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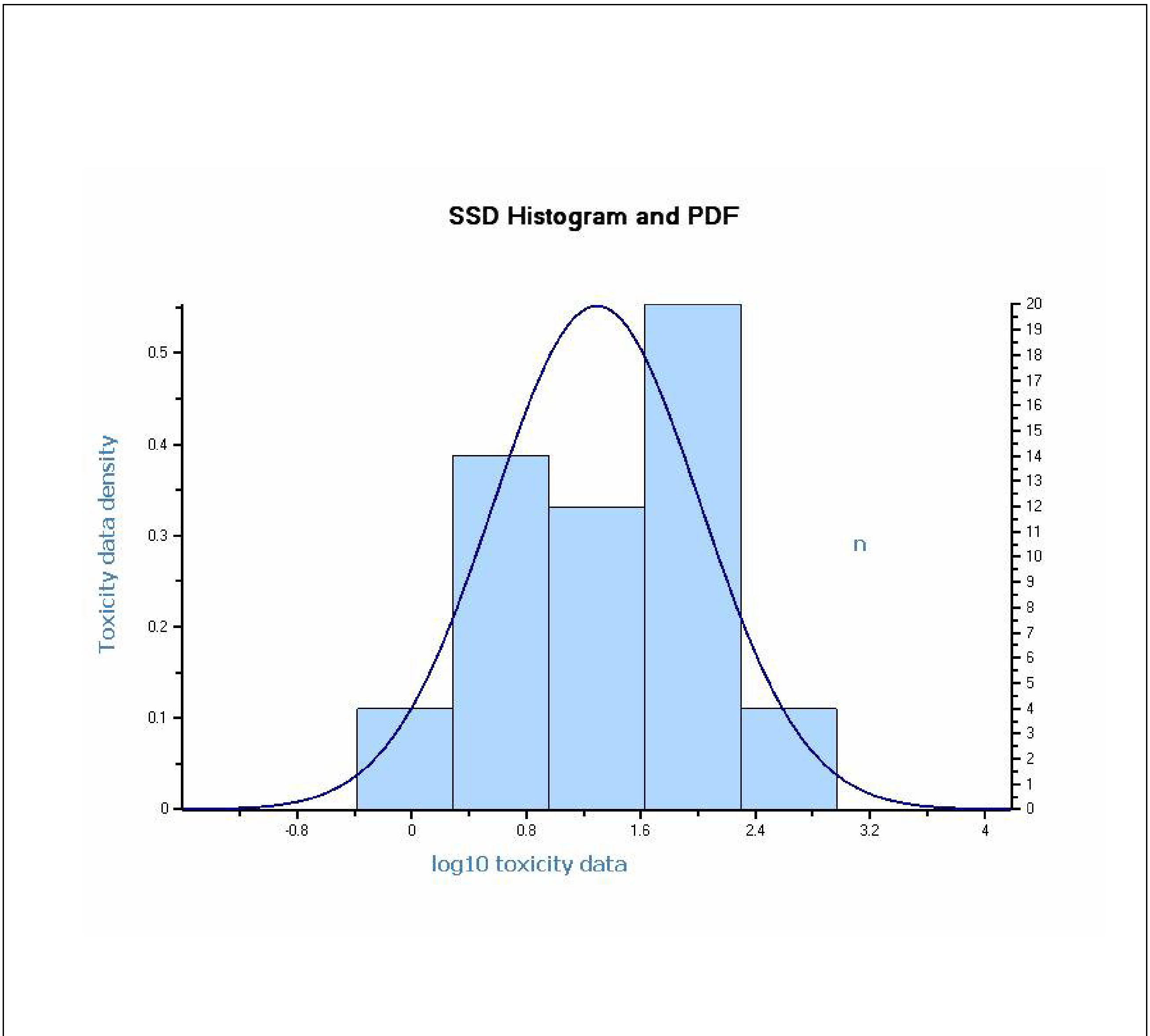
PNEC for metals in the marine environment derived from species sensitivity distributions



Norwegian Institute for Water Research
– an institute in the Environmental Research Alliance of Norway

REPORT

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Abstract

Predicted No Effect Concentrations (PNEC) for the metals cadmium, copper, nickel, mercury, lead and zinc have been calculated using statistical analysis of species sensitivity distributions. From the analysis, the median 5 percentile of the distribution (HC_5) was derived and used as a basis for PNEC. Assessment factors were applied to the HC_5 in order to take account for uncertainty. The following PNECs for the marine environment were derived: Cadmium: 0.18 µg/l, copper: 0.64 µg/l, mercury: 0.04 µg/l, nickel: 1.53 µg/l lead: 2.49 µg/l, and zinc: 3.07 µg/l.

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2. Toksisitet	2. Toxicity
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PNEC for metals in the marine environment derived from species sensitivity distributions

Preface

The Norwegian Oil Industry Association (OLF) contracted the Norwegian Institute for Water Research (NIVA) in October 2005 to review the Predicted No Effect Concentrations (PNEC) used for calculation of Environmental Impact Factors in the North Sea. A Draft Report was delivered in December 2005. The report has been revised in January 2006 to include new data that has been made available through the European Union programme on Risk Assessment of existing chemicals and the development of Environmental Quality Standards under the Water Framework Directive.

The work has been performed by Torsten Källqvist with assistance from August Tobiesen.

Oslo, 30.01.2007

Torsten Källqvist

Contents

Summary

Predicted No Effect Concentrations (PNEC) for the metals cadmium, copper, nickel, mercury, lead and zinc have been calculated using statistical analysis of species sensitivity distributions (SSD). No Observed Effect Concentrations (NOEC) from studies of chronic toxicity of metals to freshwater and marine organisms were compiled from different sources and analysed for statistical distribution according to a log normal model. From the analysis, the median 5 percentile of the distribution (HC_5) was derived and used as a basis for PNEC. Separate analyses were performed on freshwater data, saltwater data and a combination of the two sets of data. Depending on the number of marine species and taxonomic groups represented, and the fit of the log normal distributions, either the HC_5 for the saltwater data or the combined fresh-and saltwater data was used as a basis for PNEC for the marine environment. For nickel it has been proposed to base the PNEC on the freshwater data only. An Assessment factor was applied to the HC_5 in order to take account for uncertainty. The result of the analyses and the proposed PNECs are shown below. The PNECs represent total concentrations, i.e. including background concentrations.

Metal	HC_5 (µg/l)	AF	PNEC (µg/l)
Cd	0.61	3.34	0.18
Cu	1.9	3.01	0.64
Hg	0.15	3.67	0.04
Ni	5.1	3.34	1.53
Pb	7.5	3.01	2.49
Zn	8.2	2.67	3.07

1. Introduction

The Environmental Impact Factor (EIF) is used to express the impact of discharge of produced water from the Norwegian Sector of the North Sea. The determination of the EIF includes PEC/PNEC calculations for various components discharged with the produced water. PNEC-values for 14 organic and metallic components have been calculated from available data on toxicity in accordance with the Technical Guidance Document (EC 1996) and reported by Frost (2002). In 2004, TNO, the Netherlands were contracted to revise the PNECs in according with the recommendations in 2nd. edition of the TGD for derivation of PNEC for the marine environment (EC 2003). The proposed, revised PNECs were lower than the original PNECs for five of six metals and near or even below the background concentrations (Holthaus et al. 2004). For this reason the PNECs were not operational in the EIF context and another attempt to revise the PNECs for metals using a probabilistic approach was suggested.

2. Acronyms

A list of acronyms used in the report is given below.

AF	Assessment Factor
C_b	Background concentration
EC_{50}	Concentration causing 50% effect (i.e. growth reduction)
EIF	Environmental Impact Factor
HC_5	"5 % Hazardous Concentration", The 5th percentile of the SSD
LC_{50}	Concentration causing 50% lethality
MPA	Maximum Permisssible Added Concentration
MPC	Maximum Permissible Concentration
NOEC	"No Observed Effect Concentration"
PNEC	Predicted Negligible Effect Concentration
QS	Quality Standard (Under Water framework Directive)
RAR	Risk Assessment Report
SSD	"Species Sensitivity Distribution"
TGD	"Technical Guidance Document" (EC 2003)

3. Background

The procedure used for calculation of PNEC in the original TGD was developed with anthropogenic organic chemicals in mind. The procedure involves screening and evaluation of available data on acute and chronic toxicity to various organisms. The PNEC is derived from the lowest relevant acute effect concentration (EC_{50}, LC_{50}) or, preferably the lowest No Observed Effect Concentration (NOEC) in chronic toxicity tests. An assessment factor (AF), depending on the amount and quality of the data available is applied on the selected lowest effect or no-effect concentration to derive the PNEC. Typically, when only acute toxicity data are available for minimum one representative species of each of the groups algae, crustacean and fish, the lowest $L(E)C_{50}$ is divided by a AF of 1000 to obtain the PNEC for the freshwater environment. When chronic toxicity data are available for algae, crustacean and fish, the lowest NOEC is divided by the assessment factor 10. In the 2nd edition of the TGD also criteria for derivation of marine PNEC was included. This involves the use of even higher assessment factors unless additional data on toxicity to various marine taxonomic groups are available. The argument for this is the higher taxonomic diversity of the marine ecosystems, which means that a broader basis of toxicity data is required for risk assessment. This **deterministic procedure** for derivation of PNEC is by purpose conservative and appropriate for risk assessment of anthropogenic chemicals. When applied on naturally occurring substances or elements, such as metals it may however produce PNECs which are lower than natural background levels.

The 2nd edition of TGD (EC 2003) includes an alternative procedure for calculation of PNEC for chemicals with a large data base on toxic effects. For these chemicals a **probabilistic approach** based on a **statistical extrapolation technique** may be used. This technique involves an analysis of the distribution of sensitivities among species. The method is based on the proposal of Aldenberg and Slob (1993) and is based on the assumption that the sensitivity of different species, as expressed by their log(NOEC) or some other endpoint, can be described as a statistical distribution. Furthermore it is assumed that the available NOECs represent random samples of this distribution. In this case, the concentration of the toxicant that affects a specified percentile (e.g. 5 %) of all species can be estimated from the sensitivity distribution. Different distribution models, e.g. log normal, log-logistic and triangular have been proposed. The TGD recommends the log-normal distribution as a pragmatic choice because of the available description of its mathematical properties. Tests for goodness of fit (e.g. Kolmogorov-Smirnov and Anderson-Darling tests) can be used as criteria for choice of a parametric distribution for comprehensive data. The Anderson-Darling goodness-of-fit test highlights differences between the tail of the distribution and the input data, while the Kolmogorov-Smirnov test focuses on differences in the middle of the distribution and is not very sensitive to discrepancies of fit in the tail of the distribution. Confidence levels may be attached to the estimated 95 percentile in accordance with Aldenberg and Slob (1993), Adelberg and Jaworska (2000) and the 50% confidence level of the 5th percentile (HC_5) is used as a basis for PNEC. According to the TGD, an assessment factor (AF) should also be applied when PNECs are derived from HC_5. The AF should be in the range 1-5, and a number of criteria have been defined for the choice of appropriate AF (EC 2003).

Risk Assessment (RA) monographs based on the TGD have recently been drafted for several metals (Cd, Cu, Ni, Pb and Zn). For all these metals the available toxicity data bases have been considered sufficient for the use of sensitivity distributions as a basis for PNEC for the freshwater environment. PNEC for the marine environment has so far only been proposed for Pb (EU 2005b), and in this case it is based on combined freshwater and marine toxicity data. The draft RA for Zn (EU 2004a) includes a separate calculation of a HC_5 from the sensitivity distribution of marine NOECs adopted from a Dutch database (Janus 1993). However, a marine PNEC has so far not been proposed. In the RA monographs of Cd, Cu and Ni only freshwater data are included, and no attempts to derive a marine PNEC have been made.

Recently, Environmental Quality Standards for Priority Substances have been developed for the Water Framework Directive. The metals Cd, Ni, Hg and Pb are among the substances for which such Quality Standards (QS) have been proposed. The criteria for derivation of QS for water (freshwater and saltwater) are in close agreement with the methodology for derivation of PNEC as described in the TGD, and SSD analysis have been used as a basis for QS for the metals. Background documents for the QSs are published as Substance Data Sheets by the Fraunhofer Institute (2005a-d).

The use of SSD-analysis requires a sufficient number of data points. The TGD recommends at least 10 NOECs from species representing at least 8 taxonomic groups. Merging of freshwater and marine data in order to perform a sensitivity distribution analysis can only be defended if there is no general difference in sensitivity among organisms in the two environments. Such differences could occur due to differences in physico-chemical properties of fresh and marine waters, which may affect the bioavailability of the metals, or due to physiological differences between the organisms in the two environments. In a Dutch project to develop Maximum Permissible Concentrations (MPC) for metals (Crommentuijn et al. 1997), the available data on toxicity of metals were analysed for significant differences in sensitivity between freshwater and marine organisms. No such differences were found for any of the metals Cd, Cu, Hg, Ni and Zn. The HC_5 for the combined freshwater and marine data was therefore used as a basis for the MPCs. Also the Quality Standards for Pb and Hg in the Water Framework Directive have been based on combined freshwater and marine data. For nickel, however it was noted that marine organisms appeared to be less sensitive than freshwater organisms and a combination of the data was not recommended (Fraunhofer Institut 2005c). In this case the QS for saltwater has been based on freshwater data only, since not enough data was available to perform a SSD-analysis of marine NOECs.

