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European Union
Risk Assessment Report

CAS: 7440-66-6 **EINECS No: 231-175-3**

ZINC METAL

Zn

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The mission of the JRC-IHCP is to protect the interests and health of the consumer in the framework of EU legislation on chemicals, food, and consumer products by providing scientific and technical support including risk-benefit assessment and analysis of traceability.

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ZINC METAL

Part I – Environment

CAS-No.: 7440-66-6
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RISK ASSESSMENT

Final report, May 2008

The Netherlands

Rapporteur for the risk evaluation of zinc metal is the Ministry of Housing, Spatial Planning and the Environment (VROM) in consultation with the Ministry of Social Affairs and Employment (SZW) and the Ministry of Public Health, Welfare and Sport (VWS). Responsible for the risk evaluation and subsequently for the contents of this report is the rapporteur.
The scientific work on this report has been prepared by the Netherlands Organization for Applied Scientific Research (TNO) and the National Institute of Public Health and Environment (RIVM), by order of the rapporteur.

This Risk Assessment Report is the responsibility of the Member State rapporteurs. In order to avoid possible misinterpretations or misuse of the findings in this draft, anyone wishing to cite or quote this report is advised contact the Member State rapporteurs beforehand.

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Final report: **2008**

Foreword

We are pleased to present this Risk Assessment Report which is the result of in-depth work carried out by experts in one Member State, working in co-operation with their counterparts in the other Member States, the Commission Services, Industry and public interest groups.

The Risk Assessment was carried out in accordance with Council Regulation (EEC) 793/93[1] on the evaluation and control of the risks of "existing" substances. "Existing" substances are chemical substances in use within the European Community before September 1981 and listed in the European Inventory of Existing Commercial Chemical Substances. Regulation 793/93 provides a systematic framework for the evaluation of the risks to human health and the environment of these substances if they are produced or imported into the Community in volumes above 10 tonnes per year.

There are four overall stages in the Regulation for reducing the risks: data collection, priority setting, risk assessment and risk reduction. Data provided by Industry are used by Member States and the Commission services to determine the priority of the substances which need to be assessed. For each substance on a priority list, a Member State volunteers to act as "Rapporteur", undertaking the in-depth Risk Assessment and recommending a strategy to limit the risks of exposure to the substance, if necessary.

The methods for carrying out an in-depth Risk Assessment at Community level are laid down in Commission Regulation (EC) 1488/94[2], which is supported by a technical guidance document[3]. Normally, the "Rapporteur" and individual companies producing, importing and/or using the chemicals work closely together to develop a draft Risk Assessment Report, which is then presented at a meeting of Member State technical experts for endorsement. The Risk Assessment Report is then peer-reviewed by the Scientific Committee on Health and Environmental Risks (SCHER) which gives its opinion to the European Commission on the quality of the risk assessment.

If a Risk Assessment Report concludes that measures to reduce the risks of exposure to the substances are needed, beyond any measures which may already be in place, the next step in the process is for the "Rapporteur" to develop a proposal for a strategy to limit those risks.

The Risk Assessment Report is also presented to the Organisation for Economic Co-operation and Development as a contribution to the Chapter 19, Agenda 21 goals for evaluating chemicals, agreed at the United Nations Conference on Environment and Development, held in Rio de Janeiro in 1992 and confirmed in the Johannesburg Declaration on Sustainable Development at the World Summit on Sustainable Development, held in Johannesburg, South Africa in 2002.

This Risk Assessment improves our knowledge about the risks to human health and the environment from exposure to chemicals. We hope you will agree that the results of this in-depth study and intensive co-operation will make a worthwhile contribution to the Community objective of reducing the overall risks from exposure to chemicals.

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1 O.J. No L 084, 05/04/199 p.0001 – 0075

2 O.J. No L 161, 29/06/1994 p. 0003 – 0011

3 Technical Guidance Document, Part I – V, ISBN 92-827-801 [1234]

PREFACE

For zinc metal (CAS No. 7440-66-6), zinc distearate (CAS No. 557-05-1 / 91051-01-3), zinc oxide (CAS No.1314-13-2), zinc chloride (CAS No.7646-85-7), zinc sulphate (CAS No.7733-02-0) and trizinc bis(orthophosphate) (CAS No.7779-90-0) risk assessments were carried out within the framework of EU Existing Chemicals Regulation 793/93. For each compound a separate report has been prepared. It should be noted, however, that this risk assessment on zinc metal contains specific sections (as well in the exposure part as in the effect part) that are relevant for the other zinc compounds as well. For these aspects, the reader is referred to this risk assessment report on zinc.

0 OVERALL CONCLUSIONS/RESULTS OF THE RISK ASSESSMENT

CAS Number: 7440-66-6
EINECS Number: 231-175-3
IUPAC Name: Zinc

(X) i) There is need for further information and/or testing

(X) ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

(X) iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

(X) iii*) A conclusion applied to local scenarios in which the local scenario merits conclusion (ii) but where (possibly) due to high regional background concentrations a local risk cannot be excluded.

The PNEC values for zinc metal have been derived in this report solely for the purposes of this risk assessment. They must not be used for other purposes, such as setting environmental quality standards or sanitation levels, without further in-depth consideration as to whether they are fit for that purpose. In every case the bioavailability correction, which has been used in the present RAR, should be incorporated as an essential part of the process.

LOCAL
Conclusion (ii) is drawn for all local scenarios, including secondary poisoning, except those listed below.
Conclusion (iii) or (iii*) is drawn for the specified scenarios, because:
STP

- the PEC_{STP} exceeds the $PNEC_{add}$ for microorganisms for a number of the production sites of zinc metal listed in Table 1.105 and a number of the processing scenarios of zinc metal listed in Table 3.105 **(conclusion iii)**.

Surface water

- the calculated $Clocal_{add}$ in water is greater than the $PNEC_{add}$ in surface water for a number of the production sites of zinc metal listed in Table 3.105 and a number of the processing scenarios of zinc metal listed in Table 3.105 **(conclusion iii)**. For some of the production sites of zinc metal the conclusion is based on surface water monitoring data**.**
- the $Clocal_{add}$ / $PNEC_{add}$ ratio is between 0.5 and 1 for a number of the processing scenarios of zinc metal listed in Table 3.105 (**conclusion ii)**, but a potential risk at the local scale cannot be excluded due to the possible existence of high regional background concentrations **(conclusion iii*)**.

Sediment

- the $Clocal_{add}$ in sediment exceeds the $PNEC_{add}$ in sediment for a number of the production sites of zinc metal listed in Table 3.105 and a number of the processing scenarios of zinc metal listed in Table 3.105 **(conclusion iii)**. For some of the production sites of zinc metal the conclusion is based on surface water monitoring data.

- the $Clocal_{add}$ / $PNEC_{add}$ ratio is between 0 and 1 for the remaining production sites of zinc metal and processing scenarios of zinc metal listed in Table 3.105 **(conclusion ii**), but a potential risk at the local scale cannot be excluded due to the possible existence of high regional background concentrations **(conclusion iii*)**.
- the sediment risk characterization at one processing site of zinc metal determined by the SEM/AVS method points to a potential risk for sediment-dwelling organisms **(conclusion iii**).

Soil
- $PEClocal_{add}$ / $PNEC_{add}$ ratios >1 exist for the terrestrial compartment at some processing scenarios of zinc metal listed in Table 3.105 **(conclusion iii).**

Annex 3.4.3 contains recent local exposure information for a number of zinc producers and users. (Disclaimer: Industry Annex 3.4.3 was found by the Rapporteur to be useful to risk management because it sheds further light on the recent local exposure data. Annex 3.4.3 has not been formally approved by either the Rapporteur or TC NES.)
REGIONAL, INCLUDING LINE SOURCES

Conclusion (i) is drawn, because:

- some measured or calculated zinc concentrations in surface waters and sediments alongside motorways in the EU exceed the corresponding $PNEC_{add}$. Due to a number of uncertainties additional information is needed to refine this part of the risk assessment.

Conclusion (ii) is drawn because:

- the risk assessment shows that risks related to terrestrial road borders, zinc accumulation in regional soils and all remaining regional scenarios (including aquatic) of zinc metal, except those listed below, are not expected.

Conclusion (iii) is drawn, because of:

Aquatic ecosystem, including sediment
- measured surface water concentrations indicated that the $PNEC_{add,\ aquatic}$ is exceeded in some, but not all, regional waters in the EU **(conclusion iii).** Sediment PEC_{add} / $PNEC_{add}$ ratios for some, but not all, EU regions point to a potential risk for sediment-dwelling organisms **(conclusion iii)**. This conclusion is based on both calculated and measured data, including SEM/AVS measurements for the Flanders region.

In regions where conclusion iii) is drawn, it is strongly recommended that the available information on known and potential sources of zinc emissions, and region-specific natural background concentrations of zinc are carefully taken into account before taking decisions about risk reduction measures. Annex 3.2.5 already provides some useful information from the side of industry on possible sources of zinc emissions for some regions where a conclusion iii**)** is drawn. **(Disclaimer: Industry Annex 3.2.5 was found by the Rapporteur to be useful to risk management because it sheds further light on the possible sources of zinc and zinc compounds that contribute to regional concentrations from monitoring studies. Annex 3.2.5. has not been formally approved by either the Rapporteur or TC NES.)**

The findings of this report are that the current uses of zinc and zinc compounds do not per se lead to the elevated regional levels found in surface water and sediment.

The elevated zinc levels in those waters and sediments, where they are found, may be caused by a combination of zinc and zinc compounds. The elevated levels come from various emission sources, including local industrial point sources, historical contamination, mining activities, geology and diffuse sources. The contribution of each of these sources may vary between regions.

Local industrial point sources may include industrial processes that use and emit zinc and zinc compounds, as well as other processes that are unintentional sources and are not directly connected with the zinc producing or using industries. These other processes are not examined in this report, but may nevertheless have emissions of zinc.

CONTENTS

Tables

Note: Annexes to this RAR on Zinc Metal are presented in a separate document.

1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS Number: 7440-66-6
EINECS Number: 231-175-3
IUPAC Name: Zinc (min.)
Molecular formula: Zn
Structural formula: Zn
Molecular weight: 65.38
Synonyms: Zinc

Purity/impurities, additives
Purity: >= 94% w/w
Impurity: According to the European Standard EN 1179, all hydrometallurgical Zn production (>80% of all EU Zn production) is of category Z1 (highest purity). Pyrometallurgically produced zinc is of category Z1 and Z2. In the table below, the Z2 type composition of metallic zinc is indicated:

Impurity	Cas-No.	Quantity (% w/w)
Lead	7439-92-1	< 0.005
Iron	231-096-4	< 0.003
Tin	7440-31-5	< 0.001
Copper	7440-50-8	< 0.002
Cadmium	7440-43-9	< 0.005
Total of elements non-Zn	-	<0.01%

Additives: None

Physico-chemical properties

In table 1.1 the physico-chemical properties are summarised.

Table 1.1 Physico-chemical properties of metallic zinc

Property	Result	Comment
Physical state	solid	*
Melting point	420ºC	*
Boiling point	908ºC	*
Relative density	7.14 (20ºC)	*
Vapour pressure	31 Pa at 450ºC	*
Surface tension	0.750 N/m (liquid, 500ºC)	**
Water solubility	insoluble	*
Solubility in other solvents	soluble in acid, alkali, acetic acid	**
Partition coefficient n-octanol/water (log value)	not applicable	*
Flash point	not applicable	****
Flammability	not flammable	***
Autoflammability temperature	not applicable	****
Explosive properties	not explosive	*****
Oxidizing properties	no oxidizing properties	****

* More than one apparently independent source. No methods are specified.

** One source.

*** Industry had showed that stabilised zinc powder was not flammable and have provided the required tests.

**** Conclusion based on theoretical, structural considerations.

**** Explosive properties are strongly tied with type of operation and particle size. At normal conditions not explosive.

These data are mainly derived from (Material) Safety Data Sheets of Metal Europ, Norzink, Sogem and Union Minière, and from CRC Handbook of Chemistry and Physics (1995), Sax's Dangerous Properties of Industrial Materials (1984), and Ullmann's Encyklopädie der Technischen Chemie (1983). For an extended description see HEDSET.

Conclusion:

Data on partition coefficient were not provided. In view of the nature of the substance determination of this parameter is considered to be irrelevant. Information on explosive properties and oxidising properties is not available. However, on theoretical considerations the compound is concluded to be not explosive and not oxidising. Particle size for this compound is expected to depend on the industrial activity involved and may vary largely between industries. Flammability of the compound depends strongly on particle size and extent of blockage by an oxidised outer layer. Therefore, there is no labelling required for zinc massive or zinc powder. All other required physico-chemical data were submitted. None of these data is based on test results, substantiated with reports. However, the data are considered as sufficiently reliable to fulfil the Annex VIIA requirements.

It was agreed at the CMR meeting of September 2002 not to classify stabilised zinc powder for physical-chemical properties and health effects. Classification for environment was already agreed as N; R50-53. The labelling would then be with the Symbol: N, R-phrases:

50/53 and S-phrases: 61. The current classification in Annex I will be replaced by this classification. The classification of pyrophoric zinc powder would remain unchanged in Annex I.

1.2 ENVIRONMENTAL CLASSIFICATION AND LABELLING OF ZINC METAL

1.2.1 General introduction on classification and labelling of metals

This section focuses on the environmental classification and labelling of the EU priority-list chemical zinc metal. The sections 1.2.1 and 1.2.2, however, are also relevant for the other EU zinc compounds, i.e. zinc oxide, zinc phosphate, zinc distearate, zinc sulphate and zinc chloride. Zinc chloride and zinc sulphate, both having a high water solubility, were already classified by the EU Environment Effects Working Group (Classification and Labelling) (EC, 1998). For zinc metal, zinc oxide, zinc phosphate and zinc distearate a new approach was followed for their classification and labelling. This was necessary, as the standard toxicity tests are not appropriate for insoluble metals and sparingly soluble metal compounds. Therefore, the effect concentrations need to be compared to the solubility of the insoluble metals and sparingly soluble metal compounds, for which a "solubility test" or "dissolution test" was developed within the international risk assessment community.

In section 1.2.2 a short general description is given of the "dissolution test". It is also indicated how the dissolution test results should be used for classification purposes. Section 1.3 describes the results of the application of the procedure for zinc metal powder and massives.

1.2.2 Dissolution test for metals and sparingly soluble metal compounds

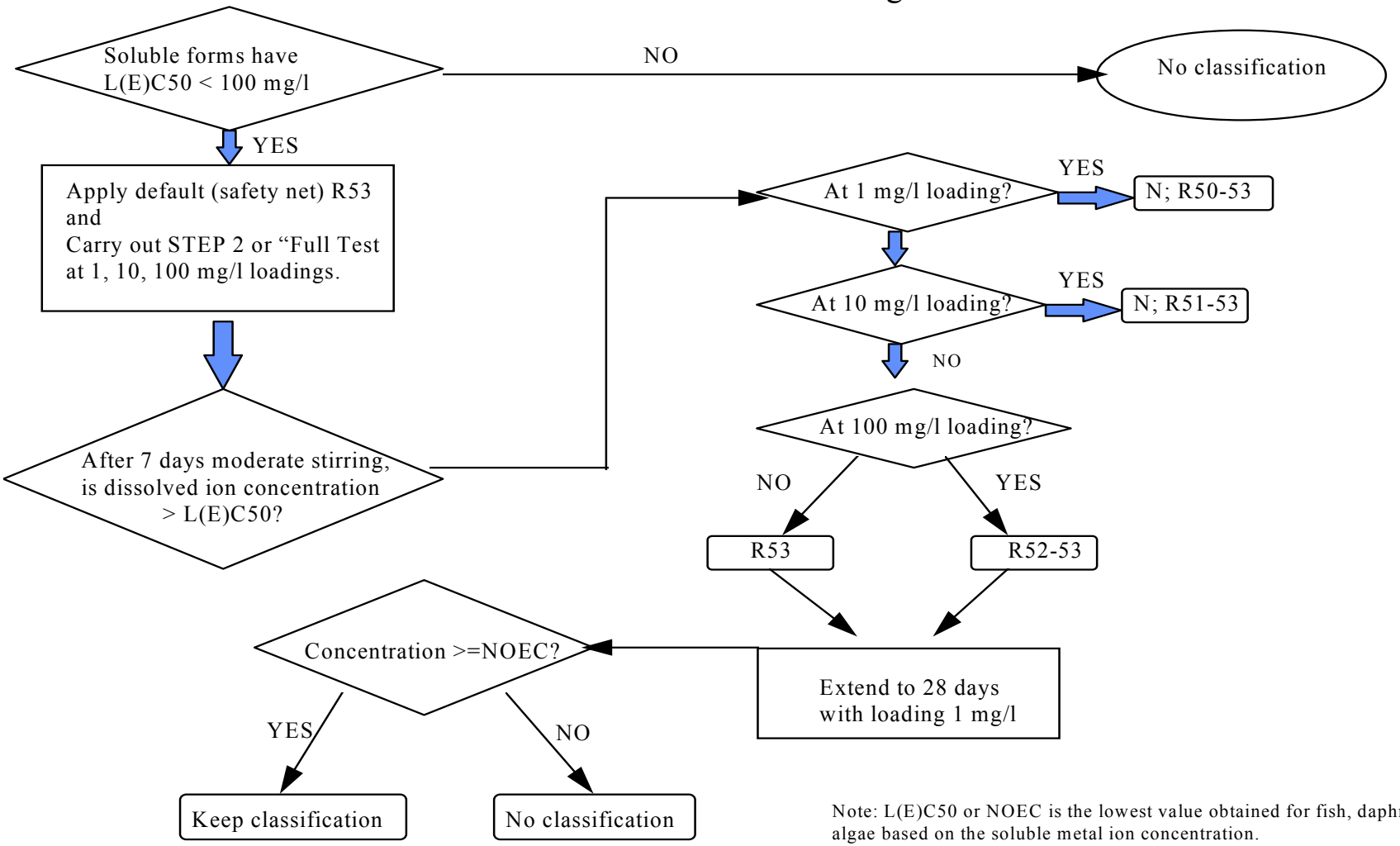
A number of problems are encountered when toxicity tests are carried out with insoluble metals and sparingly soluble metal compounds (OECD Ottawa workshop, 1995). For these compounds, at the beginning of 1997 the dissolution test was developed by EU Member States and industry, in co-operation with the OECD. The discussions resulted in two documents. One document (ECB/TM/9(97) Rev.3) describes the method of the dissolution test and the second document (ECBI/61/95-Add.51-Rev 4) introduces the testing strategy. The latter document also includes guidance on how to use the dissolution test results for classification and labelling purposes. Subsequently, in the EU Environment Effects Working Group (Classification and Labelling) a list was compiled, which presented the metals and metal compounds of Annex I. Those metals and metal compounds should be tested in such a dissolution test for their classification and labelling. The list includes, among others, zinc and zinc oxide (ECBI/61/95 - Add. 60-Rev 1., April 1997).

Description of the dissolution protocol

The aim of the dissolution or transformation test is to determine the rate and extent to which metals or sparingly soluble metal compounds can produce toxic bioavailable forms and whether this rate and extent of formation is of concern and should lead to classification. This protocol should be carried out under standard environmentally relevant conditions (OECD Ottawa workshop, 1995). For metal compounds two types of tests are available: a 24 hours "screening" test and a 7 or 28 days "full" test. The function of the screening test is to identify those metal compounds that undergo dissolution or rapid transformation such that they are indistinguishable from soluble forms. Metal compounds that do not behave in this way are then subjected to a "full" test. For metals only a "full" test is relevant. A 24 hours screening test would be unnecessary since metals can be regarded as insoluble. Massive forms of metals should be tested at a default of 1 mm particle size. Metals in powder form are to be tested at the smallest representative particle size on the market. The most important test parameters for the dissolution protocol are particle size, loading rate, pH and mixing.

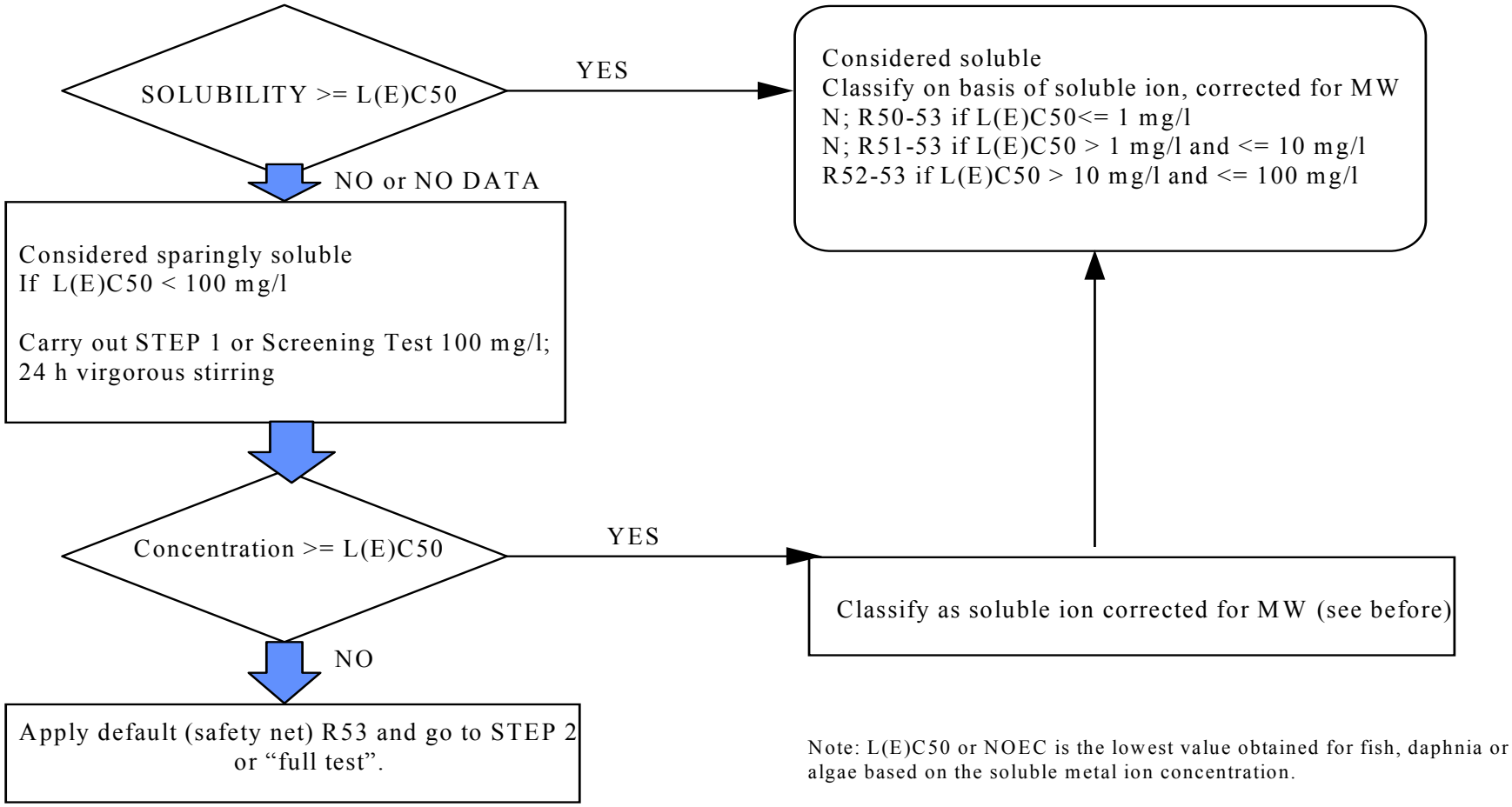
The testing strategy document indicates which dissolution test or test duration should be selected for metal compounds and metals. In addition, guidance is given on the interpretation of the test results for classification. The dissolution test results should be related to the acute toxicity results of soluble metal salts. The crucial question for classification is: at which loading rate is the concentration of the dissolved metal ion greater or equal to the lowest L(E)C50, based on the soluble metal ion concentration? If possible, the ecotoxicity data used for comparison with the dissolution data should be from a test conducted at the same pH as the dissolution test i.e. comparing 'like with like'. The diagrams 1.1 and 1.2 show in detail the testing strategy and the classification route for metals and sparingly soluble metal compounds.

TESTING STRATEGY: Diagram 1.1. METALS



Note: L(E)C50 or NOEC is the lowest value obtained for fish, daphnia or algae based on the soluble metal ion concentration.

TESTING STRATEGY: Diagram 1.2. Sparingly Soluble Metal Compounds



Note: L(E)C50 or NOEC is the lowest value obtained for fish, daphnia or algae based on the soluble metal ion concentration.

1.3 ZINC METAL

1.3.1 Dissolution test results

Transformation testing on zinc metal particles was performed at pH 8 (LISEC 1997), and pH 6 (LISEC 2002). The LISEC study of 1997 includes several tests with different media and four different particle sizes of zinc. The four different particles sizes with median diameter of 7, 207, 469 and 1400-2000 µm are identified as follows: very fine zinc powder (dust), medium sized zinc powder 1 and 2 and coarse zinc powder, respectively. The test with very fine zinc powder is relevant for classification for zinc as powder. The test with coarse zinc powder (1-2 mm) could be used for the classification for zinc in massive form. The LISEC study of 2002 was performed with particle size between 1.4-2 mm in diameter. For reasons of comparison the 2002 test set up included also a check of the transformation of zinc at pH 8, performed under the OECD (2001) protocol conditions.

Results for very fine zinc powder

The test in the LISEC report of 1997 conducted with very fine zinc powder (6.85 µm) in algae medium is considered as "the key study" for the classification of zinc metal as powder. Very fine zinc powder is the smallest representative particle size on the market.

The test parameters and results of all tests in the LISEC-report are presented in Annex 1.3.1. The parameters that deviated from the recommended dissolution are also indicated in this Annex 1.3.1. The LISEC test results with very fine zinc powder are briefly described below.

The metal ion concentration after 7 days, which corresponds with the duration of a full test, is taken into consideration for classification. However, the duration of the "key test" was 16 days and analyses were performed at different time intervals, but not at 7 days. The 7 days concentration was therefore calculated from the measured data with a model that fitted the experimental data. Both the measured and calculated dissolved zinc concentrations after 8 and 7 days, respectively, are presented in Table 1.2 The presented zinc concentrations are at loading rates 1, 10 and 100 mg/l. These loading rates correspond with the recommendations for a full dissolution test. The dissolved zinc concentration at mass loadings of 1 and 10 mg/l increased with time, approaching equilibrium within 2 days. At 100 mg/l, the dissolved zinc concentration increased with large fluctuations, without reaching a steady state after 16 days. The dissolved zinc concentration increased with an increasing mass loading. The results in Table 1.2 for very fine zinc powder will be used for the classification of zinc as powder.

Table 1.2 Measured and calculated dissolved zinc ion concentration after 8 and 7 days, respectively, at loading rates of 1, 10 and 100 mg zinc powder/l (LISEC, 1997).

Very fine zinc powder Particle size: 6.85 µm[1]	Measured concentration after 8 days (mg/l)	Calculated concentration after 7 days (mg/l)[2,3]
Loading rate (mg/l)		
1	0.422	0.459
10	0.949	0.95
100	2.137	-[4]

1. Test was conducted in sterilised algae medium (OECD Guideline 201).
2. The time-dependent concentration (C_t) was calculated with the following first-order equation: $C_t = C_\infty(1-e^{(-kt)})$, C_∞ is the steady-concentration , k is a first-order rate constant, and t is time. The parameters of this formulae were determined by statistical analysis of the results.
3. The calculated concentrations after 7 days are slightly higher than the measured concentrations after 8 days; due to variation in the experimental data points not every point will be exactly predicted.
4. The results could not be fitted in a first order model because of the great variations in the measured dissolved zinc ion concentrations.

Results of zinc with different particle sizes

The results of the LISEC studies with particles size between 1.4 – 2 mm in diameter performed are summarised in table 1.3.

Table 1.3 Transformation over 7 days of zinc metal particles (1.4-2 mm diameter) at different pH.

Mass loading mg Zn/l	Zn dissolved (mg/l) pH 6 (LISEC 2002)	Zn dissolved (mg/l) pH 8 (LISEC 2002)	Zn dissolved (mg/l) pH 8 (LISEC 1997)	pH 6/pH 8
3.3	0.233	/	0.018	12.9
10	0.552	0.066	0.050	8.4 - 9.5
30	1.30	/	0.172	7.6
90	4.34	/	0.440	9.9

From these results, it was concluded that the transformation at pH 6 was significantly higher than at pH 8**.** However, it should be noted that the transformation data presented at pH 6 and 5.5 were obtained under conditions of CO_2-concentration (5%). The OECD guidance (2001) prescribes 0,5 % CO_2 for keeping the pH at 6. For this reason tests have been repeated on the transformation of zinc at pH 6 under OECD recommended conditions of CO_2 (0.5%). The results are presented in table 1.4, and a comparison is made with the results obtained under 5% CO_2.

Table 1.4 Transformation (7 days) of zinc metal particles (1.4-2 mm diameter) at pH 6, under 0,5% CO_2 (according to OECD 2001), and under 5% CO_2.

Mass loading (mg/l)	Zn concentration (mg/l) at 0.5% CO_2.	Zn concentration (mg/l) at 5% CO_2.	Ratio between results obtained at 0.5% and 5 % CO_2
3	0.103	0.233	0.49
10	0.323	0.552	0.59
30	0.69	1.30	0.53
90	3.09	4.34	0.71

The summarised description of the test parameters in the LISEC studies and the measured dissolved zinc concentrations are presented in Annex 1.3.1 A-D.

1.3.2 Results of acute toxicity tests with soluble zinc compounds

1.3.2.1 General

As described in section 1.2.2 the lowest L(E)C50 result should be related to the dissolution test results for obtaining a classification proposal for insoluble zinc and sparingly soluble zinc compounds. There is a large database on short-term toxicity of soluble zinc for a variety of organisms, including the major taxonomic groups i.e. algae, crustaceans and fish.

The short-term toxicity for fish and algae data were mainly obtained from two reviews (WHO, 1996; U.S. EPA, 1980). An additional literature search was performed on CDROM Toxline Plus (1996 to June 1998) and in the database Biosis (1990 to 1998) for the three major taxonomical groups. This search did not reveal any new, relevant data. An overview of all obtained short term toxicity tests, test conditions and references is presented in Annex 1.3.2a-b (Table 1.4). For all three taxonomic groups the industry submitted a full data base including reliability indices. The procedure followed for selecting reliable tests is described separately within this section. The available toxicity data for fish and algae can be considered as limited, but sufficient for classification purposes. The L(E)C50's of these tests are summarised in section 1.3.2.2 (see Table 1.5). The toxicity tests were mainly performed with either zinc chloride or zinc sulphate. Toxicity tests with zinc powder and zinc oxide are also included in Table 1.5 because the effects were based on measured concentrations. However, it should be noted that such toxicity tests with insoluble metals or sparingly soluble metal compounds are not propagated for classification purposes. The results are expressed as the soluble metal, i.e. in mg Zn/l. For chronic toxicity data, see Chapter 3.3 Effect Assessment.

Selection of toxicity data

The rapporteur checked the database submitted by industry. For each reference a reliability index was assigned. The criteria used for selection of short-term toxicity tests largely follows the described criteria for long-term toxicity tests. Information on the used reliability criteria for long-term toxicity tests is described in section 3.3.1.1 and 3.3.2.1. Special attention was paid to zinc as an essential element by accepting only tests where the background concentration of dissolved zinc in culture and test conditions are similar. The rejected tests for crustaceans are presented in a separate table in Annex 1.3.2a (Table 2). The tests are mainly rejected by the rapporteur because of a lack of data on the test method, physical-chemical test conditions (e.g. pH and hardness) or because the endpoint was not considered relevant. A

more critical approach was taken for non-standard tests. It should be noted that tests with nominal test concentrations are considered acceptable. The reliable tests are presented in Annex 1.3.2a (Table 1). In table 1.5 of this section the results of these tests are given.

1.3.2.2 Overview of short-term toxicity results with soluble zinc compounds

Table 1.5 shows a compilation of the short-term toxicity values of different species algae, crustaceans and fish. Details are presented in Annex 1.3.2. The use of geometric or arithmetic mean values within one species group is not applicable because test were conducted under different test conditions. For each species the lowest available L(E)C50 and the range for all toxicity data are given in Table 1.5.

The majority of the available EC50 and LC50 values for crustaceans and fish are below 1 mg/l. Crustaceans seem to be more sensitive to soluble zinc ions than fish and algae. It should be noted that short-term tests conducted with a low hardness (50 mg/l $CaCO_3$), as recommended in the dissolution test, show lower L(E)C50's values than tests with higher hardness concentrations (see Annex 1.3.2). In section 3.2.1.1.3 the influence of hardness on zinc toxicity is explained.

Selected acute EC50-and or LC50-values

The lowest EC50- and LC50-values are selected from the data of *Daphnia magna*, *Oncorhynchus mykiss* and *Selenastrum capricornutum*. The data of these species are selected because they are recommended test species by EC-guidelines. Furthermore, the number of results for these species is large. The lowest L(E)C50-values are selected and presented in bold in Table 1.5.

Table 1.5 Overview of short toxicity of zinc to algae, crustaceans and fish.

Species	Lowest L(E)C50s (mg/l)	Range of L(E)C50s (mg/l)
Algae (n=2)	0.136	0.136-0.150
Selenastrum capricornutum (n=2)	**0.136**	0.136-0.150[1]
Crustacea (n=15)	0.07	0.07-0.86
Daphnia magna (n=10)	**0.07**	0.07-0.86
Daphnia pulex (n=2)	0.11	0.11-0.5
Ceriodaphnia reticulata (n=1)	0.08	-
Ceriodaphnia dubia (n=2)	0.09	0.09-0.36
Pisces (n=15)	0.14	0.14-7.8
Oncorhynchus mykiss (n=5)	**0.14**	0.14-2.6
Cyprinus carpio (n=1)	7.8	7.8
Oncorhynchus kisutch (n=3)	0.82	0.82-1.81
Pimephales promelas (n=4)	0.33	0.33-2.61
Thymallus arcticus (n=2)	0.14	0.14-0.17

[1] Two tests were not conducted with soluble zinc compounds but with zinc metal powder and zinc oxide.

1.3.3 Conclusion and discussion

Zinc as powder

The measured (8 days) and calculated (7 days) dissolved zinc concentrations at a loading rate of 1 mg/l of very fine zinc powder exceed the lowest 48-hour EC50 for *Daphnia magna,* 96-hour LC50 for fish (*Oncorhynchus mykiss*) and 96-hour EC50 for algae (*Selenastrum capricornutum)*. On the basis of these results zinc powder will be classified with N; R50-R53.

The same conclusion can be drawn when a "like with like approach" is taken, thus only comparing toxicity results and dissolution results at a same pH. The pH range in the dissolution protocol was 7.7-8.2. Although zinc powder was not tested at pH 6 in the LISEC study of 2002, it is to be expected that the zinc concentration will be higher as been observed with zinc particles with a size between 1.4-2 mm. This would thus not affect the overall conclusion for the classification and labelling of zinc metal as powder.

The classification and labelling within Annex 1 (67/548-EC) of metal as powders, is based on the smallest representative particle size on the market (see above). It has been proposed to use the critical surface area approach (i.e. the area of a substance needed to deliver the L(E)C50 to

the aqueous medium) in order to avoid labelling of metal powders. Annex 1.3.2c contains some more details on the critical surface area approach. For a full description of this approach reference is made to an OECD-document, which is still under preparation (OECD, 1998).

Zinc in massive form

At present DG environment is consulted for advice on classification and labelling of zinc in massive form. At the time the RAR was finalised, the classification of zinc in massive form was still under discussion.

Classification and labelling (human health, environment and physico-chemical)
At the September 2002 meeting, it was agreed no longer to classify stabilised zinc powder for physical chemical properties (flammability), but to keep the current classification for physical chemical properties (flammability) for pyrophoric zinc powder. It was agreed not to classify the powders for health effects. For zinc massive it was agreed not to classify for physical chemical properties and health effects.

Annex 1 of Directive 67/548/EEC contains a list of harmonised classifications and labellings for substances or groups of substances, which are legally binding within the EU. For zinc metal the current Annex 1 classification and labelling (29th ATP, 2004) is as follows:

Pyrophoric zinc metal powder and dust

Classification

F; R15-17

N; R50-53

Labelling

F; N

R15-17-50/53

S(2-)43-46-60-61

Stabilised zinc metal powder and dust

Classification

N; R50-53

Labelling

N;

R50/53

S60-61

2 GENERAL INFORMATION ON EXPOSURE

2.1 PRODUCTION

Primary ores

Zinc metal production plants in the European Union (EU) with a volume of more than 1000 t/y are presented in Table 2.1.

Table 2.1 Production plants of zinc metal (>1000 t/y) in the EU (Information from industry, 1996/1998)

Company	Location
Rezinal [1)]	Zolder, Belgium
Umicore	Balen, Belgium
Umicore	Overpelt, Belgium
Kokkola Zinc OY (owner New Boliden)	Kokkola, Finland
Metaleurop Nord SAS [2)]	Fontenay-sous-Bois Cedex, France
SA Mapral Sar. [1) 2)]	Fécamp, France
Umicore	Auby, France
Harzer Zinkoxyde Heubach GmbH & Co	Langelsheim, Germany
Metaleurop Weser Zinc GmbH (owner Xstrata Zinc)	Nordenham, Germany
Sudamin MHD	Duisburg-Wanheim, Germany
Ruhr Zink	Datteln, Germany
Enirisorse [3)]	Roma, Italy
Boliden Odda AS	Odda, Norway
Asturiana de Zinc S.A. (owner Xstrata Zinc)	Aviles, Spain
Espanola del Zinc S.A.	Cartagena, Spain
Budel Zink	Budel-Dorplein, The Netherlands
Brittania Zinc Ltd [2)]	Avonmouth, UK

1. Production plant of secondary zinc;
2. Production plant is closed;
3. No production anymore, put “on care and maintenance”.

Table 2.2 Production and consumption of zinc metal within the EU (ILZSG, 1996)

	1993	**1994**	**1995**
Production (tonnes)	2,123,000	2,059,000	2,095,000
Consumption (tonnes) [1)]	1,794,000	1,908,000	2,004,000
Surplus (tonnes)	329,000	151,000	91,000

It is not clear if the total consumption mentioned in this table includes recycled zinc.

The estimated EU production and consumption of zinc is presented in Table 2.2 (ILZSG, 1996). Table 2.2 shows that in the EU from 1993 to 1995 the zinc production is almost equal

to the zinc consumption. From this it can be derived that it is rather unlikely that zinc metal will be imported or exported from outside the EU. There is no detailed information submitted or available about the imported or exported volumes of zinc in the EU. The total production volume of primary zinc metal in the EU used in this report is about 2,193,000 tonnes (see Table 3.12). This figure is based on individual submitted industrial production volumes of 1995.

In the Western World the mine production of zinc was 4,730,000 tonnes in 1990, while 1,940,000 tonnes of zinc were produced from secondary sources (see Table 2.3). Although the figures in Table 2.3 are not up to date, they present a rough indication of the tonnages distributed over the different zinc products.

Table 2.3 Overall zinc production in the Western World1) in 1990 (Nilsson, 1996)

Source of zinc	Amount (tonnes)
Primary zinc production from ores	4,730,000
Zinc produced from recycled zinc containing waste (secondary ores) - of which is:	1,940,000
Primary zinc	470,000
Secondary alloys	210,000
Secondary zinc alloys	200,000
Direct use of scrap	430,000
Zinc in brass	630,000
Total 2)	6,670,000

1) Western World is defined here as the world without the former East Block countries

2) Of this amount 700,000 tonnes are immediately recycled as new or process scrap

Secondary ores

Most zinc is recycled from zinc containing scrap or residues (secondary ores). After a special treatment, the zinc is applied in other processes and products. For a few applications the recycling amount is presented in Table 2.4. Recycling rates for new scrap are about 100% (total 1500000 tonnes of zinc). The wide range of product lifetimes makes a precise calculation of old scrap recycling rates difficult. However, a reasonable evaluation can be made by considering the average lifetime of each of the main products in which zinc is used, and the historical tonnage of zinc in each of these products. Such a calculation suggests that close to 3,000,000 tonnes of zinc should arise from old scrap each year. Of this total a volume of 2,100,000 tonnes are available for recycling and 1,400,000 tonnes (66%) are indeed recycled. Combining the recycling rates for old and new scrap, 80% of the zinc available for recycling is recycled.

Table 2.4 Quantities of zinc recycled from the five dominant fields of zinc application in the Western World (IZA-Europe, 1996)

Field of application	Recycled amount (tonnes) in 1996[1)]
Galvanising	1,000,000
Brass	1,200,000
Die casting alloys	400,000
Semis/sheets	200,000
Chemicals/other	100,000

1) Integrates quantity of zinc recycled from galvanising residues and from steel industry filter dust

The total utilisation of zinc scrap and residues in 1996 was 30% of the total consumption of the metal in the Western World.

The following examples of secondary zinc can be used as raw material in the furnace:

- manufacturing residues (zinc ashes and dross) from hot dip galvanisers
- manufacturing residues (zinc ashes and residues from machining) from manufacturing of zinc die castings
- manufacturing residues (zinc ashes and residues from machining) from manufacturing of sacrificial anodes
- old zinc scrap like zinc plates used in roofs and other constructions and scrapped die castings.

2.1.1 Production process

Zinc is mined using both underground mining and open pit mining (ATSDR, 1994). Underground mining accounts for 62.5% of the mining activities, open pit mining for 14%, mixed underground and open pit for 15% and unspecified for the remaining 8.5% (MG, 1994). Major deposits of zinc ores are found in Canada, Peru and Australia. Zinc ore usually contains more than 4% of zinc. Figures of more than 10% are not unusual (Nilsson, 1996). The average zinc concentration of ore mined at present can be estimated at 10% (MG, 1994). Zinc ore is concentrated at the mine to 50-60% zinc (Cleven et al., 1993; Nilsson, 1996).

In Europe zinc mining is found in Sweden, Spain and Ireland. The quantity of ore mined in EU accounts for about 10% of the zinc concentrate that is refined by EU zinc producers. Sweden has no smelting facilities. All the concentrated zinc ore from Sweden is exported and smelted abroad, mainly in Norway, Finland and Belgium. The Swedish mining production is about 2.8% of the total world production.

In Table 2.5 an overview is given of the total zinc reserves in the world and the quantity which is economically extractable.

Table 2.5 Reserves of zinc in the world (Nilsson, 1996)

Part of the world	Total mineral reserves (tonnes of zinc)	Economically extractable reserves (tonnes of zinc)
North America	118,000,000	56,000,000
South America	18,000,000	12,000,000
Europe	53,000,000	39,000,000
Africa	22,000,000	19,000,000
Asia	40,000,000	25,000,000
Australia	49,000,000	18,000,000
Total	300,000,000	169,000,000

The 169,000,000 tonnes economically extractable reserves given by Nilson represent proven extractable reserves. The real reserves available in the future are determined by price and technological capabilities to extract complex ores. Total technical reserves are difficult to estimate but are much higher. Compared to the Nilsson figure the technical reserve for zinc of 3,400 million tonnes, mentioned by the Dutch Wetenschappelijke Raad voor Regeringsbeleid (WRR, 1994) is much larger.

Two processes are used to retrieve metallic zinc from the ore concentrate:

- the hydrometallurgical process
- the pyrometallurgical process.

a) The hydrometallurgical process

The hydrometallurgical zinc winning process involves four stages: roasting, leaching, purification and electrolysis. In roasting, the raw zinc concentrate is burned in the presence of air to convert it to zinc oxide. In the leaching step, the roasted product (calcine), is dissolved into weak sulphuric acid, in which a solution of zinc sulphate is formed. Iron, also dissolving during the leaching step, is removed by precipitation. The solution is clarified and impurities are removed (and recovered), after which the purified solution is electrolysed, and zinc is deposited at the cathodes, melted and casted. Different kinds of Fe-residues (jarosite, hematite or goethite) are produced during this process, which are stored in waste dump deposits (Cleven et al., 1993; industry information).

b) The pyrometallurgical process

The pyrometallurgical zinc winning process involves the following stages: roasting, sintering, blast furnacing, condensing and refining/casting. The roasting step of the pyrometallurgical process is similar to the one of the hydrometallurgical process. In the next step the zinc oxide is sintered to produce a high strength, porous, low impurity zinc oxide. At 1100°C carbon is oxidised to yield metallic zinc vapour and carbon monoxide. The zinc vapour is subsequently condensed to liquid and drained into moulds. The zinc product from retorting may be subject to various upgrading techniques to remove residual amounts of cadmium and lead (WHO, 1996).

The hydrometallurgical process comprised approximately 83% of the primary zinc production in 1993. Hydrometallurgical and pyrometallurgical processes are used to retrieve zinc from intermediate products, as for instance leach residues, retort residues and zinc-rich slags (WHO, 1996).

Distinct zinc releases from EU mining activities will not be taken into account in the current EU Risk assessment. It is recognised that environmental emissions from mining waste, and

waste in general, can be substantial, but a suitable instrumentarium on how to handle such emissions in a consistent way in the risk assessment is lacking. Some information on actual environmental releases from mining (Sweden, Spain and US) and zinc production is given in sections 3.2.5.3.1 and 3.2.5.2.2, respectively. The influence of (former) mining activities on zinc surface water and sediment concentrations in some EU regions is discussed in the section on risk characterisation (section 3.4.3).

2.2 USE PATTERN

2.2.1 General

Zinc metal is mainly used for coatings and in brass. Zinc metal is further used in die casting alloys, rolled/wrought zinc, pigments and chemicals and for the production of other zinc compounds (see other risk assessment reports on zinc compounds). The quantitatively estimated use percentages for each zinc industry branch are presented in Table . Only the most recent use percentages based on information from industry are further used in this report. The other use percentages according to Nilsson (1996) are only mentioned to support the industrial based percentages. Table shows the industrial and use categories of zinc metal. The industrial and use categories of this table are only a summary of the information from industry mentioned in the HEDSET. One should realise that some use categories are probably more relevant to compounds made from zinc than to zinc metal itself, because it is not always possible to draw a clear border between those two options (e.g. chemicals, pigments). Only the industry branches mentioned in Table will be further used for the local exposure assessment. The two main types of use categories for zinc can be characterised as non dispersive use and use resulting in inclusion into or onto matrix.

Table 2.6 Use percentages for each industry branch (data from International Lead and Zinc Study Group ILZSG and IZA-Europe)

No.	Branch of industry	Use percentage 1997 (information from EU industrial)
1	Galvanising [1)]	38.8%
2	Zinc in brass	25.5%
3	Die casting alloy	12.4%
4	Rolled/wrought zinc	11.8%
5	Zinc powder/dust	2.9%
6	Others (production other zinc compounds)	8.6%

1 New data on galvanisers (ILZGS) are not in conformity with those mentioned in this table (38.8% = 851000 t/y.): 990000 t/y in 1997 and 925000 t/y in 1994. See Table 3.17.

Table 2.7 Industrial and use categories of zinc metal in the EU

Industrial category	EC no.	Use category	EC no
Chemical Industry: basic chemicals	2		
Chemical industry: chemicals used in synthesis	3	Intermediates Laboratory chemicals	33 34
Electrical/electronic engineering industry	4	Conductive agents	12
Personal/domestic	5	Absorbents and adsorbents	1
Metal extraction, refining and processing industry	8	Electroplating agents Others: Production of brass and other zinc alloys	17 55
Paints, lacquers and varnishes industry	14	Absorbents and adsorbents Colouring agents Corrosion inhibitors Reprographic agents	1 10 14 45
Others: Basic metal used in metal industry	15	Corrosion inhibitors Others: Pyrotechnical use	14 55

Others not classified:

Reducing agents

In the following sections short descriptions of the various processes are given.

2.2.2 Galvanising industry

For the galvanising industry a distinction can be made between three different processes. Galvanising can be a hot dip batch process (usually called 'general' galvanising), a continuous hot dip process and a continuous electroplating process.

