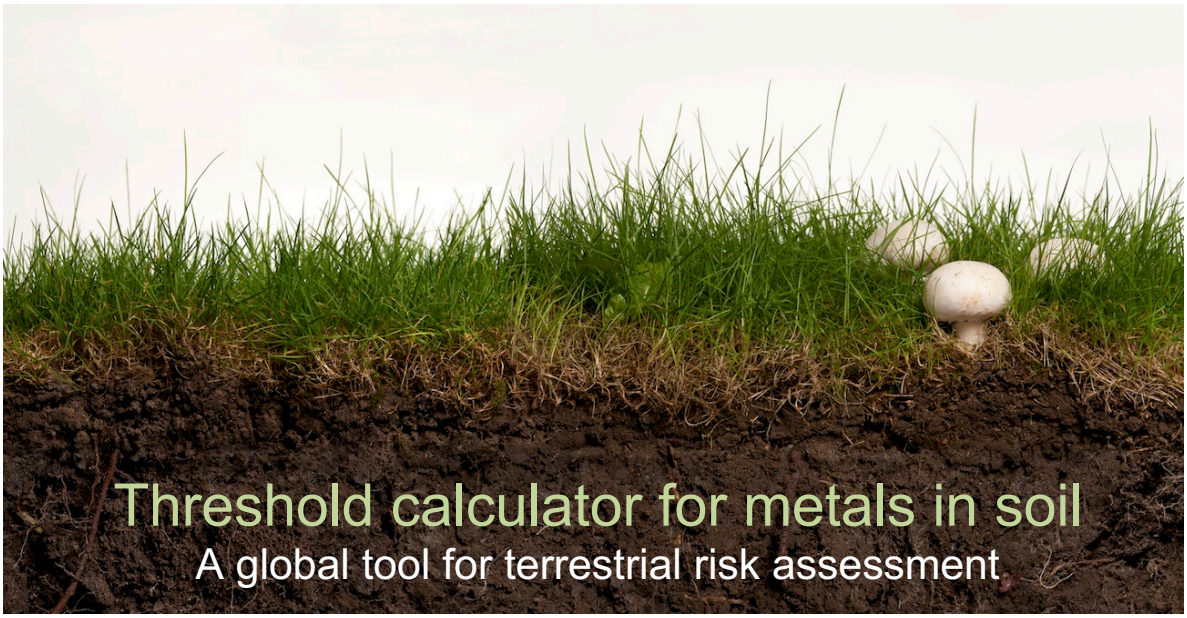

BACKGROUND DOCUMENT FOR VERSION 3.1

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Koen Oorts, PhD

ARCHE Consulting

Liefkensstraat 35D

B-9042 Wondelgem, Belgium

koen.oorts@arche-consulting.be

www.arche-consulting.be

Tel: +32 16 28 49 01

Disclaimer

This tool uses soil ecotoxicity data extracted from the REACH dossiers of the metals arsenic, cadmium, cobalt, copper, lead, molybdenum, nickel, and zinc ("the dataset"), which is the intellectual property of the International Lead Association, the International Zinc Association, the Cobalt Institute, the European Copper Institute and the Copper REACH Consortium, the International Molybdenum Association, NiPERA respectively ("the Associations"). Permission has been granted by the Associations for use of the dataset within this tool. Third parties shall not copy or change this dataset or use it for any other purpose without the express written permission of the Associations. To the maximum extent permitted by law, the Associations hereby exclude all liability arising in contract or otherwise for any direct, indirect or consequential loss or damage sustained by any direct or indirect user of the tool and its embedded dataset.

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1. INTRODUCTION

The Threshold Calculator is a flexible risk assessment tool for metals in soil and it can be used in various parts of the world to derive soil type-specific ecotoxicological thresholds for different protection goals. This tool calculates ecotoxicological threshold concentrations for the metals As, Cd, Co, Cu, Pb, Mo, Ni and Zn based on chronic toxicity data for their direct effects to soil organisms (plants, invertebrates and microbial processes) and, where relevant, for secondary poisoning to mammals and birds through bioaccumulation in the food chain. All threshold values are expressed as (pseudo-)total (i.e., aqua-regia extractable) metal concentrations in soil (mg/kg dry weight). The goal of this tool is to maximise the available toxicity data and bioavailability models for the derivation of soil quality standards for specific protection goals, jurisdictions, regions, or sites. Several options are available to calculate metal soil threshold concentrations for various goals (e.g., risk assessment, or setting of remediation thresholds for different land uses): selection of organism groups or species to be considered, selection of effect levels of original toxicity data (EC_{10}, EC_{20}, NOEC, LOEC, MATC, …), general probability level in distribution of effect levels, etc. In addition, except for Cd, site specific toxicity thresholds can be calculated based on information on the soil properties of the site of interest.

All metals in this tool have a wide range of chronic toxicity data for direct effects to soil organisms, covering all major groups of soil organisms and allowing the use of a statistical extrapolation approach for derivation of a threshold concentration with a species sensitivity distribution (SSD). The soils used for ecotoxicity testing for each metal cover a wide range of soil properties, making the results globally representative. The results are expressed both as total metal concentrations (i.e., including the background concentration in soil) and as added concentrations (i.e., only based on the added doses, without the contribution of the background concentration of the metal in soil). The former is a measure of the total metal concentration a soil can contain before ecotoxicological effects reach the protection level and can be directly compared with total measured concentrations in a soil assessed. The added approach provides a measure of how much metal can be added to soil before the allowed effects are reached. In the latter case, one must add a measured or predicted ambient background concentration for the soil type assessed for comparison of the threshold with total metal concentrations measured in soil.

The potential for secondary poisoning of mammals and birds via the food chain is only included for As, Cd, Pb and Ni. This pathway is not relevant for Co, Cu, Mo and Zn due to their essentiality and the strong homeostatic control of their internal concentrations in organisms. Soil quality standards for this pathway are all expressed as total metal concentrations and calculated following two approaches: either based on the critical metal concentration in food (in mg/kg diet) and the metal-specific bioaccumulation factor (BAF) in earthworms, or based on the critical metal intake rate (mg/kg body weight/day) and assumptions on food intake rate and the BAF in earthworms. For Pb, sufficient information is available allowing selection of effect level (x in EC_x), the use of NOEC, MATC or LOEC values in case no reliable EC_x values are available, and the selection of probability level in the species sensitivity distribution (p in HC_p) to determine the thresholds for

metal concentration in food and the metal intake rate. The bioaccumulation of Pb in earthworms varies with soil properties and a soil-specific BAF for Pb is calculated based on information on the soil properties of the site of interest.

2. DATA SELECTION

2.1. DIRECT TOXICITY TO SOIL ORGANISMS

The soil effect thresholds are based on relevant and reliable chronic toxicity data for terrestrial organisms (plants, invertebrates, and microbial processes) derived from scientific literature or research projects. All data were thoroughly screened for their relevance and reliability. Acceptance criteria are summarised in Table 1.

The datasets developed for the European REACH dossiers (Registration, Evaluation, Authorisation and Restriction of Chemicals; Regulation EC No 1907/2006) were the basis for this data collection, but all studies were re-evaluated and reliable data outside the specific scope of REACH were also included. There were no restrictions on the relevance of soil properties or species towards specific regions. Only high quality data, corresponding to categories 1 ("Reliable without restrictions") and 2 ("Reliable with restrictions") according to the Klimisch and CRED scoring systems (Klimisch et al., 1997, Moermond et al., 2016) are considered. In order to optimize this tool to meet the needs of all potential users and to maximize acceptance, a comprehensive and transparent database was developed, including information on test substance, test organism, study reference, soils used, test conditions and toxicity data.

Table 1. Main relevance and reliability criteria for selection of terrestrial ecotoxicity data.

Relevance	Reliability
• Test substance: high purity soluble metal salts • Test medium: only data from observations in natural and artificial (e.g., OECD) soil media • Test species: primary producers (plants), consumers (invertebrates) and decomposers (microbial mediated processes), relevant for the area under consideration • Toxicological endpoints: direct effects at population level, e.g., mortality, growth and reproduction for plants and invertebrates, or functional variables such as C- and N-	• Type of test: standard test (e.g., ISO, OECD) or not, endpoint used, test conditions • Description of test material and methods: e.g., test set-up, measuring chamber/device, spiking method, test organism, including size (age), origin, number of organisms per replicate, test design (# replicates used), type of food given • Description of the test soil: e.g., soil type, location, pH, organic carbon, clay content, CEC • Chemical analysis: test concentrations during the test are measured or evidence that the nom-

Relevance	Reliability
mineralisation for soil microbial endpoints • Exposure duration: tests focusing on sensitive life stages (e.g., root elongation) or from "chronic exposure" (e.g., growth, reproduction).	inal concentrations are close to actual concentrations • Concentration-effect relationship: acceptable control response (mortality, reproduction, growth, etc.), tested concentration range is reported, at least 2 different concentrations tested besides the control, a clear concentration related response, sound statistics used to derive a suitable EC_x or NOEC/LOEC value

2.2. SECONDARY POISONING OF MAMMALS AND BIRDS

Data on oral toxicity were only considered relevant and reliable when they were based on sub-chronic and chronic studies (≥ 21 days) and the endpoint is ecologically relevant (e.g., growth, reproduction) and not merely a biomarker for metal exposure. At least two concentrations above the control must have been applied. Mixed metal feeding studies, studies where the test substance was injected in test animals and tests where it was administered through drinking water or as metal pellets were all considered not relevant and excluded. In case low doses of metal were added to the diet and toxicity was observed at doses close to natural background concentrations, the metal concentration in the diet of the control animals must have been measured and quality control of these measurements reported. Unbounded toxicity data (i.e., significant effects observed at the smallest dose or no significant effect observed at the largest dose tested) were not considered.

The metal bioaccumulation in earthworms was assessed based on a compilation of literature data where the bioaccumulation factor, calculated as the ratio of the metal concentration in earthworm over the metal concentration in the soil, is based on measured concentrations in soil and biota from field observations (Sample et al., 1999). The following qualification criteria were applied in the assessment. The metal concentration in soil had to be expressed as "total" soil metal (e.g., measured after *aqua regia* destruction) and results based on extractable fractions (e.g., water- extractable metal) were not considered reliable. Earthworms must have been rinsed and soil voided from the intestinal tract prior to analysis. It must be reported if the bioaccumulation factors were expressed on a dry or wet weight basis. Data were only considered relevant and reliable if the data came from field studies or laboratory studies using soil and biota collected at the same field site. This ensured that metal burdens in biota were in a steady state with soil metal concentrations and it eliminated the need to correct for differences in metal availability between laboratory-spiked soils and field-contaminated soils. Data from laboratory studies where the metals were added to the soil as a salt are hence excluded.

3. DERIVATION OF TOXICITY DATA

Wherever possible, all dose-response curves were refitted according to a 3-parameter log-logistic dose-response curve (Figure 1) according to:

$$Y = \frac{Y_0}{1 + e^{4S(logX - logEC_{50})}}$$

where

- Y is the response at metal concentration X,
- Y_0 is the control response,
- S is the slope of the curve and
- log EC_{50} is the logarithm of the EC_{50} value.

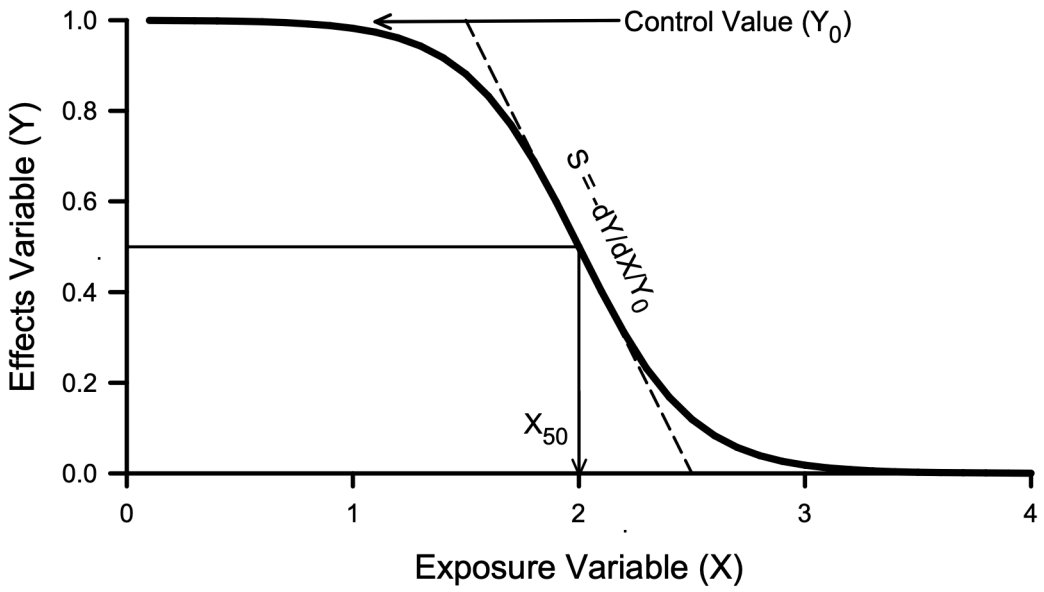

Figure 1. Standard log-logistic dose-response curve (X is the log10 of the metal concentration).

This curve is consistently fitted with the US EPA TRAP program, version 1.30a (https://archive.epa.gov/med/med_archive_03/web/html/trap.html). Depending on the available data in the original publications and reports, dose-response curves were fitted based on (in order of preference): (i) the raw data, (ii) the average response per treatment or (iii) the reported EC_x values (if ≥2 reported).

The parameters of the log-logistic curve (log EC_{50}, S, and Y_0) are reported in the database, together with the source data used, and are used to calculate the EC_x based on the effect level (x) selected according to:

$$\log EC_x = \log EC_{50} + \frac{\ln\left(\frac{x/100}{1-x/100}\right)}{4S}$$

Next to the EC_x levels derived by the log-logistic dose-response model, also NOEC (highest no observed significant effect concentration), LOEC (lowest observed significant effect concentration) and MATC (maximum acceptable toxicant concentration, = geometric mean of NOEC and LOEC) are reported in the database, together with the statistical method used to derive them.

EC_x values are always preferred over NOEC, LOEC, or MATC values. Only EC_x values within the tested concentration range, i.e., between the lowest and highest added dose tested, are considered as reliable and taken forward for the calculation of the soil threshold concentration. In case no reliable EC_x value can be derived (because no reliable dose-response curve or value outside the tested concentration range), the user can choose to use a NOEC, MATC or LOEC if such value is available and enter the upper effect levels attributed to these values (e.g., NOEC can be used equivalent up to EC_{10}, MATC up to EC_{25} and LOEC up to EC_{40}).

4. BIOAVAILABILITY CORRECTIONS

Metal toxicity and bioaccumulation not only depends on the total metal dose, but also on the time since contamination and on physico-chemical soil properties. For some metals, models are available for correction of the data on direct toxicity to soil organisms for differences in bioavailability and toxicity of the metal between the test soils and the target soil of the site of interest (Smolders et al., 2009, OECD 2016). This bioavailability correction is based on

i) correction for the differences in bioavailability between metal contamination in laboratory and field conditions, via the so-called lab-field factor (i.e. an empirically derived factor used to account for the reduced toxicity of metals observed in the field as compared to the same 'total' concentration in laboratory toxicity tests with soluble metal salts), and

ii) normalisation of toxicity thresholds or bioaccumulation factors towards soil properties of the target soil based on regressions of toxicity or bioaccumulation data with these soil properties.

The bioavailability corrections for metal toxicity to soil organisms are the result of comprehensive research projects, where various toxicity assays were performed in a range of soils after various spiking treatments.

4.1. CORRECTION FOR CONTAMINATION IN LABORATORY VERSUS FIELD CONDITIONS

The lab-field factor (L/F factor) relates the differences in metal dose required between soils tested after spiking with a soluble metal salt and corresponding field-contaminated or laboratory-spiked, leached and aged soils to produce a same toxicity effect in a specific soil:

$$L/F\ factor = \frac{EC_{x,add}\ field\ contaminated\ or\ aged}{EC_{x,add}\ after\ freshly\ spiking}$$

This factor addresses the differences in toxicity between tests on soils spiked in the lab and tests on field contaminated soils using single species or micro-organisms functional tests due to differences in ionic strength and pH or equilibration (ageing) of metals in soil. Because natural metal background concentrations are already "aged", the derivation of the L/F factors is based on added concentrations. All assessments are based on actual measured metal concentrations in soil to correct for potential losses due to leaching.

Lab-field factors selected in the calculator tool are reported in Table 2. The studies for derivation of the lab-field correction factors used are summarised in Annex 1. The changes in metal toxicity with long-term equilibration and leaching were typically studied for 6 to 10 endpoints in 3 to 7 different soils, while changes in metal chemistry were studied in up to 19 soils. The selection of the lab-field factor is based on a weight of evidence taking into account both the changes in metal toxicity with long-term equilibration (ageing) or leaching excess ions, and changes in metal behaviour (pore water concentrations, E-values, etc.) in soil.

Table 2. Bioavailability corrections for metal toxicity to soil organisms implemented in European REACH dossiers.

Element	Lab-field factor	Soil properties for data normalisation
Arsenic	/	pH and % clay
Cadmium	/	/
Cobalt	1.2-3.5 (increasing as a function of pH)	eCEC[b]
Copper	2.0	eCEC, % organic carbon, % clay and pH[c]
Lead	4.0 / 2.0[a]	eCEC
Molybdenum	2.0	pH and % clay
Nickel	1.0-4.0 (increasing as a function of pH)	eCEC
Zinc	3.0	eCEC, pH and background Zn

[a] lab-field factor for correction for both ageing and leaching processes or for ageing processes only

[b] eCEC: effective cation exchange capacity = CEC at prevailing soil pH

[c] pH measured in 0.01 M $CaCl_2$ suspension

4.2. CORRECTION FOR VARIATION IN SOIL PROPERTIES

Regression models to account for the effect of soil properties on metal bioavailability and toxicity in soils have been derived for a wide range of European, Australian and Chinese soils in the framework of corresponding risk assessment processes in these regions (OECD, 2016, Table 3, Annex 3). These regression models vary with toxicity endpoint, e.g., the model found for toxicity of a metal to plants may not be the same model as for the same metal on an earthworm. The models correct for effects of soil properties via a "factor change" of the threshold, this factor is based on the slope of a regression between the log transformed threshold and the soil property. These slopes are applied to all toxicity data of species of the same group. The 6 major groups considered are monocotyledonous plants, dicotyledonous plants, 'hard-bodied' soil invertebrates (e.g., arthropods), 'soft-bodied' soil invertebrates (e.g., earthworms), microbial carbon transformation and microbial N transformation.

Table 3. Global availability of normalisation models for metals.

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

It must be noted that when several models are available for taking into account the effect of soil properties on metal bioavailability and toxicity for the same endpoint, they may have identified other soil properties as best predictor of metal toxicity for this endpoint. For example, three different regression models exist for effect of soil properties on toxicity of Cu to the microbial nitrification process in soil in three geographic areas. The three models identify different soil properties as the best predictor of Cu toxicity: eCEC (European model), pH (Australian model) or total calcium concentration (Chinese model). Review of these models shows that differences are mainly due to differences in methodology for soil analyses (e.g., CEC vs eCEC) and endpoints measured (e.g., nitrification at limited or unlimited substrate availability). There is no indication that the applicability of the models is restricted to a specific region or soil types. Because the models developed in the framework of European risk assessments cover most metals and most species and are based on soils with a large range of soil properties, which is also relevant for most other (temperate) regions in the world, these EU models are selected for this tool. These models are based on comparative studies where the effect

of soil properties on metal bioavailability and toxicity in soils was tested for 6 to 11 different toxicity assays in 8 to 19 different soils covering a wide range in soil types and soil properties (Annex 2). Soils were sampled in Europe, except for Co, where 1 soil from Canada and 2 soils from the USA were included in the research project. The range in soil types and soil properties covered is however representative for most regions in the world (see e.g., Annex 4). For each metal and species tested, regression equations between metal toxicity and soil properties were derived in order to normalise the data for the varying soil properties.

Similar as for toxicity, field-based bioaccumulation factors of Pb in earthworm are also significantly correlated with the eCEC of the soils (Annex 5). This correlation can be use in the derivation of soil quality standards for protection of wildlife (mammals and birds) against secondary poisoning through exposure via the food chain.

The input parameters required are dependent upon the metal under consideration and are generally readily available soil parameters likely to be determined in routine soil analyses (Table 2). For compatibility with the bioavailability models, soil properties must be measured according (or equivalent) to the following methods

- **pH**: measured in a 0.01 M $CaCl_2$ soil suspension (e.g. ISO 10390:2005).
  In case different methods were used for analyses of pH, the available results for pH in the toxicity database are corrected towards pH in 0.01M $CaCl_2$ according to the following equations:
  *pH 0.01 M $CaCl_2$ = -0.54 + 1.00 * pH H_2O (based on data for set of 86 Dutch soils, R^2=0.88)*
  *pH 0.01 M $CaCl_2$ = 0.79 + 0.89 * pH 1 M KCl (based on data for set of 86 Dutch soils, R^2=0.91)*

- **Organic carbon content (%)**: as determined by e.g. a dry combustion method (e.g. ISO 10694:1995).
  When only information on the soil organic matter (SOM) content is available, the soil organic carbon (SOC) content can be calculated as follows: % SOC = %SOM x 0.58.

- **Clay content (%)**: fraction of mineral soil particles <2 µm, as determined through sieving and sedimentation after a complete dispersion of the soil (e.g. ISO 11277:2009).

- **effective Cation Exchange capacity (eCEC, $cmol_c$/kg)**: CEC measured at prevailing pH of the soil (as opposed to the CEC measured at a buffered pH value, usually pH 7.0) (e.g. ISO 11260:1994, Pleysier and Juo, 1980).
  If no eCEC value is reported, the eCEC can be predicted based on data for pH, clay and organic carbon (OC) content of the soil with the following equation (Helling et al. 1964):
  eCEC ($cmol_c$/kg) = (30 + 4.4xpH) x %Clay/100 + (-59 + 51xpH) x %OC/100

- **Background Zn content (mg/kg)**: the total or pseudo-total Zn concentration in an unpolluted reference soil with the same physico-chemical properties (as measured after digestion of the soil with aqua regia or similar strong acids, e.g. ISO 11466:1995).

When information on these soil properties for a site of interest is available and entered in the tool, soil-specific threshold concentrations are calculated. When no information on soil properties is entered, only a generic threshold, corrected for differences in bioavailability between laboratory and field conditions, will be calculated.

It must be noted that for bioavailability corrections of Zn, information is required on the natural background concentration of Zn in the target soil, while such background metal concentrations are not required for bioavailability corrections for other metals. In case the added metal concentration approach is selected, the background concentration is however needed for all metals of interest.

4.3. IMPLEMENTATION BIOAVAILABILITY INTO DERIVATION OF ECOLOGICAL THRESHOLD CONCENTRATIONS

4.3.1. DIRECT TOXICITY TO SOIL ORGANISMS

The general framework for implementation of bioavailability into derivation of ecological threshold concentrations is described in Smolders et al. (2009) and OECD (2016), and is presented schematically in Figure 2.

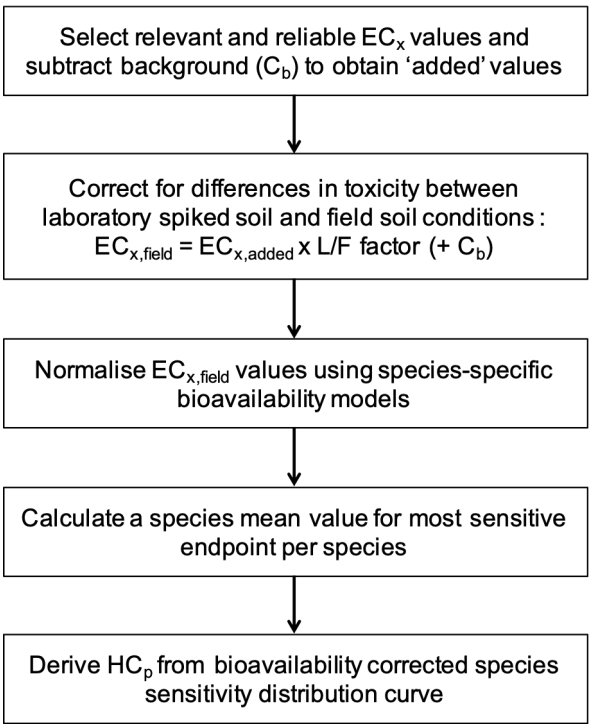

Figure 2. Flow chart for the implementation of bioavailability factors into derivation of soil thresholds.

In summary, the following steps are followed. After selection of the reliable EC_x values, the added EC_x values are derived by subtracting the background of the tested control soils from the EC_x values based on total measured concentrations. In a second step, toxicity thresholds are corrected for the discrepancy in toxicity between freshly-spiked soils in laboratory conditions and field-contaminated soils, by multiplying all individual added EC_x values with the metal-specific lab-field factor (Table 2, Annex 1). For the "total metal" approach, the corresponding metal background concentration from each individual test soil is then added again in order to calculate the total aged EC_x values. The correction with lab-field factor is omitted for toxicity data that are obtained after more than 120 days equilibration of a metal in the soil before the start of the toxicity assay since added metals are already considered as "aged" in such studies.

In the following step, the toxicity data were corrected for differences in metal bioavailability among soils, allowing calculation of a specific threshold concentration for the soil under investigation. Each "field" or "aged" EC_x value is normalised towards the soil properties of a specific target soil, using the slope of the respective regression function (log-log based, Table 2 ,Annex 2) and following equation:

$$EC_{x,reference} = EC_{x,test} \left[\frac{abiotic\,factor_{reference}}{abiotic\,factor_{test}} \right]^{slope}$$

where *reference* is the soil for which the soil threshold concentration must be derived, *test* is the soil used in the ecotoxicity test, and *abiotic factor* is the soil property with which toxicity is correlated. Normalisation of the individual EC_x data towards specific soil properties reduces the within species-variation in EC_x values for most organisms.

In case multiple data are available for the same species or microbial process, a species/process mean value is calculated as the geometric mean from all data for the most sensitive endpoint for each species or process. This species/process mean approach is preferred for normalised data, where the remaining variation among data for a given species/process can be mainly attributed to intra-species variation in sensitivity. This is however not the case for non-normalised data, where variation between toxicity data is also caused by differences in bioavailability among soils. Finally, a species sensitivity distribution (SSD) is fitted on the normalised, aged species/process mean EC_x values and the median hazardous concentration for p% of the species ($HC_{p\text{-}50}$) is derived as the median p^{th} percentile of this distribution (see paragraph 5).

4.3.2. SECONDARY POISONING OF MAMMALS AND BIRDS

Soil quality standards (SQS) for metals are calculated based either on the critical metal concentration in food (in mg/kg diet) and the bioaccumulation factor in food or on the critical metal intake rate ($mg/kg_{body\ weight}$ /day) and assumptions on food intake rate and the bioaccumulation factor in food according to the following equations.

Based on threshold concentration in food:

$$SQS = \frac{Threshold\ metal\ concentration\ in\ food\ (\frac{mg}{kg}\ food[wet\ weight])}{BAF}$$

with SQS = soil quality standard (mg/kg soil [dry weight]), and BAF = (soil-specific) bioaccumulation factor for earthworms (kg soil [dry weight] / kg worm [wet weight]) (ECHA, 2008).

Based on intake rate:

$$SQS = \frac{Threshold\ metal\ intake\ rate\ (mg/kg\ body\ weight/day)}{FIR * (P_s + BAF)}$$

with SQS = soil quality standard (mg/kg soil [dry weight]), FIR = food ingestion rate (kg food [dry weight]/ kg body weight / day), P_s = Proportion of diet that is soil, and BAF = (soil-specific) bioaccumulation factor (kg soil [dry weight] / kg food [dry weight]) (U.S. EPA, 2005). The assumptions on food intake rate and proportion of the diet that is soil are taken from the guidance for developing ecological soil screening values (Table 4).

Table 4. Parameterisation of the Eco-SSL wildlife exposure model (U.S. EPA, 2005 and Sample et al., 2019).

Receptor group	Food ingestion rate (FIR) Kg dw/kg body weight/day	Soil Ingestion (P_s)
Mammalian Herbivore	0.0875	0.032
Mammalian Ground Insectivore	0.209	0.030
Mammalian Carnivore	0.130	0.043
Avian Grainivore	0.190	0.139
Avian Ground Insectivore	0.214	0.164
Avian Carnivore	0.0353	0.057
Avian Omnivore (mixed diet)	0.159	0.200

5. DERIVATION OF SOIL THRESHOLD CONCENTRATIONS ACCORDING TO THE STATISTICAL EXTRAPOLATION METHOD (SPECIES SENSITIVITY DISTRIBUTION)

When a large data set for different taxonomic groups is available, as is the case for these metals, an ecological threshold concentration can be calculated using the statistical extrapolation method in which the susceptibility of a set of species for a given toxicant can be described by some statistical distribution (i.e.,

Species Sensitivity distribution or SSD). A SSD can be visualised as a cumulative distribution function (Figure 3). The cumulative distribution function curve follows the distribution of the sensitivity data obtained from ecotoxicological testing, plotting effect concentrations derived from the toxicity tests.

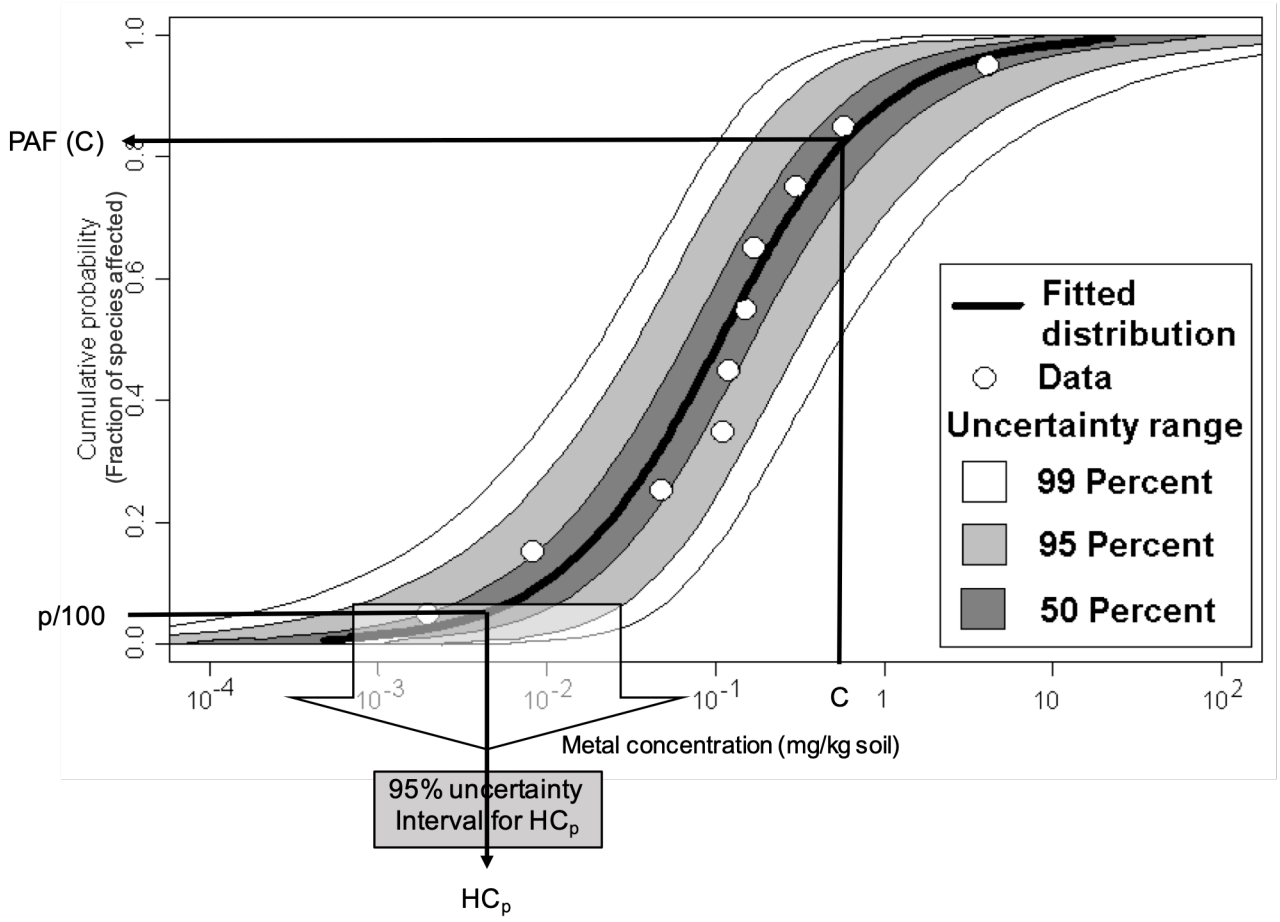

Figure 3. Example of a SSD (Species Sensitivity Distribution) with uncertainty band and its HC_p (Hazardous Concentration at p %) and potentially affected fraction (PAF) for a metal concentration of C mg/kg soil.

A cut-off percentage p is chosen (to protect 1-p percent of species), and the desired "safe" concentration HC_p is calculated. For example, the 5th percentile of a chronic toxicity distribution has been chosen under the EU REACH Regulation as a concentration that is protective for most species in a community (namely 1-p %). The median hazardous concentration for p% of the species ($HC_{p\text{-}50}$) is derived as the median pth percentile of the fitted log-normal distribution. Lower (5%) and upper (95%) confidence limits of the $HC_{p\text{-}50}$ values are calculated according to Aldenberg and Jaworska (2000). Extrapolation factors for combinations of protection level p and sample size of the SSD not reported by Aldenberg and Jaworska (2000) were linearly interpolated between the closest factors reported.

When the metal concentration for a specific soil or site is known, this program also calculates the Potentially Affected Fraction (PAF) of the selected terrestrial organisms at the given metal concentration in soil and the effect level selected based on the fitted log-normal distribution.

It is generally accepted that an SSD should contain at least 10 NOECs (preferably more than 15) for different species covering several taxonomic groups. Based on the experience of metal risk assessments under the EU REACH regulation, it is recommended that the following taxonomic groups should be covered in an SSD to be representative for the diversity in terrestrial organisms: at least 2 species of dicotyledonous plants, belonging to different families, one monocotyledon plant, an arthropod, an annelid worm and microbial processes relating to the carbon and nitrogen cycle.

All toxicity data (for selected trophic levels and effect level) are grouped in a species sensitivity distribution. Two options are followed here:

- Including all individual data in the SSD
- Only one value per species or microbial process. This value is calculated as the geometric mean of all data for the most sensitive endpoint.

This species/process mean approach is preferred for normalised data, where intra-species variation can be considered as the main source of variation among data for a given species/process. However, this is not the case for non-normalised data where variation between toxicity data is also caused by differences in bioavailability among soils and, therefore, results for the distribution of all individual data are presented when no normalisation for soil properties can be performed.

For the derivation of dietary threshold values (as concentration in diet or intake rate) for mammals and birds, a species mean approach is always preferred since results are not depending on metal bioavailability in environmental media.

6. SELECTION OF JURISDICTION

Apart from the toxicity data according to the selections on effect and probability levels made by the users, one can also select the toxicity data used in specific jurisdictions. For direct toxicity to soil organisms, only the respective EU REACH Regulation (Regulation (EC) No 1907/2006) dossier for these metals are included. In this case, the outcome is the PNEC value (= predicted no effect concentration), which is based on the 5% probability level (5% Hazardous Concentration or HC_5) of the reliable EC_{10} or NOEC values selected in the dossiers submitted to the European Chemical Agency (ECHA, status January 2017), divided by an addi-

tional assessment factor (AF) between 1 and 5 depending on the uncertainty on the HC_5 concentration. The AF deliberated for each metal under EU REACH is also reported in the output of the tool when the EU REACH regulation is selected under jurisdiction. Because an assessment factor is depending on the specific regulatory framework, no assessment factor is considered and the HC_p value are reported when no jurisdiction is selected ("open (global)" option under jurisdiction). It should be noted that when selecting the EU REACH Regulation in the jurisdiction, only PNEC, derived as 5th percentile of NOEC and EC_{10} values from the REACH dossiers, will be used. Therefore, changing the effect level (EC_x) or probability level (HC_p) in the input field will not affect the calculations. In order to select any EC_x or HC_p option, one must make sure the "open (global)" option is selected under jurisdiction.

Although the set of NOEC and EC_{10} data are identical, correcting errors in soil property data and the recalculating pH based on measurements in 0.01 M $CaCl_2$, can result in slightly different PNEC values compared to the results from the PNECsoil calculator (http://www.arche-consulting.be/metal-csa-toolbox/soil-pnec-calculator/) under the same soil conditions.

The HC_5 of EC_{10} or NOEC data can also slightly deviate between calculations for the EU REACH data and results when no specific jurisdiction ("open (global)" option) is selected. These deviations are due to some differences in the toxicity data selected:

- consistent preference for EC_x values in the database for "open (global)" threshold derivations, in contrast to the use of NOECs in some REACH dossiers
- predicted ECx values below the lowest dose added are consistently considered as not reliable and hence not used for derivation of threshold values in this database
- including some additional reliable toxicity data not covered by the REACH dossiers

For direct toxicity to soil organisms, there is also the option to select the toxicity data and thresholds used in the respective REACH dossier for these metals. In addition, the ecological soil screening levels (Eco-SSL) for wildlife toxicity derived by the US EPA (https://www.epa.gov/chemical-research/ecological-soil-screening-level) can also be consulted.

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ANNEX I: DERIVATION OF LAB-FIELD FACTORS

The derivation of the lab-field (L/F) correction factor is for each metal further explained in detail below. The available data for derivation of the lab-field correction factors are summarised in the Table A1.1.

Derivation of L/F factor for arsenic

No lab-field factor is derived for effect of As on terrestrial organisms.

Derivation of L/F factor for cadmium

No lab-field factor is derived for effect of Cd on terrestrial organisms.

Derivation of L/F factor for cobalt

Data availability:

- Difference in Co toxicity to 3 plants, 3 invertebrates and 3 microbial processes between 3 soils freshly spiked with $CoCl_2$ and corresponding experimentally aged soils
- Difference in Co toxicity to 3 plants, 3 invertebrates and 3 microbial processes between 3 soils freshly spiked with $CoCl_2$ and corresponding leached soils
- Changes in lability (isotopically exchangeable fraction) of Co with increased equilibration time after spiking, tested in 14 soils from Europe, North America and Australia with contrasting soil properties and land use, spiked with $CoCl_2$ at the EC_{10} of a nitrification assay.

Derivation of L/F factor:

A semi-mechanistic model was constructed to describe the long-term change in the isotopically exchangeable Co fitted the available data. It was found that long-term ageing of added Co could be described by the following relationship:

$$E_{corr-add}(\%) = A - \frac{B}{10^{(pK^{\phi} - pH)} + 1} \times t^{C/t} - F \times \ln(t)$$

where *A* is a coefficient which represents the *E* value of added Co at time zero (i.e. $E_{corr\text{-}add}$ = 100%); *B* is a coefficient which is considered to be related to the effect of precipitation/nucleation; t is incubation time in days; *pK°* is the first hydrolysis constant of Co on soil surfaces; *pH* is measured in aerated deionised water; and *F* is a coefficient which is considered to be related to the effect of micropore diffusion. The EC_{10} based parameterised model is as below:

$$E_{corr-add}(\%) = 84.1 - \frac{59.1}{10^{(6.06-pH)}+1} \times t^{0.00097/t} - 3.64 \times \ln(t)$$

The change in the labile pool is the basis to calculate the factor by which the Co is aged. The modelled ageing factor is the ratio of the fraction of added Co that is labile at day 15 ('freshly spiked') and day 365.