Differences in sensitivity between marine and freshwater organisms are also discussed in a document produced by EURAS (2005). It is concluded that comparing sensitivities should be performed based on the bioavailable fraction rather than on total or dissolved metal concentrations. However, data on bioavailable metal fractions are generally lacking, which hampers thorough sensitivity comparisons between freshwater and saltwater species. Still a relatively good agreement between fresh- and saltwater fish is found. This is partly due to the fact that fish represent only one taxon. For invertebrates, less agreement is found, but this is probably due to the fact that several taxa are included in "invertebrates" (contrary to fish). In general, however, differences in sensitivity between similar freshwater and saltwater species within a taxon seem to be smaller than differences between species belonging to different taxa. Hence, it is likely that extrapolation from freshwater to saltwater species will introduce less uncertainty than extrapolating between non-related taxa (EURAS 2005).

For metals, which are naturally occurring elements, background concentrations are always present in the environment. Furthermore, some elements e.g. Cu, Ni and Zn are essential for biochemical processes, which means that organisms have a minimum requirement for them. It is also known that organisms are able to adapt to different regional background concentrations of metals. In those situations it may be relevant to derive an Additional Predicted No Effect Concentration ($PNEC_{add}$), which, if it is exceeded may cause environmental effects. This $PNEC_{add}$ may be added to the Background concentration (C_b) to obtain $PNEC_{total}$. The added risk approach has been adopted in the RA for zinc but not in the other EU risk assessments of metals (Cd, Cu, Ni and Pb). The added risk approach was also used for deriving the Dutch MPCs . In this case the data on toxicity caused by added concentrations were used to derive a Maximum Permissible Addition (MPA), which was added to the C_b to obtain the MPC. Whether the "Total" or "Added" risk approach is most appropriate will depend on what data is available and for which purpose the risk assessment is performed as discussed by EURAS (2005). If exposure and effect concentrations can be expressed as bioavailable fractions, or if data on background concentration are available, the total risk concept is generally recommended by EURAS (2005). If this is not the case, and if risk management is involved, the added risk approach is recommended. The Water Framework Quality Standards for Cd, Pb, Hg and Ni are based on the added risk approach and the QSs are defined as MPA+background concentrations.

4. Proposed approach to derive PNEC for EIF

The EU risk assessments of metals have shown that the deterministic approach to derive PNEC from the lowest NOEC using Assessment Factors tend to produce PNECs which are unrealistically low. The preferred alternative is therefore derive PNECs by statistical extrapolation. The results obtained using the two approaches are shown in **Table 1**. The PNECs derived using statistical extrapolation have been calculated from the HC_5s using an Assessment Factor. Different AFs (1-3) have been used depending on the uncertainty involved. The appropriate AFs have been subject of intense discussions. Criteria for selection of an appropriate AF are included in the TGD, but the interpretation of these are not always straightforward. It is probably not coincident that the two risk assessment documents where AF=1 has been used have been drafted by industrial organisations.

For comparison the Maximum Permissible Concentrations and Maximum Added Concentrations for metals proposed for the Netherlands by Crommentuijn et al. (1997) are shown in **Table 2**. The MPAs are all calculated from sensitivity distribution according to Aldenberg Slob (1993). This means that MPA = HC_5 (i.e. no AFs are applied).

The proposed Maximum Added Concentrations for derivation of Quality Standards (QS) for metals are shown in **Table 3**. The MPAs are based on SSD analysis of NOEC for freshwater organisms, marine organisms or a combination of the data sets. An Assessment Factor (2 or 3) have been applied on the HC5 to derive the MPA.

Table 1. Preliminary PNECs calculated using a deterministic approach and by the statistical extrapolation technique in draft Risk Assessments of metals performed under EU's program for risk assessment of existing chemicals.

Metal	PNEC concept	PNEC Determ.	HC_5	AF	PNEC Stat. ext.	Comment
Cd (fresh)	Total	0.021	0.38	2	0.19	
Cu (fresh)	Total	1.0	8.2	1	8.2	1
Ni (fresh)	Total	0.4	6.43	3	2.1	
Pb (fresh)	Total	1.2	14.5	1	14.5	
Pb (marine)	Total	1.0	8.3	1	8.3	2
Zn (fresh)	Added	1.7	15.6	2	7.8	
Zn (marine)	Added	1.0	6.1	-	-	3

1) All NOECs normalised to standard abiotic scenario (realistic worst case). HC_5 calculated from an inverse Gaussian distribution
2) $PNEC_{marine}$ obtained by merging freshwater and marine data
3) No marine PNEC proposed because the data used (Janus 1993) have not been updated and checked for reliability

Table 2. Maximum Permissible Concentrations (MPC) , Maximum Permissible Additions (MPA) and Background concentrations (C_b) of metals proposed for the Netherlands (from Crommentuijn et al. 1997).

Metal	MPA	C_b (fresh)	MPC (fresh)	C_b (marine)	MPC (marine)
Cd	0.34	0.08	0.42	0.025	0.37
Cu	1.1	0.44	1.5	0.25	1.4
Hg	0.23	0.01	0.24	0.0025	0.23
Ni	1.8	3.3	5.1	-	-
Pb	11	0.15	11	0.02	11
Zn	6.6	2.8	9.4	0.35	7.0

The suggested approach for calculation of PNECs for metals to be used in EIF is to perform sensitivity distribution on available chronic NOEC-values. The HC_5 obtained from the distribution model will be used as a basis for PNEC (total approach).

Table 3. Maximum Permissible Added Concentrations (MPA) of metals derived as basis for Quality Standards ($QS_{freshwater}$, $QS_{saltwater}$) for the Water Framework Directive. (QS = MPA + background concentration)

Metal	HC5	AF	MPA	Comment
Cd (fresh)	0.38	2	0.19	
Cd (marine)	0.42	2	0.21	
Ni (fresh and marine	5.1	3	1.7	Based on freshwater data only
Pb (fresh and marine)	6.4	3	2.1	Based on combined freshwater and marine data
Hg (fresh and marine)	0.142	3	0.047	Based on combined freshwater and marine data

4.1 Data collection

Toxicity data (chronic NOECs) have been adopted from the Substance Data Sheets for Quality Standards or from RA monographs when available. The data included in these documents have been screened according to quality and relevance criteria as described in the TGD. With a few exceptions no attempt has been done in this project to re-evaluate the data and selections made by the authors of the RA monographs or QS Substance Data Sheets.

For Cu, the draft EU RAR does not include marine toxicity data and no marine PNEC or QS have been proposed. The marine toxicity data have therefore been taken from the database used for setting the MPCs for Netherlands (Crommentuijn et al. (1997) and references therein). It is realised that the criteria for relevance and reliability of data used in the EU RAs are stricter than those used for the Dutch database. However, it has not been possible within the framework of this project to perform a review of the original data. One criterion which is generally adopted in the EU RAs (in accordance with TGD) is that the exposure concentration should be verified by analytical measurements. The

Dutch database contains several data expressed as nominal (not verified) concentrations. The requirement for measured concentrations is, however, more important for organic chemicals, which may be removed from test solutions by adsorption, degradation or volatilisation than for readily soluble metal salts. In the RA for zinc it is noted that nominal concentrations were close to measured concentrations in those studies where both were reported, and also some data based on only nominal concentrations were included. Thus, in the present project NOECs based on nominal, not verified concentrations have been included as marine toxicity data. Another aspect that has been taken into consideration for all the marine toxicity data is that in studies with essential elements it can theoretically occur that the effects observed are caused by element limitation instead of other toxic effects. To prevent this, special attention has been paid to studies resulting in extreme low NOEC values. Studies resulting in extremely low NOEC values have been evaluated and only studies in which there is a concentration-effect relationship have been accepted. This criterion was applied to be sure that NOECs are based on toxic effects instead of limitation (Crommentuijn et a. 1997).

A literature search for additional marine toxicity data has been performed in Cambridge Scientific Abstracts. The search was restricted to publications later than 1994 since earlier publications would be covered by the Dutch database. Finally, data compiled by Holthaus et al. (2004) were checked against the data obtained from the previously mentioned sources and NOEC from tests that were classified as "valid" were added to the data if they were not already included.

In accordance with the TGD, only one NOEC value per species is used. If more than one value for a certain species is available, the most sensitive response is selected (e.g. growth or reproduction). If more than one value is available for the same response, the geometric mean value of the original NOECs has been calculated.

Most of the RA monographs report NOECs as "total concentration", i.e. "added concentration" + "background concentration" in the medium used in the toxicity test. However, when the "Added approach" is used, NOECs should be expressed as "added concentrations". This is the case in the Dutch database used for calculation of MPC (Crommentuijn et al. 1997). Also the RA for zinc has adopted the "added risk approach" and NOECs are listed as "added concentrations". In most cases, the difference between total and added concentration is negligible, but when NOEC is close to the background concentration the total concentration is usually significantly higher than the added concentration and it is necessary to account for the background concentration. For the data used to derive the Dutch MPCs, the background concentrations listed by Crommentuijn et al. (1997) have been added to all NOECs that are less than ten times higher than the background concentration. Thus all concentrations in the present report represent total concentrations..

It has not been possible within the framework of the current project to individually correct all NOECs expressed as "added concentrations" to "total concentrations", since it would require a check of all original data sources. Furthermore, the background concentrations are in many cases not reported. The procedure that has been used for correction of $NOEC_{add}$ to $NOEC_{total}$ is to use default background concentrations (C_b). Since most marine data have been obtained from the Dutch database, where NOECs represent added concentrations, the marine C_bs for the metals Cd, Cu, Ni, Hg, Pb and Zn (See table 2) proposed by Crommentuijn et al (1997) for calculation of MPC from MPA have been added to those NOECs that are less then a factor 10 higher than the C_b. The only freshwater data that are expressed as $PNEC_{add}$ are those for zinc. In this case the lowest end of the range of European freshwater background concentration (3 µg/l) suggested in the RA monograph was added to all $PNEC_{add}$s that were <30 µg/l.

The data included in the analyses are compiled in appendix 1.

4.2 Data analysis

The sensitivity distribution of NOEC-values have been analysed using a log-normal model (Aldenberg and Jaworska 2000) with software ETX$^{2.0}$ obtained from RIVM, the Netherlands (van Vlaardingen et al. 2004). The output includes tests for normal distribution of data and the median 5th percentile of NOECs (HC_5) as well as the lower and higher 95% CI. A graphic presentation of the median curve fitted to the data is also provided.

Goodness-of- fit tests are included to indicate if the assumption of normal distribution is fulfilled. The results of the tests are reported as the level of significance (1, 2.5, 5 and 10 %) at which normal distribution assumption is accepted. This means that “Accepted at ≤10 %” indicates a better fit than “Accepted at ≤5 %”. In case the assumption of normality is not accepted at the 1 % level this is indicated as “Rejected at 1 %”

Separate SSD-analyses have been performed on the freshwater data, salt water data and the combined (freshwater+ salt water) data. Depending on the results of the analyses, including the normality test and difference between the freshwater and salt water data sets, a HC_5 from either the marine or the combined data have been selected as a basis for the marine PNEC. The following criteria have been used:

The HC_5 is derived from the SSD of salt water NOECs when

- No. of marine species (NOECs) ≥10, and
- No. of marine taxonomic groups ≥7, and
- Anderson-Darling test shows acceptance of normality at ≥10 % significance level

If these criteria are not fulfilled, the SSD is analysed on the combined freshwater and salt water data.

5. Statistical Sensitivity Distributions (SSD)

5.1 Cadmium

The fresh water data which were obtained from the draft RA for cadmium includes 28 NOECs (EU 2002). The data selected for the calculations include NOEC data of effects in freshwater, where species geometric mean values have been calculated when more than one NOEC was available for the same species and endpoint.[1] . An additional NOEC for *Hydra* has been added to the data. The marine toxicity data (16 NOECs representing 7 taxonomic groups) have been taken from the Quality Standard Substance Data Sheet for Cd (Fraunhofer Institut 2005a). The data have been selected from the Dutch database adopting data quality criteria developed for the Quality Standards. Three NOECs found in recent scientific journals have been added. The concentrations in the Dutch database represent added concentrations. The marine background concentration (C_b)according to Crommentuijn 1997 (0.025 µg/l) has therefore been added to all marine NOECs which are <10 x C_b. (This did not apply since the lowest NOEC was 0.56 µg/l).

Table 4. Summary of data used for SSD analysis of Cd

	Fresh water	Salt water
No of species (NOECs)	29	19
No. of taxonomic groups	7	7
Source of data	EU RA-Cd + article (1)	QS-Document 6, Cd (Fraunhofer Institut 2005a) + 3 from recent scientific publications

Table 5. Result of SSD analysis of NOEC-values for Cd

	Freshwater	Marine	Combined
HC5 (median)	0.50 µg/l	**0.61 µg/l**	0.24 µg/l
HC5 (upper)	0.79 µg/l	2.07 µg/l	0.49 µg/l
HC5 (lower)	0.27 µg/l	0.101 µg/l	0.097 µg/l
A-D normality test	Accepted at ≤5 %	Accepted at ≤10 %	Rejected at 1 %
K-S normality test	Rejected at 1 %	Accepted at ≤10 %	Rejected at 1 %
Goodness of fit	Acceptable	Very good	Unacceptable

[1] In the EU RA for cadmium the sensitivity disribution was analysed on different sets of data; all individual NOECs, geometric NOECs for each species and "case-by-case geometric mean calculations". In this report the geometric mean for each species (one species, one value) was selected in accordance with the principles used for all other metals.

The HC_5 for the freshwater data is different from the HC_5 reported in the EU RAR for cadmium (See **Table 1**). This is because geometric mean values for NOECs were used for all species and not on a case-by-case basis as in the EU RAR (See footnote 1). The difference in HC_5 between fresh water and salt water is rather small. The combined data set yields a lower HC_5 but the fit of the log-normal model is poor. The number of salt water species and taxonomic groups are sufficient to base the analysis on salt water data only, and the criteria for normality are fulfilled. Thus a HC_5 of 0.61 µg/l is suggested as a basis for $PNEC_{marine}$ for cadmium.

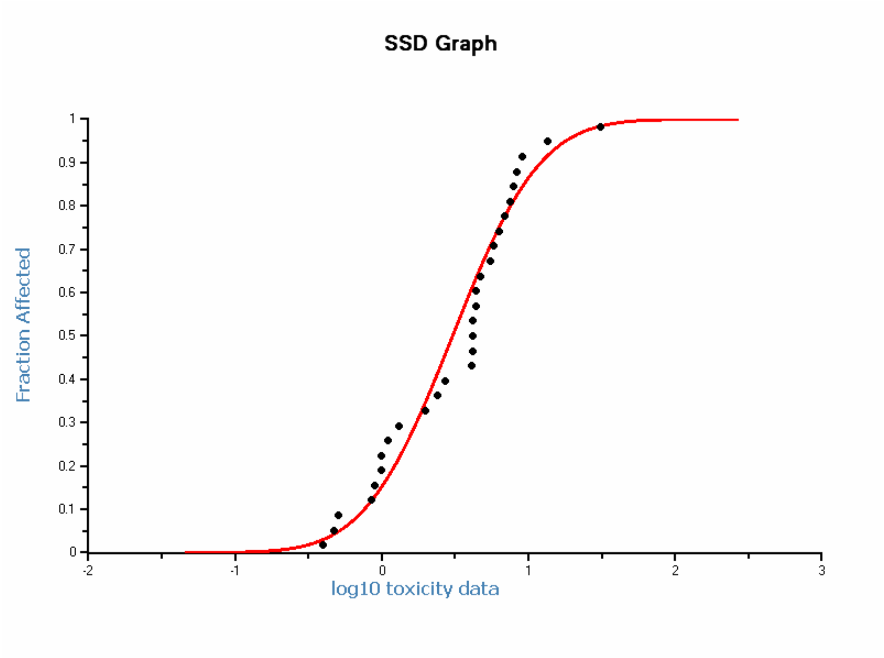

Figure 1. Distribution of NOECs for cadmium – freshwater data

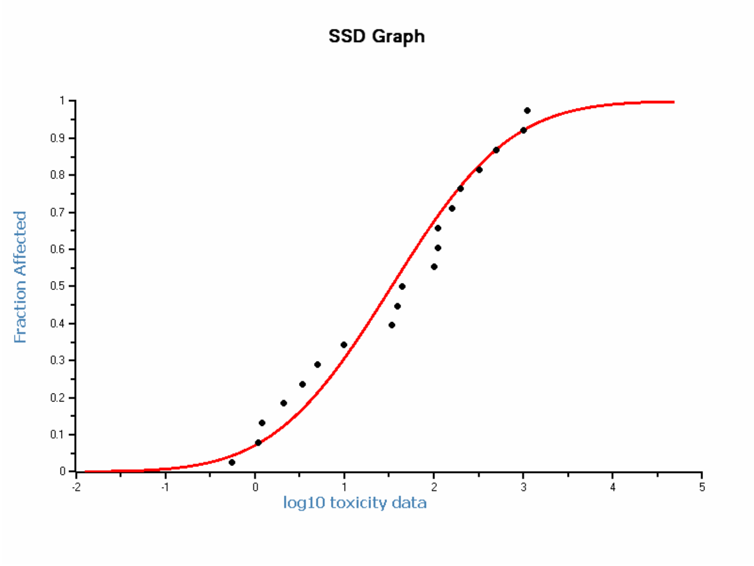

Figure 2. Distribution of NOECs for cadmium – salt water data

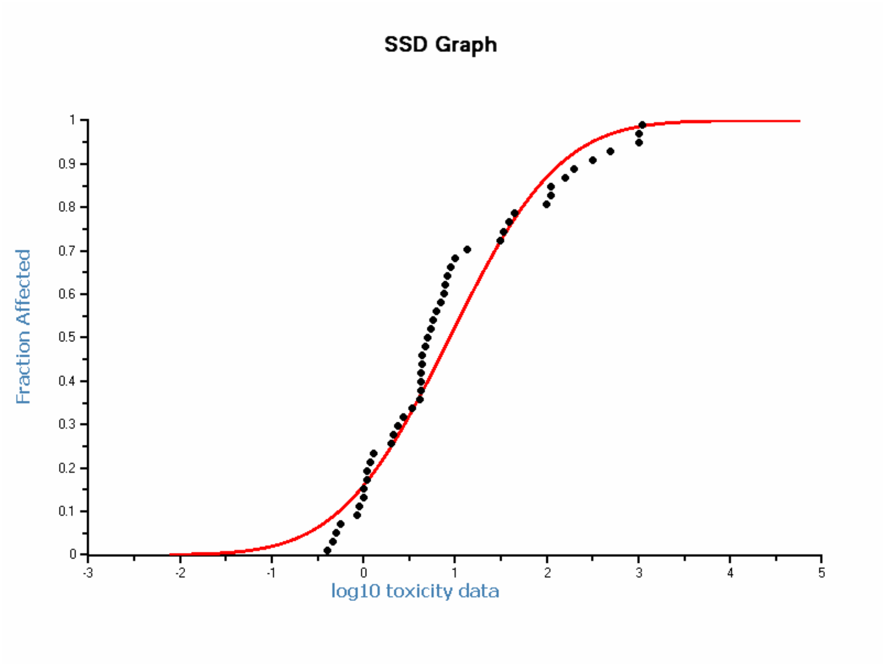

Figure 3. Distribution of NOECs for cadmium – combined freshwater and salt water data.

5.2 Copper

The draft EU RAR for copper includes only freshwater data and a PNEC for saltwater has not yet been proposed. The freshwater NOECs have been normalised to a "realistic worst case freshwater scenario" in terms of abiotic factors that affect the bioavailability of copper.

The salt water data from Crommentuijn et al. (1997) Have been used as a basis for calculation of a saltwater PNEC for EIF. A marine C_b (0.25 µg/l) have been added to NOECs that are <10 x C_b. Five additional marine NOECs found in recent scientific publications have been added.

Table 6. Summary of data used for SSD analysis of Cu

	Fresh water	Salt water
No. of NOECs	22	54
No. of taxonomic groups	7	9
Source of data	EU RAR Cu (EU 2005a)	Crommentuijn et al 1997 + scientific articles (5)

Table 7. Result of SSD analysis of NOEC-values for Cu

	Freshwater	Marine	Combined
HC_5 (median)	8.40	1.25	**1.92**
HC_5 (upper)	11.9	2.11	2.89
HC_5 (lower)	5.13	0.65	1.22
A-D normality test	Accepted at 10 %	Accepted at 1 %	Accepted at 10 %
K-S normality test	Accepted at 10 %	Rejected at 1 %	Accepted at 5 %
Goodness of fit	Very good	Unacceptable	Good

The analysis of the marine NOECs show a much lower HC_5 than for fresh water. However, the data are not normally distributed. For the combined fresh water and salt water data the assumption of normality is fulfilled at 10% significance level according to the Anderson_Darling test and the 5 % level according to the Kolmogorov-Smirnov test. The HC5 for the combined data is much lower than for the freshwater data, and although as much as 54 marine species are included only 3 NOECs (all for algae) are below the HC5. This is close to 5% of the marine species which would be the expected proportion of all species at the true HC5. It is therefore suggested to base the the PNEC for saltwater on the HC5 for the combined data sets (1.92 µg/l).

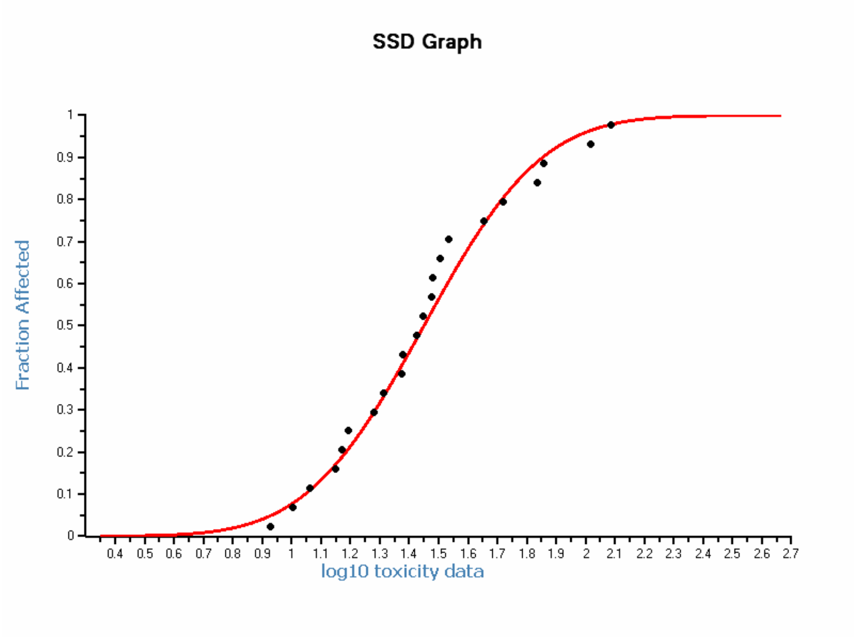

Figure 4. Distribution of NOECs for copper – fresh water data

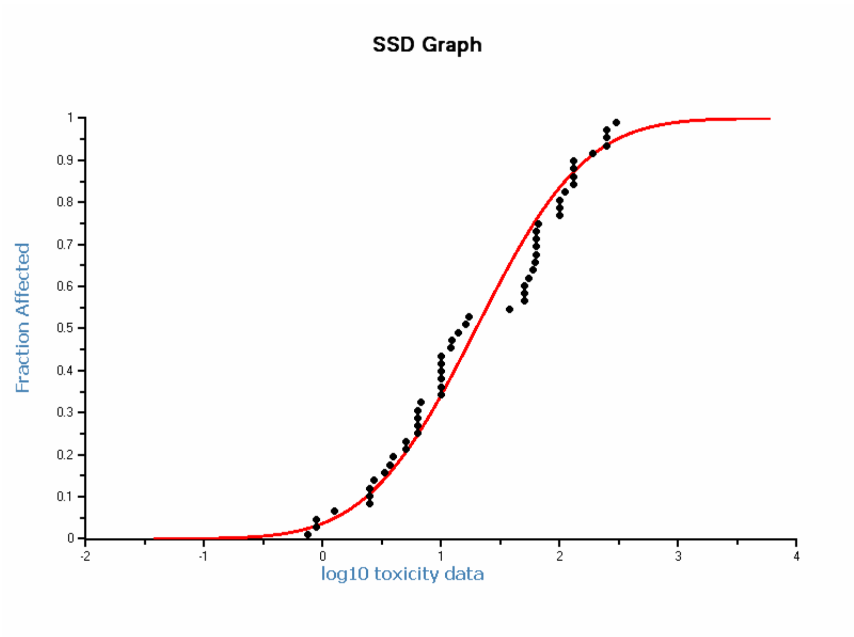

Figure 5. Distribution of NOECs for copper – salt water data

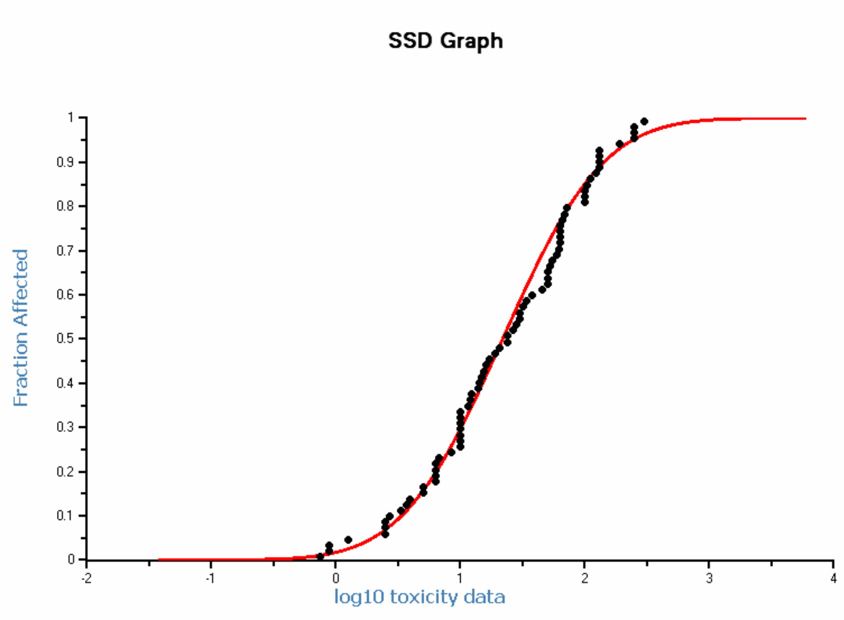

Figure 6. Distribution of NOECs for copper – combined freshwater and salt water data

5.3 Mercury

A European RA monograph has not been produced for mercury but Quality Standards have been proposed for the Water Framework Directive. The data selected for derivation of QS include chronic NOECs for 14 freshwater species (7 taxonomic groups) and 16 marine species (7 taxonomic groups). In the Substance Data Sheet it is noted that "fish and crustaceans appear to be the most sensitive groups in freshwater whereas in saltwater molluscs and coelenterata appear to be even more sensitive than the before mentioned groups". However, since no difference in the lower limit of sensitivity range of freshwater and saltwater species was found, it was suggested to derive the quality standards applicable to freshwater and saltwater environments from the combined data set.

The data selected as basis for the QS have been adopted also for derivation of PNEC for EIF. Results of the SSD-analyses are shown in table 8. The HC5 based on marine and combined marine/freshwater data are very similar, but since the number of marine species and taxonomic groups are sufficient according to the criteria, and the assumption of normal distribution is accepted at the 10 % significance level, it is suggested to base the $PNEC_{marine}$ on the HC_5 of marine NOECs, i.e. 0.146 µg/l.

Table 8. Summary of data used for SSD analysis of Hg

	Fresh water	Salt water
No. of NOECs	14	16
No. of taxonomic groups	7	7
Source of data	QS-Document Hg (Fraunhofer Institute 2005b)	QS-Document Hg (Fraunhofer Institute 2005b)

Table 9. Result of SSD analysis of NOEC-values for Hg

	Freshwater	**Marine**	**Combined**
HC_5 (median)	0.172	**0.146**	0.142
HC_5 (upper)	0.329	0.352	0.282
HC_5 (lower)	0.022	0.038	0.056
A-D normality test	Accepted at 10 %	Accepted at 10 %	Accepted at 10 %
K-S normality test	Accepted at 10 %	Accepted at 10 %	Accepted at 10 %
Goodness of fit	Very good	Very good	Very good

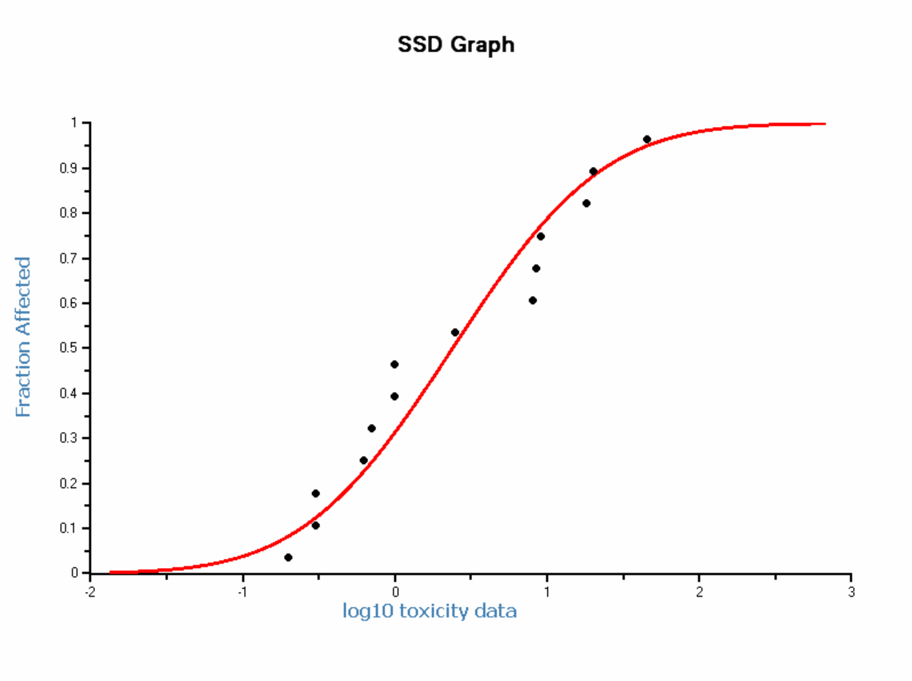

Figure 7. Distribution of NOECs for mercury – freshwater data

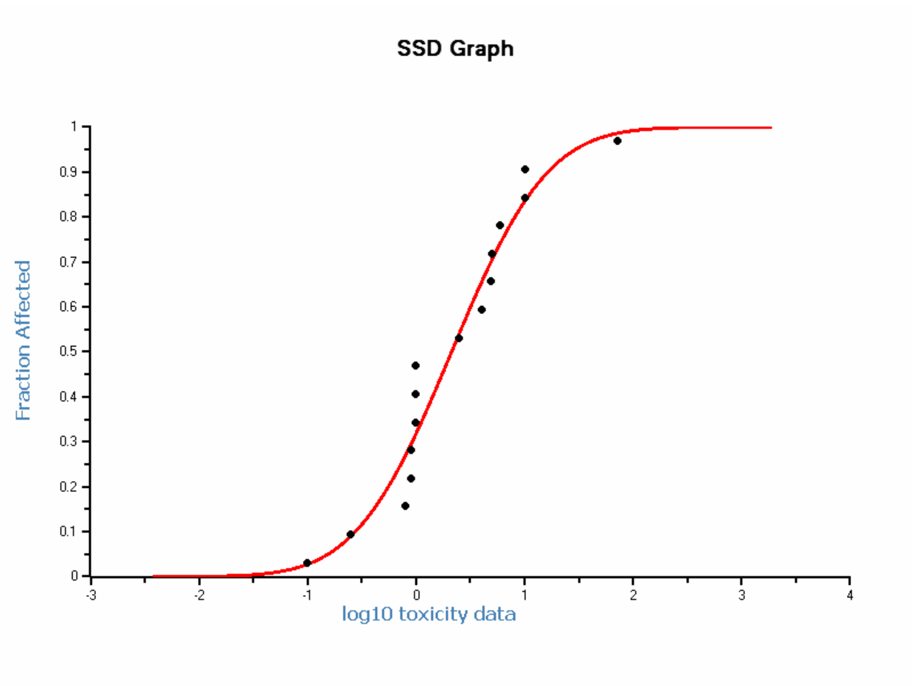

Figure 8. Distribution of NOECs for mercury – salt water data

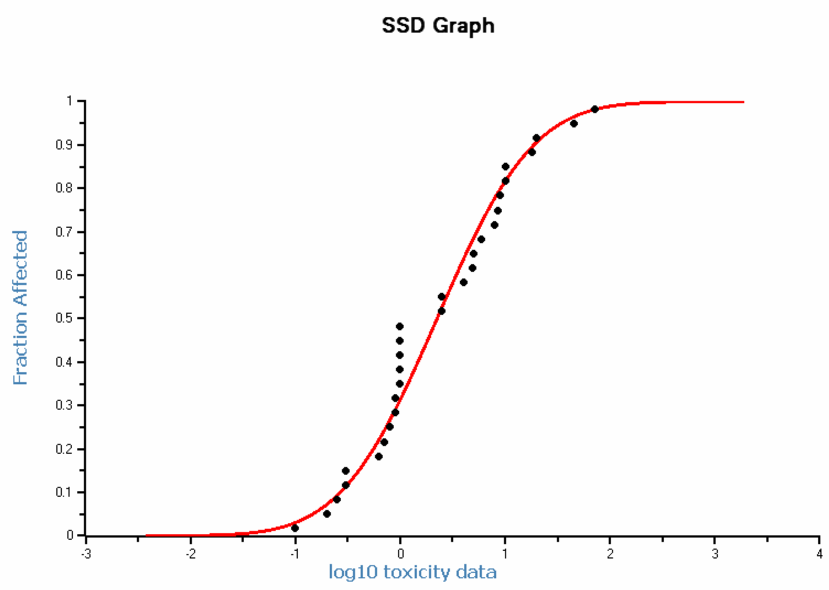

Figure 9. Distribution of NOECs for mercury – combined freshwater and salt water data

5.4 Nickel

Freshwater data on nickel toxicity have been derived from EU's draft RA monograph (EU 2004b) and the Quality Standard Substance Data Sheet for Ni. Only six marine NOECs are included in the latter document. These data indicate that marine organisms are less sensitive than freshwater organisms. Because of lack of data to perform a SSD analysis of marine NOECs it was proposed to apply the same QS for freshwater and seawater. (MPA 1.7 µg/l). The data selected for the European Quality Standards have been adopted as a basis for PNEC derivation for EIF. Since the number of data is too low for an analysis of the sensitivity distribution of salt water NOECs, the SSD analyses have been performed for the fresh water data and the combined data only. The results are shown in table 10. Even if the marine toxicity data indicate that marine organisms are less sensitive than freshwater organisms, the combined data give a lower HC5 than the freshwater data. Furthermore, the normality tests show a lower level of significance for the combined data. Therefore the proposal is to follow the approach used for the Quality Standards, i.e. to apply the freshwater HC_5 and PNEC also in the marine environment.

Table 10. Summary of data used for SSD analysis of Ni

	Fresh water	**Salt water**
No. of NOECs	21	9
No. of taxonomic groups	8	5
Source of data	EU RAR Ni (2004b)	Quality Standards Substance Data Sheet 23 (Fraunhofer Institut 2005c). and Holthaus et al. (2004) (3)

Table 11. Result of SSD analysis of NOEC-values for Ni

	Freshwater	**Marine**	**Combined**
HC_5 (median)	**5.1**	-	4.3
HC_5 (upper)	10.7	-	8.5
HC_5 (lower)	1.77	-	1.7
A-D normality test	Accepted at 10 %	-	Accepted at 1 %
K-S normality test	Accepted at 10 %	-	Accepted at 1 %
Goodness of fit	Very good	-	Unacceptable

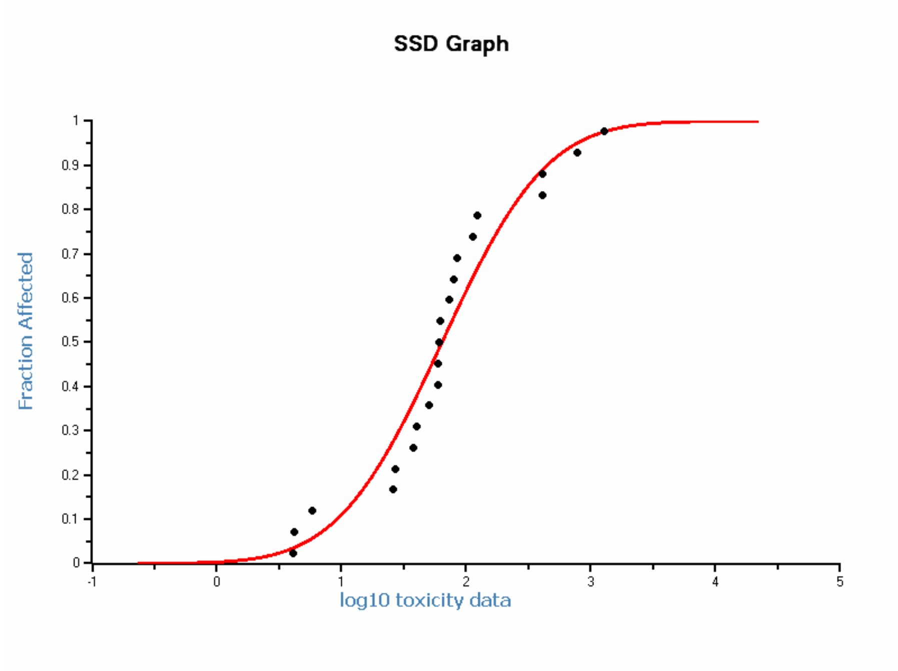

Figure 10. Distribution of NOECs for nickel – freshwater data

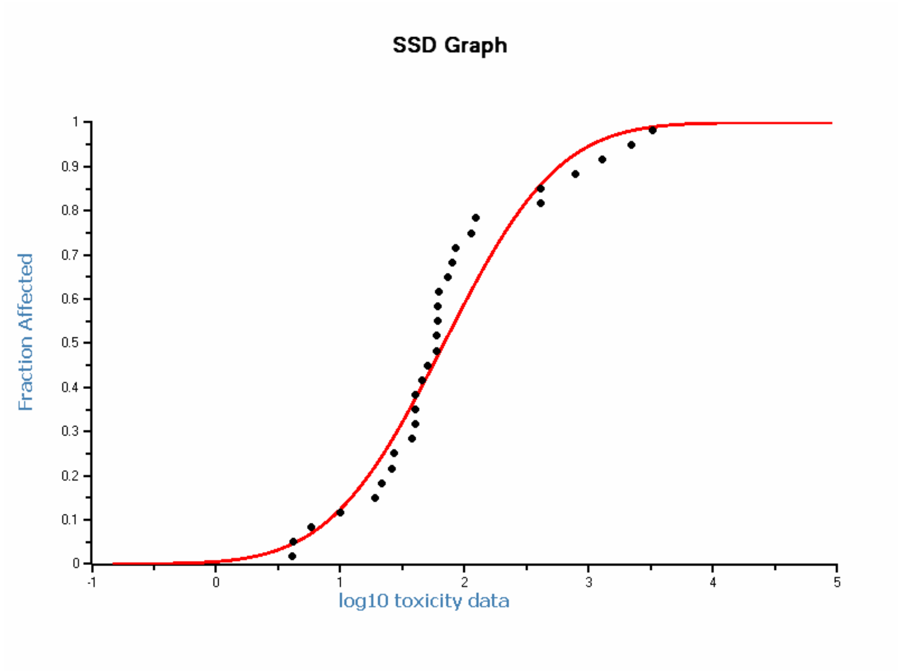

Figure 11. Distribution of NOECs for nickel – combined freshwater and salt water data

5.5 Lead

Freshwater toxicity data have been adapted from t the Quality Standards Substance Data Sheet for lead. (Fraunhofer Institut 2005). These sources contain NOEC for 24 freshwater and 9 marine species. In the QS-Document SSD-analysis have been made for the freshwater, marine and combined datasets even if the marine data includes only 9 data points representing four taxonomic groups. The analyses indicate no significant difference in sensitivity between freshwater and marine organisms and the QS have been based on the HC5 for the combined data sets.

It is proposed to base the PNEC for EIF on the same data as in the QS-document with minor modifications as shown in the appendix . SSD-analyses have been made also for the marine data to show the distribution of sensitivities although the number of taxonomic groups represented are only four. It is proposed to base the PNEC on the HC5 for the combined data sets

Table 12. Summary of data used for SSD analysis of Pb

	Fresh water	**Salt water**
No. of NOECs	24	9
No. of taxonomic groups	8	4
Source of data	EU RAR Pb (EU 2005b), QS-Document 20 (Fraunhofer Institut 2005d)	QS-Document 20. (Fraunhofer Institut 2005d)

Table 13. Result of SSD analysis of NOEC-values for Pb

	Freshwater	**Marine**	**Combined**
HC_5 (median)	9.2	3.78	**7.5**
HC_5 (upper)	16	13	12.5
HC5 (lower)	4.4	0.38	3.76
A-D normality test	Accepted at 10 %	Accepted at 10 %	Accepted at 10 %
K-S normality test	Accepted at 10 %	Accepted at 10 %	Accepted at 10 %
Goodness of fit	Very good	Very good	Very good

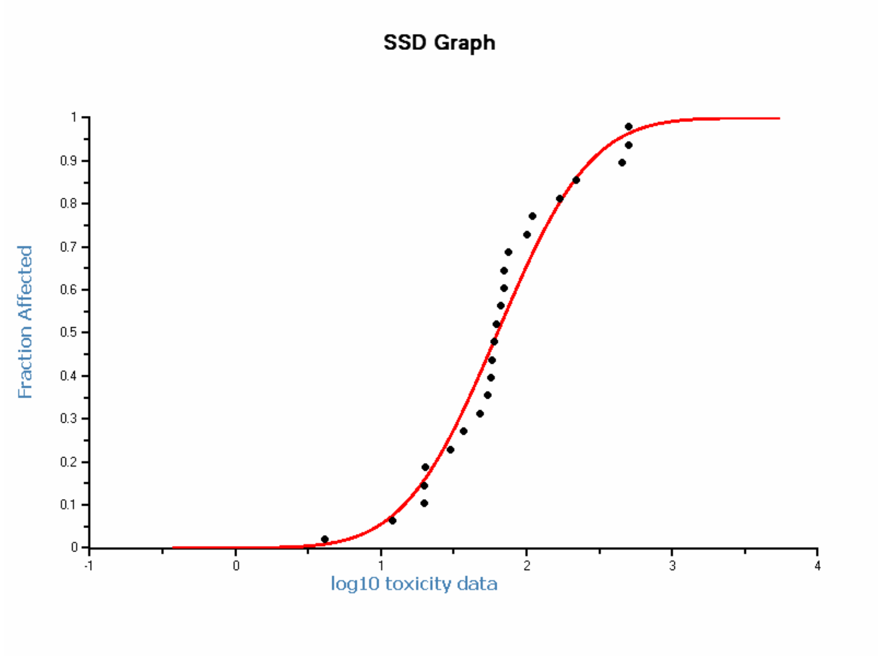

Figure 12. Distribution of NOECs for lead - fresh water data

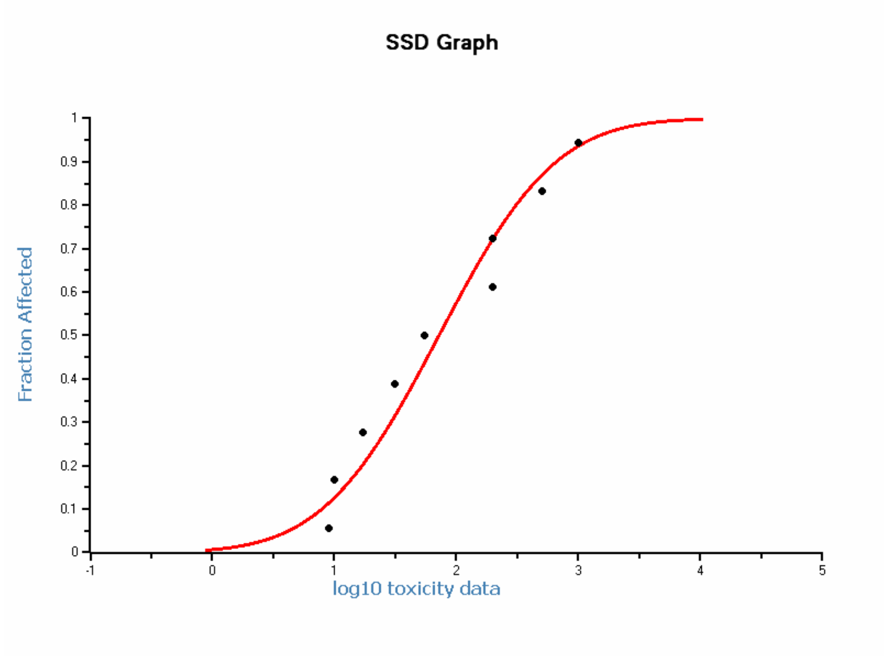

Figure 13. Distribution of NOECs for lead - salt water data

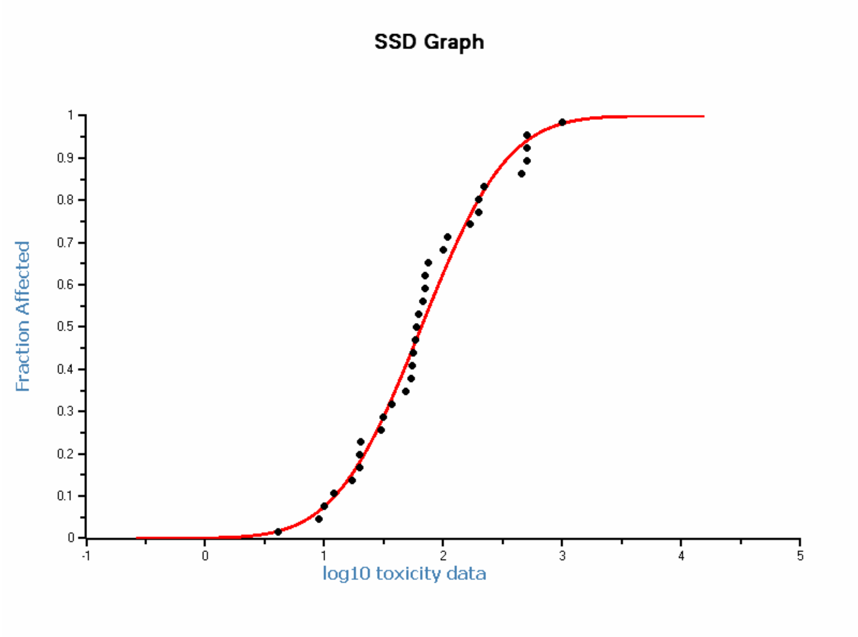

Figure 14. Distribution of NOECs for lead - combined freshwater and salt water data

5.6 Zinc

The toxicity data for zinc has mainly been adopted from the draft EU RAR (EU 2004a). This source contains a revised and updated set of data for toxicity in the fresh water. The marine data that is included has been derived from Janus (1993), which is the same source as was used to calculate the Dutch MPCs in Crommentuijn et al. (1997). The marine data were, however, not scrutinised to the same extent as the freshwater data and therefore no specific PNEC for saltwater was derived in the RA report. The added approach ($PNEC_{add}$) was used in the RA for zinc, and, thus all NOECs should represent the added concentration above the background concentrations. In the present project, the NOECs derived from the RA were corrected by addition of a C_b in case the NOECs were $<10 \times C_b$. The C_b for freshwater was set as 3 µg/l which is the lower range of background concentrations for European freshwaters as suggested in the RA monograph. For saltwater, the C_b = 0.35 µg/l was adopted from Crommentuijn et al. (1997).

Table 14. Summary of data used for SSD analysis of Zn

	Fresh water	**Salt water**
No. of NOECs	20	32
No. of taxonomic groups	7	6
Source of data	EU RAR Zn (EU 2004a)	Crommentuijn et al. (1997)

Table 15. Result of SSD analysis of NOEC-values for Zn

	Freshwater	**Marine**	**Combined**
HC_5 (median)	16	5.67	**8.2**
HC_5 (upper)	26	11.0	12.9
HC_5 (lower)	7.6	2.30	4.58
A-D normality test	Rejected at 1 %	Accepted at 5 %	Accepted at 10 %
K-S normality test	Accepted at 2.5 %	Accepted at 5 %	Accepted at 10 %
Goodness of fit	Unacceptable	Acceptable	Very good

The number of salt water species represented in the chronic toxicity database is high (32) but only 6 taxonomic groups are represented. Furthermore, the Anderson-Darling test shows acceptance of normality only up to 5 %. It is therefore suggested to base the SSD analysis on the combined fresh water and salt water data which are normally distributed. This analysis yields a HC_5 = 8.2 µg/l which is proposed a basis for $PNEC_{marine}$.