The general hot dip galvanising is used to coat pre-fabricated steel products (e.g. small ones, such as nails, or larger ones such as lattice grates, steel section and profiles) after their surfaces have been prepared by degreasing, pickling and fluxing (the fluxing step enables the zinc to "wet" the surface of the steel and so permits the galvanising reaction). The plant comprises a series of treatment baths into which the pieces are dipped. The actual galvanising bath ("kettle") is made of steel or a ceramic material. It contains molten zinc into which the fluxed steel fabrications are slowly lowered by overhead cranes. Small products are immersed in a perforated steel basket. The surface of the steel fabrications reacts with the zinc to form a coating consisting of several zinc-iron alloy layers with an outer layer of pure zinc. The immersion time varies from a few up to 30 minutes. The kettle is typically 7m long, 1.4m wide and 2.6m deep, but longer (up to 20m) and deeper (to 4m) kettles may be used. After galvanising, the steel fabrications are removed from the bath and excess zinc is removed (by wiping or "rattling" all articles except tubes and pipes from the outside of which the excess is blown off with compressed air, and steam is used to remove the excess from the inside of pipes). The excess zinc is recovered and is either returned to the zinc bath or sent for zinc recovery in the secondary industry. Zinc-enriched dross and bath skimmings are also either re-utilised in the plant or by the secondary zinc industry. Ammonium chloride, a component of the flux, sublimes at temperatures below the melting point of zinc. This, together with other ongoing reactions, causes fume generation during hot dipping. Hence, the zinc kettles are

either situated in a ventilated enclosure or are ventilated by a lip extraction system. The ventilation air is cleaned in bag filters (Industrial information EGGA).

In the continuous hot dip process a steel sheet/strip/coil is continuously unwound and passed through molten zinc in a fully automated process. The coils are automatically welded end-to-end to give a truly continuous process. Continuous hot dip coating lines for sheet involve surface cleaning (chemical and/or thermal treatment), heat treatment, zinc coating and finishing. The galvanising bath consists of one or more tanks, usually made of a ceramic material, which contain molten zinc. The steel passes through the bath and its surface is coated with some iron-zinc alloys, but because of the speed of the strip (up to 180 m/min) and the short exposure time, the coating consists mainly of zinc. When the strip leaves the bath, gas (air or nitrogen) "knives" wipe off the excess zinc. An automatic gauge that measures the thickness of the coating, using e.g. X-ray measurement technology controls the system. Ventilation is by an extraction system. The ventilation air is cleaned in bag filters and the zinc is recovered. The strip is gradually cooled by air coolers after leaving the bath, quenched in a water tank, dried, "finished" (to give the desired surface properties and appearance), edge-trimmed if necessary, cut to the required length and re-wound. "Galvannealing" is a special after treatment in which the galvanised strip is heated again to allow the formation of a zinc-iron alloy (10% iron) on the surface, giving a particularly smooth appearance. It is possible to vary the system so that just one side of the strip is coated. Coils of finished galvanised steel are large and very valuable products that have to meet very stringent quality requirements. They are always stored under cover, usually in areas with temperature and humidity control. They are also always protected against oxidation by a chromate rinse layer, an oil film, plastic wrap or interleaved paper or a combination of some of these. In addition, they are always transported on covered truck beds. Therefore, there is no possibility of zinc-containing run-off from these products while at the plant or during transport (Industrial information Eurofer, 2001).

In the electroplating process a zinc coating is applied to steel. Before the actual electrogalvanising the steel is cleaned and generally pre-annealed. In the electroplating process a zinc salt solution is used to electrolytically deposit a layer of zinc on steel. The electrolyte is generally a zinc sulfate or chloride solution, whose acidity is adjusted to obtain high current densities without causing excessive corrosion of the plant. The zinc, which forms the coating on the strip (cathode), is supplied either by the anodes (soluble anodes) or by an external source (insoluble anodes), in both cases via the electrolyte. In the first case, when soluble anodes are used, electroplating is performed with high purity (99.99 %) zinc anodes, whose shape is adapted to the electrolysis tank. In the second case the anodes can be made from metals much nobler than zinc (oxide coated titanium anodes, which are more inert). Since zinc is consumed at the cathode, its concentration in the electrolyte must be continuously re-adjusted (sulfate bath). For the majority of sheet applications, electrogalvanizing is performed on cold rolled strip, the steel being directly coated with high purity zinc. Electrolysis ensures a perfectly uniform coating, of constant thickness in both the transverse and longitudinal directions. If required, it is possible to obtain a different thickness on opposite faces (information from industry).

In Table 2.8 an overview is given of the different methods used for surface coating of steel with zinc and the annually consumed amounts in the EU.

Table 2.8 Use of different types of zinc coatings in the EU (data from IZA-Europe, 1994)

Zinc coatings	Use (tonnes)
Continuous hot dip galvanising	425,000
General galvanised steel (hot dip galvanising of steel parts and constructions)	321,000
Tubes / Wires	99,000
Mechanical plating	30,000
Zinc rich paint	20,000
Zinc spraying	20,000
Electroplating	10,000
Total	925,000

2.2.3 Zinc in brass

Brass is not only manufactured by melting copper and zinc (and alloy), but also by reprocessing brass scrap. The melted copper, zinc and brass scrap is casted to produce brass slabs and brass billets. The brass slab is successively preheated, hot rolled, scalped, cold rolled, annealed and finished to produce brass flats. Brass billets are successively preheated, extruded, drawn cold, annealed, pickled and finished to produce brass rods, bars, wires, sections and tubes. The zinc content in brass (copper-zinc) ranges between 15% and 45% (information from industry).

In Table 2.9 an overview is given of the different use types of zinc in brass in the EU.

Table 2.9 Use of different types of zinc in brass in the EU (data from IZA-Europe, 1994)

Zinc in brass	Use (tonnes)
Rods / Bars / Section	300,000
Sheet / Strip	80,000
Wires	30,000
Tubes	40,000
Other	40,000
Total	490,000

Table 2.10 shows the end use market for brass in the United States of America.

Table 2.10 End use markets for brass in the US during the 1980s (Nilsson, 1996)

End use market	Share of the total use (%)
Construction industry	45
Transportation	23
Machinery	12
Electrical uses	10
Chemical and other uses	10

2.2.4 Die casting alloy

The feedstock of zinc die casting operations is high purity zinc alloy ingot made. In-house alloy manufacture or molten metal delivery can be practised but is rare. The zinc alloy ingots are melted and taken to die casting machines by a variety of mobile ladle systems or by a launder. Casting by direct injection is from the holding furnace into steel moulds. Once the casting is solid the die opens, the casting is ejected and the cycle is repeated. In the trimming step the sprue and runner, originating from thin gates to connect the running system to the cast component, is broken of by hand, by trimming press, or by barrelling. The sprue and runners are usually returned to the melting furnace for direct recycling. Generally no further operations are required and the trimmed casting can be packed and shipped. Many die casters will have in-house facilities for secondary operations like polishing, machining or coating of castings (information from industry).

In Table 2.11 an overview is given of the different use types of zinc in alloys in the EU.

Table 2.11 Use of different types of zinc in alloys in the EU (data from IZA-Europe, 1994)

Zinc in alloys	Use (tonnes)
Automotive applications	95,000 (34%)
Builders: domestic hardware	50,000 (18%)
Tools	35,000 (13%)
Electric: electronic component	20,000 (7%)
Toys	10,000 (3%)
Others	70,000 (25%)
Total	280,000 (100%)

In Table 2.12 a survey is given on the die casting end use market in the US for the years 1981 and 1989.

Table 2.12 The end use markets for zinc die casting in the US in 1981 and 1989 respectively (Nilsson, 1996)

Die casting end use market	1981 (%)	1989 (%)
Automotive components	35.7	28.0
Builders and domestic hardware	22.7	23.6
Electrical components	8.7	17.8
Commercial and office equipment	7.3	12.8
Domestic appliances	8.0	12.8
Power generation equipment	5.0	3.0
Others	12.0	2.0

2.2.5 Rolled/wrought zinc

Rolled/wrought zinc is successively produced by melting and casting of zinc, rolling in mills with holding furnaces, finishing, smoothing and (edge)cutting and finally coiling the zinc sheet.

2.2.6 Zinc powder / dust and other zinc containing chemicals or products

Zinc powder is manufactured by air, water or centrifugal atomisation, or by a combination of these processes, of a stream of molten zinc metal. Subsequent operations are screening and packing. Typical particle sizes range from around 50 µm, up to 750 µm for battery applications. The upper limit for metal powders in general is defined as particles ≤ 1000 µm diameter (DIN 30900).

Zinc dust is normally produced by condensation of zinc vapour in an inert atmosphere. The feedstock can be primary zinc or secondary zinc scraps and drosses, depending on the process used to generate vapour. Those processes can be gas or oil fired retorts, electrothermal furnace, Larvik process, or distillation column. Subsequent operations involve screening, classifying, blending and packing. The mean particle sizes typically range from 3 to 10 µm.

The standard manufacturing process for paint grade metallic zinc dust is the vaporisation of zinc, where metallic zinc is heated to the vaporisation temperature above 906°C in a closed system. The zinc vapour will then be cooled down in a condensing system to condense as a fine zinc dust. A molecular layer of zinc oxide is formed on the surface of each dust particle, thus stabilising the zinc dust. The metallic particles of zinc may have sizes of typically less than 10 µm. There is an overlap in the particle size from the two processes, because some zinc dust can also be produced by atomisation.

Of the 180,000 tonnes of zinc used as zinc pigments and in chemicals in the EU, the main part is used as zinc oxide in rubber (90,000 t), ceramics (25,000 t), animal feed (10,000) or other applications (35,000 t).

In the next paragraphs additional information is presented about a few major uses of zinc powder and other zinc containing chemicals or products. The data in these paragraphs refer to the Swedish situation. It is unknown to what extent they can be extrapolated to other EU countries.

2.2.6.1 Zinc containing chemicals

Zinc containing substances have a lot of applications. In Table 2.13 for the most common chemicals the field of application is mentioned.

Table 2.13 Some important zinc chemicals and their fields of application in Sweden (Nilsson, 1996)

Substance	Field of application
Zinc oxide	Vulcanisation accelerator, colouring pigment
Zinc chloride	Fluxing material
Zinc sulphide	Colouring pigment, fire-resistant additive material
Zinc phosphate	In colours, in lacquer
Zinc sulphate	In metal coating materials, flotation agent
Zinc borate	Fireproofing, electrolytic coating material, ceramics

In Table 2.14 for several zinc containing chemicals an overview is given of the quantities used in Sweden in 1992.

Table 2:14 The use of zinc chemicals in Sweden in 1992 (Nilsson, 1996)

Substance	Interval for quantity used (tonnes)
Zinc naphthenate	47-58
Zinc oxide	2,192-2,238
Zinc sulphide	144-147
Zinc borate	<10
Zinc dibutyldithiocarbamate	24-36
Zinc 2-ethylhexanoate	<10
Zinc dimethyldithiocarbamate	<10
Zinc diethyldithiocarbamat	10-11
Zinc mercaptobenzothiazole	<10
Zinc octaadecanoate (stearate)	313-320
Zinc cyanide	<10
Zinc chloride	148-180
Zinc sulphate	17
Zinc phosphate	255
Total	3,150-3,312

2.2.6.2 Sacrificial anodes

There are no production facilities in Sweden. All anodes are imported from Denmark.

Zinc anodes contain more than 99% zinc. The use quantity in Sweden is 200 tonnes per year. 300 tonnes is imported; 100 tonnes is re-exported. (Nilsson, 1996).

2.2.6.3 Batteries

The consumption of batteries in Sweden is 80 million with a weight of 4000 tonnes. About 25% is ZnO which corresponds with a zinc amount of 800 tonnes per year. There are no production facilities in Sweden, so all batteries are imported. (Nilsson, 1996).

3 ENVIRONMENT

3.1 GENERAL INTRODUCTION

The presence of zinc in the environment due to natural processes (resulting in a natural background concentration of zinc in all environmental compartments, incl. organisms), the chemical processes that will affect the speciation of zinc in the environment, and the fact that zinc is an essential element have implications for the environmental exposure and effect assessment of zinc and thus for the risk characterisation of zinc.

Since the Technical Guidance Document (TGD) does not provide detailed information on how to deal with (essential) elements that have a natural background concentration in the environment, such as zinc, the "added risk approach" (according to Struijs et al., 1997 and Crommentuijn et al., 1997) has been used in this risk assessment report on zinc. In this approach both the "Predicted Environmental Concentration"(PEC) and the "Predicted No Effect Concentration" (PNEC) are determined on the basis of the added amount of zinc, resulting in an "*added* Predicted Environmental Concentration" (PEC_{add}) and "*added* Predicted No Effect Concentration" ($PNEC_{add}$), respectively. The use of the added risk approach (a method that in principle can be used for all naturally occurring substances) implies that only the anthropogenic amount of a substance, i.e. the amount added to the natural background concentration, is considered to be relevant for the effect assessment of that substance. Thus, a possible contribution of the natural background concentration to toxic effects is ignored.

In the present environmental exposure assessment (section 3.2), the use of the added risk approach implies that the PEC_{add} values have been calculated from zinc emissions due to anthropogenic activities. Thus, the PEC_{add} is the anthropogenic part of the zinc concentration in the environment. By focusing only on the anthropogenic part of zinc, the problem of the great variety of natural background concentrations of zinc over the different geographic regions is eliminated. Of course it is realised that comparison of the PEC_{add} with measured environmental concentrations must take into account that the latter values comprise the natural background concentration (Cb) and the anthropogenic part.

In the environmental effects assessment (section 3.3), the use of the added risk approach implies that the $PNEC_{add}$ has been derived from toxicity data that are based on the added zinc concentration in the tests. Thus, the $PNEC_{add}$ is the maximum permissible addition to the background concentration. From the background concentration (Cb) and the $PNEC_{add}$, the PNEC can be calculated: $PNEC = Cb + PNEC_{add}$.

Finally, in the environmental risk characterisation (section 3.4), the use of the added risk approach implies the evaluation of the PEC_{add} / $PNEC_{add}$ ratios. In case measured environmental concentrations are used in the risk characterisation, either the background concentration has to be subtracted from the measured environmental concentration (resulting in a "PEC_{add} / $PNEC_{add}$" ratio) or the background concentration has to be added to the $PNEC_{add}$ (resulting in a traditional "PEC / PNEC" ratio)

3.2 EXPOSURE ASSESSMENT

3.2.1 General

General information about zinc is available in many publications, e.g. the 'Integrated Criteria Document Zinc' (Cleven et al., 1993) and in the 'Environmental Health Criteria for Zinc' (WHO, 1996). In the present report only a summary of the available information is given. In the sections 3.2.2, 3.2.3 and 3.2.4 general characteristics are described which are relevant for the release and fate of zinc in the environment. These sections are also relevant for the risk assessment reports of all other current EU priority zinc compounds (zinc oxide, zinc chloride, zinc distearate, zinc phosphate and zinc sulphate).

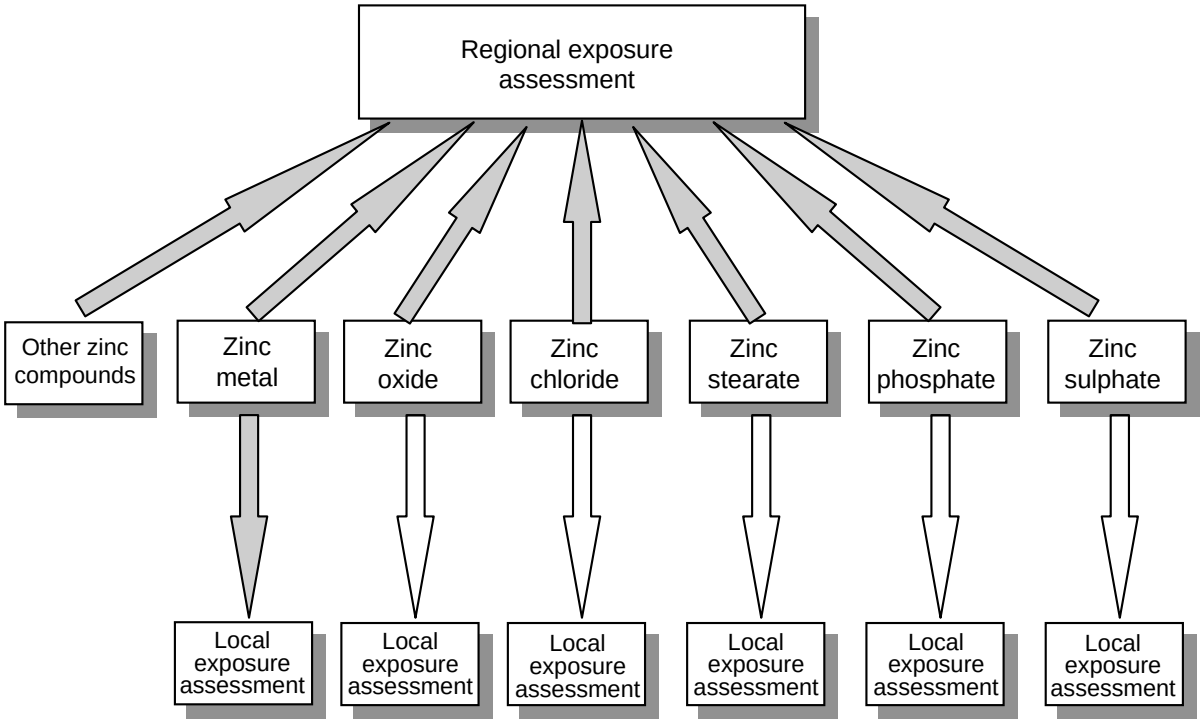


Figure 3.1 Theoretical outline for the regional and local exposure assessment for zinc metal (and other zinc compounds).

Section 3.2.5 presents the added (Predicted Environmental) Concentrations ((PE)C_{add}) for several exposure scenarios for zinc metal. The (PE)C_{add} are derived from either modelling or measured exposure data. The local exposure assessment for the production and use of zinc metal is presented in section 3.2.5.2. This local exposure assessment is focused on the emissions of industrial point sources. A regional exposure assessment is described in section 3.2.5.3. The regional exposure assessment includes the industrial and diffuse emissions of all current EU priority zinc compounds. In case of diffuse emissions it is not possible to distinguish between emissions from current EU priority zinc compounds and non-EU priority list zinc compounds. The diffuse emissions may thus also comprise emissions from other zinc compounds (Figure 3.1). For the local exposure assessment of the other zinc compounds the reader is referred to those separate reports.

As stated in section 2.1.1 the environmental releases from waste, including mining waste, are not taken into account in the current risk assessment. The Rapporteur recognises that those

releases can be significant, but the general instrumentarium is currently lacking on how to deal with this type of emissions (mostly landfills).

3.2.2 Aquatic compartment (including sediment)

3.2.2.1 Release and fate

Zinc enters the aquatic environment via industrial wastewater, atmospheric deposition and runoff from agricultural soils, solid industrial waste and sewage sludge.

Zinc in fresh water or seawater can occur in both suspended and dissolved forms and is partitioned over a number of chemical species. Zinc in freshwater can be divided in several classes (Table 3.1), as for instance hydrated zinc ions, zinc ions complexed by organic ligands (humic and fulvic acids), zinc oxy ions and zinc adsorbed to solid matter. In the principal Dutch fresh surface waters (characterised by a particulate matter content of ± 30 mg/l, a hardness of ± 200 mg $CaCO_3$/l and a pH of ± 8), about 25% of the total-Zn concentration is dissolved and 75% is adsorbed to particulate matter (Cleven et al., 1993). A similar finding was recently reported. In different European river waters in The Netherlands, Sweden and Spain the distribution over free zinc and zinc complexes is found to be roughly 30% and 70%, respectively (Jansen et al., 1998).

Dissolved zinc in two major river inputs and low salinity zones from the UK Humber estuary appears to be more or less exclusively associated with naturally-occurring organic complexing material. At higher salinities, the assessment of predominant zinc species is dependent on uncertainties in estimates of the metal ligand stability constant. The fraction really dissolved zinc varies between 10-20% at low salinity and between 30-40% at high salinity (Gardner, 1999).

Table 3.1 Possible forms (speciation) of dissolved zinc in surface water (Cleven et al., 1993).

Form	Examples	Approximate diameter (nm)
Particulate matter	retained by 0.45 μm filter	>450
Hydrated metal ion	$Zn(H_2O)^{2+}_6$	0.8
Labile inorg. Complexes	$Zn(H_2O)_5Cl^+$, $Zn(H_2O)_5OH^+$	1
Labile org. complexes	Zn-citrate, Zn-glycinate	1-2
Stable inorg. Complexes	ZnS, $ZnCO_3$, Zn_2SiO_4	1-2
Stable org. complexes	Zn-humate, Zn-cysteinate	2-4
Adsorbed on inorg. Colloids	Zn^{2+}-Fe_2O_3, Zn^{2+}-SiO	10-500
Adsorbed on org. colloids	Zn^{2+}-humic acid, Zn^{2+}-org. detritus	10-500

The dominant forms of a number of zinc compounds in fresh water are presented graphically as a function of acidity (pH) and redox potential (Eh) (Figure 3.2). The dissolved zinc concentration has a very limited influence on the position of the crossings between the different speciations (Fikkert 1997).

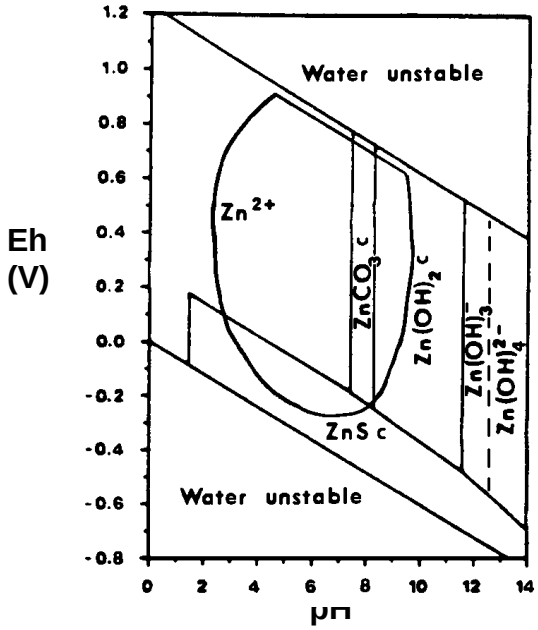


Figure 3.2 Fields of stability of solid (c) and dissolved zinc species in the system $Zn+CO_2+S+H_2O$ at 25°C and 1 atm. pressure in relation to redox potential (Eh) and acidity (pH). Dissolved zinc activity, 10^{-5} moles/l; dissolved carbon dioxide and sulphur species, 10^{-3} moles/l (Hem, 1972). The area within the closed curve encompasses Eh-pH combinations most commonly found in the environment (Cleven et al., 1993).

Possible chemical forms of zinc in seawater are presented in Table 3.2. In this table the variation in the percentages of total zinc can for instance be explained by analytical differences or by the different ion strengths of the examined seawaters.

Table 3.2 Possible chemical forms (speciation) of dissolved zinc in seawater (Cleven et al., 1993).

		Percentage of total zinc		
Zn species	**Zirino and Yamamoto (1972)**	**Dyrssen and Wedborg (1974)**	**Morgan and Sibley (1975)**	**Florence and Batley (1977)**
Zn^{2+}	17	16.1	12.5	5.7
$ZnCl_n^{2-n}$ (n:1-4)	11.4	63.7	79	17.8
$ZnOH^+$, $ZN(OH)_2$	62.2	2.3	0.6	71.8
$ZnCO_3$	6	3.3	1.6	2.4
$ZnHCO^+_3$	0.7	0.3	-	0.2
ZnOHCl	-	12.5	-	-
$ZnSO_4$	4	1.9	1.6	2.2

Adsorption at suspended matter and bed sediment is also an important factor for the behaviour of zinc in aquatic systems. Several phosphates (WHO, 1996), hydroxides, clay minerals and organic matter are important for the adsorption of zinc in aerobic waters (Cleven et al., 1993). The efficiency of these materials in removing zinc from the solution varies according to their concentrations, pH, redox potential (Eh), salinity, nature and concentrations of complexing ligands and the concentration of zinc. The metal carriers in sediments are clay minerals, silicates (quartz, feldspars), calcium carbonate and organic matter. The accumulation of zinc in the sediments from surface water increases with decreasing size of the sediment particles (Cleven et al., 1993).

Precipitation of soluble zinc compounds appears to be significant only under reducing conditions in waters with high zinc concentrations, particularly when the pH is higher than 8 (Cleven et al., 1993). Low pH is important to maintain zinc in solution, generally as the free ion, although the control of maintaining zinc in solution is related to the dissolved organic matter. Under anaerobic conditions and in the presence of sulphide ions, precipitation of zinc sulphide limits the mobility of zinc. Under aerobic conditions, desorption of zinc from sediments occurs at increasing salinity due to the displacement of adsorbed zinc ions by alkali and alkaline earth cations, which are abundant in brackish and saline waters (WHO, 1996). An increase in the dissolved and suspended fractions of zinc in estuarine water was reported in the mixing zone between fresh and brackish water, mainly due to increased residence time in estuaries (WHO, 1996).

The speciation of zinc in the aquatic compartment is of high complexity and depends highly on abiotic factors, such as pH, (dissolved) organic matter content, redox potential, etc. It is assumed that speciation is very relevant for the migration of zinc through sediment, for the distribution of zinc among its truly dissolved and non-dissolved forms, and for the uptake of zinc by some aquatic and sediment organisms. However, for other organisms the speciation of zinc in sediment or water is less relevant for uptake, since those organisms take up zinc mainly directly from detritus or sediment. Bioavailability of the different forms of zinc varies not only as a function of the physical-chemical speciation of zinc but also as a function of the organism. These bioavailability aspects are further elaborated in sections 3.3.2.1.1 and section 3.3.2.2.1.

Partition coefficients

Bockting et al. (1992) derived for zinc a similar partition coefficient for the distribution between solid particulate matter and water (Kp_{susp}) of 5.04 (log value) as described in Stortelder et al. (1989). The value is based on measurements in Dutch surface water (Table 3.3). Other $logKp_{susp}$ values described by Bockting et al. (1992) based on measurements in North American rivers are somewhat lower, with values between 3.43 and 4.48 (Table 3.3).

Table 3.3 Solids-water partition coefficients ($logKp_{susp}$) in suspended matter derived from measurements over the period 1983-1993.

$LogKp_{susp}$	Location	Reference
4.28	North America, Rio Grande	Popp, Laquer 1980
4.48	North America, Rio Puerco	Popp, Laquer 1980
3.43	North America, Rio Salado	Popp, Laquer 1980
4.00	North America, Hudson River	Li et al. 1984
3.66	North America, Hudson River + sea water	Li et al. 1984
5.04	Netherlands 4 locations fresh surface water; 1983-1986	Stortelder et al. 1989
5.03	Netherlands 7 locations fresh surface water; 1988-1992	Venema, 1994
4.73	Netherlands 3 locations fresh surface water; 1992-1994	Koelmans and Radovanovic, 1997
5.04	North sea, Wadden sea; 1995	Yland, Smedes, 1996
4.80	North sea, Wadden sea corrected for background conc. ; 1995	Yland, 1996

The principal Dutch rivers are characterised by a particulate matter content of ± 30 mg/l (=0.030 kg/m^3), a hardness of ± 200 mg $CaCO_3$/l and a pH of ± 8. Based on zinc measurements in these waters in the period 1983-1986, a median Kp_{susp} of 110,000 l/kg has been derived (Stortelder et al., 1989). The Kp for the distribution between sediment and water (Kp_{sed}) is estimated from that for particulate matter, as follows: $Kp_{sed} = Kp_{susp} / 1.5$, based on the average difference in concentrations of zinc and other metals in both media. For zinc this

results in a Kp_{sed} of 73,000 l/kg. The difference in metal concentration in particulate matter and sediment is attributable to the difference in adsorption capacity, mainly due to the difference in clay and organic matter content (particulate matter: 40% clay and 20% OM; sediment: 25% clay and 10% OM; standard values used for Dutch surface waters (Stortelder et al., 1989)).

More recent data on Kp_{susp} values for zinc in Dutch surface waters are presented in Table 3.4 (Venema, 1994) and Table 3.3. These values are not significantly different from those derived by Stortelder et al. (1989). In the present RAR the used Kp values between water and suspended matter and between water and sediment are, respectively 110,000 ($logKp_{susp}$=5.04) l/kg and 73,000 ($logKp_{sed}$=4.86) l/kg. In addition to these values also the impact of varying Kp_{susp} values on the results of the aquatic regional exposure assessment will be demonstrated (see section 3.2.5.3.3). For this both the mean (110,000 l/kg), highest (176,000 l/kg) and lowest (64,000 l/kg) Kp_{susp} values from Venema (1994) will be used.

Table 3.4 The median solids-water partition coefficients (Kp_{susp}) in suspended matter calculated with measured data over the period 1988-1992 in the Netherlands(Venema, 1994).

Location	Kp value (l/kg)
Rhine	84,000
Meuse	176,000
Scheldt	56,000
Lake IJssel	±134,000
Haringvliet	146,000
Nieuwe Waterweg	64,000
Northsea Canal	85,000
Mean (7 locations)	106,000

On the basis of monitoring data the following Kp_{susp} values are available for Germany (UBA, 1994): Rhine (at Lobith, mean value period 1983-1986): 81,000 l/kg; Rhine (1988, vertical section: 91-863 km): 113,000 l/kg and Bavarian flowing waters: 10,000 – 100,000 l/kg. These German data further support the selection of Kp values as mentioned above.

Partition coefficients for the distribution of metals between water and suspended matter are used to calculate the dissolved concentrations from total concentrations in surface water. Partition coefficients for the partitioning of metals between water and sediment are used to calculate the concentration in sediment from the dissolved concentration in water.

In large rivers (Rhine, Meuse) it appears that the solids-water partition coefficients in suspended matter increases with decreasing total zinc concentrations. It is further mentioned that in rivers with a low pollution level, the proportion of zinc transported on suspended sediment is relatively high (Cleven et al., 1993; WHO, 1996).

3.2.2.2 Ambient[4] and natural[5] background concentrations

The concentrations of zinc in seawater and fresh surface water are dependent on natural conditions: it is almost impossible to determine experimentally a natural background concentration in Europe. Due to geochemical differences, the natural background

4 Ambient = the concentration that is present due to natural background plus the imission of metals from diffuse sources of human origin

5 Natural = the concentration that is present due to natural sources only.

concentrations will differ in Europe. In addition, since the concentrations that are measured in the environment is the sum of an anthropogenic and a 'natural' source, one cannot simply distinguish the 'natural' part from anthropogenic the part. Hence, background concentrations are not measured, but estimated or determined with other methods. When in this section it is not specifically mentioned whether the background concentrations are natural or ambient and total or dissolved, then this was also not distinghuished or unclear in the corresponding reference. Below a number of differerent estimates for zinc background values in both sea water and fresh water are summarised.

Reported values for the natural concentrations in coastal seas are 0.5 and 1 µg/l. Lower natural zinc levels are reported for open oceans (surface) with values of 0.001-0.06 µg/l (ICME, 1996). The dissolved background concentration for the Atlantic Ocean is reported to be 0.1±0.4 µg/l (Laane, 1992).

The background concentration for the river Rhine and the North Sea is estimated to be 2.6 µg/l and 1 µg/l, respectively (Cleven et al., 1993). Referring to another source, Cleven et al., (1993) reported also a natural background level of 9 µg/l for total zinc and 2 µg/l for dissolved zinc. The natural background level determined for Dutch surface water is 2.6 µg/l for total zinc and 1 µg/l for dissolved zinc according to Van de Meent (1990). The natural average background concentration in surface water based on other sources varies from 5 or 6 to 35 µg/l for dissolved zinc (mean = 20 µg/l). In this case the lower concentration is based on a value of 2.6 µg/l calculated with a model of Van der Weijden for the Rhine and a contribution of 3.3 µg/l for the supply from Switzerland. The upper concentration is based on an estimated natural zinc supply and water flow of the Meuse (Van Tilborg, Van Assche, 1995). According to a recent report on zinc of the Dutch Health Council, the pre-industrial zinc concentration for the river Rhine basin is estimated to be <1 µg/l, and other waters are in the same order of magnitude (Gezondheidsraad, 1998). A value of 0.8 µg/l (dissolved) has been deduced for the Rhine recently by Van den Berg and Zwolsman (2000).

In a German review (LAWA 1997) on heavy metal concentration of unpolluted waters the following mean values are given for Germany:

Table 3.5 German review (LAWA 1997) on heavy metal concentration of unpolluted waters

Source	IKSR (1989)	IKSR (1989)	Wachs (1989, 91)	Salomons & Förster (1984)	Merian (1984)
	soluble	Total	0.45 µm filtrated		
Zn (µg/l)	1.3	5.5	<3	5 - 10	7

For Germany, a mean natural background concentration for natural water of 3.5 µg/l Zn-total is derived on the basis of various studies (LAWA 1997). This concentration is based on 1 µg/l for the dissolved fraction and 2.5 µg/l for the particulate fraction (if 25 mg/l suspended matter is assumed, no further correction or normalization for other parameters).

In Sweden natural background concentrations for rivers and lakes have been estimated to 1-3 µg Zn/l (total-conc.) (Naturvårdsverket, 1999a; Swedish Environmental Protection Agency, 1993).

For France a zinc concentration of 3-13 µg/l was reported from a reference station in the south western part of the country (region Adour Garonne; Reseau National des Donnees sur l'Eau , Office Internationale de l'Eau, F-87065 Limoges Cedex, www.rnde.tm.fr).

In Finland natural background concentrations of 1.5 – 25 µg/l (90 P) have been measured for total zinc in stream waters (median 3.6 µg/l; Lahermo et al. 1996). The highest regional background values (10-20 µg/l) are found in lowland areas in western Finland where natural soils may have a very low pH (<4).

A survey of 985 randomly selected lakes in Norway, carried out in 1995 showed total zinc concentrations ranging from 0.08 to 139 µg/l (NIVA, 1999). The Norwegian authorities propose that the 75 percentile of a subset for data in the northern part of the country (relatively unpolluted) may be considered as a tentative natural background concentration. This leads to a Norwegian natural background level of 1.2 µg/l.

Zuurdeeg (1999) reported 10^{th}-percentile and 90^{th}-percentile total-Zn concentrations in lowland brooks in unpolluted areas in Northern Europe of 4 µg/l and 35 µg/l, respectively. Because the metal concentrations in these brooks are considered to be partly from anthropogenic sources, the geometric mean value of the ambient background concentrations, 12 µg/l, is considered by Van den Hoop (1995a 1995b)[6] to be the "best guess" estimate for the natural background concentration of total-Zn in fresh surface waters in The Netherlands (and, likewise, in other lowland European areas This total-Zn concentration is equivalent to 2.8 µg dissolved-Zn/l, using a Kp_{susp} of 110,000 l/kg and a particulate matter content of 30 mg/l.

It can be concluded that there are several estimates available for a natural background value of zinc in fresh waters in a number of EU countries. Most data fall within the range of 2.5 to 12 µg total-Zn/l. In the present report on zinc a pragmatic approach is followed rather than selecting one particular natural background value by using both the lower limit of 3 µg total-Zn/l and the upper limit of 12 µg total-Zn/l for correcting the available EU monitoring data in the risk characterisation. The rapporteur is aware that some of the current sources refer to lower mean natural background values (around or below1 µg/l). Higher (> 12 µg/l) natural levels may be relevant as well in some cases. In general, if available monitoring data can unequivocally be linked with a particular natural background value in an area, preference should be given to that specific background value. An example of this is the Meuse river. There are indications that for this river, at least the part entering the Netherlands, the natural background is slightly higher than for other major surface waters in the Netherlands (pers. comm. RIZA, 2001). Therefore, rather than using the range 3-12 µg/l, a range of 6-12 µg/l is considered more appropriate for the Meuse in the current risk characterisation.

Reported natural background loads for sediments are 70 mg/kg to 95 mg/kg (Cleven et al., 1993). According to 'Desire for Levels' (Van de Meent, 1990) the provisionally natural background level for zinc in Dutch sediment can be set on a value of 68 mg/kg. For the Rhine a natural background level is defined as the concentration suspended matter with a value of 100 mg/kg (Buijs, 1995). (In the same reference the concentration suspended matter of 100 mg/kg is converted to a total concentration in surface water of 4.5 µg/l). In the Netherlands the applied natural background concentration for sediments is set at 140 mg/kg dry weight. This is the estimated background concentration for Dutch 'standard' sediment (25% lutum and 10% organic matter). Further information about this background value, which is set equal to that for soil, is presented in paragraph 3.2.3.2. For suspended matter a natural background concentration of 100 mg Zn/kg is given for Germany (LAWA, 1997). A sediment background concentration of 100 mg/kg dw has recently been estimated for Swedish lakes (Naturvårdsverket, 1999a). In an earlier report by the Swedish Environmental Protection Agency (1993) a 'preliminary background' concentration (based on upper quartile of

6 For soil and groundwater, Van den Hoop (1995a; 1995b) proposed to set the natural background concentration in these compartments at the 90th-percentile concentration of the measurements in (unpolluted areas) in the Netherlands, see also 3.1.3.2.

available data from pre-industrial sediment layers) of 175 mg/kg dw was given. In Finland natural background concentrations of total Zn for stream sediments between 20 and 140 mg/kg (90P) are measured with a median value of 46 mg/kg (Lahermo et al. 1996).

Sediment data have been collected in France between 1996 and 1998 in reference stations. The statistics of these data are presented below (values in mg/kg):

Table 3.6 Statistics of Sediment data collected in France between 1996 and 1998

Number of data		39
Mean		499.8
Standard Error		1366.9
Percentiles	10	20
	50	80
	90	1080

A survey in Norway of metal concentrations in lake sediments was carried out in 1996-97. Samples were taken from 231 lakes distributed over the whole country. Analysis of Zn were performed on the upper part of the sediment and at 30-50 cm depth. The deep-sediment samples are considered to reflect pre-industrial background levels. The range of Zn concentrations was 22-919 mg/kg in the upper sediment and 13-884 in the deep sediment. The median values were 136 and 106 mg/kg respectively. The variation reflects local geological variations. The Norwegian authorities propose that for lake sediments, the background and natural background concentrations may be derived from the 75 percentile for near surface and deep sediments respectively (similar to water). This leads to a natural background value of 150 mg/kg in sediment.

In conclusion, all currently available natural background data for sediment are more or less in the same order of magnitude (range 70-175 mg/kg dwt). Based on the data from several EU-regions (see above) the value of 140 mg/kg dwt will be used as a natural background for correcting the EU sediment monitoring data. If available monitoring data can unequivocally be linked with a particular natural background value in an area, preference should be given to that specific background value.

More (ambient) concentrations measured in surface water, suspended matter and sediment are presented in section 3.2.5.3.4.

3.2.3 Terrestrial compartment

3.2.3.1 Release and fate

Anthropogenic sources of zinc entering the terrestrial compartment are corrosion of galvanised structures, agriculture (manure, fertilisers, pesticides, sewage sludge), traffic (tires, oils, grease), atmospheric deposition and solid industrial waste (Cleven et al., 1993). The major natural source of zinc in soils are zinc sulphide minerals (WHO, 1996).

In the present section, information will be given on the speciation of zinc in soil, the influence of pH and 'ageing' on the speciation, and the subsequent consequences for mobility and bioavailability.

Speciation of zinc in soil

In soils, zinc interacts with various reactive soil surfaces. The most important in this respect are soil organic matter, amorphous soil oxides (Al, Fe, Mn) and clay minerals. The major process by which metals are bound to these surfaces is adsorption. Although adsorption ultimately can be considered as a rather simple process based on charge differences between positive metal ions and negatively charged surface sites, chemical differences between metals exist as a result of which differences in the bonding strength occur (Römkens and Groenenberg, 2001).

Other processes including precipitation of carbonate type minerals can occur but are, in non- and moderately polluted soils, unlikely to control the solubility of metals in soils. An exception to this is the formation of sulphide minerals that are formed, in the presence of sulphate under reducing conditions (Römkens and Groenenberg, 2001).

The magnitude of the sorption process is governed by what is called both *capacity* and *intensity* factors in the soils. The capacity factor is a sum of the properties that ultimately control the degree to which a soil potentially can bind metals like the amount and kind of organic matter, the amount and type of clay present in the soil etc. Intensity factors are properties that, given a certain capacity of the soil to bind metals, control the actual adsorption like pH and the concentration of competing cations, such as Al, Ca, Mg, and other micro-elements ((Römkens and Groenenberg, 2001).

Zinc in soil is distributed between the following fractions (WHO, 1996; Van Riemsdijk, 2001):

- Dissolved in pore water (which includes many species)
- Exchangeable, bound to soil particles
- Exchangeable, bound to organic ligands (of which a small part in the dissolved fraction and the major part in the solid fraction)
- Present in secondary clay minerals and metal oxides/hydroxides
- Present in primary minerals

Zinc bound to organic matter can be discriminated in chemically bound to reactive groups and a-specifically bound in the double layer. The latter fraction is susceptible to ion-exchange and thus contributes to fraction 2 (Van Riemsdijk, 2001).

Sequential extraction, as suggested by Goselink and van Erp (2001), to determine the distribution of zinc in soil, is prone to many artifacts (Van Riemsdijk, 2001) and may not result in bioavailable and non-bioavailable fractions (Römkens and Groenenberg, 2001). Sequential extraction uses operationally defined extraction solutions to identify fractions like "organically bound" or "oxide bound" and links the amount of metals extracted by each step to a certain degree of availability, ranging from low to high, with an increase in steps (e.g. Ma and Rao, 1997).

According to Römkens and Groenenberg (2001), metals are bound to surface sites in the soil. The amount of energy needed to break this adsorption type bond, i.e. to release the metal ion to the solution, can vary between very little, e.g. the energy needed to release Na from a

sorption site on a clay mineral, to very high, e.g. Pb bound to organic matter. For risk assessment, it is this energy needed to release metals that is crucial as to whether an element will be available in the future or not. It is much less important to what kind of material the element is bound, e.g. to an amorphous iron oxide, to a dissociated site on a clay mineral or organic matter. If the energy needed to release this metal is the same for these sites, it is, environmentally speaking, therefore irrelevant to what matrix zinc is bound (Römkens and Groenenberg, 2001).

The binding strength is known to vary between different types of matrices but from the point of view of risk assessment this difference is not necessarily related to matrix types only. It does, therefore make not much sense to use sequential extraction (Römkens and Groenenberg, 2001). With any solution applied onto a soil, some metals can be released at a certain 'pressure' level, whether they are bound to variable charge sites on organic matter or on permanent charge sites on clay minerals. Therefore, it would make more sense to come up with a range in solutions representing 'potential pressures', i.e. releasing step by step (in different soil samples, ***not*** sequential) the fraction of metals that can be released at a certain pressure. To illustrate this, the three different extractions that are currently used by various research groups are summarised in Table 3.7 to identify the 'exchangeable' (fraction I), 'potentially exchangeable' (fraction II) and 'total metal content' (fraction III) in the soil. The rationale behind this scheme is that some part of the metals never will contribute to the 'available pool' whereas other parts of the metals can, depending on conditions (like pH etc.).

Table 3.7 Overview of metal pools in soils as obtained by separate extractions (Römkens and Groenenberg, 2001).

Fraction	Type of binding strength	Examples of extraction solution
I	'Exchangeable': immediately available, 'loosely' adsorbed – low energy sites	0.002 or 0.01 M $CaCl_2$
II	'Potentially exchangeable': potentially available upon 'pressure', high energy sites	EDTA or 0.43 N HNO_3
III	'Total metal content'	HF or, less 'total' Aqua Regia

Metals released with a dilute salt solution (i.e. - part of - Fraction I) are considered to resemble the amount of metal in the soil solution in natural conditions. Results from experimental studies indeed reveal good correlations between the plant metal content and the amounts extracted in extraction solutions like NH_4NO_3, Na_2EDTA and $CaCl_2$ (Jackson and Alloway, 1991; Gray et al., 1999).

In conclusion: zinc is present in the soil in various forms, with varying degree of extractability.

The influence of pH and 'ageing' on the speciation of zinc in soil

Zinc tends to be more sorbed and complexed at higher pH (pH > 7) than at lower pH. Below pH 7, the amount of zinc in solution was reported to be inversely related to soil pH (Janssen et al., 1997). The pH of the soil not only determines the degree of complexation and adsorption of zinc, but also the solubility of the various zinc minerals. The solubility of zinc in soil decreases with increasing pH (Cleven et al., 1993).

A very important point is the assumption with respect to the relationship between pH changes, zinc loading and bioavailable zinc. Industry (Industry comments of May, 2001) states that "a natural low pH of a soil is related to a natural high zinc concentration. If zinc has been added

slowly during long periods of time (so that ageing is allowed to occur) and subsequently pH-control (liming) is discontinued, it means that the ultimate equilibrium zinc concentration will not deviate substantially from that of a similar uncontaminated soil at the same pH" (Industry comments of May, 2001). This statement has not a general validity (Van Riemsdijk, 2001). It is also in conflict with the "state of the art" paper by Smolders et al. (2001), where it is stated that 'ageing' processes are assumed to be reversible with pH. If 'ageing' would be due to the formation of secondary phases like double hydroxides, it is clear that the solubility of these secondary phases will increase if the pH decreases (Van Riemsdijk, 2001). These phases are only stable at near neutral pH, a pH drop can thus easily lead to a complete dissolution of such phases and are a great potential risk if the soil acidifies. It is argued that slow solid diffusional processes into mineral phases is important (Smolders et al, 2001; Goseling and van Erp, 2001). However, recent laboratory experiments with zinc transport through soil columns in Switzerland (Voegelin, 2001), show that a slow increase in sorption at near neutral pH is of relevance, but almost all of the zinc accumulated during the slow sorption phase is released when the pH is lowered (Van Riemsdijk, 2001).

After addition of a metal to a soil, often a slow decrease in the soil solution concentration, or the available fraction as determined in an extraction solution (e.g. by $CaCl_2$) decreases as a result of (presumably) slow diffusion processes of metals into the matrix of the reactive surfaces. It is this process, or sum of as of yet poorly defined slow processes, that can be defined as 'ageing' (Römkens and Groenenberg, 2001).

When a chemical species like zinc is added to a soil it will thus redistribute itself over various physical chemical forms. This redistribution will take a shorter or longer time and the solution concentration will decrease during the redistribution process. For a full chemical equilibrium one can in principle calculate the equilibrium zinc activity in the solution phase and its distribution over the various chemical forms (species) provided that one can quantitatively deal with all reactions involved (Van Riemsdijk, 2001):

- With respect to zinc binding via cation-exchange on constant charge clay minerals one can make reasonable estimates, where competition with ions like calcium or aluminium (at low pH) are important factors.

- With respect to binding to soil organic matter like humic and fulvic acids an enormous progress has been made at the quantitative interpretation and description of the competitive pH dependent binding of metal ions to this important soil constituent. This field has been developed simultaneously by Tipping and co-workers (e.g. Tipping and Hurley, 1992; Tipping, 1998), resulting in model V and more recently in model VI, and by van Riemsdijk/Kinniburgh and co-workers in the NIC(C)A model (e.g. Koopal et al., 1994; Kinniburgh et al., 1999). Both models account for chemical heterogeneity, multicomponent competitive binding both specifically by reaction with reactive groups and aspecifically via cation-exchange in the diffuse layer around the molecules.

- Also with respect to ion binding to metal(hydr)oxides an enormous progress has been made in the last twenty years. This progress is due to progress in spectroscopic techniques which can identify the structure of the adsorbed species (Bargar et al., 1997; Spadini et al., 1994), as well as with respect to modelling (Hiemstra et al., 1989a; Hiemstra et al., 1989b; Hiemstra et al., 1996, Hiemstra and van Riemsdijk, 1996).

The challenge is to develop models that scale from the molecular level to the field scale. Applying the fundamental knowledge on metal ion binding to metal(hydr)oxides to field soils is at present difficult because of the complicating factor of the effect of organic matter which may be adsorbed on the metal(hydr)oxdes which is expected to influence the metal ion binding (Van Riemsdijk, 2001).