These 'chemical' correction factors are compared with toxicity-based lab-field factors in Figure A1.1. Median lab-field factors per soil are 1.3 (pH 4.7), 2.0 (pH 7.0) and 1.9 (pH 7.5). The pH dependent chemical lab-field factors based on Co ageing in 14 different soils were selected for the risk assessment of Co in soil. This model is generally a conservative (lower) estimate of the change in toxicity in all 27 comparisons made (9 toxicity tests in 3 soils) where ageing effects on EC_{50}^{add} or on EC_{10}^{add} are not significantly lower from the values predicted by the semi-mechanistic model with one exception.

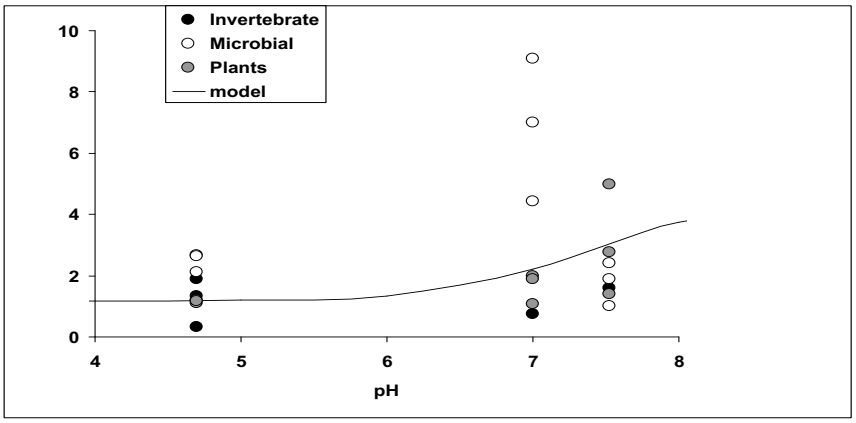

Figure A1.1. The lab-field ("ageing") factors based on toxicity (symbols) and the predicted factor changes in labile pool of Co in soil (line) according to the semi-mechanistic model. Toxicity changes are estimated from EC_{50}^{add}. Some values are unbounded and are a lower estimate of the true change in toxicity.

Derivation of L/F factor for copper

Data availability:

- Difference in Cu toxicity to 2 plants, 2 invertebrates and 3 microbial processes between soils freshly spiked with $CuCl_2$ and corresponding experimentally aged soils (3) and field contaminated soils (4)
- Difference in Cu toxicity to 2 plants, 2 invertebrates and 3 microbial processes between soils freshly spiked with $CuCl_2$ and corresponding leached soils (3)
- Changes in lability (isotopically exchangeable fraction) of Cu with increased equilibration time after spiking, tested in 19 European soils with contrasting soil properties and land use, spiked with $CuCl_2$ at the EC_{10} of a plant assay.

Derivation of L/F factor:

The frequency distribution of the 37 L/F factors available for Cu is shown in Figure A1.2. The L/F factors range 0.5-30, a 10th percentile of 1.5 and a median value of 2.8. These percentiles are still underestimates as many of the L/F factors are unbounded values, i.e. the true L/F factor is above the value indicated. In total 25 from the 37 ED_x (x≥10) based L/F factors are significantly larger than 1.0, i.e. toxicity is significantly lower in fields contaminated or artificially leached and aged soils compared to corresponding freshly spiked soils. None of the L/F factors smaller than 1.0 are significantly different from 1.0, i.e. the suggested trend of increased toxicity upon ageing is statistically non-significant. The overall evidence shows that Cu toxicity is almost consistently smaller in aged soils than in freshly spiked soils.

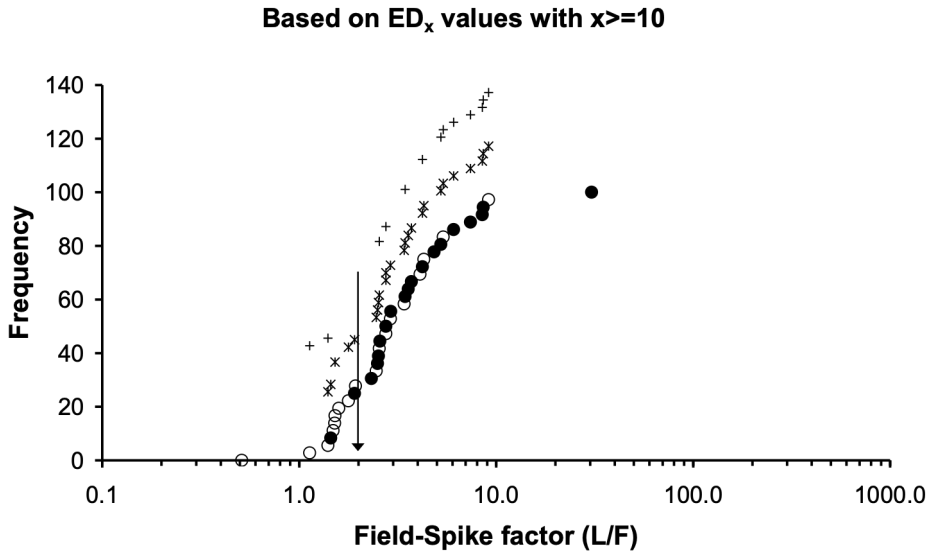

Figure A1.2: Selection of a generic L/F factor for Cu based on the frequency distribution of all individual lab-field factors for Cu. Open symbols refer to L/F factors derived from bounded toxicity thresholds, closed symbols refer to lower estimates of the L/F factors as they are derived from unbounded toxicity data in the field contaminated soils. Values marked with an asterisk or cross on top are significantly different from 1.0 and 2.0, respectively. The selected L/F factor of 2.0 is indicated with the arrow.

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

only a generic L/F factor can be used in the risk characterisation. A generic lab-field factor of 2.0 is proposed based on the following considerations:

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

Derivation of L/F factor for lead

Data availability:

- Difference in Pb toxicity to 2 plants, 2 invertebrates and 2 microbial processes between soils freshly spiked with $PbCl_2$ and corresponding experimentally aged soils (3) and field contaminated soils (3)
- Difference in Pb toxicity to 2 plants, 2 invertebrates and 2 microbial processes between soils freshly spiked with $PbCl_2$ and corresponding leached soils (3)

Derivation of L/F factor:

The factor difference in toxicity due to ageing processes varies between 0.2 and >66, with a median of 3.2. When taking both the effect of leaching and ageing processes into account, the factor difference in toxicity ranges between >1.1 and >530, with a median of 6.0. Although some ageing factors are smaller than 1, none of these values are significantly different from 1, i.e. toxicity did never increase significantly after ageing. In contrast, most of the factors for the effects of ageing and leaching+ageing are significantly larger than 1, i.e. ED_x values generally significantly increased and toxicity significantly decreased after leaching and ageing of soils. It must be noted that most of the ageing and leaching/ageing factors are unbounded. These unbounded factors are a conservative, lower estimate for the real value of these ageing or leaching/ageing factors. The overall distribution is therefore biased towards lower (conservative) values. No significant effect of either soil properties or endpoints on the laboratory-to-field factors could be identified. Therefore, a generic constant correction factor was selected for the effect of ageing or of leaching + ageing and the resulting difference in Pb toxicity between laboratory and field exposure conditions.

Based on the overall weight of evidence, a value of 2.0 was chosen for the laboratory-to-field correction factor for the effect of ageing and a factor 4.0 was selected to correct for leaching and ageing processes:

- The ageing factor of 2.0 corresponds to approximately the 27th percentile of the distribution of observed factors change in toxicity due to ageing reactions (Figure A1.3) and matches with a mean isotopically exchangeable fraction of 58% for Pb in field contaminated soils (Degryse et al., 2007, Europ. J. Soil Sci., 58: 1-7; 58% lability in field contaminated soils means that the ageing decreased the availability by a factor of 1/0.58 = 1.7).
- The leaching-ageing factor of 4.0 highlights the importance of salt stress for Pb toxicity in freshly spiked soils and corresponds to approximately the 40th percentile of the, mainly unbounded, factors change in toxicity of Pb in soils due to the combined effect of leaching and ageing after spiking with a soluble Pb source (Figure A1.3).

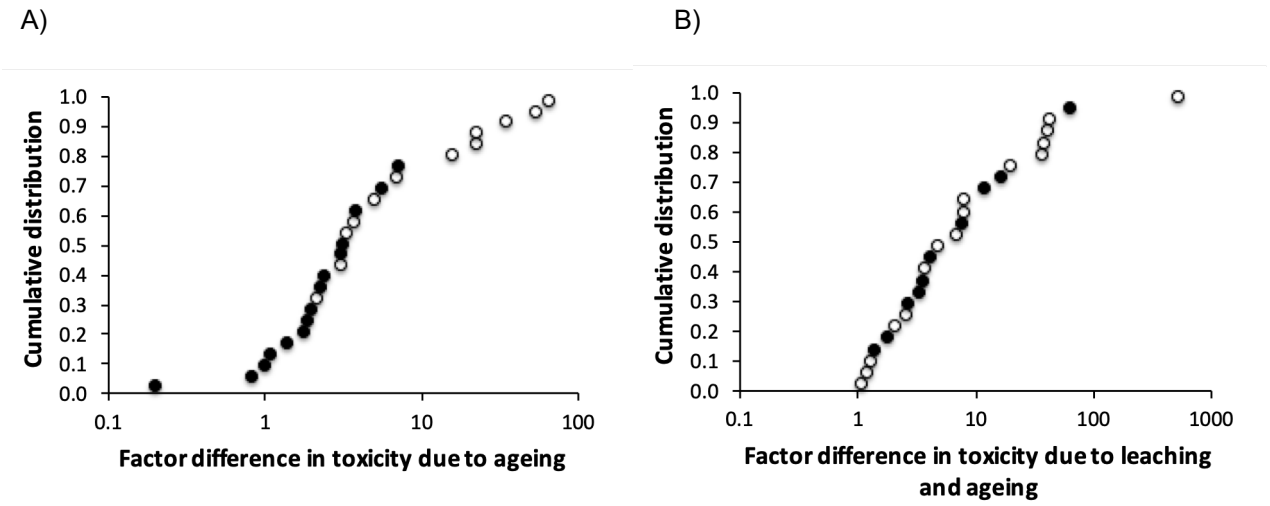

Figure A1.3. Distribution of factors difference in toxicity due to A) ageing and B) leaching-ageing. The difference in toxicity is quantified as the ratio of ED_x values from soils with realistic exposure for field conditions, i.e. removal of excess ions (leaching) and long-term equilibration (ageing), to corresponding values from soil A) spiked with soluble Pb salts and leached or B) soils spiked with soluble Pb salts without leaching or ageing. Closed symbols refer to bounded values and open symbols indicate unbounded values, i.e. lower estimate of real lab-field factor.

Derivation of L/F factor for molybdenum

Data availability:

- Difference in Mo toxicity to 4 plants, 3 invertebrates and 3 microbial processes between 3 soils freshly spiked with Na_2MoO_4 and corresponding experimentally aged soils
- Changes in lability (isotopically exchangeable fraction) of Mo with increased equilibration time after spiking, tested in 15 soils from Europe and Australia with contrasting soil properties and land use, spiked with Na_2MoO_4 at the EC_{10} of a plant assay.

Derivation of L/F factor:

Comparison of toxicity of molybdate between freshly spiked soils and soils aged for 11 months indicated that long-term equilibration of Mo in soils under field conditions generally decreased its toxicity to soil organisms. In total, eight out of 14 ED_{50}- based lab-field factors were significantly larger than one (i.e. significant decrease in toxicity with aging), whereas no lab-field factor was significantly smaller than one (i.e. increase in toxicity with aging). The median decrease in toxicity after leaching and aging processes was a factor of 5.4. There were no significant correlations between the L/F factors and soil type or type of endpoint. This means that only a generic L/F factor can be used in the hazard assessment and risk characterisation. A generic lab-field factor of 2.0 is selected based on the following considerations:

- This factor corresponds to the 32nd percentile of the individual lab-field factors values based on ED_{50} values.
- The factor of 2.0 is approximately equal to the product of the median factor found for chemical fixation of molybdate in several soils (factor 1.4) and the median factor for the effects of leaching on the toxicity thresholds for other metals (factor 1.3–2.0).
- The factor 2.0 further corresponds to the 2.1- and 2.0-fold decrease in solution Mo concentrations between freshly spiked and 11-month equilibration observed for two of the three test soils respectively, at a total soil concentration of approximately 50 mg Mo /kg dry soil.

Derivation of L/F factor for nickel

Data availability:

- Difference in Ni toxicity to 2 plants, 2 invertebrates and 3 microbial processes between 3 soils freshly spiked with $NiCl_2$ and corresponding leached and experimentally aged soils
- Changes in lability (isotopically exchangeable fraction) of Ni with increased equilibration time after spiking, tested in 16 European soils with contrasting soil properties and land use, spiked with $NiCl_2$ at the EC_{10} of a plant assay.

