Development of a PNEC sediment of nickel for the freshwater environment

Final report

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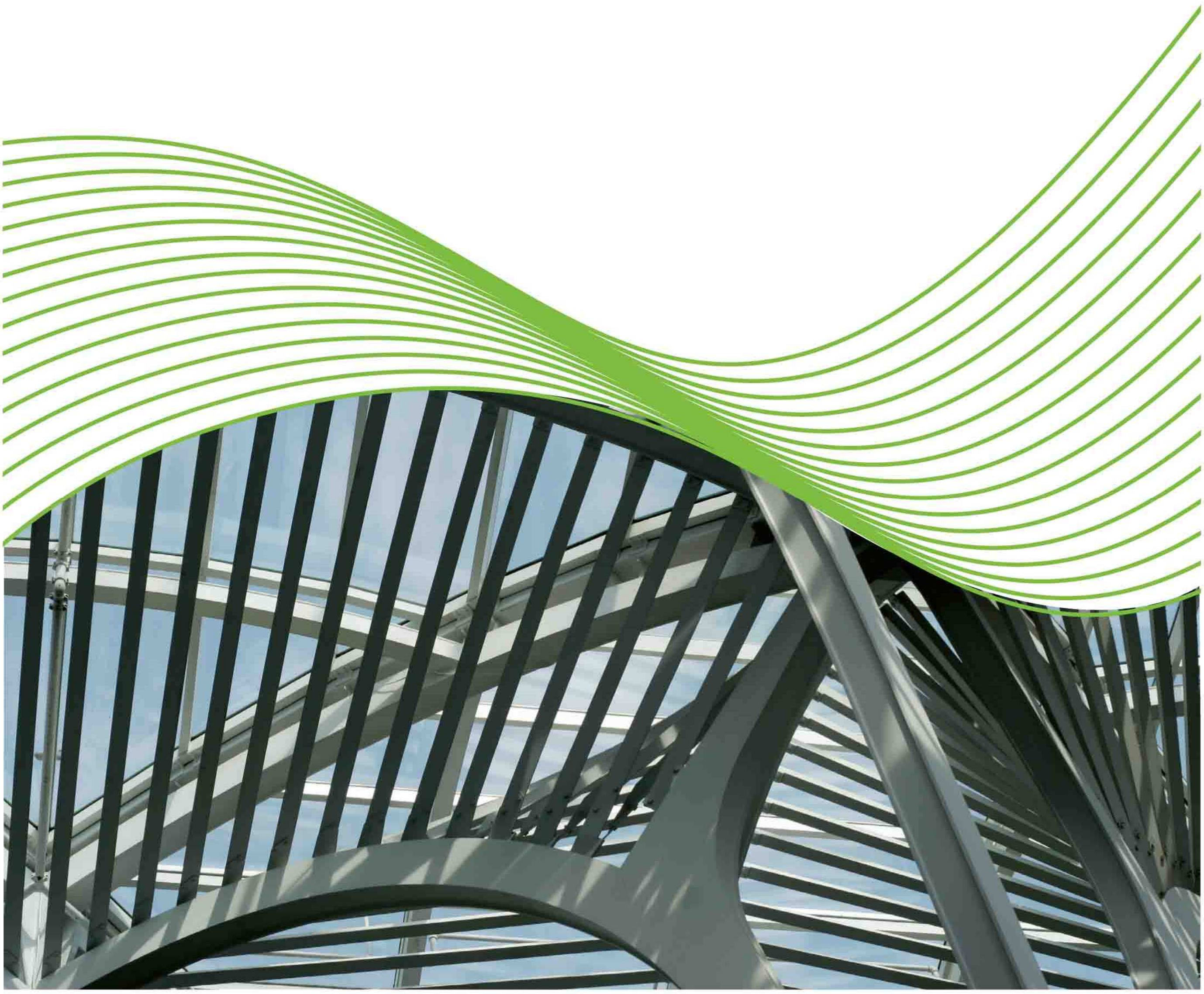
Marnix Vangheluwe & Frederik Verdonck



0. Executive summary

The work presented in this report builds further upon the work already conducted for nickel in the framework of the Existing Substance Regulation EEC 793/93. The data gaps identified in the earlier sediment conclusion i) program and discussed extensively at the technical conclusion workgroup TCNES III'07 were taken as a starting point. In short during the earlier research program the attempt to generate a robust sediment effects database for nickel failed due to the observation that spiking methods were not optimal resulting in an additional exposure to nickel from the overlying water. Although the laboratory test results could not be used for the final PNEC derivation, the laboratory results (Vandegehuchte et al, 2006) and the results of the nickel field recolonization study (Nguyen et al, 2011) did indicate the importance of certain sediment parameters (e.g. Acid Volatile Sulfides, AVS) as possible mitigating factors for nickel toxicity. An extensive program called "The Conclusion i) Sediment Research Program" was subsequently set up by NiPERA to address the remaining issues. This multi-component study had the goals of deriving Predicted No Effects Concentrations for sediment-associated nickel (PNECsed), and for identifying relationships between important sediment parameters and the toxicity of nickel to sediment-dwelling organisms.

Overall the Ni conclusion i) work progressed our general understanding on how to estimate chronic Ni toxicity to sediment organisms substantially and resulted in a more robust sediment toxicity database containing 8 species including amphipods (*Hyalella azteca*, *Gammarus pseudolimnaeus*), mayflies (*Hexagenia sp.*), oligochaetes (*Tubifex tubifex*, *Lumbriculus variegatus*), mussels (*Lampsilis siliquoidea*) and midges (*Chironomus dilutus*, *Chironomus riparius*). However, four insensitive species resulted in censored data (> NOEC or EC_{10} values) and hence the species sensitivity distribution could only be constructed using the other four non-censored data points. This yielded a Reasonable Worst Case (RWC) $HC_{5\text{-}50}$ of 94 mg Ni/ kg dry wt. The benefits of increasing the number of data points using alternative approaches such as the EP method and Kernell/MLE do not seem to outweigh the substantial increase in uncertainty by applying these methods. Hence the preference is be given to the use of the whole sediment toxicity data base even though only 4 bounded data points are available. Part 1 of this report and respective annexes describe the different approaches taken in this context.

Part 2 of the report explores the possibility of developing predictive models for predicting bioavailability and chronic toxicity of nickel in freshwater sediments. Meaningful bioavailability relationships were obtained between three nickel sensitive sediment species and the sediment parameters AVS and Fe. For some species Total Organic Carbon (TOC) and Cation Exchange Capacity (CEC) also were also important parameters. However, due to co-variance none of the considered sediment parameters could be singled out as being the predominant parameter. Normalizations toward the different sediment parameters reduced the inter sediment variability in a significant way (up to 77 % reduction) for the amphipod species. However, the bioavailability relationships were less outspoken for the mayfly *Hexagenia*. It is not clear what contributed to this observation. One hypothesis could be the specific life stage of the mayflies forming burrows that they actively ventilate with overlying water creating a micro-habitat which has less resemblance with the overall sediment environment that other species see. Another possible explanation is dietary exposure. Since *Hexageni*a is one of the more sensitive species of the distribution the final effect on the HC_{5-50} of normalising the SSD towards the conditions prevailing in the different sediments (representing the 10-90th percentile of conditions encountered in the EU) is rather limited (factor 1.6-2.2). The HC_{5-50} values obtained for the different bioavailability scenarios range with the AVS model from 126-281 mg/kg dry wt. A similar range is observed when using Fe based models with ranges of 143-265 mg/kg dry wt

Finally, the Robustness of the HC_{5-50} estimate concerning the SSD for chronic toxicity of nickel to sediment organisms, the uncertainty analysis taking also into account the results of the performed mesocosm studies and an assessment factor analysis are discussed in the last part of the report. The final decision on an assessment factor for Ni has not been made, but a discussion of the arguments for considering AFs of 3, 2, 1.5 and 1 is being presented.

1. General introduction

An extensive program called "The Conclusion i) Sediment Research Program" has been conducted by NiPERA to address the data gaps created when a "conclusion i)" was determined for the sediment compartment under the Existing Substances Risk Assessment of Nickel. This multi-component study had the goals of deriving Predicted No Effects Concentrations for sediment-associated nickel (PNECsed), and for identifying relationships between important sediment parameters and the toxicity of nickel to sediment-dwelling organisms.

Task 1 of the program focused on developing spiking procedures that would create a more realistic exposure and reduce the diffusional loss of soluble nickel from the sediment phase into the overlying water in laboratory sediment toxicity tests. The purpose of Task 2 was to provide the ecotoxicity data set necessary to populate a species sensitivity distribution (SSD) and to derive a PNEC sediment for the freshwater compartment. Sediment toxicity tests were conducted with 9 species, including amphipods (*Hyalella azteca*, *Gammarus pseudolimnaeus*), mayflies (*Hexagenia sp*.), oligochaetes (*Tubifex tubifex*, *Lumbriculus variegatus*), mussels (*Lampsilis siliquoidea*,) midge (*Chironumus dilutus*, *Chironomus riparius*), and nematodes (*Caenorhabditis elegans*) on two sediment types (Spring River (low AVS/TOC) and West Bearskin Lake (high AVS/TOC) representing sediments with low and high nickel binding capacity, respectively.

The development of a bioavailability model to normalize total nickel sediment concentrations was the main objective of Task 3 where six additional sediments spanning the 10^{th} to 90^{th} percentiles for AVS and TOC in EU sediments were tested with four invertebrate taxa (*H. azteca*, *Hexagenia sp*., *T. tubifex* and *G. pseudolimnaeus*). These additional data have been combined with results of Task-2 tests to develop a bioavailability model for nickel in sediment. These models will have the benefit of normalizing a RWC-PNEC towards prevailing local, site-specific conditions.

Finally field studies have been conducted where the short-term toxicity and recolonization of sediments, with varying nickel contamination were followed over a timeframe up to 56 days for five different sediment types.

For detailed descriptions of the procedures followed and the results obtained the reader is referred to the individual research reports of the institutes/universities involved in the Conclusion i) Sediment Program.

The current report used the available information to propose PNEC values that can be used for regulatory purposes (e.g., REACH, Water Framework Directive) and explores the possibility of developing bioavailability models in order to incorporate the bioavailability concept within the EU risk assessment framework. This work as well as all research results were presented and discussed in depth at the review panel meetings organized with the Technical Conclusion i) group on the PNECsediment derivation for Nickel, consisting of representatives of Academia, Member States and industry (NIPERA). Denmark, The Netherlands and Germany attended all or most working group meetings/ telephone conferences. Spain, UK and France participated in some telephone conferences/ working group meetings.

The current report reflects as much as possible the general consensus reached at these discussions but should still be looked at as an independent report expressing the view of the authors.

The information on PNEC setting provided here consists of four parts:

- Derivation of a Reasonable Worst Case (RWC) $HC_{5\text{-}50}$ sediment value of nickel for the freshwater environment.
- Development of predictive models of bioavailability and toxicity of nickel in freshwater sediments
- Derivation of $HC_{5\text{-}50}$ sediment values of nickel for the freshwater environment for different bioavailability scenarios.
- Robustness of the $HC_{5\text{-}50}$ estimate: uncertainty analysis and AF derivation

In annexes more information is given on other approaches that were evaluated but were finally not retained as basis for the final PNEC derivation.

2. Derivation of a Reasonable Worst Case (RWC) HC_5 sediment of nickel for the freshwater environment

2.1 Approach and selection of toxicity values for RWC-HC5 derivation

The REACH process requires the generation of generic exposure scenarios (GES) which identify generic operating conditions that result in safe use. To ensure that the GES are appropriately conservative to cover a wide range of conditions, a Reasonable Worst Case (RWC) PNEC (expressed as total recoverable nickel (TR Ni)) is used in risk calculations to assess the potential environmental risks of nickel to benthic species within this framework. Furthermore, a RWC-PNEC can be applied when the data necessary for performing bioavailability correction (e.g., acid volatile sulfide (AVS) are not available.

The ecotoxicological data used were derived from the final reports on the Conclusion i) Sediment Research Program (performed by USGS) investigating the bioavailability and ecotoxicity of nickel spiked into in natural sediments (Besser et al, 2011).

According to REACH guidance, a RWC-PNEC should reflect conditions that represent the 10th percentile of parameters controlling nickel bioavailability. Therefore, sediment toxicity data from Task 2 and Task 3 sediments in which the presence of AVS or other mitigating factors (organic carbon (OC), and iron (Fe)) did not represent a RWC for bioavailable nickel were excluded from the RWC PNEC calculation. For example the sediment of West Bearskin was not used since it contained 36 µmol AVS/g dry weight, which represents the 90th percentile of the AVS distribution in EU sediments.

From the Task 2 sediments Spring River (SR) sediment was selected as the sediment with the highest bioavailability (AVS < 1 µmol/g dry weight, organic carbon 0.42%, Fe 7,753 mg/kg dry wt.)) and hence the best candidate to derive a realistic worst-case (RWC) PNEC for the freshwater sediment compartment. A Task-3 sediment with similar characteristics was Dow Creek sediment. In Table 1 a comparison is made between the AVS-TOC concentrations measured in these sediments and the 10 % values found in the EU for these parameters.

Table 1: Comparison AVS and TOC concentrations of Spring River and Dow Creek sediment with the RWC conditions in the EU (10th percentiles)

Sediment	AVS (µmol/g dry wt.)	TOC (%)
Test sediments		
Spring River	1.1	0.4
Dow Creek	1.0	1.2
10th percentiles		
Belgium (Flemish region) (n=200)	0.8	0.3
Netherlands (n= 28)	1.3	1.5
Finland (n= 25)	1.0	2.3
United Kingdom (n= 16)	0.3	2.0
EU database (n= 335)	0.5	NA

NA: not available

Concentrations of AVS for both Spring River and Dow Creek are higher than the 10th percentile of all EU sediments (0.5 µmol/g), whereas AVS from these systems lie between the highest (1.3 µmol/g) and lowest (0.3 µmol/g) observed for specific EU Member States. Concentrations of TOC in both Spring River and Dow Creek are below the 10th percentile for three out of four Member States. AVS concentrations are near the 10th percentile of most Member States and TOC concentrations are below the 10th percentile for most Member States. It can be concluded that the combinations of low AVS and low TOC represent RWC conditions for the EU.

Table 2 summarizes the results of the chronic toxicity tests for several freshwater species from Task 2 and Task 3 conducted with Spring River or Dow Creek Sediments. The endpoints presented represent the most sensitive endpoint for the given species.

Table 2: Species EC_{10}-NOEC values (total recoverable Ni, mg Ni/kg dry wt.) for the most sensitive endpoint for all sediment dwelling organisms for the Spring River and Dow Creek sediments.

		SR sediment	**DOW sediment**	
Organism	**Most sensitive endpoint**	**Species EC_{10}-NOEC (mg total Ni/kg dry wt)**	**Species EC_{10}-NOEC (mg total Ni/kg dry wt)**	**Geometric mean (mg total Ni/kg dry wt)**
Hyalella azteca	Biomass	160[a] (49-609)	139 (76-252)	**149.1**
Gammarus pseudolimnaeus	Biomass	Test failed[b]	228 (107-486)	**228**
Hexagenia species	Biomass	371 (94-1,463)	151 (32-710)	**236.7**
Lumbriculus variegatus	Abundance	554 (169-1,816)	/	**554**
Chironomus dilutus		> 762[c]	/	> 762[c]
Chironomus riparius		> 762[c]	/	> 762[c]
Lampsilis siliquoidea		> 762[c]	/	> 762[c]
Tubifex tubifex		> 762[c]	>1,372[c]	> 762[c]
Caenorhabditis elegans		Test failed	/	/

[a] *mean of two tests: EC_{10} values and CL = 82 (95 % CL: 45-149) and 337 (95 % CL: 53-1,069) mg total Ni/kg dry wt.*
[b] *unacceptable control mortality*
[c] *unbounded NOEC*
/ test not conducted
bold data: used for the HC_{5-50} calculation

Although the use of statistical extrapolation methods for calculation of a PNEC for sediment organisms is embedded in the ECHA guidance when sufficient data are available, clear guidance on the minimum sample size for this compartment is lacking. For the aquatic compartment confidence can be associated with a PNEC derived by statistical extrapolation if the database contains at least eight taxonomic groups. For the sediment compartment no specific requirements have yet been defined.

The sediment effects data set (eight species) for nickel (Table 2) is representative of different sediment exposure pathways, as well as a variety of feeding strategies and taxonomic groups. In short, the nickel sediment toxicity database is representative of benthic ecosystems, thus fulfilling one of the characteristics that should be considered when evaluating whether or not the use of the SSD approach is appropriate.