The concept of 'ageing' deals with a range of processes that link one or more pools in Table 3.7. Soil chemical processes are often described as equilibrium processes. The fact that there is a continuous decrease in the dissolved metal concentration after addition of metals to the soils already indicates that this is not entirely true. There always will be a slow but continuous shift in the bonding energy of metals, especially if the matrixes to which the metals are bound are unstable, e.g. under variable redox conditions (Römkens and Groenenberg, 2001).

Various conditions can be mentioned where either the binding capacity or the chemical conditions change to such an extent that the equilibrium between solid and solution phase will be changed completely. Therefore, it is not correct to use the term 'ageing' in these conditions, since they are fundamentally different from the previously mentioned topic of slow re-equilibration and diffusion and only occur under special conditions (Römkens and Groenenberg, 2001):

- *Changes in redox potential.* Due to changes in the redox potential, new mineral phases can be formed. Upon wetting of a system, the formation of sulphide minerals (if sufficient SO_4 is present) will reduce the solubility of most metals drastically. If conditions remain anoxic for prolonged periods, eventually all amorphous iron will be converted to Fe(II) and ultimately FeS or FeS_2. A large part of the metals in fraction I and II will then either be included in the matrix of the iron sulphides or will form metal sulphides like CdS or PbS. Upon oxidation of the reduced system, however, a significant release of metals will occur and amorphous Fe(III) hydroxides will be formed. During this re-oxidation, part of the metals previously adsorbed onto the surface, and thus exchangeable, can be incorporated into the matrix of the hydroxides and thus unavailable for exchange reactions.
- *Addition of reactive minerals to the soil.* The addition of chemically unstable products to the soil, e.g. to improve the soil quality by reducing the availability of metals, can cause a substantial decrease in the immediate availability of metals. Addition of Beringite to a Cd- and Zn polluted sandy soil for example has been shown to increase soil pH and, as a result, to substantially reduce the solubility of both metals. Apart from a pH effect it has been assumed that recrystallisation of the unstable matrix of the Beringite leads to incorporation of Cd and Zn into the inaccessible matrix. However, work by Oste et al. (2001) shows that addition of reactive like Beringite had similar effects as the addition of lime and no additional immobilising effect was observed. As of now, no evidence has been put forward that recrystallisation and metal scavenging indeed occurs and affects long-term metal availability. Only in volcanic areas it can be expected that the nature of the soil parent material is such that new phases are indeed being formed and incorporation of metals into the mineral lattice could play a role.

In conclusion: Zinc is present in soil in various forms, which quantitatively can be described if sufficient information is available. Recent developments have resulted in a better understanding and various models. However, modelling the zinc speciation in soil, and in particularly the role of organic matter, needs further study. The soil pH is an important parameter that affects the speciation and the distribution of the zinc species over the soil and

the solution. Furthermore, with respect to the effect of 'ageing' on the speciation of zinc in soil, it must be concluded that there are:

- various definitions of 'ageing',
- various processes involved, such as leaching, incorporation in soil matrices, effects of changing redox conditions and changing minerals, etc., and
- various concepts with widely varying time-scales that lead to 'ageing'.

Mobility of zinc in soil

The mobility of zinc in soil depends on the degree of adsorption and the solubility of the species. These factors in turn depend on the composition of the soil solution and the properties of the soil material (Cleven et al., 1993). As mentioned earlier, pH increase may decrease the mobility of zinc through the soil. Recent laboratory experiments with Zn transport through soil columns in Switzerland (Voegelin, 2001), show that a slow increase in sorption at near neutral pH is of relevance. However, all of the zinc accumulated during the slow sorption phase is released when the pH is lowered (Van Riemsdijk, 2001).

In conclusion: the mobility of zinc in soil is affected by e.g. the degree of adsorption, and thus also by parameters such as pH. To be able to quantitatively describe the mobility of zinc in soil thus highly depends on how well the degree of adsorption of zinc in soil can be quantitatively described.

Bioavailability of zinc in soil

In section 3.3.3.1.1 bioavailability of zinc will be discussed in more detail, but the present section will deal with some more fundamental issues. One of the currently used assumptions is that only the concentration in the pore water is readily bioavailable. Another assumption is that the fraction of zinc that is incorporated in the soil matrix, which in turn may be the result of 'ageing' processes, is less bioavailable (Goselink and van Erp, 2001).

Several studies showed that bioavailability and toxicity of zinc in soil change in time. For example, Smit (1997) found a 4-9 fold lower toxicity of zinc towards springtails in an 'aged' sandy soil from a zinc smelter when compared to 'freshly added' zinc. Posthuma (1994) found similar effects for earthworms, where toxicity of zinc was 3-fold less in 1 year 'aged' soil compared to zinc that was 'freshly added' to soil.

Furthermore, the extractability of zinc from soil was shown to decrease to approximately 10% of the applied dose in a 7 year study (Boawn, 1976). One of the reasons for the reduced extractability and the lower toxicity in time is that zinc may become increasingly entrapped in soil mineral lattices with time (Elgabaly, 1950; Tiller and Hodgson, 1962; Reddy and Perkins, 1974).

In another study it was shown that the extractability of zinc increased during the years due to a decrease in pH and organic carbon. Sewage sludge amended soils exhibited inhibitory effects on nitrogen fixation and on the growth of clover many decades after the last application of metal containing sewage sludge (McGrath, 1995). The inhibition of nitrogen fixation occurred at concentrations between 26 and 325 mg Zn per kg soil in different long term field tests (McGrath, 1995). These experiments, however, refer to multi-metal studies, and it is therefore difficult to attribute the observed toxicity to one metal.

After application, zinc may thus sometimes become more and more entrapped in the soil matrix and be less extractable, and possibly becomes less bioavailable. A few questions, however, still remain unanswered to adequately deal with this time-dependent process of

encapsulation of zinc in soil for a generic risk assessment. Firstly, quantitative information on the rates of exchange of zinc between the various soil fractions was lacking. Secondly, quantitative information on the distribution percentages of zinc over these soil fractions is currently not known. Thirdly, the effects of environmental conditions (especially pH, but also temperature, redox potential, organic matter, etc.) on these time related processes is unknown. Fourthly, plants in the micro-environment around their roots and soil organisms in their gastro-intestinal tract alter the pH and other soil properties, which limits our understanding of the actual bioavailable fraction of zinc in soil. Fifthly, it is presently unknown whether the effect of the redistribution of zinc in soil is following diffuse, ongoing deposition.

Since it is technically difficult to obtain soil solutions from soils, a good surrogate extraction that resembles the amount in the soil solution is essential. Even more so since the chemical bioavailability is often thought to be comparable to the biological availability, for example in case of plant uptake. Although there is still considerable debate as to whether even for plants uptake there is more than just this solution fraction (or even a small part of it, the free metal ion activity, Parker and Pedler, 1997), it has been shown that the uptake of various elements can be described quite well with either the free metal activity (e.g. for Cu, Temminghoff, 1998) or the amounts of metals extracted by $CaCl_2$ (such as Cd and Zn uptake by lettuce).

As for now it is still unclear whether or not the extraction solutions (Table 3.7) are used really reflect the total exchangeable pool. Furthermore, the difference between the exchangeable pool (fraction II, Table 3.36) and the total metal content can be regarded as inert. Metals stored in this inert pool can be considered not relevant for risks related to uptake, leaching etc. (Römkens and Groenenberg, 2001).

One of the many problems related to risk assessment is the comparison between field data and laboratory data (Römkens and Groenenberg, 2001). Numerous examples exists where toxic levels found in laboratory are (much) lower than those in field systems, i.e. the critical level found in laboratory studies is apparently lower than those in 'real systems'. This phenomenon can be explained to a large extent to the difference in availability between metals in the laboratory system and those in the field. If a 'clean' experimental soil is spiked with e.g. zinc in the form of a Zn-salt solution, the actual availability is almost equal to this addition. In a field system with the same total concentration of zinc, the actual availability may be (much) less. Apart from the question whether the experimental soil resembles that of the field soil (which of course should be the case), it is therefore necessary to compare the *actual* availability in both systems. This again is only possible if we have an extraction solution, which is able to extract that fraction which equals the exchangeable fraction (see section 3.3.3.1.1).

As yet it is unknown to what degree changes in bioavailability are reversible. This means that changes in bioavailability as measured by changes in the exchangeable metal pool (which has been considered the basis for ‘ageing’) do not reflect changes in bioavailability for organisms that accumulate metals other than the ones from the soil solution alone (Römkens and Groenenberg, 2001; Oste et al., 2001; see also section 3.3.3.1.1).

In conclusion: bioavailability of zinc in soil is clearly not a single function of the speciation of zinc in soil. A single, clear relationship between a chemically defined ‘available’ concentration in the soil solution and the real, ‘biological’ availability as experienced by plants and invertebrates and micro-organisms, etc., can at the moment not be provided. However, section 3.3.3.1.1 will discuss how various relationships can be used between on the one hand abiotic parameters and on the other hand the toxicity of zinc to plants or invertebrtaes or microbial endpoints. Section 3.3.3.1.1 will also explain how these various relationships can be used in the terrestrial risk assessment of zinc.

Conclusions on speciation and risk assessment

Van Riemsdijk (2001) concluded that the quantitative relationship between zinc speciation and bioavailability for different soil species and processes needs further study. Such studies should as much as possible be based on fundamental chemical and biological process knowledge and as little as possible on empirical/correlative approaches (Van Riemsdijk, 2001).

Following van Riemsdijk (2001), an integrative research program has been conducted aiming to reveal the relevant information required for using bioavailability corrections within the framework of the terrestrial risk assessment. This is shown in section 3.3.3.1.1. Thus, it is realised that long-term distribution is an important process that affects the distribution of zinc and bioavailability in soil and toxicity towards soil species. Based on recent studies and a recent evaluation of older studies, this 'ageing' phenomenon is quantitatively taken into account in the present RAR (see section 3.3.3.1.1).

Partition coefficients

According to Bockting et al. (1992) the used Kp values should be based on experiments where adsorption processes control the aqueous concentrations. The metal concentrations should be at equilibrium or in a steady state situation. Further, Kp values should be based on the fraction of the metal content that can actually exchange with the aqueous phase and not on the total metal content. Because of these conditions Kp values resulting from batch experiments are preferred. For zinc Bockting et al. (1992) derived a solids-water partition coefficient for soil of 2.2 (log value) as described in Buchter et al. (1989), which are based on batch experiments with 11 American soils.

For Dutch soils near background concentration Van den Hoop (1995b) determined a field-based logKp for zinc of 3.07 (Kp=1175 l/kg). Because of the variation in soil and pore water composition, the variability of the Kp values was high (the standard deviation was approximately equal to the mean value). Janssen et al. (1996) determined a field-based partition coefficient for Dutch polluted soils comparable to Van den Hoop, with a log value of 3.22.

The partition coefficients reported by Van den Hoop and Janssen are based on total metal contents in the solid phase and pore water. The logKp value of 2.2 of Buchter et al. (1989) is used in this RAR because it is based on the part of the metals that can actually exchange and is to be assumed in equilibrium with the water phase. Furthermore, experimental Kp values are thought to be better suited than those that are field based. It must be noted that the partition coefficients of Van den Hoop and Janssen are in the same order of magnitude as the value of Buchter et al. (1989).

3.2.3.2 Ambient and natural background concentrations

The natural zinc concentrations in soils are highly variable and dependent on the native soil material and the present soil characteristics, especially the clay and organic matter content (Cleven et al., 1993; WHO, 1996). The data for European countries as reported in Table 3.8 show that the mean ambient zinc concentrations in unpolluted soils are usually between 50 and 100 mg/kg, with a total range of 1 to 8900 mg/kg (Angelone and Bini, 1992). Specific information about soil type, sampling method, analyses etc. are not mentioned by Angelone and Bini (1992).

Table 3.8 Mean and ranges of total concentrations (mg/kg) of zinc in soils of unpolluted areas in Europe (Angelone and Bini, 1992)

	Belgium	Denmark	Germany a)	England & Wales a)	France	Italy a)	The Netherl.	Norway	Austria	Portugal a)	Scotland	Spain	Sweden a)
Mean	57	7	83	78.2	16	89	72.5	60	65	58.4	58	59	182
Range	14-130	7-15			5-38		9-1020[b)]	40-100	6-8900[b)]		0.7-987	10-109	

1. No range indicated
2. Highest value not taken into account for calculating the mean value

More detailed and recent soil concentrations in various EU countries are presented in the text below.

For the Netherlands there are many data on the zinc concentration in unpolluted soils in rural areas, especially for soils in nature reserves and in agricultural areas not influenced by local emission sources[7]. These data show that the zinc concentrations are mainly related to the soil type, i.e. the lowest concentrations are found in sandy soils (having a low clay and organic matter content) and the highest in clay and peat soils (having a high clay and/or organic matter content. The data on these soils show the following soil type-related zinc concentrations (range of mean values from a number of studies; rounded figures): 20-45 mg/kg in sandy soils, 55-140 mg/kg in peat soils and 70-150 mg/kg in clay soils. The concentrations in soil are usually measured in the upper (0-10 cm) mineral soil layer (Cleven et al., 1993).

In the environmental policy in the Netherlands, a soil type-related "target" value for zinc has been derived from zinc measurements in Dutch soils in nature reserves, combined with measurements of the lutum (clay; particles <2 μm) content and the humus (organic matter) content. These measurements resulted in the following "reference line": *[Zn] = {50 + 1.5 (2L + H)} mg/kg dry soil,* in which "L" is the weight percentage of lutum and 'H' is the weight percentage of humus. The reference line represents the 90th-percentile value of all measurements. Furthermore, the zinc concentrations in these soils may have been influenced to some extent by atmospheric deposition of zinc from anthropogenic sources. Thus, the reference line estimates the maximum value of the soil type-related natural background concentration. Based on this reference line, the natural background concentration of zinc in Dutch standard soil (defined as a soil containing 25% lutum and 10% humus) is 140 mg/kg dwt. (Cleven et al., 1993; Van den Hoop, 1995; see also TGD Chapter 3 - Appendix VIII). It is emphasised that this is in fact a theoretical value. It has to be corrected 'before use' according to the above-mentioned equation with the specific lutum and humus contents of the soil type under investigation. In a recent comment on data and conclusions in the Integrated Criteria Document Zinc, a commission of the Dutch Health Council estimated the pre-industrial concentrations in soil and sediment to be lower than 100 mg/kg. The value of 100 mg/kg represents the maximum value for soils having a very high lutum content. Thus, according to the commission, the natural background concentration in soils with similar properties as a (Dutch) "standard" soil (25% L; 10% H) will be considerably lower than 100 mg/kg (Gezondheidsraad, 1998).

Several national Swedish reports give estimations of ambient (!) background concentrations in different types of soils in Sweden:

7 It is noted that soils from nature reserves and agricultural areas will be influenced to some extent by atmospheric deposition.

- From 1988 through 1995, 3,100 plough layer samples (0-20 cm) and 1,700 subsoil samples (40-60 cm) were collected from sampling sites randomly distributed throughout the agricultural areas in Sweden. Among other parameters Zn was analysed in these samples. The median concentrations in the plough-layer samples and the subsoil samples were 54 and 48 mg/kg dw, respectively. The 10^{th} and 90^{th} percentiles for the plough-layer samples were 25 and 99 mg Zn /kg dw, respectively (Eriksson et al. 1997).
- Another survey (Naturvårdsverket, 1997) reports results from the ongoing geochemical mapping (conducted by The Geological Survey of Sweden) of the c-horizon (about 1 m) in till soil from rural areas. More than 18 000 soils have been sampled and analysed (XRF) between 1983 and 1995. The values for the 10^{th}, 50^{th} and 99^{th} percentiles of these results were 30, 51 and 81 mg Zn/kg dw, respectively.
- The median zinc concentrations in mor layers of forest soils was (1983-84) estimated to 55 mg/kg dw (150 samples) (Naturvårdsverket, 1999b).

Above-mentioned data refer to ambient background concentrations in Sweden, which implies that natural background values are, or can be lower.

Background (ambient or natural not especified) concentrations for different federal states in Germany are given in the table below. Figures represent the topsoil (A horizon) and do not refer to the parent rock material (Source : LABO 1998).

Table 3.9 Background concentrations of zinc in soils for different federal states in Germany (source LABO 1998)

Federal state	Grassland	Agricultural soil	Forest soil
	50th perc./90 th perc. (n)	50 th perc./90 th perc. (n)	50 th perc./90 th perc. (n)
Baden-Württemberg	72/108 (344)	60 /107 (344)	37/64 (225)
Brandenburg	17/29 (56)	15/25 (598)	-/-
Bremen	132/246 (517)	31/134 (111)	35/250 (45)
Rheinland-Pfalz	-/-	Type II 54/81 (125) Type III 71/118 (257)	Type II 34/73 (103) Type III 77/146 (196)

Type II: regions with >150 inhabitants/km^2 and one centre with at least 100,000 inhabitants
Type III: regions with <150 inhabitants/km^2

Measures of "natural" soil zinc concentrations in metals are available for France (see Table below; http://www-sescpf.orleans.inra.fr/public/etm/)). The analysed samples are taken from surface and deep soils. The deeper layers often have a higher contents in clay than surface soils in France and therefore have a higher concentration in some of the metals, including zinc, analysed.

	Zn (mg/kg)
Number of data	(804)
Minimum	< 5
10th percentile	31
25th percentile	49
50th percentile	80
Mean	149

75th percentile	132
90th percentile	275
Maximum	3820

For zinc, concentrations from 10 to 100 mg/kg (<2mm air dried) soil are frequently observed in France. Soils with 100 to 250 mg/kg (<2mm, air dried) are observed in high mineralised zones at the contact between crystalline massifs and sedimentary basins, in particular in Yonne and Côte d'Or and in clay soil above calcareous, as Bourgogne or Jura. Soils with higher levels, 250 to 3800 mg/kg (<2mm, air dried) are observed in high mineralised zones at the contact between crystalline massifs and sedimentary basins, in particular in Yonne and Côte d'Or and in the department of Indre.

In a Danish monitoring program 393 sampling sites were monitored for heavy metals in Denmark (Bak et al., 1997). Urban areas and known contaminated areas were excluded. The zinc concentrations were: 0.3 (min), 5.8 (5P), 26.8 (50P), 59.7 (95) and 135 mg/kg dwt (max.). The authors concluded that these values can be regarded as natural background concentrations.

There are some background values (ambient or natural not especified) of zinc for soils in different regions of Northern and Mediterranean regions in Spain. The data are presented below:

Table 3.10 Background values (ambient or natural not specified) of zinc for soils in Nothern and Mediterranean regions in Spain

Area	Soil concentration (in mg/kg)	Reference
Galicia (North-west Spain)	5-159 (total range) forestry soils 5-133 (granites) 11-63 (shales) 22-132 (schists) 21-129 (anfiboles) 36-159 (gabbros)	Calvo et al. (1996)
Catalonia (North-east Spain, Mediterranean area)	67 (mean) 15 (min) 239 (max)	Junta de Residus (1995); Tobias et al. (1997(
Valencia (Southern Spain, Mediterranean area)	40-50 (mean) 10 (min) 300 (max)	Andreu (1991)

Conclusion

From the available soil data for a number of EU countries it is clear that there is a large variation in the natural zinc background concentrations. This variation is related to soil characteristics like humus and lutum. This relationship between natural background levels and various soil parameters is obvious. A quantification, however, of the exact natural background level for a specific EU soil type is at present still an extremely difficult and complex issue.The abovementioned reference line for soils in the Netherlands, which is based on background concentrations of zinc in Dutch soils, may be considered a useful screening tool for estimating

(natural) background zinc concentrations in soils in other European countries as well. In the present report, however, available soil monitoring data will only be used in the risk characterisation when a correction with the natural zinc background concentration(s) that are typical for that soil type is possible.

More (ambient) zinc concentrations measured in different soil types and areas, strongly polluted soils included, are presented in section 3.2.5.3.4.

3.2.4 Atmosphere

3.2.4.1 Release and fate

The most important anthropogenic sources of zinc entering the atmosphere are traffic and transport (tyres), the base metal industries and coal and fuel combustion (RIVM, 1996, Auweraert, 1997). Zinc may naturally enter the atmospheric compartment by windborne soil particles, igneous emissions, forest fires, biogenic emissions and seasalt sprays (WHO, 1996).

Zinc in the atmosphere is primarily bound to aerosols. Gaseous zinc accounts for less than 1% of total atmospheric zinc concentration. The mass median diameter of zinc-containing aerosol particles range from 0.3 μm to 5 μm. In rural areas zinc is primarily present in the finest fraction (70% of the zinc mass <1 μm, WHO, 1996), in urban and industrial areas the particle size can be as large as 5 μm. The proportion of water-soluble zinc on atmospheric particulate matter collected from a rural area was 26%, with a range of 12-48 % (WHO, 1996). Under conditions pertaining in The Netherlands, the average rate of removal by dry and wet deposition is 0.5% and 1.5% per hour, respectively (Cleven et al., 1993). The dry deposition velocity of atmospheric bound zinc ranges from 0.05 to 0.66 cm/s (WHO, 1996). The residence time of a zinc aerosol in the atmospheric is about 2 days (Cleven et al., 1993). In the atmosphere zinc-bearing particles may undergo chemical transformation before deposition. It is difficult to draw conclusions regarding the speciation of zinc in the atmosphere (ATSDR). Zinc particles (species) found in air are zinc sulphide, ferrous zinc, zinc phosphide, zinc chloride and metallic zinc (WHO, 1996).

3.2.4.2 Ambient and natural background concentrations

In ambient air the concentration of zinc is usually below 1 $\mu g/m^3$. As a background concentration in the Netherlands a value of 0.07 $\mu g/m^3$ has been reported. The level in the Netherlands is somewhat lower than that in Germany or Belgium (Cleven et al., 1993). Background levels given for all areas over the world range from 0.01 to 0.2 $\mu g/m^3$. For urban-industrial areas measured concentration range from 0.01 to 1.0 $\mu g/m^3$. More (ambient) concentrations measured in air are presented in section 3.2.5.3.4 (page 123).

3.2.5 Exposure scenarios

3.2.5.1 General

The objective of this exposure assessment is to determine the emissions, pathways and rates of movement and of transformation of zinc. This in order to estimate the added predicted

environmental concentration (PEC_{add}) for zinc in the different environmental compartments at a local and a regional scale. The EU-Technical Guidance document (TGD, 1996) and the European Union System for the Evaluation of Substances (EUSES 1.0) are used as a guideline to achieve this objective. The entry for estimating the environmental concentrations is, when available, the submitted information from industry, including monitoring data, and/or information gathered from other non-industrial sources. Otherwise PEC_{add} values will be calculated according to the TGD. Deviations from the TGD are mentioned in the text. For modelling the behaviour of zinc in the environment, the octanol-water partitioning coefficient (Kow) and the water solubility are not appropriate. Measured Kp values are used instead for sediment and suspended matter (TGD (Ap. VIII), 1996). See section 3.2.2 for more information about the used Kp values. The vapour pressure has been fixed at a low value of 1.10^{-10} Pa and the biotic and abiotic degradation rates have been minimised (TGD (Ap. VIII), 1996).

In the local exposure assessment the agricultural soil concentrations are calculated accounting for accumulation for 10 consecutive years. One should realise that this TGD defined period of 10 years is of lesser relevance to metals than to most organic chemicals. For zinc no steady state will be reached within 10 years. Unless stated otherwise, the input sources to the agricultural soil compartments are the application of sludge and the airborne deposition. For zinc the only removal or output from the agricultural soil compartment is by leaching to deeper soil layers. It is emphasised that other input or output sources, e.g. the use of manure or the crop offtake, are not taken into account for zinc in the local scenarios In the regional exposure assessment steady state agricultural soil concentration are calculated, accounting for the input sources deposition from air, sludge application, corrosion, manure and fertilisers and the output sources leaching to deeper soil layers and offtake via crops. The reason that factors like manure input and removal via crops have been applied in the regional calculations and not in the local modelling are pragmatic: there are reliable, average estimates available for these parameters at a regional level.

The mentioned concentrations ($(PE)C_{add}$) in surface water are mostly expressed as dissolved zinc concentrations. In the exposure scenarios the concentrations effluent water are expressed as total zinc concentrations. Only in the risk characterisation the total effluent concentrations are converted to dissolve effluent concentrations. The concentrations in sediment and soil are initially expressed on a wet weight (wwt) basis. Only when it is explicitly mentioned concentrations are dry weight (dwt) based.

Depending on the information submitted to the rapporteur, the $(PE)C_{add}$ calculations start at a different level. The different levels are presented in the flowchart of Figure 3.3. A generic scenario is used when no specific industrial emission information is available. In that case the EU (production) tonnage is the starting point for calculating the $(PE)C_{add}$ (entry 1). When a regional tonnage or an EU emission is available, which can be possible for the formulating and processing stages, the starting point is subsequently entry 2 or entry 3. With a regional tonnage, regional emissions can be derived by multiplying it with the appropriate release fractions (A-Tables, TGD, 1996). An EU emission can be divided by 10 to derive a regional emission. The justification of the use of the 10% rule in the emission estimation is explained in the paragraphs concerning the use categories of zinc metal. Also a submitted regional emission can be an entry for the $(PE)C_{add}$ calculation (entry 4). With this regional emission a local emission can be derived by multiplying it with the appropriate fraction of main source (B-Tables, TGD, 1996). With a local tonnage (entry 5) also local emissions can be derived by multiplying it with the appropriate release fractions (A-Tables, TGD, 1996). A site specific scenario can be used when local emissions are submitted by the industry (entry 6).

The risk characterisation, i.e. the comparison of the (PE)C_{add} with the corresponding PNEC, should be based on the most realistic exposure information. For this, the calculated local (PE)C_{add} values are compared with measured local concentrations, if available (entry 7). This step includes a possible correction for bioavaialability. In the next sections reference is made to Figure 3.3 for a better understanding of the procedures followed and entry points of the exposure assessment.

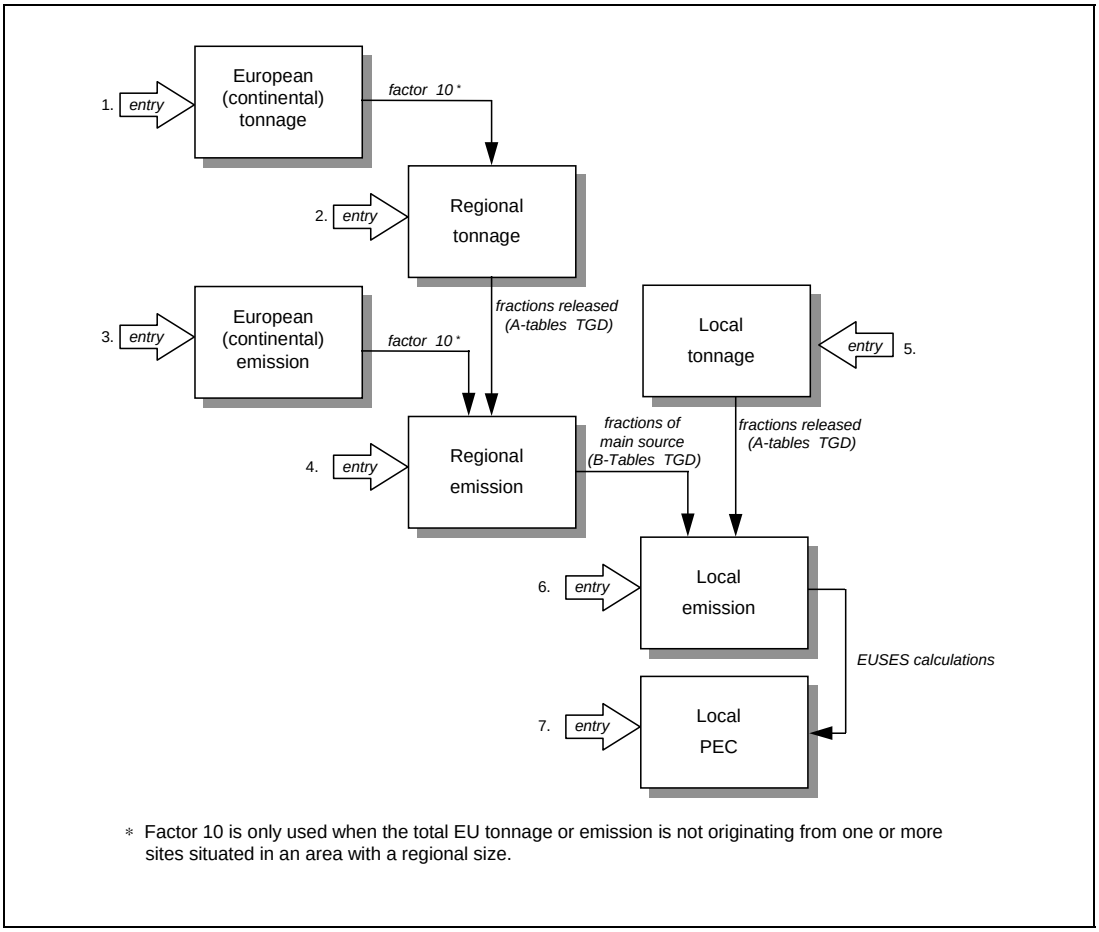


Figure 3.3 Flowchart for calculating the (PE)Cadd: the entry for the calculations is depending on the submitted information.

3.2.5.2 Local exposure assessment

3.2.5.2.1 General

The local environmental exposure assessment of zinc metal is based on the industrial releases of zinc during the following life cycle stages:

1. Production of zinc metal
2. Processing of zinc in galvanising industry
3. Processing of zinc in brass
4. Formulation in zinc alloy and processing of zinc die casting
5. Processing of rolled and wrought zinc

6. Production of zinc powder and dust

7. Production of zinc compounds

For the production stage site-specific emission scenarios could be used for calculating the local (PE)C_{add}'s in the various compartment. Also for most of the use categories (processing stages) actual local site-specific emissions were submitted. The submitted aquatic emissions mentioned in this report are assumed to be emissions to the surface water (net values). This means that these emissions are determined after treatment in a local waste water treatment plant (WWTP) or municipal sewage treatment plant (STP), unless it is otherwise mentioned. Generic scenarios are only used if data are missing from either the industry or other sources in order to carry out a representative local exposure assessment.

3.2.5.2.2 Production of zinc metal

For the production stage site specific emission scenarios could be used for calculating the local (PE)C_{add}'s in the various compartments (entry 6, Figure **3.3**). The submitted emissions per annum are corrected for the number of production days. Most of the producers submitted the number of production days, which are presented in Table 3.11. The emissions per year are transformed to emission per day with the number of production days, which are presented in Table 3.11. If no number of production days is available, it is assumed that the companies produce 300 days per year. Production tonnages, aquatic, atmospheric and waste emissions submitted by the zinc producing companies in the EU are presented in Table 3.11. With this information emission factors (ratio emission versus production) are calculated which are presented in Table 3.12. Table 3.12 illustrates that, except for the emission factors for waste, the difference between the calculated emission factors of individual companies is about two orders of magnitude. In addition, a comparison can be made between site-specific emission factors and the default ones from the TGD. These default emission factors for water and air are, respectively, 3.10^{-3} and 1.10^{-5} (Table A1.1). This implies that the average emission factor based on 'real world' data for air ($1.4.10^{-4}$) is higher than the default value. For water is the site-specific emission factor of $1.7.10^{-5}$ lower than the default value.

Additional aquatic information submitted by the zinc producing plants is presented in Table 3.13. This additional information is used for calculating the (PE)C_{add} values for surface water.

Table 3. 11 Production tonnages, aquatic, atmospheric and waste emission rates of the zinc producing industry in the EU for 1995 (information from industry).

Company number [11]	Production tonnage (t/y)	Number of production days	Emission to Air [10] (kg Zn/year)	Emission to water [10] (kg Zn/year)	Waste Emission [7] [10] (kg waste/year)
1	319,000	365	12,400	4,900	181,192,000
3 [13]	100,000	365	59,018	1,999	87,300,000 [1]
4	207,615	365	9,600	4,600 [6]	120,000,000
8 [2]	275,000	335	45,270	700	207,084,000
9	43,555	330	1,921	22.5	32,230,700
12 [12]	18,000	-	1,200 [12]	0	n.a.
15 [13]	107,800	365	33,849	6,791	62,540,000 [1]
16	121,503	365	575	9.93	43,376,571
18	82,000	330	11,808	820	53,500,000
20 (1995)	130,737	365	39,824	5,550	70,467,243 [5]
(2002)	146,328	365	15,683	4,567	96,133,000
21 (1995)	176,583	365	40,100 [3]	2,200 [3]	112,130,205
(2002)	235,337	335	23,906	243	169,272,000
22 [8]	24,860	322	251	46.4	0
23	95,000	365	5,000	180	1,030,300 [4]
24 [8] [13]	16,777	-	1,580	0	-
26	211,300	350	2,600	0	105,266,000
27	33,513	300	19,000 [9]	231 [9]	5,233,000
28	230,005	365	1,236	13,012	132,084,000
Total	2,193,248	-	246,722	41,062	$1.21.10^9$

1) Waste from pyrometallurgical process;
2) No production anymore, put “on care and maintenance”.
3) 35,700 kg Zn emitted to air and 770 kg zinc emitted to water in 1998;
4) This is no Fe-residue, but other wastes with zinc content <0.1% e.g. gypsum from waste water treatment;
5) Main waste stream of jarosite (4.6% zinc) dumped in mountain caverns;
6) This figure includes also historical emission, without this historical contribution an emission level of 1000 to 1500 kg might be expected;
7) Zinc content in waste 2.3% - 9.8%;
8) secondary zinc producer;
9) These data are only emissions of the production of zinc metal. The total emission values of this site including those of the separate activities of zinc alloys, zinc calots (semis) and zinc powders are 1330 kg/y to water and 21212 kg/y to air.
10) These emissions are not corrected for the number of production days mentioned in the third column
11) Some companies (numbers 2, 5, 6, 7, 10, 11 13, 14, 17, 19 and 25) indicated not to be a zinc metal producer and therefore no information is presented for these companies.
12) Unknown if the emissions of the seperate activity of the production of zinc powder are included in this figure.
13) Production plant is closed;

n.a not available, indicated by company
- unknown, no information submitted

Table 3. 12 Emission factors (emission / production) for the zinc metal producing industry in the EU for 1995 (calculated from Table 3. 13).

Company number	**Emission factor** Air (kg Zn/y / kg Zn/y)	**Emission factor** Water (kg Zn/y / kg Zn/y)	**Emission factor** Waste (kg waste/y / kg Zn/y)
1	3.89E-05	1.54E-05	0.568
3	5.90E-04	2.00E-05	0.873
4	4.62E-05	2.22E-05	0.577
8	1.65E-04	2.55E-06	0.753
9	4.41E-05	5.17E-07	0.740
12	6.67E-05	0	-
15	3.14E-04	6.30E-05	0.580
16	4.73E-06	8.17E-08	0.357
18	1.44E-04	1.00E-05	0.652
20 (1995)	3.05E-04	4.25E-05	0.539
(2002)	1.07E-04	3.12E-05	0.656
21 (1995)	2.27E-04	1.25E-05	0.635
(2002)	1.02E-04	1.03E-06	0.719
22 [1)]	1.01E-05	1.87E-06	-
23	5.26E-05	1.89E-06	0.0108
24 [1)]	9.42E-05	-	-
26	1.23E-05	-	0.498
27	5.67E-04	6.89E-6	0.156
28	5.37E-06	5.66E-05	0.574
Minimum	4.73E-06	8.17E-08	0.0108
Maximum	5.90E-04	6.30E-05	0.873
Average	1.58E-04	1.71E-05	0.537

1) Secondary zinc producer

- No emission or emission factor could not be calculated

Table 3.13 Additional aquatic information for zinc producing plants in the EU for 1995 (information from industry).

Company number [5]	Emission amount to water (kg/y)	Effluent discharge rate (m^3/day)	Efficiency local STP (%)	Flow rate or type of receiving water (m^3/day)
1	4,900	5,000	99.9	n.a.
3 [8]	1,999	3,400	99.98	1,074,400 [1]
4	4,600	7,200	>99	21,600
8 [9]	700	400	90	Sea
9	22.5	250	90	Sea
12	0	-	-	-
15 [8]	6,791	24,432	98.2	864,000
16	9.93	971	>99	38,880,000 [2]
18	820	9,600	-	172,800,000[6]
20 (1995)	5,550	2,331	99.9	sea [4]
(2002)	4,567	2,331	99.9	sea [7]
21 (1998)	770	3,700	>99	sea
(2002)	243	4036	>99	sea
22	46.4	240	-	n.a.
23	180	500-650 [3]	99-99.5	1,700,000
24 [8]	0	-	-	-
26	0	824	>90	closed circuit
27	1,330	2,000-6,000	>90	84,300
28	13,012	6,300	99.5	648,000 canal

n.a not available, indicated by company
- unknown, no information submitted
1) sea, flow rate calculated from measured dilution factor
2) mean value
3) without any comment the industry revised this value to 1080 m^3/day, the value of 500 m^3/day is further used in this report
4) According to national authorities the dilution factor varies from 16-50. Most of the values are >20. The dilution factor of 16 is used for this report.
5) Some companies (numbers 2, 5, 6, 7, 10, 11 13, 14, 17, 19 and 25) indicated not to be a zinc metal producer and therefore no information is presented for these companies.
6) Based on low water level flow of 2000 m^3/s.
7) According to Norwegian Inst. for Water Reaseach the dilution factor varies from 15-30. The dilution factor of 16 is used for this report.
8) Production plant is closed;
9) No production anymore, put “on care and maintenance”.

Air

For all major zinc producers in the EU the site-specific emission data are used for calculating the (PE)C_{add} values in air (entry 6, Figure **3.3**). Emissions into air arise from the transhipment of ore (as dust) and the production process (Cleven et al., 1993). Fabric filters are the most commonly used system to reduce particulate emissions during the production process (IHE, 1991). The annual average local atmospheric C_{add} values at a distance of 100 meters from a point source are calculated from the daily amounts released to air (TGD, 1996).

The emission amounts during emission episode and the calculated local annual average concentrations of zinc in air are presented in Table 3.14. The range of these calculated local C_{add} values in air is **0.178-36.9 µg/m^3.**

Water

The zinc metal producing industry submitted aquatic emissions as effluent water emissions after treatment in a local (industrial) waste water treatment plant (WWTP). The waste waters of none of the production plants are treated in a municipal sewage treatment plant (STP). The zinc emissions to effluent water are reduced when industrial waste water is treated in a WWTP. Adsorption is the most important removal process. Other removal processes (evaporisation, degradation) are considered not to be relevant for zinc. In a WWTP the adsorbed fraction is mainly removed by precipitation. More information about zinc in WWTP or STP sludge is presented further on in this section. Other information about the suspended and dissolved forms of zinc is presented in section 3.2.2.1.

For all major zinc producers in the EU the site-specific emission data are used for calculating the (PE)C_{add} values in water (entry 6, Figure **3.3**).

The submitted aquatic emissions for all production sites are emissions to surface water (net values). This means that these emissions are determined after a WWTP has treated the industrial waste waters.

The daily releases to surface water and the effluent discharge rates are the input for calculating the concentration in the WWTP effluent. For 15 production companies (incl. secondary producers) the submitted effluent discharge rate (Table 3.13) is used instead of the default value of 2000 m^3/d.

The concentration of zinc in the effluent of an STP is calculated with the equation:

$$C\,local_{effluent} = \frac{EMISSION\,local}{EFFLUENT\,local_{STP}}$$

Clocal$_{effluent}$: concentration in effluent water (kg/m^3)
EMISSIONlocal: local emission rate to waste water (kg/d)
EFFLUENTlocal$_{STP}$: effluent discharge rate of local STP (m^3/d)

Only one company (number 9) submitted an effluent concentration with a value of 0.3 mg/l.

For six production companies the default dilution factor of 10 could be overwritten and is calculated with the submitted effluent discharge rate of the STP and the flow rate of the river (Table 3.13) according to the following equation:

$$D = \frac{EFFLUENTlocal_{STP} + FLOW}{EFFLUENTlocal_{STP}}$$

D: dilution factor
EFFLUENTlocal$_{STP}$: effluent discharge rate of local STP (m^3/d)
FLOW: flow rate of the receiving river (m^3/d)

For calculating the local concentrations of zinc in water emitted to estuaries or lakes a default dilution factor of 10 is assumed, unless otherwise mentioned.

Subsequently, from the effluent concentration in the STP the local concentration in the receiving surface water during the emission episode can be calculated with the following equation. Dilution in the receiving surface water and sorption to suspended solids are taken into account.

$$C_{add}local_{water} = \frac{C local_{effluent}}{(1 + Kp_{susp} * C_{susp}) * D}$$

$C_{add}local_{water}$: local added concentration in water during emission episode (kg/m^3)
Kp_{susp}: solids-water partition coefficient of suspended matter. For zinc 110 m^3/kg (see Partition coefficients (Stortelder et al., 1989))
C_{susp}: concentration of suspended matter in river water (0.015 kg_{dwt}/m^3, TGD)
D: dilution factor (default = 10)

The emission amounts during emission episode and the calculated local concentrations of zinc in water are presented in Table 3.14. The range of calculated local C_{add} values in water starting from a submitted emission to water is **$2.64.10^{-4}$-165 µg/l,** sources with no emission to water excluded.

Sediment

The local concentrations in sediment (wet weight) during the emission episode can be estimated from the local C_{add} values in water, the suspended matter-water partition coefficient and the bulk density of suspended matter. The local concentrations in sediment during emission episode are calculated according to the following equation:

$$C_{add}local_{sed} = \frac{K_{susp-water}}{RHO_{susp}} * PEC_{add}local_{water}$$

$$where: \quad K_{susp-water} = Fwater_{susp} + Fsolid_{susp} * Kp_{susp} * RHOsolid$$

$C_{add}local_{sed}$: added concentration in sediment during emission episode (kg/kg_{wwt})
$K_{susp\text{-}water}$: suspended matter-water partition coefficient (calculated $2.75.10^4$ m^3/m^3)
RHO_{susp}: bulk density of suspended matter (1150 kg_{wwt}/m^3)
$Fwater_{susp}$: fraction of water in suspended matter (0.9)
$Fsolid_{susp}$: fraction of solids in suspended matter (0.1)
Kp_{susp}: solids-water partition coefficient of suspended matter. For zinc 110 m^3/kg (see Partition coefficients (Stortelder et al., 1989))
RHOsolid: density of solid phase (2500 kg/m^3)

The calculated local concentrations of zinc in sediments are presented in Table 3.14. The range of calculated local C_{add} values in sediment is **$6.31.10^{-3}$- 3,949 mg/kg_{wwt}**, sources with no emission to the aquatic compartment excluded.

Table 3.14 Summary of the local production tonnages, emission rates and calculated C_{add} values.

Company name [1]	Production tonnage (t/y)	Emission air (kg Zn/d)	Emission water (kg Zn/d)	C_{add} air ($\mu g/m^3$)	Concentr. effluent STP (total) ($\mu g/l$)	C_{add} water (dissolved) ($\mu g/l$)	C_{add} Sediment (mg/kg_{wwt})
1	319,000	34.0	13.4	7.76	2,685	101	2,423
3 [4]	100,000	162	5.48	36.9	1,611	1.92	45.9
4	207,615	26.3	12.6	6.0	1,750	165	3,949
8 [5]	275,000	135	2.09	30.9	5,224	197	4,714
9	43,555	5.82	0.0682	1.33	273	10.3	246
12	18,000	4.0	0	0.913	0	0	0
15 [4]	107,800	92.7	18.6	21.2	762	7.90	189
16	121,503	1.58	0.0272	0.360	28.0	$2.64.10^{-4}$	$6.31.10^{-3}$
18	82,000	35.8	2.48	8.17	259	$5.43.10^{-3}$	0.130
20 (1995)	130,737	109	15.2	24.9	6,523	154	3,679
(2002)	146,328	43.0	12.5	9.81	5,367	127	3,027
21 (1998)	198,900	97.8	2.11	22.3	570	21.5	514
(2002)	235,337	65.5	0.666	15.0	42.7	1.61	38.5
22	24,860	0.778	0.144	0.178	600	22.7	542
23	95,000	13.7	0.493	3.13	986	0.109	2.62
24 [4]	16,777	5.27	0	1.20	0	0	0
26	211,300	7.43	0	1.70	0	0	0
27 [2]	33,513	63.3	0.77	14.5	385	3.37	81
27 total [3]		70.7	4.43	16.1	2217	19.4	464
28	230,005	3.38	35.6	0.773	5,659	20.6	492

- unknown, no information submitted

Some companies (numbers 2, 5, 6, 7, 10, 11 13, 14, 17, 19 and 25) finally indicated not to be a zinc metal producer and therefore no information is presented for these companies.

Only zinc metal production separated from the other activities at this site

Total emission values and concentrations of this zinc metal production site, including those at the production of zinc alloys, zinc calots (semis) and zinc powders.

4) Production plant is closed;

5) No production anymore, put "on care and maintenance".

Soil

According to the TGD (1996) both the application of STP sludge on agricultural soil and the deposition from air are taken into account for calculating the zinc levels in the terrestrial compartment. For zinc production companies the STP sludge is either partially reused into the process or disposed off in controlled landfill sites (information from industry). Hence, only the emission to air, followed by a distribution and deposition model, is used for calculating soil concentrations. In this case the local emissions to air are the only input for calculating soil concentrations. The calculated concentrations of zinc in agricultural soils calculated at a local scale are presented in Tabel 3.15. For production companies the range of calculated local C_{add} values in agricultural soil are **$6.75.10^{-2}$ – 14.0 mg/kg_{wwt}**.

Sludge

The industrial waste waters can be treated in a local (industrial) waste water treatment plant (WWTP) or in a municipal sewage treatment plant (STP). In a WWTP (and STP) the adsorbed fraction is mainly removed by precipitation. The precipitate (sludge), which is separated during the cleaning process, is either partially reused into the process or disposed off in controlled landfill sites (information from industry). The waste water releases ($Elocal_{water}$) are calculated from the effluent water releases in which it is assumed that zinc is removed in the WWTP for the percentage presented in Table 3.13.

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The concentration in dry sewage sludge can be calculated according to the equation:

$$C_{sludge} = \frac{Fstp_{sludge} * Elocal_{water}}{SLUDGERATE}$$

where: $$SLUDGERATE = \frac{2}{3} * SUSPCONC_{inf} * EFFLUENT_{STP} + SURPLUSsludge * Nlocal$$

C_{sludge}:	concentration in dry sewage sludge (kg/kg_{dwt})
$Fstp_{sludge}$	fraction directed to sludge by STP (see

)

$Elocal_{water}$:	local emission rate to waste water during episode (kg/d)
SLUDGERATE	rate of sewage sludge production (calculated: 710 kg/d)
$SUSPCONC_{inf}$:	concentration of suspended matter in STP influent (0.45 kg/m^3)
$EFFLUENT_{stp}$:	effluent discharge rate of local STP (2000 m^3/d)
SURPLUSsludge	sludge per inhabitant equivalent (0.011 kg/d.eq)
Nlocal:	Number of inhabitants feeding local STP (10,000 eq)

The calculated concentrations in dry sewage sludge range from 0 to 22.6 kg/kg_{dwt} (data not shown). The calculated concentrations in sludge are very high (unrealistic) for a few plants (3.5-22.6 kg/kg_{dwt}), mainly as a result of the very high submitted efficiency rate of the WWTP (99.5%-99.98%), a relative small default STP-size and a rather high zinc emission to water. Further it must be mentioned that the equation above is probably not appropriate for industrial WWTPs. The rapporteur realises that the above mentioned sludge concentrations exceed the theoretical maximum. The issue, however, is not relevant for zinc producers, as their sludge is not used on agricultural soils.

Waste (see note in section 2.1.1)

The submitted total waste emission in the EU is about 1,210,000 t/y (Table 3.11). The total zinc emission is about 55,000 t/y, calculated with a reported average zinc content in waste of about 4.8%, with a range of 2.3% to 9.8% (see Table 3.11, footnote 7). The waste factor (ratio tonne waste versus tonne zinc produced) for the production of zinc metal varies from 0.01 – 0.87 (see Table 3.12).

Waste from the hydrometallurgical process

Zinc is mainly produced according to the hydrometallurgical process (>80% of total production). At the production of zinc by the hydrometallurgical process Fe-residue is generated, due to the Fe-content in the zinc concentrates. These Fe-residues are stored as jarosite or goethite in monodeposits, specially designed for this purpose. The zinc content of jarosite waste (about 2-5%) is in general lower than the zinc content of goethite waste (about 7-8%). Jarosite and goethite waste is permanently stored on landfill sites and storage basins, because there is no prospect that it can be processed in the future. Only in a few cases alternatives to storage in ponds are used, e.g. deposits in mountain caverns or the use in road constructions. Other wastes are neutralised waste water residues (gypsum, about 5% zinc content), filter material and rubble (debris), as well as small quantities of specific waste (containing Hg, As, Cd, Pb etc.), which are treated in a specific way. For most waste disposal sites it is known that they are monitored on regular basis, however actual concentrations in ground water and surface water are not available or unknown. Only one site reported upstream and downstream concentrations in ground water, from which was concluded that both concentrations did not significantly differ.

Zinc emissions can arise from old storage ponds, which were not properly designed. In those cases contamination of surrounding groundwater and surface water is as far as possible prevented by specific installation systems. From a large storage basin in The Netherlands (build in 1973) of 650,000 tons of jarosite it is known that it is leaking. At this site the emission of zinc to the soil compartment as a result of leaking was about 300 t/y. To prevent migration of the contaminant, leaking water is collected with a drainage system. According to the industry, this figure is no longer relevant since this jarosite pond has been sealed off recently. At another company in Finland around a 37 ha storage pond (build in 1974) a 10 meters deep HDPE/bentonite barrier has been constructed to receive the contaminated percolating water. Before the construction of this barrier, an emission of 16.7 t/y to sea and 6.7 t/y to ground was estimated. Data from after the construction of the barrier are not yet available.

Waste from the pyrometallurgical process

At the production of zinc by the pyrometallurgical process mainly granulated zinc blast furnice slag (zinc sinter reduction) is generated, which is deposited on landfill storage sites. Other waste, e.g. hydroxides from the WWTP are directly recycled in the plant or directed to other sites for metals recovery. In the EU only three plants produce zinc according to a pyrometallurgical process. Two plants have submitted more detailed information on their waste. The reported zinc concentrations in the blast furnace slag is 10.9% and 4-8%. At one site surface water is monitored at 2 points (1.9 mg/l) and ground water at three points (average 3.5 mg/l). At the second waste disposal site only ground water is monitored (<10 µg/l).

Table 3.15 Summary of the local emission rates and calculated C_{add} values for agricultural soils

Company number [1]	**Emission** Air (kg Zn/d)	**C_{add}** Agricultural soil (30 d.) (mg/kg_{wwt})
1	34.0	2.94
3 [4]	162	14.0
4	32.0	2.28
8 [5]	135	11.7
9	5.82	0.504
12	4.0	0.346
15 [4]	92.7	8.03
16	1.58	0.136
18	35.8	3.10
20 (1995)	109	9.45
(2002)	43.0	3.72
21 (1998)	97.8	8.47
(2002)	65.5	5.67
22	0.778	$6.75.10^{-2}$
23	13.7	1.19
24 [4]	5.27	0.456
26	7.43	0.643
27 [2]	63.3	5.48
27 total [3]	70.7	6.12
28	3.38	0.293

1) Some companies (numbers 2, 5, 6, 7, 10, 11 13, 14, 17, 19 and 25) finally indicated not to be a zinc metal producer and therefore no information is presented for these companies.

2) Only zinc metal production seperated from the other activities at this site

3) Total emission values and concentrations of this zinc metal production site, including those at the production of zinc alloys, zinc calots (semis) and zinc powders.

4) Production plant is closed;

5) No production anymore, put “on care and maintenance”.

3.2.5.2.3 General information on the use categories of zinc in the EU

The distribution and EU tonnages of the different use categories of zinc metal are presented in Table 3.16. For the use categories specific emissions or emissions factors were submitted.

Table 3.16 Distribution and total zinc tonnages for the different use categories of zinc in the EU (information from industry).

No.	Branch of industry	Fraction [1)]	EU Tonnage (tonnes/year) [2)]
1	Galvanising	38.8%	± 851,000
2	Zinc in brass	25.5%	± 560,000
3	Die casting alloy	12.4%	± 273,000 [3)]
4	Rolled/wrought zinc	11.8%	± 258,000
5	Zinc powder/dust	2.9%	± 63,000
6	Others (production zinc compounds)	8.6%	± 188,000
	Total	100%	2,193,000

1) The fractions are based on detailed analyses of the International Lead and Zinc Study Group (ILZSG) and IZA-Europe, slightly adjusted for the new submitted EU tonnage of brass

2) The tonnages for each branch of industry are calculated according to the fractions and the total of 2,193,000 t/y

3) Based on later submitted information this figure might be an underestimation, see section 3.2.5.2.6.

Not only for the zinc production stage, but also for the processing stages the submitted aquatic emissions are determined after a WWTP or STP has treated the industrial waste waters (net values). For all use categories emissions after local WWTP are reported. The local C_{add} values for water and sediment are calculated as described in the production section 3.2.5.2.2.

For soil the application of WWTP sludge on agricultural soil is not taken into account, according to information from the industry. Hence, for all use categories only the deposition from air is taken into account for calculating the concentration in agricultural soils. For all use scenarios the emissions from incineration or storage of zinc containing sludge are not taken into consideration.

3.2.5.2.4 Processing in the galvanising industry

Because of the nature of galvanising a distinction is made between three different processes (see also section 2.2.2). Galvanising can be a hot dip batch process (usually called ‘general’ galvanising), a continuous process (continuous hot dip galvanising) and an electroplating process (electro galvanising). The most recent zinc tonnages for the different use categories of galvanising in the EU are presented in Table 3.17. These figures are slightly different from those mentioned in Table 3.16, because the sources (and probably calculation methods) are not identical. In the general galvanising pretreated steel is immersed in liquid zinc until the liquid zinc has reacted with the solid surface of the steel fabrication. In the continuous hot dip galvanising a fused metal coat is applied to zinc strip on a continuous basis. In the electro galvanising a zinc salt solution is used to electrolytically deposit a layer of zinc on steel. More information about the galvanising processes is presented in section 2.2.2.

Also zinc chloride is used in the general galvanising industry as a constituent of a flux coating to make the steel surface capable of wetting by liquid zinc. It is not possible to make a clear distinction between the zinc emission from either metallic zinc or zinc chloride. Hence, this chapter is also applicable to the use of zinc chloride in the galvanising industry.

Table 3.17 Zinc tonnages for the different use categories of galvanising in the EU.

	Tonnage ILZSG (1997)	**Tonnage**	**Tonnage covered in submission** [3]	**Percentage covered**
General Hot Dip Galvanising	358,000		unknown	unknown
Continuous Hot Dip Galvanising	632,000	579,200 [1]	428,352	74%
Electro Galvanising		52,800 [2]	45,213	86%
Total	990,000			

1. Calculated from the submitted values of 632,000-52,800=579,200;
2. Submitted data februari 2001 (Eurofer);
3. See Table 3.22 and Table 3.23;

Separate exposure scenarios are carried out for calculating local environmental concentrations from the galvanising industry. The scenario for the General Hot Dip Galvanising (GHDG) is based on aggregated site-specific emission data from individual countries. The scenario for the Continuous Hot Dip Galvanising (CHDG) and Electro Galvanising (EG) is based on submitted site specific information for individual companies.

General Hot Dip Galvanising (GHDG)

The wastes generated during the general galvanising processes are used flux solutions (3,000 t Zn/y), retained filter dust (600 t Zn/y), ashes (66,000 t Zn/y) and dross, a Fe-Zn alloy (66,000 t Zn/y). The estimated total EU emission to air of the total general galvanising industry is about 50 t/y. In Table 3.18 for the GHDG industry local emission values are presented, based on information from the European General Galvanizers Association (EGGA, 1998). For the GHDG industry emission data to air and water was received from the UK, France and Germany. The data covers about 312 out of a total of about 650 general galvanising plants. Only emission ranges for specific countries were received, data are lacking concerning separate companies in the EU. From the general galvanising plants in the Netherlands only the emission data to water was received. According to the industry there are no emissions to the aquatic compartment from general galvanising, except the use of quench water at three UK plants. The use of quench water is responsible for an estimated discharge of 64 g ZnO/y (=48 g Zn/y). For the French general galvanisers a threshold level of 0.3 kg/d has been mentioned, but this refers to the lower limit of emission below which no reporting is required..

In Table 3.18 the concentrations in air are calculated with the minimum and maximum emissions to air, which are based on the submitted data for the mentioned countries.

Table 3.18 Input data and results for the local exposure assessment for processing in the GHDG industry, based on information from the European General Galvanizers Association (EGGA).

	General hot dip galvanising
Based on	UK / Germany / France / Netherlands
Industrial category / use category	15/14
Number of days	300
Fraction of main source (B-tables, TGD)	Not relevant
Local amount released to air (kg/d)	0.0023-0.149
Local amount released to water (kg/d)	0
Size of STP (m^3/d)	2,000
Dilution factor	10
Results:	
Conc. Effluent STP (µg/l)	0 (<150[1)])
C_{add} water (µg/l)	0 (<5.66[1)])
C_{add} air (µg/m^3)	$5.25.10^{-4}$-$3.40.10^{-2}$
C_{add} sediment (mg/kg_{wwt})	0 (<135[1)])
C_{add} agricultural soil (mg/kg_{wwt})	$1.99.10^{-4}$ – $1.29.10^{-2}$

1. The values between brackets are calculated with the submitted threshold level of < 0.3 kg/d to water. This is not an actual release.

According to industry information (SDV, 2000) zinc emission may occur in waste water streams from galvanisers due to the following reasons: 1) deposition of zinc discharged by the air filter of the plant, 2) draining water from polluted soils on site and 3) wash off from the galvanised steel present in the yard of the plant. According to research of the EGGA (2001) the zinc emissions in waste water streams from general galvanisers in the Netherlands and Germany due to the above mentioned reasons are much lower (10.5 kg/y) than the SDV (2000) estimate. The EGGA data were, however, only submitted as yearly average figures. The zinc emissions according to the above mentioned reference are summarised in Table 3.19.

In 2002 the SDV submitted an updated review of the emissions to surface water based on 20 GHDG plants in the Netherlands (SDV, 2002). The main sources for zinc emission to the environment are 1) spills and used pre-treatment liquids, 2) dust and roof runoff and 3) corrosion and runoff of stored product. Because none of the 20 plants in the Netherlands discharge any process liquid or process water to the sewer or the environment, only the second and third source is further quantified.

The SDV determined the yearly zinc emissions to air to quantify the dust and roof runoff. They applied a realistic worst case approach were a 90^{th} percentile value of the emission factor (46.28 g/ton) of the most accurate method is applied on each plant. The result shows a range of 8.33 to 121.82 kg zinc/y emitted to air. Based on an existing computer model of TNO (1998) a realistic worst case deposition of 0.5% is used to determine the runoff from local deposition at the industrial area of a GHDG plant. Therefore, the largest dust and roof runoff is 122 kg/y * 0.005 = 0.61 kg/y.

To estimate the emission from corrosion and runoff a theoretical approach has been used, because actual data from a monitoring system for storm water was not available. Several assumptions were made for the theoretical approach (SDV, 2002): the amount of stored product is equal to a daily production volume (220 days/y), an avarage outer surface of 32 m^2 per ton steel, a corrosion rate of 3 g/m^2 and a correction factor of 0.3 for the exposed surface area as a result of stacking. Based on these assumptions an emission factor of 0.13 g zinc per tonne of steel was calculated. With the local processing tonnage of steel a range of 0.20 to 4.46 kg zinc per year is calculated for corrosion and runoff.

The largest total zinc release is therefore 0.61 + 4.46 = 5.07 kg/y. Based on 86 days per year of rainfall larger than 2 mm (rainfall < 2 mm will not generate runoff) the total release is 0.0589 kg zinc/d. The release per rainy day may differ, but accurate methods to quantify these differences are lacking and therefore the yearly load is divided equally over the 86 days. The largest concentration in surface water without treatment of a STP is 0.0589/18,000 m^3 = 3.27 µg/l. With STP treatment the largest concentration in surface water is 0.29 µg/l (flow STP = 2000 m^3/d, removal = 74%, Kp_{susp} = 110 m^3/kg and dilution = 10). The zinc emissions are summarised in Table 3.19. The calculated concentrations in effluent water, surface water and sediment are presented in Table 3.20.

Table 3.19 Reported emissions to public sewer from other sources than processing in the GHDG industry.

	GHDG Netherlands and Germany: about 185 plants (EGGA, 2001) [1)]	**GHDG** Netherlands (SDV, 2002)
1. Deposition of zinc discharged by the air filter of the plant to C_{add} water (kg/y)	3 (annual average)	0.042-0.61 [3)]
2. Draining water from polluted soils at site (kg/y)	no emissions	Unknown
3. Wash off from the galvanised steel present in stock of the plant (kg/y)	6 (annual average)	0.20-4.46 [3)]
4. Other sources (kg/y)	1.5 (annual average)	no estimate
Total of all sources (kg/y)	10.5 (average)	0.24-5.07 [3)]

1. No data submitted for separate sites. No range available.
2. Maximum emission value at galvano sites
3. 86 days per year of rainfall are used for calculating the amount emitted per day

Table 3.20 Reported waste water concentrations and emissions and calculated water and sediment concentrations for GHDG plants in the Netherlands and Germany.

	GHDG NL and D: (EGGA, 2001) [4]	**GHDG** Netherlands (SDV, 2002)
Number of days (d)	300	220
Dilution factor in public sewer system	46 [1]	Not applicable
Size of STP (m^3/d)	2000	2000
Removal of zinc in municipal STP	74%	74%
Dilution factor (from effluent to river)	10	10
Reported emissions to public sewer from other sources than processing (kg/y)	10.5 [5]	0.24-5.07 [3]
Reported concentration in waste water discharges to municipal STP (mg/l)	Not applicable	Not applicable
Results:		
Calculated concentration in waste water to municipal STP based on total of all sources (µg/l)	790 [2]	-
Calculated conc. effluent STP (µg/l)	12.7	0.36-7.67
Calculated C_{add} water (µg/l)	0.480	0.014-0.29
Calculated C_{add} sediment (mg/kg_{wwt})	11.5	0.33-6.92

1. Average, based on average water flow from gavanising sites of 44 m^3/d (=19,000 m^2 * 0.700 m = 13,300 m^3/y) and a default sewage water flow of 2000 m3/d;
2. Calculation based on average site area of 19,000 m^2 and a rainfall of 700 mm/y ;
3. See Table 3.19
4. Double counting possible with the NL emissions listed in the next column.

The Rapporteur is aware that the site-specific information on the GHDG industry is limited to only a few EU countries. The available data are, however, considered to be representative for the EU. This because the process itself does not result in zinc emissions (holds for EU in general) and the releases from 'non-process sources' are expected not to be significantly different between the various EU countries. The only point may be that the additional sewage treatment step is lacking in some EU countries, but there is no information available on that issue. Only the SDV (2002) data will be further used in the risk characterisation for the GHDG industry.

Continuous Hot Dip Galvanising (CHDG)

The industry submitted site specific information for 31 continuous hot dip galvanising companies in the EU which are presented in Table 3.22 (numbers 1-31). The total production volume of these companies is about 428,352 t/y (Table 3.22), which is equal to about 74% of the total EU tonnage used in CDHG industries (see Table 3.17). In future the production levels of CDHG galvanised steel continues to rise (Information from industry). For France additional site specific emissions to water were submitted for 19 unknown CHDG plants, which are also presented in Table 3.22. Some of those 19 CHDG plants in France are already covered by the survey for separate companies and therefore some companies are double counted in Table 3.22. Emissions are lacking for the other remaining CHDG sites in the EU.

The results for the local exposure assessment, based on site specific information for continuous hot dip galvanising are presented in Table 3.24. The methodology used to obtain local C_{add} values is described in section 3.2.5.2.3. In contrast to GHDG, in the CHDG industry, the coils of finished galvanised steel are always stored under cover (see section 2.2.2). Therefore, there is no possibility of zinc-containing run-off from these stored products (Industrial information Eurofer, 2001).

Electro Galvanising (EG)

The industry submitted site specific information for seven electro galvanising companies in the EU which are presented in Table 3.23 (numbers 1-12). The total production volume of these companies is about 45,213 t/y (Table 3.23), which is equal to about 86% of the total EU tonnage used in electro galvanising industries (see see Table 3.17). In future the production levels of EG galvanised are tending to fall (Information from industry). Emissions are lacking for the remaining EG sites in the EU (about 14% of the total EU tonnage). The results for the local exposure assessment, based on site specific information for electro galvanising, are presented in Table 3.25. The methodology used to obtain local C_{add} values is described in section 3.2.5.2.3.

Additional exposure assessment for CHDG and EG industries

About 26% and 14% of the CHDG and EG plants, respectively, is not covered by the submitted site specific information. According to industry the submitted information comes from industries in EU countries where zinc releases have to be reported on a legal basis (permits). Industry further indicated that, implicitly, no information was presented for CHDG and EG industries in EU countries without such 'zinc regulation'. From the reported data it can be seen that high emissions occurred before 1998. Most probably local emission reduction measures have actually led to this decrease in emissions. However, because 1) a considerable part of the plants is not covered, 2) information is only available for 'zinc regulated' countries and 3) high water emissions occurred before actual measures were taken, indicating that high emissions from the process itself may occur, it was felt that an additional exposure assessment for the CHDG and EG sector is still needed. This to represent the sites in countries with no specific regulations for zinc emissions. The assumption is that industrial emission factors from before 1998 in the regulated countries may still be relevant for the non-regulated ones. This additional realistic worst case scenario starts with the largest known local CHDG and EG tonnage and calculates emissions to air and water with the largest site-specific release factor from before 1998 (entry 5, Figure **3.3**). This release factor is determined from the tonnages and emissions of the known sites (Table 3.22 and Table 3.23). Table 3.21 contains the input data and results of the local exposure assessment for processing in the CHDG and EG galvanising and coating industry according to this realistic worst case scenario.

Table 3.21 Used input data and results for the additional local exposure assessment for processing in the CHDG and EG galvanising industry.

	Continuous hot dip galvanising	**Electro-galvanising**
Largest local production tonnage, see Table 3.22, Table 3.23(t/y)	57,000	7,000
Fraction released to air (see Table 3.22, Table 3.23)	0	$6.0.10^{-6}$ 1)
Fraction released to water (see Table 3.22, Table 3.23)	$8.1.10^{-5}$ 2)	$4.5.10^{-4}$ 3)
Number of days	300	300
Calculated local amount released to air (kg/d)	0	0.14
Calculated local amount released to water (kg/d)	15.4	10.4
Size of STP (m^3/d)	2,000	2,000
Dilution factor	10	10
Results:		
Conc. effluent STP (µg/l)	7,686	5,203
C_{add} water (µg/l)	290	196
C_{add} air (µg/m^3)	0	0.032
C_{add} sediment (mg/kg_{wwt})	6,935	4,695
C_{add} agricultural soil (mg/kg_{wwt})	887	601

1) Based on company F as presented in Table 3.23;

2) Based on company H as presented in Table 3.22;

3) Based on company D as presented in Table 3.23.

Table 3.22 Submitted data for continuous hot dip galvanising (CHDG) sites in the EU.

No	Company	Additional information	Total Zn processed in 1998	No. of working days	Emission to air	Emission to water (1998)	Largest emission to water (before 1998)	Flow local WWTP	Flow receiving water
			t Zn/y	*day/year*	*kg Zn/y*	*kg Zn/y*	*kg Zn/y*	*m^3/d*	*m^3/d*
1	Company A	2 lines	26,096	-	no data	11.32	18.25 (1997)	14.88	lake
2	Company B	1 line	14,960	-	0	21.3	-		sea
3	Company C	4 lines	40,072	320	negl. [4]	60.5	147.8 (1995)	1,700	16,243,200
4	Company E1	line 1	14,433	-	negl. [5]	0	-	No WWTP	500
5	Company E2	line 2	8,200 [1]	-	idem	196 [1]	-	No WWTP	1,300
	Company E2	line 2	9,300 [14]	-	idem	12.9 [14]	-	No WWTP	1,300
6	Company G1	line 1	7,214	-	< det limit [3]	30 [7]	-	430 [9]	large river
7	Company G2	line 2	11,089	-			-		
8	Company H	1 plant: 4 lines	22,984	365	0	938.82	1858 (1995)	18,353	-
9	Company I	1 plant	12,000	-	no data	5.04	-	unknown [13]	-
10	Company J1	3 lines	57,077	-	0 [2]	0	-	unknown [13]	-
11	Company K1	line 1	9,126	315	0 [2]	15	-	240	187,500
12	Company K3	3 lines	23,600	317	52.2 [6]	175	306 (1997)	900	1,000,000
13	Company L1	line 1	13,615	348	32	3.5	7.8	8	1,200
14	Company L2	line 2		348	32	3.5		96	28,800
15	Company M1	3 lines	26,422	-	no data	0	-	no data	-
16	Company M3	-	9,716	-	no data	0	-	no data	-
17	Company M4	-	9,708	-	no data	0	-	no data	-
18	Company M5	2 lines	24,297	-	no data	0	-	no data	-
19	Company M6	-	16,600	-	no data	0.01	-	no data	-
20	Company M7	-	8,952	-	no data	5.6	-	no data	-
21	Company O	1 line	9,300	326	no data	4.3	1,060 (1993)	2,642	large river
22	Company P	1 line	7,439	325	not measured	29.2	43.2 (1997)	101	2000
23	Company Q	1 line	2,825	184	not measured	360	800 (1997)	3700	sea
24	Company R	2 lines	1,083	335	0.02	0	-	2,278	500,000 [16]
25	Company T	1 line	8,710	271	0	2.873	2,873	21.5	172,800,000
26	Company U	2 lines	20,000	353	not measured	1.147	1,147	25	2,000
27	Company V	1 line	6,576	365	no data	124 [8]	-	4,500	-
28	Company W	1 line	7,500	-	no data	no data	750 [15]	3,950	sea
29	Company X	1 line	7,658	304	0	552	-	3,950	sea
30	Company Y1	line 1	20,700	334	no data	<11 [7]	-	no data	no data
31	Company Y2	line 2			no data		-	no data	no data
	France [12]	19 plants	unknown	-	unknown	171 – 573 (avg. 306) [10]	-	-	-
	Total tonnage CHDG sites:		**428,352**						

- no information submitted or available negl. negligible

WWTP Waste Water Treatment Plant

1) Data for 1995;
2) No stacks, no emissions
3) Detection limit unknown
4) Emissions in the vicinity of the galvanising baths are so low (range: <2-6.7 ug Zn/m3) that there are only negligible releases to ambient air;
5) The temperature of the hot dip galvanising pots of 460-490˚C is much lower than melting point (907˚C), therefore the emissions to air are negligible;
6) Data for 1999;
7) Combined emission for two CHDG lines;
8) Data for 1997;
9) Mean flow municipal STP;
10) Based on values in aqeous effluent submitted with a unit kg per day and 300 emission days per year
11) Calculated with the estimated maximum percentage in steel (1.5%; EG) and the total production of steel for this company (447,850 t/y)
12) Companies unknown, therefore double counting possible with above-mentioned companies.
13) No local WWTP, flow municipal STP unknown;
14) New submitted data (year unknown);
15) Combined emission for two CHDG plants and one EG plant;
16) The actual flow of the receiving water ranges from 350,000 – 12,000,000 m^3/d, 500,000 m^3/d used for risk assessment.

Table 3.23 Submitted data for electro galvanising (EG) sites in the EU.

No	Company	Additional information	Total Zn processed in 1998	No. of working days	Emission to air	Emission to water (1998)	Largest emission to water (before 1998)	Flow local WWTP	Flow receiving water
			t Zn/y	*day/year*	*kg Zn/y*	*kg Zn/y*	*kg Zn/y*	*m^3/d*	*m^3/d*
1	Company D [5]	3 lines	6,718 [1]	270	unknown [9]	348	2,997	720	16,000,000
2	Company F [5]	1 plant	4,660	-	28	90	-	855 [8]	44,000
3	Company G3 [5]	1 line	4,482	-	20	50.41	-	430 [3]	-
4	Company J2 [7]	2 lines	4,059	-	0	450	-	unknown	-
5	Company K2 [6]	1 line	4,810	311	no data	409	1,060	1,000	187,500
6	Company K4 [6]	1 line	5,015	317	no data	167	816	640	-
7	Company M1	2 lines	5,857	-	no data	242	-	no data	-
8	Company M2	-	no data	-	no data	no data	-	no data	-
9	Company M4	2 lines	no data	-	no data	45	-	no data	-
10	Company M6	-	2,900	-	no data	45	-	no data	-
7	Company N [5]	1 line	2,616	354	no data	408	-	350	950,000
8	Company W+X [7]	1 line	5,005	304	no data	552 [2]	750 [2]	3,950	sea
	Total tonnage EG sites:		**45,213**						

- no information submitted or available

1) Calculated with the estimated maximum percentage in steel (1.5%) and the total production of steel for this company (447,850 t/y)

2) Combined emission for two CHDG plants and one EG plant;

3) Mean flow municipal STP;

4) -;

5) Company uses insoluble anodes;

6) Company uses soluble anodes;

7) Unknown what type of anodes are used by this company, possibly soluble;

8) Water output after waste water treatment;

9) Below detection limit (detection limit not specified).

Table 3.24 Input data and results for the local exposure assessment for continuous hot dip galvanising (CHDG).

No	Company	Emission to air	Emission effluent water [1]	Used dilution factor	C add air (100m)	Conc. effluent WWTP	C add water episode	C add sediment	C add agricultural soil
		kg/d	*kg/d*	*-*	*µg/m³*	*µg/l*	*µg/l*	*mg/kg$_{wwt}$*	*mg/kg$_{wwt}$*
1	Company A	0 [3]	0.0377	10	0	2,536	95.7	2,288	0 [7]
2	Company B	0 [2]	0.071	10	0	35.5	1.34	32.0	0 [7]
3	Company C	0 [3]	0.189	11,765	0	111	0.00356	0.0853	0 [7]
4	Company E1	0 [3]	0	10	0	0	0	0	0 [8]
5	Company E2	0 [3]	0.653	10		327 [5]	12.3	295	0 [8]
	Company E2	0 [3]	0.043	10	0	21.5	0.811	19.4	2.48
6	Company G1	0 [3]	0.1	10	0.0152 [4]	233 [4]	8.78 [4]	210 [4]	26.9 [4]
7	Company G2	0 [3]							
8	Company H	0 [2]	2.57	10	0	140	5.29	126	16.2
9	Company I	0 [3]	0.0168	10	0	8.4	0.317	7.58	0.970
10	Company J1	0 [2]	0	10	0	0	0	0	0
11	Company K1	0 [2]	0.0476	782	0	198	0.0957	2.28	0 [7]
12	Company K3	0.165 [2]	0.552	1112	0.0376	613	0.208	4.98	0.0140 [7]
13	Company L1	0.0920 [2]	0.0101	151	0.0210 [4]	1,257	3.14	75.1	0.00782 [7]
14	Company L2		0.0101	301		105	0.131	3.14	0 [7]
15	Company M1	0 [3]	0	10	0	0	0	0	0
16	Company M3	0 [3]	0	10	0	0	0	0	0
17	Company M4	0 [3]	0	10	0	0	0	0	0
18	Company M5	0 [3]	0	10	0	0	0	0	0
19	Company M6	0 [3]	$3.33.10^{-5}$	10	0	0.0167	$6.29.10^{-4}$	0.015	0.00192
20	Company M7	0 [3]	0.0187	10	0	9.33	0.352	8.42	1.08
21	Company O	0 [3]	0.0132	10	0	4.99	0.188	4.51	0.576
22	Company P	0 [2]	0.0898	20.8	unknown	890	16.1	386	103
23	Company Q	0 [2]	1.96	10	unknown	529	20.0	477	60.0
24	Company R	$5.97.10^{-5}$ [2]	0	220	$1.36.10^{-5}$	0	0	0	$5.08.10^{-6}$
25	Company T	0 [2]	0.0106	10	0	493	$2.32.10^{-5}$	$5.54.10^{-4}$	56.9
26	Company U	0 [2]	0.00325	81	unknown	129	0.606	14.5	15.0
27	Company V	0 [3]	0.340	10	0	75.5	2.85	68.1	8.72
28	Company W	0 [3]	no data	10	0	no data	no data	no data	no data
29	Company X	0 [2]	1.82	10	0	460	17.3	415	53.1
30	Company Y1	0 [3]	no data	10	0	16.5	0.621	14.9	1.90
31	Company Y2	0 [3]	no data	10	0				
	France [9]	unknown	0.57-1.91 (avg. 1.02)	10	unknown	285-955 (avg. 510)	10.8-36.0 (avg. 19.2)	257-862 (avg. 460)	32.9-110 [6] (avg. 58.9)

1) Based on submitted number of working days. The used number of production days is 300 if no value is available;
2) Submitted emission value (divided by the number of production days);
3) Calculated with an emission factor of 0, based on the information of the CHDG sites with known tonnages and emissions as presented in Table 3.22.
4) All emissions of the same company and site (CHDG and EG) are added to calculate one environmental concentration. See also Table 3.25.
5) Waste water concentration; this site has no WWTP;
6) Only based on the calculated emission to waste water (74% to sludge);
7) STP or WWTP sludge is not used on agricultural soils (disposed, recycled);
8) No STP/WWTP and therefore the use of sludge on agricultural soils is not taken into account;
9) Companies unknown, therefore double counting possible with above-mentioned companies.

Table 3.25 Input data and results for the local exposure assessment for electro galvanising (EG).

No	Company	Emission to air	Emission effluent water [1]	Used dilution factor	C add air (100m)	Conc. effluent WWTP	C add water episode	C add sediment	C add agricultural soil
		kg/d	*kg/d*	*-*	*µg/m³*	*µg/l*	*µg/l*	*mg/kg$_{wwt}$*	*mg/kg$_{wwt}$*
1	Company D	0.149 [3]	1.29	22,223	0.0341	1,790	0.0304	0.727	0 [5]
2	Company F	0.0933 [2]	0.3	52.5	0.0213	351	2.52	60.4	40.5
3	Company G3	0.0667 [2]	0.168	10	0.0152 [4]	391	14.7	353	45.1
4	Company J2	0 [2]	1.5	10	0	750	28.3	677	86.6
5	Company K2	0.0753 [3]	1.32	189	0.0172	1,315	2.63	63.0	0 [5]
6	Company K4	0.0949 [3]	0.527	10	0.0217	823	31.1	743	0 [5]
7	Company M1	0.117 [3]	0.807	10	0.0267	403	15.2	364	46.6
8	Company M2	no data	no data	10	no data	no data	no data	no data	no data
9	Company M4	no data	0.15	10	no data	75	2.83	67.7	no data
10	Company M6	0.058 [3]	0.15	10	0.0132	75	2.83	67.7	8.66
11	Company N	0.0443 [3]	1.15	2715	0.0101	3,293	0.458	10.9	380
12	Company W+X	0.0987 [3]	1.82	10	0.0226 [4]	460 [4]	17.3 [4]	415 [4]	53.1 [4]

1) Based on submitted number of working days. The used number of production days is 300 if no value is available;

2) Submitted emission value (divided by the number of production days);

3) Calculated with an emission factor of $6.0.10^{-6}$, based on the largest emission factor of the EG company F (number 7) as presented in Table 3.23;

4) All emissions of the same company and site (CHDG and EG) are added to calculate one environmental concentration. See also Table 3.24;

5) STP or WWTP sludge is not used on agricultural soils (disposed, recycled);

6) Only based on the calculated emission to waste water (74% to sludge);

3.2.5.2.5 Processing of zinc in brass

The sources of the releases of zinc to air at brass production are the melting of zinc and the transfer of the melt to the casting ladle or machines. The brass production process generates waste in the form of filter dust, filter cake, slag, and spent pickling baths (Cleven et al., 1993). The total EU production tonnage of 560.000 t Zn / y is based on the total semimanufactured production of 498,000 t Zn/y and the use of zinc in brass castings of 62,500 t Zn/y (25%[8] zinc in 250,000 t brass casting / y). Industry has submitted emissions for all EU brass producers, companies 1-12 (see Table 3.26),

The scenario used to obtain local C_{add} values is described in section 3.2.5.2.3. Table 3.26 contains the production and emission data and the results of the local exposure assessment for the processing of zinc in the brass industry.

8 Later in this section a percentage of 36% of zinc in brass is mentioned.

Table 3.26 Local emissions and concentrations from EU brass producers and the generic scenario (Information from industry)

	Total brass production	Brass production; (as zinc used in brass)	Yearly emission to air	Yearly emission to water	Local amount released to air	Local amount released to water	Cadd air (100m)	Conc. effluent STP	Cadd water dissolved	Cadd sediment	Cadd agricultural soil
	(kt brass/y)	(ktonnes Zn/y)	(kg Zn/y)	(kg Zn/y)	(kg Zn/d)	(kg Zn/d)	(µg/m^3)	(µg/l)	(µg/l)	(mg/kg$_{wwt}$)	(mg/kg$_{wwt}$)
Company 1	78 [1)]	28.1 [9)]	1 [2)]	0	$3.33.10^{-3}$	0	$7.61.10^{-4}$	0	0	0	$2.89.10^{-4}$
Company 2	130 [10) 11)]	52 [10) 11)]	1,000	97	3.33	0.323	0.761	162	6.10	146	0.289
Company 3	[11)]	[11)]	630	49	2.10	0.163	0.479	81.7	3.08	73.7	0.182
Company 4	[11)]	[11)]	280	25	0.933	$8.33.10^{-2}$	0.213	41.7	1.57	37.6	$8.08.10^{-2}$
Company 5	40 [10)]	14.4 [9)]	3,000 [3)]	61.6 [3)]	10	0.205	2.28	103	3.87	92.6	0.866
Company 6	40 [10)]	14.4 [9)]	542 [4)]	800 [4)]	1.81	2.67	0.413	1,333	50.3	1,203	0.157
Company 7	20 [10)]	7.2 [9)]	109 [5)]	58 [5)]	0.363	0.193	$8.29.10^{-2}$	96.5	3.64	87.1	$3.14.10^{-2}$
Company 8	46 [10)]	15.1 [10)]	2325	500	7.75	1.67	1.77	833	31.4	752	0.671
Company 9	106 [10)]	38.2 [9)]	1,590 [6)]	23.3 [6)]	5.30	$7.77.10^{-2}$	2.21	38.9	1.47	35.1	0.459
Company 10	375 [10)]	135 [9)]	290 [7)]	300 [7)]	0.967	1	0.221	500	18.9	451	$8.37.10^{-2}$
Company 11	220 [10)]	88 [10)]	unknown	22	-	$7.33.10^{-2}$	-	36.7	1.38	33.1	-
Company 12	615 [10)]	221 [9)]	2,030 [8)]	3,383 [8)]	6.77	11.3	1.54	5,640	213	5,090	0.585

1) Estimated

2) For this company the emission figures of the previous year (1994) were 95 kg/y to air and 0 kg/y to water

3) Company 5: Based on a production tonnage of 40000 and an emission factor to air and water of 0.075 kg Zn/t and 1.54 g Zn/t, respectively

4) Company 6: Based on an estimated production tonnage 40000 and an emission factor to air and water of 0.01688 kg ZnO/t (=0.01356 kg Zn/t) and 0.01999 kg/t, respectively

5) Company 7: Based on a production tonnage of 21,528 and an emission factor to air and water of 5.06 g Zn/t and 2.69 g Zn/t, respectively

6) Company 9: Based on a production tonnage of 105984 and an emission factor to air and water of 0.015 kg Zn/t and 0.22 g Zn/t, respectively

7) Company 10: Based on a production tonnage of 100,000 and an emission factor to air and water of 2.9 g Zn/t and 3 g Zn/t, respectively

8) Company 12: Based on a production tonnage of 615,000 and an emission factor to air and water of 3.3 g Zn/t and 5.5 g Zn/t, respectively

9) Calculated (zinc = 36% of brass)

10) Reported

11) Total tonnage of company 2, 3 and 4

3.2.5.2.6 Formulation in zinc alloy and processing of zinc die casting

For this use category a distinction is made between the production of zinc alloy ingots, which are used for die casting, and the die casting foundries where the ingots are re-melted and the zinc alloys are casted to form the die cast pieces. As a rule these two separate activities take place at different industrial plants (information from industry).

Zinc alloy producers (formulation)

The total zinc alloy production in France, Germany, Italy and the UK is about 223,500 t Zn/y (1997). During the melting and alloying zinc oxide emissions to air are generated. The emissions values for seven EU zinc alloy producers are presented in Table 3.28. The processing volume for these companies are about 270,000 tonnes zinc / year, as mentioned in Table 3.28. This covers almost the total estimated production volume of about 273,000 tonnes zinc / year (see Table 3.16). The figure of 273,000 t Zn/y might be a slight underestimation, which is based on the recently submitted volume of 314,000 t Zn/y (1997 data) of the International Lead and Zinc Study Group (2002).

For estimating the emission to air and water for the formulation of zinc alloy at site 7 (no emission values submitted), the maximum emission values are used of company 1 (realistic worst case). The scenario used to obtain local C_{add} values is described in section 3.2.5.2.3. Table 3.28 contains the input data and results of the local exposure assessment for formulation of zinc in zinc alloy producing plants.

Table 3.27 Emissions from EU zinc alloy producers (information from industry)

Company	Zinc alloy production (t/y)	Zinc contained (t/y)	Emission to air (kg/y)	Emission to water (kg/y)	Emission in waste (kg Zn/y)
1	170,000	153,000	250	1065	2,238
2	0 [1)]		- [2)]	-	- [3)]
3	35,000	31,500	0	0	0
4	25,000	22,500	365	25 [7)]	0
5	34,400 [4)]	32,560	32	0	-
6	12,300 [4)]	11,642	17.7	0	-
7	20,000	18,000	- [6)]	-	-
Total	296,700	269,802			

1) No production anymore of die casting alloy zinc since august 1999. Production was 51,055 t/y before 1999.

2) Main emissions are from the zinc production. Separate emissions for zinc alloy production unknown.

3) All zinc emissions to waste are from zinc production.

4) Production 1994/1995

5) This emission to air is a part of the total zinc emission already declared in section 3.2.5.2.2

6) Release of total particulate matter is 433 kg/y (1998)

7) Amount is released into the sewer and treated in a municipal sewage treatment plant

- unknown, no information submitted

Table 3.28 Input data and results for the local exposure assessment for zinc alloy producers and other formulation site(s).

	Company 1 Site specific	**Company 3 Site specific**	**Company 4 Site specific**	**Company 5 Site specific**	**Company 6 Site specific**	**Company 7 Site specific**
Local tonnage (t/y)	170,000	35,000	25,000	34,400	12,300	20,000
Industrial category / use category	15/55 (MC=2)	15/55 (MC=2)	15/55 (MC=2)	15/55 (MC=2)	15/55 (MC=2)	15/55 (MC=2)
Fraction released to air	not appl.	not appl.	not appl.	not appl.	not appl.	$1.47.10^{-6}$ 2)
Fraction released to water	not appl.	not appl.	not appl.	not appl.	not appl.	$6.26.10^{-6}$ 2)
Fraction of main source (B-tables TGD)	not appl.	not appl.	not appl.	not appl.	not appl.	not appl. (1 site)
Number of days	300	300	300	300	300	300
Local amount released to air (kg/d)	0.833 1)	0	1.22 1)	0.107 1)	0.059 1)	0.098 (calculated)
Local amount released to water (kg/d)	3.55 1)	0	0.083 1) 4)	0	0	0.418 (calculated)
Zinc removal rate in STP (%)	not appl.	not appl.	80% 7)	not appl.	not appl.	74% 3)
Size of STP (m^3/d)	2,000	2,000	230,000 6)	2,000	2,000	2,000
Dilution factor	10	10	752 5)	10	10	10
Results						
Conc. effluent STP (µg/l)	1,775	0	0.072	0	0	208
C_{add} *water (µg/l)*	67.0	0	$3.64.10^{-5}$	0	0	7.88
C_{add} *air (µg/m^3)*	0.190	0	0.278	0.0244	0.0135	0.0224
C_{add} *sediment (mg/kg_{wwt})*	1,602	0	$8.7.10^{-4}$	0	0	188
C_{add} *agricultural soil (mg/kg_{wwt})*	$7.22.10^{-2}$	0	0.105	$9.24.10^{-3}$	$5.11.10^{-3}$	$8.49.10^{-3}$

not appl.: not applicable

1) Calculated from the value of Table 3.27 and the number of production days

2) Based on emission factor of company 1

3) This percentage is based on measured influent and effluent concentrations of communal STPs. The average removal of zinc in the examined STPs was about 74% (RIZA, 1996).

4) Amount not directly discharged to any surface water body, but is released into the sewer to a municipal sewage treatment plant. This amount is treated as waste water emission of municipal STP;

5) The dilution factor is based on the dilution in the river (flow rate of 172,800,000 m^3/d) at the discharge point of the municipal STP;

6) Based on a sewage flow rate of 83,500,000 m^3/y and 365 days/year;

7) Removal of municipal STP, based on a measured influent concentration of 362 µg/l and an effluent concentration of 70.6 µg/l.

Zinc die casting plants or foudries (processing)

The total zinc die castings production in Austria, Belgium, France, Germany, Greece, Italy, The Netherlands, Portugal, Spain, Sweden, Switzerland and the UK is 314,000 t Zn/y (1997 data). Hence, the figure of 273,000 t Zn/y, which is used for the exposure assessment and is mentioned in Table 3.16, might be a slight underestimation. The sources of the releases of zinc to air are the melting of zinc and the transfer of the melt to the casting ladle or machines. Except for produced waste material as filter dust there is no further information available about produced waste (Cleven et al., 1993).

The submitted industrial emissions to water for four different die casting foundry sites in the UK are: 135, 160, 11 and 4 g/day (fact file «zinc (alloy) die casting», June 1996). The emission to water for another UK foundry is <22 to 76 g zinc per tonne of casting (average 43 g/tonne). These values are in accordance with the emission factors to water in the UK of 20 to 80 g zinc per tonne of casting, mentioned in Atkins (2000). The total releases to air from die casting in the UK range from 70 to 280 g/t of ingot (Atkins, 2000). For Germany the industry submitted data which is considered representative for the foundry sector with a production of 72,000 t/y. New data is received based on a "most representative" and a "non-representative" company. The more representative data is based on a company with a central melting facility and a crucible melting furnace. The non representative data is based on a company melting their returns in a shaft furnace and is used for not more than 5-10% of the German die casters. For both type of companies together the total emission factor to air ranges from 0.36 g/t (representative, 90-95% of the German production) to 2.1 g/t (non-representative, 5-10% of the German production). The weighted average of the emissions is therefore 0.53 g/t. In addition to the casting process itself the German survey also showed emission factors (including water emissions!) for further processing. Roughly 35% of the cast parts are tumbled with water and grinding particles (slide grinding) and the emission factor to water for this process is 0.14 g Zn/t cast. This figure is based on the maximum allowed zinc emissions in Germany, the actual emissions can therefore be even lower. Heat treatment is used for 3-5% of the cast parts and the emission factor to air is 9 g/t. In Germany about 60% of the cast parts are finished by punching, but there are no emissions to air or water during this process.

In Sweden a total of 4,000 tonnes of die casting is produced in 1998. For Sweden the total estimated emission factor to air for the whole foundry sector (18 foundries) is 5 g per tonne produced (information from the Swedish Foundry Association). There are no emissions to water in Sweden (forbidden).

New air and water emission factors were received from pressure die casting plants in France. According to the industry the data may be considered as average, regarding production and their environmental performance. The emission factor to air from a pressure die casting machine is 0.74 g/t. The emission factor from a central sprout remelting furnace is comparable with a range of 0.49 to 0.77 g/t. This last technology is not very widespread in France. The total plant output for emissions to water of one company ranges from 1.6 to 2.4 g/t, based on machining the cast, cleaning of the dies and all peripheric shops. For vibro polishing the discharge emission factor to water can be estimated as 0.25 g/t. The treated castings cooling water emission factor has been found to be 0.07 g/t.

For Italy emission data is available for three die casting plants. Based on the production volume of two of them, only a few percent (about 3%) of the total Italian die casting volume is covered by these sites. Therefore it is questionable if the submitted emission data are representative for the Italian die casting sector. The submitted emission factors to air are 0, 0.09 and 132 g/t zinc. The emissions to water are zero for two of the Italian sites. The emission factor to water for the remaining site is < 0.000015 g/t zinc.

An overview of all the emission factors based on the data from the UK, Germany, Sweden and France is presented in Table 3.29.

Table 3.29 Emission factors for die casting plants or foundries in the different EU countries.

Emission factor to:	UK	Germany	Sweden	France	Italy
Air	70-280 g/t [1)]	0.36-2.1 g/t (weighted average = 0.53 g/t) [2)]	5 g/t	0.74 g/t [3)] 0.49-0.77 g/t [4)]	0-132 g/t [7)]
Water	20 - 80 g/t [1)]	No emissions	No emissions	1.6-2.4 g/t	0-<0.000015 [7)]
Emission factor for further processing of cast parts:					
Air: Heat treatment		9 g/t (weighted average = 0.45 g/t) [5)]			
Water: Vibro-polishing				0.25 g/t	
Water: Slide grinding		0.14 g/t (weighted average = 0.049 g/t) [6)]			
Water: Castings cooling water (after WWTP)				0.07 g/t	

1) Based on a review of zinc in the UK (Atkins, 2000);

2) The weighted average emission factor to air is calculated from a company with a central melting facility and a crucible melting furnace (representative, 0.36 g/t, 90% of the German production) and a company melting their recycling of returns in a shaft furnace (non-representative, 2.1 g/t, 10% of the German production) ;

3) The emission factor to air from a pressure die casting machine;

4) The emission factor from a central sprout remelting furnace; not very common.

5) Weighted average is calculated from 9 g/t emissions concerning 3-5% of the German production. This factor is based on the maximum allowed zinc emission in Germany;

6) Weighted average is calculated from 0.14 g/t emissions concerning 35% of the German production;

7) Based on emission data of only three Italian sites covering about 3% of the total Italian die casting volume.

In summary, based on the submitted information from the UK, Germany, Sweden and France the emission factor to air ranges from 0.36 to 280 g per tonne produced. For the UK actual emissions to water were submitted ranging from 4 to 160 g/day (see Table 3.30). The emission factor to water in the UK ranges from 20 to 80 g zinc per tonne of casting. Based on the data from France the maximum (averaged) emission factor to water is 2.72 g/t, including the emissions at further processing of cast parts. There are no emissions to water in Germany and Sweden, but emissions at further processing of cast do occur. Italy is the largest user of zinc alloys in the EU, with a volume of 104,600 t/y (1997 data). For only a few percent (about 3%) of the total Italian die casting volume emission data is available. Emissions are lacking for other zinc die casting counties in the EU including Italy. For the die casting plants only emissions factors are available and it is unknown which part of the total processing tonnage is covered by the industrial submissions. Therefore realistic worst case scenarios are carried out based on the estimated total EU production volume of 273,000 t/y (Table 3.16). The first realistic worst case scenario is based on the UK emission factors. For the second and third scenario the highest submitted emission factor to air and water are used based on data for Germany and France (see Table 3.29). Besides these scenarios an assessment is carried out based on the submitted emissions to water for four UK plants.

Applying the 10% rule for extrapolation from EU to region is justified because information was submitted on the number of sites and their geographical distribution over the EU. The scenario used to obtain local C_{add} values is described in section 3.2.5.2.3. Table 3.30 contains the input data and results of the local exposure assessment for processing of zinc in die casting plants.

Table 3.30 Input data and results for the local exposure assessment for die casting plants or foundries.

	UK data site specific (4 sites)	**UK data**	**German data**	**France data**
Regional tonnage (t/y)	not appl.	27,300 [6]	27,300 [6]	27,300 [6]
Industrial category / use category	15/55	15/55	15/55	15/55
Maximum fraction released to air	not appl.	0.00028 [1]	0.0000021 [2]	0.00000077 [4]
Maximum fraction released to water	not appl.	0.000080 [1]	0.00000014 [3]	0.00000272 [5]
Fraction of main source (B-tables TGD)	not appl.	not appl.	not appl.	not appl.
Number of days	300	300	300	300
Local amount released to air (kg/d)	not appl.	25.48	0.191	0.070
Local amount released to water (kg/d)	0.004-0.16	7.28	0.0127	0.248
Size of STP (m^3/d)	2,000	2,000	2,000	2,000
Dilution factor	10	10	10	10
Results				
Conc. effluent STP (µg/l)	2-80	3,640	6.37	124
C_{add} water (µg/l)	$7.55.10^{-2}$ - 3.02	137	0.24	4.67
C_{add} air (µg/m^3)	not appl.	5.82	0.0436	0.0160
C_{add} sediment (mg/kg_{wwt})	1.80 - 72.2	3,285	5.75	112
C_{add} agricultural soil (mg/kg_{wwt})	not appl.	2.21	0.0165	0.0061

not appl.: not applicable

1) Maximum emission factor is based on a review of zinc in the UK (Atkins, 2000) (see Table 3.29);

2) Emission factor is based on the maximum site specific emission factor to air of 2.1 g/t, based on data from Germany (see Table 3.29);

3) Emission factor is based on the maximum allowed site specific emission factor to water for further processing of cast parts by slide grinding of 0.14 g/t, based on data from Germany (see Table 3.29);

4) Emission factor is based on the maximum site specific emission factor to air of 0.77 g/t, based on data from France (see Table 3.29);

5) Emission factor is based on the maximum site specific emission factors to water, based on data from France and includes vibro-polishing and casting cooling water (2.4 g/t + 0.25 g/t + 0.07 g/t = 2.72 g/t; see Table 3.29);

6) This tonnage is lower than the recently submitted regional figure of 31,400 t/y.

3.2.5.2.7 Processing of rolled and wrought zinc

According to the industry there are 5 major manufacturers of rolled and wrought zinc in the EU. For four manufacturers emissions are available, which are presented in Table 3.31. There is no solid waste generation of zinc at all sites. The site with unknown emissions covers only a small part (2%) of the total production tonnage of this category. Additionally, the rapporteur is aware of the fact that the emissions calculated with the default generic scenario would result in unrealistic high emissions compared to the submitted site specific emissions. For these two reasons, for the remaining site the emissions are estimated by using the highest (calculated) emission factor of the four sites with known emissions.

Diffuse emissions to surface water and soil occur by the wash off from zinc sheet surfaces, exposed to atmospheric conditions (information from industry). The scenario used to obtain local (PE)C_{add} values is described in section 3.2.5.2.3.

, with a known removal of 80%, the emission to (effluent) water is 5 kg/y.

Same site as alloy producer no. 4.

Table 3.32 contains the input data and results of the local exposure assessment for processing of zinc in rolled and wrought zinc.

Table 3.31 Local emissions from two EU rolled and wrought zinc producers (information from industry)

Company	Rolled/wrought zinc production (t/y)	Emission to air (kg/y)	Emission to water (kg/y)	Emission in waste (kg Zn/y)
1	120,000	7	50	0
2	85,000	15.2	0	0
3	20,000	47	0	0
4 [4)]	12,000	365	25 [3)]	unknown
5	5,000	152 [1)]	2.1 [2)]	unknown
Total	242,000			

1) Emissions to air for company 5 are estimated with the highest calculated emission factor (emission per ton produced) of company 4.

2) Emissions to water for company 5 are estimated with the highest calculated emission factor (emission per ton produced) of company 1 and 4.

3) Amount is released into the sewer and treated in a municipal sewage treatment plant, with a known removal of 80%, the emission to (effluent) water is 5 kg/y.

4) Same site as alloy producer no. 4.

Table 3.32 Input data and results for the local exposure assessment for processing of rolled and wrought zinc.

	Company 1 Site specific	**Company 2 Site specific**	**Company 3: Site specific**	**Company 4: Site specific**	**Company 5: Site specific**
Local tonnage (t/y)	not appl.	not appl.	not appl.	not appl.	not appl.
Industrial category / use category	15/55	15/55	15/55	15/55	15/55
Number of days	240	300	300	300	300
Fraction of main source (B-tables TGD, 1996)	not appl.	not appl.	not appl.	not appl.	not appl.
Fraction released to air (A-tables TGD)	not appl.	not appl.	not appl.	not appl.	not appl.
Fraction released to water (A-tables TGD)	not appl.	not appl.	not appl.	not appl.	not appl.
Local amount released to air (kg/d)	0.0233 1)	0.0507 1)	0.157 1)	1.22 1)	0.51 1)
Local amount released to water (kg/d)	0.208 1)	0	0	0.083 1) 2) 3)	0.0080 1)
Size of STP (m^3/d)	2,000	2,000	2,000	230,000	2,000
Dilution factor	10	10	10	752 2)	10
Results					
Conc. effluent STP (μg/l)	104	0	0	0.072	3.50
C_{add} *water (μg/l)*	3.93	0	0	$3.64.10^{-5}$	0.132
C_{add} *air (μg/m^3)*	$6.66.10^{-3}$	$1.16.10^{-2}$	$3.58.10^{-2}$	0.278	0.116
C_{add} *sediment (mg/kg_{wwt})*	94.0	0	0	$8.7.10^{-4}$	3.16
C_{add} *agricultural soil (mg/kg_{wwt})*	$2.53.10^{-3}$	$4.39.10^{-3}$	$1.36.10^{-2}$	0.105	0.044

not appl. not applicable

1) Calculated from the values of Table 3.31 and the number of production days;

2) Amount not directly discharged to any surface water body, but is released into the sewer to a municipal sewage treatment plant. This amount is treated as waste water emission of municipal STP;

3) Removal of municipal STP is 80%, which is based on a measured influent concentration of 362 μg/l and an effluent concentration of 70.6 μg/l;

4) The dilution factor is based on the dilution in the river (flow rate of 172,800,000 m3/d) at the discharge point of the municipal STP.

3.2.5.2.8 Production of zinc powder and dust

Eight zinc powder and dust producers have submitted their site specific emission data (Table 3.33) to the rapporteur. In the EU there are known to be 10 zinc powder and dust producers, with a total volume of 65,000 tonnes (This figure is slightly different from the total production tonnage as mentioned in Table 3.16). Emission data is available for 94% of the EU total. For the remaining two sites, both with a production volume of 2000 t/y, the emissions are estimated by using the highest (calculated) emission factor of one of the eight sites with known emissions. For most producers all zinc waste is recycled (information from industry). The zinc contaminated waste of one company is disposed off in an authorised landfill.

The company's specific information of the two sites with the lowest and highest emission to air and water (except the 0 emission values) are used for calculating the local (PE)Cs to air and water as presented in Table 3.34. The scenario used to obtain local (PE)C_{add} values is described in section 3.2.5.2.3. Table 3.34 contains the input data and results of the local exposure assessment for the production of zinc powder and dust.

The emission scenarios for further downstream use of powder and dust are not addressed in the current local risk assessment. Among others, zinc powder is used for the formulation of paints. It is assumed that this scenario is already covered by the zinc oxide industry, where zinc powder is oxidised for the production of zinc oxide. See also next paragraph.

Table 3.33 Local industrial emissions from six EU zinc powder and dust producers (information from industry, 1995). The company numbers refer to zinc metal production plants.

Company number (**see** Error! Reference source not found.)	**Production** (t/y)	**Number of production days per year**	**Emission to air** (kg/y)	**Emission to water** (kg/y)	**Emission to waste** (t/y)
Company 2	3820	330	350	0	- [1]
Company 7 [3]	1920	unknown	69.2	0	- [1]
Company 10	2400	250	0	0	- [1]
Company 11 [3]	6000	unknown	29	15	- [1]
Company 12	4000	unknown	450	0	- [1]
Company 14 (1995)	11000	365	2800	0	- [1]
Company 14 (2002)	-	365	132	0	- [1]
Company 27	8700	300	12	51	100 [2]
Company A	23237	unknown	6258	0	?
Company B	2000	unknown	unknown	unknown	Unknown
Company C [3]	2000	unknown	unknown	unknown	Unknown
TOTAL	65,077				

1) Recycled;

2) Waste refractory materials from furnaces, slightly contaminated with zinc, disposed off in authorised landfill;

3) Production plant is closed.

Table 3.34 Input data and results for the local exposure assessment for production of zinc powder and dust.

	Min-Max Air [3] **Companies 27 and A Site specific**	**Min-Max Water** [4] **Company 11 and 27 Site specific**	**Remaining two companies with unknown emissions**
Local tonnage	not appl.	not appl.	2,000
Industrial category / use category	15/10	15/10	15/10
Number of days	See **Error! Reference source not found.**	See **Error! Reference source not found.**	300
Fraction of main source (B-tables)		not appl.	not appl.
Fraction released to air	not appl.	not appl.	$2.69.10^{-4}$ [2]
Fraction released to water	not appl.	not appl.	$5.86.10^{-6}$ [2]
Local amount released to air (kg/d)	0.04-20.9 [1]	not appl.	1.80 [2] (calculated)
Local amount released to water (kg/d)	not appl.	0.05-0.17 [1]	$3.91.10^{-2}$ [2] (calculated)
Size of STP (m^3/d)	not appl.	2,000	2,000
Dilution factor	not appl.	10	10
Results			
Conc. effluent STP (μg/l)	not appl.	25-85	19.5
C_{add} *water (μg/l)*	not appl.	0.943-3.21	0.74
C_{add} *air ($\mu g/m^3$)*	$9.13.10^{-3}$ – 4.76	not appl.	0.41
C_{add} *sediment (mg/kg_{wwt})*	not appl.	22.6-76.7	17.6
C_{add} *agricultural soil (mg/kg_{wwt})*	$3.46.10^{-3}$ – 1.81	not appl.	0.155

not appl. not applicable

1) calculated from the values of Table 3.33 and the number of production days

2) estimated by using the highest (calculated) emission factor of one of the other 8 sites with known emissions

3) Based on the company's specific data of the two sites with the lowest and highest emission to air (except 0) as presented in Table 3.34.

4) Based on the company's specific data of the two sites with the lowest and highest emission to water (except 0) as presented in Table 3.34.

3.2.5.2.9 Production of zinc compounds

The submitted emission rates and local exposure assessment for the production of the other zinc compounds is available in the separate risk assessment reports of zinc oxide, zinc phosphate, zinc sulphate, zinc chloride and zinc distearate.

3.2.5.2.10 Measured local zinc concentrations in the environment

Water

Measured local zinc concentrations in surface water, which is receiving industrial effluent water, for a number of zinc producing plants are presented in Table 3.35. It is assumed that all data refer to total zinc levels. The range of the measured concentrations in surface water, thus

including the ambient background level, is **<1-1,000 µg/l**. There are five sites where zinc levels higher than 150 µg/l were measured. It must be noted that the (natural) background concentrations are very low when surface water concentrations of 1 µg/l or smaller are measured. In some cases these low values could be explained by the fact that they refer to sea monitoring data (lower background than freshwater).

The measured zinc concentration in waste water of a brass producing plant is 1 mg/l (further details unknown).

The total zinc concentration in the river at a continuous hot dip galvanising and electro galvanising plant (company G) was reported to be 20 µg/l (range 10-50 µg/l) for 1998. The effluent concentration of the WWTP of the same site was 200 µg/l (range 0-1300 µg/l) for 1998. The measured concentrations in a local river near a galvanising plant (company K) were 10 µg/l (CHDG and EG) and 20 µg/l (CHDG). The zinc concentration in sea water (port) of a CHDG site (company B) was about 15 µg/l. The zinc concentration in sea water of another CHDG plant (company H) was 3.4 µg/l (range 0-25 µg/l).

In 2002 the freshwater zinc concentration near the discharge point of rolled zinc company 1 was measured to be 16 µg/l (upstream concentration < 1.0 µg/l).

Near the freshwater discharge point of the sewage treatment plant of alloyer company 4 and rolled and wrought zinc company 4 (same site) a dissolved concentration was measured of 34 µg/l downstream and 2.7 µg/l upstream (data of 4 March 2003). In theory, the rise in local zinc concentration from 2.7 µg/l to 34 µg/l should be explained by the discharge of a municipal STP, because no other emission sources are available between these two monitoring points. However, the daily influx from that STP must be unrealistically high to account for such a rise. As a consequence, the measured concentration of 2.7 µg/l must be considered as an artefact. This is also based on the remark in Euras (2004) report that the observed value of 2.7 µg/l is not in accordance with other reported zinc concentrations for sites in the proximity of the sampling site. According to information from alloyer 4 / rolled and wrought 4, the zinc contribution of this company to the receiving water is negligible, i.e. about 0.9% (Euras, 2004). Therefore, these actual measurements are not taken into account in the local risk characterisation of alloyer company 4/ rolled zinc company 4.

Table 3.35 Measured local zinc concentrations in the receiving surface waterat zinc production and use plants (information from industry).

Company number	Concentration receiving surface water
	(µg/l [4])
1 [a]	300
2 [c]	not applicable
3 [a]	25 [2]
4 [a]	180 (100-600) 100 (downstream)
5	-
6	-
7 [c]	not applicable
8 [a]	10-50 [2]
	-
9 [a]	n.a. [1]
10 [c]	-
11 [c]	-
12 [a] [c]	-
13 [c]	-
14 [c]	n.a.
15 [a]	200 202 (131 dissolved) [7]
16 [a]	45.6
17	-
18 [a]	-
19	-
20 [a]	10-40 [3]
	8.0-15.2 (2003)
21 [a]	1-34 [2]
	1.0-43.0 (mean 5.69) [5]
22 [b]	-
23 [a]	42
24 [b]	-
25 [c]	<1
26 [a]	-
27 [a] [c]	400-1,000
28 [a]	200-400 149 (91 dissolved) [8]

n.a not available, indicated by company; - unknown, no information submitted;

1) Zinc producer (primary)

2) Zinc producer (secondary)

3) Zinc use companies (i.e. zinc dust/powder, brass, zinc alloys)

4) concentration in effluent water is 0.3 mg/l

5) sea

6) sea; representative concentration, due to an acute discharge at the time of sampling also a value is reported of 1372 mg/l

7) It is assumed that all data refer to total zinc levels.

8) Data from 1998-2000 (sea). Samples are taken three times per year at 18 sample points in an area of about 16x13 km near the site.

9) The upstream concentration is higher with a value of 130 µg/l (dilution)

10) Measured in 2002 after point of discharge. Before point of discharge a value of 164 µg/l (86 µg/l dissolved) was measured.

11) Measured in 2002 after point of discharge. Before point of discharge a value of 238 µg/l (41 µg/l dissolved) was measured.

Sediment

Several companies provided measured local sediment concentrations. In 2002 the downstream zinc concentration near the discharge point of producer 4 was measured to be 190 mg/kg dry weight. The upstream concentration at the same site was higher with a value of 890 mg/kg dry weight. The higher concentrations in the upstream sediment are a result of historical pollutions.

For several sample points near the point of discharge of producer 28 sediment concentrations were measured in the receiving canal of 1,600, 2,240, 5,100 and 8,740 mg/kg dwt (2000/2002). In the same canal near the point of discharge of producer 15 sediment concentrations were measured of 5,930 mg/kg dwt (2002) and 8,790 mg/kg dwt (2002).

The zinc concentration in marine sediment in the vicinity of production site number 20 is about 1,700 mg/kg dry weight (1996). There is a decreasing trend near this site in sediment concentrations from 1985-1996. In the vicinity of production site number 21 a concentration was measured of 100-500 mg/kg dry weight at 5 sample points.

Near the discharge point of rolled zinc company 1 a concentration was measured of 54 mg/kg dry weight downstream and 68 mg/kg dry weight upstream (data of 2002). Near the freshwater discharge point of the sewage treatment plant of alloyer company 4/rolled zinc company 4 (same site) a concentration was measured of 130 mg/kg dry weight downstream and 110 mg/kg dry weight upstream (data of 4 March 2003) (Euras, 2004).

Air

Measured local zinc concentrations in air around zinc producing plants are presented in Table 3.36.

Table 3.36 Measured local atmospheric zinc concentrations around zinc production and use plants(information from industry).

Company number	Recent concentrations in ambient air around company sites
	(μg/m3)
1 a)	n.a.
2 c)	-
3 a)	2.5 3)
4 a)	0.59 and 0.155
5	-
6	-
7 c)	1)
8 a)	0.2-0.7
9 a)	0.109
10 c)	
11 c)	-
12 a) c)	-
13 c)	2)
14 c)	0.4 (0.03-6.00)
15a)	0.03-9.23 (1993) 0.03-12.32 (1994) 0.03-6.00 (1995) 4)
16a)	0.86-1.8 7) (350m) 10)
17	-
18 a)	- 5)
19	-
20 a)	0.122
21 a)	0.0193-0.243 8)
22 b)	-
23 a)	0.22 (1995)
24 b)	-
25 c)	n.a.
26 a)	<0.04 (100m.)10)
27 a) c)	0.04-1.88 arithm.mean: 0.31 (1996) 9)
28 a)	0.12 (100m) 0.08 (400m) 0.07 (1km) 10)

- unknown, no information submitted

A.Zinc producer (primary)

B.Zinc producer (secondary)

C. Zinc use companies (i.e. zinc dust/powder, brass, zinc alloys)

1. The stack emissions are monitored at two release points, with a range of 500-2100 µg/m^3 (mean: 1200 µg/m^3)
2. Zinc concentration in waste gas (stack emission) is 1000 µg/m^3
3. Representative measured value at the site boundary using 3 monitors
4. Range of annual minimum and maximum values from daily measurements, measured between 1 km and 2 km from the emission point at four different wind directions. Average values are ranging from 0.30-0.65 (1993), 0.31-0.63 (1994) and 0.29-0.54 (1995) in µg/m^3.
5. The following data: 3500 (1993) 4200 (1994) 3200 (1995) are related to deposited zinc (mg/m^2.d).
6. Daily value measured once in 1994
7. Range of monthly average value for 1997
8. Actual data range for all measurements (3 sampling stations) for nearest inhabited areas, 2-5 km. distance from zinc plant
9. In 1996 this point (downwind) was measured for 365 days (24 h/d). The 50P value was 0.18 µg/m^3 and the 90P value was 0.72 µg/m^3. Data for 1997 are comparable (Vlaamse Milieumaatschappij, 1997).
10. Distance from emission point.

The range of these measured concentrations in air, including the ambient background level, is **0.019 – 12.3 µg/m^3**. According to the industry most of the presented ranges are based on daily measurements and consequently subject to high variability induced by different meteorological conditions.

In the HEDSET a maximum value of up to 17.6 µg zinc/m^3 was reported directly near an industrial point in Belgium, for the period 1989-1990. More recent data are available for this point (zinc powder producer). A continued emission control effort has been undertaken since 1990 which is reflected in the local atmospheric concentrations. Levels (yearly averages) decreased from 9.2 and 10.9 µg zinc/m^3 in, respectively, 1991 and 1992 towards 2.5 µg zinc/m^3 in both 1999 and 2000. Average zinc levels of 0.39-1.02 µg/m^3, with a maximum level of 14.62 µg/m^3 in air were systematically measured near industrial point sources in Flanders in the eighties.

The zinc concentrations in air related to one die casting plant in the UK, containing three foundries, are ranging from 20 to 660 µg/m^3 (average 240 µg/m^3). However, according to the industry these figures (from about 1990) are not relevant anymore for the sector in the UK. Currently, a measuring programme undertaken by the UK die casting sector will generate new figures.

Soil

There are almost no measured local soil concentrations submitted or available for sites influenced by the zinc production or use activities.

In the Netherlands soil concentrations around a production site vary from about 200 mg/kg dwt to almost 1800 mg/kg dwt, measured at distances ranging from 2.6 km to 0.4 km from the factory site. The soil characteristics pH (KCl), organic matter and clay content are about 4.5, 2.6% and 1.5%, respectively (Posthuma et al., 1998). Other soil concentrations reported for the same site are 948 mg/kg (near site) and 203 mg/kg (area) (Posthuma, 1992) and 1340 mg/kg (Van Straalen et al., 1987).

Table 3.37 Summary of results for the local exposure assessment

Company	Conc. effluent STP (total) (µg/l)	C_{add} water episode (dissolved) (µg/l)	C_{add} sediment episode (mg/kg_{wwt})	C_{add} agricultural soil (mg/kg_{wwt})	C_{add} air (100m) ($\mu g/m^3$)
PRODUCTION COMPANIES: 1)					
Company 1	2,685	101	2,423	2.94	7.76
Company 3	1,611	1.92	45.9	14.0	36.9
Company 4	1,750	165	3,949	2.28	6.0
Company 8	5,224	197	4,714	11.7	30.9
Company 9	273	10.3	246	0.504	1.33
Company 12	0	0	0	0.346	0.913
Company 15	762	7.90	189	8.03	21.2
Company 16	28.0	$2.64.10^{-4}$	$6.31.10^{-3}$	0.136	0.360
Company 18	259	9.77	$5.43.10^{-3}$	0.13	8.17
Company 20 (1995)	6,523	154	3,679	9.45	24.9
Company 20 (2002)	5,367	127	3,027	3.72	9.81
Company 21 (1998)	570	21.5	514	8.47	22.3
Company 21 (2002)	42.7	1.61	38.5	5.67	15.0
Company 22	600	22.7	542	$6.75.10^{-2}$	0.178
Company 23	986	0.109	2.62	1.19	3.13
Company 24	0	0	0	0.456	1.20
Company 26	0	0	0	0.643	1.70
Company 27 2)	385	3.37	81	5.48	14.5
Company 27 total 3)	2217	19.4	464	6.12	16.1
Company 28	5,659	20.6	492	0.293	0.773
GALVANISING:					
GHDG: aqueous discharges from run-off, reported waste water concentrations for 20 plants in the Netherlands	0.363-7.66	$1.37.10^{-2}$ - 0.289	0.327-6.92	not appl	not appl
Continuous Hot Dip Galvanising (CHDG): additional assessment	7,686	290	6,935	887	0
CHDG Company A	2,536	95.7	2,288	0	0
CHDG Company B	35.5	1.34	32.0	0	0
CHDG Company C	111	$3.57.10^{-3}$	$8.53.10^{-2}$	0	0
CHDG Company E1	0	0	0	0	0
CHDG Company E2	21.5	0.811	19.4	2.48	0
CHDG Company G1 + G2	233	8.78	210	26.9	0.0152
CHDG Company H	140	5.29	126	16.2	0
CHDG Company I	8.40	0.317	7.58	0.970	0
CHDG Company J1	0	0	0	0	0
CHDG Company K1	198	$9.57.10^{-2}$	2.29	0	0
CHDG Company K3	613	0.208	4.98	$1.40.10^{-2}$	0.0376

Company	**Conc. effluent STP (total) (µg/l)**	**C_{add} water episode (dissolved) (µg/l)**	**C_{add} sediment episode (mg/kg_{wwt})**	**C_{add} agricultural soil (mg/kg_{wwt})**	**C_{add} air (100m) ($\mu g/m^3$)**
CHDG Company L1	1,257	3.14	75.1	$7.83.10^{-3}$	0.0210
CHDG Company L2	105	0.131	3.14	0	
CHDG Company M1	0	0	0	0	0
CHDG Company M3	0	0	0	0	0
CHDG Company M4	0	0	0	0	0
CHDG Company M5	0	0	0	0	0
CHDG Company M6	$1.67.10^{-2}$	$6.29.10^{-4}$	$1.50.10^{-2}$	$1.92.10^{-3}$	0
CHDG Company M7	9.33	0.352	8.42	1.08	0
CHDG Company O	4.99	0.188	4.51	0.576	0
CHDG Company P	890	16.1	386	103	0
CHDG Company Q	529	20.0	477	61.0	0
CHDG Company R	0	0	0	$5.08.10^{-6}$	$1.36.10^{-5}$
CHDG Company T	493	$2.32.10^{-5}$	$5.54.10^{-4}$	56.9	0
CHDG Company U	130	0.606	14.5	15.0	0
CHDG Company V	75.5	2.85	68.1	8.72	0
CHDG Company W	no data	no data	no data	no data	0
CHDG Company X	460	17.3	415	53.1	0
CHDG Company Y1 + Y2	16.5	0.621	14.9	1.90	0
CHDG France	285-955 (avg. 510)	10.8-36.0 (avg. 19.2)	257-862 (avg. 460)	32.9-110 (avg. 58.9)	unknown
Electro Galvanizing (EG): additional assessment	5,203	196	4,695	601	0.032
EG Company D	1,790	0.0304	0.727	0	0
EG Company F	351	2.52	60.4	40.5	0.0213
EG Company G3	391	14.7	353	45.1	0.0152
EG Company J2	750	28.3	677	86.6	0
EG Company K2	1,315	2.63	63.0	0	0
EG Company K4	823	31.1	743	0	0
EG Company M1	403	15.2	364	46.6	0.0267
EG Company M2	no data	no data	no data	no data	no data
EG Company M4	75.0	2.83	67.7	no data	no data
EG Company M6	75.0	2.83	67.7	8.66	0.0132
EG Company N	3,293	0.458	10.9	380	0.0101
EG Company W+X	460	17.3	415	53.1	0.0226
BRASS:					
Brass company 1	0	0	0	$2.89.10^{-4}$	$7.61.10^{-4}$
Brass company 2	162	6.10	146	0.289	0.761

Company	Conc. effluent STP (total) (µg/l)	C_{add} water episode (dissolved) (µg/l)	C_{add} sediment episode (mg/kg_{wwt})	C_{add} agricultural soil (mg/kg_{wwt})	C_{add} air (100m) ($\mu g/m^3$)
Brass company 3	81.7	3.08	73.7	0.182	0.479
Brass company 4	41.7	1.57	37.6	$8.08.10^{-2}$	0.213
Brass company 5	103	3.87	92.6	0.866	2.28
Brass company 6	1,333	50.3	1,203	0.157	0.413
Brass company 7	96.5	3.64	87.1	$3.14.10^{-2}$	$8.29.10^{-2}$
Brass company 8	833	31.4	752	0.671	1.77
Brass company 9	38.9	1.47	35.1	0.459	2.21
Brass company 10	500	18.9	451	$8.37.10^{-2}$	0.221
Brass company 11	36.7	1.38	33.1	-	-
Brass company 12	5,640	213	5,090	0.585	1.54
ALLOY AND DIE CASTING					
Alloy production: company 1	1,775	67	1,602	$7.22.10^{-2}$	0.19
Alloy production: company 3	0	0	0	0	0
Alloy production: company 4	0.072	$3.64.10^{-5}$	$8.7.10^{-4}$	0.105	0.28
Alloy production: company 5	0	0	0	$9.24.10^{-3}$	0.0244
Alloy production: company 6	0	0	0	$5.11.10^{-3}$	0.0135
Alloy production: company 7	208	7.88	188	$8.49.10^{-3}$	0.0224
Die casting: UK data (4 sites) water emissions	2-80	$7.55.10^{-2}$ - 3.02	1.80 - 72.2	not appl.	not appl.
Die casting: UK data	3,640	137	3,285	2.21	5.82
Die casting: German data	6.37	0.24	5.75	0.0165	0.0436
Die casting: France data	124	4.67	112	$6.07.10^{-3}$	0.0160
ROLLED/WROUGHT ZINC					
Rolled/wrought zinc: company 1	104	3.93	94	$2.53.10^{-3}$	$6.66.10^{-3}$
Rolled/wrought zinc: company 2	0	0	0	$4.39.10^{-3}$	$1.16.10^{-2}$
Rolled/wrought zinc: company 3	0	0	0	$1.36.10^{-2}$	$3.58.10^{-2}$
Rolled/wrought zinc: company 4	0.072	$3.64.10^{-5}$	$8.7.10^{-4}$	0.105	0.28
Rolled/wrought zinc: company 5	3.5	0.132	3.16	0.044	0.116
ZINC POWDER/DUST					
Zinc powder/dust: companies 27 and A min and max emission air	not appl.	not appl.	not appl.	3.46.10-3 – 1.81	9.13.10-3 – 4.76
Zinc powder/dust: companies 11 and 27 min and max emission water	25-85	0.943-3.21	22.6-76.7	not appl.	not appl.
Zinc powder/dust: remaining two companies with unknown emissions	19.5	0.74	17.6	0.155	0.41

1. Some production companies (numbers 2, 5, 6, 7, 10, 11 13, 14, 17, 19 and 25) finally indicated not to be a zinc metal producer and therefore no information is presented for these companies.
2. Only zinc metal production separated from the other activities at this site

3. Total emission values and concentrations of this zinc metal production site, including those at the production of zinc alloys, zinc calots (semis) and zinc powders.

not appl Not applicable

3.2.5.2.11 Comparison of local monitoring and calculated data

Water and sediment

The measured concentrations, including natural and ambient background concentrations, range from <1 to 1,000 µg/l for zinc production sites (Table 3.35). The range of calculated local C_{add} values in water is 0.00026 - 200 µg/l. A more in-depth comparison of these local monitoring data for sites emitting to surface water with the calculated local PEC_{add} values of the corresponding sites (including background correction) shows that the difference is usually within one order of magnitude (either higher or lower).

The measured data for a rolled zinc company and some CHDG and EG companies are within the same order of magnitude as the corresponding calculated values for these sites. The maximum measured effluent concentration of 1.3 mg/l clearly exceeds the calculated value for an EG site, but similar, high effluent concentrations have been calculated for other CHDH/EG sites.

The measured zinc concentrations in sediment are within the same range as the calculated values and the difference is usually within one order of magnitude (either higher or lower). The measured and calculated sediment concentrations near production company 4 and alloy producing company 4 / rolled zinc company 4 deviate more than one order.

Air

For a number of the production companies, measured zinc concentrations in ambient air around the site were reported. The measured concentrations, including ambient background concentrations, range from 0.019 – 12.3 µg/m^3 (Table 3.36). The range of calculated local C_{add} values in air is 0.18 - 36.9 µg/m^3. For corresponding sites a comparison of these local monitoring data with the calculated local C_{add} values shows that the calculated C_{add} is usually one order of magnitude higher than the measured concentration at the respective site. For one site the calculated C_{add} is lower than the measured concentration.

The measured zinc concentrations in air near a die casting plant in the UK, ranging from 20 to 660 µg/m^3 (average 240 µg/m^3) are much higher than the calculated $C's_{air}$ for this use category.

Soil

For one company the measured values in soil range from 200 mg/kg$_{dwt}$ to 1800 mg/kg$_{dwt}$. The calculated value for this same site is 2.77 mg/kg$_{wwt}$. Irrespective of the chosen background concentration, the measured PEC_{add} is much higher than the calculated concentration. Historical pollution may well explain this difference.

Both calculated and measured concentrations will be taken into account in the local risk characterisation. In general preference is given, however, to measured data provided that they are reliable and representative for that particular local scenario.

3.2.5.3 Regional exposure assessment (including line source emissions)

3.2.5.3.1 Regional releases

General

In accordance with Appendix VIII from the TGD, it is assumed that the individual zinc compounds are all transformed into the ionic species. Another assumption for the regional exposure assessment is that all emissions are diffuse. The industrial emissions mentioned in the previous paragraphs and in the RAR's of other zinc compounds are not directly used for the current regional exposure assessment. This because at present in a number of cases it is not known which companies, either producing or processing one of the six zinc compounds, are located in a particular region. Default generic scenarios are therefore being used which may not reflect the 'real' situation in a region. Another reason is that good alternative estimates for the regional (national) industrial emissions are available from other sources (e.g. national emission registrations).

Emission data are available for The Netherlands (1999), Belgium (1995), Sweden (1990-1995), Germany (1998) and UK (1999 and 2000).

I. The Netherlands

Collection of data

Emission data in the Netherlands are gathered at a regular basis from all source categories, being industry, public utilities, traffic, households, agriculture and natural sources. Agreement about definitions, methods and emission factors, based on reports by expert groups, is achieved in the national Co-ordination Committee for the Monitoring of Target Groups (CCDM). The data presented here may be considered as the most recent data for the zinc emissions in The Netherlands for the year 1999, based on official publications (CBS/RIVM, 2000; CCDM, 2000).

Table 3.38 presents the overall zinc emissions in the Netherlands in 1999 (CCDM, 2000). The underlying data can be found in Annex 3.2B (1998 data).

Table 3.38 Zinc emissions to water, soil and air in the Netherlands (1999) (in t/y).

	Waste water	Surface water	Soil	Air
Agriculture	4	4	2240 [5)]	
Industry	63	31		64
Waste treatment	4	-		
Traffic	140	54[1)]	150	22
Consumers	212	8	4	5
Trade and Services [3)]	37	2		
Effluents STP	-	95		
Others	0.4	50[2)]	238[4)]	
Atmospheric deposition	-	8	90	
			2	
Total	**460**	**254**	**2720**	**91**

1) Original CCDM figure of 84 t/y is corrected for new (preliminary) estimates for emissions from ship anodes (7 t/y in stead of 23.9 t/y) and anodes on lock gates (14 t/y in stead of 27.7 t/y)

2) Including emissions from a.o. overflows and separated (rainwater) sewer.

3) Trade and Services (HDO in Dutch) comprises emissions from a.o. car trade, storage firms, educational institutes, medical care, government agencies, recreation and sports and catering industry

4) Emissions from 'Others' to soil mainly comprises emissions from composted/re-used or incinerated sewage treatment sludge (see Annex 3.2B).

5) Soil is the primary receiving compartment for zinc emissions from agricultural activities. It has to be noted, however, that owing to runoff etc. a significant part of this load will end up in surface water. A reliable ('official') quantitative estimate is still lacking (see text for further details).

In Table 3.38 the overall emissions are given for each individual target group. In the text below additional information will be given on the major contributing factors.

Emissions from atmospheric corrosion contribute to the total load of zinc to water and soil. Emission from corrosion can be estimated with the total exposed area that is being potentially exposed to corrosion and the so-called run-off rate. The latter is mainly determined by the concentrations of acid pollutants (SO_2 in particular) in the atmosphere. During the last 30 years ambient SO_2 concentrations in Europe have considerably decreased. Run-off rates are therefore considerably lower than several years ago. According to a recent TNO-report (TNO, 1999a) the empirical equation of Odnevall et al. (1998) is at present the best descriptor for the run-off rate. The formula is as follows:

$$run\text{-}off\ rate\ (g/m2/y) = 1.36+0.16\ [SO_2],$$

where [SO2] is a measured regional year-average concentration level in $\mu g/m^3$.

By multiplying the estimated total exposed surface area with the run-off rate(s) the total emission from corrosion is obtained. For the zinc emission estimates from corrosion in the Netherlands two different SO_2 concentrations have been used. One for objects being exposed in an a area with a relatively high SO_2 concentration (area 1: run-off rate is 2.96 g/m2/y) and one for an area with lower SO_2 concentrations (area 2: run-off rate is 2.08 g/m2/y). The next step is the distribution of these total zinc emission(s) over the various compartments (soil, sewage or direct to surface water). Table 3.40 gives a summary of the zinc corrosion estimates

in the Netherlands for 1999 (RIZA/MEWAT, 2000). The table shows that a total tonnage of 73 t/y is being released from corrosion to surface water. This amount is about 29% of the total emission to surface water in the Netherlands (254 t/y see Table 3.38). The RIZA/MEWAT report (2000) indicated that there can be large uncertainties in the zinc emission estimates from atmospheric corrosion. Major items to be further elaborated are:

- run-off of zinc under field conditions;
- check on actual area of roofing and gutters of houses and utility buildings
- check on percentage of exposed surface area, correction factor for growth of zinc market, percentage of coating being applied on different products of galvanised steel ;
- check on distribution of zinc emissions over soil, water and sewer

The corrosion emissions (Table 3.40) are used in combination with other, non corrosion zinc emission to estimate the zinc emissions from the various target groups in Table 3.38. Corrosion of greenhouses contributes to zinc emissions from Agriculture (soil and water), although to a minor extent. Corrosion of crash barriers and lampposts forms (a minor) part of the emissions of Traffic (soil (non-agricultural) and water). Within the target groups Consumers, Effluent STP and Others a significant part of the zinc emissions is due to corrosion of roofing and gutters of houses etc.

Major zinc emissions from Traffic, other than corrosion of crash barriers etc., are related to wearing of tyres and brakes and emissions from fuel and oil. Emissions from these transport sources, including road surface wearing, for 1999 are presented in Table 3.39.

Table 3.39 Estimated zinc emissions from transport for the Netherlands in t/y (Emissieregistratie 1999 data).

Source	Air	Soil	Water	Total
Exhaust gass	0.3	0	0	0.3
Tyre wearing	6.3	88	73.9	168.2
Brake lining wearing	0.2	0	0	0.2
Road surface wearing	0	0.3	0.3	0.6
Oil leakage, motor gasket	0	0.4	0.4	0.8
Oil leakage, piston rings	13.6	2.6	1.9	18.1
Total	20.4	91.3	76.5	188.2

Table 3.39 shows that wearing of tyres is a major source with an release figure of 168 t/y in the Netherlands. An alternative and slightly lower emission figure for tyre emissions has recently been suggested by industry (Haskoning, 2003). Based on newly obtained empirical data on tyre wearing (field tests) and zinc contents in tyre tread, the total emissions of zinc into the environment in the Netherlands is estimated at 140 t/y. This amount is subsequently distributed as follows:

- 64.4 t/y in the urban area, which may be assumed going almost completely to an STP and
- 75.9 t/y in non urban areas.

The alternative data and exposure assessment are not further used, however, in the current risk assessment. This because the influence of these alternative values on the regional exposure assessment are considered to be marginal. The alternative local exposure assessment for road borders is built on a number of assumptions for which no guidance and/or consensus has been reached yet at EU level. Furthermore the Rapporteur feels that for the local road border scenario, due to all the uncertainties around the alternative exposure model, preference should be given to the large and representative set of measured data of zinc in road borders (see section 3.2.5.3.4).

Zinc emissions from sacrificial anodes on e.g. lock gates and ships also contribute to water emissions under Traffic. In the CCDM (2000) report emissions from lock gates and ships were estimated at 27.7 and 23.9 t/y, respectively. This total of about 50 tonnes, which are direct emissions to surface water, amounts to 60% of the total traffic emissions to surface water (84 t/y) and about 20% of the total zinc emissions to water (284 t/y). It is recognised that this older estimate for the zinc emissions from sacrificial anodes for the Netherlands is too high. This because of several reasons: 1) increased application of alternative technologies on ships, 2) inappropriate functioning of zinc anodes in fresh water environments and 3) allocation of total zinc emissions to fresh water, whereas considerable part will end up in marine environment in the Netherlands. Results from a recent research in the Netherlands indicate that for ships the emissions are at a maximum of 7 t/y (RIZA, 2001). Also the emissions from lock gates will become significantly lower. A recent estimate from RIZA amounts to 14 t/y based on the (rough) assumption that of the original 27.7 t/y 50% will go to the marine environment and 50% will end up in the freshwater (RIZA, 2001). The exposure assessment will thus deviate from the official CCDM (2000) figures and will use the upper limit of 7 t/y for zinc emissions from ship anodes in stead of the figure of 23.9 t/y and 14 t/y in stead of 27.7 t/y for lock gates. As a result of these changes the total water emission have become 254 t/y.

Table 3.40 Emissions to water and soil from atmospheric corrosion in the Netherlands 1999 (RIZA/MEWAT, 2000).

	Surface area (km2)	Emission (t/y)[1)]	Fraction to soil	Fraction to water direct	Fraction to sewer [2)]	Soil (t/y)	Water direct (t/y)	Effluent STP (t/y)	Overflow and rain water sewer (t/y)	Surface water total (t/y)
Roofs and gutter housing	32.6	76.3	0.03	0.07	0.90	2.3	5.4	15	14.4	34.8
Roofs and gutter utility buildings	3.0	8.9		0.05	0.95		0.4	1.2	4.2	5.7
Skeletons	8.9	26.4		0.05	0.95		1.3	3.7	11.7	16.8
Greenhouses	1.9	5.7	0.75	0.25		4.3	1.4			1.4
Crash barriers	8.4	25.1	0.90	0.10		22.6	2.5			2.5
Lampposts	0.07	0.2	0.03	0.07	0.90	0.006	0.013	0.04	0.03	0.08
Other[3)]	11.2	26.1	0.03	0.07	0.90	0.79	1.8	5.1	5	11.9
Total	**66**	**168**				**29.9**	**12.8**	**25**	**35.3**	**73.2**

1. Emission is calculated from total surface area and run-off rates in two areas (1 and 2) in the Netherlands with different SO2 concentrations (see text).
2. Total of emissions to separated (rainwater) sewer and mixed (standard) sewer.
3. Including: 1. transport: boat trailers, small trailers, etc., 2. fasteners: application in steel constructions, almost entirely used indoors and 3. other: not well defined. Important machinery parts, playing ground tools, household appliances, tubing for ships, grids, fences.

Agricultural activities represent the largest source of zinc emission to soil, mainly caused by excretion from animals (manure). The CCDM (2000) gives a total agricultural zinc emission to soil of 2220 t/a for 1999. The estimate is mainly based on total usage of animal feed, its zinc contents and absorption rates of zinc in animals. The difference of 20 t/y between this figure and the figure of 2240 t/y in Table 3.38 is due to minor emissions via corrosion of greenhouses, hunting and fishing. In essence the same approach for estimating the zinc emissions via animal feed was followed in an alternative assessment conducted by industry. The agricultural release values were calculated using European statistics on animal feed sales (FEFAC/ EUROSTAT/ ZOPA). The estimates for the EU countries are presented in Annex 3.2A. These data are compiled by the industry. Either zinc oxide or zinc sulphate is added to animal feed as a source of essential trace element zinc. A large proportion of the feed given to the animals is not absorbed (20-50%). This fraction will pass straight into the manure. From the absorbed fraction a large portion will be excreted after transformation in the animal body (20-50%). The remaining fraction (app. 15%) will be concentrated in the various animal tissues. It is assumed that 85% of the zinc in animal feed will end up in the manure (FEFAC/EUROSTAT/ZOPA, 1999). Based on this release assumption and the animal feed sales the zinc release from agriculture in the Netherlands is estimated to be 1,366 tonnes/y, or 8% of the EU total of 17,049 tonnes. The figure of 1,366 t/y also includes an environmental release of 70 tonnes zinc via fertiliser and pesticides. The industry estimate of about 1400 t/y is lower than the CCDM (2000) estimate of 2220 t/y which is due to a.o. different assumptions about retention times of zinc in animals (5% absorption in CCDM (2000) versus 15% in industry estimate), but mainly to the fact that the CCDM figure contains both concentrates ("krachtvoer") and roughage (green maize, hay and meadow grass) as zinc emission sources. Roughage represents about 550 t/y. The CCDM figure further includes a higher estimate (170 t/y) for emissions from fertilisers and agrochemicals than the industry's estimate (70 t/y) for these sources. Finally, the CCDM estimate comprises the natural background of zinc in animal feed (concentrates) in contrast to the industry figure. This natural background of zinc in animal is not quantified, however.

As stated above, Annex 3.2A gives an overview per EU country of the animal feed sales (in tonnes zinc), the zinc releases via pesticides/fertilisers, total zinc releases to soil and the amounts of zinc emitted per hectare. The amounts of agricultural zinc release per hectare are useful to get an impression on the relative intensity of agricultural activities in the individual EU countries. From this it is evident that the Netherlands have the highest yearly input, 694 g zinc/ha, followed by Belgium/Luxembourg (453 g/ha) and Denmark (283 g/ha). It should be noted that these data refer to industry estimates that are different from CCDM values (see above). The relative pattern, however, i.e. large differences between individual EU member states, will be more or less identical for both estimates.

As stated above, agricultural activities form the largest input of zinc to soil. One should realise that besides this zinc input to soil, there is also a zinc output, consisting of crop uptake and leaching. Leaching to groundwater is a parameter that is being dealt with in the EUSES-model. This in contrast, however, to the uptake (and subsequent removal) of zinc through plants. In the report 'Input and output balances of heavy metals in Dutch agricultural soils' (IKC, 1996) it is concluded that 'the uptake through crops is (much) smaller than the input'. Similar conclusions can be drawn from a series of RIVM-reports on the National Soil Monitoring Network of the Netherlands (Groot et al., 1996; Groot et al., 1997; Groot et al., 1998). The ratio between the overall Dutch zinc soil output via crops and input is estimated at 0.1 in the IKC-report. Both lower and higher ratios (range 0.05 - 0.4) were found in the RIVM-reports, depending on the use and type of soil. The CCDM (2000) report gives a net emission (thus corrected for harvest removal) of 1620 t/y to soil. As the IKC-report gives an

overall figure for the Netherlands, the correction factor of 0.1 can be used in the alternative exposure assessment from industry (net emission NL: 1366 - 136= 1230 t/a and for EU: 17,049 – 1705 = 15,344). Further aspects of accumulation are discussed in section 3.2.5.3.3.

According to CCDM (2000) both leaching and run-off from agricultural (and other) soils seems to contribute significantly to zinc emissions to surface water in the Netherlands (direct emissions). Preliminary estimates indicated that emissions are occurring in the range of several hundreds of tonnes zinc per year. A research on this topic has been started in the Netherlands (RIZA and others) in order to further quantify this emission route. This research has not been completely finalised yet, but it seems to confirm the earlier findings. In Dutch agricultural areas leaching of zinc (and cadmium) is responsible for about 40% of the water pollution of these chemicals (CIW, 2003). One should realise that this aspect is already dealt with in the regional modelling as EUSES takes into account both leaching and run-off from soils (only antropogenically added amounts) into surface water.

Note: the recently conducted Alterra study (De Vries et al., 2004) on zinc balances in agricultural soil points to lower estimates of the total zinc emissions in the Netherlands compared to the CCDM (2000) data. Based on the same source as the Alterra report, the RIVM Milieucompendium 2004 (RIVM, 2004) presents lower total zinc emissions for 2001 and 2002 (app. 1600 t/y). The Alterra study now forms the basis for the agricultural soil scenarios in the current RAR (see section 3.2.5.3.3.). For the regional EUSES modelling, however, the original set of emission data from CCDM (2000) was used for reasons of consistency (except for ship anodes). Moreover, the years 1999 and 2000 were selected as reference year in the present RA.

The emissions from the Target Group Consumers account for a relatively large part of the zinc emissions to waste water. Table 3.38 shows that 212 t/y on a total amount of 460 t/y (= 46%) comes from consumers. Emissions from consumers are built up of three main sources, i.e. corrosion from housing roofs (± 40 %), private sewage from consumers (± 50 %) and other applications (± 10 %). Private sewage emissions, thus responsible for about 50% of the zinc emissions to water for consumers, are calculated by using an estimate for the yearly zinc emission per capita. Table 3.41 presents the various sub-sources for emissions from private usage according to the SPEED document (1993). It is clear that human excretion comprises 53% of the total private sewage zinc emissions and thus contributes for about 25% to the total consumer emissions to water.

The total yearly zinc emission in the Netherlands from private sewage is calculated by multiplying the number of inhabitants in the Netherlands (app. 15.5 million) with the emitted amount per capita. For the latter a figure of 8100 mg/inh/y (slightly different from SPEED, 1993) estimate was used. More recently (1998 and 1999 estimates in CCDM report), however, this figure was reduced to 7400 mg/inh/y due to lower corrosion emissions (use of PE water pipes) and softening of water (Haskoning, 2000).

Table 3.41 Split-up of zinc emissions (in mg/inhabitants/y) from private sewage (SPEED, 1993)

Source	mg Zn/inh./y
Faeces and urine (food)	4400
Tap water (from waterworks)	300
Corrosion water system (stagnant)	300
Corrosion water system (streaming)	1000
Corrosion water system (water heaters)	500
Products (cosmetics, deteregents etc.)	1800
Total	8300

A comparison of the figure of 4400 mg Zn/inh/y as reported in the SPEED document (1993) can be made with more recent (1998) estimates for the daily intake of zinc via food (see section 4.1.1.4 Man indirectly exposed via the environment). The average daily intake amounts to 9.4 mg zinc. On a yearly basis this gives a total intake estimate of 3431 mg per capita. Assuming 0% absorption (steady state) and thus an emission of 3431 mg/inh/y, means that this recent estimate is lower than the SPEED (1993) figure. The conclusion, however, is the same, i.e. faeces and urine contribute to a relatively large extent to the total consumer zinc emissions. Major part of the zinc emissions via food will be determined by the natural background levels in those food products.

STP balance

From the Dutch emission data an estimate can be made for the total zinc influent of STPs in the Netherlands. The calculated zinc load amounted to app. 270 t/y for the year 1997. There are also measured data available for STP influents in the Netherlands from which also a total zinc load can be estimated. The STP zinc load for 1997 is 367 t/y. The difference between the calculated and the measured load is about 100 tonnes. This difference may be due to uncertainties in the calculations. In addition, other potential zinc sources may be present that have not be taken into account yet. One of these sources is for example the emission due to plant (leaf) decay. Tree leaves have an average zinc concentration of 222 mg/kg dwt and a recent TNO study (TNO, 2000) has estimated that this could result in a total load of 11 t/y to STP influents. The margins of uncertainty of this natural flux of zinc are not quantified in the TNO-study. Additional possible sources are rainwater and soil erosion (see above). Only for rain water a quantitative estimate of 5 t/y is available.

Sludge application

Since 1993 the sludge of communal STPs in the Netherlands is no longer used on agricultural soils. This because for a number of chemicals, including zinc, the current quality criteria are exceeded. In 1990 the zinc emission via sludge from communal STPs to soil was 74 t/a (CBS, 1999). Most of the sludge from private STPs is still being used on agricultural soil in the Netherlands. The total zinc load of this sector, however, is very small, 4 t/a in 1997.

Deposition

Although deposition is a significant emission source of zinc in the Netherlands, it is not a primary source (rather a flux). Important information is available on zinc deposition in the Netherlands. Zinc deposition occurs via both dry and wet deposition. Deposition has been calculated in the Netherlands based on measured zinc concentrations in air and rainwater and deposition rates. For 1998 a deposition of 44 g/ha/y is estimated (RIVM/LLO, 1999). The total load of zinc to the Netherlands via deposition was 154 tonnes in 1998 (RIVM/LLO,

1999). Loads in 1995, 1996 and 1997 were, respectively 184, 191 and 153 t/a. The deposition load in 1986 was 234 t/a. According to Cleven et al. (1993) the contribution of foreign countries to the total deposition of zinc in the Netherlands amounts to 69%.

II. Belgium

Zinc emissions (estimates) for Belgium are presented in Table 3.36. It should be emphasised that these data are the original data from the MNZ report (1995). More recent release data for traffic and agriculture, which could be deduced from the alternative estimates for these two categories that were made by industry in the previous section (Appendices A and B), have not been incorporated.

Table 3.42 Emissions in Belgium to air and water in 1995 [1)] (MNZ, 1995)

Emission source	Emission to air (tonnes/year)	Emissions to water (tonnes/year)
Industry	314[2)]	126
Households		105
Retail, etc.		
Agriculture		11
Traffic[3)]	50	
Waste disposal industry	72	
Others	4	
Diffuse emissions		408
Total	440	650 [4)]

1. no data are available on the emission to soil, for example as a result of agricultural activity and the corrosion of zinc containing articles.
2. of which 223.6 tonnes from Ferro- and steel industry (60% comes from “blast furnaces”), 77.8 from non-ferro industry and 12.8 tonnes from glass industry
3. of which 45.2 tonnes comes from emission by combustion of gasoline and diesel and 4.6 tonnes comes from the wearing of car tyres
4. the actual emission of the waste water treatment is 527.0 tonnes

III. Sweden (including Spain and US for mining)

Zinc emissions into air from different industrial branches in Sweden in 1995 are presented in Table 3.43. Only point sources have been taken in account.

Table 3.43 Emission of zinc to air from different sources in Sweden, 1995 (SCB).

Branch	Amount of zinc (tonnes/year)
Mines	0.8
Cement industry	0.56
Iron- and Steelworks	24
Ferro-alloy works	1.4
Metal works	12
Foundries	4.1
Engineering industry	50
Combustion of waste	0.28
Central heating	2.7
Combustion (for heating?) :	
Industry	18
Households	24
Total	140

In Table 3.44 an overview is given of the industrial emission to water in Sweden. Here also, only point sources have been taken in account.

Table 3.44 Emissions of zinc into water from different sources in Sweden, 1995 (SCB)

Branch	Amount of Zinc (tonnes/year)
Mines	14
Mining waste	360
Rayon industry	49
Paper and pulp industry	90
Refineries	0.29
Iron and-steelworks	6.9
Metalworks	2
Engineering industry	4.9
Municipal sewage treatment works	52
Total	580

Landner and Lindeström (1998) roughly estimated the yearly total emissions of zinc from the technosphere to the environment in Sweden (figures from 1990-1995)(Table 3.45)

Table 3.45 Total zinc emissions from the technosphere in Sweden (1990-1995; Landner and Lindeström, 1998)

Sources	Emissions (t/y)
Point sources	
Emissions to air	230
Emissions to water	260
Diffuse sources	
Corrosion	750
Tyres	230
Asphalt	120
Breaks	100
Untreated sewage	40
Mining waste	400
Total	2100

In Sweden a research programme "Metals in the Urban and Forest Environment" (financed by the Swedish Environmental Protection Agency) has been conducted (Water, Air and Soil Pollution (2001): Focus/Vol. 1 issue 3-4). One main project within this programme focuses on stocks and flows (emissions) of metals within the Urban environment. The city of Stockholm is used as study object. One conclusion from the project is that the traffic sector (tyres, brakes, car washes, street surface abrasion) corresponds to approximately half the total goods emissions. The dominating diffuse sources of zinc from goods in Stockholm (1995) were: tyres (10 t/y), galvanised goods 7.1 t/y, and sacrificial anodes 1.5 t/y. The emissions from breaks were 0.9 t/y and from street surface abrasion, 1 t/y. In Sweden asphalt is usually made of granite or gneiss, which have rather high zinc contents. Therefore, emissions from street surface abrasion cannot be neglected in Sweden (Bo Bergbäck, pers. com).

In the Landner and Lindeström (1998) report a total annual emission of 750 t from corrosion has been given. This is about half of the amount (1300 t/y) that has been given as a worst case estimate by Nilsson (1996) for zinc emissions from corrosion in Sweden. A very recent estimate from industry for corrosion estimate which is based on recent marketing figures and a run-off rate of 3 $g/m^2/y$, resulted in a total amount of about 85 t/y. This much lower estimate is based on general galvanised steel only, but emissions from continuously galvanised steel and rolled zinc will not contribute to significant amounts in Sweden.

Mining waste

There are two types of mining waste: waste from the iron ore deposits and waste from sulphide ore deposits. Waste for sulphide ore contains leachable metals in large quantities. Mining has been going on for more than thousand years, but because of the intensification of the mining in the second half of the 20th century, most waste is produced in this later period (70% in the last 30 years). Because of poor information on leaching of heavy metals, no reliable estimation of the pollution by zinc containing mining waste is available.

Every year approximately 30 million tonnes of mining waste is stored in waste dumps or dumping-grounds in Sweden (Nilsson, 1996). In 1993, about 14,000 tonnes of zinc were deposited or used as backfill material as a result of mining activity in Sweden. Beside the 14,000 tonnes about 1000 tonnes of zinc in slag are generated in the slag-fuming plant in the Rönnskär Works in 1993. In 1993 a total amount of zinc concentrates of 303,000 tonnes were

produced in Sweden (waste/concentrates ratio is therefore 0.046). By means of this ratio, from a total concentrate production of 7,886,000 tonnes in Sweden in the period 1962-1992, a total deposition of zinc in waste can be calculated to be 360,000 tons in the last 30 years. (Nilsson, 1996).

On the basis of the above mentioned it can be concluded that considerable emissions from mining waste storage sites can not be excluded. An estimate of 400 t/y zinc emission from mining waste is given by (Landner and Lindeström, 1998; see Table 3.38)

Information is lacking to get a broader view of these emissions in the EU. For Spain (Aznalcollar mine), however, it is known that significant zinc emissions to surface water occur(ed) both before and after the great spill in 1998.

For comparison some information is presented on mining waste in the US. The TRI database provides data on zinc releases from metal mining in the US in 1999 (TRI, 2001). Table 3.46 shows that atmospheric and aquatic emissions amount to app. 110 t/y and 17 t/y, respectively. Releases to land (on-site) by far exceed the emissions to air and water.

Table 3.46 Zinc releases from mining in the US (TRI, 2001).

Compartment	Release (pounds/y)
Air	224,000
Surface water	34,000
Underground injections	21,000
On-site land	658,000,000
Off-site land	8,000
Total	**678,000,000**

The zinc releases from metal mining contribute to a large extent (app. 70%) to the total zinc releases in the US (1,000,000,000 pounds/y).

As mentioned in section 2.1.1 the releases from waste (including mining waste) are not addressed in this risk assessment, although it is recognised that such emissions can be substantial in some regions.

IV. Germany

Arpaci (1998) has made an assessment for the zinc emssions to aquatic ecosystems in Germany (Table 3.47).

Table 3.47 Zinc emissions to surface water in Germany (Arpaci, 1998)

	Total emission (t/a)	**Fraction to surface water (t/a)**
Diffuse sources		
Atmospheric emissions	**7,190**	550
Traffic, total	**1,850**	150
tyre wear	825	
street abrasion	660	
fuel	220	
others	145	
Corrosion	**3600**	1,300
Direct sources		
Communal waste water, total	**4300**	1,250
Non-industrial	2370	
Street run off	1,500	
industrial	430	
Industrial	430	430
Other sources		1,500
Total		**5200**

In the Arpaci report (1998) a rather large zinc emission via the street surface abrasion has been mentioned for Germany, i.e. 660 t/a compared to 825 t/a from tyre debris. Arpaci based this conclusion on an original paper from Muschak (1990). The Rapporteur has reviewed the Muschak-paper, but did not find convincing evidence that such a large proportion of zinc emissions on roads could indeed be attributed to street surface wearing. This because no clear distinction could be made between zinc originating either from road wearing, tyre debris, dry and wet deposition and corrosion from crash barriers. In addition, Muschak mentions the usage of 'studded tyres', which is considered not to be representative at a regional scale.

Arpaci also estimated the output of zinc in Germany via rivers etc. at 5,200 t/a, resulting in a net balance of + 100 t/a for the aquatic ecosystem.

The zinc emissions to agricultural soils in Germany have been investigated by Crössmann (1998). Table shows that a total input of about 9000 t/a is estimated. The total output due to harvest (1,050 t/a), run-off (2,550 t/a) and animal products (250 t/a) amounts to 3,850 t/a according to the author, resulting in a net balance of about + 5,000 t/a in Germany.

Table 3.48 Zinc input into German agricultural soils (Crössmann, 1998)

Source	Input to agricultural soil (t/y)
Atmospheric deposition	3,400
Fertilisers	950
Sludge	890
Animal feed	3,430
Total	8,670

A much higher estimate can be calculated for manure and dung input in Germany based on the data from Wilcke and Döhler (1995). They reported an average input of around 600 g/ha/a due to manure and dung application. Using this value and multiplying it with the total arable area in Germany (16,950,000 ha, status 1992) results in a total input figure of about 10,000 tonnes.

V. England and Wales

Nicholson et al. (1999) estimated the zinc inputs to agricultural soils in England and Wales (Table 3.49). No other data are (yet) available for UK emissions (e.g. water and air).

Table 3.49 Sources of zinc to agricultural land in England and Wales (Nicholson et al., 1999).

Source	Input (t/y)
Atmospheric deposition	2337
Animal manures	1992
Sewage sludge	385
Fertilisers and lime	290
Agricultural chemicals	26
Irrigation water	4
Industrial wastes	210
Total	5244

A more recent inventory from Nicholson et al. (2003) point to more or less the same estimate (5038 t/a) for the annual zinc inputs to agricultural land in England and Wales for the year 2000.

VI. Selection of EU region

For a comparison of the zinc emissions from diffuse sources between the Netherlands, Germany, Belgium, Sweden and UK an overview is presented of the emissions in these countries (Table 3.50). In this overview those emissions are excluded which are not relevant to the circumstances in other countries (e.g. mining).

It would be too speculative to draw sound conclusions on the differences between these four countries because of the imbalance in the data set, the different assessment methods etc. The available dataset of Belgium is e.g. incomplete and the Swedish dataset is rather dated and less complete compared to that of the Netherlands. The information for Germany seems to be

rather complete, although they are compiled from several sources and the reference period for the water emissions is unclear. Nevertheless data from Sweden and the Netherlands are, roughly taken, in the same order of magnitude (total volume of 3065 t/y (NL) versus 2966 (2301) t/y (S). The German data seem to support this conclusion, as the size of the country and its number of inhabitants in comparison with the Netherlands is reflected in the total emission data for zinc. Generally, the UK total zinc emission input to soil also seems to fit with the German data for soil, regarding the size of both countries.

As for the Netherlands the most recent, extended and detailed information is available, it will be selected as EU-region. The area of the Netherlands also corresponds with the area of a regional system (40,000 km^2). Finally, the zinc emissions of the Netherlands are assumed to be representative for an EU regional system, which is generally supported by the above-mentioned comparison with other EU countries. It must of course be stated that for specific emission sources (e.g. agriculture) rather large differences may occur between EU regions. The official CCDM (2000) regional input data will be used for the Netherlands. The industry alternative estimates, as given for tyre wearing and agriculture emissions, are used as background information.

An alternative approach has been followed as well for selecting an EU-region. This was done by dividing the total EU emissions by a factor of 10 (default TGD approach). This approach is further elaborated in section 3.2.5.3.2 .

Table 3.50 Comparison of total emission rates (tonnes/year) for The Netherlands, Germany, Belgium and Sweden

Compartment	Netherlands (1999)	Germany (?)	Belgium (1995)	Sweden (1990-1995)
Air	91	6640	440	230 + diffuse emissions
Water	254	5200	527	)260 + diffuse emissions
Soil	2720	8670	?	1266[1)]
Subtotal	3065	20,510	967 + ?	2966 (2301) [3)]
- specific emissions	?	?	?	400 [2)]
Total	3065+ ?	20,510 + ?	967 + ?	3366 (2701)[3)]

1. calculated from Table 3.2 in Landner and Lindeström report (1998). Total of 527 g/ha/y and area of 24,000 km^2.
2. refers to mining
3. figure in brackets is the value when lower corrosion estimate of 85 t/y instead of 750 t/y is used.

3.2.5.3.2 Continental releases and PEC calculations

The emissions used for the continental scale (foreign emissions) are defined according to the following TGD default equation:

$$Continental\ Emission = 10 * Regional\ Emission - Regional\ Emission$$

However, for zinc most continental emissions to air, water and soil are initially not calculated with this equation, because more realistic extrapolation factors are available from other sources.

For **industrial** emissions (water and air) a factor of **22** is used to extrapolate the NL data to the EU. This extrapolation factor of 22 is based on the ratio NL inhabitants (16 million) versus EU inhabitants (350 million). The assumption is that there is a relationship between the number of inhabitants and the industrial activities. The Rapporteur is aware that this is an

arbitrary choice, but the standard TGD factor of 10 is considered to be too low for zinc. This because it is known that there are a number of EU Member States with (much) higher zinc production and processing activities than the Netherlands. The EU atmospheric emission becomes about 2400 t/a (Table 3.51; Note: this value also includes traffic emissions for which another extrapolation factor is used; see below). This value is lower than the estimate available for Germany (7,190 t/a). The background of this value is unknown however, and, additionally, it is unknown to which period the German data refers. It is further known that the last decade a considerable number of emission reduction measures has been taken by industry. Some support for the current estimate of 1408 t/y for EU extrapolated air emissions from industry comes from very recent and reliable US data. The TRI database gives a total zinc atmospheric emission figure of 6,500 t/a for the US industry in 1998. The TRI database gives a total US industrial emission value of 800 t/y which is very close to the total EU estimated surface water emission of 682 t/y for industry.

For **agricultural soil** emissions a factor of **10** is used on the recent Dutch estimate of 1620 t/a (corrected for harvest removal). This is the TGD default factor, but it is supported by the ratio for the agricultural emissions based on recent European animal feed marketing figures(Annex 3.2A; FEFAC / EUROSTAT / ZOPA). The EU estimate amounts to 10 * 1620 = 16,200 t/a. This value is very close to the industry's estimate of 15,344 t/a from the data in Annex 3.2A (17,049 corrected for harvest removal factor of 0.1 = 15, 344 t/a).

For **consumers, waste treatment, trade and services, STP effluent and others** the NL/EU inhabitants correction factor of **22** is used as these sectors are all related to consumption aspects. Emissions from corrosion contribute significantly to the emissions from these target groups. The total exposed surface area of zinc amounts to 1317 km^2 (645 km^2 rolled zinc + 672 km^2 galvanised zinc: Industry information). As the exposed surface area in the Netherlands is 66 km^2 the difference NL-EU is about a factor 19-20. The extrapolation factor of 22 is therefore considered applicable to emissions from corrosion as well. Support for the extrapolation for consumers etc. on the basis of the inhabitants ratio is given by the fact that more or less similar zinc levels are monitored in the communal sewage sludge of a number of EU countries (see section 3.2.5.3.5 sludge monitoring data).

For **traffic** emissions to soil an extrapolation factor from the Netherlands to EU of **24** is used based on the ratio of driven kilometres in the Netherlands compared to the EU (OECD, 1995). The same factor of 24 is used for traffic emissions to waste water and surface water. However, a considerable part (about 20 tonnes) of the traffic emissions to surface water comes from emissions of zinc anodes. A lower factor (default) of 5 is used for emissions from anodes, as this usage is expected to be relatively high in the Netherlands.

Details and results of the calculations for the conversion of NL data into the EU are presented in Table 3.51.

Table 3.51 Conversion of the NL emission data to EU.

	Surface water **NL emission (t/y) and relevant extrapolation factor**	**EU (t/y)**	**Soil** **NL emission (t/y) and relevant extrapolation factor**	**EU (t/y)**	**Air** **NL emission (t/y) and relevant extrapolation factor**	**EU (t/y)**
Agriculture	4*10	40	1620*10	16,200		
Industry	31*22	682			64*22	1,408
Waste treatment	-					
Traffic	34*24	816	150*24	3,600	22*24	528
	20*5	100				
consumers	8*22	176	4*22	88	5*22	110
Trade and services	2*22	44				
Effluents STP	95*22	2,090				
Others	50*22	1,100	238*22	5,236		
EU total		**5048**		**25,124**		**2046**
Agricultural soil				**16,200**		
Industrial soil				**8924**		

Theoretical EU-region

In Table 3.51 the continental emissions are calculated from the NL emission data and specific NL→EU extrapolation factors. Most of the emissions to the region are less than 10% of the total continental emissions, and one may therefore argue that this approach is different from a (default) TGD region (continental emission/10). Regional NL-emissions may thus underestimate those in a theoretical EU region. On the other hand, as stated in section 3.2.5.3.1., the NL-region closely meets the definitions of a TGD region (surface area and number of inhabitants) and furthermore the NL-emissions are assumed to be rather realistic estimates. Nevertheless an alternative approach for a theoretical EU-region is carried out in the current risk assessment, but only for reasons of comparison. The NL data are used as a basis for the total emissions and then 10% of these is taken for the region. This results in the following input data (t/y):

Table 3.52 Data used as a basis for the total emissions and 10% of these results in the input data (t/y) taken for the region

	Regional	**Continental**
Air	2046/10= 205	2046-205= 1841
water	5048/10= 505	5048-505 = 4543
agr. soil	16,200/10= 1620	16,200-1620= 14,580
ind. soil	8924/10= 892	8924-892= 8032

Calculation of PEC_{add}s

As mentioned, EUSES 1.0 (according to the TGD, 1996) has been used for calculating the regional PEC_{add} values for each environmental compartment. The input for the regional assessment are the emissions to air, wastewater, surface water and agricultural soil. For modelling the behaviour of zinc in the environment, the octanol-water partition coefficient (Kow) and the aqueous solubility are not appropriate. Measured solids-water partition coefficients for sediment, suspended matter and soil (Kp values) are used instead (TGD (Ap. VIII), 1996). See section 3.2.2 and 3.2.3 for more information about the used Kp values. The impact of various Kp values on the outcomes of the aquatic regional exposure assessment (PEC water) is illustrated by carrying out the modelling with three different Kp_{susp} values. Besides the average value of 110,000 l/kg, also the lowest (64,000 l/kg) and the highest value (176,00 l/kg) being reported for the Netherlands (see Table 3.4) have been used. The Kp_{sed} values were changed accordingly, i.e. by using the ratio of 1.5 between Kp_{susp} and Kp_{sed}. The vapour pressure has been fixed at a low value of 1.10^{-10} Pa and the biotic and abiotic degradation rates have been minimised (TGD (Ap. VIII), 1996). With EUSES the regional environmental concentrations are directly calculated from the regional and continental emission input. The used regional and continental emissions are presented in Table 3.53. The distribution of the diffuse zinc emissions over the various environmental compartments in Table 3.53 is based on two additional assumptions: 1) the soil emissions from traffic are all allocated to industrial/urban soil[9] and 2) the soil emissions from consumers and others are allocated to industrial/urban soil except for the emissions from greenhouses. Emissions from greenhouses are added to the agricultural soil (negligible compared to emissions from manure etc.). The sludge application route is not taken into account in this regional assessment, because sewage sludge is not used in several countries and, additionally, it would result in an overconservative agricultural soil scenario in combination with the spread of manure over the soil.

The resulting regional PEC_{add} values (NL-region) are listed in Table 3.53. The influence of the different Kp's on the estimated water levels (PEC total) is also given in the table: 10.8 µg/l (Kp of 176,000 l/kg), 12.2 µg/l (Kp of 110,000 l/kg) and 14.4 µg/l (Kp of 64,000 l/kg). Increasing the Kp value with a factor of about 3, results in a 1.4 times lower total regional water concentration. Dissolved PECs are 3.0 µg/l (Kp of 176,000 l/kg), 4.6 µg/l (Kp of 110,000 l/kg) and 7.4 µg/l (Kp of 64,000 l/kg), logically showing a decrease with a more pronounced factor (2.5) with increasing Kp. The partitioning pattern due to different Kp's is also reflected in different sediment concentrations: 201 mg/kg wwt (176,000 l/kg), 194 mg/kg wwt (110,000 l/kg) and 181 mg/kg wwt (64,000 l/kg).

9 Zinc emissions from traffic to soil/ water are addressed in the line source scenarios (section 3.2.5.3.4).

It is stated that the PEC_{add} values are not corrected for the *natural* background concentrations in surface water, sediment and soil.

In section 3.2.5.3.1 the influence was mentioned of zinc emissions to agricultural soil on the surface water concentrations by leaching and run-off (CIW, 2003). This aspect can be further investigated quantitatively in the EUSES calculations by varying the various emission input routes (e.g. estimation of PEC water with zinc emissions to agricultural soil set at zero, etc.). The impact of agricultural zinc emissions on the regional PEC water is found to be significant (app. 60%), which is within the same order of magnitude as the preliminary conclusions of the CIW (2003) report (40%). Emissions to industrial soil (mainly from traffic) have a smaller, but still substantial (app. 20%) impact on the PEC water.

Table 3.53 Input data and results of the regional exposure assessment (all data refer to NL-region).

Input Regional:	
Amount released to air	91 t/y
Amount released to surface water	254 t/y
Amount released to agricultural soil	1620 t/y
Amount released to industrial/urban soil	392 t/y
Input Continental:	
Amount released to air	2046-91= 1955 t/y
Amount released to surface water	5048-254 = 4794 t/y
Amount released to agricultural soil	16,200-1620= 14,580 t/y
Amount released to industrial/urban soil	8924-392= 8532 t/y
Results Regional:	
PEC_{add} air	0.006 µg/m^3
PEC_{add} surface water (total) K_p 110,000 l/kg	12.2 µg/l [1)]
(PEC_{add} surface water (total) K_p 64,000 l/kg)	(14.4 µg/l)
(PEC_{add} surface water (total) K_p 176,000 l/kg)	(10.8 µg/l)
PEC_{add} sediment	194 mg/kg$_{wwt}$ (504 mg/kg$_{dwt}$)
PEC_{add} agricultural soil	56.5 mg/kg$_{wwt}$ (64 mg/kg$_{dwt}$)
PEC_{add} natural soil	0.5 mg/kg$_{wwt}$ (0.6 mg/kg$_{dwt}$)
PEC_{add} industrial/urban soil	38 mg/kg$_{wwt}$ (43 mg/kg$_{dwt}$)

1) This value is calculated with a default suspended matter concentration of 15 mg/l. With a suspended matter level of 30 mg/l the value is 20 µg/l.

EU-region

The PECs that are based on emissions from a theoretical EU-region, i.e. continental emissions divided by 10, are presented in Table 3.54. For comparison also the PECs from the NL-region calculation are also given in Table 3.54. PECs in the theoretical EU-region are found to be higher than in the NL-region, except for the agricultural soil.

Table 3.54 Calculated PEC_{add}s in theoretical EU-region and NL-region.

	EU-region	NL-region
PEC_{add} air (µg/m³)	0.01	0.006
PEC_{add} water (total; µg/l)	16.8	12.2
PEC_{add} sediment (mg/kg wwt)	268	194
PEC_{add} soil agricultural (mg/kg wwt)	56.8	56.5
PEC_{add} soil natural (mg/kg wwt)	0.9	0.5
PEC_{add} soil industrial (mg/kg wwt)	86	38

Share of transboundary input

Based on the EUSES calculations the continental contribution to the regional PEC_{add} values is 29 % for water and 42 % for air. This is calculated based on the ratio between the regional and continental concentrations for water and air.

Data are available on the total foreign zinc load via major rivers into The Netherlands. These transboundary inputs are calculated from the measured surface water/suspended matter concentrations and the total flow of the rivers. The total zinc load (sum of Rhine, Meuse and Scheldt) in 1998 amounted to 2868 tonnes (Hoogeveen, 1999). Loads in 1995, 1996 and 1997 were, respectively, 3033, 2044 and 1916 tonnes (CIW, 1998). Much higher zinc loads were found in the early seventies, e.g. 20,000 t/a in 1974 (Rhine and Meuse) (Buijs, 1995). One should realise that these loads comprise both a natural and a anthropogenic part. Comparing the measured surface water concentrations as given in Figure 3.4 with the natural background concentration of 12 µg/l (section 3.2.2.2) a very rough estimation can be made that at least 50% of the load is from anthropogenic origin (>1400 tonnes in 1998). This ratio of >50% is supported by a figure of 75% as given for the Meuse by RIZA (1992). This means that the share of the transboundary input of zinc emissions is >75% (254 t inland sources and >1400 t foreign input). The foreign share of the zinc load in the aquatic environment based on 'measured' loads is higher than the prediction of EUSES.

As reported in the previous section, 69% of the atmospheric zinc deposition in the Netherlands comes from foreign emissions (1993 estimate). This figures correlates fairly well with the continental contribution to the regional air concentration (42 %).

3.2.5.3.3 Accumulation in soil

As discussed in the previous section there are a large number of input parameters (emission sources) for zinc into the environment. On the other hand there is also an output (e.g. crop uptake/removal). An important question is whether accumulation of zinc takes place, i.e. whether via a complex interaction of processes the input of zinc exceeds the output. Soil and sediment are expected to be the most relevant compartments for zinc accumulation. This because the retention times of zinc for these compartments are large compared to retention times in air and water.

Relevant data on zinc accumulation in agricultural soils are available for the Netherlands, Germany and UK.

The Netherlands

In the Netherlands a number of studies estimated the accumulation of zinc in soils. In the report 'Input and output balances of heavy metals in Dutch agricultural soils (IKC, 1996) zinc balances have been calculated for five different agricultural branches (arable farming, dairy farming, field vegetables, bulb growing and successive maize growing). For each branch twelve fertilisation scenarios were applied that are commonly used in the Netherlands. The general conclusion was that for almost all scenarios a zinc surplus was found, mostly in the range of 500 to 2,000 g/ha/y. It should be noted that the branch balance surpluses in the IKC-report pertain to the agricultural balance and do not include leaching and deposition.

Next to balances for different branches, also an overall zinc balance for the Netherlands has been made in the IKC-report. The total input from manure, fertiliser sludge and deposition is estimated at 1,885 tonnes per annum in 1994. The removal via crop uptake is estimated at 160 t/a, which leaves a yearly total surplus of 1,725 t zinc in the Netherlands. It should be mentioned that leaching has not been taken into account in this national IKC balance. (In the current zinc exposure assessment more recent RIVM data are used for the total emissions to soil in the Netherlands. The difference, however, is only marginal.)

In a series of RIVM-reports on the National Soil Monitoring Network of the Netherlands (Groot et al., 1996; Groot et al., 1997; Groot et al., 1998; Groot et al., 1999) the accumulation of zinc was estimated for various types of soil with different uses. The objective of the National Soil Monitoring Network (LMB) is to determine the changes in soil quality in the Netherlands over time and (in the case of heavy metals) explain these changes with quantitative information on input and output of heavy metals. Within LMB, ten categories are distinguished (each category is a different combination of soil type and land use). Each category has 20 sampling locations (generally a farm). The average heavy metal contents in the topsoil (0-10 cm depth), the subsoil (30-50 cm) and the uppermost groundwater (ca. 1 meter depth) are determined for each of the 200 locations. Furthermore, at each location the zinc (and other heavy metal) balance is determined as follows: input (manure, fertilizer, atmosferic deposition, food, etc.) and output (manure, milk, crops, meat, etc.) are determined in kg product per year and multiplied with default values for the zinc contents of the specific products. In this way the net application of zinc to the soil surface is determined. The leaching of zinc from the soil is estimated by multiplying the yearly net precipitation with the measured zinc content of the groundwater. Table 3.55 indicates that zinc accumulation is expected to occur in all soil types, except for forest. The accumulations are in the range of 200 to 700 g/ha/y, which is somewhat lower than the accumulation rates found in the IKC-report. This can be explained by the fact that in the IKC report in some cases less realistic fertilisation scenarios have been used and, in addition, leaching has not been taken into account.

Moolenaar and Lexmond (1998) estimated net zinc balances between 55 and 800 g/ha/y for various types of ecological and conventional arable farming systems in the Netherlands. These data are in line with the RIVM estimates of 200 and 700 g/ha/y.

Table 3.55 Estimated average zinc accumulation in various types of soil with different uses (g/ha/y). Data from Groot et al. (1996; 1997;1998 and 1999).

	Surplus*	Leaching	Accumulation	Reference
Cattle farms, sandy soil (extensive) (1993)	547	332	215	Groot et al. (1996)
Cattle farms, sandy soil (intensive) (1993)	662	384	278	Groot et al. (1996)
Cattle farms (1994)	913	245	668	Groot et al. (1997)
Forest (1994)	41	1258	-1217	Groot et al. (1997)
Arable farm, sandy soil (1995)	512	163	349	Groot et al. (1998)
Cattle farm, peaty soil (1995)	417	97	320	Groot et al. (1998)
arable farms – marine clay soil (1996)	419	41	378	Groot et al. (1999)
cattle farms – river clay soils (1996)	743	43	700	Groot et al. (1999)

* Surplus is defined by the authors as input (manure, deposition etc.) minus output (uptake crops). Leaching is treated separately.

Alterra report (De Vries et al., 2004)

In a very recent report from Alterra research centre (De Vries et al., 2004) a prediction was made on the (potential) risks of zinc accumulation in agricultural soils in The Netherlands. Additionnally the report discussed the relevancy of the data for The Netherlands for other EU Member States.

Using geo statistical data for The Netherlands and applying the most recent zinc input figures (Delahaye et al., 2003) the average zinc fluxes were estimated for the year 2000. This was done both for various land uses (arable land and grassland) and various soil types ((calcareous) sand, (calcareous) clay, loess and peat). A zinc mass balance model was applied to the whole of The Netherlands using 4647 so-called STONE plots, limited to agricultural land use only. These plots consisted of one or more 500m x 500m grid cells with a unique combination of land use, soil type and ground water characteristics. For further details of the study reference is made to the original report. The results of the zinc fluxes are presented in Table 3.56. In all types of soil a net accumulation of zinc is estimated for both arable land and grassland. Net accumulation rates are shown to be within the same order of magnitude as reported in earlier Dutch studies. Major sources of zinc inputs varied only slightly between different land uses and soil types. In grassland animal manure contributes most (more than 90%) to the input of zinc. Fertilisers are a comparatively small source of zinc, whereas atmospheric deposition is also limited. Other sources, mainly compost and pesticides are a substantial source in arable land (approximately 15%). The Alterra report further concludes that the zinc input data for The Netherlands are high in comparison with EU countries having a less intensive agriculture activity. On the other hand they are shown to be representative for regions in North-Western Europe characterised by a similar, intensive agriculture.

The above mentioned data on net accumulation rates (in g/ha/yr) have to be converted into 'annual added' concentrations (in mg/kg soil) before allowing a comparison with the PNEC soil (in mg/kg soil) for the purpose of risk characterisation. Such conversion can either be done via a linear extrapolation or by dynamic modelling. Linear extrapolation has been conducted in the SCAN report and the Nicholson et al (2003) study (both see below). A

dynamic approach takes into account the various processes (e.g., all inputs to the soil, i.e. by fertilisers, animal manure, atmospheric deposition and other sources, and all outputs in terms of plant uptake and leaching) and their inherent fluctuations. The Alterra study contains such a dynamic modelling approach that is based on the most actual data and scientific insights. Time periods before reaching a steady state level and/or the critical zinc level (PNEC soil) were estimated according to the method of De Vries (2002). Additionally, estimation is made of the number of geographic areas where exceedings may occur in The Netherlands. These results of the Alterra report calculations will be presented and discussed in the risk characterisation (section 3.4.3.2).

Table 3.56 Average fluxes of Zn for the various land use and soil types in 2000. Both leaching and accumulation refer to the plough layer (0-10 cm for grassland and 0-30 cm for arable land) (De Vries et al., 2004).

Land use	Soil type	Zn flux ($g.ha^{-1}.yr^{-1}$)			
		Input	Uptake	Leaching	Accumulation
Grass	Sand	938	700	228	10
	Sand calcareous	853	510	66	277
	Clay	969	474	34	460
	Clay calcareous	885	390	16	479
	Loess	1013	636	117	260
	Peat	889	455	126	308
Arable	Sand	1039	392	377	271
	Sand calcareous	868	319	86	463
	Clay	911	347	43	521
	Clay calcareous	899	238	19	642
	Loess	993	405	178	410
	Peat	836	317	271	248
All		926	425	152	349

Germany

The zinc budget for agricultural soils in Germany is given in Table 3.57. It should be mentioned that this estimate is based on rather old deposition figures (before 1990) and also the sludge concentration used (1,318 mg/kg dwt; period 1983-1985) is higher than current figures.

Table 3.57 Zinc budget for agricultural soils in Germany (Wilcke and Döhler, 1995).

	flux (g/ha/y)
Weathering	2.3
Deposition	540.0
inorganic fertilizer	65.6
Irrigation	4.1
sewage sludge	55.0
Compost	0.1
imported food	3.6
remaining inputs via manure without imported food and self cultivated food	551.4
Import total	1,222.1
Leachate	240
Erosion	338
Harvest	69.9
soil sticking to the harvest	0.3
animal food	15.9
Export total	664.1
Net budget (Import-Export)	558

The net budget of 558 g/ha/y is in the same range as values reported for the Netherlands.

UK (adapted from letter McGrath 13-4-2000)

In the UK data are available on the trend of zinc concetrations in agricultural soils for a period of 140 years (Jones et al. 1987). The results of the comparison of the Zn levels in control and farm yard manure (FYM)-treated plots of the Broadbalk and Barnfield experiments (1980) are presented in Table 3.59. It can be estimated from these data that 35 t/ha FYM added an average of ~50 mg/kg to the plough layer soil over 140 years, indicating a net increase of 0.35 mg/kg per year in the soil, and this equates to 1000 g/ha/yr.

Control plots allow estimation of net inputs from the atmosphere, and because these have been subtracted when estimating FYM inputs, the *total* inputs when FYM is added are shown in

Table 3.58 Effect of adding FYM at 35 t/ha/yr for 140 years on soil Zn concentrations (Jones et al, 1987).

Experiment/treatment	Zn (mg/kg dry soil)
Broadbalk	
Control	80
FYM treated	118
Barnfield	
Control	83
FYM treated	142

Table 3.59 Net increases in Zn, estimated from Rothamsted long-term experiments.

Source	Zn mg/kg/yr	Zn kg/ha/yr
Atmospheric deposition	0.1	0.3
*FYM**	0.35	1.0
*Deposition + FYM**	0.45	1.3

* 35 t DM/ha/yr

This long-term trend information can be compared with modern data and application rates. Average applications of FYM to tillage land in the UK have been estimated from survey data as 23 t/ha (Nicholson, 1998). Also, farmers do not apply FYM to each field every year. So, it is likely that each field only receives manure on a rotational basis, perhaps every three years, making the annual average application equivalent to 7.7 t/ha. (Note: This is definitely not the case for many Dutch agricultural grounds, which in some cases (grassland) receive manure several times a year !). This means that the applications on the long-term experiments are about 4.5 times greater than current practice on average. It is therefore likely that additions of Zn to soils receiving both atmospheric deposition and FYM at current practice are 100g Zn/kg or 300 g Zn /ha per year. Because atmospheric deposition is now lower than in the pre 1980 period (Rautengarten, 1993), inputs could in fact be less than this. On the other hand, above-mentioned UK figures are based on averages which means that there will be some situations that receive lower inputs and other with more.

UK

Nicholson et al (2003) calculated in their inventory of heavy metal inputs to agricultural soils in England and Wales the time required to raise soil zinc concentrations from background to limit concentrations. This was done for various types of manure etc. (Table 3.60). The limit concentration was set at 200 mg/kg dwt. As background level an average value of 88 mg/kg dwt was used in this study (pers. comm. Prof Chambers ADAS Gleadthorpe Research Centre UK; January 2004). It should be stated that this UK extrapolation does not refer to a real net accumulation estimate, as it does not account for potential zinc losses via crop offtake or leaching. So in that respect it is a rather worst case situation, also because no bioavailability correction was used. On the other hand the reported times would be lower if soil zinc levels were already above background values or if more than one material was applied to a field each year.

Table 3.60 Zinc addition rates (g/ha/y) for various agricultural input sources and time (y) required to raise zinc soil levels from background to limit concentrations (from Nicholson et al., 2003).

Source	Zn addition rates (g/ha/yr)	Time (years)
Sewage sludge	4557	80
Layer manure	2734	130
Pig slurry	2321	151
Pig FYM	2120	164
Broiler litter	1142	281
Cattle slurry	1063/1214	358
Catlle FYM	718	408
Atmospheric deposition	221	1733
Paper sludge	1380	239
Fertilisers and lime	90	1234
Irrigation water	39	1473

Scientific Committee for Animal Nutrition on the use of Zinc in feedingstuffs (EU SCAN)

On 14 March 2003 the SCAN adopted their opinion on the use of zinc in feedingstuffs. Their conclusions were based on calculations of the annual load of zinc onto agricultural soil via different manure applications. Zinc concentrations were calculated in soil after one and 20 years (Table 3.61). The soil concentration was calculated after one-year application for the top soil layer (5 cm thick) assuming a default soil density of 1.5 g/cm³. For long term application of manure (20 years) maximum accumulation of zinc in soil is referred to a depth of 20 cm of soil (the minimum layer involved in tillage).

The estimates accounted for two different nitrogen levels, i.e 170 and 350 kg/ha/y, respectively, for vulnerable and non-vulnerable areas. In this study zinc loss routes in agricultural soil, like leaching, harvest and degree of erosion, are not taken into account. From that point of view the calculations can be considered as worst case estimates.

The data show that soil concentrations increased between 4 and 14 mg/kg dwt after one year (top 5 cm). After 20 years this increase is ranging from 15 to 70 mg/kg dwt (top 20 cm).

Table 3.61 Values of zinc annual load and resulting metal concentrations in soil after one year and 20 years application of animal manure for different animals. Calculations are performed for two levels of nitrogen application on soil: 170 and 350 kg/ha/y, respectively for vulnerable and not vulnerable areas. From SCAN (2003).

	Calculation based on application on soil of two levels of nitrogen : 170 and 350 kg/ha/y, respectively, for vulnerable and non vulnerable areas					
	170	350	170	350	170	350
	Zinc annual load on soil (g/ha/y)		**Increase zinc soil concentration (mg/kg) over one year (upper 5 cm)**		**Increase zinc soil concentration (mg/kg) over 20 years (upper 20 cm)**	
Veal valves	3682	7580	4.9	10.1	24.5	50.5
Replac. calves	2863	5895	3.8	7.9	19.1	39.3
Fattening steers	3819	7864	5.1	10.5	25.5	52.4
Replac. heifers	3800	7824	5.1	10.4	25.3	52.2
Dairy cow	3744	7708	5.0	10.3	25.0	51.4
piglets	3477	7159	4.6	9.6	23.2	47.7
Fattening pigs	3434	7071	4.6	9.4	22.9	47.1
sows	2179	4487	2.9	6.0	14.5	29.9
Sheep-goats	3704	7626	4.9	10.2	24.7	50.8
Fattening limbs	5166	10637	6.9	14.2	34.4	70.9
Broilers 5 wks	4575	9420	6.1	12.6	30.5	62.8

Conclusion

Cleven et al. (1993) already reported that ´values for addition of zinc with various fertilization scenarios, and for removal by crop plants vary widely in literature, chiefly because of uncertainties in the concentrations of zinc in animal feeds and grass. Nevertheless, every known calculation shows a net accumulation´. They presented a net zinc accumulation of more than 530 g/ha/year in agricultural soil.

The more recent accumulation figures as presented in the current RAR, including the Alterra study, also show that net zinc accumulation is expected to occur in various agricultural soils with average ranges between 200 and 700 g/ha/y. The data refer to the situation in the Netherlands, Germany and UK. Thus approximately the same net accumulation figures are found for agricultural soils as the ones mentioned by Cleven et al. (1993).

For the risk characterisation the accumulation rates are converted into (future) zinc concentrations in soil. The Alterra report contains the most advanced (dynamic) model for this extrapolation and on top of that it refers to the most recent input data. For these reasons the Alterra report will be used as the key study in the current risk assessment (see section 3.4.3.2).

3.2.5.3.4 Measured regional data in the environment.

In this section measured zinc concentrations in various environmental compartments will be presented. Monitoring data related to particular diffuse zinc sources (i.c. corrosion and/or

traffic) are discussed in a separate part at the end of this section. Strictly speaking, these data refer to local situations.

The zinc industry executed an analysis on the available regional monitoring data for surface water and sediment as presented below and more detailed in Annex 3.2.5. Besides more technical correction steps, e.g. an outlier analysis, industry also made a selection of data that are assumed to be influenced by point sources and historical industrial activities (mining). For further details on the (possible) application of this analysis, see section 3.4.4.1. (Disclaimer: The Industry annex 3.2.5. was found by the Rapporteur to be useful to risk management because it sheds further light on the possible sources of zinc and zinc compounds that contribute to regional concentrations from monitoring studies. Annex 3.2.5. has not been formally approved by either the Rapporteur or TC NES.

Water

Measured total zinc concentrations in water and suspended matter of major rivers in the EU are presented in Table 3.62 and Table 3.63. For the Netherlands the total zinc levels in surface waters in the period 1985-1998 are presented in Figure 3.4. Levels in Rhine, Meuse, Scheldt, state waters and regional waters have been collected in extensive, regular monitoring programmes of CIW/RIZA. Data refer to average 90 percentile values, i.e. the average of the 90 percentile values (based on monthly data) for the various, individual sampling stations in a particular water. 'State waters' are defined as the group of major Dutch rivers (incl. Rhine, Meuse and Scheldt) and other large inland surface waters. 'Regional waters' represent approximately 250 differerent sampling stations spread over the Netherlands. These regional sampling stations are selected on the basis that they are not influenced by local point sources (industry, STP effluent etc.). Figure 3.7 presents zinc levels in suspended matter from 1985-1998 in Rhine, Meuse and Scheldt according to the CIW/RIZA monitoring programme.

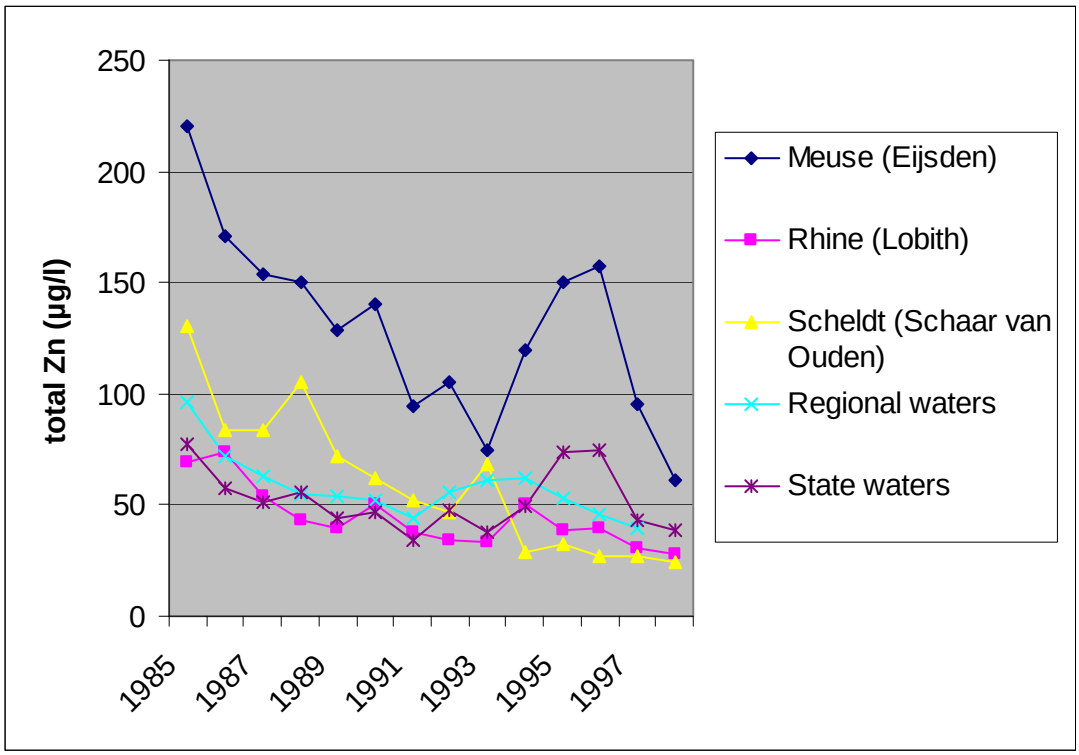


Figure 3.4 Total zinc concentrations (average 90th percentile values) in Dutch surface waters during the period 1985-1998 (RIVM/CBS 2000). Original data from RIZA/CIW).

Recently years measurements of zinc in Swedish lakes and watercourses have been compiled (Landner and Lindeström, 1998). None of the sampling stations is situated in the immediate vicinity of a major source of metal emissions. A summary of the data is given in Table 3.62.

Much higher zinc concentrations were measured in areas near major point sources in Sweden. In the vicinity of 'traditional' mining districts leaching and erosion of mining waste leads to levels of e.g. 710 µg/l (average value) in a lake near Gruvsjön, Garpenberg during the period 1990-1996. Much lower zinc concentrations (no data given) are found outside point sources in Sweden where activities first began during 'modern' times.

Table 3.62 contains a large number of zinc concentrations (90 P values) in German surface waters (LAWA, 1998). In general, the LAWA monitoring net is designed to measure the ambient overall pollution of surface waters. Data from the LAWA monitoring net are used repeatedly in Germany for assessing and reporting the general water quality within the frame of the European environmental laws, e.g. under the Directive 76/464/EEC. The sampling sites are not used for compliance monitoring of plant permits. Zinc levels in Germany range from 3-291 µg/l. Much higher zinc values were reported from the period 1977-1983 in surface waters of old mining districts in Germany, e.g. in the Harz Mountains (max. 1300 µg/l Zn), in the Rheinische Schiefergebirge (max. 11,700 µg/l), near Maubach and Mechernich at the North edge of the Eifel, and near Bodenmais in Bavarian Forest (max. 10,000 µg/l) (Fauth et al.,1985).

Recent (1996-1998) zinc surface water concentrations have been reported for France (see Table 3.62). The 90 P values for various regions in France ranged from 30 – 99 µg/l. The value of 99 µg/l is for the Rhin-Meuse region. More recent data for France (2000-2002) on the same and other regions are discussed in the section Regional risk characterisation.

Monitoring data are available from two Belgium monitoring networks, i.e. the Walloon Region and Flanders (Table 3.62). In the Walloon Region the network contains 179 sampling locations spread over various Walloon surface waters. About 10%[10] of the sampling locations has a zinc concentration exceeding 100 µg/l zinc (90P), 13% between 50 and 100 µg/l, 18% between 25 and 50 µg/l and for 56% of the locations the zinc level is below the detection limit of 25 µg/l. The Walloon region is characterised by 3 river basins, the Scheldt, the Meuse-Seine and the Meuse. It is further important to note that the measured zinc concentrations in Walloon Region do not refer to total zinc levels, but to 'zinc extractible'. This 'in house' analytical technique is based on AAS and flame analysis after acidification (HNO3, pH<2), settling and decanting of the water samples (based on EPA method 7000, Sept. 1986; EPA method 7950, Sept. 1968 and Standard Methods 20th ed). A limited internal comparison of the results of analysis based on 'zinc extractible' and total zinc showed that total zinc levels tend to be (slightly) higher, but the difference is not more than 30%.

The monitoring network in Flanders contains a large number of sampling locations distributed over various types of surface waters in Flanders (670 sampling points in 1999 and 805 sampling points in 2000). Total zinc levels have been analysed and the data show that average 90P values amount to 146 and 110 µg/l for 1999 and 2000, respectively. Zinc concentrations above 100 µg/l are found in 41% (1999) and 27% (2000) of the locations. In 11% (1999) and 8% (2000) of the sampling locations the zinc concentrations are found to be above 200 µg/l. An important conclusion is that the regional zinc levels in surface water for Flanders are substantially higher than those for the Netherlands (90 P value of 41 µg/l). It should be noted,

[10] The number of sampling locations may vary from 1 for small surface waters to about 10 for large rivers like the Meuse. The % coresponds to the % of sampling stations (all rivers pooled) within a certain value of 90P.

however, that the sampling set in Flanders includes locations heavily influenced by point sources, whereas the Dutch data is mainly influenced by diffuse sources.

Figure 3.5 presents an overview of zinc concentrations in the Meuse during its course from the French-Belgium border (Heer-Agimont) through Belgium (until Eijsden, the Netherlands) and then further alongside the Dutch/Belgian border (until Kinrooi). The data points refer to sampling during the period 1995-2001 (90 P values). Zinc levels in this Meuse transect are found to range from 29 µg/l (Dave) to 129 µg/l (Engis). High levels are also measured in Liege and Kinrooi (both 106 µg/l). It is emphasised that these data refer to average zinc concentrations of several years (1996-2000). Data for the individual years per sampling station therefore show both lower and higher values. For example: zinc levels of 188 and 163 µg/l are measured in 1997 in, respectively, Engis and Liege. At the Belgian sampling point Kinrooi levels around 190 µg/l were recorded in both 1996 and 1998.

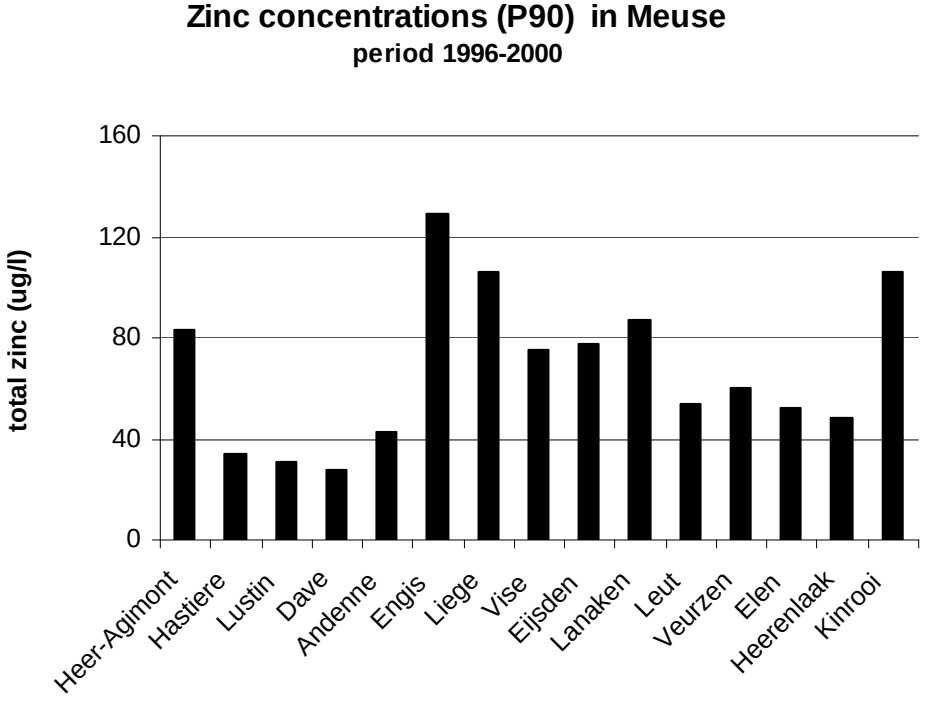


Figure 3.5 Zinc concentrations in surface water at various sampling points downstream the Meuse river (Belgium and the Netherlands). Data refer to the average 90P value during the period 1996-2000. Source: p.m.

In the report 'Revised Proposal for a List of Priority Substances in the Context of the Water Framework Directive (COMMPS Procedure)' from Denzer et al. (1999) monitoring data (water and sediment) were collected for a large number of chemicals (including zinc) in the EU. Data are from 1994-1998. For zinc dissolved measurements were received from sampling stations in Austria, Germany, Spain, UK, Italy and the Netherlands. For zinc total the database contains measurements from sampling stations in Austria, Belgium, Germany, Finland, UK, Ireland, Portugal and Sweden. Sediment data are from Austria, Belgium, Germany, France and UK (2854 measurements from 495 sampling stations). From the total number of 11,948 measurements (340 sampling stations) for total zinc ultimately 10,809 (306 sampling stations) were used for the aggregated 90P calculation. Data were discarded if the zinc concentration was found to be below the detection limit in combination with a relatively high detection limit for that particular sampling station (for details see original report). This

implies that 10% of the measurements and sampling stations were removed from the database before estimating the 90P value for total zinc. For dissolved zinc 2528 (170 sampling stations) from the original 3144 (300 sampling stations) had been discarded based on similar criteria concerning the detection limit. About 80% of the measurements and 60% of the sampling stations were thus left out for dissolved zinc. This means that, especially for dissolved zinc, a bias may occur towards a relatively higher overall 90P value due to omitting a significant number of sampling stations with (relatively) low zinc levels. It has to be noted, however, that data below the detection limit in combination with a (relatively) low detection limit remained in the data set. For sediment hardly any data were removed from the original data set (app. 1%). The results, i.e. 90P values from the aggregated data base are presented in Table 3.62 and Table 3.64.

The following general considerations can be made on the use of the Denzer et al. database in the zinc risk assessment:

- It is an 'officially approved' EU database that had played an important role in the Water Framework Directive priority setting activities (COMMPS). The database underwent a number of statistical and other quality checks on possible outliers etc. It has to be noted, however, that priority setting differs from a risk assessment at regional scale.
- There is an overlap between the data in the Denzer et al database and information directly received from the various EU regions. The Denzer et al. database in fact constitutes a meta database on EU data for zinc in the environment. German data in Denzer et al mainly refer to Rhine, Weser en Elbe, Dutch data to the Meuse river and comparable data were also directly received from these two individual Member States. Also the Swedish and Belgian (e.g. Scheldt river) Denzer et al. data are covered in the regional data sets for those countries. Germany, the Netherlands, Sweden and Belgium together constitute to a large extent to the Denzer database. Recent UK zinc surface water monitoring data became available and are discussed in section 3.4.4.1.
- The database is heterogenous especially with respect to different detection limits applied among the various countries. Compiling all this information into one 90P value introduces a relatively large uncertainty (bias, see above).

In conclusion: it is recognised that the Denzer et al. database shows (large) uncertainties and overlaps with available data from individual EU regions, but it does give information at an EU meta level. Therefore the Denzer et al. will only be used as 'indicative' in the current risk assessment. It will not be used for drawing the final conclusions on potential risks of zinc at regional scale. Although above-mentioned considerations mostly relate to surface water (especially the detection limit issue), also the sediment data from Denzer et al. will only be used as 'indicative' in the present risk assessment. Preference is given to sediment data from the individual EU regions.

Seasonal variation in surface water concentrations

Industry has investigated the Dutch CIW monitoring data for surface water in further detail. They concluded that there is "a clear pattern of seasonal variability in surface waters, except the lakes". High zinc values are consistently being found in winter, whereas levels drop to minimum values during the summer months. Such seasonal patterns could be explained by the natural biological cycling of the essential element zinc in surface waters. Winter values are high because of zinc containing leaf litter causing a high zinc input in autumn/winter, and on top of that, there is a degradation of biota (mainly algae) resulting in a release of zinc in

autumn and winter from biota into the water phase. In spring there is subsequently a strong uptake of the essential element of zinc by biota resulting in lower zinc levels in water.

Industry concludes that if the PEC regional assessment would be based on 90percentile values of regional monitoring data it should be interpreted with great caution, because;

- 90 percentile values observed for these waters are consistently winter values;
- Higher winter values and lower summer values are consistently found in all NL waters, including the big ("State") waters and in other EU waters;
- High winter values cannot be explained by physicochemical factors, nor by inputs from diffuse zinc releases, e.g. corrosion run-off. But they follow closely the seasonal cycle of uptake (depletion of zinc in water) of the essential element zinc by biota in spring/summer, followed by the degradation of biota in autumn and the input of zinc in autumn/winter by leaf litter decay;
- It can be calculated that the influence of this natural input on zinc concentration in the water can be significant.

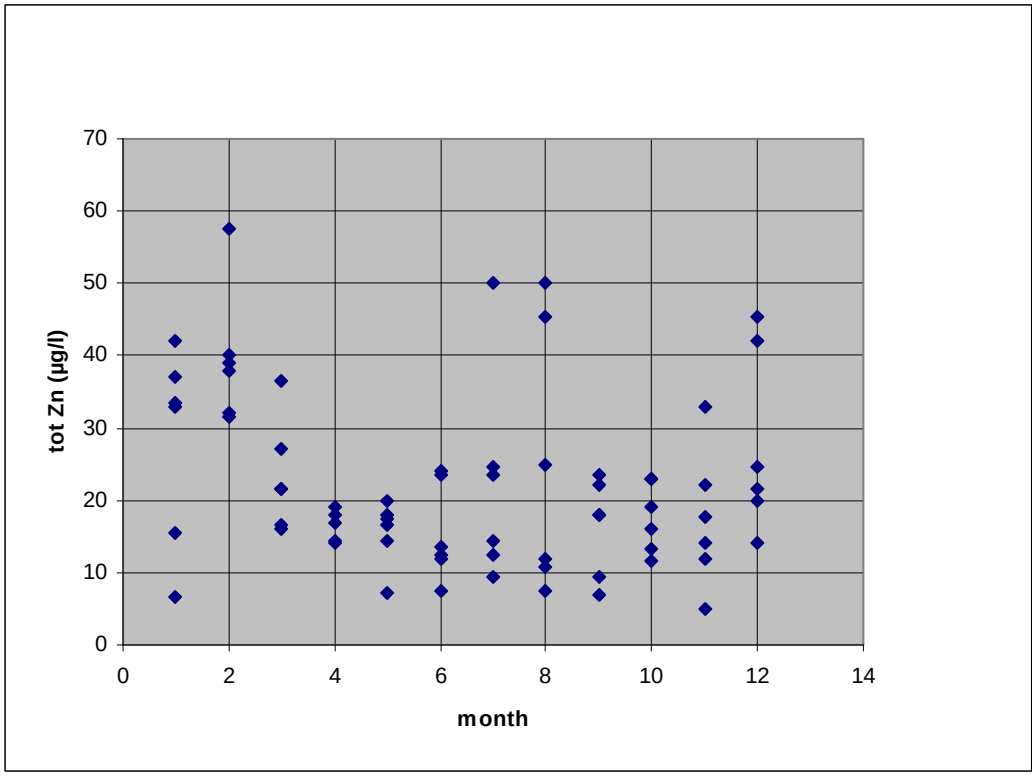


Figure 3.6 Total zinc levels in Rhine (Lobith) in the period 1990-1995.

The arguments brought forward by industry have been examined by the Rapporteur. The first issue is whether a seasonal pattern in zinc concentrations in surface waters is really observed, and the second one is, if such relationship is found (either weak or strong), what could be the plausible factors causing this phenomenon?

There is a great scattter in the data making this phenomenon possibly less obvious as stated by industry. As an example of this large scatter the data for the Rhine (1995-2000) are given (see Figure 3.6). In addition, the CIW monitoring data refer to total values. There may be a clear relationship between the (total) zinc concentration and the suspended matter concentration. High zinc concentrations in winter could then be explained by higher suspended matter concentrations. It should be borne in mind that all CIW monitoring data are normalised to a suspended matter concentration of 30 mg/l. If, after correction for suspended matter, there still

is/seems to be (some) seasonal variation then a combination of the following factors could (also) explain the seasonal variation in zinc concentrations:

- a higher frequency of sewage treatment overflows in winter (more rainfall);
- increased zinc leaching/run-off from agricultural soils and paved surface areas in winter (more rainfall);
- increased re-mobilisation of zinc from sediments due to change in redox zones. The (sub)oxic toplayer of sediments in winter is somewhat deeper (1-2 cm) than in summer. This results in an oxidation of metal sulphides in the toplayer. Metal release from sediment is known to be increased in winter (Van de Berg, 1998);
- the relation between (dissolved) zinc and pH. In the period April-September the pH is relatively large due to algae blooms which causes dissolved zinc concentrations to decrease to very low values. This relation between pH and zinc levels has been clearly demonstrated in several waters, incl. rivers (Salomons and Mook, 1980: Zwolsman, 1990 and Shiller and Boyle, 1985);
- the (possible) decrease of the zinc concentration in the Rhine in the period February-June can be explained by the fact that in winter the Rhine water is mostly dominated by groundwater from France and Germany, whereas in spring and summer it is dominated by melting water from the Alps (Van der Weijden and Middelburg, 1989). In comparison with the catchment basins in Germany and France, the Alps are relatively calcium rich and therefore have low zinc levels (Van der Weijden and Middelburg, 1989).

Resuming: 1) the seasonal variation of zinc concentrations in surface waters may be less pronounced as stated by industry, and 2) besides the biological cycle, a number of other explanations (both antropogenical, geological and physico-chemical) could be given to explain this phenomenon (if observed). As up to now no sound and quantitatively underpinned arguments are available that only non-antropogenical factors cause the (possible) seasonal variation of zinc concentrations in surface water, there is no reason to exclude the 90P values in the current zinc risk characterisation.

Table 3.62 Measured zinc concentrations in water (see also Figure 3.4).

Location	Concentration (µg/l)	Source
Rhine (Schmitter, CH), 1977-1984	13 (arithmetical mean, total Zn)	Weijden, Middelburg, 1989
Rhine (Rekingen, CH), 1975-1984	10 (arithmetical mean, total Zn)	Weijden, Middelburg, 1989
Aare (Brugg, CH), 1975-1984	12 (arithmetical mean, total Zn)	Weijden, Middelburg, 1989
Rhine (Village Neuf, D), 1977-1984	22 (arithmetical mean, total Zn)	Weijden, Middelburg, 1989
Neckar (Mannheim, D), 1976-1984	43 (arithmetical mean, total Zn)	Weijden, Middelburg, 1989
Main (Kostheim, D), 1976-1984	152 (arithmetical mean, total Zn)	Weijden, Middelburg, 1989
Mosel (Koblenz, D), 1975-1984	88 (arithmetical mean, total Zn)	Weijden, Middelburg, 1989
Emscher (Duisburg, D), 1977-1984	104 (arithmetical mean, total Zn)	Weijden, Middelburg, 1989
Swedish watercourses, 1989-1995	12 (90 P; total) total Swedish watercourses 3.6 (90P; total) Lakes Northern Sweden 6.4 (90P) Lakes Southern Sweden	(Landner and Lindeström, 1998)
France, 1996-1998 various regions	99 (90P) Rhin Meuse (North east France) 30 (90P) Seine Normandie	« Réseau National des Données sur l'Eau », Office International de l'Eau, F - 87065 LIMOGES Cedex,

Location	Concentration (µg/l)	Source
	40 (90P) Adour Garonne (South west)	www.rnde.tm.fr)
Germany, 1998 various regions	All 90 P values. More data per water refer to different sampling stations Aland : 10 Aller: 169, 93 Altbach: 19.7 Argen: 24.4 Bille: 11.8 Bongsiel Kanal: 17 Donau: 19.6, 20.1, 10.8, 7.0, 10.0, 10.0 Elbe: 30, 50, 45, 65, 52, 70,8 57.3, 72,0 Elde: 3,7 Ems: 88, 36, Erft: 66,2 Freib.Mulde: 140 Fulda: <50 Grosse Ohe: 10 Grosse Roder: 28 Hase: 38 Havel: < 25, 14.8, <25, 27 Hunte: 104 Iller: 5 Ilm: 102,9 Ilmenau: 12 Inn: 29.3, 10, 10, 60, 56 Lausitzer Neisse: 62.4 Lech: 6 Leine: <30 Lenne: 90 Lippe: 36.7 Main: 10, 30.8, 31.4, 53 Mosel: 52 Mulde: 114 Naab: 30 Nahe: 35 Neckar: 22.3, 32.7, 21, 21.8, 24, 29.3	LAWA, 1998

Location	Concentration (µg/l)	Source
	Neisse: <50 Nidda: 70 Niers: 50 Oder: 60, 50 Peene: 3.9 Pleisse: 12,0 Prims: 20.7 Radolfz.-Aach: 17.8 Regnitz: 20 Rhein: 8.2, 10.0, 12,9, 19.7, 29.0, 15.2, 17.2, 46.0, 32.2, 44.6 Rotach: 19.4 Ruhr: 61.4 Rur: 179.1, 119.2 Saale: 11.5, 29, 35, 135 Saar: 25.5, 29, 68 Sachs.Saale: 60 Salzach: 20 Schussen: 33.7 Schwalm: 50 Schwarzenbach: 60 Schwarze Elster: 36, 30 Schwentine: 8 Sieg: <50, 46, 166 Spree: <25, <25, 29 Steinach: 17.1 Stever: 82 Stor: 17.3 Sude: 3.9 Swist: 30 Teltowkanal: 49 Tollense: 6.9 Trave: 10.1 Treene: 6.9 Uecker: 5.3 Unstrut: 72.7, 49 Vechte: 33 Vereinig. Mulde: 291	

Location	Concentration (µg/l)	Source
	Warnow: 3.3 Weisse Elster: 42, 39, 90 Werra: 18.2 Weschitz: 36.5 Weser: 30, 39, 181 Wipper: 47.6 Wupper: 51.2 Zwick.Mulde: 86	
Belgium, Walloon Region (2001)	2001 (179 measuring points; 90P values of 'zinc extractible': see text*) <25 µg/l: 102 points 25-50 µg/l: 32 points 50-100: 24 points 100-200: 15 points > 200: 4 points min-max: <25-1354	DGRNE, 2001
Belgium, Flanders (1999 and 2000)	1999 (670 measuring points; 90 P values of total zinc) < 50 µg/l: 130 points 50-100 µg/l: 261 points 100-200 µg/l: 179 points 200-500 µg/l: 74 points > 500 µg/l: 24 points min-max: 0.1-3722 average 90P: 146 µg/l 2000 (805 measuring points; 90 P values) < 50 µg/l: 288 points 50-100 µg/l: 291 points 100-200 µg/l: 156 points 200-500 µg/l: 51 points > 500 µg/l: 15 points min-max: 5-3831 average 90 P: 110 µg/l	VMM, 2003
Major Norwegian rivers (1998)	River: min-mean-max value (in µg/l of total zinc) Glomma: 1.9-6.9-43.8 Drammenselva: 2.2-3.0-4.2 Nummedalsagen: 3.6-9.5-26.2 Skienselva: 2.1-2.5-3.1	NIVA, 1999 (Report no. 4116-99)

Location	Concentration (µg/l)	Source
	Otra: 2.9-3.8-5.1 Orreelva: 0.6-2.1-4.3 Suldalslagen: 1.6-1.2-1.9 Orkla: 7.2-17.5-38.5 Vefsna: 0.4-11.8-50.2 Alta: 0.2-0.4-0.8	
NORDIC countries (lakes) (1995)	Finland: 4.4 µg/l (90P of total zinc) Norway: 5.9 µg/l (90P of total zinc) Sweden: 5.3 µg/l (90P of total zinc) Denmark: 12.6 µg/l (75P of total zinc)	NIVA, 1999 (Report no. 4039-99)
EU-level 90th percentile of monitored water concentrations, 1994-1998	*59.2 µg/l (total, n=10,809)* *82.5 µg/l (dissolved, n=2528)1)* *Data only' indicative' (see text for details)*	*Denzer et al., 1999*

The data sets for total and dissolved zinc levels are (partly) different. This explains the fact that dissolved value is higher than total value.

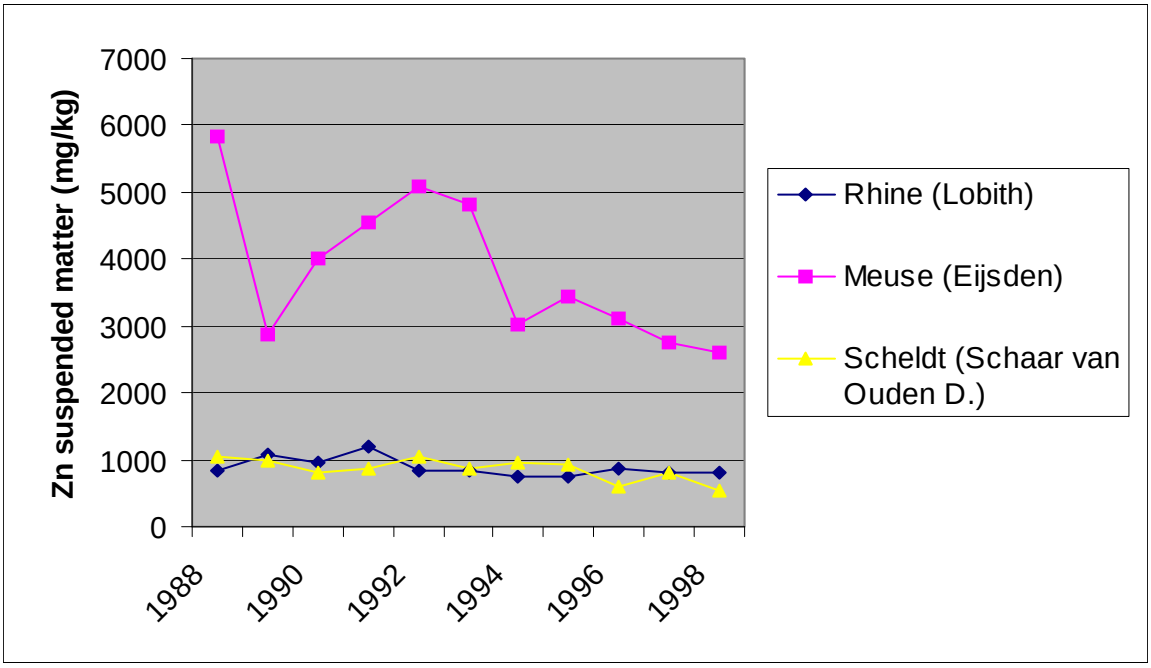


Figure 3.7 Zinc concentrations (90th percentile values) in suspended matter in Dutch surface waters during the period 1985-1998 (RIVM/CBS, 2000). Original data from RIZA/CIW.

Table 3.63 Measured zinc concentrations in suspended matter (see also Figure 3.7)

Location	Concentration (mg/kg_{dwt})	Source
Scheldt (1980-1989)	1090 (river) 190 (estuary)	van Eck et al., 1991
Rhine (1980-1989)	960 (river) 200 (estuary)	van Eck et al., 1991
Elbe (1980-1989)	700 (river) 210 (estuary)	van Eck et al., 1991
Weser (1980-1989)	1030 (river) 130 (estuary)	van Eck et al., 1991
Gironde (1980-1989)	870 (river) 190 (estuary)	van Eck et al., 1991
Clyde (1980-1989)	590 (river) 260 (estuary)	van Eck et al., 1991
Adige (1980-1989)	270 (river) 330 (estuary)	van Eck et al., 1991
Meuse Belgium (1999-2000) *Dave* *Andenne* *Vise*	 432 and 500 (n=2) 505 and 678 (n=2) 1875 and 2534 (n=2)	DGRNE (Laboratoire ISSeP) (2001)

Sediment.

A number of sediment monitoring data throughout the EU are reported in Table 3.64. The sediment data from the Denzer et al (1999) database (see also section on water) result in a 90 P value of 1367 mg/kg dwt (period 1994-1998). A number of recent (1998) German data have been included as well in the table (LAWA, 1998). They range from 216 to 3230 mg/kg dwt (90 P values). As the Denzer et al. database comprises German monitoring data as well, it may be possible that both references refer to (partly) the same data. The Denzer et al. database will, however, only be used as indicative in the current zinc risk assessment (see considerations in section on surface water).

Sediment data from Sweden have been reported as well (see Table 3.64). Median values are 150 and 240 mg/kg dwt for Norther Sweden and Southern Sweden, respectively.

Table 3.64 Measured zinc concentrations in sediment (see also Table 3.67)

Location	Concentration (mg/kg$_{dwt}$)	Source
Dutch State waters, (1989-1990)	< 140 (28%) >2500 (4%)	Cleven et al. 1993
Dutch regional waters, (1989-1990)	< 140 (32%) >2500 (1%)	Cleven et al. 1993
Rhine (NL), 1982-1989	1389 (mean) 392/3300 (10/90th percentile)	Cleven et al. 1993
Meuse (NL), 1982-1989	1289 (mean) 549/1665 (10/90th percentile)	Cleven et al. 1993
Lake IJssel (NL), 1982-1989	627 (mean) 3/1512 (10/90th percentile)	Cleven et al. 1993
Scheldt estuary 1987-1988	157±145 (mean, bulkconc.) 600±100 (mean, fraction <63μm)	Alsenoy et al., 1990 (A10)
Northsea coast near Scheldt, '87-'88	50±14 (mean, bulkconc.) 175±17 (mean, fraction <63μm)	Alsenoy et al., 1990 (A10)
Northsea near Scheldt 1987-1988	9±3 (mean, bulkconc.) 214±43 (mean, fraction <63μm)	Alsenoy et al., 1990 (A10)
Rhine estuary (year unknown)	240-760	van Eck et al., 1991
Elbe estuary (year unknown)	42-570	van Eck et al., 1991
Weser estuary (year unknown)	43-1432	van Eck et al., 1991
Mersey estuary (year unknown)	7-684	van Eck et al., 1991
Tamar estuary (year unknown)	195-1150	van Eck et al., 1991
Loire estuary (year unknown)	14-279	van Eck et al., 1991
Gironde (year unknown)	7-464	van Eck et al., 1991
Wadden Sea (year unknown)	100-350	van Eck et al., 1991
Eems/Dollard (year unknown)	150±25	van Eck et al., 1991
Scheldt (year unknown)	3-1325	van Eck et al., 1991
Lower/Middle Rhine (1985)	1125 (mean, <2 μm fraction)	Müller, 1987
Upper Rhine (1985)	515 (mean, <2 μm fraction)	Müller, 1987
Elbe (1985)	1818 (mean, <2 μm fraction)	Müller, 1987
Donau (1985)	365 (mean, <2 μm fraction)	Müller, 1987
Weser (1985)	611 (mean, <2 μm fraction)	Müller, 1987
Ems (1985)	727 (mean, <2 μm fraction)	Müller, 1987
Main (1985)	1094 (mean <2 μm fraction)	Müller, 1987
Neckar (1985)	460 (mean, <2 μm fraction)	Müller, 1987
Rhine (Lobith, NL), 1993-1997	770 (90th percentile, n=4)	RIZA, 1998
EU waters (1994-1998)	*1367 (90th perc., n=2833)*	*Denzer et al. 1999*

Location	Concentration (mg/kg$_{dwt}$)	Source
	Data only' indicative' (see text for details)	
Germany, various regions, 1998	90 P values. More data per water refer to different sampling stations Aller: 1500 Elbe: 820, 900, 1696, 1693, 988, 476 Ems: 480 Lausitzer Neisse: 680 Main: 403 Mosel: 1029 Mulde: 3230 Nahe: 398 Neckar: 416, 452, 404 Rhein: 216, 296, 356, 546 Saale: 2519 Saar: 585, 593 Swarzbach: 1557 Schwarze Elster: 1033 Spree: 1010 Vereinig. Mulde: 1600 Warnow: 465 Weser: 300, 879	LAWA, 1998
Swedish sediments (unaffected by point sources)	150 (median value) Northern Sweden 240 (median value) Southern Sweden	Landner and Lindeström, 1998
Belgium, Flanders (1994-2001)	1083 sampling points (single measurements) < 200 mg/kg dwt: 662 points 200-500 mg/kg dwt: 277 points 500-1000 mg/ kg dwt: 108 points >1000 mg/kg dwt: 36 points min-max: 2-13,400 mg/kg dwt average 279 mg/kg dwt 90 P value: 604 mg/kg dwt 50 P value: 145 mg/kg dwt	VMM, 2003
Norway (lakes) 1996-1997	Data from 231 Norwegian lakes: 361 mg/kg (90P) at surface 195 mg/kg (90P) at 30-50 cm	Rognerud et al. (1999)

Location	Concentration (mg/kg$_{dwt}$)	Source
	136 mg/kg (50P) at surface 106 mg/kg (50P) at 30-50 cm	
France (various region), 1996-1998	90P values: Artoie Picardie: 1200 mg/kg dwt Rhin Meuse: 1908 mg/kg dwt Seine Normandie: 463 mg/kg dwt Loire Bretagne: 989 mg/kg dwt Adour Garonne: 340 mg/kg dwt Rhone Mediterranee Corse: 372 mg/kg dwt	Réseau National des Données sur l'Eau »
Meuse (Walloon Region) Belgium (1999-2000) *Dave* *Andenne* *Vise*	 334 and 319 (n=2) 528 and 697 (n=2) 907 and 818 (n=2)	DGRNE (Laboratoire ISSeP) (2001)

Some individual Belgian data are available for zinc sediment concentrations in the Meuse (Walloon Region) in Table 3.64. A much larger set of sediment is available from the Belgium Flanders monitoring network (VMM, 2003). A total of 1083 sampling locations distributed over various water types have been monitored in Flanders during the period 1994-2001. Data are reported in Table 3.64. The figures show that 61% (n=662) of the sampling stations have a zinc sediment level lower than 200 mg/kg dwt. About 25% (n=277) have a zinc concentration between 200 and 500 mg/kg dwt, in about 10% (n=108) of the waters a sediment zinc level between 500 and 1000 mg/kg dwt has been reported and in 3% (n=36) of the cases the zinc sediment levels exceeds 1000 mg/kg dwt. Another sediment data set is available for Flanders which is based on less sampling stations (n=200), but it contains more recent data from 2002. A 90P zinc value of 535 mg/kg dwt is reported, which only slightly differs from the value of 604 mg/kg dwt in the other Flanders data set. The importance of the 2002 Flanders sediment database is that total zinc measurements are accompanied by SEM/AVS measurements. The latter data will be used in the sediment risk characterisation for Flanders (see section 3.4.3).

A number of monitoring data have been reported for sediments in France (« Réseau National des Données sur l'Eau », Office International de l'Eau, F – 87065 LIMOGES Cedex, www.rnde.tm.fr). These France data are also presented in Table 3.64, but some further details are given below:

All French sediment data:

Table 3.65 Statistics of measured zinc concentrations in sediment in France

Number of data		996
Mean		335.4
Standard error		790.2
Percentiles (mg/kg dwt)	10	52.7
	50	130
	90	583.9

Split-up of French sediment data according to their origin:

Table 3.66 Split-up of French sediment data according to their origin

Region		**Artoie Picardie**	**Rhin Meuse**	**Seine Normandie**	**Loire Bretagne**	**Adour Garonne**	**Rhone Méditéranée Corse**
Number of data		249	48	141	62	233	263
Mean		494	682.1	277.7	366.6	238.3	231.7
Standard error		920.7	942.9	682.2	508.4	838.3	641.1
Percentiles (mg/kg dwt)	10	74.5	158	61.1	50.3	44.4	38.4
	50	164.4	339.3	150	152.5	110	97
	90	1200	1908.5	462.8	989.7	340	372

In the RWS-report (1998) zinc levels in sediment are presented from Hollandsch Diep and Dordtsche Biesbosch in the Netherlands. Both waters can be characterised as large sedimentation areas from the rivers Rhine and Meuse. In the RWS report zinc concentrations in Hollandsch Diep have been measured at various depth-layers of the sediment (‘ historical profile’). These depth-layers correspond to three different time/sedimentation periods (1: before 1972; 2: 1972-1985; 3: 1985-1993. The results of the RWS-study are presented in Table .

Table 3.67 Zinc levels (mg/kg dwt) in different sediment-layers of Hollandsch Diep and Dordtsche Biesbosch in the Netherlands (RWS, 1998).

Location	1995/1997	Before 1972	1972-1985	1985-1993
Hollandsch Diep (deeper layers)		465 (min) 770 (av) 3391 (max)	40 (min) 1145 (av) 3777 (max)	104 (min) 589 (av) 1100 (max)
Hollandsch Diep East (freshly deposited layers)	41 (min) 1001 (av) 2089 (max)	-	-	-
Hollandsch Diep West (freshly deposited layers)	22 (min) 293 (av) 4003 (max)	-	-	-
Dordtsche Biesbosch clay	242 (min) 1131 (av) 2802 (max)	-	-	-
Dordtsche Biesbosch sand	46 (min) 663 (av) 1904 (max)	-	-	-

Sludge and STP effluent

There is a lot of information available on the quality of sludge from both communal and private (mostly industrial) STPs in the Netherlands (CBS, 1999). Mean zinc concentrations in communal and private STP sludge amount to, respectively, 865 mg/kg dwt and 143 mg/kg dwt in 1997. Much higher levels were found in the early eighties: 1739 mg/kg dwt in communal STPs and 617 mg/kg dwt in private STPs (1981 data). Figure 3.8 gives the distribution of the sludge in Dutch communal STPs into four zinc concentration classes in 1997 and 1981.

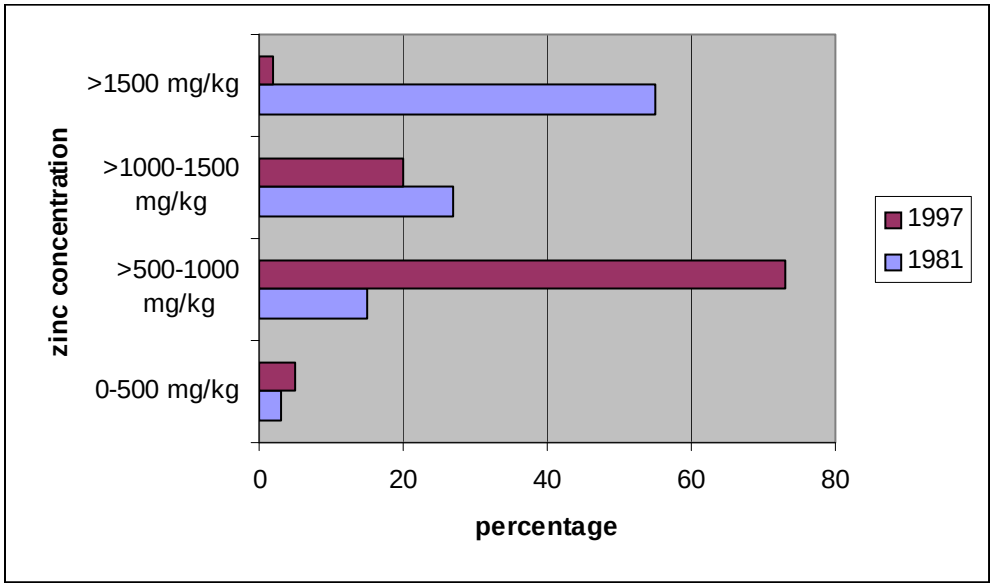


Figure 3.8 Zinc concentrations (classes) in sludge from communal waste water treatment plants in the Netherlands in 1981 and 1997 (after CBS, 1999).

UK data (1996/7) are available on the quality of sludge used in agriculture: the median zinc contents is 559 mg/kg dwt and the 90th percentile-value is 1,076 mg/kg dwt (Environment Agency). Median sludge levels in 1982/3 and 1990/1 were, respectively, 1205 and 889 mg/kg dwt, indicating a decrease in zinc levels during the period 1982-1997. The decreasing trend seems to be similar to the situation in the Netherlands. The same holds for Germany. In the years 1982 and 1983-85 the Zn contents in sewage sludge used for agriculture were 1480 and 1318 mg/kg/dwt, respectively (WAII4, 199a and Wilcke and Döhler, 1995). More recent German data amounts to 863 (1995), 831 (1996) and 809 (1997) mg/kg dwt, pointing to a clear decrease in zinc levels in German sewage sludge from 1982 to 1997.

For Denmark the calculated load of zinc as a result of normal sewage sludge application in 1997, as a worst case situation in 1997 and 2000, are 3040 g/ha, 16,000 g/ha and 12,000 g/ha respectively (Henning Krogh 1997). All figures are calculated based on the latest sludge directive in Denmark of 1996. In 1997 the weighted mean for zinc in Danish sewage sludge was 760 mg/kg dw for all sludges and 678 mg/kg dwt for sludges used to amend soils. The 90 P values were 1068 and 1069 mg/kg dwt , respectively (Miljøstyrelsen, 1999).

In conclusion, the current zinc sewage sludge concentration in various EU countries (the Netherlands, Germany, UK and Denmark) have all decreased clearly during the last decades and they are now found to be at more or less the same levels (Table 3.68). Reduced corrosion run-off rates, due to lower SO_2 levels may be a good explanation for the decreasing trend. The observation of approximately the same (absolute) levels nowadays in different countries may point to a more or less similar zinc consumption pattern (at least via the sewage sludge route) in these EU countries.

Table 3.68 Former and recent zinc sewage sludge concentrations in various EU countries.

	Sludge concentration (mg/kg dwt)	
	Former data	Recent (1997)
The Netherlands	1739 (mean: early eighties)	865 (mean)
Germany	1480 (mean ?; 1982)	809 (mean ?)
U.K.	1205 (median; 1982/3)	559 (median)
Denmark	-	760 (mean)

RIZA (1999) reports measured effluent concentrations from a large number of (communal) sewage treatment plants in the Netherlands in the range of 25-160 µg/l (respectively 5 and 95 percentile).

Air

The available zinc concentrations in air are reported in Table 3.69.

Table 3.69 Measured zinc concentrations in air.

Location	Concentration (μg/m³)	Source
The Netherlands (1995)	0.037-0.054 (annual mean, 4 locations)	Monitoring data LML (1995)
The Netherlands (1992)	0.038-0.057 (annual mean, 4 locations)	Aben et al. (1994)
Bilthoven (NL), 1990/1992	0.08 / 0.043 (annual mean) 0.160 (98%)	CCRX, 1991/1994
Vlaardingen (NL), 1990/1992	0.08 / 0.057 (annual mean) 0.210 (98%)	CCRX, 1991/1994
Houtakker (NL), 1990/1992	0.07 / 0.054 (annual mean) 0.210 (98%)	CCRX, 1991/1994
Belgium (1989/1990)	0.03-42.0 / 0.03-1.56 (monthly averages)	IDE (B), 1991 (A3)
Flanders (B), 1992-1993	0.07-1.02 (mean) 7.75-14.62 (maximum)	Vlaamse Milieumij, 1993 (A4)
The Netherlands	0.065 (calculated annual mean)	Cleven et al, 1993
North Limburg (B)	1-2 (mean)	Cleven et al, 1993
The Netherlands 1996-1998	0.05 (annual mean 1996) 0.04 (annual mean 1997) 0.04 (annual mean 1998)	RIVM, 1999
Beerse and Engis (B) 1985/1986	3 (annual mean)	Cleven et al, 1993

Soil and groundwater

The available zinc concentrations in soil and groundwater are shown in Table 3.70. Zinc concentrations in soil are strongly related to the nature of the soil material. When available the soil type is mentioned in Table 3.70.

Table 3.70 Measured zinc concentrations in soil and groundwater.

Location	Concentration (mg/kg$_{dwt}$)	Source
World, natural background	10-300 (range)	WHO, 1996
Belgium	14-130 (range), 57 (average)	Angelone, Bini, 1992
Denmark	7-15 (range), 7 (average)	Angelone, Bini, 1992
Germany	83 (average)	Angelone, Bini, 1992
England and Wales	78.2 (average)	Angelone, Bini, 1992
France	5-38 (range), 16 (average)	Angelone, Bini, 1992
Italy	89 (average)	Angelone, Bini, 1992
The Netherlands	9-1020 (range), 72.5 (average)	Angelone, Bini, 1992
Norway	40-100 (range), 60 (average)	Angelone, Bini, 1992
Austria	6-8900 (range), 65 (average)	Angelone, Bini, 1992
Portugal	58.4 (average)	Angelone, Bini, 1992
Scotland	0.7-987 (range), 58 (average)	Angelone, Bini, 1992
Spain	10-109 (range), 59 (average)	Angelone, Bini, 1992
Sweden	182 (average)	Angelone, Bini, 1992
England (North of Somerset) *'normal' background values*	33-60	Davies, Ballinger, 1990
Poland (range and average) *-Podzol:* *-Luvisol:* *-Cambisol:* *-Fluvisol:*	 3-762, 36.87 (n=31) 12-120, 34.36 (n=34) 24-725, 58.02 (n=51) 46-110, 83.58 (n=8)	Kabata-Pendias e.a., 1992
Poland (n=293)	36 (average)	Czarnowska, Gworek, 1990
Nature reserves (NL) sandy loam	6.4-62	Edelman 1984
Nature reserves (NL) sandy loam	28-189	Edelman 1984
Nature reserves (NL) clay	81-153	Edelman 1984
Nature reserves (NL) peaty clay	62-150	Edelman 1984
Nature reserves (NL) peat	62-150	Edelman 1984
Agricultural soil (NL), clay soil	117	Driel, Smilde 1981
Agricultural soil (NL), sandy soil	44	Driel, Smilde 1981
Agricultural soil (NL), loess	86	Driel, Smilde 1981
Agricultural soil (NL), fen peat soil	101	Driel, Smilde 1981
Agricultural soil (NL), peat/sand mixture	25	Driel, Smilde 1981
1067 soil samples in The Netherlands	48.1 (mean) 8.1 / 130 (10% / 90%)	Cleven et al. 1993
clay (NL)	81 (mean) 1300 (maximum)	Cleven et al. 1993
peat (NL)	55 (mean) 320 (maximum)	Cleven et al. 1993
sand (NL)	25(mean) 400 (maximum)	Cleven et al. 1993

Location	Concentration (mg/kg$_{dwt}$)	Source
Sandy loam (NL)	52(mean) 89 (maximum)	Cleven et al. 1993
Plombière (B)	5890 (near mine)	Van Straalen, 1987
Plombière (B)	4900 (sampling site) 24,700 - 29,100 (dense turf)	Posthuma, 1992
Kempenland (NL)	Max. 642	Kreis, 1992
North-East Belgium (polluted area of 136 ha in 1989)	Range 1500-16,000 (topsoil (0-20 cm)	Vangronsveld et al., 1991
National Soil Monitoring Network, 1993 (NL)	13.5 (min) – 52.2 (max) cattle farm	Groot et al., 1996
National Soil Monitoring Network, 1994 (NL)	18.6 (min) – 53.1 (max): cattle farms 37.5 (min) – 317.7 (max) forest locations	Groot et al., 1997
National Soil Monitoring Network, 1995 (NL)	54.1 (10 perc) – 193.3 (90th perc): 0-10 cm; peat soil, cattle farm 29.8 (10 perc) – 116.8 (90th perc): 30-50 cm; peat soil, cattle farm 20.3 (10 perc) – 50.3 (90th perc): 0-10 cm; sandy soil, arable farm 8.1 (10 perc) – 20.3 (90th perc): 30-50 cm; sandy soil, arable farm	Groot et al., 1998
Sweden	54 (plough-layer) median 48 (subsoil) median 25 (plough layer) 10th P 99 (plough layer) 90th P	Eriksson et al. 1997
France (11,161 ploughed soils and 1084 cultivated soils)	0.4 (min) 68 (mean) 2707 (max) 102 (90 P)	http://www-sescpf.orleans.inra.fr/public/etm/
Groundwater (in µg/l)		
National Soil Monitoring Network, 1993 (NL) *Upper groundwater*	< 10 (min) – 1100 (max) cattle farm	Groot et al., 1996
National Soil Monitoring Network, 1994 (NL) *Upper groundwater*	13.1 (min) – 294.3 (max): cattle farms > 65 (min) – 3120 (max) forest locations	Groot et al., 1997
National Soil Monitoring Network, 1995 (NL) *Upper groundwater*	13.1 (min) -58.9 (max); peat soil, cattle farm 13.1 (min) -98.1 (max); sandy soil, arable farm	Groot et al., 1998

Note: Zinc levels in various soils from the Spanish peninsula (period 2001-2003) are presented in Annex 3.2.5a. These very recently received data are not further used in the current risk assessment (illustration purposes only).

Monitoring data related to particular sources (corrosion and/or traffic)

A number of monitoring data are available at locations which are predominantly influenced by zinc emissions from traffic and/or corrosion. The data comprise both emissions from point sources, line sources and diffuse sources. They are discussed below.

Line sources

Road borders 1: soil

Lijzen and Franken (1994) collected data from a number of investigations on the zinc soil concentrations below crash barriers of Dutch motorways. They concluded that zinc levels in soil under crash barriers were higher (up to 1500 mg/kg dwt) than in the vicinity of comparable roads without crash barriers. Groot and Van Swinderen (1993) carried out a research to determine the environmental quality of soil and groundwater along motorways. Samples of forest litter, soil and shallow groundwater were taken at five motorway roadside sites. The samples were taken at two distances from the motorway: nearby (app. 8 meters) and far away (app. 80 meters). In forest soil the zinc concentrations were found to be significantly higher near the motorway than at larger distance. The highest concentration in litter nearby the motorway was 218 mg/kg. For sandy soil and groundwater no difference was found between nearby and far away samples. In a more recent KIWA report (1998) a literature survey was carried out for zinc concentrations in road borders. They reported average zinc levels of 346, 171 and 130 mg/kg dwt in the top soil at a distance to the road of 0-1, 1-5 and >50 m, respectively. Levels exceeding the so-called 'intervention value' of 720 mg/kg dwt in the Netherlands have been observed as well. Lower (absolute) zinc values were found in a detailed research of KIWA near a road in Arnhem, the Netherlands: 79 mg/kg dwt (0-1 m), 46 mg/kg dwt (3 m) and 6 mg/kg dwt (50m). The conclusion is similar, however, to the literature survey data, i.e. a significant increase of zinc in top soil levels near roads with a clear relationship between zinc levels and the distance to the road.

The above-mentioned conclusion is confirmed by a number of recent reports on this topic. Royal Haskoning (Blok, 2002) made an extensive inventory of zinc levels in soil road borders. A total number of 40 studies is described from all over the world concerning roads without a drainage system for the runoff. An overall compilation of the data is given in Figure 3.9. A distinction is made between the road intensity (Average Daily Traffic Intensity (ADTI)), the distance from the road and the depth of the soil measurement. All data from the Blok (2002) report were grouped into those classes, where for the ATDI the recently EU agreed (CA decision 2003, document JM/56/2003) categories/definitions were used: highways: ADTI > 60,000; regional roads: > 14,000 and urban roads: > 1,000. It is well realised that such grouping of the total data set is a very rough way of presenting the information, but nevertheless it does show the general trend in a concised way. The overall picture shows a clear accumulation of zinc in a rather thin top soil layer and a exponentially decreasing concentration over the distance from the curb of the road. Moreover, Figure 3.9 also shows that zinc levels are found to decrease with decreasing road intensity.

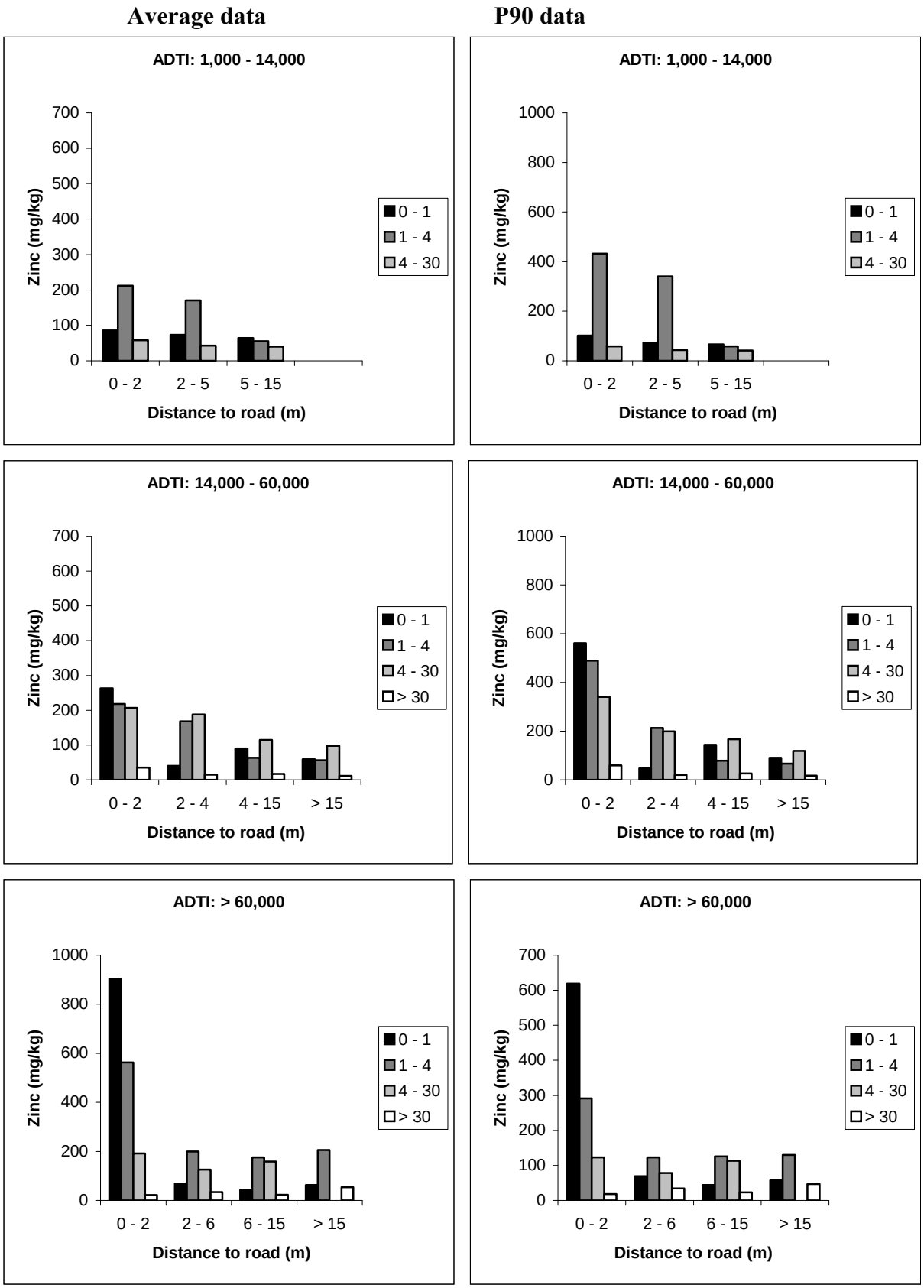


Figure 3.9 Zinc concentrations in soil road borders at various depths (boxes; depth ranges in centimeters) and distances from the road. Compilation of data from Blok (2002).

Additional recent data from German roads point to the same trends as observed in the overall picture from the Blok (2002) data (TU Berlin, 2002). 10 gives data for both German motorways (n=4) and regional roads (n=4). The motorways have ADTI-values ranging between 50,000 and 90,000. The ADTI for all four regional roads lie between 15,000 and 20,000. The zinc concentrations in the upper 10 cm soil are presented. Here again, zinc levels are correlated with both ADTI (difference between motorways and regional roads) and distance from the road.

A similar picture is observed from recent Dutch road border data (CIW, 2002; data not shown).

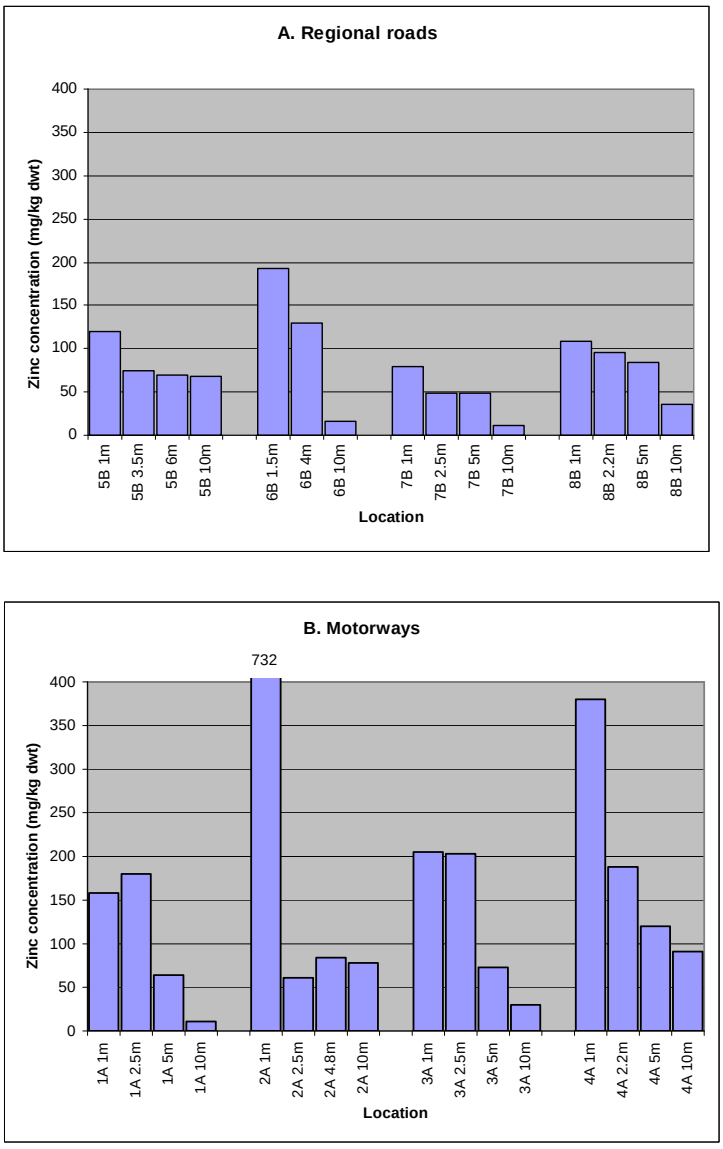


Figure 3.10 Zinc concentrations in top soil (0-10 cm) alongside German roads (TU Berlin, 2002). A. Regional roads and B. Motorways. Per road type four different roads were monitored at several distances from the road itself.

Road borders 2: runoff and sediment

Recent zinc concentrations in motorway run-off varying between 18 and 1500 µg/l were reported in the Netherlands (RIZA, 1996). Further zinc run-off data from Dutch roads are given in the recent CIW report (2002). Levels between 18 and 900 µg/l are found and the run-off levels from porous asphaltic concrete (PAC) roads are lower than those from dense asphaltic concrete (DAC) roads (Table 3.71Table). The latter observation is supported by several other sources. The use of PAC is mainly restricted to the Netherlands.

Table 3.71 Zinc run-off values (in µg/l) at various types of roads in the Netherlands (CIW 2002). (DAC = dense asphaltic concrete; PAC = porous asphaltic concrete).

	Median (µg/l)	**Range (µg/l)**
Motorway (DAC)	452	225-530
Motorway (PAC)	47	18-133
Regional road N-Z Holland (DAC)	152 (avg)	22-700
Regional road La Cabine (DAC)	181	111-313
Local road Lelystad (DAC)	248	52-900
Local road Breda (DAC)	135	-
Rain	15	-

The CIW (2002) states that several studies have shown that serious contamination occurs in sediments from ditches receiving untreated road runoff. However, further details are lacking on this study.

The effects of motorway runoff on freshwater ecosystems were investigated in a UK field study by Maltby et al. (1995). Zinc motorway runoff levels up to 489 µg/l were detected. Concentrations of several metals (including zinc) were significantly elevated in run-off contaminated sediment at some of the sampling sites (upstream concentration: 137 mg/kg dwt and down stream: 338 mg/kg dwt). The ecotoxicological aspects of this study will be discussed in the risk characterisation.

Additional UK data come from a recent Highway Agency/Environment Agency (HA/EA) study on highway runoff from six different UK motorways (Moy et al., 2002). The efficiency of various treatments to reduce contamination from runoff was investigated. Zinc was characterised in this study as one of the so-called 'key determinands' in runoff. Zinc runoff levels were found to range from 53 to 222 µg/l with an overall mean value of 140 µg/l. Zinc sediment concentrations were additionally measured at various sampling points at each location (a.o. upstream and downstream receiving water system; see Table 3.72). The authors concluded that sediment analysis showed little significant accumulation of contaminated sediments downstream of highway runoff discharges in watercourses. For zinc this conclusion is (weakly) substantiated by the data presented in Table 3.72. In most cases the zinc downstream concentrations seem to be higher than the upstream levels, but the pattern is not very clear. On the other hand the '- differences' are all lower than the '+ differences' in absolute terms. The highest observed accumulation levels are found to be around 60 mg/kg dwt (River Frome and Souldern Brook). There seems to be no relation with the presence or absence of any treatment of the runoff.

Table 3.72 Zinc concentrations in sediment (in mg/kg dwt) from UK waters receiving motorway runoff (Moy et al., 2002).

Location	**Zinc concentration (mg/kg dwt)**		
	upstream	downstream	difference
Brinkw. Brook (no treatment)	140 (1997) 134 (1997)	130 (1997) 155 (197)	- 10 + 21
River Frome (treatment)	113 (1998) 70 (1999)	175 (1998) 60 (1999)	+ 62 - 10
Souldern Brook (treatment)	67 (1999) 44 (2000)	113 (1999) 80 (2000)	+ 56 + 36
Newbury Bypass (treatment)	155 (2001) 200 (2002)	155 (2001) 181 (2002)	0 - 19
River Ray (treatment)	180 (1997) 134 (2000)	168 (1997) 155 (2000)	- 12 + 21
Gallos Brook (no treatment/ filter drain)	26 (2000) 22 (2003)	52 (2000) 64 (2003)	+ 26 + 42

From the same study an average zinc sediment concentration of about 720 mg/kg dwt can be estimated for sediment in the drainage systems. This value can be considered as an indicative value of potential zinc runoff sediment contamination before any form of treatment.

In addition to the chemical analyses, biological surveys were undertaken in the HA/EA study at five sites receiving either treated or untreated highway drainage (No survey was undertaken at the A34/Newbury site due to the culverting of the receiving watercourse). In each case, a spatial control/impact survey design has been employed with one or more control sites located upstream of the discharge and one or more impact sites downstream of the discharge. Wherever possible, sites have been located on a similar substrate within the constraints of accessibility and within the supposed zone of effect. Samples have been sorted and results presented in a standard way (BMWP, ASPT biotic scores) which allows cross-comparison between sites and sampling occasions.

The observed results suggest that:

- Macro-invertebrate communities located below the range of treatment options available at the five sites are not affected by treated runoff.
- Macro-invertebrate communities located below discharges of untreated runoff may be marginally affected but that changes are too small to draw firm conclusions. It has not been possible to eliminate the possibility that confounding effects such as changes in macro-invertebrate habitat quality and life cycle induced changes in community composition are responsible for the observed changes.

It should be borne in mind that the HA/EA study is from non urban highway locations in the South of England and may not be representative for the full range of sediment and climate conditions and highway characteristics that may be found throughout the UK and EU. In addition, sediment quality was a relatively minor part of the study and only a small number of sediment samples were collected.

Electricity pylons

Lijzen and Franken (1994) reported zinc concentrations around electricity pylons (galvanised steel). They referred to available studies from the Netherlands, UK and Canada. In the Netherlands zinc levels were found ranging from 200 to 650 mg/kg dwt in the topsoil close to these pylons. The UK and Canadian studies confirmed that very high zinc concentrations (up to 17,400 mg/kg dwt) can occur in the surface soil near electricity pylons. Differences can be mainly attributed to sampling site (e.g. distance to pylon and prevalent wind direction) and duration of emission (age of pylon).

Urban areas

In Sweden a research has been performed in which zinc soil levels in urban areas were compared with those in satellite municipalities and rural areas (in: Landner and Lindeström, 1998). The median zinc concentration is higher in Stockholm's inner city, about 2.5 times the level in the satellite municipalities (median values of 160/145 mg/kg (Stockholm) versus 60 mg/kg (satellite municipalities)). On the basis of the results of this and another study Landner and Lindeström (1998) concluded: " some general elevation of zinc concentrations in soils in the country's medium-sized conurbations can be expected (in the order of 10-70%), and up to a two-fold elevation in the inner city of the biggest cities." The authors further mention that future zinc input in 'technoland' may be lower due to lower corrosion run-off rates (decreasing SO_2 levels).

3.2.5.3.5 Comparison of measured and calculated regional zinc concentrations

The risk characterisation should be based on the most realistic exposure information. Hence, it must be decided whether calculated regional concentrations or monitoring data are more useful for the exposure assessment. In this section a comparison is made between the measured concentrations of zinc in the various environmental compartments (section 3.2.5.3.4) and the corresponding calculated PEC_{add} values (section 3.2.5.3.2). It must be noted that measured concentrations can only directly be compared with calculated concentrations when the natural background concentration is added to the calculated values.

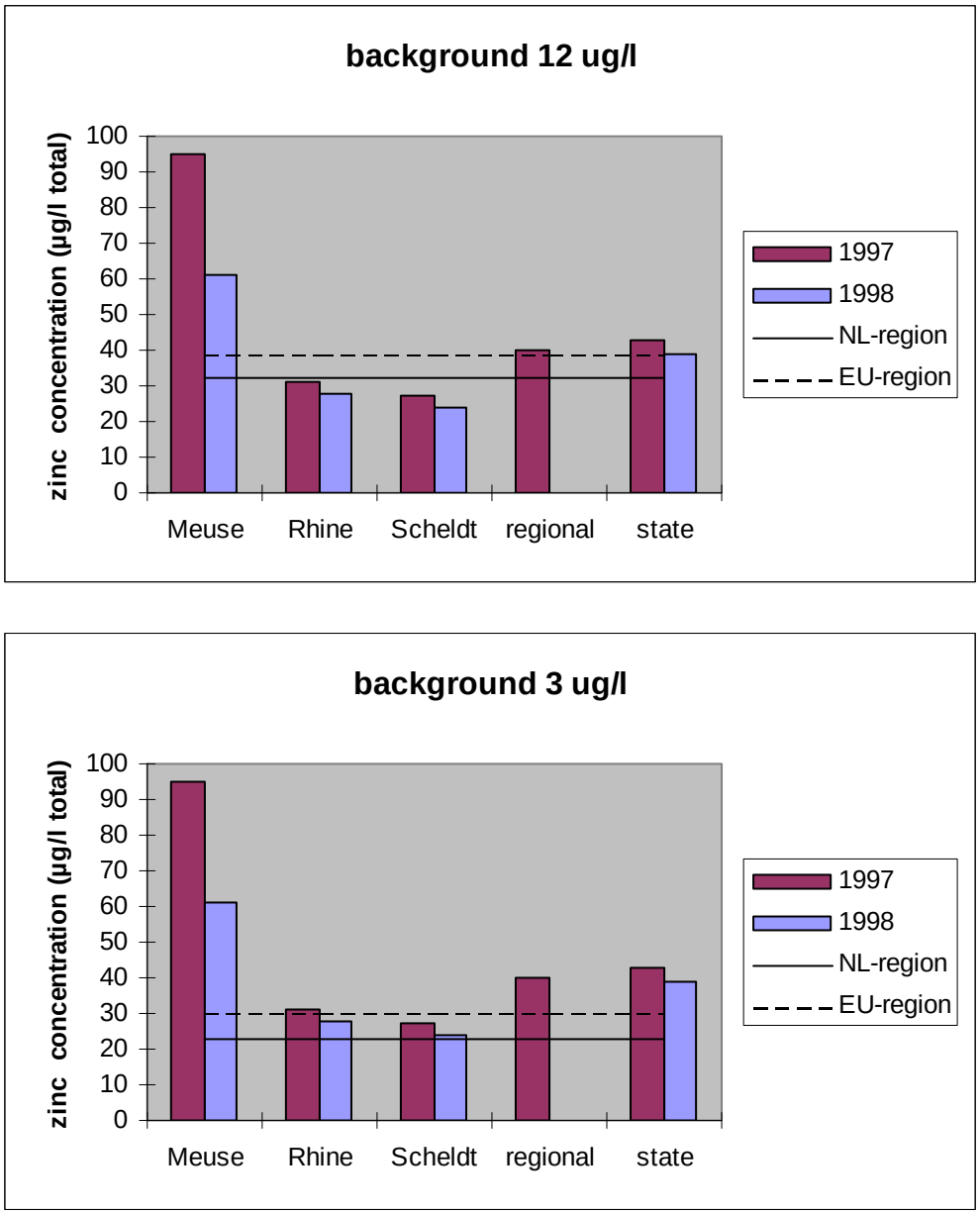


Figure 3.11 Comparison of 1997-1998 zinc monitoring data for Dutch surface waters with calculated regiona PECs (NL-region and theoretical EU-region). (Background levels of both 3 (bottom) and 12 µg/l (top) are added to calculated PEC.)

Water

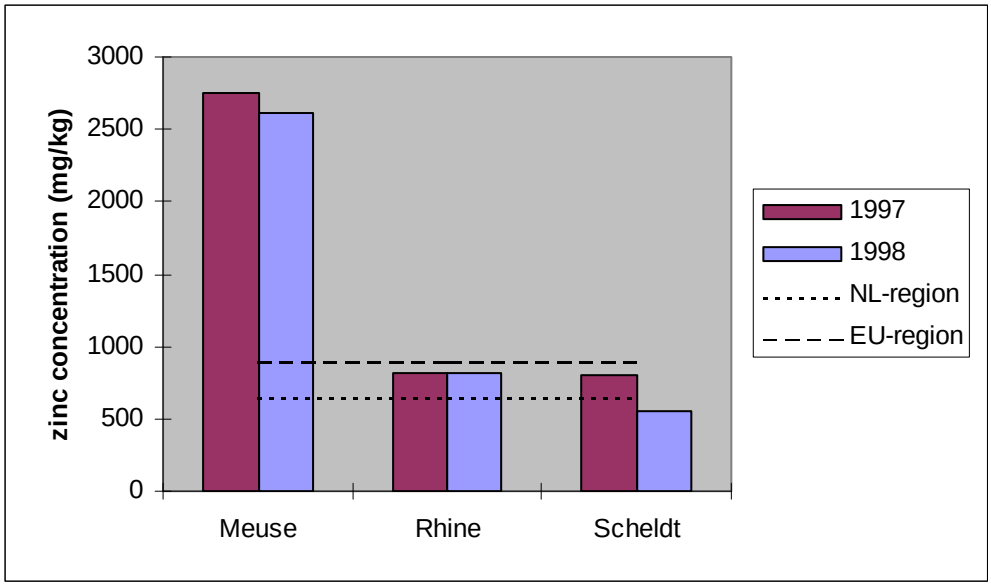
The calculated regional (NL region) concentrations (PEC_{add}) of zinc in surface water are 12.2 µg/l ($C_{susp.}$ = 15 mg/l) and 20 µg/l ($C_{susp.}$ = 30 mg/l). For the theoretical EU region the values are, respectively, 16.8 and 27 µg/l. A meaningful comparison of measured and calculated data is possible, because a large set of reliable monitoring data of zinc concentrations in surface water is available. Figure 3.11 gives both the 1997-1998 monitoring data for Dutch surface waters (extraction from Figure 3.4) and the calculated regional PECs. The value of 20 µg/l and 27 µg/l are taken for this comparison as the suspended matter concentration of 30 mg/l mostly reflects the Dutch situation. Natural background values of 3 and 12 µg/l (see section 3.2.2.2) are added to the calculated concentrations. From this comparison it can be concluded

that the difference between measured and calculated data is very narrow. Only the Meuse data[11] are substantially higher than the calculated PECs (factor 2-3).

The 'indicative' EU concentrations (59.2 µg/l) according to Denzer et al. (1999) are higher than the calculated PECs, irrespective of the chosen background (3 or 12 µg/l). A considerable number of the reported surface water concentrations (90 P values) for various regions in France and Germany in Table 3.62 also exceed the calculated PECs, again irrespective of the chosen background (either 3 or 12 µg/l). The same is true for the large and representative monitoring data set for Flanders Belgium. The 90 P values of 146 and 110 µg/l for, respectively, 1999 and 2000 clearly exceed the calculated PECs. With the exception of some extreme values the exceeding of the calculated PECs is about a factor of 2-5. Furthermore a value of 12 µg/l zinc (total) is given as the 90 P value for Swedish water courses in Table 3.62. This value is below the calculated PEC.

Sediment

The calculated regional (NL-region) concentrations (PEC_{add}) of zinc in sediment is 510 mg/kg_{dwt} (196 mg/kg_{wwt}), excluding a natural background level (provisional) of 140 mg/kg_{dwt}. For the theoretical EU-region the sediment concentration amounts to 700 mg/kg_{dwt} (269 mg/kg_{wwt}). Also for sediment a meaningful comparison of measured and calculated data is possible. Monitoring data of sediments in the Netherlands are found in the same order of magnitude or higher (e.g. maximum values of 2089 mg/kg and 4003 mg/kg in Hollandsch Diep for freshly deposited layers). The 'indicative' 90th percentile sediment concentration from the collected EU data amounts to 1367 mg/kg dwt (Dentzer et al., 1999) which is also higher than the calculated values, including an added natural background estimate of 140 mg/kg dwt. The same is true for a considerable number of sediment monitoring data (90 P-values) from France and Germany as given in Table 3.64. The exceeding of the calculated



PECs is not greater than a factor of 2-3, with the exception of some extreme values.

Figure 3.12 Comparison of 1997-1998 zinc monitoring data for suspended matter in Dutch surface waters with calculated regional PECs (NL-region and theoretical EU-region). (Background level of 140 mg/kg dwt is added to calculated PECs.)

[11] Strictly speaking for the Meuse in Figure 3.11 a minimum natural background value of 6 µg/l should have been added to the calculated PECs rather than a value of 3 µg/l. This because the natural background range for this river was set at 6-12 µg/l (see section 3.2.2.2). However, the difference is marginal.

A comparison of the 1997-1998 suspended matter zinc concentrations in major Dutch rivers (extraction from Figure 3.7) with the calculated PEC sediment is shown in Figure 3.12. Similar to water, the difference between measured and calculated data is found to be narrow, except for the Meuse river (a factor 3.5 higher).

Air

The calculated regional concentrations (PEC_{add}) of zinc in air are 0.006 (NL-region) and 0.01 (EU-region) $\mu g/m^3$ based on the calculations as described in section 3.2.5.3.1. Recent monitoring data of the Netherlands (0.04 $\mu g/m^3$, annual average values for the Netherlands in 1997 and 1998) are found to be within the same order of magnitude, but nevertheless substantially higher than the calculated PEC_{add}s (around a factor of 5 higher). Available Belgian monitoring data are up to 2 or 3 orders of magnitude higher than the calculated PEC_{add}s, but the Belgian data are less recent and include monthly avarages, see Table 3.69.

Soil

The calculated regional concentrations (PEC_{add} NL region) of zinc in agricultural and natural soils are respectively 56.5 mg/kg_{wwt} (64 mg/kg_{dwt}) and 0.5 mg/kg_{wwt} (0.6 mg/kg_{dwt}). A comparison of these values with monitoring data is performed in the regional risk characterisation on agricultural soil.

In the risk characterisation both calculated and measured data will be used for the regional scale, but the emphasis will be on the large number of measured data from various EU regions. Only monitoring data from after 1995 will be used as they reflect the most representative situation. The reference year is 1998/1999 for the current zinc exposure assessment. Some very recent monitoring data (after 2000) will, however, be discussed in the regional risk characterisation (section 3.4.3) to indicate the most recent trends in zinc levels in the environment.

3.3 EFFECTS ASSESSMENT

3.3.1 General introduction

As mentioned in section 3.1, the "added risk approach" has been used in this risk assessment report (RAR) on zinc, both in the exposure assessment and effects assessment. With respect to the effects assessment the added risk approach implies that the PNEC is derived from toxicity data that are based on the added zinc concentration in the tests. This results in an "*added* Predicted No Effect Concentration" ($PNEC_{add}$).

The calculation of the $PNEC_{add}$ values is in agreement with the calculation of PNEC values (for substances with no background concentration) as described in the TGD, i.e. the $PNEC_{add}$ values are derived from toxicity data (either NOEC values or LC50 and EC50 values from laboratory tests), using assessment factors or statistical extrapolation.

Bioavailability

For zinc as well as for other metals, it would be important to define the actual or bioavailable concentration, which is important for toxicity, both in the laboratory tests and in the real environment. Due to several physico-chemical processes, zinc will exist in different chemical forms, some of which are more bioavailable than others will. It is thus realised that the bioavailability of metals in both laboratory tests and in the environment may be affected by several physico-chemical parameters, such as pH, alkalinity and hardness. Until recently,

adequate information was lacking how to quantitatively determine or estimate the bioavailable fraction in either the laboratory tests or the environment. The results of a recent extensive research program, however, lead to adequate information and to quantitative ways of taking into account bioavailability of zinc in water, sediment, and soil. In the sections 3.3.2.1.1, 3.3.2.2.1 and 3.3.3.1.1 on abiotic factors this is further explained.

The added risk approach, as applied in this report, takes into account only the amount of zinc that is added due to anthropogenic activities. The approach assumes that both in the laboratory tests and in the real environment this added amount is bioavailable, i.e. may contribute to toxic effects (see also section 3.1).

Essentiality

Zinc is an essential element, which implies that organisms will have a minimum requirement for zinc that supplies the needs, and a maximum concentration above which zinc is toxic. The minimum requirement is necessary because zinc plays an essential role in organisms (e.g. Clarkson, 1986; Begon et al., 1990; Cleven et al., 1993; Rainbow, 1993). The range between the minimum and maximum is often called the window of essentiality (Hopkin, 1993). Organisms have evolved mechanisms to supply their needs independent of the external concentration by regulating an essential element to a constant internal level (Rainbow and Dallinger, 1993). However, when an organism is exposed to such a low level of an essential element at which it no longer can regulate its internal concentration to cope with its needs, effects due to deficiency may occur.

The use of the added risk approach implies that there is no risk for deficiency at the "Predicted No Effect Concentration", as the $PNEC_{add}$ derived in this approach is defined as the maximum permissible addition to the background concentration. The background concentration in a given ecosystem is partly bioavailable and provides the organisms in that ecosystem with sufficient essential metals, thus contributing to the existing biodiversity.

Metallo-regions

Application of the added risk approach as described in section 3.1 will result in $PNEC_{added}$ values for the various environmental compartments that are independent of the magnitude of the natural background concentration (in absolute terms). Thus a terrestrial $PNEC_{add}$ can be used both for a soil with a natural background level of e.g. 10 mg/kg d.w., and for a soil with a background of e.g. 100 mg/kg d.w. On theoretical grounds, however, it can be argued that organisms that live in an environment with relatively low background concentrations might be more sensitive to the addition of a certain amount of zinc than organisms that live in an environment with much higher natural background concentrations. This idea is related to the "metallo-region" concept as has been discussed in a workshop on environmental risk assessment methodologies for metals and metal compounds (ICME, 2000). A metallo-region can be described as any portion of the Earth's surface having common geological and biological characteristics and must be defined in terms of the physico-chemical characteristics and boundaries of terrestrial and aquatic environments as well as in terms of their representative species. The concept, although attractive from a theoretical point of view, currently lacks validation and further research is needed to characterise regions and to attribute the relevant ecotoxicity data to them (ICME, 2000). The currently available data for zinc do not show a clear dependence of the sensitivity of aquatic organisms to the natural background concentrations of the media they were tested in (see section 3.3.2). Therefore in the current risk assessment report for zinc no specific effort was made to define metallo-regions to which the $PNEC_{added}$ should apply. On the other hand, however, specific selection criteria have been developed for both the aquatic and the terrestrial effects data that pay attention to the relevancy aspect of the data. These criteria refer to the most relevant abiotic

factors that may influence the toxicity such as the natural background concentration, pH, hardness, organic matter content and clay content.

3.3.1.1 Sources and selection of ecotoxicological data

The ecotoxicological data that have been used to derive generic PNECadd values for surface water, STP-effluent, sediment and soil are listed in Table 3.3.2.a–Part I (freshwater organisms), Table 3.3.2.b-Part I (saltwater organisms) and Table 3.3.2.c (aquatic microorganisms) in Annex 3.3.2.A, Table 3.3.2.e–Part I (freshwater benthic macroinvertebrates) in Annex 3.3.2.D, and in Table 3.3.3.a– Part I (microbe-mediated processes in soil), Table 3.3.3.b–Part I (terrestrial invertebrates) and Table 3.3.3.d–Part I (terrestrial plants) in Annex 3.3.3.A. The data in these tables partly originate from Janus (1993) which is the ecotoxicological Appendix to the Integrated Criteria Document Zinc (ICDZ: Cleven et al., 1993); more recent data were retrieved from extensive literature searches conducted by the industry and rapporteur in 1999 [12]. In addition, the most recent data are from new studies that were conducted in the framework of this risk assessment report, especially the studies from the "Conclusion i" program that was performed in 2000 and 2001 and the soft water testing program that was performed in 2002. The aquatic toxicity data from the soft water testing program, used to derive the 'water effect ratio' (WER) for the derivation of the soft water PNECadd, aquatic from the generic freshwater PNECadd, aquatic, are listed in Annex 3.3.2.C. The data included in the aquatic and terrestrial effect assessments focus on chronic toxicity studies (long-term studies) that used soluble, inorganic zinc salts as test compound and from which No Observed Effect Concentrations (NOEC values) for relevant toxicological endpoints (in particular survival, growth and reproduction) could be derived, as chronic NOEC values are used rather than acute LC50 or EC50 values to derive PNEC values.

The data listed in Table 3.3.2.c (aquatic microorganisms) in Annex 3.3.2.A originate from the $ZnCl_2$-IUCLID data sheet (Goldschmidt-version of 24 March 1996) and the $ZnSO_4$-IUCLID data sheet (Grillo-version of 7 March 1997), which are provided by the industry, and from a limited literature search. One study selected from these data was used to derive the $PNEC_{add}$ for STP effluent.

The data listed in Table 3.3.2.d (aquatic organisms - tests with metallic zinc powder as test compound) in Annex 3.3.2.A were provided by the industry. The study by Van Woensel (1994a) was originally submitted in the framework of the Industry Addendum to the ICDZ (IA-ICDZ: Van Tilborg and Van Assche, 1995). The data listed in Table 3.3.2.d were not included in the IUCLID data sheet on zinc (metal) *(ECB-version of 1 March 1995).*

All aquatic and terrestrial toxicity data in this report are expressed as zinc, not as the test compound, because zinc itself is considered to be the causative factor for toxicity. For aquatic organisms, which are mainly exposed via water, especially the zinc ion and other dissolved zinc species are relevant for toxicity[13]. Thus, with respect to the aquatic toxicity data, the dissolved-Zn concentration in water is a better indicator of toxicity than the total-Zn concentration, although the dissolved fraction also may contain forms of zinc that are not or

[12] With respect to the aquatic toxicity data the literature searches for the update were focused on freshwater organisms, because the effects and risk assessment in this risk assessment report is for freshwater.

[13] This also applies for terrestrial organisms, which are mainly exposed via (pore) water, for example, earthworms. With respect to aquatic organisms it is noted that exposure via the water is not necessarily the main route of exposure: the intake of (food) particles can considerably contribute to the accumulation of zinc in a number of organisms, e.g. in filter-feeding organisms such as water fleas and mussels.

hardly bioavailable. In practice, the dissolved fraction of a substance in water is defined as the fraction that passes a 0.45 µm filter. This fraction includes a series of zinc speciations, such as (hydrated) zinc ion, labile inorganic and organic zinc complexes (such as Zn-hydroxides and Zn-citrate) and stable inorganic and organic complexes (such as ZnS and Zn-humic acid complexes). The final zinc speciation depends on the water characteristics (e.g. Cleven et al., 1993; Pham and Garnier, 1998).

In this RAR the results of the aquatic toxicity studies are expressed as either the actual (i.e. measured) concentration of zinc or, usually, as the nominal (i.e. added) concentration (Cn) of zinc. The actual concentration includes the background concentration (Cb) of zinc. Because of the use of the "added risk approach" in this RAR, the results based on actual concentrations have been corrected for the background concentration of zinc, if the latter was reported. For example, in the second accepted test with rainbow trout *(Oncorhynchus mykiss)* the NOEC based on the actual Zn concentration was 36 µg/l and the background Zn concentration (Cb) was 11 µg/l, resulting in a NOEC of 25 µg/l (being actual-Cb) listed in Table 3.3.2.a–Part I in Annex 3.3.2.A. It is noted that in a large number of accepted tests the background concentrations of Zn were negligible (or assumed to be negligible) compared to the NOEC values derived from those tests. For example, in all accepted tests performed by De Schamphelaere et al. (2003) in artificial test media with alga *Pseudokirchneriella subcapitata,* crustacean *Daphnia magna* or fish *Oncorhynchus mykiss,* the background Zn concentrations in the artificial test media were 1-3 µg/l. The NOEC values derived from these tests were not corrected for the background concentration of Zn, as the majority of the NOEC values were much higher than the background concentration of Zn.

This correction for the background concentration of zinc is based on the assumption that only the added concentration of zinc is relevant for toxicity. In case both actual and nominal concentrations were reported, the results are expressed in this report as nominal concentrations, provided the actual concentrations were within 20% of the nominal concentrations.

It is emphasised that almost all reported aquatic toxicity data (either actual or nominal) represent total-zinc concentrations, i.e. the dissolved plus particulate fraction. However, the results are regarded as being dissolved-zinc concentrations, because under the conditions that were used in the laboratory tests, it is assumed that the greater part of zinc present in the test waters was in the dissolved fraction. This is especially true for the long-term studies, e.g. by using flow-through systems, in which particulate matter (suspended inorganic material and/or organic matter) was removed from the artificial test waters or natural waters. A recent study corroborated this assumption. In static and flow-through acute toxicity studies with several saltwater species, dissolved zinc was greater than 93% of the total zinc. Dissolved zinc was defined by the concentration of zinc determined by filtering it through a 0.45 µm filter (Lussier et al., 1999). Therefore, the $PNEC_{add}$ values derived from the aquatic toxicity studies are for dissolved zinc.

The results of almost all terrestrial toxicity studies are expressed as the nominal concentration (Cn) in soil; actual concentrations were only reported in a few studies. In a number of studies the background concentration (Cb) in the test soil was reported in addition to the nominal test concentrations.

The aquatic toxicity data and the terrestrial toxicity data which might be useful for $PNEC_{add}$ derivation were evaluated (new data) or re-evaluated (data from Janus, 1993) on the basis of reliability (quality) and relevance criteria, with the exception of the data for saltwater organisms (as only a $PNEC_{add}$ for freshwater was derived). With respect to reliability and

other general rules that have been applied for the selection of the aquatic and terrestrial toxicity data used to derive $PNEC_{add}$ values, the following is noted.

- Preferably the design of the study has to be in agreement with internationally accepted guidelines such as the OECD guidelines for the testing of chemicals. However, because the OECD guidelines on ecotoxicological tests only cover a very limited number of different organisms (especially aquatic organisms), also more general guidelines for the evaluation of ecotoxicological studies were used as guidance, especially those used in the Dutch National Institute of Public Health and the Environment (RIVM; several guidelines, including standard operating procedures). The studies used for PNEC derivation include both standardised tests (tests that have been conducted according to a specific guideline, e.g. OECD 201: Alga, Growth Inhibition Test) and non-standardised tests. It has been proposed by industry to rank the toxicity tests based on reliability index RI-I to RI-IV (RI-I: highly reliable, RI-II: reliable: RI-III: not reliable; RI-IV: unknown reliability). In this industry proposal, both RI-I and RI-II studies may be used for PNEC derivation and risk assessment with important legislative consequences, such as the EU regulation, but only standardised tests (c.f. OECD and accordingly) can be ranked as RI-I; the highest possible ranking for non-standardised tests is RI-II. Although the reliability of standardised tests can be more easily determined than that of non-standardised test, a distinction between RI-I (highly reliable) for standardised tests and RI-II (reliable) for reliable non-standardised tests is not considered meaningful, because both kinds of reliable tests will be used in this RAR for PNEC derivation and risk assessment, in accordance with the TGD (EC, 2003). It is emphasised that all tests were checked for quality and had to meet certain minimum quality criteria, for example with respect to the concentration-effect relationship. Therefore, a ranking based on reliability index was not made, but standardised tests or tests conducted in conformity with a specific (OECD) guideline have been marked as such, if possible.

- For the further selection of data, especially the chronic NOEC values used to derive $PNEC_{add}$ values, the following approach has been taken:

  - Toxicological endpoints, which may affect the species at the population level, are taken into account. In general, these endpoints are survival, growth and reproduction. The toxicity results are commonly expressed as an acute LC50 or EC50 (usually derived from toxicity tests with a duration of four days or less) or as a chronic NOEC (usually derived from toxicity tests with a duration of more than four days). With respect to the NOEC values it is noted that the fact whether or not a NOEC is considered a chronic NOEC is not determined exclusively by the above exposure time limit of four days, but also by the generation time of the test species. For unicellular algae and other microorganisms (bacteria; protozoa), an exposure time of four days or considerably less already covers one or more generations, especially in water, thus for these kinds of species, chronic NOEC values may be derived from experiments during less than four days. On the other hand, for organisms that have a long generation time, for example fish, an exposure time of just over four days is much too short to derive a chronic NOEC. It will be clear that for PNEC derivation a full life-cycle test, in which all relevant toxicological endpoints are studied, is normally preferred to a test covering not a full life cycle and/or not all relevant endpoints. However, the results of a test, which is more limited than a full life-cycle test may be used, see further the points below.
  - If for one species several chronic NOEC values (from different tests) based on the same toxicological endpoint are available, these values are averaged by calculating the

geometric mean, resulting in the "species mean" NOEC. With respect to this it is noted that the NOEC values should be from equivalent tests, for example from tests with similar exposure times. However, NOEC values derived from tests with a relatively short exposure time may be used together with NOEC values derived from tests with a longer exposure time if the data indicate that a sensitive life stage was tested in the former tests.

- If for one species several chronic NOEC values based on different toxicological endpoints are available; the lowest value is selected. The lowest value is determined on the basis of the geometric mean if more than one value for the same endpoint is available (see above).
- In some cases, NOEC values for different life stages of a specific organism are available. If from these data it becomes evident that a distinct life stage is more sensitive, the result for the most sensitive life stage is selected. The life stage of the organisms is indicated in the tables as the life stage at start of the test (e.g. fish: yearlings) or as the life stage(s) during the test (e.g. eggs → larvae, which is a test including the egg and larval stage).
- Only the results of tests in which the organisms were exposed to zinc alone are used, thus excluding tests with metal mixtures.
- Only the results in unpolluted test media (water, sediment or soil) are used, thus excluding tests that were performed in media containing high to very high background Zn concentrations, i.e. in case the control media contained zinc concentrations that are clearly above Zn concentrations normally encountered in relatively unpolluted environmental compartments.
- Only the results of tests with soluble zinc salts are used, thus excluding tests with "insoluble" zinc (such as ZnO, $ZnCO_3$ or zinc metal powder). It is noted, however, that some tests with "insoluble" zinc were accepted. i.e. tests in which the results were reported as actual dissolved-Zn concentrations.
- Unbounded NOEC values (i.e. no effect was found at the highest concentration tested) are not used. Unbounded NOEC values, which are set at the level of the highest test concentration, are indicated by "≥").

Methods used for the derivation of NOEC values are discussed in sections 3.3.1.2 and 3.2.1.3. For further data on study and NOEC selection, with respect to aquatic toxicity studies or terrestrial toxicity studies specifically, see section 3.3.2 and 3.3.3, respectively. The data in these sections include both reliability criteria (quality criteria in addition to those already mentioned in this section) and relevance criteria.

Based on the quality and relevance criteria used in this report, a number of studies are considered not useful for NOEC derivation or, in case a NOEC could be derived, not useful for PNEC derivation, for example studies resulting in unbounded NOEC values. The rejected studies are included in the tables (in Part-II: studies not useful for PNEC derivation). All rejected studies that were already included in Janus (1993), the ecotoxicological Appendix to the ICDZ, in which partly other selection criteria were used (for example, unbounded NOEC values for plants were included in the data set of Janus (1993)), are included in Part II of the tables. Most studies from the 1999 update that did not meet the current selection criteria, are not included in the tables and not included in the list of references either.

3.3.1.2 Derivation of NOEC values (methods)

The methods that have been used for the derivation of NOEC values (No Observed Effect Concentrations), being "real" NOEC values or NOEC values derived from effect concentrations, are essentially the same as outlined in the TGD (Chapter 3 – Table 13).

If possible, "real" NOEC values were derived from the data reported, i.e. the NOEC is one of the concentrations actually used in the test. In order of preference:

- Statistical analysis: the NOEC is the highest concentration (in a series of test concentrations) showing no statistical significant effect (inhibition) compared to the control. Significance level: p = 0.05 (optional: the p = 0.01 level if reported instead of the p = 0.05 level).
- If no statistical analysis has been applied: the NOEC is the highest concentration that results in ≤ 10% inhibition compared to the control.

In both cases there must be a consistent concentration-effect relationship, i.e the LOEC is the concentration at which and above which statistical significant toxicity is found (1) or, when no statistical analysis has been applied (2), >10% inhibition is found.

If the "real" NOEC could not be derived from the data reported, the following procedure was used to derive the NOEC. In order of preference:

- The NOEC is set at the EC10 level.
  a) Especially in more recent references on terrestrial toxicity data there is increasing preference for the benchmark dose approach. Hence, a benchmark dose (usually the EC10) was reported in a number of references instead of the NOEC. The EC10, which is calculated from the concentration-effect relationship, is used as NOEC equivalent, unless the "real" NOEC was also reported or could be derived from the data reported. The reported EC10 values mostly refer to terrestrial studies.
  b) Furthermore, a number of EC10 values was calculated by the rapporteur; most of these calculations refer to microbial toxicity studies (see section 3.3.3 for further explanation on the method and prerequisites used by the rapporteur for the calculation of EC10 values).

2) The NOEC is derived from the LOEC
   If the EC10 was not reported and could not be calculated, the NOEC was derived from the LOEC using the following "extrapolation" factors:
   a) NOEC = LOEC/2, in case inhibition is >10% but ≤20%, e.g. LOEC = EC(15%).
   b) NOEC = LOEC/3, in case inhibition is >20% but ≤30% e.g. LOEC = EC(25%).
   If the percentage inhibition at the LOEC is >30% or in case the percentage inhibition at the LOEC is unknown, no NOEC is derived.

With respect to "rule 2b" it is noted that the TGD does not mention the derivation of a NOEC from a LOEC in case inhibition at the LOEC is >20%, while in this RAR the derivation of a NOEC from a LOEC up to 30% effect has been used in some aquatic toxicity studies and especially in terrestrial microbial toxicity studies. The use of the higher effect level is justified by the use of a higher extrapolation factor. Regarding the microbial data the use of NOEC = LOEC/3 allows the calculation of a number of "alternative" NOEC values from tests that resulted in a "real" NOEC that is considered to be unreliable. For further explanation see section 3.3.3.1 (under "Reliability").

3.3.1.3 Derivation of PNEC values using statistical extrapolation (methods)

In this report PNEC values (indicated in the other sections as $PNEC_{add}$ values, because of the use of the added risk approach) were derived from the ecotoxicity data, using the two ecotoxicological extrapolation methods that both are described in the TGD:

- The PNEC is calculated from the lowest acute LC50 or EC50 or, preferably, from the lowest chronic NOEC, using assessment factors that depend on the available toxicity data (TGD - Chapter 3).
- In case the chronic database is sufficiently large, the PNEC is calculated by means of statistical extrapolation, using all available chronic NOEC values as input (TGD - Chapter 3, Appendix V).

In the TGD preference is given to the first-mentioned extrapolation method and it is recommended to use statistical extrapolation as a "supplementary approach". However, there is increasing preference to include statistical extrapolation for the derivation of PNEC values in case of data-rich substances (such as zinc).

In general there are two main reasons for the preference of the use of statistical extrapolation for deriving PNEC values, provided the database is sufficiently large (i.e. includes a large number of chronic NOEC values for a variety of organisms from different taxonomic groups).

- Firstly, the large database allows the calculation of a reliable estimate of the distribution of species sensitivity by means of statistical extrapolation, resulting in more reliable PNEC values (see also Chapman et al., 1998).
- Secondly, statistical extrapolation uses all available NOEC values as input. The inherent consequence is that the PNEC is less dependent on a single toxicity value (the lowest chronic NOEC or acute LC50 or EC50) compared to the traditional assessment factors method. This is particularly relevant for metals and metal compounds (and even more for the essential element zinc) where a high correlation between the toxicity and some abiotic factors is sometimes observed. As a consequence there is a higher probability for outliers caused by extreme or poorly controlled abiotic conditions. Such outliers may have a stronger influence on the PNEC derived from standard assessment factors than on the PNEC based on statistical analysis of the whole data set (although it is noted that in the former case the PNEC only depends on outliers at the lower range of toxicity values, while in the latter case the PNEC depends on both the lower and higher range).

The most important assumption of statistical extrapolation is that the sensitivities of species in ecosystems can be described by a probability distribution. Several distribution functions, usually estimated from chronic NOEC values, have been proposed, viz. log-triangular, log-logistic and log-normal. From the estimated distribution a certain percentile value, usually the 5^{th} percentile value, is used as criterion, i.e. as concentration that is assumed to be "safe" for ecosystems. In this risk assessment report, PNEC values were calculated as the 5^{th} percentile value of both the log-normal distribution of NOEC values according to Aldenberg & Jaworska (2000) (using the RIVM-program *ETX 2000_1.407*; Van Vlaardingen & