Incorporation of Bioavailability in the Aquatic 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):

Daphnia magna is one of the four species for which nickel BLMs were developed.

- 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 the 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 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-based ecotoxicity tests performed with highly soluble, almost completely dissociated metal salts in "clean" laboratory standard waters. Under these test conditions most of the metal is usually present as the free ion (*e.g.*, Ni^{2+}), which is the most bioavailable and toxic form. Research has demonstrated that the bioavailability and toxicity of Ni to freshwater organisms is dependent on water chemistry parameters, such as hardness, pH, and dissolved organic carbon (DOC). Practically speaking, this means that Ni toxicity can vary considerably among different freshwater systems. Also, it means that toxicity tests with the same aquatic species that are performed under different water quality conditions can result in different toxicity endpoints. To remove the influence of chemical conditions on the outcome of toxicity tests, and to provide a way to offer the same degree of environmental protection for freshwater systems of varying water chemistry, the Biotic Ligand Model (BLM) was developed. The BLM is a mechanistically based model that is able to describe metal bioavailability and toxicity to freshwater organisms. BLMs are available for a number of metals, including Cu, Ni, and Zn.

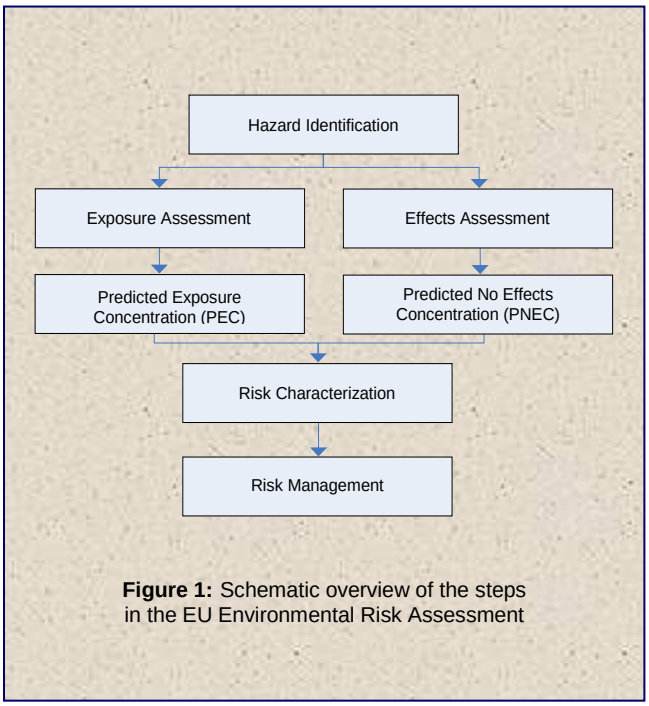


Figure 1: Schematic overview of the steps in the EU Environmental Risk Assessment

Like all BLMs, the Ni BLM integrates two fundamental chemical processes that occur with Ni in all natural waters. The first is complexation, where DOC complexes dissolved free ionic Ni^{2+}, thereby reducing the quantity of Ni^{2+} that is available to bind to the biotic ligand. The second process is competition, which describes the interaction between similarly charged ions that occur naturally in freshwater, such as calcium (Ca^{2+}), magnesium (Mg^{2+}), and protons (H^+, expressed as pH). These cations will compete with Ni for binding sites on the biotic ligand. Included in the BLM are aqueous speciation reactions that quantify the degree of DOC complexation and cation competition. Correcting for bioavailability using the BLMs is crucial to translate the toxicity of Ni to the prevailing site-specific water chemistry to generate site-specific PNECs for the freshwater environment.

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

2 AVAILABILITY OF BLMS

Chronic BLMs for Ni have been developed in laboratory experiments for three different trophic levels (for the invertebrates *Ceriodaphnia dubia* and *Daphnia magna*, the fish *Oncorhynchus mykiss*, and the green alga *Pseudokirchneriella subcapitata*) (Deleebeeck *et al.*, 2007; Deleebeeck *et al.*, 2008; Deleebeeck *et al.*, 2009). An additional BLM has been developed for the terrestrial higher plant *Hordeum vulgare* using hydroponic exposures (Lock *et al.*, 2007).