Four of the eight species yielded reliable EC_{10} values (*H. azteca*, *G. pseudolimnaeus*, *Hexagenia* and *L. variegatus*). Unfortunately, four species tested resulted in unbounded (censored) data (i.e. no effects were observed at the highest test concentration), which indicate the insensitivity of these important sediment dwelling species towards nickel. For three species of these four species no effect at all was observed at the highest tested concentration. Two tests (*G. pseudolimnaeus* and *Caenorhabditis elegans*) conducted with Spring River sediment did not pass test acceptability criteria. The *C.* exposure to SR failed due to poor performance in the controls and treatments. Most probably the physico-chemical characteristics (i.e. grain size) of the test sediment exceeded the tolerance range of the test organism. The SR sediment contains a lot of inorganic matter (largely sand) while the test organisms were isolated from compost and high organic compost soils. Similar variability results in survival were observed for the other tested sediments in task 2 and task 3. The amphipod *G. pseudolimnaeus* test with SR (yielding an EC_{10} of 138 mg Ni/kg dry wt.) failed due to low control survival, which was attributed to a mechanical problem with the system controlling the pH. However, the test with *G. pseudolimnaeus* in Task 3 with the Dow Creek sediment (similar in characteristics as the Spring River sediment) yielded a valid test (EC_{10} of 228 mg/kg dry wt.) showing that this species is quite sensitive to nickel at similar concentrations as the amphipod *H. azteca*.

2.2 Derivation of HC_{5-50} sediment using the statistical extrapolation method: whole sediment toxicity data (excluding unbounded values)

The statistical extrapolation method for the whole sediment toxicity test results from Spring River and Dow Creek was performed using the ETX program. The ETX program developed by the RIVM calculates the HC_{5-50} using the conventionally used log-normal distribution and is a conservative way to calculate the HC_{5-50}. Since the HC_{5-50} is the most important parameter the Anderson-Darling test (which puts more emphasis on the fit of the tail of the distribution) was used to evaluate the fit of the model. However, although the lower part of the curve is the one we are concerned with, the upper part of the curve also influences the HC_{5-50} values especially in balanced models such as used in the ETX program.

Figure 1 presents the lognormal function that was fitted through the four bounded data points and which was accepted at $P < 0.05$.

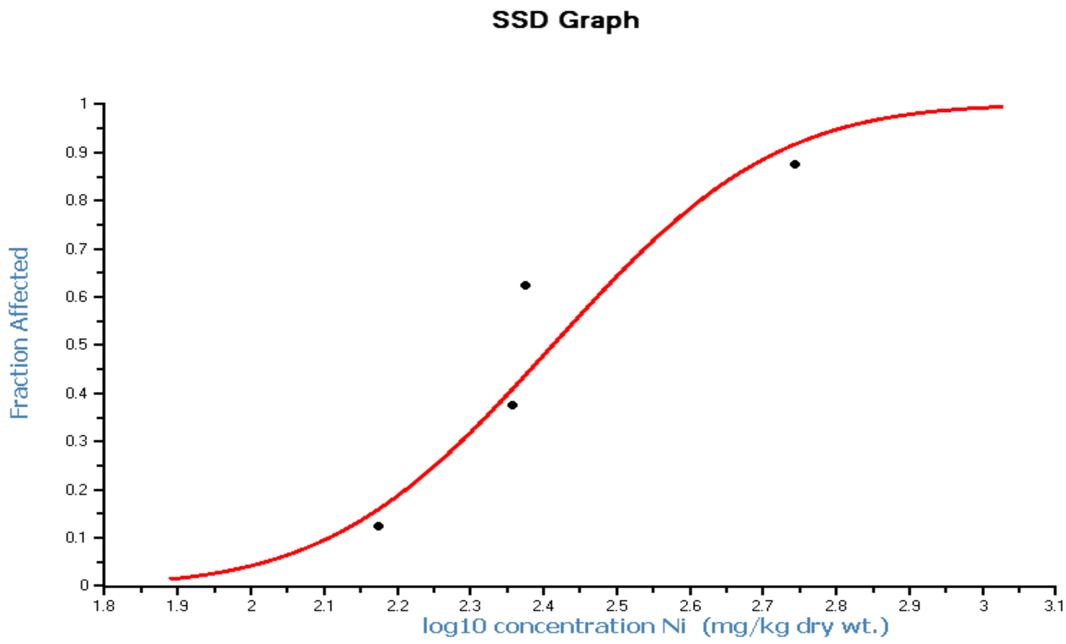


Figure 1: The cumulative frequency distributions of the NOEC/EC_{10} values (n= 4) (expressed as mg Ni/kg dry wt.) from the Ni chronic toxicity tests towards sediment-dwelling organisms – observed data and log-normal curve for the dataset fitted on the data. Unbounded NOEC values were excluded.

A summary of the estimated $HC_{5\text{-}50}$ value (with the 90% confidence bounds) for the log-normal function (calculated with ETX) is provided in Table 3.

Table 3: Calculated $HC_{5\text{-}50}$ value (mg Ni/kg dry wt.) (with the 5-95% confidence limits) Unbounded NOEC values excluded.

$HC_{5\text{-}50}$ at 50% (& 5-95% confidence limits) expressed as mg Ni/kg dry wt.	Type of best fitting model	Parameters
94 (15-172) (n = 4)	Log-normal model (ETX)	(2.41;0.239)

Since the current SSD only contains four data points other approaches were explored to increase the number of data points in the SSD or to bring additional information into the final decision making process. These approaches and the outcome of the analysis are discussed in the respective annexes attached to this report. In short the following approaches were investigated:

1) Including the fatmucket clam *L . siliquoidea* data point as a valid entry (Annex A)
2) Substituting unbounded values by the use of water only data for the insensitive species using the equilibrium partitioning (EP) method (Annex B)
3) Use of other statistical techniques in order to take the censored data into account (Annex C)

Omitting or including the *L. siliquoidea* data point in the SSD analysis has no real influence on the obtained HC_5 (Annex A) If the data point is included a HC_{5-50} of 95.8 mg Ni/kg dry wt (n = 5) is obtained compared to 94 mg Ni/kg dry wt for the SSD constructed on four data points. Please note that in any case this endpoint was not statistically significant and was determined to be invalid to include it in the SSD.

The EP method was used to increase the number of species by translating water only data for the insensitive species to whole sediment nickel toxicity thresholds (see annex B for details). The EP-method resulted in slightly lower HC_{5-50} values (79-81 mg Ni/kg dry wt) but these values are overall supportive of the HC_5 value derived based solely on whole sediment contact data. There was a general agreement in the technical conclusion i) review group that the results of this exercise was useful in terms of a weight of evidence approach but should finally not be used in the SSD given the high uncertainty surrounding some EP calculated values. Some implausible differences of intrinsic species sensitivity between the water only data and the whole sediment data were observed that generally could be explained by differences in water hardness and DOC levels between the two test set ups. The large difference i.e. the high sensitivity of *L. siliquoidea* in the water only data set and the insensitive response of the same species in the whole sediment toxicity test could, however, not be explained. Applying the EP method to metals, however, is not deemed the most scientific way forward for and introduces considerable uncertainty.

Finally, several statistical methods were explored in order to fit censored data (Annex C). The kernel distribution gives a higher HC_{5-50} (120 mg Ni/kg dry wt.). Using the maximum likelihood method results in a lower HC_{5-50} (71.6 mg Ni/kg dry wt.). Typically, no single method is unequivocally superior across all scenarios, although all of the methods may excel in one or more scenarios. Overall, a selection of a method for SSD fitting with censored data purposes would require a thorough review and comparison of the existing methods. This may feed further discussions between experts and non-experts on the best approach.

Overall preference should be given to the derivation of the HC_5 based on whole sediment toxicity data, excluding censored data, even if only 4 data points are available. This is justified because:

- Although only four data points are included in the final SSD these data points have been extracted from an extensive sediments effect database covering eight species representative of different sediment exposure pathways, as well as a variety of feeding strategies and taxonomic groups,
- the four species that yielded valid test results populate the lower part of the SSD and hence forms the basis for the SSD curve fitted to these data points,
- including the EP generated SSD data would introduce considerable uncertainty to the SSD.

3. Development of predictive models of bioavailability and toxicity of nickel in freshwater sediments

3.1 Introduction

Task 3 of the sediment conclusion i) program was aimed at the identification of key sediment parameters driving nickel toxicity in freshwater sediments. Four species (*H. azteca*, *G. pseudolimnaeus*, *Hexagenia sp* and *T. tubifex* were selected as model organisms to conduct a set of ecotoxicity tests in sediments representing a range of sediment parameters. An overview of the physico-chemical characteristics of these sediments and the reference conditions chosen for the RWC are given in Table 4.

Table 4: Overview Physico-chemical characteristics sediments task 2 and task 3 sediments.

Sediment	AVS (µmol/g dry wt.)	TOC (%)	Fe (mg/kg dry wt.)	CEC (meq/100g)
	Task 2 sediments			
Spring River	1.1	0.4	7,753	5.6
West Bearskin Lake	36	10.5	51,317	41
	Task 3 sediments			
Dow Creek	1.0	1.2	6,400	6.1
P30	12.4	1.8	15,800	18.7
RR2	6.1	4.1	10,500	14.3
RR3	8.0	8.1	14,900	27.6
STJ	3.8	1.9	22,900	10.3
STM	24.7	8.1	26,400	29.2
	RWC sediment			
RWC sediment	0.77[a]	0.5[b]	12,920[c]	8.6[d]

[a] *10th percentile AVS Flanders database (Vangheluwe et al, 2003)*
[b] *Expert judgment*
[c] *10th percentile TOC database (Vangheluwe et al, 2008)*
[d] *10th percentile CEC GEMAS soil dataset (EU 27 + Norway)*

T. tubifex was initially chosen for this task to increase taxonomic diversity; however, the data could not be used for developing relationships due to a low toxicity response. Ultimately, bioavailability models were developed for the following three sediments species:

- *Hyalella azteca*
- *Gammarus pseudolimneaus*

- *Hexagenia* species

Unfortunately, the development of a bioavailability model for the oligochaete *L. variegatus* was not within the scope of task 3. In order to normalize this species in the SSD the possibility of read-across from a bioavailability model developed with another species needs to be explored.

3.2 Endpoint selection and data analysis

Toxicity endpoints were evaluated for use in bioavailability models based on their sensitivity and variability, which is important from a statistical point of view. Endpoints with high sensitivity tended to have a wide range of responses to spiked sediments, which facilitates development of concentration-response models for many or all of the sediments tested. However, it is also important that effects concentrations have low variability (narrow confidence intervals) because it increases the confidence in differences in effects concentrations among sediments; this inter-sediment variability will be the basis for the bioavailability models. For the three sensitive species, several endpoints were less suitable for bioavailability models because they were relativity insensitive (e.g., survival of *Hexagenia*, growth of *Hyalella* and *Gammarus*) and/or sensitive, but highly variable (*Hyalella* reproduction). No endpoints from the *Tubifex* tests were sufficiently sensitive or reliable for the derivation of a bioavailability model. The survival endpoint was selected as the most robust toxicity data for bioavailability models for both *Gammarus* and *Hyalella*. For *Hexagenia* growth was selected.

Correlations and simple linear regressions between toxicity thresholds (EC_{20} values) and the sediment properties measured were calculated using the STATISTICA software package in order to identify the sediment properties that explain the greatest proportion of variation in the toxicity thresholds. In all cases, the EC_{20} value was used instead of the EC_{10} for the calculation of these relationships because the EC_{20} values were less variable than the EC_{10} values.

Multiple regressions are also calculated by a stepwise procedure in STATISTICA. This procedure identifies the parameters that explain most of the variation in the dependent variable. The threshold significance level for entry or removal of parameters in the model was set at 1.0. The sediment parameters taken into account were: Acid Volatile Sulphides (AVS, µmol/kg dry wt), Total Organic Carbon (TOC, %), pH pore water, iron, and manganese (Fe_{tot}, Mn_{tot}; mg/kg dry wt; Fe_{SEM}, Mn_{SEM}; mg/kg dry wt), CEC (Meq/100g), sand (%), silt (%), clay (%). All data, except pH, were log-transformed.

3.3 Results

3.3.1 General analysis

Single linear regressions between sediment toxicity thresholds and various sediment properties were analyzed in order to explain the inter-sediment variation in EC_{20} values. Results were similar for toxicity thresholds based on Total Ni dose (TR Ni) as for toxicity thresholds based on SEM Ni. Results for the single linear regressions for the three species (*H. azteca and G. pseudolimnaeus*, and the mayfly *Hexagenia* sp) are listed in Tables 5-7. Nickel toxicity thresholds were significantly correlated with AVS, Fe, TOC and CEC content of the sediment for all amphipod assays. For *Hexagenia* Fe and/or AVS were correlated with the toxicity values. None of the toxicity thresholds based on pore water were significantly correlated. The absence of any significant correlations of PW-EC_{20}s with sediment characteristics for any of the species tested is consistent with the equilibrium partitioning theory: sediment constituents control bioavailability by modifying Ni partitioning. In a similar way no significant correlations were identified between overlying Ni water concentrations and the observed toxicity as was seen in previous studies where overlying water confounded the test results.

Table 5: Single linear regression of *Hyalella* Ni toxicity threshold values (n = 8) and sediment parameters. Only significant variables ($p< 0.05$ two-tailed test) are shown.

Dependent variable	**Independent variable**	**R^2**	**Effect**	**P< 0.05**
Log EC_{20} total Ni (mg/kg dry wt.)	Log AVS (µmol/g dry wt.)	0.74	+	0.005
	Log Fe total (mg/kg dry wt.)	0.61	+	0.02
	Log TOC (%)	0.59	+	0.02
	Log CEC (meq/100g)	0.59	+	0.02
Log EC_{20} SEM Ni (mg/kg dry wt.)	Log AVS (µmol/g dry wt.)	0.80	+	0.0025
	Log CEC (meq/100g)	0.67	+	0.013
	Log Fe total (mg/kg dry wt.)	0.63	+	0.018
	Log TOC (%)	0.60	+	0.023
Log EC_{20} SEM Ni-AVS (µmol/g dry wt.)	Log Mn total (mg/kg dry wt.)	0.68	+	0.012
	Log Fe total (mg/kg dry wt.)	0.65	+	0.016
	Log TOC (%)	0.64	+	0.017
	Log Silt (%)	0.63	+	0.018
	Log AVS (µmol/g dry wt.)	0.62	+	0.02
	Log CEC (meq/100g)	0.57	+	0.03

Table 6: Single linear regression of *Gammarus* Ni toxicity threshold values (n = 7) and sediment parameters. Only significant variables ($p< 0.05$ two-tailed test) are shown.

Dependent variable	**Independent variable**	**R^2**	**Effect**	**P< 0.05**
Log EC_{20} total Ni (mg/kg dry wt.)	Log Mn total (mg/kg dry wt.)	0.80	+	0.006

	Log TOC (%)	0.78	+	0.0079
	Log Mn SEM (mg/kg dry wt.)	0.72	+	0.016
	Log Fe total (mg/kg dry wt.)	0.68	+	0.02
	Log CEC (meq/100g)	0.68	+	0.02
	Log Silt (%)	0.65	+	0.028
	Log AVS (µmol/g dry wt.)	0.62	+	0.03
Log EC_{20} SEM Ni (mg/kg dry wt.)	Log Mn total (mg/kg dry wt.)	0.85	+	0.003
	Log TOC (%)	0.77	+	0.01
	Log Mn SEM (mg/kg dry wt.)	0.77	+	0.01
	Log CEC (meq/100g)	0.76	+	0.01
	Log Fe total (mg/kg dry wt.)	0.71	+	0.018
	Log AVS (µmol/g dry wt.)	0.64	+	0.029
	Log Silt (%)	0.58	+	0.04
Log EC_{20} SEM Ni-AVS (µmol/g dry wt.)	Log Mn total (mg/kg dry wt.)	0.65	+	0.028

Table 7: Single linear regression of *Hexagenia* Ni toxicity threshold values (n = 6) and sediment parameters. Only significant variables (p< 0.05 two-tailed test) are shown.