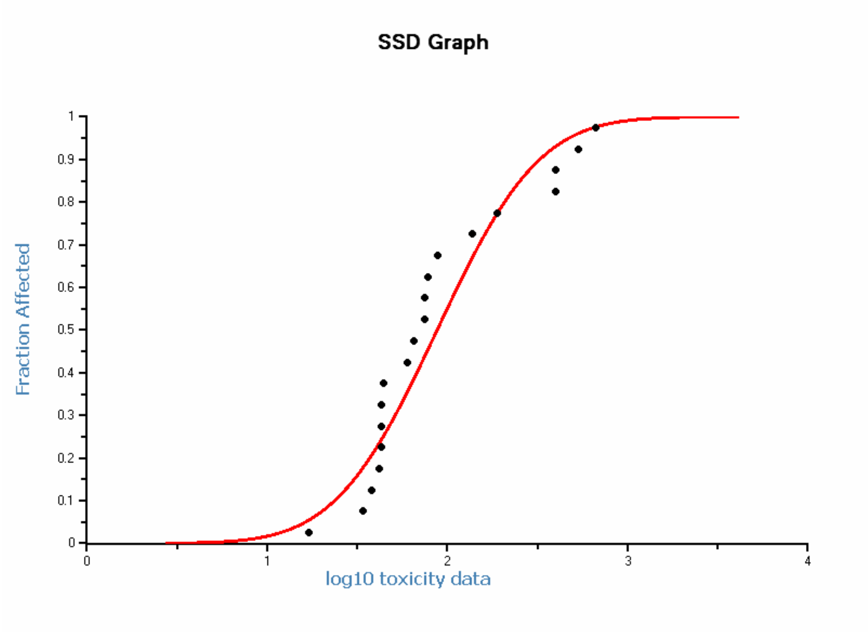

Figure 15. Distribution of NOECs for zinc - freshwater data

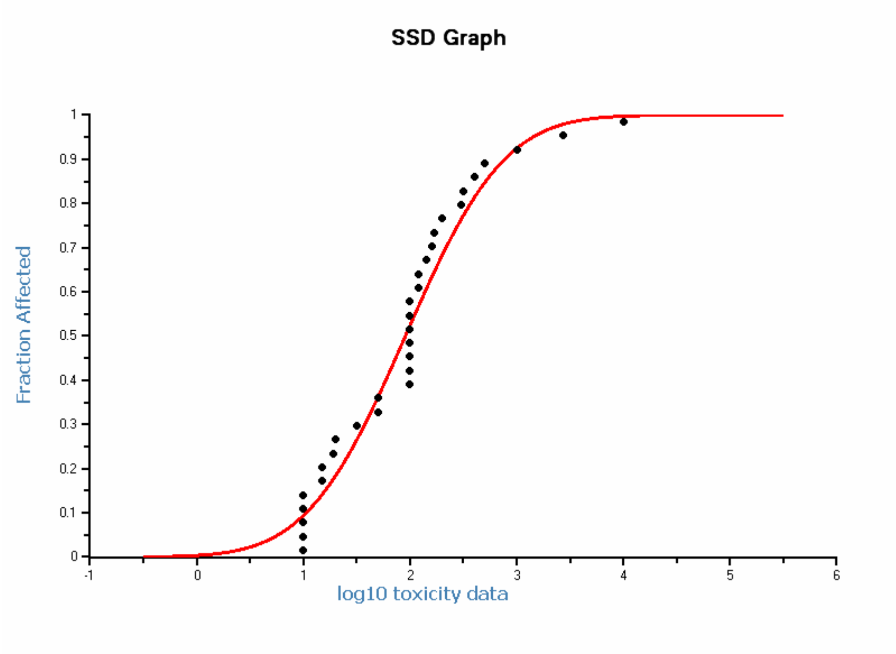

Figure 16. Distribution of NOECs for zinc – salt water data

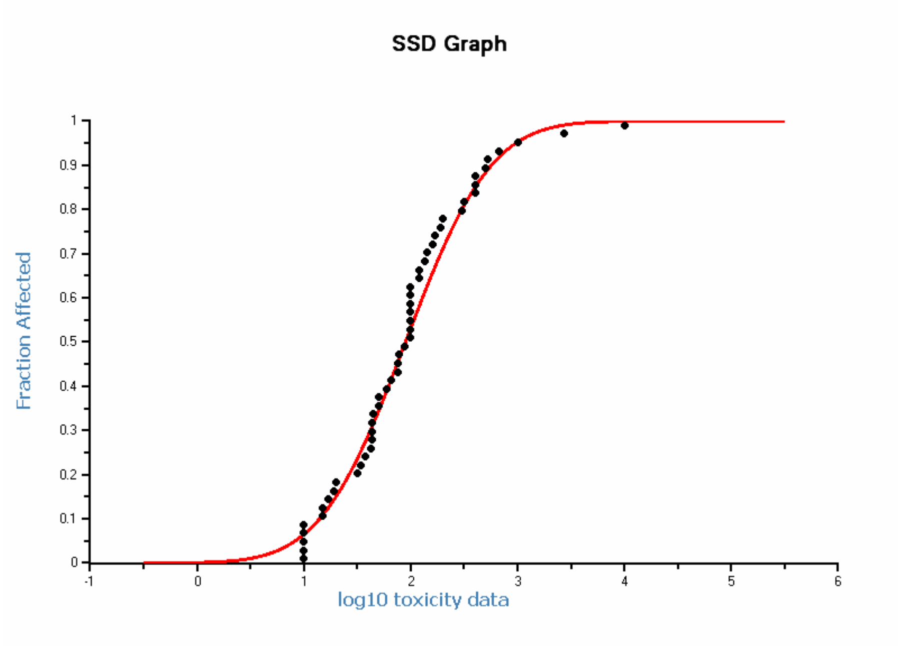

Figure 17. Distribution of NOECs for zinc – combined freshwater and salt water data

6. Calculation of PNEC

According to TGD the PNEC should be derived from the HC_5 by application of an assessment factor (AF):

$$PNEC = \frac{HC_5}{AF}$$

As regards the assessment factor, the following guidance is given in the TGD:

"AF is an appropriate assessment factor between 5 and 1, reflecting the further uncertainties identified. Lowering the AF below 5 on the basis of increased confidence needs to be fully justified. The exact value of the AF must depend on an evaluation of the uncertainties around the derivation of the 5th percentile. As a minimum, the following points have to be considered when determining the size of the assessment factor:

- *the overall quality of the database and the endpoints covered, e.g., if all the data are generated from "true" chronic studies (e.g., covering all sensitive life stages);*

- *the diversity and representativity of the taxonomic groups covered by the database, and the extent to which differences in the life forms, feeding strategies and trophic levels of the organisms are represented;*

- *knowledge on presumed mode of action of the chemical (covering also long-term exposure);*

- *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, and consideration of different levels of confidence (e.g. by a comparison between the 5% of the SSD (50%) with the 5% of the SSD (95%));*

- *comparisons between field and mesocosm studies, where available, and the 5th percentile and mesocosm/field studies to evaluate the laboratory to field extrapolation.*

A full justification should be given for the method used to determine the PNEC.

Further recommendations
NOEC values below the 5% of the SSD need to be discussed in the risk assessment report. For example if all such NOECs are from one trophic level, then this could be an indication that a particular sensitive group exists, implying that some of the underlying assumptions for applying the statistical extrapolation method may not be met;

The deterministic PNEC should be derived by applying the "standard" Assessment Factor Approach on the same database;

If mesocosm studies are available, they should also be evaluated and a PNEC derived following the TGD according to the standard method (deterministic approach).

The various estimates of PNEC should be compared and discussed and the final choice of a PNEC be based on this comparison."

Most of the chronic toxicity data which have been used as a basis for HC_5 calculation in the present report have been adopted from Risk Assessment Reports and Environmental Quality Standards Substance Data Sheets which means that the data adhere to the quality criteria laid down in the TGD. It is felt, however, that the requirement that all data should be from true chronic studies, covering all sensitive life stages is not completely fulfilled for any of the metals. Furthermore, no marine field or mesocosm studies which could be used to evaluate laboratory to field extrapolation have been found and reviewed. For these reasons it is suggested not to apply AFs less than 2 on the HC_5 derived from the present data on chronic toxicity to marine organisms.

The other criteria which are proposed for selection of an appropriate AF, i.e. diversity and representativity of taxonomic groups, statistical uncertainty of the HC_5 and the occurrence of NOECs below the estimated HC_5 have been analysed for the different metals and a scoring system is proposed to account for them in the selection of AF. The proposed scoring system, which is outlined in **Table 16**, has AF=5 as the starting point and this AF is reduced when certain criteria are fulfilled. The lowest AF that can be obtained is 2. The correction values for AF that apply for each of the metals and the final AFs in accordance with the criteria are shown in **Table 17**.

The final proposed PNECs derived from the HC_5s using AFs from **Table 17** are shown in **Table 18**

Table 16. Criteria for correction of AF. For each criterion that is fulfilled, the corresponding correction value is subtracted from the default AF (5).

Criteria	**Correction of AF (Default AF = 5)**
HC_5 based on marine species only	-0.67
No. of species >15	-0.33
No. of taxonomic groups >7	-0.33
Normal distribution at 10 % (A/D-test)	-0.33
HC_5 High/Low 5 – 10 (1)	-0.33
HC_5 High/Low <5 (1)	-0.67
>0-<5 % of NOECs < HC_5 (2)	-0.33
0 % of NOECs < HC_5 (2)	-0.67

(1) "High/Low" is the ratio of the upper and lower confidence limits of HC5 and, thus, represents the with of the HC5 distribution.
(2) This criterion is for the number of NOECs in the data base that are lower than the HC5. It is recognized that with a large data base you would find that some (ideally 5 %) of the NOECs are lower than HC5. Still, a lower number of NOECs below HC5 indicates that HC5 is conservative, and in that case a lower AF can be applied.

Table 17. Correction values that apply for the different metals for derivation of AF. The final AF is shown at the bottom row.

Criteria	**Cd**	**Cu**	**Hg**	**Ni**	**Pb**	**Zn**
marine species only	-0.67		-0.67			
No. of species >15	-0.33	-0.33	-0.33	-0.33	-0.33	-0.33
No. of taxonomic groups >7		-0.33			-0.33	-0.33
Normal distribution at 10 %	-0.33	-0.33	-0.33	-0.33	-0.33	-0.33
HC_5 High/Low 5 - 10	-0.33			-0.33		
HC_5 High/Low <5		-0.67			-0.67	-0.67
>0-<5 % of NOECs < HC_5		-0.33			-0.33	
0 % of NOECs < HC_5				-0.67		-0.67
Sum AF correction	-1.66	-1.99	-1.33	-1.66	-1.99	-2.33
AF	3.34	3.01	3.67	3.34	3.01	2.67

Table 18. Proposed PNEC for various metals obtained by applying AFs on the HC_5s.

Metal	**HC_5 (µg/l)**	**AF**	**PNEC (µg/l)**
Cd	0.61	3.34	0.18
Cu	1.92	3.01	0.64
Hg	0.146	3.67	0.04
Ni	5.1	3.34	1.53
Pb	7.48	3.01	2.49
Zn	8.2	2.67	3.07

7. Comments

For each of the three metals cadmium, copper and mercury the NOEC for one marine species in the data set is slightly below the HC_5 for the corresponding metal. When the AFs are applied, the resulting PNEC is below the lowest NOEC for all metals, which indicates that the proposed PNEC will protect all marine species included in the data base.

A recent publication (Hook and Fisher 2001) on toxic effects of Cd shows effects on reproduction of the marine copepod *Acartia* spp. when parent copepods were fed with algae that had been cultured in water with concentrations of Cd above 2 nM (0.224 µg/l). This NOEC has not been included in the SSD analysis since it does not represent direct exposure through the water phase. However, the results of the test with *Acartia* spp. indicates that exposure through food may be a significant pathway for cadmium. However, The proposed AF (3.34) yields a PNEC which is slightly below the NOEC for

Acartia fed with CD-contaminated algae. Thus the PNEC for Cd will cover also the effects on *Acartia* spp. exposed through the food.

The proposed PNECs for metals are shown together with the original PNECs calculated by Frost (2002) and by Holthaus (2004) in **Table 19**.

Table 19. The proposed marine PNECs for metals, compared to the original PNECs used for calculation of EIF (Frost 2002) and the revised PNECs proposed by Holthaus (2004).

Metal	Frost 2002	Holthaus et al. 2004	This report
Cd	0.028	0.000024	0.18
Cu	0.02	0.014	0.64
Hg	0.008	0.0005	0.04
Ni	1.22	0.6	1.53
Pb	0.182	0.004	2.49
Zn	0.46	0.11	3.07

The PNECs based on SSD-analysis are, as would be expected, higher than those calculated using a deterministic approach by applying assessment factors on the lowest NOECs according to the TGD. The differences are particularly large between the PNECs proposed by Holthaus et al. (2004) and those from the present report. In the case of cadmium, the difference is almost four orders of magnitude. The very low (preliminary) PNEC proposed by Holthaus et al. (2004) is based on a PNEC = 0.24 µg/l for Atlantic salmon (*Salmo salar*) and AF=10000. This study (Rombough & Garside 1982) was, however, performed in low hardness fresh water and the result is not considered relevant for risk assessment in salt water even if the species occurs also in the sea.

For mercury, the difference in PNECs amounts to a factor 80. The low PNEC (0.0005 µg/l) proposed by Holthaus et al. (2004) is based on a chronic NOEC = 0.5 µg/l with AF=1000. The sensitivity distribution indicates that the AF in this case is inordinately protective. The proposed Water Quality Standard for protection of pelagic communities in coastal and territorial waters is 0.047 µg Hg/l, which supports the PNEC proposed in this report (0.04 µg/l).

For lead, the PNEC based on SSD-analysis is more than a factor 800 higher than the deterministically derived PNEC by Holthaus et al. (2004) which is based on an EC_{50} = 4.4 µg/l for the marine alga *Skeletonema costatum* (Rivkin 1979) and an AF=1000. Unfortunately the data reported by Rivkin (1979) do not allow a calculation of a NOEC from that study, but one must assume that the NOEC would be <EC_{50}. The PNEC derived from the SSD is 3.25 µg/l, which is lower than the EC_{50}, but maybe not lower than the NOEC for *S. costatum.* The lowest chronic NOEC in the marine toxicity base (See annex) is 9 µg/l. In the draft EU risk assessment document for lead, the low EC_{50} for growth inhibition of *S. costatum* is not included in the data base, not even among the data considered not valid and the proposed marine PNEC, based on HC_5 with AF=1 is 8.3 µg/l (EU 2005b). It should also be noted that the proposed Water Quality Standard (WQS) for lead in coastal waters is 2.1 µg/l +C_b (Fraunhofer Institut 2005d). This is based on combined freshwater and marine data which gave av HC_5=6.4 and an AF=3.

For copper, nickel and zinc the proposed PNECs, based on sensitivity distributions deviate less from the deterministically determined PNECs, and the differences is a natural consequence of the lower assessment factors applied when PNEC is calculated from the sensitivity distribution.

The proposed PNECs are calculated according to the criteria used for PNEC in EUs Risk Assessment Reports (RAR) and for Environmental Quality Standards (QS) in the Water framework Directive. For the metals Cd, Hg, Ni, Pb and Zn, they are also mainly based on the same data as used in the Risk Assessment Reports and the Quality Standards Substance Data Sheets. Due to a few additional data and different assessment factors the proposed PNECs deviate slightly from the corresponding PNECs from the RARs and the marine QSs (See tables 1 and 3).