Derivation of L/F factor:

Clear differences in toxicity based L/F factors for Ni were observed among the 3 different soils tested Figure A1.4). The fixation factor, calculated as the change in isotopically exchangeable fraction of Ni in soil between 1 day after spiking and 540 days equilibrated in outdoor conditions, ranged 0.7-4.0 with a median fixation factor of 1.0 and shows a clear increase with pH (Figure A1.4). It is proposed to use the fixation factor, derived from an empirical chemical model as the L/F factor, i.e.

$$L/F=1+\exp^{(1.4(pH-7.0)}$$

in which pH is the pH measured in $CaCl_2$ 0.01M. This equation is calibrated on soil aged maximally 1.5 year and soil pH ranged between pH 3.6 and 7.7. That empirical model predicts almost no ageing (L/F<1.2) up to pH 6 and L/F=2 at pH 7.0 and L/F=3 at pH 7.5. The L/F factor estimated from the fixation factor only accounts for the changes in the isotopically exchangeable pool, which is the fraction of the total that buffers the free metal ion activity in solution. This factor is a conservative estimate for the changes in toxicity for Ni, as shown in Figure A1.4.

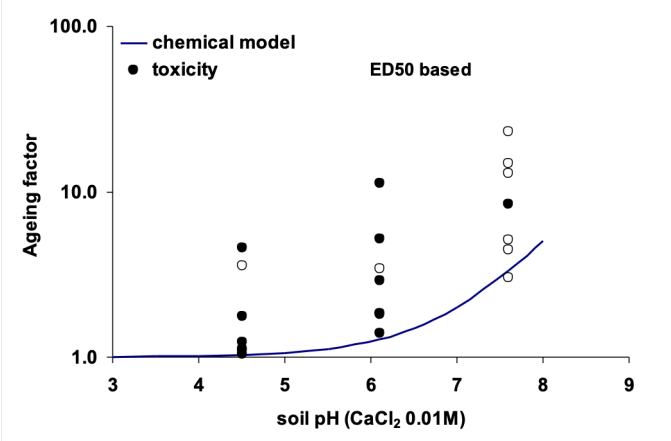

Figure A1.4: The L/F factors for Ni based on toxicity (symbols) and the predicted factor changes in labile pool of Ni in soil (line). Open symbols are 'unbounded' values and are a lower estimate of the ageing factor. None of the ageing factors are significantly lower than those predicted by the chemical model.

Derivation of L/F factor for zinc

Data availability:

- Difference in Zn toxicity to plants, invertebrates and microbial processes between soils freshly spiked with $ZnCl_2$ and corresponding experimentally aged soils (1) and field contaminated soils (3)

Derivation of L/F factor:

It was concluded that there is sufficient justification to assume that toxicity under field conditions is less than under laboratory conditions and a lab-field factor of 3 is proposed for all soils.

- The majority of the lab-field factors, i.e. 19 out of 22 values are clearly higher than 1, varying from 2 to >13. In 15 out of 22 values the ratio was higher than ≈ 3. Moreover, many of the available ratios are unbounded; in those cases no effect was found up to the highest concentration measured in the field-polluted soil. Only 3 out of the 22 values are around 1 (0.8-1.3).
- The mean ratios per test soil, based on the combined data for species and processes, are >6.5, >3.9, >5.6 and 2.7. The lowest value is for a soil that after spiking with zinc was placed in uncovered outdoor

plots for up to nearly 2 years, while the other three soils were more gradually polluted with zinc over a period of around 10 to 50 years due to corrosion of galvanised electricity transmission towers.

Table A1.1. Summary bioavailability studies: effect of leaching and ageing

Metal	Ecotoxicity					Soil chemistry			
	Species covered	# soils studied	Major soil orders WRB	Countries covered	Soil properties	# soils studied	Major soil orders WRB	Countries covered	Soil properties
As	No lab-field factor derived								
Cd	No lab-field factor derived								
Co	1. Tomato, shoot yield 2. Oilseed rape, shoot yield 3. Barley, shoot yield 4. *Eisenia fetida*, reproduction 5. *Enchytraeus albidus*, reproduction 6. *Folsomia candida*, reproduction 7. Nitrification 8. Substance induced respiration 9. Plant residue mineralisation	3	• Cambisol • Luvisol • Undetermined	• Belgium (2) • France	• pH: 4.7 – 7.5 • Org. C: 0.9 – 2.1% • Clay: 2 – 39% • eCEC: 4 – 24 $cmol_c/kg$ • Fe_{0x}: 1.9 – 3.2 g/kg • bg Co: 2 – 30 mg/kg	14	• Acrisol • Cambisol (4) • Podzol (2) • Kastanozem • Leptosol • Luvisol • Undetermined (4)	• Australia (4) • Belgium (2) • Canada • Denmark • Greece • France • Italy • United Kingdom • USA (2)	• pH: 4.3 – 7.5 • Organic carbon: 0.8 – 5.3% • Clay content: 1 – 48% • eCEC: 2 – 29 $cmol_c/kg$ • Oxalate extractable iron: 0.3 – 22.0 g/kg • Co-background: 1 – 30 mg/kg
Cu	1. Tomato, shoot yield 2. Barley, root elongation 3. *Eisenia fetida*, reproduction 4. *Folsomia candida*, reproduction 5. Nitrification 6. Substance induced respiration 7. Plant residue mineralisation	7	• Cambisol • Luvisol • Podzol • Sandy • Sandy clay loam • Loamy sand (2)	• Belgium • Denmark • Spain • The Netherlands (2) • United Kingdom (2)	• pH: 3.4 – 7.5 • Org. C: 1.1 – 4.4% • Clay: 5 – 23% • eCEC: 1 – 23 $cmol_c/kg$ • Fe_{0x}: 0.5 – 16.2 g/kg • Cu-background: 2 – 88 mg/kg	19	• Cambisol (6) • Fluvisol • Histosol (2) • Leptosol • Luvisol (5) • Podzol (2) • Regosol (2)	• Belgium (2) • France (4) • Germany • Greece (2) • Italy • Spain (2) • Sweden (2) • The Netherlands (2) • United Kingdom (3)	• pH: 3.0 – 7.5 • Organic carbon: 0.4 – 23% • Clay content: 5 – 51% • eCEC: 2 – 36 $cmol_c/kg$ • Oxalate extractable iron: 0.1 – 16.2 g/kg • Cu-background: 2 – 88 mg/kg

Metal	Ecotoxicity					Soil chemistry			
	Species covered	**# soils studied**	**Major soil orders WRB**	**Countries covered**	**Soil properties**	**# soils studied**	**Major soil orders WRB**	**Countries covered**	**Soil properties**
Pb	1. Tomato, shoot yield 2. Barley, shoot yield 3. *Eisenia fetida*, reproduction 4. *Folsomia candida*, reproduction 5. Nitrification 6. Substance induced respiration	3	• Cambisol • Luvisol (2)	• Belgium • Spain • United Kingdom	• pH: 6.1 – 7.4 • Org. C: 1.0 – 4.3% • Clay: 12 – 30% • eCEC: 8 – 27 $cmol_c/kg$ • Fe_{0x}: 1.4 – 16.7 g/kg • bg Pb: 21 – 137 mg/kg	No additional soils studied			
Mo	1. Oilseed rape, shoot yield 2. Red clover, shoot yield 3. Ryegrass, shoot yield 4. Tomato, shoot yield 5. *Enchytraeus crypticus*, reproduction 6. *Eisenia andrei*, reproduction 7. *Folsomia candida*, reproduction 8. Nitrification 9. Substance induced respiration 10. Plant residue mineralisation	3	• Cambisol • Luvisol • Podzol	• Belgium (2) • United Kingdom	• pH: 5.2 – 6.7 • Org. C: 0.9 – 3.6% • Clay: 2 – 27% • eCEC: 4 – 30 $cmol_c/kg$ • Fe_{0x}: 1.0 – 15.3 g/kg • bg Mo: 1 mg/kg	15	• Cambisol (2) • Chernozem • Histosol • Luvisol (4) • Podzol (2) • Regosol • Undetermined (4)	• Australia (4) • Belgium (3) • France (2) • Greece • Hungary, • Spain, • Sweden, • The Netherlands • United Kingdom	• pH: 4.4 – 7.8 • Org. C: 0.6 – 30.7% • Clay: 2 – 59% • eCEC: 4 – 42 $cmol_c/kg$ • Fe_{ox}: 0.1 – 15.3 g/kg • Mo: <1 – 3 mg/kg

Metal	Ecotoxicity					Soil chemistry			
	Species covered	# soils studied	Major soil orders WRB	Countries covered	Soil properties	# soils studied	Major soil orders WRB	Countries covered	Soil properties
Ni	1. Tomato, shoot yield 2. Barley, root elongation 3. *Eisenia fetida*, reproduction 4. *Folsomia candida*, reproduction 5. Nitrification 6. Substance induced respiration 7. Plant residue mineralisation	3	• Cambisol (2) • Inceptisol	• Denmark • Spain • United Kingdom	• pH: 3.4 – 7.5 • Org. C: 0.5 – 4.3% • Clay: 1 – 55% • eCEC: 2 – 35 $cmol_c$/kg • Fe_{0x}: 0.7 – 17.8 g/kg • Ni-background: 1 – 39 mg/kg	16	• Cambisol (7) • Fluvisol • Histosol (2) • Inceptisol • Luvisol (3) • Podzol • Regosol	• Belgium (2) • Denmark (2) • France (2) • Greece • Italy • Spain (3) • Sweden • The Netherlands (2) • United Kingdom (2)	• pH: 3.6 – 7.7 • Organic carbon: 0.3 – 33% • Clay content: 0.4 – 55% • eCEC: 2 – 53 $cmol_c$/kg • Oxalate extractable iron: 0.2 – 17.8 g/kg • Ni-background: 1 – 113 mg/kg
Zn	1. *Triticum aestivum*, shoot yield 2. *Trifolium pratense* 3. *Eisenia fetida*, reproduction 4. *Folsomia candida*, reproduction 5. Nitrification 6. Substance induced respiration 7. Plant residue mineralisation	4	• Histosol • Undetermined (3)	• Belgium • The Netherlands (2) • United Kingdom	• pH: 4.8 – 5.7 • Org. C: 1.4 – 10.2% • Clay: not known • eCEC: 11 – 30 $cmol_c$/kg • Fe_{0x}: 0.7 – 7.7 g/kg • Zn-background: 76 – 155 mg/kg	No additional soils studied			

ANNEX 2: NORMALISATION OF TOXICITY FOR DIFFERENCES IN SOIL PROPERTIES

A summary of the selected normalisation models for metals considered in the threshold calculator tool for metals is presented in Table A2.1. The available datasets for derivation of these models are summarised in the Table A2.2 and Table A2.3 reports the number of data points, R^2 values, slopes and range in soil properties covered for the individual regression models.

Table A2.1. Overview of selected normalisation models for metals.

Metal	Organisms/Microbial processes	Abiotic factors	Reference
As	Plants	pH and Clay	Römbke et al., 2020; Song et al., 2006; Kader et al., 2016
	Invertebrates	Clay	Romero-Freire et al., 2016; Lin et al., 2019; Römbke et al., 2020
	Microbial processes	/	/
Co	Plants	eCEC	Mico et al., 2008; Li et al., 2009
	Invertebrates	eCEC	De Schamphelaere et al., 2008
	Microbial processes	eCEC	Salpeteur et al., 2007
Cu	Plants	eCEC	Rooney et al., 2006
	Invertebrates	eCEC	Criel et al., 2008
	Microbial processes	eCEC, Organic C, clay and pH	Oorts et al., 2006
Pb	Plants	eCEC	Smolders et al., 2011
	Invertebrates	eCEC	Lanno, 2012
	Microbial processes	eCEC	Smolders et al., 2011
Mo	Plants	pH and clay	McGrath et al., 2010; Oorts et al., 2016
	Invertebrates	Clay	Van Gestel et al., 2011; Oorts et al., 2016
	Microbial processes	Clay	Oorts et al., 2016
Ni	Plants	eCEC	Rooney et al., 2007
	Invertebrates	eCEC	Van Eeckhout et al., 2005
	Microbial processes	eCEC	Oorts et al., 2006
Zn	Plants	pH and eCEC	Smolders et al., 2003
	Invertebrates	eCEC	Lock et al., 2003
	Microbial processes	Background Zn	Smolders et al., 2004

Table A2.2. Overview of bioavailability studies: effect of soil properties

Metal	Species studied	# soils studied	Major soil orders WRB	Countries covered	Land use	Range soil properties
As	1. Tomato, shoot yield 2. Oat, shoot yield 3. Barley, root elongation 4. Cucumber, yield 5. *Eisenia andrei*, reproduction 6. *Folsomia candida*, reproduction	37	• Cambisol (8) • Luvisol (6) • Histosol (2) • Regosol (2) • Fluvisol (1) • Leptosol (1) • Tenosoil (2) • Kurosoil (1) • Ferrosoil (1) • Calcarosoil (1) • Undetermined (12)	• Belgium • Germany (3) • Spain (9) • Italy • France (3) • Greece (2) • Sweden • The Netherlands (3) • United Kingdom (4) • China (10) • Australia (5)	• 14 arable soils • 9 grassland soils • 1 olive orchard soil • 3 woodland soils • 9 agriculture soil • 1 desert soil	• pH: 3.4 – 8.79 • Organic carbon: 0.38 – 23.3% • Clay: 6.9 – 51% • eCEC: 6.66 – 49.4 $cmol_c/kg$ • Oxalate extractable iron: 0.01 – 20.06 g/kg • As-background: 0.56 – 134.8 mg/kg
Cd	No comparative studies on effect of soil properties on bioavailability and toxicity of cadmium to soil organisms					
Co	1. Tomato, shoot yield 2. Oilseed rape, shoot yield 3. Barley, shoot yield 4. Barley, root elongation 5. *Eisenia fetida*, reproduction 6. *Enchytraeus albidus*, reproduction 7. *Folsomia candida*, reproduction 8. Nitrification 9. Substance induced respiration 10. Plant residue mineralisation	10	• Acrisol • Cambisol (4) • Podzol (2) • Kastanozem • Leptosol • Luvisol	• Belgium (2) • Canada • Denmark • Greece • France • Italy • United Kingdom • USA (2)	• 6 arable soils • 1 grassland soil • 3 woodland soils	• pH: 4.3 – 7.5 • Organic carbon: 0.8 – 5.3% • Clay content: 1 – 48% • eCEC: 2 – 29 $cmol_c/kg$ • Oxalate extractable iron: 0.3 – 22.0 g/kg • Co-background: 1 – 30 mg/kg
Cu	1. Tomato, shoot yield 2. Barley, root elongation 3. *Eisenia fetida*, reproduction 4. *Folsomia candida*, reproduction 5. Nitrification 6. Substance induced respiration 7. Plant residue mineralisation	19	• Cambisol (6) • Fluvisol • Histosol (2) • Leptosol • Luvisol (5) • Podzol (2) • Regosol (2)	• Belgium (2) • France (4) • Germany • Greece (2) • Italy • Spain (2) • Sweden (2) • The Netherlands (2) • United Kingdom (3)	• 9 arable soils • 3 grassland soils • 6 woodland soils • 1 orchard soil	• pH: 3.0 – 7.5 • Organic carbon: 0.4 – 23% • Clay content: 5 – 51% • eCEC: 2 – 36 $cmol_c/kg$ • Oxalate extractable iron: 0.1 – 16.2 g/kg • Cu-background: 2 – 88 mg/kg

Metal	Species studied	# soils studied	Major soil orders WRB	Countries covered	Land use	Range soil properties
Pb	1. Tomato, shoot yield 2. Barley, shoot yield 3. *Eisenia fetida*, reproduction 4. *Folsomia candida*, reproduction 5. Nitrification 6. Substance induced respiration	8	• Cambisol (2) • Histosol • Luvisol (2) • Podzol • Undetermined (2)	• Belgium (2) • Denmark (2) • Spain • The Netherlands (2) • United Kingdom	• 4 arable soils • 4 grassland soils	• pH: 4.7 – 7.4 • Organic carbon: 1.0 – 31.0% • Clay content: 2 – 60% • eCEC: 4 – 42 $cmol_c$/kg • Oxalate extractable iron: 1.2 – 16.7 g/kg • Pb-background: 15 – 137 mg/kg
Mo	1. Oilseed rape, shoot yield 2. Red clover, shoot yield 3. Ryegrass, shoot yield 4. Tomato, shoot yield 5. Barley, root elongation 6. *Enchytraeus crypticus*, reproduction 7. *Eisenia andrei*, reproduction 8. *Folsomia candida*, reproduction 9. Nitrification 10. Substance induced respiration 11. Plant residue mineralisation	10	• Cambisol (2) • Chernozem • Histosol • Luvisol (3) • Podzol (2) • Regosol	• Belgium (3) • France • Greece • Hungary, • Spain, • Sweden, • The Netherlands • United Kingdom	• 7 arable soils • 2 grassland soils • 1 orchard soil	• pH: 4.4 – 7.8 • Organic carbon: 0.6 – 30.7% • Clay content: 2 – 59% • eCEC: 4 – 42 $cmol_c$/kg • Oxalate extractable iron: 0.1 – 15.3 g/kg • Mo-background: <1 – 3 mg/kg
Ni	1. Tomato, shoot yield 2. Barley, root elongation 3. *Eisenia fetida*, reproduction 4. *Folsomia candida*, reproduction 5. Nitrification 6. Substance induced respiration 7. Plant residue mineralisation	16	• Cambisol (7) • Fluvisol • Histosol (2) • Inceptisol • Luvisol (3) • Podzol • Regosol	• Belgium (2) • Denmark (2) • France (2) • Greece • Italy • Spain (3) • Sweden • The Netherlands (2) • United Kingdom (2)	• 8 arable soils • 3 grassland soils • 3 woodland soils • 2 orchard soils	• pH: 3.6 – 7.7 • Organic carbon: 0.3 – 33% • Clay content: 0.4 – 55% • eCEC: 2 – 53 $cmol_c$/kg • Oxalate extractable iron: 0.2 – 17.8 g/kg • Ni-background: 1 – 113 mg/kg

Metal	Species studied	# soils studied	Major soil orders WRB	Countries covered	Land use	Range soil properties
Zn	1. *Triticum aestivum*, shoot yield 2. *Eisenia fetida*, reproduction 3. *Folsomia candida*, reproduction 4. Nitrification 5. Substance induced respiration 6. Plant residue mineralisation	15	• Cambisol (3) • Fluvisol • Histosol (2) • Leptosol • Luvisol (3) • Podzol (2) • Regosol • Undetermined (2)	• Belgium (3) • France • Germany • Greece (2) • Italy • Spain • Sweden • The Netherlands (3) • United Kingdom (2)	• 5 arable soils • 5 grassland soils • 4 woodland soils • 1 orchard soil	• pH: 3.0 – 7.5 • Organic carbon: 0.4 – 23% • Clay content: 5 – 51% • eCEC: 2 – 36 $cmol_c/kg$ • Oxalate extractable iron: 0.1 – 16.2 g/kg • Zn-background: 7 – 191 mg/kg

Table A2.3. Summary selected regression models (all based on total concentrations)

Metal	Species/process	Dependent variable	Soil property	# soils	Adj R^2	Slope (total)	Range soil properties covered
As	Oat, shoot yield	log EC_{50}	log clay	6	0.83	0.981	pH: 4.8 – 7.3; Org. C: 1 – 22%; Clay: 7 – 68%; eCEC: 7 – 49 $cmol_c/kg$; Fe_{ox}: 1 – 20 g/kg; As: 3 – 46 mg/kg
As	Tomato, shoot yield	log EC_{50}	log clay	5	0.68	0.712	pH: 5.2 – 7.3; Org. C: 1.8 – 22%; Clay: 7 – 68%; eCEC: 6.6 – 49 $cmol_c/kg$; Fe_{ox}: 1 – 18 g/kg; As: 3 – 12 mg/kg
As	Barley, elongation	log EC_{50}	log clay	19	0.43	1.034	pH: 3.4 – 7.6; Org. C: 0.4 – 23%; Clay: 7 – 51%; eCEC: 2 – 36 $cmol_c/kg$; Fe_{ox}: 0.1 – 16 g/kg; As: 0.1 – 152 mg/kg
As	Cucumber, yield	log EC_{50}	pH	7	0.80	-0.273	pH: 4.5 – 7.7; Org. C: 1.5 – 8.4%; Clay: 8 – 20%; eCEC: 3 – 18 $cmol_c/kg$; Fe_{ox}: 0.5 – 12 g/kg; As: 0.7 – 5 mg/kg
As	*Eisenia fetida*, reproduction	log EC_{50}	log clay	6	0.59	0.989	pH: 5.9 – 8.8; Org. C: 0.4 – 8.2%; Clay: 8.3 – 55%; eCEC: 3 – 26 $cmol_c/kg$; Fe_{ox}: 0.2 – 1.2 g/kg; As: 3 – 26 mg/kg
As	*Folsomia candida*, reproduction	log EC_{50}	log clay	6	0.89	1.074	pH: 4.9 – 8.4; Org. C: 0.6 – 22%; Clay: 7 – 68%; eCEC: 7 – 49 $cmol_c/kg$; Fe_{ox}: 0.5 – 20 g/kg; As: 0.6 – 46 mg/kg
Cd	No models derived						
Co	Oilseed rape, shoot yield	log EC_{50}	log eCEC	10	0.69	1.55	pH: 4.3 – 7.5; Org. C: 0.8 – 5.3%; Clay: 1 – 48%; eCEC: 2 – 29 $cmol_c/kg$; Fe_{ox}: 0.3 – 22.0 g/kg; Co: 1 – 30 mg/kg
Co	Tomato, shoot yield	log EC_{50}	log eCEC	9	0.61	1.40	pH: 4.3 – 7.5; Org. C: 0.8 – 5.3%; Clay: 1 – 48%; eCEC: 2 – 29 $cmol_c/kg$; Fe_{ox}: 0.3 – 22.0 g/kg; Co: 1 – 30 mg/kg
Co	Barley, shoot yield	log EC_{50}	log eCEC	10	0.70	1.33	pH: 4.3 – 7.5; Org. C: 0.8 – 5.3%; Clay: 1 – 48%; eCEC: 2 – 29 $cmol_c/kg$; Fe_{ox}: 0.3 – 22.0 g/kg; Co: 1 – 30 mg/kg
Co	Barley, root elongation	log EC_{50}	log eCEC	10	0.83	1.13	pH: 4.3 – 7.5; Org. C: 0.8 – 5.3%; Clay: 1 – 48%; eCEC: 2 – 29 $cmol_c/kg$; Fe_{ox}: 0.3 – 22.0 g/kg; Co: 1 – 30 mg/kg
Co	*Enchytraeus albidus*, reproduction	log EC_{50}	log eCEC	4	0.96	1.05	pH: 4.3 – 7.0; Org. C: 1.6 – 5.3%; Clay: 1 – 14%; eCEC: 2 – 10 $cmol_c/kg$; Fe_{ox}: 1.9 – 3.2 g/kg; Co: 1 – 7 mg/kg
Co	*Eisenia fetida*, reproduction	log EC_{50}	log eCEC	8	0.52	0.65	pH: 4.4 – 7.5; Org. C: 0.8 – 4.5%; Clay: 1 – 48%; eCEC: 2 – 29 $cmol_c/kg$; Fe_{ox}: 0.3 – 22.0 g/kg; Co: 1 – 30 mg/kg
Co	*Folsomia candida*, reproduction	log EC_{50}	log eCEC	10	0.68	0.98	pH: 4.3 – 7.5; Org. C: 0.8 – 5.3%; Clay: 1 – 48%; eCEC: 2 – 29 $cmol_c/kg$; Fe_{ox}: 0.3 – 22.0 g/kg; Co: 1 – 30 mg/kg
Co	Nitrification	log EC_{50}	log eCEC	9	0.70	1.00	pH: 4.4 – 7.5; Org. C: 0.8 – 4.5%; Clay: 1 – 48%; eCEC: 2 – 29 $cmol_c/kg$; Fe_{ox}: 0.3 – 22.0 g/kg; Co: 1 – 30 mg/kg
Co	Substance induced respiration	log EC_{50}	log eCEC	10	0.67	1.26	pH: 4.3 – 7.5; Org. C: 0.8 – 5.3%; Clay: 1 – 48%; eCEC: 2 – 29 $cmol_c/kg$; Fe_{ox}: 0.3 – 22.0 g/kg; Co: 1 – 30 mg/kg
Co	Plant residue mineralisation	log EC_{20}	log eCEC	8	0.13	0.53	pH: 4.3 – 7.5; Org. C: 0.9 – 5.3%; Clay: 1 – 39%; eCEC: 2 – 29 $cmol_c/kg$; Fe_{ox}: 0.3 – 22.0 g/kg; Co: 1 – 30 mg/kg

Metal	Species/process	Dependent variable	Soil property	# soils	Adj R^2	Slope (total)	Range soil properties covered
Cu	Tomato, shoot yield	log EC_{50}	log eCEC	17	0.74	0.96	pH: 3.4 – 7.5; Org. C: 0.4 – 23%; Clay: 5 – 51%; eCEC: 2 – 36 $cmol_c$/kg; Fe_{ox}: 0.1 – 16.2 g/kg; Cu: 2 – 88 mg/kg
Cu	Barley, root elongation	log EC_{50}	log eCEC	18	0.65	0.69	pH: 3.4 – 7.5; Org. C: 0.4 – 23%; Clay: 5 – 51%; eCEC: 2 – 36 $cmol_c$/kg; Fe_{ox}: 0.1 – 16.2 g/kg; Cu: 2 – 88 mg/kg
Cu	*Eisenia fetida*, reproduction	log EC_{50}	log eCEC	14	0.72	0.59	pH: 3.0 – 7.5; Org. C: 0.4 – 23%; Clay: 7 – 50%; eCEC: 2 – 36 $cmol_c$/kg; Fe_{ox}: 0.5 – 16.2 g/kg; Cu: 2 – 70 mg/kg
Cu	*Folsomia candida*, reproduction	log EC_{50}	log eCEC	18	0.61	0.96	pH: 3.0 – 7.5; Org. C: 0.4 – 23%; Clay: 5 – 51%; eCEC: 2 – 36 $cmol_c$/kg; Fe_{ox}: 0.1 – 16.2 g/kg; Cu: 2 – 88 mg/kg
Cu	Nitrification	log EC_{50}	log eCEC	17	0.64	1.07	pH: 3.4 – 7.5; Org. C: 0.4 – 23%; Clay: 7 – 51%; eCEC: 2 – 36 $cmol_c$/kg; Fe_{ox}: 0.1 – 16.2 g/kg; Cu: 5 – 88 mg/kg
Cu	Substance induced respiration	log EC_{50}	log Org. C and log clay	18	0.74	0.73 (log Org.C) 0.60 (log clay)	pH: 3.4 – 7.5; Org. C: 0.4 – 23%; Clay: 5 – 51%; eCEC: 2 – 36 $cmol_c$/kg; Fe_{ox}: 0.1 – 16.2 g/kg; Cu: 2 – 88 mg/kg
Cu	Plant residue mineralisation	log EC_{20}	pH and log eCEC	16	0.67	-0.34 (pH) 0.74 (log eCEC)	pH: 3.0 – 7.5; Org. C: 0.4 – 23%; Clay: 7 – 51%; eCEC: 2 – 36 $cmol_c$/kg; Fe_{ox}: 0.1 – 16.2 g/kg; Cu: 2 – 88 mg/kg
Pb	Plant shoot yield (tomato + barley)	log EC_{50}	log eCEC	5	0.49	0.55	pH: 4.7 – 7.4; Org. C: 1.0 – 31.0%; Clay: 3 – 59%; eCEC: 4 – 42 $cmol_c$/kg; Fe_{ox}: 1.2 – 11.7 g/kg; Pb: 15 – 135 mg/kg
Pb	*Eisenia fetida*, reproduction	log EC_{50}	log eCEC	5	0.95	1.70	pH: 5.2 – 7.4; Org. C: 1.4 – 5.0%; Clay: 3 – 60%; eCEC: 4 – 42 $cmol_c$/kg; Fe_{ox}: 1.2 – 16.5 g/kg; Pb: 15 – 135 mg/kg
Pb	*Folsomia candida*, reproduction	No significant model available					
Pb	Nitrification	log EC_{50}	log eCEC	6	0.83	0.95	pH: 4.9 – 7.4; Org. C: 1.0 – 5.0%; Clay: 2 – 60%; eCEC: 4 – 42 $cmol_c$/kg; Fe_{ox}: 1.2 – 12.7 g/kg; Pb: 15 – 135 mg/kg
Pb	Substance induced respiration	No significant model available					
Mo	Oilseed rape, shoot yield	log EC_{50}	pH and log clay	10	0.91	-0.61 (pH) 1.08 (log clay)	pH: 4.4 – 7.8; Org. C: 0.6 – 30.7%; Clay: 2 – 59%; eCEC: 4 – 42 $cmol_c$/kg; Fe_{ox}: 0.1 – 15.3 g/kg; Mo: <1 – 3 mg/kg
Mo	Red clover, shoot yield	log EC_{50}	pH and log clay	10	0.78	-0.50 (pH) 0.77 (log clay)	pH: 4.4 – 7.8; Org. C: 0.6 – 30.7%; Clay: 2 – 59%; eCEC: 4 – 42 $cmol_c$/kg; Fe_{ox}: 0.1 – 15.3 g/kg; Mo: <1 – 3 mg/kg
Mo	Ryegrass, shoot yield	log EC_{50}	pH and log clay	10	0.81	-0.35 (pH) 0.90 (log clay)	pH: 4.4 – 7.8; Org. C: 0.6 – 30.7%; Clay: 2 – 59%; eCEC: 4 – 42 $cmol_c$/kg; Fe_{ox}: 0.1 – 15.3 g/kg; Mo: <1 – 3 mg/kg
Mo	Tomato, shoot yield	log EC_{50}	pH and log clay	10	0.86	-0.45 (pH) 0.93 (log clay)	pH: 4.4 – 7.8; Org. C: 0.6 – 30.7%; Clay: 2 – 59%; eCEC: 4 – 42 $cmol_c$/kg; Fe_{ox}: 0.1 – 15.3 g/kg; Mo: <1 – 3 mg/kg
Mo	Barley, root elongation	log EC_{50}	pH and log clay	9	0.80	-0.28 (pH) 0.56 (log clay)	pH: 4.4 – 7.8; Org. C: 0.8 – 30.7%; Clay: 2 – 59%; eCEC: 4 – 42 $cmol_c$/kg; Fe_{ox}: 0.1 – 15.3 g/kg; Mo: <1 – 3 mg/kg
Mo	*Enchytraeus crypticus*, reproduction	log EC_{50}	log clay	6	0.84	0.72	pH: 5.0 – 7.8; Org. C: 0.9 – 3.6%; Clay: 2 – 31%; eCEC: 4 – 30 $cmol_c$/kg; Fe_{ox}: 1.0 – 15.3 g/kg; Mo: <1 – 1 mg/kg

Metal	Species/process	Dependent variable	Soil property	# soils	Adj R^2	Slope (total)	Range soil properties covered
Mo	*Eisenia andrei*, reproduction	log EC_{50}	log clay	10	0.67	0.73	pH: 4.4 – 7.8; Org. C: 0.6 – 30.7%; Clay: 2 – 59%; eCEC: 4 – 42 $cmol_c$/kg; Fe_{ox}: 0.1 – 15.3 g/kg; Mo: <1 – 3 mg/kg
Mo	*Folsomia candida*, reproduction	Not sufficient reliable EC_{50} values (3) for regression analysis					
Mo	Nitrification	log EC_{50}	log clay	8	0.64	1.17	pH: 4.4 – 7.8; Org. C: 0.6 – 30.7%; Clay: 2 – 59%; eCEC: 4 – 42 $cmol_c$/kg; Fe_{ox}: 0.1 – 1.7 g/kg; Mo: <1 – 3 mg/kg
Mo	Substance induced respiration	log EC_{50}	log clay	4	0.85	0.73	pH: 5.2 – 7.3; Org. C: 0.9 – 2.8%; Clay: 2 – 13%; eCEC: 4 – 14 $cmol_c$/kg; Fe_{ox}: 1.0 – 2.2 g/kg; Mo: <1 – 1 mg/kg
Mo	Plant residue mineralisation	Not sufficient reliable EC_{50} values (1) for regression analysis					
Ni	Tomato, shoot yield	log EC_{50}	log eCEC	16	0.64	1.27	pH: 3.6 – 7.7; Org. C: 0.3 – 33%; Clay: 0.4 – 55%; eCEC: 2 – 53 $cmol_c$/kg; Fe_{ox}: 0.2 – 17.8 g/kg; Ni: 1 – 113 mg/kg
Ni	Barley, root elongation	log EC_{50}	log eCEC	16	0.82	1.12	pH: 3.6 – 7.7; Org. C: 0.3 – 33%; Clay: 0.4 – 55%; eCEC: 2 – 53 $cmol_c$/kg; Fe_{ox}: 0.2 – 17.8 g/kg; Ni: 1 – 113 mg/kg
Ni	*Eisenia fetida*, reproduction	log EC_{50}	log eCEC	16	0.70	0.95	pH: 3.6 – 7.7; Org. C: 0.3 – 33%; Clay: 0.4 – 55%; eCEC: 2 – 53 $cmol_c$/kg; Fe_{ox}: 0.2 – 17.8 g/kg; Ni: 1 – 81 mg/kg
Ni	*Folsomia candida*, reproduction	log EC_{50}	log eCEC	15	0.68	1.17	pH: 3.6 – 7.7; Org. C: 0.3 – 33%; Clay: 1 – 55%; eCEC: 2 – 53 $cmol_c$/kg; Fe_{ox}: 0.2 – 17.8 g/kg; Ni: 1 – 113 mg/kg
Ni	Nitrification	log EC_{50}	log eCEC	15	0.57	1.00	pH: 4.1 – 7.7; Org. C: 0.3 – 33%; Clay: 0.4 – 55%; eCEC: 2 – 53 $cmol_c$/kg; Fe_{ox}: 0.2 – 17.8 g/kg; Ni: 1 – 113 mg/kg
Ni	Substance induced respiration	log EC_{50}	log eCEC	13	0.92	1.34	pH: 3.6 – 7.7; Org. C: 0.3 – 4.3%; Clay: 0.4 – 55%; eCEC: 2 – 35 $cmol_c$/kg; Fe_{ox}: 0.2 – 17.8 g/kg; Ni: 1 – 113 mg/kg
Ni	Plant residue mineralisation	log EC_{20}	log eCEC	12	0.69	1.22	pH: 3.6 – 7.7; Org. C: 0.3 – 13%; Clay: 0.4 – 55%; eCEC: 2 – 35 $cmol_c$/kg; Fe_{ox}: 0.2 – 17.8 g/kg; Ni: 1 – 113 mg/kg
Zn	Wheat, shoot yield	log EC_{50}	log eCEC and pH	14	0.81	0.11 (pH) 0.88 (log eCEC)	pH: 3.0 – 7.5; Org. C: 0.4 – 23%; Clay: 5 – 51%; eCEC: 2 – 36 $cmol_c$/kg; Fe_{ox}: 0.1 – 7.7 g/kg; Zn: 7 – 191 mg/kg
Zn	*Eisenia fetida*, reproduction	log EC_{50}	log eCEC	14	0.76	0.80	pH: 3.0 – 7.5; Org. C: 0.4 – 23%; Clay: 5 – 46%; eCEC: 2 – 36 $cmol_c$/kg; Fe_{ox}: 0.1 – 16.2 g/kg; Zn: 7 – 191 mg/kg
Zn	*Folsomia candida*, reproduction	log EC_{50}	log eCEC	15	0.84	1.12	pH: 3.0 – 7.5; Org. C: 0.4 – 23%; Clay: 5 – 51%; eCEC: 2 – 36 $cmol_c$/kg; Fe_{ox}: 0.1 – 16.2 g/kg; Zn: 7 – 191 mg/kg
Zn	Nitrification	log EC_{50}	log bg-Zn	13	0.59	0.79	pH: 4.7 – 7.5; Org. C: 0.4 – 23%; Clay: 9 – 51%; eCEC: 5 – 36 $cmol_c$/kg; Fe_{ox}: 0.1 – 16.2 g/kg; Zn: 26 – 191 mg/kg
Zn	Substance induced respiration	log EC_{50}	log bg-Zn	14	0.40	0.77	pH: 3.0 – 7.5; Org. C: 0.4 – 10%; Clay: 5 – 51%; eCEC: 2 – 36 $cmol_c$/kg; Fe_{ox}: 0.1 – 16.2 g/kg; Zn: 7 – 155 mg/kg
Zn	Plant residue mineralisation	No significant model available					

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ANNEX 3: BIOAVAILABILITY MODELS DERIVED FOR CHINA AND AUSTRALIA

Testing programmes for implementation of metal bioavailability in regulatory frameworks were also conducted in China and Australia (see e.g. Ma et al., 2012; NEPC, 2011). Copper and nickel toxicity to plants and microbial endpoints was tested in 17 Chinese soils, after different spiking treatments (with and without leaching) (Li et al., 2010, 2013). Likewise, copper and zinc toxicity to wheat and nitrification was tested in a range of Australian soils (Broos et al., 2007; Warne et al., 2008a and 2008b). Results from experiments in China and Australia also confirmed the strong variation in toxicity across soils and illustrate the need to implement the role of soil characteristics into the derivation of soil thresholds. Significant regressions between soil properties and metal toxicity were developed and it was proposed to use this information for normalisation of toxicity data in the derivation of ecological soil quality standards for these metals in soil (Table A3.1). In some cases, different soil properties were identified as best related with metal toxicity to the same endpoint for different test programmes (Table A3.1). These differences in the models can be due to either differences in soil types covered, potential differences in soil treatments (leaching and or equilibration), methodology (e.g. (e)CEC analysis), endpoints measured, etc. Next to models derived from toxicity data with Australian soils, also some 'European' models were used for the derivation of soil quality standards in Australia (Table A3.1).

Apart from normalisation for variation in toxicity due to variation in soil properties, correction factors to account for the higher toxicity observed in freshly spiked soils compared to field contaminated soils were also proposed in both China and Australia. In Australia, the lab-to-field correction factors selected for Europe (Table 2) were used. In China, soil specific leaching factors were proposed based on comparative toxicity in freshly spiked and leached Chinese soils (Table A3.2). Because there was no ageing model derived for Chinese soils, ageing models derived from European soils were used (Ma et al., 2006). In the models, an isotopic dilution technique was used to determine long-term changes in the lability of Cu added to soils leached under laboratory conditions, and for soils incubated outdoors. The ratio in isotopically exchangeable fraction at 360 and 14 days after spiking a soil with a soluble metal salt was selected as the ageing factor (Ma et al., 2012).

Table A3.1: Regression models selected for setting soil quality standards for copper in Europe, Australia and China.

Endpoint	Geographical region	Regression equation*	Reference
Barley root elongation	Europe, Australia	log EC_{50} = 1.56+0.69 log eCEC	Rooney et al., 2006
Tomato shoot yield	Europe, Australia	log EC_{50} = 1.46+0.96 log eCEC	Rooney et al., 2006

Endpoint	Geographical region	Regression equation*	Reference
Eisenia fetida (earthworm) reproduction	Europe, Australia	log EC_{50} = 1.85+0.59 log eCEC	Criel et al., 2008
Folsomia candida (springtail) reproduction	Europe	log EC_{50} = 1.63+0.96 log eCEC	Criel et al., 2008
Potential nitrification rate	Europe	log EC_{50} = 1.41+1.07 log eCEC	Oorts et al., 2006
Substrate induced respiration	Europe	log EC_{50} = 1.08+0.73 log OC+0.60 log clay	Oorts et al., 2006
Maize residue mineralisation	Europe	log EC_{50} = 3.75-0.34 pH+0.74 log eCEC	Oorts et al., 2006
Wheat grain yield (field)	Australia	log EC_{10} = 0.56+0.31 pH+1.05 log OC	Warne et al., 2008b
Folsomia candida (springtail) reproduction	Australia	log EC_{10} = 1.499+0.8475 log eCEC	NEPC, 2011
Substrate induced nitrification	Australia	log EC_{50} = 0.84+0.35 pH	Broos et al., 2007
Tomato shoot yield	China	log EC_{10} = 0.635+0.092 pH+0.873 log CEC	Li et al., 2013
Bok choy shoot yield	China	log EC_{10} = 1.554+0.706 log OC	Li et al., 2013
Barley root elongation	China	log EC_{10} = 1.18+0.159 pH+0.597 log OC+0.702 log CEC	Li et al., 2010
Substrate induced respiration	China	log EC_{10} = -2.247 + 0.565 pH + 0.283 OC	Wei, 2010
Bioluminescent bacteria	China	log EC_{10} = -0.942 + 0.411 pH + 0.033 CEC	Ma et al., 2012

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

Table A3.2: Leaching and ageing factors proposed for setting soil quality standards for copper in China.

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

*pH measured in H_2O

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ANNEX 4: REPRESENTATIVENESS OF SELECTED BIOAVAILABILITY CORRECTIONS FOR NORTH AMERICAN SOILS

The distribution of major soil properties affecting metal bioavailability in soil was studied for surface horizons from Europe and North America. The GEMAS project (Geochemical Mapping of Agricultural and Grazing Land Soils; http://gemas.geolba.ac.at/) provides high quality and comparable exposure data for natural elements and soil properties known to influence the bioavailability of metals (pH, organic carbon content, clay content and effective CEC) in agricultural (arable) and grazing land at the European scale. In total 2108 samples of agricultural soil (0-20 cm) and 2024 samples of grazing land soil (0-10 cm) were collected at an average sampling density of one site per 2500 km^2 (grid of 50 x 50 km). Sampling depth depends on land-use: 0-20 cm and 0-10 cm for agricultural (arable) land and grazing land, respectively. Because sites were sampled in a regular grid over Europe and there is no bias due to spatial heterogeneity of sampling density, it is appropriate to base the distributions of soil properties on the measured observations and not on interpolated values (i.e., point-based distributions and not area-based distributions). Access databases containing soil survey data were downloaded for each of the 50 states from the United States Department of Agriculture, Natural Resources Conservation Service website at http://datagateway.nrcs.usda.gov/GDGOrder.aspx. The STATSGO2 version was selected, which combines all the data for a given state into one downloadable file. Data tables in dBase format were downloaded for all Canadian provinces that were available from the CanSIS National Soil Database website at http://sis.agr.gc.ca/cansis/nsdb/dss/v3/index.html. The samples all come from the soil surface horizons with an average sampling depth of 20 cm. Percentiles were calculated for soil characteristics using an area-weighted scheme.

Comparison of individual soil properties among Europe, USA and Canada does not identify major differences among these regions (Table A4.1). Values for pH agree very well among the 3 datasets. USA soils generally show lower organic carbon contents compared to Europe and especially Canada. The higher organic carbon content for Canadian soils can probably be explained by the large area of forest soils, which generally have high organic carbon content in their topsoil. Data for clay content can be slightly biased by differences in dispersion methods during particle-size analysis, but results generally do agree well among Europe, North America and Canada. Differences in methodologies used certainly affect the results for CEC. Data for Europe are all based on the eCEC, i.e., the CEC at the pH of the soil, while most of the data for USA and Canada are derived by the ammonium acetate method at a buffered pH of 7. For soils with pH <6 (e.g., many forest soils), the latter method generally results in higher CEC values compared to eCEC because of the contribution of pH-dependent charge sites at the organic matter and clay (CEC generally increases with pH). This most likely explains the high CEC values observed for Canadian soils When the values for pH, organic carbon content, clay content and CEC are further compared with the typical ranges covered by the bioavailability research programmes for most metals (Table A4.1), it can be concluded the research covered the relevant range (10^{th} – 90^{th} percentile) in abiotic soil conditions in the USA and Canada.

Table A4.1. Comparison of pH, organic carbon content, clay content and (e)CEC in soils in Europe, USA and Canada and ranges covered by most bioavailability research programmes.

Soil property	Region	Percentiles						
		5	**10**	**25**	**50**	**75**	**90**	**95**
pH (0.01 M $CaCl_2$)	Europe	4.1	4.4	4.8	5.6	7.0	7.4	7.5
	USA	4.1	4.3	4.8	6.0	6.7	7.4	7.8
	Canada	3.6	4.0	5.2	6.3	7.0	7.4	7.5
	Research*	4.0 – 7.5						
Organic carbon content (%)	Europe	0.8	1.0	1.4	2.1	3.4	5.5	8.1
	USA**	0.41	0.44	0.73	1.2	2.0	4.6	37.7
	Canada	0.6	1.1	1.8	2.7	4.2	50.4	53.8
	Research	0.8 – 20						
Clay content (%)	Europe	1.0	1.0	6.2	13.8	20.6	26.2	29.3
	USA	2.0	2.5	7.5	15.0	22.5	31.0	37.5
	Canada	5	8	14	21	29	47	57
	Research	2 – 50						
(e)CEC ($cmol_c/kg$)	Europe	7.1	8.7	11.9	17.5	24.9	32.4	36.8
	USA	3.7	5.4	9.3	15	20	28	38
	Canada	7	11	19	25	37	106	125
	Research	2 – 30						

* typical ranges covered by the bioavailability research programmes for metals (see Annex 1 and 2)
** data corrected from organic matter to organic carbon by a factor 0.58.

Apart from the individual soil properties, one also should look at their combinations. Combinations of physico-chemical parameters are reflected in the soil classification because this is the result of various soil forming factors, including parent material, topography, climate, organisms etc. that also affect the soil properties. Soils selected for derivation of bioavailability correction models cover most major soil types for temperate regions (Table A4.2). Because most representative soil types and the relevant range in individual soil properties are covered, the models can be considered representative for most soil conditions in temperate regions.

Table A4.2. Soil types covered by the metal bioavailability research programmes

Co	Cu	Pb	Mo	Ni	Zn
• Acrisol	• Cambisol	• Cambisol	• Cambisol	• Cambisol	• Cambisol
• Cambisol	• Fluvisol	• Histosol	• Chernozem	• Fluvisol	• Fluvisol
• Podzol	• Histosol	• Luvisol	• Histosol	• Histosol	• Histosol
• Kastanozem	• Leptosol	• Podzol	• Luvisol	• Inceptisol	• Leptosol
• Leptosol	• Luvisol		• Podzol	• Luvisol	• Luvisol
• Luvisol	• Podzol		• Regosol	• Podzol	• Podzol
	• Regosol			• Regosol	• Regosol

ANNEX 5: NORMALISATION OF BIOACCUMULATION OF PB IN EARTHWORMS FOR DIFFERENCES IN SOIL PROPERTIES

In total, 248 reliable bioaccumulation factors for Pb in earthworms were identified, ranging from 0.01 to 22.05 $kg_{dw\ soil}/kg_{dw\ worm}$ on a dry weight (dw) basis. The median bioaccumulation factor for earthworms is 0.23 $kg_{dw\ soil}/kg_{dw\ worm}$ (10-90th percentiles are 0.06-1.19).

Results are available for several earthworm species, belonging to different ecological groups of earthworms: anecic, endogeic and epigeic earthworms. No distinct differences in bioaccumulation factors across these groups could be identified.

- Anecic earthworms (n=46): range: 0.06-1.76, median: 0.41
- Endogeic earthworms (n=108): range: 0.01-22.05, median: 0.18
- Epigeic earthworms (n=61): range: 0.02-9.15, median: 0.17
- mixed or not reported (n=33) range: 0.06-1.25, median: 0.25

Soil properties were not reported for all studies, but based on the reported data, it can be concluded that the bioaccumulation factors are derived in a wide range of soils and the data available can be considered as representative for soils from temperate regions:

- pH 3.0 – 8.4 (n=217)
- Organic carbon content 1.1 – 24.6 % (n=186)
- Clay content 4 – 53 % (n=111)
- Cation exchange capacity 5.3 – 78.8 $cmol_c/kg$ (n=114)
- Total Pb content in soil 9.4 – 16700 mg/kg (n=231)

Correlation of the bioaccumulation data for earthworms with soil properties shows that only CEC is significantly correlated with bioaccumulation values. No significant correlation of bioaccumulation factors with Pb content, pH, organic carbon content or clay content is observed. Because of the lack of any effect of Pb level in soil on the bioaccumulation factor for Pb in earthworms, also data from Pb contaminated soils could be included in the analysis.

Four field studies report CEC data for the soils where earthworms have been sampled (Beyer et al., 1982; Ernst et al., 2008; Ma, 1982; Nannoni et al., 2011). Correlations between bioaccumulation factors in earthworms and soil properties for individual studies are either non-significant or contradictory. The combined dataset shows a significant decrease of bioaccumulation factors with increasing eCEC (effective CEC) of the soil (Figure A5.1):

*Log bioaccumulation factor ($kg_{dw\ soil}/kg_{dw\ worm}$) = -0.89 * log eCEC ($cmol_c/kg$) +0.55* $R^2 = 0.16$, $P<0,01$

This regression is based on data from different studies, for 9 different earthworm species (anecic: *Aporrectodea longa*, *Lumbricus terrestris*; endogeic: *Aporrectodea caliginosa*, *Aporrectodea rosea*, *Aporrectodea*

tuberculate, *Octolasion cyaneum*, *Octolasion tyrtaeum*; epigeic: *Lumbricus rubellus*, *Dendrodrilus rubidus*) and for a wide range of Pb levels and forms in soil (from natural and various anthropogenic sources). No clear distinction could be noticed between different ecological groups of earthworms (Figure A5.1).

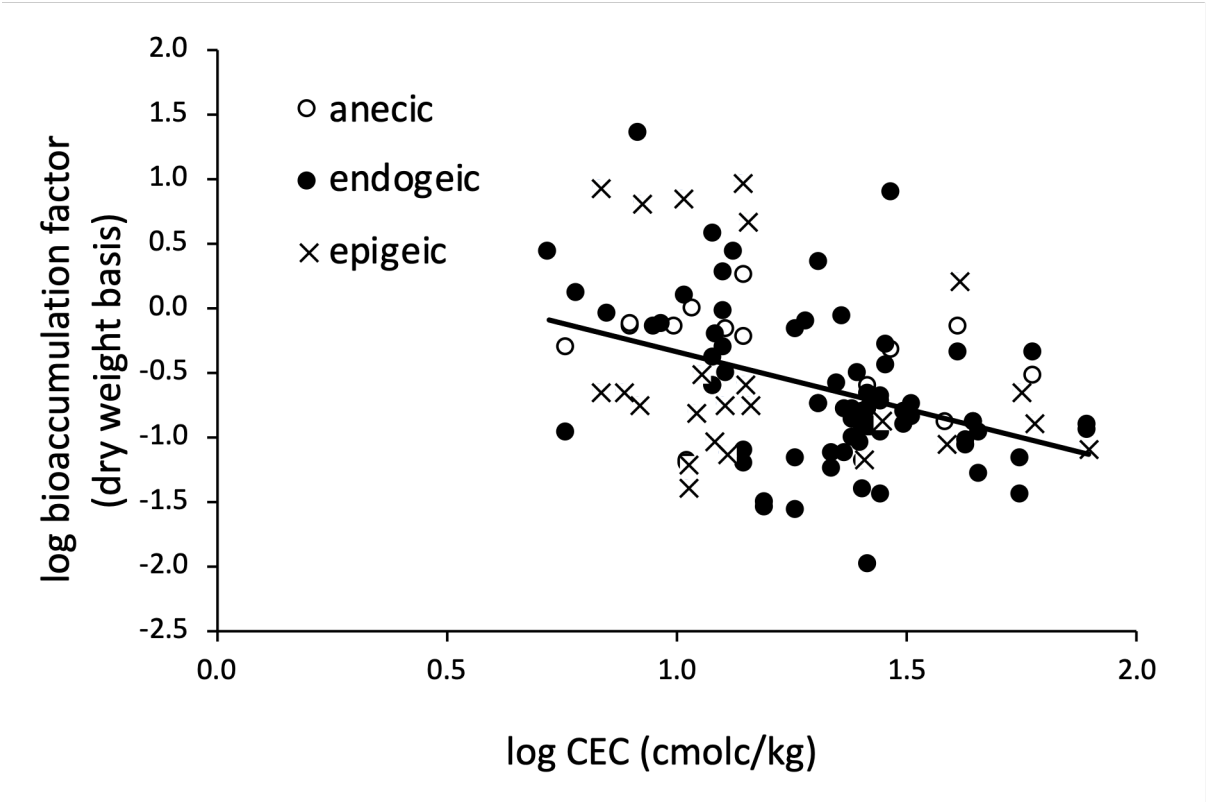

Figure A5.1. Correlation of field bioaccumulation factors for Pb in earthworms with CEC of the soil. Data from Beyer et al. (1982), Ernst et al. (2008), Ma (1982) and Nannoni et al. (2011).

The slope of this regression between bioaccumulation of Pb in earthworms and the CEC of the soil corresponds well with the results of a laboratory study on the effects of soil type on the bioavailability and toxicity of Pb salts to earthworm *Eisenia fetida* exposed for 28 days to Pb in 6 different soils spiked with $PbCl_2$ and leached with a dilute salt solution (Lanno et al., 2019). This points to a similar effect of CEC on Pb accumulation in earthworms in controlled laboratory conditions and in field conditions. The significant regression between bioaccumulation of Pb in earthworms and CEC is also consistent with the regression observed between toxicity of Pb to *E. fetida* reproduction and eCEC of the soil and is selected to calculated soil-specific bioaccumulation factors for Pb in earthworms.

References Annex 5

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