Dependent variable	**Independent variable**	**R^2**	**Effect**	**P< 0.05**
Log EC_{20} total Ni (mg/kg dry wt.)	Log Fe total (mg/kg dry wt.)	0.79	+	0.018
Log EC_{20} SEM Ni (mg/kg dry wt.)	Log AVS (µmol/g dry wt.)	0.86	+	0.007
	Log Fe total (mg/kg dry wt.)	0.69	+	0.04

Trends between the toxicity thresholds and the identified sediment parameters were similar across species but some differences were observed in the relative importance of the different parameters. Most of the toxicity thresholds for the amphipod *H. azteca* showed a clear relationship with AVS, with EC_{20} values increasing with AVS. Next to AVS, organic carbon, iron content and CEC were the main mitigating factors for *H. azteca*. Also for *G. pseudolimnaeus* multiple sediment characteristics were positively related with the observed toxicity. However, although AVS is positively correlated, AVS explained less variability than it did for *H.azteca*. Relationships between EC_{20}s and total manganese, organic carbon and iron were stronger. Note that for *G. pseudolimnaeus* no Spring River results were present (SR was a low AVS data point), which could explain this observation. AVS seemed to play a less important role in governing the nickel toxicity for *Hexagenia*. A possible explanation for this observation is the specific lifestyle of these burrowing mayflies. *Hexagenia* nymphs burrow within the top few centimeters of sediment where they create microhabitats by ventilating their burrows with overlying water. This may lead to an exposure (dissolved Ni concentrations and abiotic parameters like DOC) that is different than where measurements were made, e.g., undisturbed porewater. More accurate measurements of the nickel exposure to *Hexagenia* sp. including how the micro-habitat of this species impact bioavailability factors may help to increase the understanding of the robustness of these relationships.

Combining the Task 2 and Task 3 *Hexagenia* toxicity data for the derivation of a bioavailability model was not justified because there are clear discrepancies between the high EC_{20}s for *Hexagenia* determined for the two Task-2 sediments (SR and WB) and the lower EC_{20}s determined for the six Task-3 sediments. These differences are most probably related to the differences between the test systems used for Task 2 (1000 mL beakers with 200 mL sediment and 10 mayflies) and Task 3 (300 ml beakers with 100 mL sediment and 5 mayflies). Briefly, sediment depth was less and surface area was greater in Task 2 compared with Task 3, resulting in different conditions for establishing burrows between the two tests. Due to these differences it was decided not to pool the data for the purposes of bioavailability modeling since pooling the data obscure the identification of the underlying controls on nickel bioavailability.

Once the Task-2 and Task 3 results were separated significant relationships were derived between Fe and total Ni and AVS/Fe and SEM Ni. The slope of the relationship between toxicity (expressed as SEM Ni, mg Ni/kg) and AVS (umol/g) for Task 3 is similar to the slope observed for Task 2 (although it is noted that this was based on only 2 data points). (Figure 2). Note that these slopes (0.26-0.34) are based on the relationship between toxicity expressed as SEM Ni and AVS. These slopes are higher than the slope (0.175) based on the relationship between toxicity expressed as total Ni and AVS. The total Ni vs. AVS relationship was used in the *Hexagenia* bioavailability model (Table 7) because it is consistent with relationships used for other species, and because it provides an added layer of precaution in the HC_{5-50} determination: the lower slope yields lower normalized toxicity values for this species.

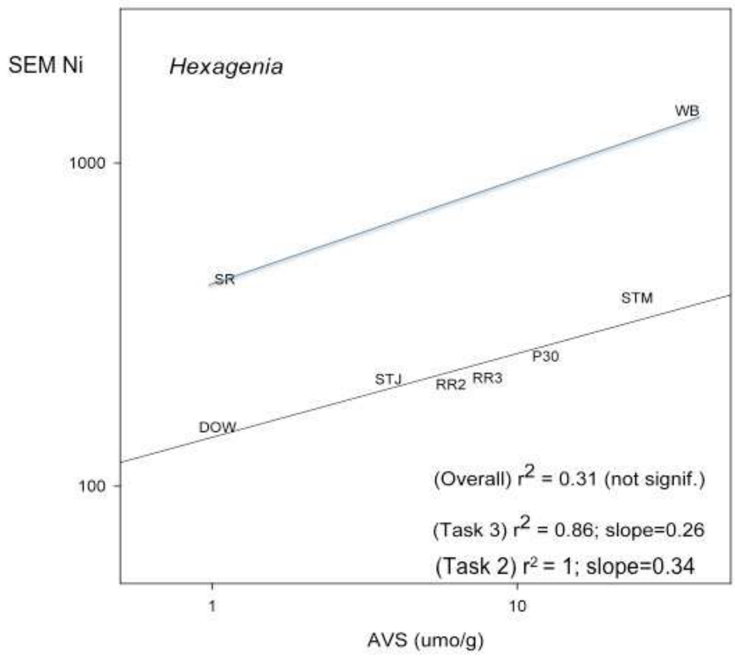


Figure 2: Linear relationship between SEM Ni (mg/kg dry wt.) and AVS (µmol/g dry

wt.) for Task 2 (SR and WB) sediments and Task 3 (DOW, STJ, RR2, RR3, P30 and STM) sediments for *Hexagenia* species

3.3.2 Overview bioavailability models

In the correlation analysis between the different sediment parameters it was observed that multiple sediment characteristics are positively related with each other (e.g. Fe co-varied with AVS). Due to the co-variation between sediment parameters, only one parameter was significant when performing a multiple regression analysis and hence no multiple regression outcomes containing more than one variable could be established. Therefore the bioavailability models have been developed for each of these sediment characteristics using single linear regression methods.

In Section 3.3.1, significant relationships between Ni toxicity and several sediment phases were identified, including AVS, Fe, OC and CEC. The relevance of these sediment phases is discussed below.

Acid Volatile Sulfides (AVS)

The basic concept behind the AVS approach is that most metals have higher solubility products than most iron and manganese mono sulfides (except for pyrite) and hence can displace iron from its sulfide complex on a mole-to-mole basis, forming insoluble sulfide complexes with minimal biological availability. The SEM-AVS model predicts that when the measured AVS concentrations exceeds the concentration of SEM (SEM -AVS difference smaller than 0) the pore water levels of dissolved metal concentrations should be very low resulting in the prediction of no toxicity. For nickel, the mitigating effect of AVS is apparent from the data but it should be acknowledged that the affinity of Ni for AVS is weaker than for other metals and that other sediment phases (organic carbon, Fe/Mn oxy hydroxides) may be equally important in controlling pore water concentrations.

Organic Carbon (OC)

The observation that metals may bind strongly to organic carbon suggests that organic carbon normalization might also reduce the variability observed in nickel toxicity. Similar to the chemical reactions that occur between dissolved metal and dissolved organic carbon in the aquatic environment, the free nickel ion can form complexes with the carboxylic, phenolic, and other (amino- and sulfidic groups) functional groups on the organic molecules . As with AVS, the amount of metal that can be complexated by these sediment-associated organic ligands, is metal-dependent. Copper is a very strong binder while for nickel the binding capacity is less strong due to the higher nickel sulfide solubility product.

Iron (oxy)hydroxides

The Fe/Mn (oxy) hydroxides component of sediments is an equally important, even dominant, repository for a wide variety of metals in the sediment compartment. The high adsorption and scavenging capacities of Fe/Mn (oxy) hydroxides can adsorb or incorporate substantial amounts of divalent metals and may play also a role for nickel.

Cation Exchange Capacity (CEC)

Finally CEC gave significant relationships with the Ni toxicity thresholds. This is consistent with the relationship between Ni toxicity to soil organisms and soil phases; CEC explained most of the variation in the bioavailability and toxicity of nickel in the soil compartment. CEC is largely determined by the pH and organic matter and clay content of the sediment.

Bioavailability relationships

Since for nickel no one parameter could be singled out as generally superior it was deemed appropriate by the TC i) group to develop also regressions using the before mentioned sediment parameters. Linear regression models explain between 59-74% of the variability of the EC_{20} for the three sediment organisms for AVS. Iron based models explained 62-79% of the observed variability. OC based models, which were significant for the amphipods only, explained 59-79 %. CEC based models 59-68 %. The OC model and CEC models with *Hexagenia* were not significant and only explained 29% and 36% of the observed variability, respectively. The lesser performance of the bioavailability models (AVS, OC and CEC models) for the Hexagenia species could, for the AVS relationship, be due to the specific life strategy of the species (formation of oxygenated burrows). No clear explanation can be found for the poor performance of the OC and CEC models. These models are not taken forward in the analysis.

Table 8 summarises the different bioavailability models developed per species and per sediment parameter.

Table 8: Overview of derived regression models relating the toxicity of nickel to several abiotic factors (AVS, TOC, Fe and CEC) in sediment.

Species	Model	R^2	Intercept	Slope
	AVS based		(S.E.)	(S.E.)
H. azteca	Log EC_{20} total Ni (mg/kg dry wt) = 2.65 + 0.492 Log AVS (µmol/g dry wt.)	0.74	2.65 (0.11)	0.492 (0.11)
G. pseudolimnaeus	Log EC_{20} total Ni (mg/kg dry wt) = 2.8 + 0.358 Log AVS (µmol/g dry wt.)	0.62	2.8 (0.13)	0.358 (0.13)
Hexagenia sp.	Log EC_{20} total Ni (mg/kg dry wt) = 2.35 + 0.175 Log AVS (µmol/g dry wt.)	0.59* (p = 0.07)	2.35 (0.06)	0.175 (0.07)*
	TOC based			
H. azteca	Log EC_{20} total Ni (mg/kg dry wt) = 2.81 + 0.513 Log OC (%)	0.59	2.81 (0.11)	0.513 (0.17)
G. pseudolimnaeus	Log EC_{20} total Ni (mg/kg dry wt) = 2.81 + 0.557 Log OC (%)	0.79	2.81 (0.09)	0.557 (0.13)
Hexagenia sp.	Log EC_{20} total Ni (mg/kg dry wt) = 2.40 + 0.164 Log OC (%)	0.29* (p = 0.26)	2.40 (0..07)	0.164 (0.13)

	Fe based			
H. azteca	Log EC20 total Ni (mg/kg dry wt) = - 0.54 + 0.854 Log Fe (mg/kg dry wt.)	0.62	-0.54 (1.15)*	0.854 (0.27)
G. pseudolimnaeus	Log EC_{20} total Ni (mg/kg dry wt) = 0.31 + 0.666 Log Fe (mg/kg dry wt.)	0.68	0.31 (0.87)*	0.666 (0.20)
Hexagenia sp.	Log EC_{20} total Ni (mg/kg dry wt) = 0.75 + 0.418 Log Fe (mg/kg dry wt.)	0.79	0.75 (0.45)*	0.418 (0.11)
	CEC based			
H. azteca	Log EC_{20} total Ni (mg/kg dry wt) = 2.11 + 0.783 Log CEC (meq/100g)	0.59	2.11 (0.32)	0.783 (0.26)
G. pseudolimnaeus	Log EC_{20} total Ni (mg/kg dry wt) = 2.28 + 0.679 Log CEC (meq/100g)	0.68	2.28 (0.26)	0.679 (0.26)
Hexagenia sp.	Log EC_{20} total Ni (mg/kg dry wt) = 2.20 + 0.0.244 Log CEC (meq/100g)	0.36* (p = 0.21)	2.2 (0.20)	0.244 (0.16)

* non-significant

Since the majority of relevant exposure data (e.g., available through national monitoring programs and site specific measurements) are only reported as total recoverable nickel) the AVS model for *Hexagenia* test, which only marginally failed the $P < 0.05$ criterion, was still considered to be appropriate to use for normalizing *Hexagenia* toxicity data based on AVS content of sediments. This can be justified because of the significant and strong relationship that was observed between toxicity expressed as SEM Ni and AVS ($r^2 = 0.82$, $p = 0.007$). In addition using the slope based on the relationship between toxicity expressed as TR-Ni and AVS (slope = 0.175) represents a precautionary approach since this slope is lower than the slope based on the SEM Ni-AVS relationship (slope = 0.26).

In the next phase of the project the normalization equations were used to translate the different ecotoxicity values towards the specific bioavailability parameters of a certain bioavailability scenario. Using these bioavailability models will, as apparent of the analysis here below, decrease uncertainty as compared to the situation where no normalization is considered. Of course, as with any model there is still residual uncertainty as can be deduced from the R^2 values and the calculated uncertainty on the slopes and intercept of the regression equations indicated between brackets (Table 8).

3.3.3 Reduction in variability

The regression models developed on sub-lethal endpoints for the amphipods and mayfly were used for normalizing all individual EC_{20} values (also based on sub-lethal endpoints), gathered in Task 2 and Task 3 and characterized by varying physico-chemical test conditions..

The normalization procedure uses the following equation:

AF = abiotic factor (AVS, TOC, Fe, CEC)

The EC_{20} values are normalised using the corresponding slopes to both reasonable worst case sediment properties (AVS: 0.77 µmol/g dry wt i.e 10th percentile Belgium (Flanders) AVS database (Vangheluwe et al, 2003); TOC: 0.5 % (expert judgment), Fe: 12,920 mg/kg dry wt., i.e 10th percentile United Kingdom database (Vangheluwe et al., 2008) and CEC: 8.6 meq/100g, i.e. 10th percentile GEMAS database

Table 9 shows the original (non-normalised) and the bioavailability normalised intra-species variability (expressed as the ratio between the highest and lowest EC_{20} from a specific species among different test sediments, i.e. max/min). Only those normalizations are shown for the bioavailability models which were significant.

Table 9: The intra-species variability (expressed as max/min ratios EC_{20}) of the normalised and non-normalised EC_{20} values, using the sediment chronic bioavailability regression models

AVS Normalisation	Ratio Non-normalised EC_{20}	Ratio Normalised EC_{20}	Variability reduction
Amphipods			
Hyalella azteca-survival	11.2	2.6	+77%
Gammarus pseudolimneaus – survival	4.5	2.6	+42%
Insects			
Hexagenia species – growth	2.1	1.4	+31%
TOC Normalisation	Non-normalised EC_{20}	Normalised EC_{20}	Variability reduction
Amphipods			
Hyalella azteca-survival	11.2	3.9	+65%
Gammarus pseudolimneaus – survival	4.5	2.0	+56%
Fe Normalisation	Ratio Non-normalised EC_{20}	Ratio Normalised EC_{20}	Variability reduction
Amphipods			
Hyalella azteca-survival	11.2	3.9	+65%
Gammarus pseudolimneaus – survival	4.5	2.2	+51%
Insects			
Hexagenia species – growth	2.1	1.3	+38%
CEC Normalisation	Ratio Non-normalised EC_{20}	Ratio Normalised EC_{20}	Variability reduction
Amphipods			
Hyalella azteca-survival	11.2	3.7	+67%
Gammarus pseudolimneaus – survival	4.5	2.5	+44%

The max/min ratios for the normalized EC_{20} data show a clear reduction in intra-species variability when compared with the non-normalised data for all bioavailability models.

- The AVS normalisation results in a reduction of intra-species variability between 31 and 77%.
- Normalisation with the Fe model reduced variability between 38 and 65%.
- TOC normalization reduced variability with 55-65 % for the amphipods.
- CEC normalisation reduced intra-species variability between 44 and 67% for the amphipods.

Since the TOC and CEC models were not significant for Hexagenia these models were not used to demonstrate a reduction in variability.

Variability among sediments means that non-normalised data may be either under-or over-protective. Normalization removes the variability part caused by differences in sediment parameters like AVS, TOC, CEC, Fe content. Although it is acknowledged that the application of the bioavailability models still inherently introduces some uncertainty, the overall picture shows that using the chronic univariate regressions reduce a large component of uncertainty within the effects assessment and could therefore be applied for setting an ecologically more relevant PNEC.

3.3.4 $HC_{5\text{-}50}$ derivation for selected bioavailability scenarios

The different bioavailability models have subsequently be used to normalize the EC_{10} results obtained in Task 2 and Task 3 towards 1) RWC conditions per sediment parameter and 2) the sediment characteristics prevailing in the different Task 3 sediments.

The normalization procedure uses the following equation:

AF = abiotic factor (AVS, TOC, Fe, CEC)

For the amphipods *H. azteca* and *G. pseudolimnaeus* bioavailability models are available for all four abiotic factors (AVS, TOC, Fe and CEC). For Hexagenia the TOC and CEC model were poor models and hence the outcome of the TOC and CEC normalizations for this species will not be used.

For the oligochaete *L. variegatus* no bioavailability model was derived and hence this is the only data point in the SSD that cannot be normalized with a species-specific bioavailability model. Different options are explored in Annex D to deal with this issue. One option is to use the non-normalized data as such in the SSD. An alternative option is still to account for the variability caused by differences in bioavailability and to normalize the data point by using one of the bioavailability models developed for another species that resembles the life strategy of *L. variegatus* the most. In practice this means either using one of the amphipod models or using the Hexagenia model.

Oligochaetes such as *L. variegatus* and other benthic worms such as Tubificids alter their immediate environment through the formation of I-shaped burrows which in contrast with Hexagenia are not irrigated with oxygenated water. Tubificids live head-down in relatively permanent vertical burrows feeding on deposits on some depth. When inhabiting soft substrates *L.variegatus* burrows also into the sediment and feeds in a similar head-down fashion. Sediment is ingested, the digestible portion is assimilated, and the undigested remainder is egested onto the sediment surface as faecal pellets (Appleby and Brinkhurst 1971). Because feeding rates are relatively high the so- called “conveyor-belt” feeding exhibited by many oligochaetes (including *L. variegatus*) results in the regular reworking of the top layer of sediment, which can have profound effects on the properties of sediments and overlying waters (Robbins 1982). Literature is replete with examples of how the sediment reworking behaviour of oligochaetes can cause significant changes to the biological, chemical and physical characteristics of sediments and overlying waters (Philips Williams, 2005). For example Davies (1974) observed a significant increase in redox potential by the burrowing activity of Tubificids. Peterson et al (1996) investigated the effect of bioturbation of the burrowing oligochaete *L. variegatus* on the oxidation of metal sulfide complexes in surficial freshwater sediments. Metal bioavailability (Cd and Zn) was determined directly by bioaccumulation in the test organisms and indirectly through analysis of interstitial (pore) water metal concentrations. Burrowing activity of the oligochaete significantly reduced AVS concentrations in surficial sediments in a density-dependent manner. The effect was more outspoken in the control sediments which is not surprising since is has been shown that iron sulfides are more prone to oxidation than cadmium sulfide and zinc sulfide that are more resistant to oxidation.

Various infaunal animals disturb the sediment structure differently depending on their specific feeding type, mobility and life cycle and care should be taken in the choice of bioavailability models since the impact on diagenic reactions may be different. With regard to Hexagenia this species forms oxygenated U shaped burrows. This micro-habitat maximizes the exchange with the overlying water and hence minimizes the mitigating capacity of a bioavailability factor such as AVS as reflected in the smaller slope of the AVS model.

Overall a closer similarity between tubificid/oligochaete worm behavior and U-shaped tube builders like Hexagenia can be expected as compared with intermittent sediment browsers like amphipods, which do not form burrows. Therefore the Hexagenia model will be used to normalize the *Lumbriculus* data. The choice of Hexagenia is considered precautionary because this species pumps in addition water through their burrows by active ventilation increasing oxygenation minimizing the mitigating effect of AVS. Anyway as can been seen in the sensitivity analysis the choice of bioavailability model does not have a major impact on the HC_5 value (Annex D). Species to species "read across", however, does imply adding some additional uncertainty. How much this type of uncertainty is compensated by decreasing the overall uncertainty in bioavailability using a normalization procedure vs using no normalization is unknown.

Figure 3-4 presents the lognormal functions normalized for AVS and Fe that were fitted through the 4 data points for the six sediments of task 3 and the RWC. All functions were accepted at $P < 0.05$.

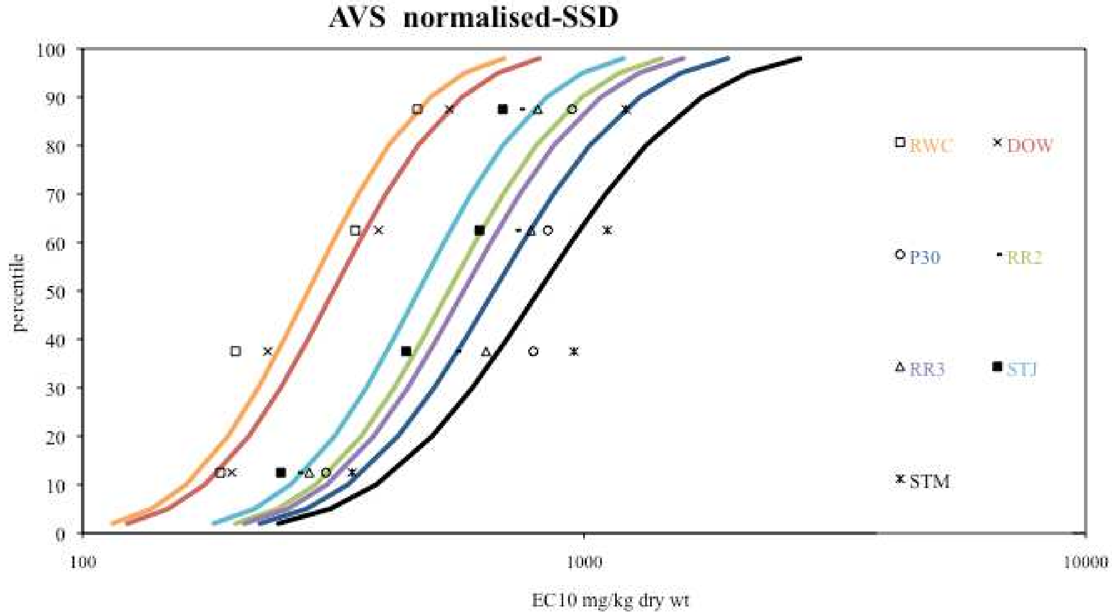


Figure 3: The cumulative frequency distributions of the EC_{10} values (n= 4) (expressed as mg Ni/kg dry wt.) from the Ni chronic toxicity tests towards sediment-dwelling organisms. Normalized towards prevailing AVS conditions – observed data and log-normal curve for the dataset fitted on the data. Unbounded/censored NOEC values were excluded.

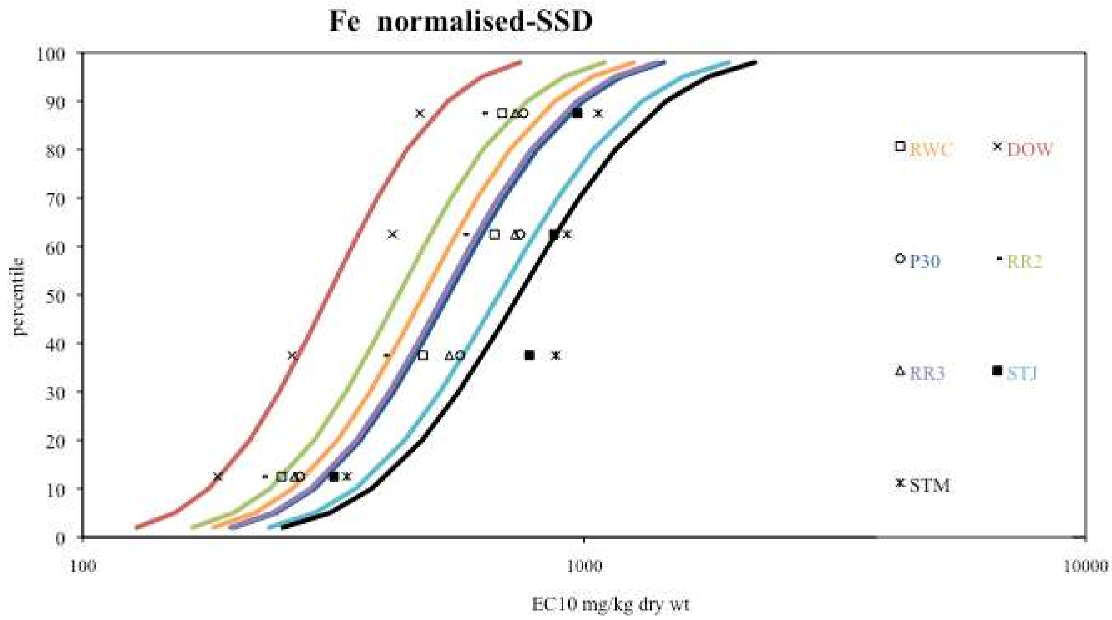


Figure 5: The cumulative frequency distributions of the EC_{10} values (n= 4) (expressed as mg Ni/kg dry wt.) from the Ni chronic toxicity tests towards sediment-dwelling organisms. Normalized towards prevailing Fe conditions – observed data and log-normal curve for the dataset fitted on the data. Unbounded/censored NOEC values were excluded.

A summary of the estimated HC_{5-50} value (with the 5-95% confidence limits) for the different log-normal distributions is provided in Table 10.

Table 10: Calculated HC_{5-50} value (mg Ni/kg dry wt.) (with the 5-95% confidence limits)

Bioavailability scenario	Model	HC_5 at 50% (5-95 % confidence limits)	Model	HC_5 at 50% (5-95 % confidence limits)
	AVS (µmol/g dry wt.)	mg Ni/kg dry wt.	Fe (mg/kg dry wt.)	mg Ni/kg dry wt.
RWC estimate	0.77	119 (24-202)	12,920	207 (53-323)
DOW	1.04	135 (29-135)	6,400	116 (14-231)
P30	12.4	255 (45-450)	15,800	225 (46-380)
RR2	6.06	224 (46-376)	10,500	184 (41-303)
RR3	7.98	237 (46-404)	14,900	219 (45-368)
STJ	3.78	201 (44-331)	22,900	265 (48-461)
STM	24.7	281 (41-529)	26,400	245 (49-496)

The HC_{5-50} values obtained for the different bioavailability scenarios range with the **AVS model from 119-281 mg/kg dry wt**. A similar range is observed when using the **Fe based model** with a reported range of **116-265 mg/kg dry wt.**

4. Robustness of the HC_5 estimate: uncertainty analysis and AF derivation

According to the London workshop on the use of statistical extrapolation methods an assessment factor between 1-5 should be applied on the derived HC_5 value. The size of the AF will depend mainly on the remaining uncertainty. It should be pointed out that the London Workshop specifically focused on considerations for the aquatic compartment. Extrapolating the London Workshop guidance to sediments may not be appropriate, so in general the intent of the London Workshop was used, and considerations that apply directly to freshwater pelagic ecosystems only were carefully evaluated.

To establish the necessity for assessment factors, a number of uncertainties must be addressed to extrapolate from single-species laboratory data to a multi-species ecosystem. The four areas that need to be considered are:

1. intra- and inter-laboratory variation of toxicity data;
2. intra- and inter-species variations (biological variance);
3. short-term to long-term toxicity extrapolation;

4. laboratory data to field impact extrapolation (additive, synergistic and antagonistic effects from the presence of other substances may also play a role here).

The use of the SSD approach with a higher number of species already reduces the uncertainty in some of the traditional areas of concern (e.g. interspecies variation). In case of the nickel sediment toxicity SSD approach, data are available for 8 species representing a general cross section of feeding behaviors that can be found in natural sediment ecosystems. Although data for detritus feeders are present, decomposers such as bacteria are not included. Data for periphyton are also not available. The reason that no data are available for these groups is mainly due to the lack of suitable standard test methods for these sediment organisms or for sediment microbial processes. These groups have also not been a traditional focus of sediment risk assessment. The organisms that are present in the database represent key functional groups, and for that reason they are important contributors to the maintenance of benthic ecosystem function, which is among the levels that risk assessment should strive to protect.

The following criteria related to the robustness of the HC_5 estimate for nickel have been considered for the derivation of the PNEC:

The overall quality of the database and the end-points covered, e.g., if all the data are generated from "true" chronic studies (e.g., covering all sensitive life stages; real chronic exposure time)

- The pooled Ni-database covers ecologically relevant endpoints. The selected endpoints are relevant for potential effects at the population level: mortality, biomass, emergence, growth and reproduction,
- Covering of sensitive life stages and 'chronic' exposure times are achieved for all sediment-dwelling organisms covered in the Ni database. All tests were performed in agreement with international agreed standard procedures (e.g., OECD, ISO, ASTM, USEPA, Environment Canada) and comprise chronic exposure times for the different organisms between 28 and 42 days. The age of the test organisms used for toxicity testing was dependent on the type of test used: i.e., the reproduction tests with oligochaetes were initiated with adult organisms while the toxicity tests with the amphipods and mussels were started with juveniles, midge tests were started with 1st instar larvae, and mayfly exposures were started with nymphs. In cases where different options were available for the age of organisms at test initiation, decisions were made by reaching consensus among the Technical Conclusion i) Group.

The diversity and representativeness of the taxonomic groups covered by the database

- High quality chronic $L(E)C_{10}$/NOEC values (Q1) are available for 8 different sediment-dwelling invertebrates, belonging to 4 different orders (i.e. oligochaetes, molluscs, crustaceans and insects) with different feeding habits and ecological niches.

The test species were *Lumbriculus variegatus* (Oligochaetae, Lumbriculidae), *Tubifex tubifex* (Oligochaeta, Tubificidae), *Hyalella azteca* (Crustacea: Amphipoda), *Gammarus pseudolimneus* (Crustacea: Amphipoda), *Chironomus dilutus* (Insecta, Diptera, 'midge'), *Chironomus riparius* (Insecta, Diptera, 'midge') *Lampsilis siliquoidea* (Mollusca) and *Hexagenia* sp (Insecta, Ephemeroptera) with different feeding habits and ecological niches:

- *Chironomus dilutus* and *C. riparius* are insects with a short generation time and inhabit eutrophic lakes, ponds and streams. *Chironomus* larvae are sediment ingesting deposit feeders and construct U-shaped burrows, which they irrigate with oxygenated water (Warren et al., 1994). As a consequence they may be exposed to sediment, pore water and water in its burrows. Burrow water metal concentrations in the micro-environment of the larvae may depend on irrigation/oxygenation rates, oxidation rates of metal sulfides, diffusion rates of lead, etc. (Warren et al., 1994).

- *Tubifex tubifex* is an oligochaete with a short generation time and is a deposit feeder constructing I-shaped burrows which do not irrigate with oxygenated water. They feed head-down, decomposing organic material present in the ingested sediment (organic detritus and its associated microflora) (Warren et al., 1994; Pennak, 1989; Pekarsky et al., 1990). By doing so they can bring sediment from deeper layers to the surface, making (metal)-sulfides susceptible to oxidation at the surface. Its tail, protruding into overlying water makes circular movements to enhance oxygen diffusion to the tail, which is the site of cutaneous oxygen uptake (Pekarsky et al., 1990). Like this, tubificids are able to withstand the anoxic conditions in deeper sediment layers.

- *Lumbriculus variegatus* is also a burrowing oligochaete with also a short generation time and are typically sub-surface deposit feeders; *Lumbriculus* is found throughout North America and Europe. It prefers shallow habitats at the edges of ponds, lakes, or marshes where it feeds on decaying vegetation and microorganisms. Favorite microhabitats include layers of decomposing leaves, submerged rotting logs, or sediments at the base of emergent vegetation, such as cattails. Although less detail is known about *Lumbriculus*, they are assumed to behave similarly as *Tubifex*. According to this life-style, these organisms may be exposed via the pore water, the overlying water, and via sediment ingestion

- *Hyalella azteca* is an amphipod (crustacean) with a short generation time and is typically an epibenthic detritivore that burrows into the sediment surface. *Hyalella* azteca and *Gammarus pulex* are bottom dwellers, mainly feeding on algae and detritus (Warren et al., 1994). It does not ingest sediment and does not construct burrows. Hyalella mainly feeds on periphyton, algae and detritus located at the sediment-water interface (Stephenson and Turner, 1993; Warren et al., 1994). A similar life strategy is observed for *Gammarus*. According to this life-style, these organisms may be exposed mainly via the overlying water, although exposure via resuspended particles (e.g. detritus) may not be excluded.

- *Hexagenia* sp. are insects (mayfly) are deposit feeders ingesting mud, detritus and organic matter. Mayflies also filter-feed seston as the nymph passes overlying water through their burrows and ingest smaller amounts of algae, diatoms, bacteria and plant debris. According to this life-style, these organisms may be exposed both through sediment ingestion and through overlying water.

- *Lampsilis siliquoidea is a fresh*water *mussel* that inhabits a variety of freshwater habitats. The juvenile life stage tested burrows in sediment and is exposed to particle-bound contaminants in sediment and pore-water contaminants in sediment.

Statistical uncertainties around the 5th percentile estimate, e.g., reflected in the goodness-of-fit or the size of confidence interval around the 5th percentile

The log-normal distribution (n= 4) based on RWC without bioavailability correction yielded a $HC_{5\text{-}50}$ of 94 mg Ni/kg dry wt. The 5-95 % confidence intervals were 15 and 172 mg Ni/kg dry wt.

The $HC_{5\text{-}50}$ values obtained for the different bioavailability scenarios range with the AVS model from 126-281 mg/kg dry wt. A similar range is observed when using the Fe based with ranges of 143-265 mg/kg dry wt. The 5-95 % confidence intervals were typically in the range of 40-500 mg Ni/kg dry wt.