The chronic BLMs were further validated in natural waters within specific boundaries selected to reflect the typical range (defined as the 10th to 90th percentile) of physico-chemical factors (pH, hardness, DOC) occurring in EU surface waters. It must be emphasized that the *H. vulgare* BLM has not been validated in natural waters, and therefore, this model should be used with caution.

The results of the BLM development/validation experiments indicated that both Ca^{2+} and Mg^{2+} (which together comprise the water quality characteristic known as "hardness"), H^+ (expressed as pH), and DOC affected Ni toxicity. The same trends were observed for all of the species tested:

- as pH ↑, toxicity ↑
- as hardness ↑, toxicity ↓
- as DOC ↑, toxicity ↓

The consistency in trends has not been observed for all metals (*e.g.*, Cu) and offers empirical evidence that the mechanisms of Ni toxicity are conserved across algae, invertebrates, and fish.

3 APPLICABILITY OF BLMS

3.1 APPLICABILITY IN SURFACE WATERS

The ranges of Ca^{2+}, Mg^{2+}, and pH used to develop and validate the BLMs represent the physico-chemical boundaries of the models. Some freshwater systems and geographic regions will be outside of these boundaries, and the use of the BLMs in these situations may be over- or under-protective. An overview of the range of physico-chemical parameters for which the chronic Ni BLMs were validated is provided in Table 1.

3.2 APPLICABILITY TO DIFFERENT SPECIES

As mentioned above, BLMs have been developed and validated for only a limited number of species, and they therefore do not cover all freshwater species included in the chronic Ni database. The chronic Ni aquatic toxicity database contains data for 31 different species (see Fact Sheet 1) while fully validated chronic Ni BLMs are available for only 4 species (*i.e.*, *C. dubia*, *D. magna*, *O. mykiss* and *P. subcapitata*). Thus, extrapolation of the BLMs to other species is necessary to develop site-specific PNECs. Much debate has centered on the validity of cross species extrapolation of BLMs. Conceptually, the extrapolation of BLMs developed for one species (*e.g.*, the invertebrate *D. magna*) to other taxonomically similar species (*e.g.*, other invertebrates, including crustaceans, molluscs, insects, etc.) should be justified as long as the mechanisms of toxicity are similar among the broader taxonomic group. With empirical evidence showing that mechanisms of Ni toxicity are similar among and between broader taxonomic groups, the following approach for cross-species extrapolation can be used:

- the *D. magna*/*C. dubia* BLMs can be used to normalize the chronic toxicity to other invertebrates;
- the *P. subcapitata* BLM can be used to normalize the chronic toxicity to other algae; and
- the *O. mykiss* BLM can be used to normalize the chronic toxicity to fish and amphibians.

While empirical evidence is important, legitimate uncertainties on the mechanisms of Ni toxicity exist, and these uncertainties raise questions about the validity of cross-species extrapolation of BLMs. To resolve these uncertainties, an agreement was reached during the EU Risk Assessment process on what information and criteria was needed to support employment of the full cross species BLM extrapolation. Sufficient information was available to convincingly demonstrate similarity in Ni toxicity mechanisms among different fish species and among different algae species, but not among different invertebrate or vascular plant species. Therefore, a "spot-check" study was undertaken to test whether or not the invertebrate and plant BLMs were able to predict chronic Ni toxicity to organisms for which no BLM had been developed.

