Incorporation of Bioavailability in the Terrestrial Compartment

The Existing Substances Risk Assessment of Nickel was completed in 2008. The straightforward explanation of the goal of this exercise was to determine if the ongoing production and use of nickel in the European Union (EU) causes risks to humans or the environment. The European Union launched the Existing Substances regulation in 2001 to comply with Council Regulation (EEC) 793/93. "Existing" substances were defined as chemical substances in use within the European Community before September 1981 and listed in the European Inventory of Existing Commercial Chemical Substances. Council Regulation (EEC) 793/931 provides a systematic framework for the evaluation of the risks of existing substances to human health and the environment.

The conceptual approach to conducting the environment section of the EU risk assessment of nickel included the following steps (Figure 1):

Barley roots exposed to nickel in two types of soil show different responses due to differences in soil chemistry.

- Emissions of nickel and nickel compounds to the environment were quantified for the whole life cycle, *i.e.*, from production, use, and disposal;
- Concentrations of nickel resulting from these emissions were determined in relevant environmental media (water, sediment, soil, tissue) at local and regional scales (PECs);
- Critical effects concentrations (PNECs) were determined for each of these relevant environmental media;
- Exposure concentrations were compared to critical effects concentrations for each of the relevant environmental media (risk characterization); and
- Appropriate corrective actions (also described as risk management) were identified for situations where exposure concentrations were greater than critical effects concentrations. Where exposure concentrations were below critical effects concentrations, there was no need for concern or action.

The EU Risk Assessments for Nickel and Nickel Compounds were developed over the period from 2002 to 2008. The Danish Environmental Protection Agency (DEPA) acted as the Rapporteur in this process, in close collaboration with the international nickel industry. EU Risk Assessment Reports (RARs) for the environment for nickel substances (metallic nickel, nickel carbonate, nickel chloride, nickel nitrate, and nickel sulfate) were submitted in the spring of 2008 after thorough review by the Technical Committee on New and Existing Substances (TCNES), which was comprised of technical representatives from the EU Member States. A final peer review was provided by the Scientific Committee on Health and Environmental Risks (SCHER) (see Section 8). The European Commission's Institute for Health and Consumer Protection published the final Risk Assessment Reports for nickel and nickel compounds in November 2009.

After the EU RARs received approval within Europe, the data sets were discussed at the international level within the Organization of Economic Cooperation and Development (OECD). The nickel ecotoxicity data sets used in the EU RARs were accepted at the OECD's SIDS (Screening Level Information Data Set) Initial Assessment Meeting (SIAM 28, October 2008), as was the use of nickel bioavailability models (BLMs) to normalize the nickel ecotoxicity data.

1 INTRODUCTION

Environmental risks are typically characterized in the risk assessment framework by comparing exposure concentrations and critical effect concentrations. In OECD countries, critical effect concentrations for metals are based on Predicted No Effect Concentrations (PNEC), which are typically derived from long-term laboratory ecotoxicity tests performed shortly after amending "clean" standard soils with highly soluble, almost completely dissociated metal salts.

Under these test conditions most of the metal is usually present in the most bioavailable and toxic form and resulting toxicity thresholds are near or below the background Ni concentrations in naturally occurring soils. Research has demonstrated that when considering the bioavailability of nickel (and other metals) in soils, the following factors are the most important in determining the ecotoxicity to soil organisms:

- Metal Form: Ni can enter the soil environment in different forms, such as soluble (associated with a high bioavailability, *e.g.*, soluble salts) or sparingly soluble compounds (associated with a low bioavailability, *e.g.*, oxides);
- Ageing: Laboratory Ni spiked soils often exhibit greater toxicity than field contaminated soils with the same Ni concentration. The greater toxicity of Ni in spiked soils compared

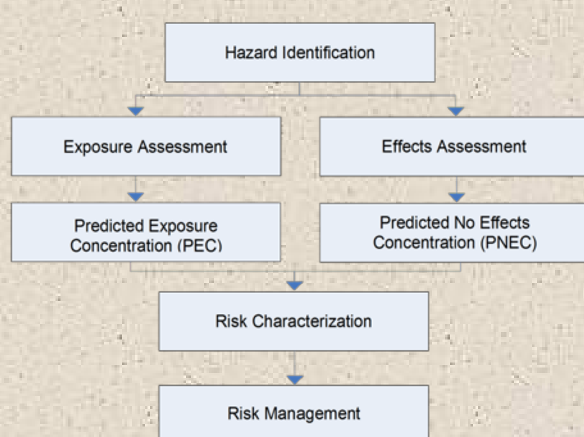


Figure 1: Schematic overview of the steps in the EU Environmental Risk Assessment

to corresponding field contaminated soils can be partly attributed to the time between the addition of nickel to soils and the measurement of toxicity. The bioavailability and toxicity of nickel in spiked soils tend to decrease with time in a manner that is dependent on soil pH (see Section 2.1); and

- Soil Characteristics: The toxicity of nickel is highly dependent on soil characteristics. Specifically, Ni toxicity to plants, invertebrates, and microbial processes decreases as the Effective Cation Exchange Capacity (eCEC)[1] of the soil increases (see Section 2.2).