Evidence of field data

Several field studies exist that examined nickel toxicity under field conditions (Costello et al, 2001, Nguyen et al, 2011). These studies cover mainly streams and a range of different sediment types, were conducted during different seasons, and were carried out in different geographical locations (Europe and North America) and in different types of systems (lotic and lentic), with varying water quality and abiotic parameters. The field studies were conducted over a time period of two months (Costello et al, 2011) to nine months (Nguyen et al, 2011) and the colonization of the deployed spiked sediments were followed over time.

The results of these studies converge in a range of effects concentrations that are protective of the toxicity results seen in the laboratory sediment testing. No evidence exists to show that field data are more sensitive than laboratory-based HC5 values. To the contrary, these data and similar data for benthic macro-invertebrates and pelagic communities show that field/mesocosm data are less sensitive than results of laboratory tests. Results of the most recent field colonization study (Costello et al. 2011), which was performed on the same sediments as the USGS study mentioned above, indicated a NOEC of 230 mg Ni/kg dry wt. A streamside experiment (Burton et al. 2009) that was performed on a low binding sediment resulted in an EC_{10} of 137 mg Ni/kg. The lowest NOEC of an earlier colonization study performed in Europe in 2005 (Nguyen et al. 2011) resulted in a NOEC of 100 mg Ni/kg dry wt. Effects in this study were observed at 500 mg Ni/kg dry wt(only three spiking levels were used – 100, 500, and 1,000 mg Ni/kg dry wt).

In the Costello et al. (2011) study, effects on recolonization (expressed with macro invertebrate indices) were measured after 28 and 56 days. Effects attributable to Ni exposure were only observed at the 28 day sampling period. Substantial amounts of Ni were lost from sediments over the course of the study, and the sediment factors corresponding to Ni partitioning changed over the course of the experiment as well. However, Ni concentrations at 56 days remained > 4,500 mg Ni/kg in some cases. Notably, no effects on the composition of the benthic communities were measured at the Day 56 sampling period. This may indicate an ageing phenomenon or a loss of weakly bound Ni over time. The consequences of this observation should be accounted for in the uncertainty analysis.

Outstanding issues: formation of micro-habitats and influence of dietary nickel exposure

1) Formation of micro-habitats

Hexagenia nymphs burrow within the top few centimeters of sediment where they create microhabitats by actively ventilating their burrows with overlying water. This may lead to an exposure (dissolved Ni concentrations and abiotic parameters like DOC) that is different than where measurements were made, e.g., undisturbed pore water. For species with well-ventilated burrows with a good exchange with the overlying water this does not necessarily have to lead to an increased metal uptake. However, for other burrowing species that interact less directly with the overlying water this may cause an increased exposure.

The issue of the formation of micro-habitats possibly resulting in lower AVS concentrations in the top few centimeters of a sediment is interesting from a scientific point of view but with regard to a risk assessment perspective the issue may not have such a major impact. Most benthic communities reside in the thin upper layer of substrate and if the source of pollution has ceased this top layer may be already relatively clean (Chapman *et al.* 1992). Under these circumstances the benthic community may actually be less exposed to toxicants than would be predicted by disruptive field sampling (sampling also the deeper more contaminated layers) and subsequent laboratory testing.

Most often the higher sediment nickel concentrations and AVS concentrations in the deeper sediment layers will still govern the overall nickel sediment toxicity. More accurate measurements of the nickel exposure to *Hexagenia* sp. including how the micro-habitat of this species impact bioavailability factors may, however, help to increase the understanding of the robustness of the observed bioavailability relationships. This in particular to the use of the Hexagenia AVS model for the normalization of other benthic species with similar life strategies.

2) Dietary exposure

Concern has been raised over the possibility that sediment-dwelling organisms are exposed to and affected by Ni via the diet, and that the contribution of dietborne Ni exposure should be evaluated within the context of the ongoing Conclusion i) Research Program on the toxicity of sediment-associated Ni. The specific concern is that the Ni $PNEC_{sediment}$ values that are being derived under the Conclusion i) Research Program may not reflect all contributions from dietborne Ni exposure.

A critical review of the literature on dietborne nickel exposure and toxicity was performed by DeForester and Fairbrother (2010). Only a few studies were found in the literature for aquatic organisms, and most of them studied the effect of diet on bioaccumulation, which although it provides useful information on exposure it can not be necessarily linked to toxicity. This makes it difficult to formulate general conclusions on the relative importance of dietborne Ni exposure on Ni toxicity to sediment organisms. In general the importance of dietary Ni exposure on toxicity for aquatic invertebrates varied depending on site-specific Ni bioaccumulation potential into food items.

Natural nickel background concentrations

Natural ambient concentrations of nickel in EU sediments from pristine areas were gathered from the FOREGS Geochemical Baseline Mapping Program. Its main aim is to provide high quality, multi-purpose environmental geochemical baseline data for Europe. Figure 6 shows that Ni-ambient concentrations ($Ni_{bc,\ aqua\ regia}$) in freshwater sediments from uncontaminated first order streams in the EU varied between 2 and 942 mg/kg dry wt (90^{th} % = 46 mg Ni/kg dry wt; 50^{th} % = 18 mg Ni/kg dry wt.).

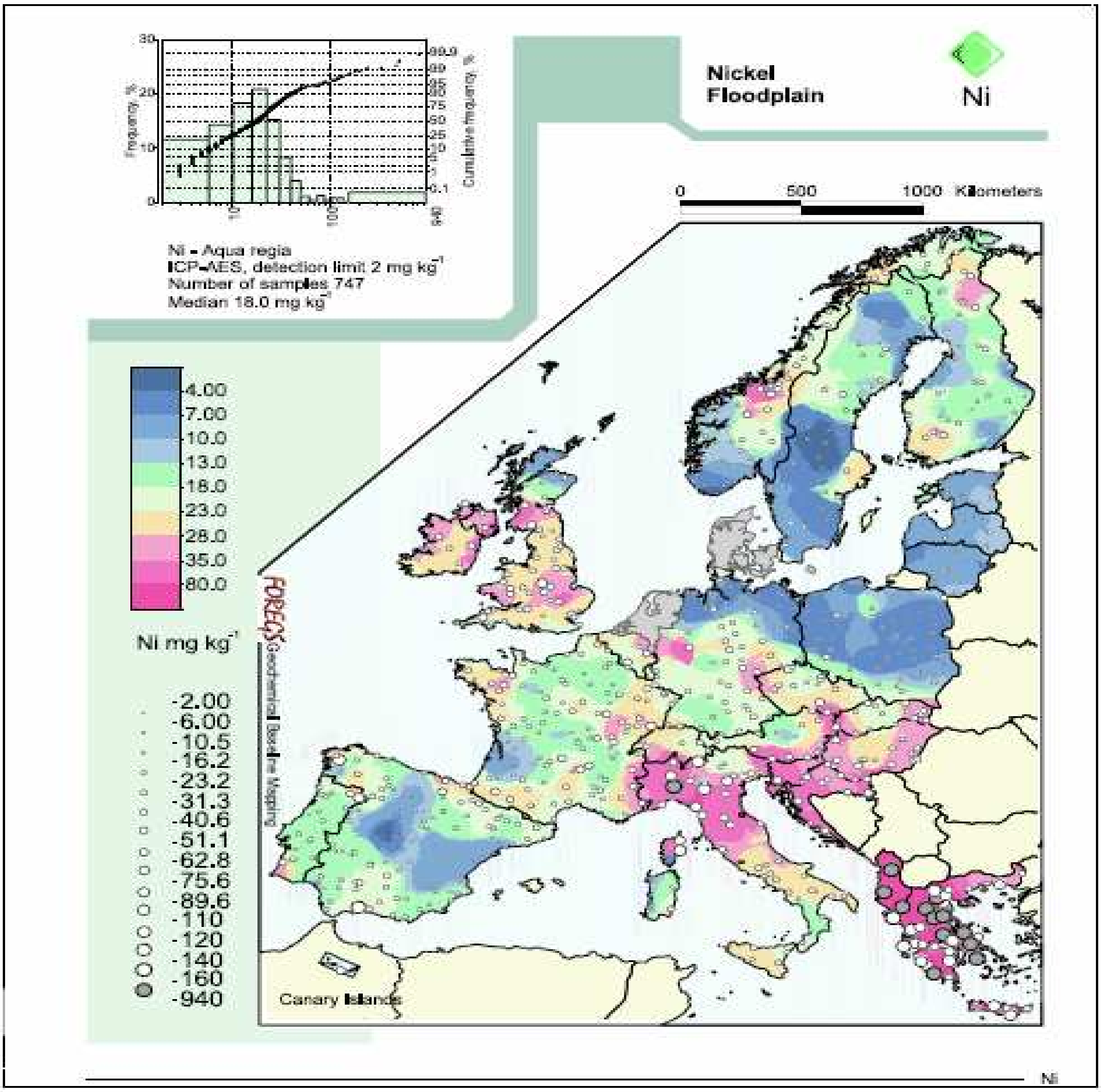


Figure 6: Range of Ni-ambient concentrations from pristine areas in EU sediments (aqua regia) (FOREGS database)

Ranges of Ni background concentrations from other sources are similar (Table 11).

Table 11: Ni-background concentrations in European freshwater sediments

Country	Background value (mg Ni/kg dry wt)	Reference
Northern Belgium	9 (3-15)	Swennen et al., 1998
Southern Belgium	36 (20-52)	
Belgium and Luxembourg	**24**	

	(6-42)	
The Netherlands + Germany (River Rhine)	**29**	Salomons (1983)
Sweden	**10**	www. naturvardsverket.se
The Netherlands	**29**	Van de Meent et al., 1990 (in: Crommentuijn et al., 1997)

5. Derivation of the RWC sediment PNEC (freshwater sediments)

According to the REACH guidance if the statistical extrapolation technique is used an AF of 5 is applied unless justification can be given to apply a lower AF (between 5-1).

An AF of 3 was proposed for the earlier conclusion Ni i) sediment program, to recognize the uncertainty with which the HC_5 was determined. Main issues included:

- Toxicity from overlying water;
- Need to back calculate to a critical sediment concentration
- Uncertainties regarding the bioavailability normalization

Arguments can be made that the current sediment toxicity database is more robust, and less uncertain:

- New sediment spiking techniques have been developed;
- More species have been tested;
- The two most sensitive species were crustaceans; pelagic crustaceans were a sensitive group within the aquatic effects assessment, and suggest that sensitive groups have been included in the database;
- More sediments were tested, covering the 10^{th} to 90^{th} percentile of the distributions of relevant sediment phases like AVS and TOC;
- Direct relationships between toxicity and relevant sediment phases, as opposed to overlying water
- Quantifiable relationships as been established for 3 out of 4 species on which chronic effects were observed between toxicity and sediment phases, which decrease uncertainty by removing the inter-sediment variability attributable to differences in sediment parameters like AVS and iron
- Additional field studies have been performed
- Field exposures using the same spiking techniques used in the laboratory showed an absence of effect after 56 days despite the observation of Ni concentrations as high as 4,500 mg Ni/kg
- Additional information on nickel toxicity in the field was obtained.

Clearly by developing a more robust sediment toxicity database and bioavailability models the uncertainty are less than after concluding the first conclusion 1) exercise.

General guidance on how to reflect the residual uncertainty in an appropriate AF is, however, lacking especially for sediment organisms. The AF should reflect the residual uncertainty proportional with the results obtained in the new conclusion 1) project i.e. the increase of knowledge since TC NES III'07 and now. Based on the description of the remaining uncertainty described above an assessment factor of 1, 1.5 and 2 could be considered, which would yield the following RWC PNEC values (Table 12).

Table 12: PNEC values (mg Ni/kg dry wt.) based on AF 1,1.5, 2,3

Scenario	Discussed in section	HC_5 at 50%	Type of fitting model	PNEC (AF 1)	PNEC (AF 1.5)	PNEC (AF 2)	PNEC (AF 3)
RWC no normalization	2.2	94	Lognormal	94	63	47	31
RWC EP[1]	Annex A	79-81	Lognormal	79-81	53-54	40-41	26-27
RWC MLE[2]	Annex C	72	MLE	72	48	36	24
RWC Kernel	Annex C	120	Kernel	120	80	60	40
RWC AVS normalization	3.3.4	119	Lognormal	119	79	60	40
RWC Fe normalization	3.3.4	205	Lognormal	207	138	104	69

[1]*: EP = equilibrium partitioning*
[2]*: MLE = Maximum likelihood estimation*

The nickel sediment effects database is the largest database for sediments that have been developed for a metal so far. In addition the use of new spiking techniques and the development of bioavailability models strongly reduced the uncertainty. Given the robust database and our increased knowledge on understanding nickel behavior and toxicity it could be argued that an Assessment Factor of 1 is more appropriate for Ni., This would result in a PNEC of 72 -205 mg Ni/kg dry wt. The range 94-126 mg Ni/kg dry wt obtained with the whole sediment data (RWC) and normalization to the AVS which is most likely the most relevant normalization factor, would be protective of the most sensitive geometric EC_{10} value (i.e 139 mg/kg dry wt.) obtained in the current study (which was obtained for *H. azteca* in the reasonable worst case Spring River and DOW river sediments). This value is also below all field based NOECs and EC_{10} values.

It should be noted that the range of PNECs using an AF of 2 is within the range of natural background concentrations, and is below the 50P distribution of Ni in sediments for several Member States, e.g., Finland (50P = 41 mg Ni/kg) and the UK (50P = 35 mg Ni/kg). Implementation of such a RWC PNEC could be used as a first tier to screen out those case where no bioavailability correction is needed. In those cases where a risk will be identified a second tier will require the use of a bioavailability-based tiered approach to avoid conclusions of risk from sediments where natural background Ni concentrations are greater than the RWC PNEC. The residual uncertainties include:

- uncertainties of HC_5 related to the fit of the SSD curve relative to the fact that the basis for that curve only included fitting to 4 data points (even though data on unbounded values on other species suggest that sensitive species have been included as the basis for the curve fitting and HC_5 estimation)

- uncertainties of the bioavailability normalization for species for which such normalization has been established. Even for species where normalization has shown to decrease uncertainty, residual uncertainty exist
- uncertainty in relation to the significantly difference in normalization between the insect larvae Hexagenia and the two crustecean species suggests that sediment species may differentially influence bioavailability but it is currently unknown how big such a difference generally is between sediment species (Furthermore the actual cause(s) for why nickel bioavailability of chronic nickel exposure is different for Hexagenia is not known even though two hypothesis in relation to this have been put forward)

The main new findings and strengths of the new chronic nickel toxicity database for sediment organisms include the points mentioned above

Based on this an AF of 2 may be argued (cf. table 12).

Understanding the residual uncertainties associated with the Ni database, e.g., the possibility that dietborne sediment exposure may play a role in the observed toxicity of some of the test organisms, the relative importance of the formation of oxygenated burrows and the use of 4 species in the SSD, an additional factor of 0.5 could alternatively be considered, i.e., to set the Assessment Factor at 1.5.

Using the log-normal HC_5 (50%) of 94-126 mg/kg, this would yield a PNECsed range of 63-84 mg Ni/kg. This is below the observed laboratory and field EC_{10}s and NOECs ranges.

6. Conclusions

The Ni conclusion i) work progressed our general understanding on how to estimate chronic Ni toxicity to sediment organisms substantially and resulted in a more robust sediment toxicity database containing 8 species including amphipods (*Hyalella azteca*, *Gammarus pseudolimnaeus*), mayflies (*Hexagenia sp.*), oligochaetes (*Tubifex tubifex*, *Lumbriculus variegatus*), mussels (*Lampsilis siliquoidea*) and midges (*Chironomus dilutus*, *Chironomus riparius*). However, four insensitive species resulted in censored data (> NOEC or EC_{10} values) and hence the species sensitivity distribution could only be constructed using the other four non censored data points. This yielded a $HC_{5\text{-}50}$ of 94 mg Ni/ kg dry wt. The benefits of increasing the number of data points using alternative approaches such as the EP method and Kernell/MLE do not seem to outweigh the substantial increase in uncertainty by applying these methods. Hence the preference is be given to the use of the whole sediment toxicity data base even though only 4 bounded data points are available.