Physico-Chemical Parameter	*P. subcapitata BLM*	*D. magna BLM*	*C. dubia BLM*	*O. mykiss BLM*
pH	5.7-8.2	5.9-8.2	6.5-8.2	5.4-8.5
Ca^{2+} (mg/L)	1.4-113	0.4-72	1.1-72	1.1-72
Mg^{2+} (mg/L)	2.4-144	1.3-88	1.3-88	3.8-110

Table 1: Physico-chemical ranges for the chronic nickel BLMs

In the spot check study, four non-BLM organisms were tested. Three invertebrates were tested, including the insect *Chironomus tentans*, the rotifer *Brachionus calyciflorus*, and the snail *Lymnaea stagnalis*. One plant species, *Lemna minor*, was also tested. Toxicity tests were performed with each species in five natural waters that represent the range of bioavailability found within typical European freshwater systems. Results showed that the BLMs were able to accurately predict Ni toxicity to the spot-check species (Schlekat *et al.*, 2010), which gave quantitative support to the cross-species extrapolation approach.

Based on the results from the spot-check exercise and other weight-of-evidence arguments (*i.e.*, the ecological relevance of the BLMs, accuracy of the BLMs, and the conservatism of the proposed cross-species approach), the following normalization approach was determined to be appropriate for the normalization of toxicity data:

- for algae, the *P. subcapitata* BLM can be used;
- for higher aquatic plants, the *D. magna* (best fitting BLM) BLMs can be used;
- for cladocerans, insects, and amphipods, the most stringent of the *D. magna* and *C. dubia* BLM can be used;
- for rotifers, the *D. magna* BLM can be used;
- for molluscs and hydra, the *C. dubia* (best fitting BLM) BLMs can be used; and
- for fish and amphibians, the O. mykiss BLM can be used.

4 ACCURACY OF BLMS

Toxicity endpoints that are measured in natural waters with different DOC, pH, and hardness will vary considerably. For example, EC_{10} values for *D. magna* showed an 8-fold difference among different natural waters. For the BLMs to work, they must explain these differences. The chronic Ni BLMs are able to predict toxicity among different waters within a factor less than 2, indicating that the models are well calibrated and are able to accurately predict chronic toxicity in natural freshwaters. Figure 2 shows an overview of the relationship between observed and BLM predicted chronic toxicity values for the *D. magna*, *C. dubia*, *P. subcapitata*, and *O. mykiss* BLMs.