Practically speaking, this means that Ni toxicity can vary considerably between laboratory-spiked and field-contaminated soils and among soils with different physico-chemical characteristics. It also means that toxicity tests with the same terrestrial species that are performed using different types of soil can result in different toxicity conclusions. Consequently, a generic PNEC may be largely over or under protective depending on the soils and procedures used for generation of toxicity data. Hence, there is a clear need for bioavailability models to account for these differences in order to generate site-specific PNECs for the terrestrial environment.

This fact sheet provides a summary of the development of Ni bioavailability models for the terrestrial compartment, as well as clear guidance on how to perform and implement bioavailability correction for these systems.

2 BIOAVAILABILITY CORRECTION FACTORS

2.1 CORRECTION FOR LEACHING AND AGEING EFFECTS

The Ni soil toxicity data used for PNEC derivation (see Fact Sheet 2) are generally based on soils spiked in the laboratory with a soluble Ni salt. Comparative studies show that toxicity tests in freshly spiked soil generally overestimate toxicity of Ni to soil organisms/microbial processes compared to field-contaminated soils or aged soils. It is observed that Ni solubility in soils appreciably decreases with increasing equilibration time after an initial rapid sorption phase. Hence it can be expected that testing soils immediately after spiking with a soluble Ni salt will also result in an overestimation of Ni toxicity compared to long-term equilibrated soils.

Moreover, spiking a soil with a soluble metal salt also changes the physico-chemical characteristics of the soil by increasing the ionic strength and decreasing the pH of the soil. These spiking artifacts can either directly affect the response of the endpoint tested or alter the Ni bioavailability and the toxicity of the soil. These issues are corrected by leaching and ageing the soil after spiking.

To correct for the ageing and leaching effects when using Ni soil toxicity data for PNEC derivation, a correction factor (*i.e.*, leaching-ageing factor) was developed. The L/A (leaching-ageing) factor[2] is the ratio of toxicity values in leached and aged soils to toxicity values in corresponding freshly spiked soils. For Ni, there is a clear effect of pH on the change in toxicity after leaching and ageing (Figure 2). Toxicity is ameliorated least in acidic soils (median ageing factor of 1.2) and most in calcareous soils (median ageing factor of 8.4). This is consistent with the differences in the amount of added Ni that is isotopically exchangeable in freshly spiked and corresponding aged soils for 16 soils sampled across Europe with contrasting soil properties and land use.

The L/A factor is estimated from an empirical model fitted to the ratio of added Ni that is (isotopically) exchangeable in freshly amended soils (1–21 days after amendment) to that after at least one year of ageing, *i.e.*, L/A = 1 + exp (1.4(pH-7.0)). This equation is calibrated on soil aged maximally 1.5 year and soil pH ranged between pH 3.6 and 7.7. This factor is a conservative estimate for the changes in toxicity.

The L/A factors vary between 1 to 4 and exhibit a positive relationship with pH, but only become significant beyond pH values of about 6.0.

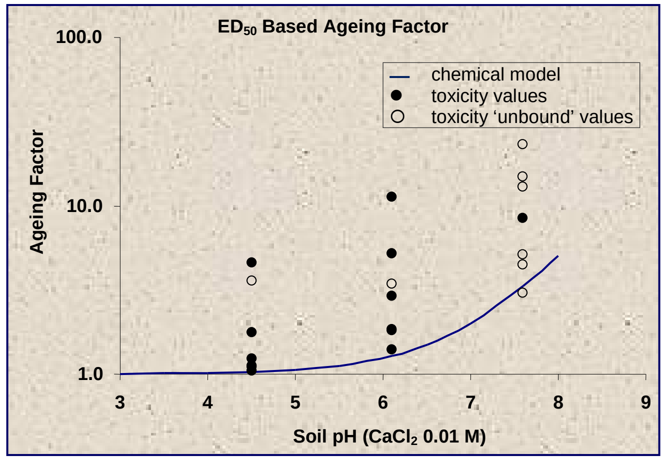


Figure 2: The ageing factors based on toxicity (symbols) and the predicted factor changes in isotopically exchangeable pool of Ni in soil (line). Open symbols are 'unbounded' values and are a lower estimate of the ageing factor

2.2 NORMALIZATION FOR SOIL TYPES

Bioavailability and chronic toxicity of Ni to soil organisms/microbial communities vary as a result of the characteristics of the soil media. In order to make comparisons between laboratory toxicity data, results must be normalized to a standardized set of conditions using bioavailability models. Bioavailability models can be used to derive site-specific HC_5 and PNEC values for sites in which appropriate soil properties have been quantified.