For copper, the marine dataset used to derive the PNEC was adopted from the Dutch database (Crommentuijn et al. 1997) since the existing draft RAR does not include a marine assessment. The SSD analysis of the marine data showed a significantly lower PNEC than the freshwater PNEC proposed in the RAR. This may indicate that marine organisms are more sensitive to Cu than freshwater organisms. However, analysis of the freshwater toxicity data for Cu in the Dutch database gives a much lower HC5 than the analysis of the smaller number of data points used in the RAR. This indicates that the more strict selection criteria used in the RAR eliminated some low NOECs included in the Dutch database. Applying the same selection criteria on the marine data may have the same effect which would give a higher marine PNEC for Cu than the one proposed in this report.

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Appendix

Toxicity data

Freshwater chronic toxicity data for Cd

Taxonomic groups	Species	NOECspecies	Comment	Reference[1]
Algae	Asterionella formosa	0.85		EU RAR Cd
Algae	Selenastrum capricornutum	2.4		EU RAR Cd
Algae	Coelastrum proboscideum	6.3		EU RAR Cd
Algae	Chlamydomonas reinhardtii	7.5		EU RAR Cd
Algae	Scenedesmus quadricauda	31		EU RAR Cd
Macrophytes	Lemna paucicostata	**7.9**	Geomean	EU RAR Cd
Hydrozoa	Hydra viridissima	0.4		Holdway et al. 2001
Annelida	Aplexa hypnorum	4.41		EU RAR Cd
Annelida	Physa integra	8.3		EU RAR Cd
Crustacean	Hyalella azteca	0.51		EU RAR Cd
Crustacean	Ceriodaphnia reticulata	**0.92**	Geomean	EU RAR Cd
Crustacean	Daphnia magna	**0.96**	Geomean	EU RAR Cd
Crustacean	Daphnia galeata mendotae	2		EU RAR Cd
Crustacean	Daphnia pulex	**2.7**	Geomean	EU RAR Cd
Crustacean	Ceriodaphnia dubia	**5.5**	Geomean	EU RAR Cd
Insect	Chironomus tetans	5.8		EU RAR Cd
Fish	Salmo salar	0.47		EU RAR Cd
Fish	Brachydanio rerio	1		EU RAR Cd
Fish	Salmo trutta	1.1		EU RAR Cd
Fish	Onchorhynchus kisutch	1.3		EU RAR Cd
Fish	Salvenius fontinalis	**3.9**	Geomean	EU RAR Cd
Fish	Jordanella floridae	4.1		EU RAR Cd
Fish	Catostomus commersoni	4.2		EU RAR Cd
Fish	Esox lucius	4.2		EU RAR Cd
Fish	Oryzias latipes	**4.24**	Geomean	EU RAR Cd
Fish	Salvenius namaycush	4.4		EU RAR Cd
Fish	Salmo gairdneri	**6.9**	Geomean	EU RAR Cd
Fish	Xenopus laevis	9		EU RAR Cd
Fish	Pimephales promelas	**13.5**	Geomean	EU RAR Cd

[1]EU RAR Cd: EU 2002.

Saltwater chronic toxicity data for Cd

$NOEC_{total}$ has been calculated by adding the background concentration (C_b = 0.025 µg/l) to the added concentration according to Crommentuijn et al, (1997) for NOECs > 10×C_b.

Taxonomic groups		NOEC	$NOEC_{total}$	Comment	Reference[1]
Cyanobacteria	Synechococcus bacillaris	3.4	3.4		QS-Document 6
Algae	Peridinium sp	0.56	0.56		QS-Document 6
Algae	Asterionella glacialis	1.1	1.1		QS-Document 6
Algae	Skeletonema costatum	34	34		QS-Document 6
Algae	Chlorella vulgaris	39	39		QS-Document 6
Algae	Dunaliella sp	1100	1100		QS-Document 6
Annelida	Ophryothroca labronica	200	200		QS-Document 6
Annelida	Capitella capitata	320	320		QS-Document 6
Annelida	Neantes aranaceodentata	160	160		TNO (4007)
Nematoda	Monohystera microphtalmata	500	500		QS-Document 6
Crustacea	Allorchestes compressa	2.1	2.1		QS-Document 6
Crustacea	Mysodopsis bahia	**1.2**	**1.2**	Geomean, immobilisation	QS-Document 6
Crustacea	Balanus amphitrite	10	10		Wu et al. 1997
Mollusca	Crassostrea virginica	5	5		QS-Document 6
Mollusca	Mytilus edulis	110	110		QS-Document 6
Fish	Clupea harengus	100	100		QS-Document 6
Fish	Mugil cephalus	**44.7**	**44.7**	Geomean	QS-Document 6
Fish	Pleuronectes flesus	1000	1000		QS-Document 6
Fish	Girella punctata	112	112		TNO (5774)

[1] QS Document 6: Substance Data Sheet 6, Cd, Fraunhofer Institut 2005a
TNO = Holthaus et al. (2004). The numbers in paranthesis are the reference numbers of Holthaus et al. (2004).

Freshwater chronic toxicity data for Cu

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Taxonomic groups	Species	NOEC	Comment	Reference[1]
Algae	Pseudokirchneriella subcapitata	23.8		EU-RAR draft Cu
Algae	Chlorella vulgaris	28		EU-RAR draft Cu
Algae	Chlamydomonas reinhardtii	32.1		EU-RAR draft Cu
Higher plants	Lemna minor	68.8		EU-RAR draft Cu
Molluscs	Campeloma decisum	15.6		EU-RAR draft Cu
Molluscs	Juga plicifera	8.5		EU-RAR draft Cu
Crustacean	Ceriodaphnia dubia	19.1		EU-RAR draft Cu
Crustacean	Daphnia magna	20.5		EU-RAR draft Cu
Crustacean	Daphnia pulex	23.7		EU-RAR draft Cu
Crustacean	Gammarus pulex	29.9		EU-RA draft Cu
Crustacean	Clistoronia magnifica	14.9		EU-RAR draft Cu
Crustacean	Chironomus riparius	72.1		EU-RAR draft Cu
Crustacean	Paratanytarus parthenogeneticus	122.1		EU-RAR draft Cu
Crustacean	Hyalella azteca	103.9		EU-RAR draft Cu
Fish	Ictalurus punctatus	10.1		EU-RAR draft Cu
Fish	Oncorynchus kisutch	11.5		EU-RAR draft Cu
Fish	Oncorynchus mykiss	14.1		EU-RAR draft Cu
Fish	Salvenius fontinalis	30.2		EU-RAR draft Cu
Fish	Pimephales promelas	26.6		EU-RAR draft Cu
Fish	Pimephales notatus	45.3		EU-RAR draft Cu
Fish	Perca fluviatilis	34.2		EU-RAR draft Cu
Fish	Noemacheilus barbatulus	52.5		EU-RAR draft Cu

[1] EU-RA draft Cu = EU 2005a

Saltwater chronic toxicity data for Cu

$NOEC_{total}$ has been calculated by adding the dissolved background concentration (C_b = 0.25 µg/l) to the added concentration according to Crommentuijn et al, (1997) for NOECs < 10×C_b.

TAXONOMIC GROUPS		NOEC	$NOEC_{total}$	Comment	Reference[1]
Cyanobacteria	Synechococcus bacillaris	0.64	0.89	C_b added	RIVM 601501 001
Cyanobacteria	Anabaena variabilis	5	5		RIVM 601501 001
Cyanobacteria	Synechococcus sp	**12.4**	**12.4**	Geomean	RIVM 601501 001
Algae	Cyclococcolithina leptopora	0.64	0.89	C_b added	RIVM 601501 001
Algae	Macrocystis pyrifera	1	1.25	C_b added	RIVM 601501 001
Algae	Chaetoceros sp	2.5	2.5		RIVM 601501 001
Algae	Champia parvula	2.73	2.73	MATC/1.5	TNO (10178)
Algae	Bacteriastrum hyalinum	6.4	6.4		RIVM 601501 001
Algae	Peridinium sp	**6.4**	**6.4**	Geomean	RIVM 601501 001
Algae	Torachosphaera sp	**6.4**	**6.4**	Geomean	RIVM 601501 001
Algae	Umbilicosphaera hulburtiania	6.4	6.4		RIVM 601501 001
Algae	Laminaria saccharina	10	10		RIVM 601501 001
Algae	Symbiodinium microadriaticum	12.1	12.1	MATC/1.5	TNO (45184)
Algae	Prorocentrum sp.	**14**	**14**	Geomean	RIVM 601501 001
Algae	Chlorella vulgaris	17	17		RIVM 601501 001
Algae	Enteromorpha flexuosa	50	50		Andrade et al. 2004
Algae	Ecklonia radiata	62.5	62.5		Bidwell et al. 1998
Algae	Asterionella glacialis	64	64		RIVM 601501 001
Algae	Gephryrocapsa oceanica	64	64		RIVM 601501 001
Algae	Rhizosolenia stolterfothii	64	64		RIVM 601501 001
Algae	Streptotheca tamensis	**64**	**64**	Geomean	RIVM 601501 001
Algae	Emiliana huxleyi	**66**	**66**	Geomean	RIVM 601501 001
Algae	Hymenomonac carterae	**110**	**110**	Geomean	RIVM 601501 001
Algae	Bacteriastrum delicatulum	130	130		RIVM 601501 001
Algae	Hemiaulus sinensis	130	130		RIVM 601501 001
Algae	Rhizosolenia setigera	130	130		RIVM 601501 001
Algae	Umbilicosphaera sibogae	130	130		RIVM 601501 001
Algae	Gymnodinium sp	190	190		RIVM 601501 001
Algae	Biddulphia moluliensis	250	250		RIVM 601501 001
Algae	Dithylum brightwelli	250	250		RIVM 601501 001
Algae	Lithodesmium undulatum	250	250		RIVM 601501 001
Algae	Skeletonema costatum	**301**	**301**	Geomean	RIVM 601501 001
Annelida	Neanthes arenaceodentata	3.9	3.9		RIVM 601501 001
Annelida	Ctenodrilus serratus	50	50		RIVM 601501 001
Annelida	Nereis diversicolor	100	100		RIVM 601501 001
Annelida	Ophryotroca diadema	100	100		RIVM 601501 001
Chordata	Ciona intestinalis	16	16		Bellas et al. 2001
Coelenthreata	Hydra littoralis	2.5	2.5		RIVM 601501 001
Coelenthreata	Campanularia flexuosa	10	10		RIVM 601501 001
Coelenthreata	Eirene viridula	10	10		RIVM 601501 001

Crustacean	Cancer anthonyi, embryo	3.3	3.3		RIVM 601501 001
Crustacean	Allorchestes compressa (larvae)	3.7	3.7		RIVM 601501 001
Crustacean	Pandalus danae	10	10		RIVM 601501 001
Crustacean	Mysodopsis bahia	38	38		RIVM 601501 001
Crustacean	Calianassa australiensis	60	60		RIVM 601501 001
Echinodermata	Paracentrotus lividus	50	50		His et a. 1999
Fish	Atherinops affinis (embryo)	55	55		RIVM 601501 001
Mollusca	Mytilus edulis (larva)	0.5	0.75	C_b added	RIVM 601501 001
Mollusca	Agropecten viradians	2.5	2.5	LOEC/2	TNO (215287)
Mollusca	Mercenaria mercenaria	5	5		RIVM 601501 001
Mollusca	Pecten maximus	6.7	6.7		RIVM 601501 001
Mollusca	Crassostrea virginica	10	10		RIVM 601501 001
Mollusca	Saccostrea commercialis	10	10		RIVM 601501 001
Mollusca	Busicon canaliculatum	100	100		RIVM 601501 001

[1] RIVM 601501001 = Crommentuijn et al. 1997. TNO = Holthaus et al. (2004). The numbers in paranthesis are the reference numbers of Holthaus et al. (2004).

Freshwater chronic toxicity data for Hg

$NOEC_{total}$ has been calculated by adding the dissolved background concentration (C_b = 0.01µg/l) to the added concentration according to Crommentuijn et al, (1997) for NOECs <10×C_b.

Taxonomic groups	Species	NOEC	$NOEC_{total}$	Comment	Reference[1]
Cyanobacteria	Microcystis aeruginosa	2.5	2.5		QS-Document 21
Algae	Scenedesmus acuminatus	0,2	0,2		QS-Document 21
Algae	Scenedesmus capricornutum[2]	1	1		QS-Document 21
Macroalga	Chara vulgaris	20	20		QS-Document 21
Protozoa	Entosiphon sulcatum	9	9		QS-Document 21
Protozooa	Chilomonas paramecium	8	8		QS-Document 21
Mollusca	Viviparus bengalensis	**45**	**45**	Geomean	QS-Document 21
Crustacea	Daphnia magna	**0.7**	**0.7**	Geomean	QS-Document 21
Crustacea	Ceriodaphnia dubia	8.5	8.5		QS-Document 21
Crustacea	Cyclops sp.	18	18		QS-Document 21
Crustacea	Hyalella azteca	0,62	0,62		QS-Document 21
Mollusc	Viviparus bengalensis	45			QS-Document 21
Fish	Pimephales promelas	**0.3**	**0.3**	Geomean,	QS-Document 21
	Salvenius fontinalis	0,29	0.30		QS-Document 21
	Brachydanio rerio	1	1		QS-Document 21

[1]QS-Document 21: Substance Data Sheet 21, Fraunhofer Institute 2005b.

[2] The correct species is probably *Selenastrum capricornutum* (= *Pseudokirchneriells subcapitata*).

Saltwater chronic toxicity data for Hg

$NOEC_{total}$ has been calculated by adding the background concentration (C_b = 0.0025 µg/l) to the added concentration according to Crommentuijn et al, (1997) for NOECs <10×C_b.

Hg – Salt water

Taxonomic groups	Species	NOEC	NOECtotal	Comment	Reference[1]
Algae	Streptotheca tamensis	0.9	0.9		QS-Document 21
Algae	Skeletonema costatum	1	1		QS-Document 21
Macroalga	Fucus serratus	0.9	0.9		QS-Document 21
Macroalga	Laminaria saccharina	1	1		QS-Document 21
Protozoa	Cristigera sp	2.5	2.5		QS-Document 21
Crustacea	Mysidopsis bahia	0.8	0.8		QS-Document 21
Crustacea	Gallinectes sapidus	4.9	4.9		QS-Document 21
Crustacea	Penaeus indicus	6	6		QS-Document 21
Colenterata	Clavopsella micheali	0.1	0.1		QS-Document 21
Molluscs	Crepidula fornicata	0.25	0.25		QS-Document 21
Molluscs	Mercenaria mercenaria	4	4		QS-Document 21
Molluscs	Crassostrea virginica	1	1		QS-Document 21
Annelida	Ctenodrilus serratus	10	10		QS-Document 21
Annelida	Ophryotrocha diadema	71	71	Geomean	QS-Document 21
Fish	Fundulus heteroclitus	10	10		QS-Document 21
Fish	Clupea harengus membras	5	5		QS-Document 21

[1] QS-Document 21: Substance Data Sheet 21, Hg, Fraunhofer Institute 2005b

Freshwater chronic toxicity data for Ni

Taxonomic groups	Species	NOEC	Comment	Reference[1]
Algae	Pseudokirchneriella subcapitata	**74**	Geomean	QS- Document 23
Plants	Lemna gibba	62.5		QS- Document 23
Radiata	Hydra littoralis	60		QS- Document 23
Mollusca	Juga plicifera	124		QS- Document 23
Crustacean	Ceriodaphnia dubia	**4.2**	Geomean	QS- Document 23
Crustacean	Daphnia magna	**26**	Geomean	QS- Document 23
Crustacean	Hyalella azteca	5.8		QS- Document 23
Insect	Chironomus riparius	780		QS- Document 23
Insect	Clistoronia magnifica	**60**	Geomean	QS- Document 23
Amphibia	Gastrophryne caroliensis	4.1		QS- Document 23
Amphibia	Ambystoma opacum	60.4		QS- Document 23
Amphibia	Xenopus laevis	84.5		QS- Document 23
Amphibia	Bufo fowleri	407		QS- Document 23
Amphibia	Bufo arenarum	1270		QS- Document 23
Fish	Onchorynchus mykiss	**27.1**	Geomean	QS- Document 23
Fish	Ictalurus punctatus	38		QS- Document 23
Fish	Brachydanio rerio	40		QS- Document 23
Fish	Salmo salar	50		QS- Document 23
Fish	Pimephales promelas	**79**	Geomean	QS- Document 23
Fish	Micropterus salmoides	113		QS- Document 23
Fish	Carassius auratus	414		QS- Document 23

[1] QS Document 23: Substance Data Sheet 23, Ni, Fraunhofer Institut (2005c).

Saltwater chronic toxicity data for Ni

Taxonomic groups		NOEC	Comment	Reference[1]
Algae	Glenodinium halli	40		TNO (5557)
Algae	Isochrysis galbana	40		TNO (5557)
Algae	Gracilaria tenuistipitata	2200		TNO (18453)
Mollusc	Haliotis rufescens	21.5		QS Document 23
Crustacean	Mysidopsis intii	19		QS-Document 23
Crustacean	Mysidopsis bahia	61		QS-Document 23
Crustacean	Portunus pelagicus	10		QS Document 23
Annelid	Ctenodrilius serratus	45		QS Document 23
Fish	Atherinopos affinis	3240		QS Document 23

[1] TNO = Holthaus et al. (2004). The numbers in paranthesis are the reference numbers of Holthaus et al. (2004).
QS-Document 23: Substance Data Sheet 23, Ni, Fraunhofer Institut (2005c).

Freshwater chronic toxicity data for Pb

Taxonomic groups		NOEC	Comment	Reference[1]
Algae	Chlorella vulgaris	60		QS-Document 20
Algae	Ankistrodesmus sp.	500		QS-Document 20
Algae	Scenedesmus obtusiusculus	500		
Algae	Pseudokirchneriella subcapitata (Selenastrum, Raphidocelis)[2]	100	Geomean of 10 and 1000	QS-Document 20
Cyanobacteria	Microcystis aeruginosa	450		QS-Document 20
Protozooa	Chilomonas paramecium	220		QS-Document 20
	Enthosiphon sulcatum	20		QS-Document 20
	Uronema parduozi	70		QS-Document 20
Rotifera	Brachionus calyciflorus	67		QS-Document 20
Molluscs	Lymnaea palustris	12		QS-Document 20
Crustacean	Ceriodaphnia dubia	**56.5**	Geomean	QS-Document 20
Crustacean	Ceriodaphnia reticulata	**36.7**	Geomean	QS-Document 20
Crustacean	Daphnia magna	**61.8**	Geomean	QS-Document 20
Insect	Chironomus tetans	109		QS-Document 20
Fish	Cyprinus carpio	20.2		QS-Document 20
Fish	Brachydanio rerio	30		QS-Document 20
Fish	Onchorynchus mykiss	4.1		QS-Document 20
Fish	Salmo salar	20		QS-Document 20
Fish	Salvenius namaycusch	48		QS-Document 20
Fish	Salvenius fontinalis	58		QS-Document 20
Fish	Lepomis macrochirus	70		QS-Document 20
Fish	Lepomis gibbonus	54		QS-Document 20
Fish	Ictalurus punctatus	75		QS-Document 20
Fish	Pimephales promelas	**168**	Geomean	QS-Document 20

[1] QS Document 20: Substance Data Sheet 20, Pb, Fraunhofer Institut 2005d. (The same data is used for derivation of PNEC in the EU RAR (EU 2005b))

[2] The NOECs for *Selenastrum capricornutum* and *Raphidocelis subcapitata* have been combined to a geometric mean as these refer to the same species (*Pseudokirchneriella subcapitata*)

Saltwater chronic toxicity data for Pb

$NOEC_{total}$ has been calculated by adding the background concentration (C_b = 0.02 µg/l) to the added concentration according to Crommentuijn et al, (1997) for NOECs > $10 \times C_b$.

Pb- Salt water

Taxonomic groups	Species	NOEC	$NOEC_{total}$	Comment	Reference[1]
Algae	Champia parvula	9.1	9.1		QS-Document 20
Algae	Asterionella japonica	**54.6**	**54.6**	Geomean of 60 and 49.7	QS-Document 20
Mollusca	Crassostrea virginica	200	200		QS-Document 20
Mollusca	Crassostrea gigas	10	10		QS-Document 20
Mollusca	Mercenaria mercenaria	200	200		QS-Document 20
Annelida	Ctenodrillus serratus	500	500		QS-Document 20
Annelida	Ophryotrocha diadema	1000	1000		QS-Document 20
Crustacean	Cancer anthonyi[2]	31	**31**	Geomean of 10 and 100	QS-Document 20
Crustacean	Mysidopsis bahia	17	17		QS-Document 20

[1] QS Document 20: Substance Data Sheet 20, Pb, Fraunhofer Institut 2005d. (The same data is used for derivation of PNEC in the EU RAR (EU 2005b)).
[2] In QS Document 20, the two individual NOECs for this species were used in the analysis, here the geomean has been calculated

Freshwater chronic toxicity data for Zn

$NOEC_{total}$ has been calculated by adding the background concentration (C_b = 3 µg/l) to the added concentration according to EU RA Draft Zn (EU 2004a).

Taxonomic groups	**Species**	**NOEC**	**$NOEC_{total}$**	**Comment**	**Reference**[1]
Algae	Pseudokirchneriella subcapitata	**17**	20	Geomean, C_b has been added	EU RA Draft Zn
Algae	Cladophora glomerata	60	60		EU RA Draft Zn
Cnidaria	Hydra viridis	38	38		Holdway et al. 2001
Porifera	Ephydatia fluviatilis	43	43		EU RA Draft Zn
Porifera	Ephydatia muelleri	43	43		EU RA Draft Zn
Porifera	Eunapius fragilis	43	43		EU RA Draft Zn
Porifera	Spongilla lacustris	65	65		EU RA Draft Zn
Molluscs	Potamopyrgus jenkinsi	75	75		EU RA Draft Zn
Molluscs	Dreissena polymorpha	400	400		EU RA Draft Zn
Crustacean	Ceriodaphnia dubia	**34**	**34**	Geomean	EU RA Draft Zn
Crustacean	Hyalella azteca	42	42		EU RA Draft Zn
Crustacean	Daphnia magna	**88**	**88**	Geomean	EU RA Draft Zn
Insect	Chironomus tetans	137	137		EU RA Draft Zn
Molluscs	Potamopyrgus jenkinsi	75	75		EU RA Draft Zn
Molluscs	Dreissena polymorpha	400	400		EU RA Draft Zn
Fish	Jordanella floridae	**44**	**44**	Geomean	EU RA Draft Zn
Fish	Pimephales promelas	78	78		EU RA Draft Zn
Fish	Onchorynchus mykiss	**189**	**189**	Geomean	EU RA Draft Zn
Fish	Salvenius fontinalis	530	530		EU RA Draft Zn
Fish	Brachyodanio rerio	**666**	**666**	Geomean	EU RA Draft Zn

[1] EU RA Draft Zn = EU 2004a

Saltwater chronic toxicity data for Zn

$NOEC_{total}$ has been calculated by adding the background concentration (C_b = 0.02 µg/l) to the added concentration according to Crommentuijn et al, (1997) for NOECs > $10 \times C_b$.

Zn – sea water

Taxonomic groups		NOEC	$NOEC_{total}$	Comment	Reference[1]
Algae	Chaetoseros compressum	10	10		EU RA Draft Zn
Algae	Schroederella schroedri	10	10		EU RA Draft Zn
Algae	Thalassiosira rotula	10	10		EU RA Draft Zn
Algae	Asterionella japonica	**15**	15	Geomean	EU RA Draft Zn
Algae	Rhizosolenia spp.	15	15		EU RA Draft Zn
Algae	Nitzschia closterium	**20**	20	Geomean	EU RA Draft Zn
Algae	Skeletonema costatum	**32**	32	Geomean	EU RA Draft Zn
Algae	Amphidinium carteri	100	100		EU RA Draft Zn
Algae	Prorocentrum micans	100	100		EU RA Draft Zn
Algae	Scrippsiella faeroense	100	100		EU RA Draft Zn
Algae	Laminaria hypernborea	100	100		EU RA Draft Zn
Algae	Thalassiosira pseudonana	**141**	141	Geomean	EU RA Draft Zn
Algae	Macrocystis pyrifera	170	170		TNO (2349)
Algae	Thalassiosira guillardii	200	200		EU RA Draft Zn
Algae	Gymnodinium splendens	500	500		EU RA Draft Zn
Algae	Phaeodactylum tricornutum	**2714**	2714	Geomean	EU RA Draft Zn
Coelenterates	Eirene viridula	300	300		EU RA Draft Zn
Echinoderms	Arbacia lixula	10	10		EU RA Draft Zn
Molluscs	Haliotes rufescens	10	10		TNO (17224)
Molluscs	Haliotis refercens	19	19		EU RA Draft Zn
Molluscs	Crassostrea gigas	50	50		EU RA Draft Zn
Molluscs	Mecenaria mecenaria	50	50		EU RA Draft Zn
Molluscs	Scrobicularia plana	1000	1000		EU RA Draft Zn
Annelida	Ctenodroillus serratus	**100**	100	Geomean	EU RA Draft Zn
Annelida	Nereis arenceodenata	100	100		EU RA Draft Zn
Annelida	Ophryotrocha diadema	**100**	100	Geomean	EU RA Draft Zn
Annelida	Neanthes arenaceodentata	160	160		TNO (4007)
Annelida	Capitella capitata	320	320		EU RA Draft Zn
Crustacean	Holmesimysis costata	120	120		EU RA Draft Zn
Crustacean	Mysidopsis bahia	120	120		EU RA Draft Zn
Crustacean	Callianassa australiensis	400	400		EU RA Draft Zn
Crustacean	Limulus polyphemus	10000	10000		TNO (19470)

[1] EU RA Draft Zn = EU 2004a. TNO = Holthaus et al. (2004). The numbers in paranthesis are the reference numbers of Holthaus et al. (2004).