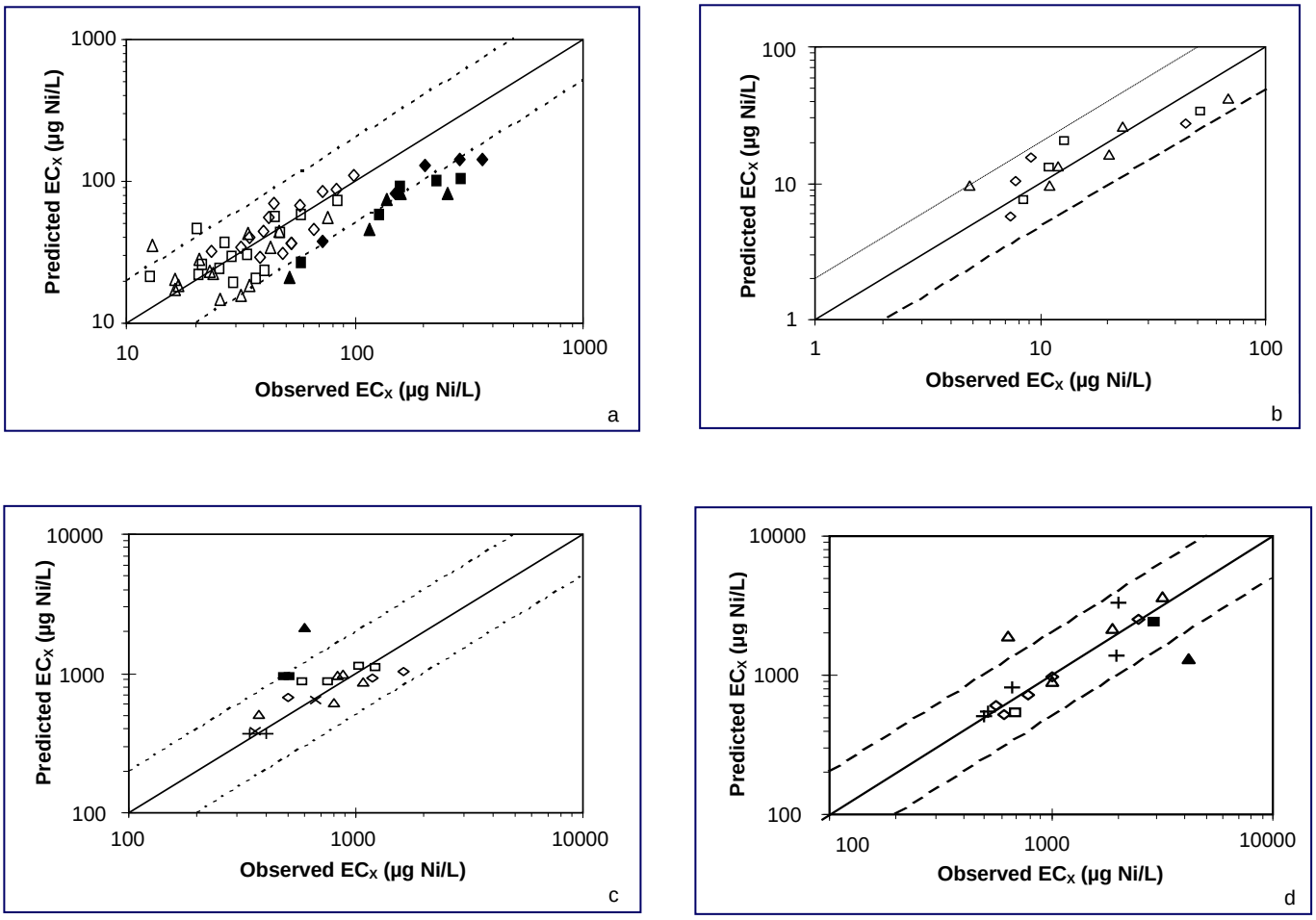


Figure 2: Overview of the relationship between observed and BLM predicted chronic toxicity values for the (a) *D. magna*, (b) *C. dubia*, (c) *P. subcapitata*, and (d) *O. mykiss* BLMs
Note: Logarithmic scales are used for the Y- and X- axis

5 INCORPORATION OF BLMS

The correction for bioavailability is applied to the effect concentrations (NOECs or EC_{10} values) in the nickel aquatic toxicity database (see Fact Sheet 1).

The following steps need to be accomplished in order to incorporate bioavailability for the derivation of 'bioavailability-based' PNECs of Ni for the freshwater compartment (Figure 3).

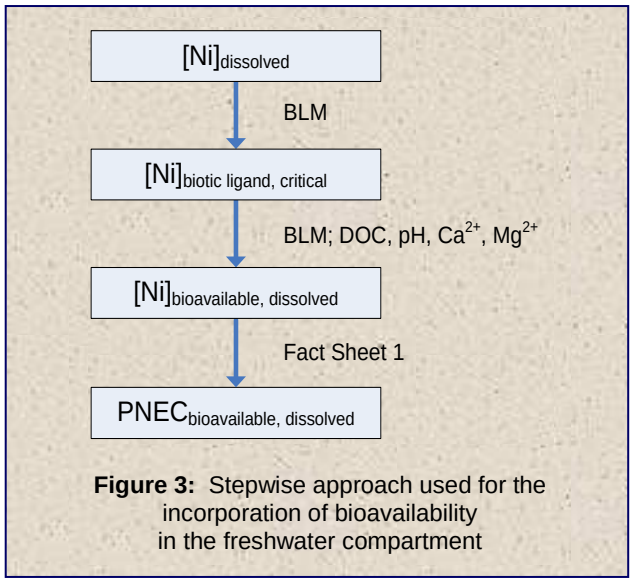


Figure 3: Stepwise approach used for the incorporation of bioavailability in the freshwater compartment

The first step in using a toxicity related bioavailability model (*i.e.*, the BLM) is the determination of a critical biotic ligand accumulation ($[Ni]_{biotic\ ligand\ critical}$[1]) calculated from the experimentally generated organism specific toxicity values ($[Ni]_{dissolved}$[2]) in the ecotoxicity database. Organism-specific bioavailability models should be used as much as possible for that purpose (see Section 3 for further guidance).

In the second step of the approach each organism specific critical biotic ligand accumulation ($[Ni]_{biotic\ ligand\ critical}$[3]) is translated into a critical bioavailable dissolved concentration ($[Ni]_{bioavailable,\ dissolved}$) for a specific area under investigation characterized by a specific set of water-quality conditions (DOC, Ca^{2+}, Mg^{2+}, and H^{+}).

Subsequently, these critical bioavailable dissolved concentrations ($[Ni]_{bioavailable,\ dissolved}$) are further used to calculate the bioavailable $PNEC_{bioavailable\ dissolved}$[4] values according to the approach described in Fact Sheet 1.

Incorporation of the bioavailability concept using the chronic BLMs will result in the derivation of different Species Sensitivity Distributions (SSDs) and PNEC values for the freshwater environments under assessment. An overview of the SSDs derived for the different selected eco-regions in Europe as defined in Fact Sheet 1 is provided in Figure 4.

The water chemistry and median HC_5/PNEC values calculated for the different selected eco-regions in EU surface waters are summarized in Table 2. The PNEC values for Ni in common EU surface waters vary between 3.6 and 21.8 µg/L.

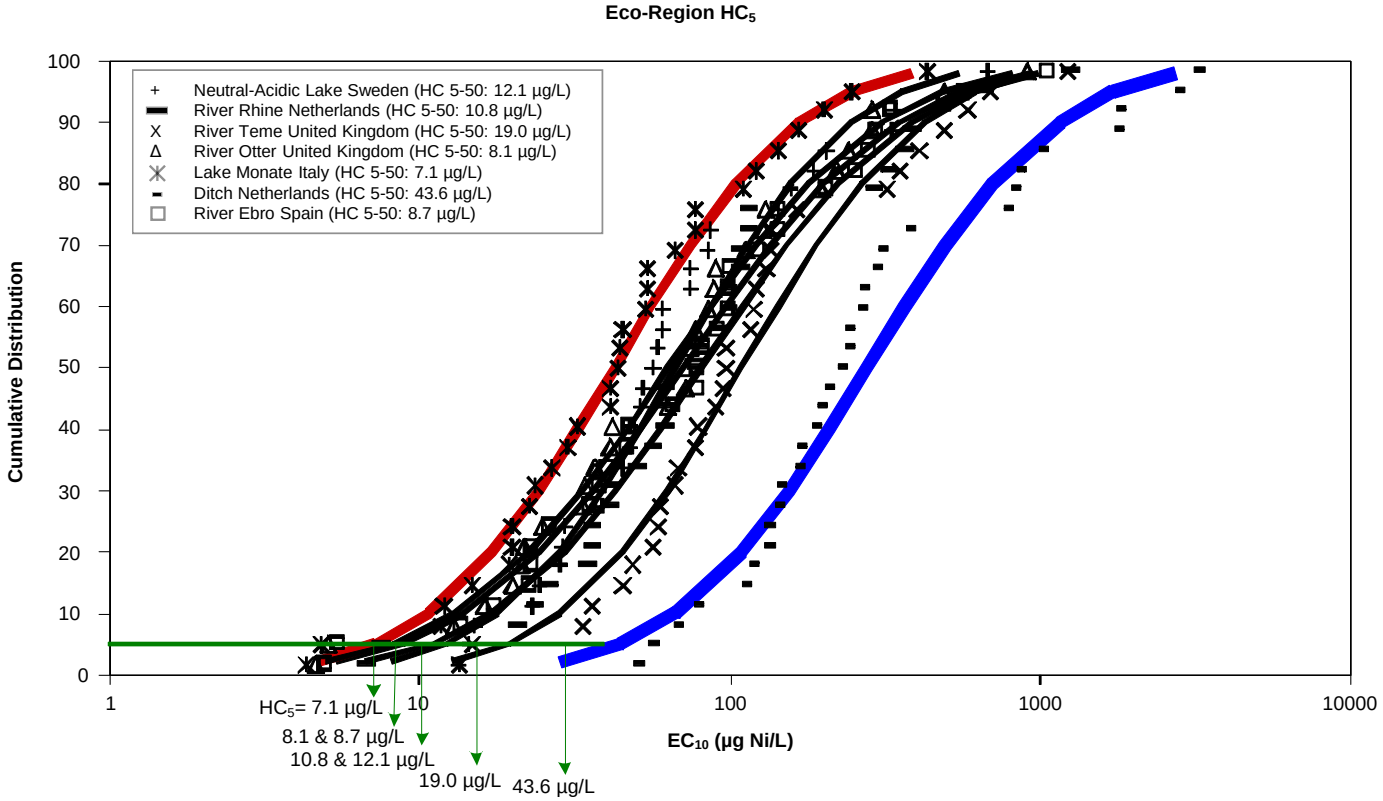


Figure 4: Overview of the SSD and HC_5 for the different selected freshwater eco-regions

Eco-Region	*Water Chemistry*	*Median HC_5 (µg/L)*	*PNEC (µg/L)*[5]
Ditch in The Netherlands	pH 6.9, H 260 mg/L, DOC 12.0 mg/L	43.6	21.8
River Otter in the United Kingdom	pH 8.1, H 165 mg/L, DOC 3.2 mg/L	8.1	4.1
River Teme in the United Kingdom	pH 7.6, H 159 mg/L, DOC 8.0 mg/L	19.0	9.5
River Rhine in The Netherlands	pH 7.8, H 217 mg/L, DOC 2.8 mg/L	10.8	5.4
River Ebro in Spain	pH 8.2, H 273 mg/L, DOC 3.7 mg/L	8.7	4.4
Lake Monate in Italy	pH 7.7, H 48.3 mg/L, DOC 2.5 mg/L	7.1	3.6
Neutral-Acidic Lake in Sweden	pH 6.7, H 27.8 mg/L, DOC 3.8 mg/L	12.1	6.1

Table 2: Overview of the water chemistry and median HC_5/PNEC values for the different selected EU eco-regions