Chronic regression bioavailability models for Ni have been developed using laboratory experiments for three different trophic levels, *i.e.*, for microbial function [using nitrification (PNR), substrate induced respiration (SIR), and maize respiration (MR)], for higher plants (using the tomato *Lycopersicon esculentum* and barley *Hordeum vulgare*), and for both hard-bodied (using the collembole *Folsomia candida*) and soft-bodied (using the worm *Eisenia fetida*) invertebrates.

The chronic regression models for Ni were developed/calibrated based on soils that represent the full range in physico-chemical parameters (pH, clay, OM, eCEC) that represent soil conditions in the EU. Accounting for differences in soil properties significantly explained variation in Ni toxicity to all endpoints tested, and it was observed that chronic Ni toxicity was best correlated with the eCEC of the soils.

The same trends were observed for all of the species tested:

as eCEC ↑, toxicity ↓

Linear regression models (log EC_{50} (mg/kg) = a + b log eCEC) were developed to allow for normalization based on differences in Ni toxicity between soils with different properties. An overview of all significant regression models is presented in Table 1.

Organism or Microbial Function	Regression Model[3]	R^2
Invertebrates		
Eisenia fetida	log[Ni] = 0.95 log (eCEC) + 1.76)	0.72
Folsomia candida	log[Ni] = 1.17 log (eCEC) + 1.70)	0.71
Higher Plants		
Hordeum vulgare	log[Ni] = 1.12 log (eCEC) + 1.57)	0.83
Lycopersicon esculentum	log[Ni] = 1.27 log (eCEC) + 1.06)	0.67
Microbial Community		
Nitrification	log[Ni] = 1.00 log (eCEC) + 1.42)	0.60
Substrate induced respiration	log[Ni] = 1.34 log (eCEC) + 1.38)	0.92
Maize induced respiration	log[Ni] = 1.22 log (eCEC) + 1.37)	0.72

Table 1: Overview of all significant regression models relating the toxicity of nickel ([Ni] in mg/kg_{dw}, after correction for ageing) to eCEC

3 APPLICABILITY OF BIOAVAILABILITY CORRECTION

3.1 PHYSICO-CHEMICAL RANGES

The ranges of soil physico-chemical conditions used to develop the bioavailability models for all soil organisms/functions represent the physico-chemical boundaries of the models. An overview of the range of physico-chemical parameters for which the chronic bioavailability models for Ni were developed is provided in Table 2.

Physico-Chemical Parameter	Range
pH	3.6 – 7.7
Organic carbon	2.5 – 330.5 g/kg
Organic matter	0.4 – 56.8%
Effective cation exchange capacity	1.8 – 52.8 $cmol_c$/kg
Clay content	0 – 55%
Ni background content	1 – 113 mg/kg

Table 2: Overview of the ranges of soil physico-chemical conditions used to develop the bioavailability models

3.2 APPLICABILITY TO DIFFERENT SPECIES

As mentioned above, the bioavailability models have been developed for only a limited number of species/functions, and they therefore do not cover all soil species/functions included in the chronic Ni database. The chronic Ni aquatic toxicity database contains data for 43 different species/functions (see Fact Sheet 2) while chronic Ni bioavailability models are available for only 4 species (*i.e.*, *F. candida, E. fetida, H. vulgare, L. esculentum*) and 3 microbial functions (nitrification, substrate induced respiration, maize induced respiration).

The application of bioavailability models to species for which no model exists requires a cross-species extrapolation. A cross-species extrapolation is justified based on the following evidence:

- A reduction in intra-species variability after normalization with the regression equations was achieved; and
- The regressions for the different species/functions are similar (see Table 1).

For all species and functions the eCEC of the soil is the primary driver for Ni bioavailability and the slopes for all the regression equations are similar (vary between 0.95 and 1.34), indicating a cross-species extrapolation is warranted. Moreover, the eCEC relationship has a robust mechanistic explanation, *i.e.*, the higher the eCEC, the lower the proportion of free Ni^{3+} in soil pore water, which is the assumed most toxicologically relevant nickel species.

The following approach can be used for the normalization of all soil Ni toxicity data:

- for higher plants other than *L. esculentum*, the *H. vulgare* model can be used;
- for hard-bodied invertebrates, the *F. candida* model can be used;
- for soft-bodied invertebrates, the *E. fetida* model can be used;
- for microbial processes related to the N-cycle, the model for nitrifying micro-organism can be used;
- for all respiration processes using natural substrate or basal soil respiration, the model for maize respiration model can be used;
- for microbial biomass, the substrate induced respiration model can be used; and
- for all other indicators of microbial assays, the model for nitrifying micro-organism can be used.

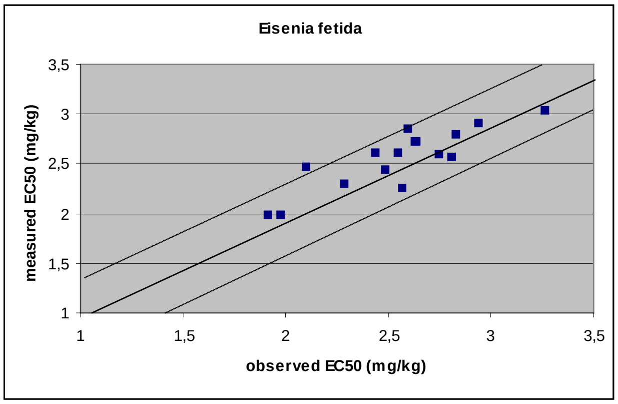
4 ACCURACY OF BIOAVAILABILITY MODELS

Based on eCEC alone, chronic Ni toxicity in soils could be predicted within a factor of 2 for soils with a wide range of physico-chemical parameters. Currently, there are no independent datasets available for these species/functions to validate the models. However, the models are calibrated for soils that represent the full range in physico-chemical parameters (pH, clay, OM, eCEC) for the EU, and therefore it is expected that for soils in the same range, predictions will show the same uncertainty (*i.e.*, factor of 2).

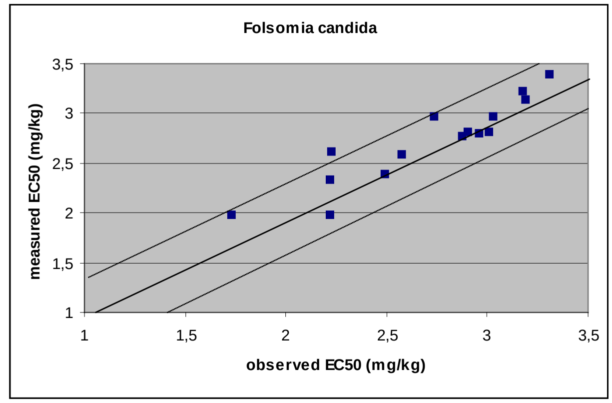
Figures 3, 4, and 5 show an overview of the relationship between the observed and predicted chronic Ni-toxicity values for microbial processes, invertebrates, and higher plants.

5 INCORPORATION OF BIOAVAILABLTILY MODELS

The correction for bioavailability is applied to the effect concentrations [No Observed Effects Concentration (NOEC) and 10% Effects Concentration (EC_{10})] in the nickel terrestrial toxicity database (see Fact Sheet 2). The steps below need to be accomplished in order to incorporate bioavailability for the derivation of

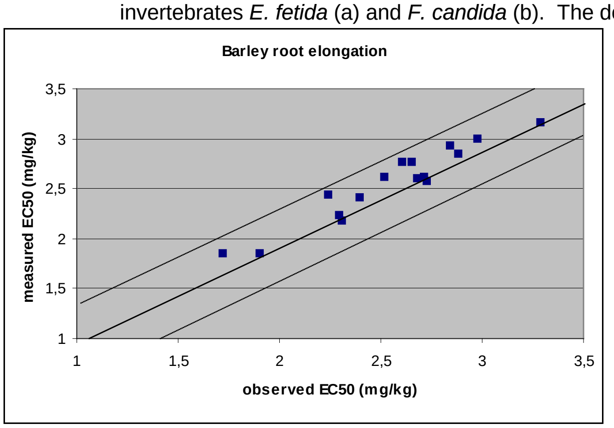


a.



b.

Figure 3: Overview of the relationship between observed and predicted chronic toxicity values for the invertebrates *E. fetida* (a) and *F. candida* (b). The dotted lines represent a factor of 2 difference from the solid 1:1 line.



a.

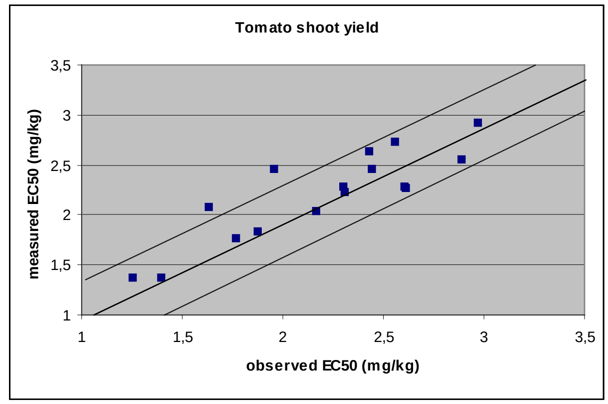


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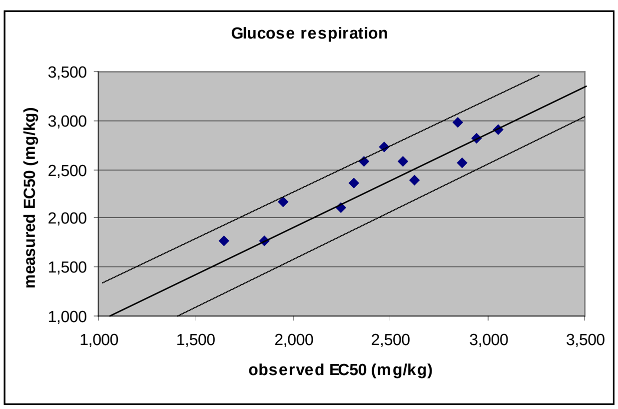
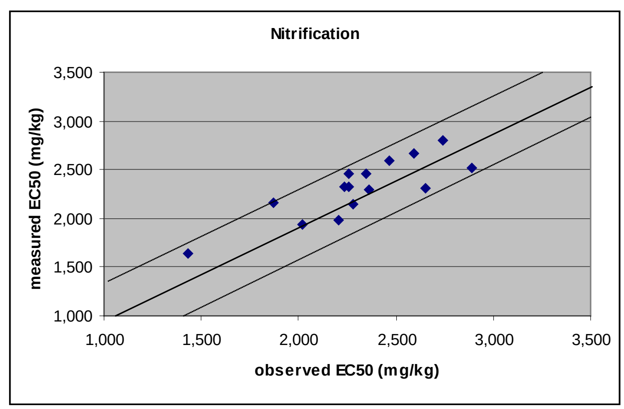
Figure 4: Overview of the relationship between observed and predicted chronic toxicity values for the higher plants *H. vulgare* (a) and *L. esculentum* (b). The dotted lines represent a factor of 2 difference from the solid 1:1 line.



a.



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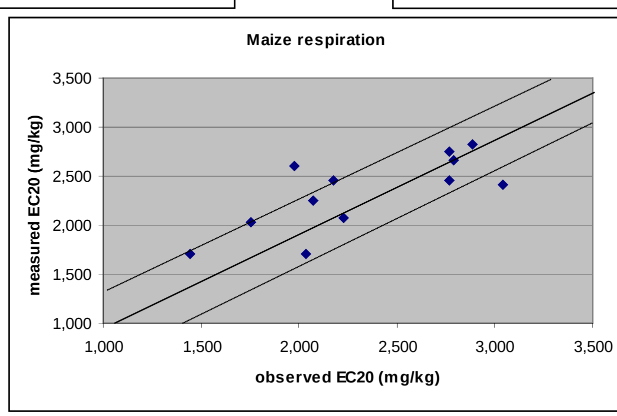

c.

Figure 5: Overview of the relationship between observed and predicted chronic toxicity values for the microbial processes, *i.e.*, glucose respiration (a), nitrification (b), and maize respiration (c). The dotted lines represent a factor of 2 difference from the solid 1:1 line.

bioavailability-based' PNECs of Ni for the terrestrial compartment for use in risk characterization (Figure 6).

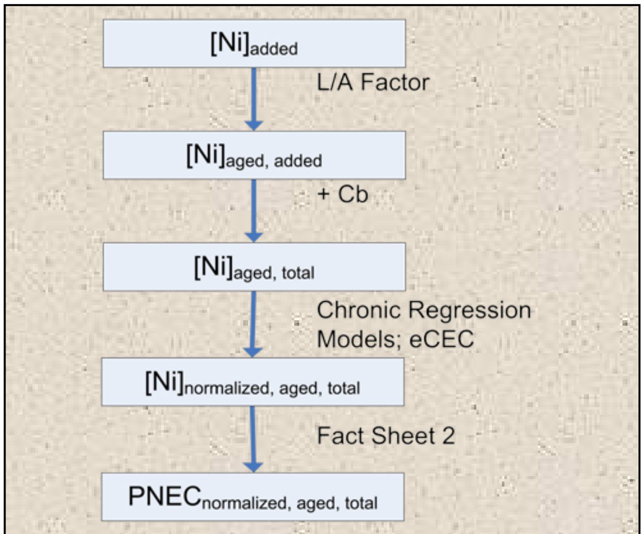

Figure 6: Stepwise approach used for the incorporation of bioavailability in the freshwater compartment

In the first step, the added Ni toxicity values, *i.e.*, $[Ni]_{added}$[4], are calculated by subtracting the Ni background concentration (Cb) in the test media from the reported total toxicity values, *i.e.*, $[Ni]_{total}$.

In the second step the $[Ni]_{added}$ toxicity values are corrected for the difference in Ni bioavailability between laboratory conditions (Ni freshly added as soluble salts) and field conditions, through the application of the L/A factor[5]. Because the background concentration is assumed to be already aged, the pH dependent L/A factor should only be applied to the $[Ni]_{added}$ which result in $[Ni]_{aged,\ added}$[6] values. The individual Ni background concentration (Cb) from the control soil is subsequently added to the $[Ni]_{aged,\ added}$ values resulting in an $[Ni]_{aged,\ total}$[7] (= $[Ni]_{aged,\ added}$ + Cb). The derivation and application of the L/A factor is discussed in Section 2.1.

The third step is the correction of the $[Ni]_{aged,\ total}$ values towards the specific soil properties of a given site, since it is demonstrated that abiotic factors (*i.e.*, soil properties) affect Ni toxicity in soil. This normalization is based on the slopes of the organism specific regression models between toxicity thresholds and the driving abiotic factor (*i.e.*, eCEC). This approach is discussed in Section 2.2 and result in the calculation of $[Ni]_{normalized,\ aged,\ total}$[8].

Subsequently, these $[Ni]_{normalized,\ aged,\ total}$ concentrations are further used to calculate the $PNEC_{normalized,\ aged,\ total}$[9] values according to the approach described in Fact Sheet 2.

Incorporation of the bioavailability concept as described above will result in the derivation of different Species Sensitivity Distributions (SSDs) and PNEC values, depending on the physico-chemical characteristics (eCEC) of the terrestrial environments under assessment.

An overview of the SSDs derived for the different selected soil eco-regions in Europe as defined in Fact Sheet 2 is provided in Figure 7.

The soil physico-chemistry and median HC_5 and PNEC values calculated for the different selected eco-regions in EU soils are summarized in Table 3.

6 BIOAVAILABILITY SOFTWARE

6.1 INTRODUCTION

In 2009, ARCHE developed a user-friendly calculator to facilitate the practical application of bioavailability normalization for use in the field of different soil policies such as the European Soil Framework Directive (COM(2006) 232). This bioavailability tool is based on the corrections for leaching and ageing and for variation in physico-chemical soil properties and estimates of risks associated with the presence of Ni in a particular terrestrial environment, based on a limited set of routinely screened input parameters. It can be downloaded from http://www.arche-consulting.be/Metal-CSA-toolbox/soil-pnec-calculator.

The tool is an Excel-based tool consisting of three main pages, *i.e.*, introductory, input, and output (results). The introductory page gives the user information on how to use the tool, on which input parameters are needed to run the bioavailability tool, and on which output (results) are generated by the model. The tool calculates PNEC values for: i) a generic scenario; ii) a standard soil type (eco-regions); or iii) site-specific properties, depending on the level of information available. The site specific information required to run the tool is listed below.

- eCEC, as $cmol_c/kg_{dw}$[11]
- pH[12]
- organic matter (OM) content, as %
- clay content, as %
- total Ni concentration as mg/kg_{dw}
- Ni background concentration as mg/kg_{dw}[13]

Eco-Region	Soil Use	Soil Physico-Chemistry	L/A Factor	Median HC_5 (µg/L)	PNEC (µg/L)[10]
Acid Sandy Soil in Sweden	Arable Land	pH 4.8, OM 2.8%, clay 7%, eCEC 2.4 cmol/kg	1.05	8.50	4.3
Loamy Soil in The Netherlands	Arable Land	pH 7.5, OM 2.2%, clay 26%, eCEC 20 cmol/kg	3.01	99.20	49.6
Peaty Soil in The Netherlands	Grassland	pH 4.7, OM 40%, clay 24%, eCEC 35 cmol/kg	1.04	186.3	93.2
Acid Sandy Soil in Germany	Forest Land	pH 3.0, OM 9%, clay 7%, eCEC 6 cmol/kg	1.00	25.00	12.5
Clay Soil in Greece	Woodland	pH 7.4, OM 4.5%, clay 46%, eCEC 36 cmol/kg	2.75	192.3	96.2
Soils of Different Types in Denmark	Arable & Forest Land	pH 6.3, OM 0.6%, clay 8.9%, eCEC 10.4 cmol/kg	1.38	47.10	23.6

Table 3: Overview of the soil chemistry, L/A factors, and median HC_5 and PNEC values for the different selected EU eco-regions

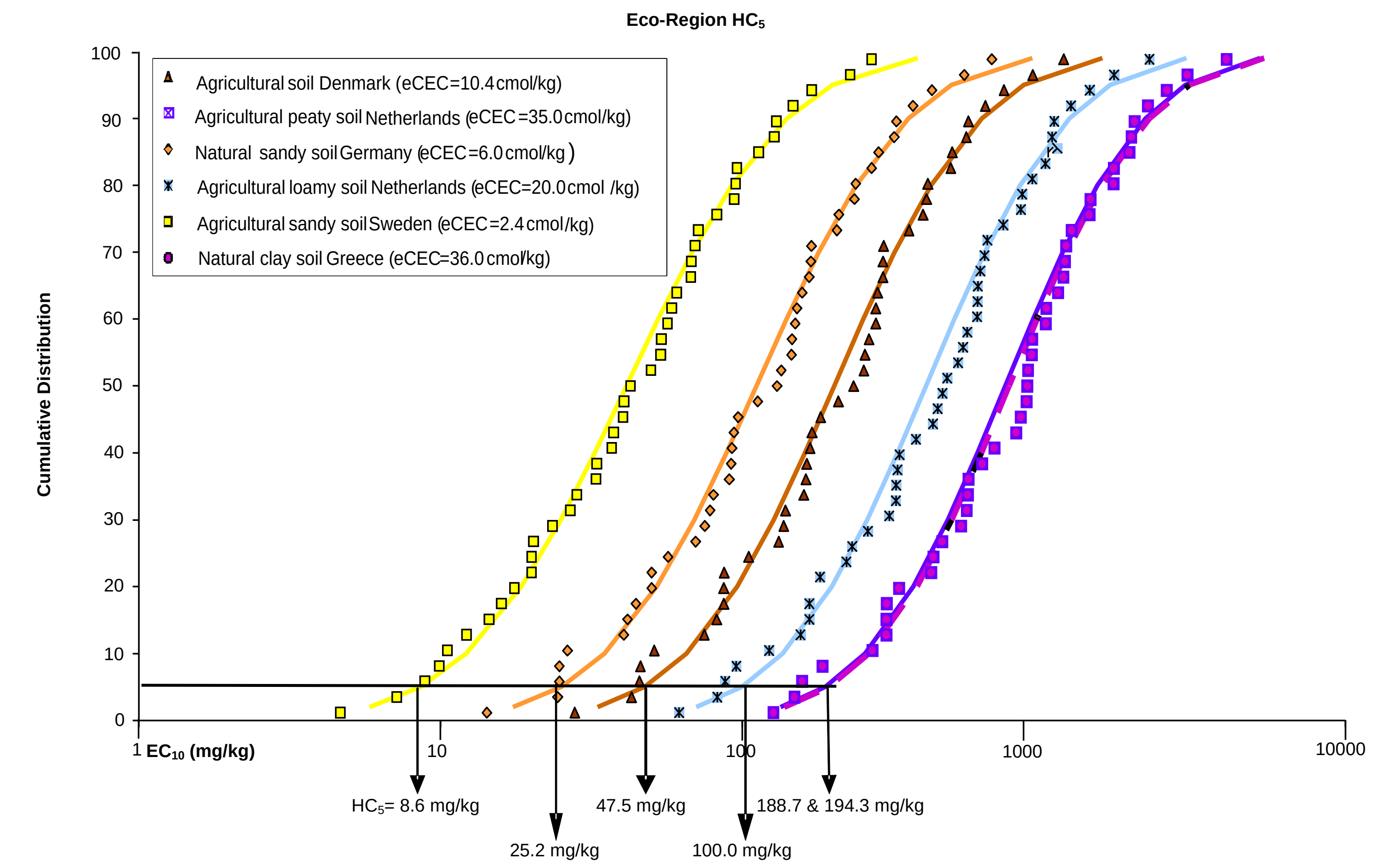

Figure 7: Overview of the SSD and HC$_5$ for the different selected soil eco-regions

6.2 EXAMPLE

An example of the application of the soil bioavailability tool for Ni is presented in Figures 8 and 9. In this example, the total Ni soil concentration is 45 mg/kg$_{dw}$, the Ni background concentration 22.0 mg/kg$_{dw}$, the pH 5.1, the OM 3.2, and the clay content 35%. The pH, %OM, and %clay are used to calculate the eCEC, *i.e.*, 22.1 cmol$_c$/kg$_{dw}$ (Figure 8).

Entering the required input parameters for the soil bioavailability tool resulted in the calculation of the PNEC$_{normalized,\ aged,\ added}$ value (as mg/kg$_{dw}$), the PNEC$_{normalized,\ aged,\ total}$ value (as mg/kg$_{dw}$), the PEC$_{added}$ value (as mg/kg$_{dw}$), the PEC$_{total}$ value (as mg/kg$_{dw}$), and the RCR (risk characterization ratio) (Figure 9).

7 CONCLUSIONS AND NEXT STEPS IN RISK ASSESSMENT

When considering Ni toxicity in soils, it is important to account for factors such as ageing and the physico-chemical characteristics of the soils (eCEC, OM, pH, clay). Bioavailability models have been developed to normalize ecotoxicity data to a common site condition when deriving HC$_5$ and PNECs or soil standards. This fact sheet presents the background information on the available soil bioavailability tools and demonstrates how this information can be used to estimate site-specific bioavailable PNEC values. The availability of soil physico-chemistry data, such as eCEC or pH, OM, and clay content, allows site-specific PNEC values to be calculated and a more accurate, site-specific risk characterization to be conducted.

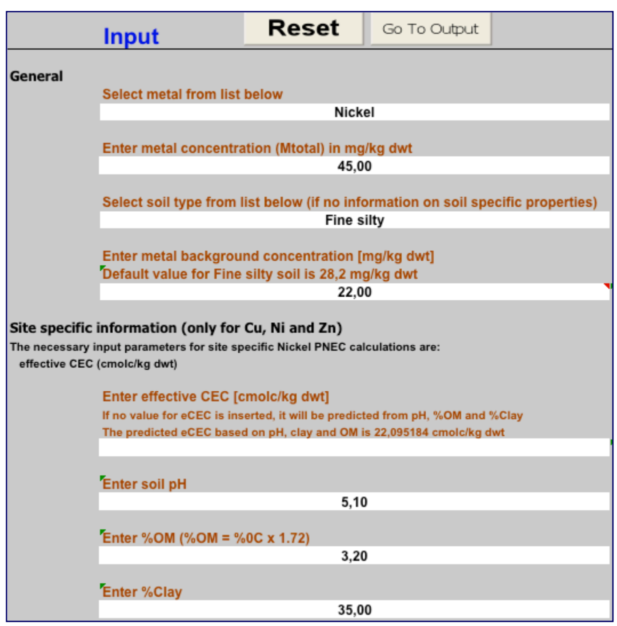

Figure 8: Example of selected input data in the soil bioavailability tool

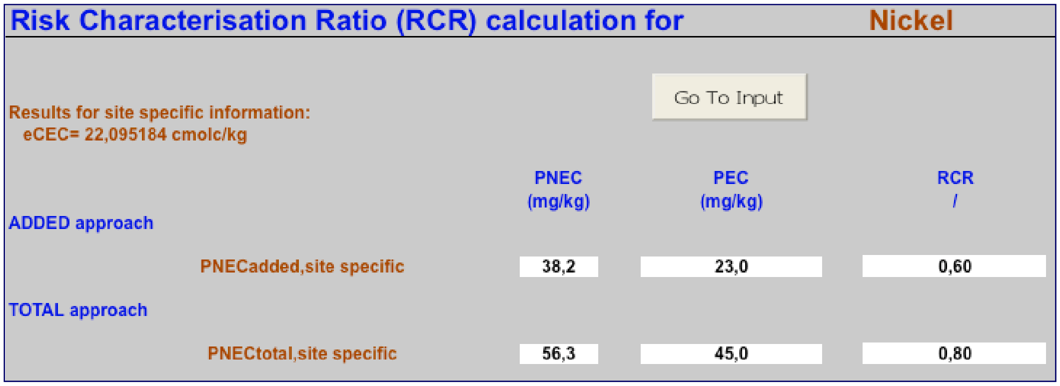

Figure 9: Example of results calculated in the soil bioavailability tool

8 LINK TO NICKEL EU RISK ASSESSMENT DOCUMENTS

The final report on the Environmental Risk Assessment of Nickel and Nickel Compounds can be retrieved from the following website:
http://ecb.jrc.ec.europa.eu/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/nickelreport311.pdf

The opinion of the SCHER can be found at the following address:
http://ec.europa.eu/health/ph_risk/committees/04_scher/docs/scher_o_112.pdf.

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1 eCEC: effective cation exchange capacity = CEC measured at the native pH of the soil (in contrast to CEC measured at a fixed, buffered pH). This is a measure for the sum of exchangeable cations plus extractable acidity held on or near the surface of negatively charged material, such as clay or organic matter, at native soil pH. It is usually expressed in centimoles of charge per kilogram of exchanger ($cmol_c$ /kg).

2 L/A factor: the term leaching-ageing factor (L/A factor) refers to the combined effect of leaching (due to changing ionic strength) and ageing (due to long-term reactions) on Ni bioavailability and toxicity in soil.

3 The slopes of the bioavailability models were based on regressions of EC_{50} with soil properties, because EC_{50} values are more robust and less sensitive to experimental error compared to the NOEC or EC_{10} values.

4 $[Ni]_{added}$ = Ni concentrations in the soils beyond which toxicity occurs after subtraction of the Ni background in the test medium.

5 The L/A factor could be derived from the following empirical chemical model as, *i.e.*, $L/A = 1 + \exp(1.4(pH-7.0)^4$. This equation is calibrated on soil aged maximally 1.5 year and soil pH ranged between pH 3.6 and 7.7. This empirical model predicts almost no ageing (L/A < 1.2) up to pH 6 and L/A = 2 at pH 7.0 and L/A = 3 at pH 7.5.

6 $[Ni]_{aged, added}$ = Ni concentrations in the soils beyond which toxicity occurs after subtraction of the Ni background in the test medium and correction for the L/A effect.

7 $[Ni]_{aged, total}$ = Ni concentrations in the soils beyond which toxicity occurs after correction for the L/A and addition of the Ni background in the test medium.

8 $[Ni]_{normalized, aged, total}$ = Ni concentrations in the soils beyond which toxicity occurs after correction for the L/A and normalization for differences in toxicity between different soil types.

9 $PNEC_{normalized, aged, total}$ = integration of aged and normalized EC_{10}/NOEC values from the terrestrial Ni toxicity database via the SSD (see Fact Sheet 2).

10 PNEC is calculated using an assessment factor of 2.

11 If no value for eCEC is inserted, it will be predicted from pH, %OM, and % clay.

12 Data for pH, organic matter content, and clay content are not strictly required for the calculation of a site-specific PNEC for Ni if data for the eCEC of the soil are available.

13 Only required for calculation of added risks. If no Ni background concentration is inserted, a default value will be predicted from the soil type information.

Fact Sheets on the European Union Environmental Risk Assessment of Nickel

This is the fifth in a series of fact sheets addressing issues specific to the environment section of the European Union's Existing Substances Risk Assessment of Nickel (EU RA). The fact sheets are intended to assist the reader in understanding the complex environmental issues and concepts presented in the EU RA by summarizing key technical information and providing guidance for implementation.

NiPERA welcomes questions about the concepts and approaches implemented in the EU RA. For inquiries, please contact:

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