Bioavailability relationships were obtained between three nickel sensitive sediment species and the sediment parameters AVS and Fe. However, due to co-variance none of the considered sediment parameters could be singled out as being the predominant parameter. Normalizations toward the different sediment parameters reduced the inter sediment variability in a significant way (up to 77 % reduction) for the amphipod species. However, the bioavailability relationships were less outspoken for the mayfly *Hexagenia*. It is not clear what contributed to this observation. One hypothesis could be the specific life stage of the mayflies forming burrows that they ventilate with overlying water creating a micro-habitat which has less resemblance with the overall sediment environment that other species see. Another possible explanation is dietary exposure. Since *Hexageni*a is one of the more sensitive species of the distribution the final effect on the HC_{5-50} of normalising the SSD towards the conditions prevailing in the different sediments (representing the 10-90th percentile of conditions encountered in the EU) is rather limited (factor 1.6-2.2). The HC_{5-50} values obtained for the different bioavailability scenarios range with the AVS model from 126-281 mg/kg dry wt. A similar range is observed when using Fe based models with ranges of 143-265 mg/kg dry wt

The final decision on an assessment factor for Ni has not been made, but a discussion of the arguments for considering AFs of 3, 2, 1.5 and 1 is being presented.

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ANNEX A: Derivation of $HC_{5\text{-}50}$ sediment using the statistical extrapolation method: whole sediment toxicity data set expanded with *L. siliquoidea* data point.

For the fatmucket clam *L . siliquoidea* the technical conclusion i) review group asked for specific clarification in relation to the calculation of the effects concentrations. More specifically there was a 6.6 % growth reduction (expressed as mean length) observed at the highest concentration (762 mg/kg dry wt. in Spring River sediment), which could be potentially extrapolated to an EC_{10} value contingent on the assessment and confirmation from the experts of the USGS laboratory who executed the test. According to their evaluation no dose-response was observed and a 3.7 % increase in biomass was even observed at the highest test concentration. Furthermore the observed difference was not statistically significant ($p = 0.30$) and USGS could not support extrapolating to an EC_{10} value based in a single non-significant value. Following these arguments the *L. siliquoidea* value is still considered as un unbounded NOEC value.

In order to evaluate the importance of adding the *L. siliquoidea* point on the $HC_{5\text{-}50}$ value a lognormal function was fitted through the four bounded data points and the *L. siliqoidea* point (which showed 6 % effect but was deemed not statistical significant) (Table A1).

Table A1: Species EC_{10}-NOEC values (total recoverable Ni, mg Ni/kg dry wt.) for the most sensitive endpoint for all sediment dwelling organisms used in the SSD

Organism	**Most sensitive endpoint**	**Geometric mean EC_{10}/NOEC (mg total Ni/kg dry wt)**
Hyalella azteca	Biomass	**149.1**
Gammarus pseudolimnaeus	Biomass	**228**
Hexagenia species	Biomass	**236.7**
Lumbriculus variegatus	Abundance	**554**
Lampsilis siliquoidea	Growth	**762***

** a non significant effect on growth (6%) was observed for the L. siliquoidea data and is here used as a substitute for a real NOEC value*
bold data: used for the $HC_{5\text{-}50}$ calculation

Figure A1 presents the lognormal distribution which was accepted at $P < 0.05$.

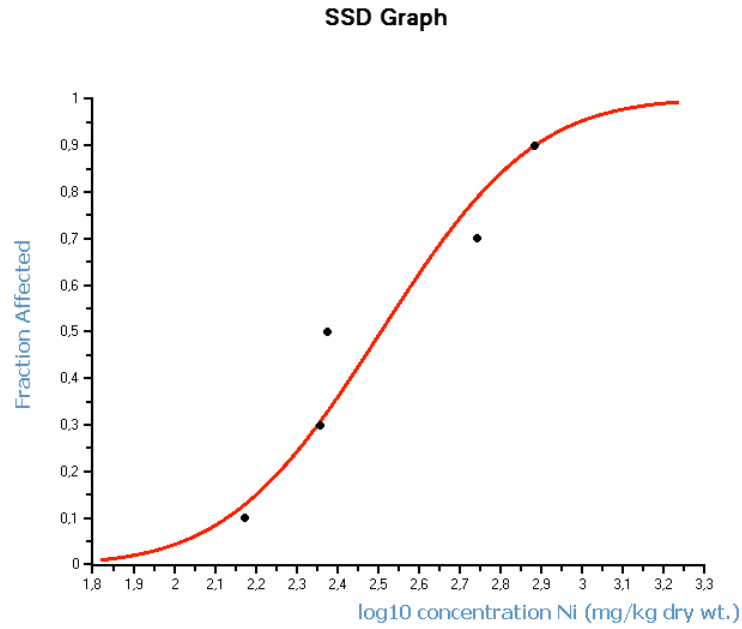

Figure A1: The cumulative frequency distributions of the NOEC/EC_{10} values (n= 5) (expressed as mg Ni/kg dry wt.) from the Ni chronic toxicity tests towards sediment-dwelling organisms – observed data and log-normal curve for the dataset fitted on the data. The unbounded NOEC value for *L. siliquoidea* was added as a surrogate NOEC

A summary of the estimated $HC_{5\text{-}50}$ value (with the 90% confidence bounds) for the log-normal function (calculated with ETX) is provided in Table A2.

Table A2: Calculated $HC_{5\text{-}50}$ value (mg Ni/kg dry wt.) (with the 5-95% confidence limits)

$HC_{5\text{-}50}$ at 50% (& 5-95% confidence limits) expressed as mg Ni/kg dry wt.	**Type of best fitting model**	**Parameters**
95.8 (19-184) (n = 5)	Log-normal model (ETX)	(2.51;0.295)

<u>Conclusion:</u>

Adding the addition test species *L. siliquoidea* data point has no influence on the $HC_{5\text{-}50}$ value = 95.8 mg Ni/kg dry wt. (n = 5) vs 94 mg Ni/kg dry wt. (n = 4)

ANNEX B: Derivation of $HC_{5\text{-}50}$ sediment using the statistical extrapolation method: whole sediment toxicity data (excluding unbounded values) and unbounded values substituted by the Equilibrium partitioning (EP) method

In order to increase the overall number of data points used in the SSD the possibility of replacing the unbounded values with the results of water only ecotoxicity data for these species, translated to whole sediment concentrations using the Equilibrium Partitioning Approach for a RWC sediment is explored in detail here below.

B.1 Translating water only data with whole sediment toxicity data

In parallel with the whole sediment toxicity tests, water only toxicity tests were also conducted with all species. Table B1 presents the EC_{10} values of this exercise.

Table B1: Species EC_{10}-NOEC values (total Ni) for the most sensitive endpoint for all sediment dwelling organisms-water only exposures.

Organism	**Most sensitive endpoint**	**Species EC_{10}-NOEC (µg total Ni/L)**
Hyalella azteca	Biomass	6.5
Gammarus pseudolimnaeus	Biomass	56
Hexagenia species	Growth	53
Lumbriculus variegatus		> 494 (unbounded LOEC)
Chironomus dilutus	Emergence	204
Chironomus riparius	Emergence	893
Lampsilis siliquoidea	Biomass	32
Tubifex tubifex		> 494 (unbounded LOEC)
Caenorhabditis elegans	Larval production	349

According to ECHA, the results of whole sediments tests are preferred since both the dietary and aqueous routes exposure pathways are covered in these experimental designs. However, the results of the equilibrium partitioning approach can be used to estimate sediment effect concentrations (expressed in mg/kg dry wt.) from aquatic effects data (expressed in µg/L) using a partitioning coefficient to replace the unbounded values in the whole sediment toxicity tests (i.e; *L. siliquoidea*, *C. dilutus*, *C. riparius* and *T. tubifex*) with the results of water only ecotoxicity data for these species.

In order to transform the water only toxicity data towards whole sediment toxicity data the following equation is used:

$$EC_{10} \text{ sediment EP (mg Ni/kg dry wt.)} = EC_{10} \text{ water only (}\mu g/L\text{) X Kd (L/kg) x } 10^{-3}$$

Both Spring River and Dow Creek are representative of low binding sediment and have Kd values of 3,643 L/kg and 3,603 L/Kg, respectively. Since most of the species in the RWC SSD have been derived using Spring River sediment the value of 3,643 L/kg has been used for the calculations. This Kd represents a geomean of the five Ni treatments and one control. The Kd in the control was 3,668 L/kg and 3,680 L/kg in the highest Ni spike demonstrating that the Kd does not need to be modified to account for saturation effects at high Ni concentrations as observed in the Kd values of West Bearskin sediment.

Table B2 summarises the outcome of this exercise. In case both bounded sediment and EP values are available the “true” sediment values have been chosen.

Table B2: Calculated EC_{10} values using the EP approach and a RWC Kd of 3,643 L/kg.

		Water only	**EP approach**	**Whole sediment test**
Organism	**Most sensitive endpoint**	**Species EC_{10} (µg/L)**	**(Species EC_{10} (mg/kg dry wt.)**	**(Species EC_{10}/NOEC (mg/kg dry wt.)**
Hyalella azteca	Biomass	6.5	23.6	**149.1**
Gammarus pseudolimnaeus	Biomass	56	204	**228**
Hexagenia species	Growth	53	193	**236.7 (biomass)**
Lumbriculus variegatus	Abundance	/	/	**554**
Chironomus dilutus	Emergence	204	**743**	> 762
Chironomus riparius	Emergence	893	**3,253**	> 762
Lampsilis siliquoidea	Biomass	32	116.6	> 762
Caenorhabditis elegans	Larval production	394	**1,435**	/

Bold values are used for the SSD
/ failed

From the comparison between the toxicity data obtained with the EP approach and the whole sediment toxicity data it is clear that the EP approach (using the same endpoints) creates a situation in which the EP normalized sediment toxicity data yields lower toxicity values for *H. azteca* and *L. siliquoidea*. Specifically, the EC_{10} value for *H. azteca* from the EP approach is 3.5 times lower than the EC_{10} from an actual sediment toxicity test for *H. azteca*. Furthermore, the EC_{10} value for *L. siliquoidea* that was estimated by EP is even 6.5 times lower than the highest test concentration from actual sediment toxicity tests performed with this species. The actual sediment toxicity test data demonstrate that the “real” EC_{10} value should be certainly greater than the EP estimate of 116.6 mg/kg dry wt. because the NOEC is >762 mg/kg dry wt.

In the whole sediment toxicity test with *L. siliquoidea* only a slight length decrease was observed (6 %) in the highest test concentration but data were insufficient to derive a meaningful EC_{10} value. There is no clear explanation of the discrepancy in sensitivity between the whole sediment test results and the water only test results. Both tests were performed on the same life stage- juvenile organisms about two months old. One possibility is that the animals receive additional stress from being exposed without sediments for 28 days.

In water only tests with *L. siliquoidea* and *H. azteca* on sulphate toxicity the latter was 3 times more sensitive than the bivalve (Soucek et al, presentation). Other studies confirm that *H. azteca* is an intrinsically sensitive organism (Phipps et al. 1995), which again does not support that *L. siliqoidea* would be more sensitive than *H. azteca*. Similar results were obtained in as study evaluating the sensitivity of mussel glochidia and juveniles. The results suggested that mussel glochidia and juveniles are less sensitive to chlorpyrifos (48h EC_{50} for *L. siliqoidea* is 0.43 mg/L) than the amphipod *H. azteca* (48h LC_{50} for *H. azteca* is 0.1 mg/L) (Bringolf et al, 2007).

The uncertainty that is produced from adding these apparently invalid data is extensive and it was decided to reject this data point from the database. If the only merit that is obtained from adding these data to the SSD is to increase the size of the database, it does not seem to counteract the increase in uncertainty.

For *G. pseudolimnaeus* and *Hexagenia* sp. toxicity values derived with EP are comparable. The results with *C. riparius* and *C. dilutus* are in the line of the expectations and can be used for the SSD. That is, for water-only exposures, chironomids are relatively insensitive when compared with crustaceans. This consistency supports the use of the chironomid data in the SSD.

None of the whole sediment tests with the nematode *C. elegans* gave adult survival data which were above the test acceptability criterion (90%). However, the water only tests were valid where the larval production endpoint was the most sensitive endpoint with an EC_{10} value of 394 µg/L which equals a whole sediment concentration of 1,435 mg Ni/kg dry wt.

In any case it should be noted that for all tested species the conditions in the water only tests do not resemble the conditions that are typically seen in pore water. Although DOC concentrations were not measured during the Ni water-only tests, they were probably <1 mg/L based on data from other water-only tests with fish and invertebrates that used essentially the same test water as the nickel water-only tests. Thus, they were substantially lower than PW-DOC measured in sediments from the Spring River (11-43 mg/L in Task 1; 3-32 mg/L in Task 2) or other Ni-spiked sediments. The same is true for hardness in pore water compared to that of the test media used in water-only tests. For pore water in the SR sediments, hardness ranged from 330 to > 1,000 mg $CaCO_3$/L, which is well above the hardness of 100 mg $CaCO_3$/L that was used in water-only tests.

Replacing the unbounded whole sediment toxicity values for both chironomid species and the nematode species with toxicity values obtained with the EP approach extends the number of species in the SSD to 7. The values used in the hybrid SSD are marked in bold in Table B2. Figure B1 presents the lognormal function that was fitted through the 6 data points and which was accepted at P < 0.05.

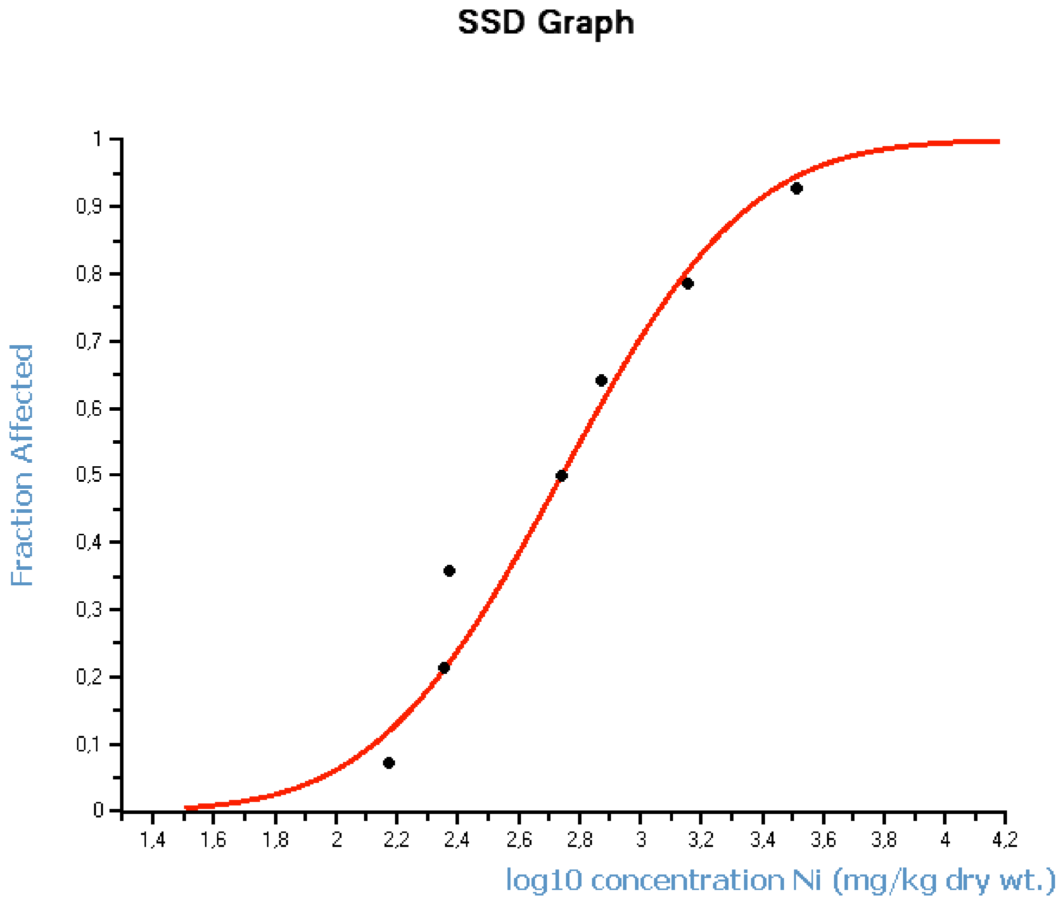

Figure B1: The cumulative frequency distributions of the EC_{10} values (n = 7) (expressed as mg Ni/kg dry wt.) from the Ni chronic toxicity tests towards sediment-dwelling organisms – observed data and log-normal curve for the dataset fitted on the data. Unbounded NOEC values were substituted by the EP method.

A summary of the estimated $HC_{5\text{-}50}$ value (with the 5-95% confidence limits) for the log-normal function (calculated with ETX) is provided in Table B3.

Table B3: Calculated $HC_{5\text{-}50}$ value (mg Ni/kg dry wt.) (with the 5-95% confidence limits)

$HC_{5\text{-}50}$ at 50% (& 5-95% confidence limits) expressed as mg Ni/kg dry wt.	Type of best fitting model	Parameters
81 (13-199) (n =7)	Log-normal model (ETX)	(2.74; 0.48)

Although the number of species in the SSD increased from four to seven the addition of three insensitive species i.e, 743, 1,435 and 3,253 mg/kg dry wt results in a lower HC_{5-50} than the HC_{5-50} derived with the whole sediment test results only which yielded a HC_{5-50} of 94 mg/kg dry wt (n= 4 species). Although more species are retained in the SSD the 95 % CL (13-199) were not smaller than the 95 % CL observed with the whole sediment test results (15-172). The advantage of having more data points is counter balanced by the increase in variability.

The lower HC_{5-50} obtained with the EP approach can be explained by the inherent assumption of proportionality embedded in the log-normal model, i.e. that there are species that are proportionally as sensitive as the insensitive species. The assumption that EC_{10} values are proportionately distributed is not supported by the whole sediment toxicity data where the effect data of the more sensitive species (i.e. *Hyalella*, *Gammarus* and *Hexagenia* are close together (i.e. EC_{10} values that range from 149-237 mg/kg dry wt). This point is further supported by the fact that the organisms sensitive to sediment exposure (amphipods) were shown in water only tests to be also in the sensitive part of the water only distribution indicating that *H. azteca* is indeed an intrinsically sensitive organism. Other sources of information support this observation. For example, Phipps et al. (1995) showed that *H. azteca* was consistently among the most sensitive organisms to metals. The expectation that there are organisms with even greater sensitivity is questionable, especially given the background concentrations of Ni in sediments is in the range of 9-36 mg/kg dry wt., and that Ni concentrations in non-spiked test sediments reached 51 mg Ni/kg (West Bearskin Lake). Accepting the assumption that the sensitivities of benthic organisms are proportionally distributed according to the log-normal model would mean that there are groups of organisms that are substantially more sensitive than *H. azteca*, and that effects to these organisms could occur at Ni concentrations that occur naturally in typical freshwater sediments. Based on the data from this and other studies, *H. azteca* is representative of very sensitive benthic species, and that the SSD analysis should reflect this.

B.2 Effect of adding Clistoronia data

The water only data obtained in the current research project could possibly extended with other benthic species retained in the aquatic SSD for Ni. For example the data for the caddisfly *Clistoronia magnifica* present in the aquatic nickel SSD could be a candidate to be included. However, caddisflies cannot be strictly considered benthic species. They typically form cases from small pieces of wood or mineral particles (e.g., gravel) and attach the cases to hard substrata. This behaviour may separate the organism from direct exposure to porewater sediment phases. However, again they may ingest suspended particles, which may include sediments.

The entry in the aquatic SSD for this species has a water only NOEC of 66 µg/L which yields a EP value of 240 mg/kg dry wt using the Spring river sediment Kd value of 3,643 L/kg. Replacing the unbounded whole sediment toxicity values for both chironomid species with toxicity values obtained with the EP approach and adding the *C. magnifica* data extends the number of species in the SSD to eight. Figure B2 presents the lognormal function that was fitted through the eight data points and which was accepted at $P < 0.05$.

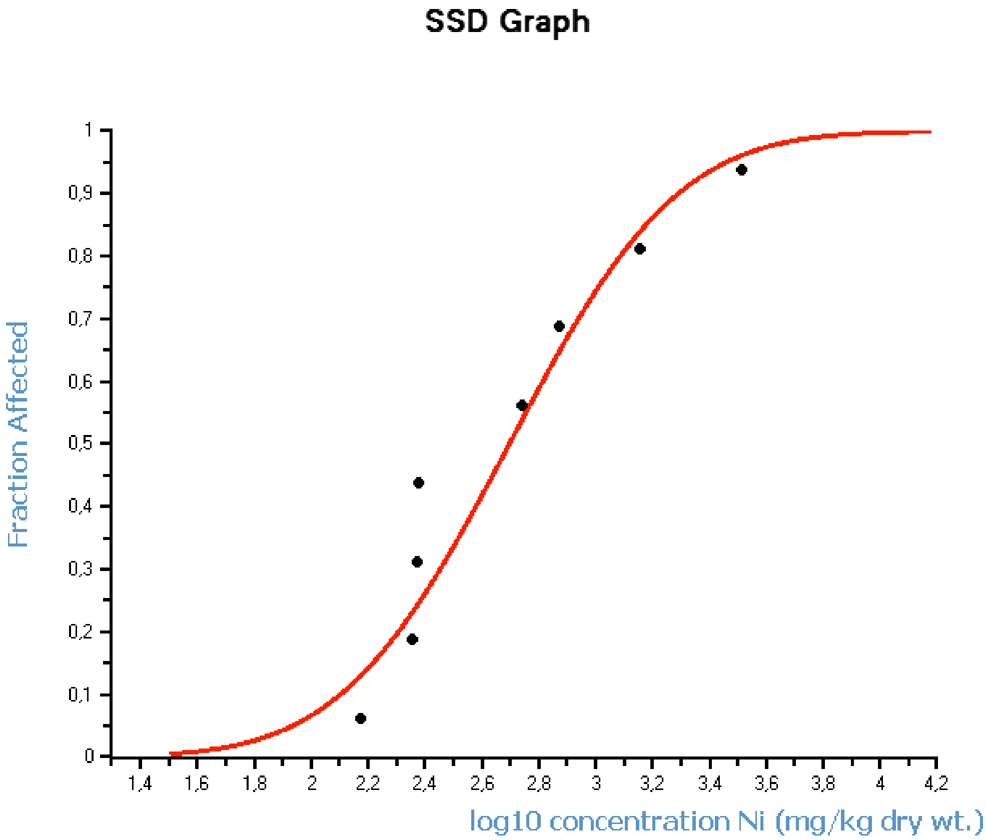

Figure B2: The cumulative frequency distributions of the EC_{10} values (n = 8) (expressed as mg Ni/kg dry wt.) from the Ni chronic toxicity tests towards sediment-dwelling organisms – observed data and log-normal curve for the dataset fitted on the data. Unbounded NOEC values for chironomids were substituted by the EP method. An additional data point (*Clistoronia magnifica*) has also been added using the EP method.

A summary of the estimated HC_5 value (with the 5-95 % confidence limits) for the log-normal function (calculated with ETX) is provided in Table B4.

Table B4: Calculated $HC_{5\text{-}50}$ value (mg Ni/kg dry wt.) (with the 5-95% confidence limits)

$HC_{5\text{-}50}$ at 50% (& 5-95 % CL% confidence bounds) expressed as mg Ni/kg dry wt.	Type of best fitting model	Parameters
79 (17-11792) (n = 8)	Log-normal model (ETX)	(2.7; 0.46)

Adding this species to the SSD yields an $HC_{5\text{-}50}$ of 79 mg/kg dry wt.

B3 Pros and cons of applying the EP approach for metals

The relevance of the use of the EP approach for metals need to be carefully evaluated together with the potential benefits of using the data (e.g., adding additional species to the SSD, weight of evidence), which needs to be weighed against possible uncertainties introduced by this approach. The EP approach was originally developed for non ionic organic compounds where it was shown that in absence of real sediment toxicity data the method could be used to predict sediment toxicity data from water only exposures taking into account the partitioning behavior of the compound and assuming that the pore water is the primary route of exposure. Since sediment toxicity data for some metals are also still lacking this EP approach has also been applied to metals. However, validation studies of this concept to metals are scarce. Van Beelen et al (2003) studied the validity of the EP-method for both organic compounds as metals to predict soil toxicity values. The results showed that the EP-method can give significant over-or underestimations, due to inaccurate partitioning coefficients or differences in species sensitivities (aquatic versus terrestrial species). The $HC_{5\text{-}50}$ values derived using the EP-method were in 5% of the cases more than 20 times higher than the corresponding $HC_{5\text{-}50}$ values that were derived directly from soil toxicity tests (Van Beelen et al 2003).

In general the following factors may contribute between a deviation between EP values and “true” sediment toxicity values:

1. Differences between used species sensitivity distributions in water and sediments: is not the case here since the SSD of water only data only consists of the same benthic species except for the caddis fly *C. magnifica*

2. Differences in exposure conditions during the toxicity tests in water and sediments: from a scientific point of view, which is also supported by the guidance provided by ECHA, results of whole sediments tests are preferred since both the dietary and aqueous routes exposure pathways are covered in these experimental designs. In the EqP approach only the pore water route is considered as the primary route of exposure. Furthermore differences in DOC and hardness between water only experiments and pore water may have a large influence here. This is illustrated by the pore water data from the USGS study, where pore water DOC in SR sediments ranged from 3 to 32 mg DOC/L, and hardness ranged from 330 to > 1,000 mg $CaCO_3$/L. These values are substantially higher than the media used in water-only tests, suggesting that bioavailability of Ni in pore-water will be much, much lower than in water only tests. Predictions of Ni toxicity in sediment phases will most likely be overestimated when using the EP approach.

3. Selection of the Kp value for the metal of concern: has been minimized here since the Kd values of the Spring river are being used. But in general the use of the EqP approach for metals is hampered by the large variability in Kd values. The Dutch Health Council stated that the EP-method was only suited for organic, apolar and not for very hydrophobic substances and not for metals (gezondheidsraad, 1995) since the variation in different partition coefficients for a single metal is large introducing quite some uncertainty to the system. Because of this wide variability, the use of Kp values for the purpose of equilibrium partitioning was not recommended for the derivation of ecotoxicological risk limits for metals in sediment (Verbruggen et al., 2001).

Taken all factors in consideration the SSD based on the whole sediment toxicity tests only, without substitution, gives the most realistic HC_5 estimate (i.e. 94 mg/kg dry wt.). Although the EP approach has the benefit of increasing the number of species in the SSD, the approach also adds substantial uncertainty. Furthermore the introduction of the two insensitive data points shifts the SSD towards lower HC_{5-50} values that are not supported by the whole sediment data set (based on a RWC sediment type with very low binding capacity). In addition using EP data excludes the use of a bioavailability normalization model unless one is willing to accept a great number of assumptions. The above general formulated criticisms are still present today and raise questions about the validity of the EP approach for metals.

<u>Conclusion:</u>

Applying the EP method to metals, is not deemed the most scientific way forward and introduces considerable uncertainty. There was a general agreement in the technical conclusion i) review group that the results of this exercise was useful in terms of context but should not be used as such in the SSD given the high uncertainty surrounding the EP calculated values. However, overall the EP-derived HC_5 values are supportive of the HC_5 value derived based solely on whole sediment contact data.

ANNEX C: Derivation of HC_{5-50} sediment using the statistical extrapolation method: whole sediment toxicity data (including unbounded/censored values)

In order to preserve the fact that some species did not respond at a specific measured total recoverable Ni concentrations the database was also analyzed using different methods for distribution fitting with censored data. Fifty percent of the Ni sediment ecotoxicity data are right-censored data ('greater-thans') or unbounded data. This complicates SSD fitting and HC_{5-50} derivation. In the current section some of the methods to circumvent the issues of censored data are explored.

C.1 Statistical methods for distribution fitting with censored data

Aldenberg (2011) referred to statistical literature (Helsel, 2005) specifically dedicated to the issue of dealing with censored data. More specifically a maximum likelihood method was proposed in order to fit a normal distribution to log-transformed data of which a portion of the data is censored. This method yields a HC_{5-50} of 71.6 mg/kg dry wt (95 % CL = 2.7-171.7 mg/kg dry wt) (Table C1).

However, there are also variations to the used maximum likelihood method (such as more robust versions) and there are also other methods given in statistical literature to deal with censored data such as log-probit regression methods, substitution methods, several non-parametric quantile methods (such as Kaplan-Meier method). In literature, several attempts are made to compare all these methods for analyzing censored data: non-exhaustive examples: Hewett & Ganser, 2007; Serasinghe, 2010; Kuttatharmmakul et al., 2001. These studies investigate statistical inference for varying standard deviation, varying sample size, varying degree of censoring, varying underlying distribution types, etc… Typically, no single method is unequivocally superior across all scenarios, although all of the methods may excel in one or more scenarios. For example, Helsel and Lee (2006) would not recommend MLE in case of small sample sizes.

C.2 Use of accepted methods for distribution fitting and extension to censored data

Alternatively, one may rely on existing and already accepted methods for SSD fitting with an extension for the censored data issue. In the Ni dossier, kernel density estimation was already proposed as a sophisticated SSD fitting method to deal with the marine aquatic toxicity data. The "flexible kernel density estimation" (Aldenberg, 2007) is a semi-parametric approach that attempts to fit a distribution to all empirically derived data (censored and non-censored). The underlying assumption is a log-normal distribution between the curve and the most influential points. The semi-parametric nature of the approach means that classical Goodness-of-Fit tests are not relevant for evaluating the distribution.

Kernel width in Gaussian kernel is sometimes called kernel bandwidth or kernel window. Wider kernel bandwidth will span to larger domain. One can imagine kernel width as the width of a window center at the data point and give weighing value to any points located in the window. These weights will be used as local average for all points within that window. Consequently, the kernel density estimation method allows to include all ecotoxicity data (censored and non-censored) in one single SSD fit without having the right-censored data at the higher end influencing too much the HC_5 estimation at the lower end. This is the strength of kernel density estimation. A proposal could be to estimate the optimal bandwidth based on the non-censored data and conduct the kernel fitting on all data (censored and non-censored). Applying this approach to the Ni sediment data (149.1 [*H. azteca*], 228 [*G. pseudolimnaeus*], 236.7 [*Hexagenia* sp.], 554 [*L. variegatus*], >762, >762, >762, >762 [*C. dilutis, C. riparius, L. siliquoidea,* and *T. tubifex*]) gives a bandwidth of 0.17 (normal adaptive bandwidth based on non-censored data only). The kernel fitting on all data with right-censored replaced by their lower limit (149.1, 228, 236.7, 554, 762, 762, 762, 762) gives an $HC_{5\text{-}50}$ estimation of 120 mg/kg dry wt (Figure C1). The kernel fitting on all data with right-censored data replaced by arbitrary chosen high values (i.e. 760; 1,000; 5,000 and 10,000) gives the same $HC_{5\text{-}50}$ estimation of 120 mg/kg dry wt. (Figure C2). This demonstrates that the $HC_{5\text{-}50}$ estimation is a robust estimate and does not deviate even when additional insensitive values are added to the species sensitivity distribution due to the estimation the optimal bandwidth based on the non-censored data.

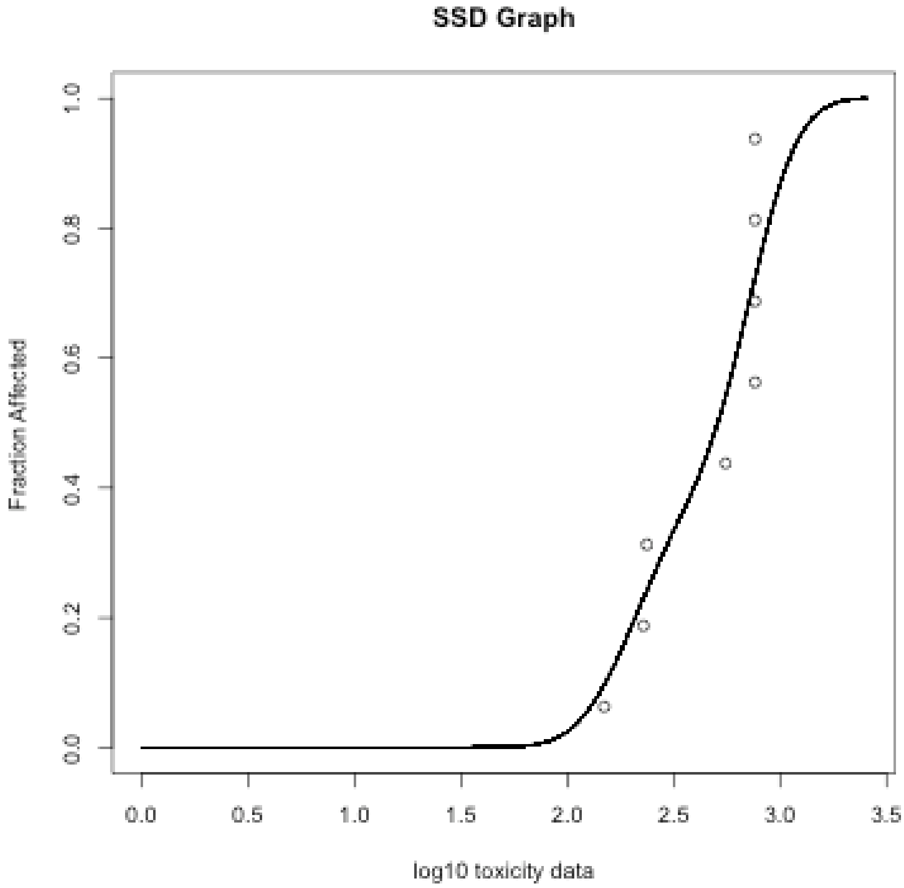

Figure C1: The cumulative frequency distributions of the NOEC values (n = 8) (expressed as mg Ni/kg dry wt.) from the Ni chronic toxicity tests – observed data and kernel curve for the dataset fitted on the data.