6 BLM SOFTWARE

6.1 INTRODUCTION

In 2010, ARCHE and Watts Crane Associates developed a user-friendly BLM tool to facilitate the practical application of BLM normalization for different regulatory applications, such as the European Water Framework Directive (WFD, 2000/60/EC). The BLM tool is based on the chronic BLMs for Ni and estimates of risks associated with the presence of Ni in a particular freshwater environment, based on a limited set of routinely screened BLM input parameters. Because the full BLM calculations are not rapid, a quick and easy to use Microsoft Excel™-based tool was developed to allow the calculation of many BLM parameters simultaneously.

The BLM tool consists of two main pages, *i.e.*, an introductory page and an input/output (results) page. The introductory page gives the user information on how to use the tool, on which input parameters are needed to run the BLM tool, and on which output (results) are generated by the model. The ambient water quality information required to run the BLM tool is listed below.

- pH
- Dissolved Organic Carbon (DOC) as mg/L
- Ca^{2+} as mg/L or Hardness as (mg/L $CaCO_3$)
- Dissolved Ni concentration as µg/L

It is important to note that the BLM tool provides accurate predictions only within well-defined water chemistry boundaries. Accurate predictions are only estimated for Ca^{2+} concentrations between 3.8 and 88 mg/L, and for pH between 6.5 and 8.2.

6.2 EXAMPLE

An example of the application of the BLM screening model for Ni is presented in Figure 5. In this instance, three examples have been selected (Example 1, 2, and 3). The dissolved Ni concentrations vary between 2 and 10 µg/L, the pH between 6.5 and 8, the Ca^{2+} concentration between 20 and 40 mg/L, and the DOC between 1 and 10 mg/L.

Entering the required input parameters for the BLM screening tool resulted in the calculation of the bioavailable PNEC value (as µg/L), the BioF[6] for Ni, the bioavailable Ni (as µg/L), and the risk characterization ratio (RCR).

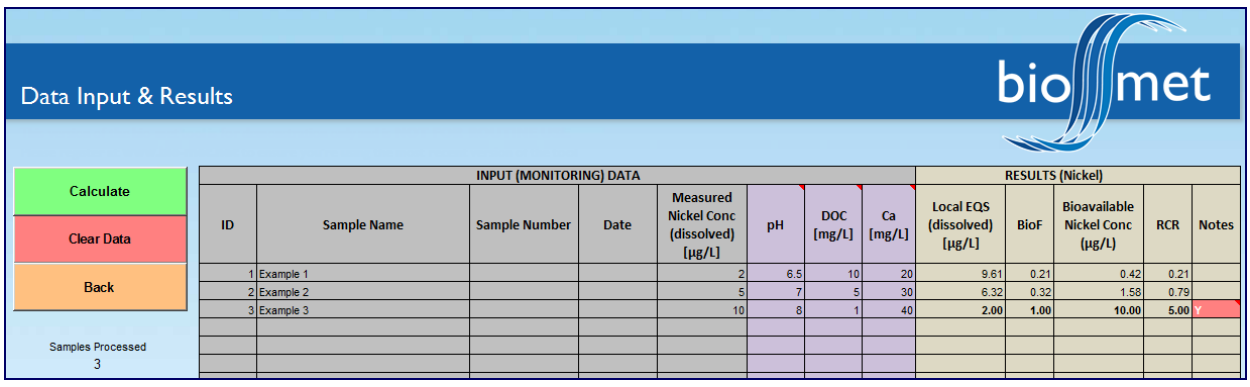
The site-specific PNEC value is estimated using the approach as described in this fact sheet. Through the use of a BioF, differences in bioavailability are accounted for by adjustments to the monitoring data but the environmental quality standard (EQS) remains the same. The BioF is calculated as follows:

BioF = Generic PNEC[7]/site-specific PNEC

The model also provides bioavailable predicted environmental concentrations (PEC) values which are calculated as follows:

bioavailable PEC = Dissolved Ni concentration * BioF

Finally the screening model also provides calculations of the risks associated with the presence of Ni in the freshwater environment. The RCR is calculated as the ratio between the PEC and the PNEC values.



				INPUT (MONITORING) DATA				RESULTS (Nickel)				
ID	Sample Name	Sample Number	Date	Measured Nickel Conc (dissolved) [µg/L]	pH	DOC [mg/L]	Ca [mg/L]	Local EQS (dissolved) [µg/L]	BioF	Bioavailable Nickel Conc (µg/L)	RCR	Notes
1	Example 1			2	6.5	10	20	9.61	0.21	0.42	0.21	
2	Example 2			5	7	5	30	6.32	0.32	1.58	0.79	
3	Example 3			10	8	1	40	2.00	1.00	10.00	5.00	Y

Figure 5: Example of results calculated in the BLM screening tool[8]

7 CONCLUSIONS AND NEXT STEPS IN RISK ASSESSMENT

The ecotoxicity of Ni is largely affected by the physico-chemistry of the freshwater (pH, hardness, DOC); hence, it is highly recommended to normalize the ecotoxicity data for HC_5/PNEC derivation using the available bioavailability models. This fact sheet presents the background information on the developed/validated BLMs and how this information can be used to estimate site-specific bioavailable PNEC values. The collection of water chemistry data such as pH, hardness, and DOC allow the calculation of different bioavailable PNEC values which enables a more accurate, site-specific risk characterization to be conducted.

8 LINK TO 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://echa.europa.eu/documents/10162/cefda8bc-2952-4c11-885f-342aacf769b3
(last accessed July 2015)

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
(last accessed July 2015)

9 REFERENCES

Deleebeeck, N.; K. De Schamphelaere; and C. Janssen. 2007. A bioavailability model predicting the toxicity of nickel to rainbow trout (*Oncorhynchus mykiss*) and fathead minnow (*Pimephales promelas*) in synthetic and natural waters. Ecotox. Environ. Safe. Volume 67, Issue 1, Pages 1-13.

Deleebeeck, N.; K. De Schamphelaere; and C. Janssen. 2008. A novel method for predicting chronic nickel bioavailability and toxicity to *Daphnia magna* in artificial and natural waters Environ. Toxicol. Chem. Volume 27, Issue 10, Pages 2097-2107.

Deleebeeck, N.; K. De Schamphelaere; and C. Janssen, 2009. Effects of Mg^{2+} and H^+ on the toxicity of Ni^{2+} to the unicellular green alga *Pseudokirchneriella subcapitata*: Model development and validation with surface waters. Science of The Total Environment, Volume 407, Issue 6, Pages 1901-1914.

Lock, K.; H. Van Eeckhout; K. De Schamphelaere; P. Criel; and C. Janssen. 2007. Development of a Biotic Ligand Model (BLM) predicting nickel toxicity to barley (*Hordeum vulgare*). Chemosphere Volume 66, Issue 7, Pages 1346-1352.

Schlekat, C.; E. Van Genderen; K. De Schamphelaere; P. Antunes; E. Rogevich; and W. Stubblefield. 2010. Cross-species extrapolation of chronic nickel Biotic Ligand Models. Science of the Total Environment. Volume 408, Pages 6148–6157.

1 Biotic ligand = the toxicologically relevant receptor within the organism. When a critical Ni concentration is achieved at the biotic ligand, its function is disrupted, and toxicity ensues. In freshwater fish, the gill has been determined to be the biotic ligand: when sufficient metal accumulates within gill proteins that are responsible for maintaining physiologically critical levels of essential cations like Ca, Na, and K, systemic toxicity occurs. For other organisms like cladocerans, the biotic ligand is conceptually the same.
$[Ni]_{biotic\ ligand}$ = Ni concentrations accumulated at the biotic ligand. These concentrations can be calculated using the BLM.
$[Ni]_{biotic\ ligand,\ critical}$ = Ni concentrations at the biotic ligand beyond which toxicity occurs.

2 $[Ni]_{dissolved}$ = dissolved Ni concentrations as gathered in the Ni toxicity database beyond which toxicity occurs.

3 $[Ni]_{bioavailable,\ dissolved}$ = dissolved Ni concentrations that are necessary to reach $[Ni]_{biotic\ ligand,\ critical}$ in a specific water. Water quality parameters that affect Ni dissolved concentrations (*e.g.*, DOC) and that compete with Ni for accumulation at the biotic ligand (*e.g.*, Ca^{2+}, Mg^{2+}, and H^+) are used to determine the critical dissolved concentration that corresponds with $[Ni]_{biotic\ ligand,\ critical}$ for a specific water. These concentrations can be calculated using the BLM.

4 $PNEC_{bioavailable,\ dissolved}$ = integration of normalized EC_{10}/NOEC values from the aquatic Ni toxicity database via the SSD (see Fact Sheet 1).

5 PNEC is calculated using an assessment factor of 2.

6 The BioF is the bioavailability factor is based on a comparison between the expected bioavailability at the reference site and that relating to site-specific conditions.

7 Generic PNEC is a PNEC estimated using realistic worst case conditions, representing a PNEC of 'high bioavailability.'

8 The Local EQS (dissolved) (µg/L) is the calculated dissolved concentration of metal that is equivalent the $EQS_{bioavailabe}$ at the local water conditions at the site. This can be considered to be equivalent to a site-specific PNEC for dissolved nickel.

Fact Sheets on the European Union Environmental Risk Assessment of Nickel

This is the fourth 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:

NiPERA, Inc.
2525 Meridian Parkway, Suite 240
Durham, NC 27713, USA
Telephone: 1-919-595-1950

Chris Schlekat, Ph.D., DABT
cschlekat@nipera.org

Emily Garman, Ph.D.
egarman@nipera.org

This fact sheet was prepared by Patrick Van Sprang of ARCHE, Stapelplein 70, b 104, B-9000 Gent, Belgium.
patrick.vansprang@arche-consulting.be