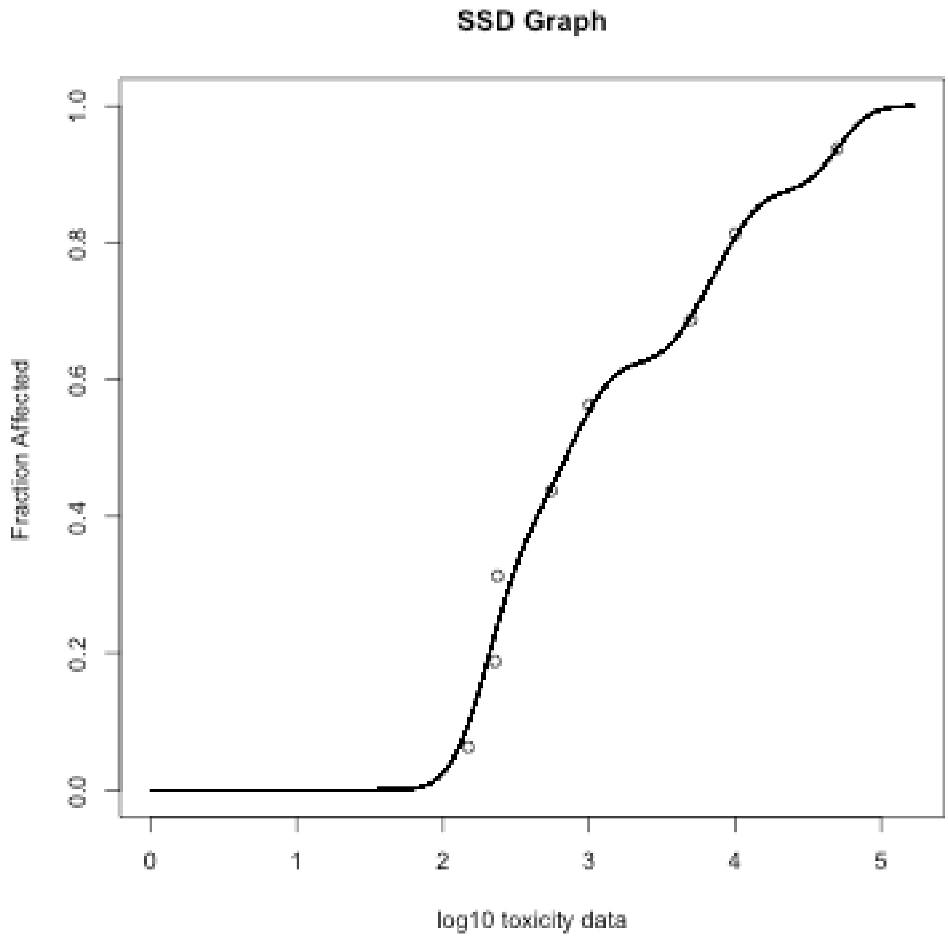

Figure C2: The cumulative frequency distributions of the NOEC values (n = 8) (expressed as mg Ni/kg dry wt.) from the Ni chronic toxicity tests – right censored data replaced by high values. Observed data and kernel curve for the dataset fitted on the data.

A summary of the estimated $HC_{5\text{-}50}$ value for the Kernel distribution and Maximum Likelihood method (calculated by Aldenberg) is provided in Table C1.

Table C1: Calculated $HC_{5\text{-}50}$ value (mg Ni/kg dry wt.) (with the 5-95% confidence limits)

$HC_{5\text{-}50}$ at 50%) expressed as mg Ni/kg dry wt.	**Type of best fitting model**	**Parameters**
120 (n= 8)	Kernel distribution	
71.6 (n = 8)	Maximum Likelihood method	

Conclusion:

Attempts to take the censored data into account using the kernel distribution gives a higher $HC_{5\text{-}50}$ (120 mg Ni/kg dry wt.). Using the maximum likelihood method results in a lower $HC_{5\text{-}50}$ (71.6 mg Ni/kg dry wt.) Typically, no single method is unequivocally superior across all scenarios, although all of the methods may excel in one or more scenarios. For example, Helsel and Lee (2006) would not recommend MLE in case of small sample sizes. Overall, a selection of

a method for SSD fitting with censored data purposes would require a thorough review of the existing methods or at least a review of the comparison papers. This may feed further discussions between experts and non-experts on the best approach.

ANNEX D: Sensitivity analysis- read across bioavailability models for normalization of the *L. variegatus* data point.

The development of a bioavailability model for the oligochaete *L. variegatus* was not within the scope of this project. However, since there is a valid effect concentration for this species in the SSD different options are explored to see if this data point could still be normalized using read across towards to bioavailability models developed for other species. The individual non-normalized EC_{10} value for *L. variegatus* is 554 mg/kg dry wt and was compiled for this species in Task 2 for the Spring River.

The choice of the most appropriate bioavailability model to apply to *L. variegatus* should not be based on the fact if the model gives the most conservative individual value or not. As can be seen in Table D1 depending on the reference conditions towards one is normalizing a different model could give the most precautionary outcome (Table D1). Specifically, the smallest slope should be used when normalizing to higher reference conditions and the highest slope when normalizing to lower reference conditions in order to obtain the lowest values. However, using the most conservative slope (and hence obtain the lowest *L. variegatus* value) does not imply that the most stringent HC_5 will be derived. By using the lognormal model shifting the *L.variegatus* value for example to a higher value would increase the overall steepness of the SSD and would render a lower HC_5.

Table D1: Sensitivity analyses slope selection in normalizing the *L. variegatus* data of 554 mg/kg dry wt.

Parameter	Initial test conditions	Model	Slope	Normalisation to lower value (RWC or hypothetical value)	Normalization to higher value (STM sediment)
AVS	1.1 µmol/g dry wt			*0.8*	*24.7*
		Hexagenia	0.175	521	955
		Hyalella	0.492	465	2,561
		Gammarus	0.557	459	2,880
Fe	7,753 mg/kg dry wt.			*5,000*	*26,400*
		Hexagenia	0.418	461	925
		Hyalella	0.854	381	1,577

The choice for the most appropriate bioavailability model to be applied on the *L. variegatus* data point, as explained in section 3.3.4 of the main report should instead be based on the closer similarity between the life style and behavior of tubificid/oligochaete worm and U-shaped tube builders like Hexagenia as compared with intermittent sediment browsers like amphipods which do not form burrows. Therefore the Hexagenia model has been selected to normalize the *Lumbriculus* data.

As a kind of sensitivity analysis Figures D1-2 represent the lognormal function that was fitted through the four bounded data points and which was accepted at $P < 0.05$. Figure D1 represents the SSD where the Hexagenia model was used to normalize the *L. variegatus* data point. For Figure D2 the *H. azteca* model was used.

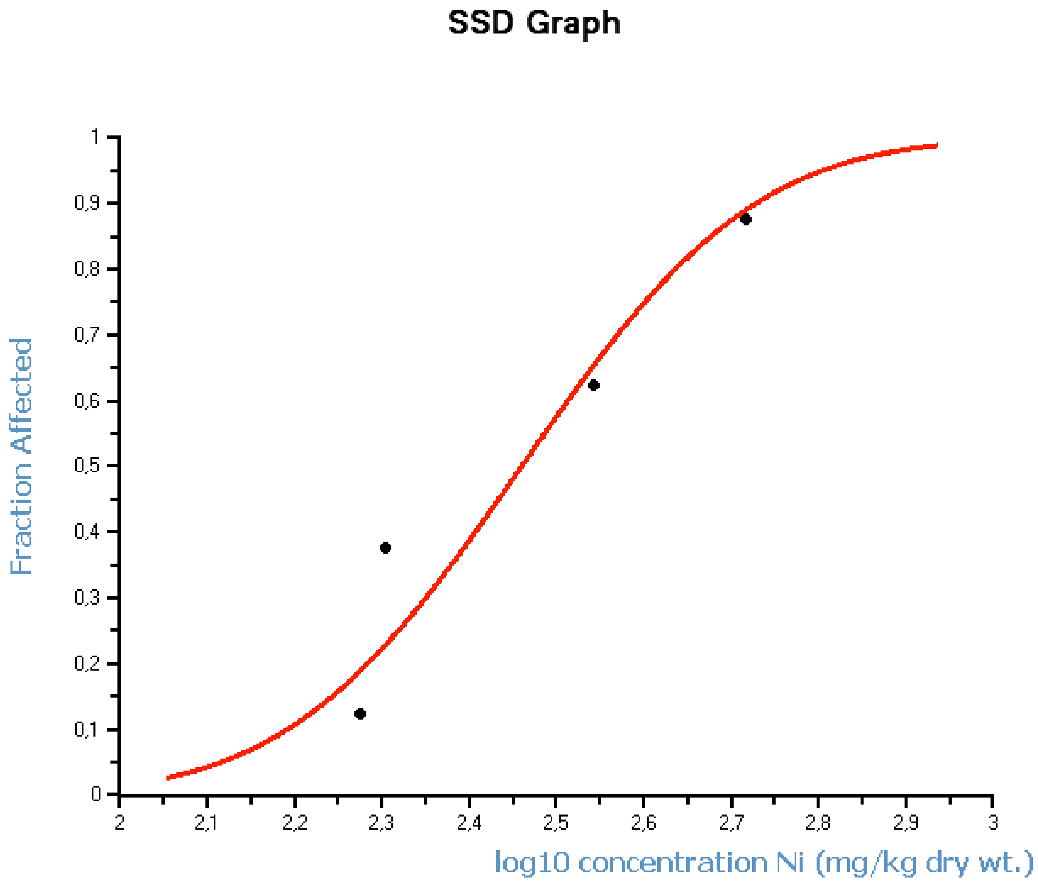

Figure D1: The cumulative frequency distributions of the EC_{10} values (n= 4) (expressed as mg Ni/kg dry wt.) from the Ni chronic toxicity tests towards sediment-dwelling organisms – observed data and log-normal curve for the dataset fitted on the data normalized to a reference conditions of 0.77 µmol AVS/g dry wt. Unbounded NOEC values were excluded. *L. variegatus* data point was normalized using the *Hexagenia* bioavailability model

A summary of the estimated $HC_{5\text{-}50}$ value (with the 90% confidence bounds) for the log-normal function (calculated with ETX) is provided in Table D2.

Table D2: Calculated $HC_{5\text{-}50}$ value (mg Ni/kg dry wt.) (with the 5-95% confidence limits) Unbounded NOEC values excluded.

$HC_{5\text{-}50}$ at 50% (& 5-95% confidence limits) expressed as mg Ni/kg dry wt.	Type of best fitting model	Parameters
119 (13.8-230) (n = 4)	Log-normal model (ETX)	(2.46;0.21)

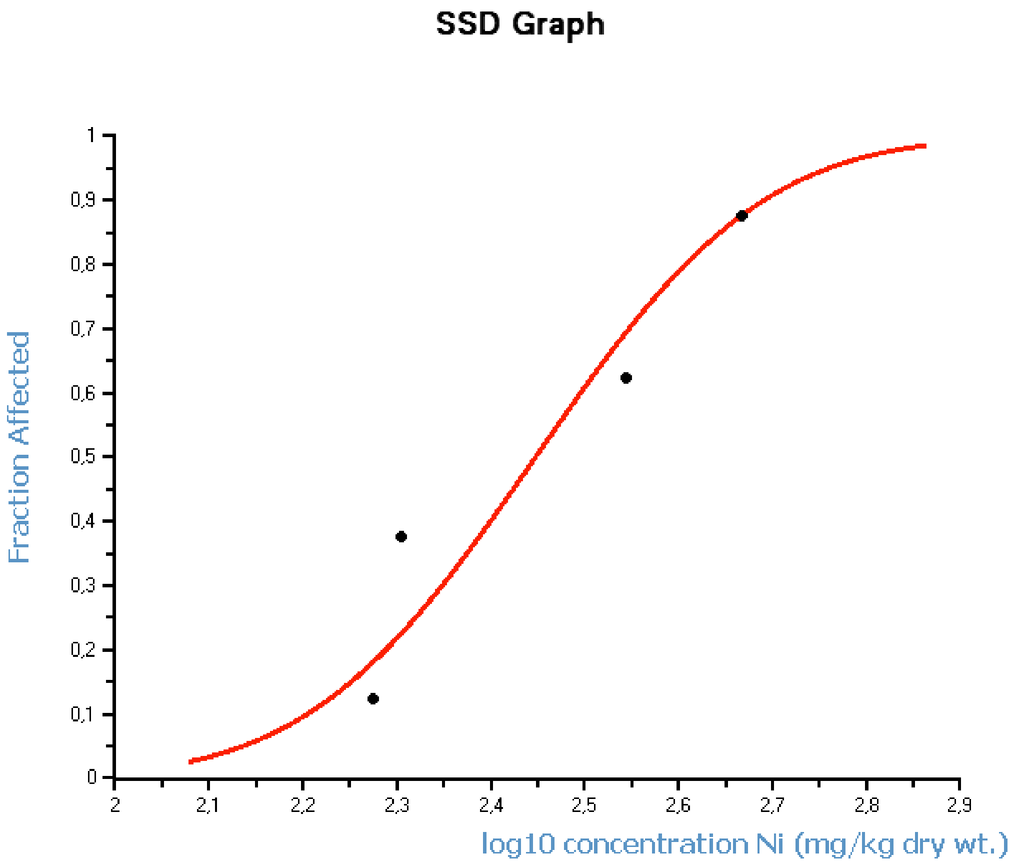

Figure D2: The cumulative frequency distributions of the EC_{10} values (n= 4) (expressed as mg Ni/kg dry wt.) from the Ni chronic toxicity tests towards sediment-dwelling organisms – observed data and log-normal curve for the dataset fitted on the data normalized to a reference conditions of 0.77 µmol/g dry wt. Unbounded NOEC values were excluded. *L. variegatus* data point was normalized using the *H. Azteca* bioavailability model

A summary of the estimated $HC_{5\text{-}50}$ value (with the 90% confidence bounds) for the log-normal function (calculated with ETX) is provided in Table D3.

Table D3: Calculated $HC_{5\text{-}50}$ value (mg Ni/kg dry wt.) (with the 5-95% confidence limits). Unbounded NOEC values excluded.

$HC_{5\text{-}50}$ at 50% (& 5-95% confidence limits) expressed as mg Ni/kg dry wt.	Type of best fitting model	Parameters
126 (29.6-202) (n = 4)	Log-normal model (ETX)	(2.45;0.19)

Similar normalizations towards higher AVS reference conditions were performed together with normalizations for the sediment parameter Fe. For comparative reasons the $HC_{5\text{-}50}$ was also calculated using the non-normalized data point for *L.variegatus.* The results are presented in Table D4.

Table D4: Summary table calculated HC_{5-50} values (mg Ni/kg dry wt.) (with the 5-95% confidence limits) derived using different bioavailability models.

	HC_{5-50} at 50% (& 5-95% confidence limits) expressed as mg Ni/kg dry wt.		
AVS (mmol/g dry wt.)	**Non-normalized**	**Hexagenia model**	***H. azteca* model**
0.77	115	119	126
24.7	238	281	229
Fe			
12,920	210	207	197
26,400	250	280	252

From the analysis above it is clear that the choice of the bioavailability model does not influence the HC_{5-50} to a large extent when the *L. variegatus* data point is normalized toward RWC conditions. The range is 119-126 mg Ni/kg dry wt. for AVS normalization (non-normalized = 115) and 197-207 for Fe normalization (non-normalized = 210). Normalizing towards conditions with higher AVS and Fe concentrations results in slightly broader range. 229-281 (non-normalized = 238) for AVS and 252-280 (non-normalized = 250).

Conclusion:

The choice of bioavailability model to normalize the *L. variegatus* data point only has a marginal influence on the derived HC_{5-50} value. Based on similarity of life style the choice has been made to use the Hexagenia model to normalize the *L. variegatus* data.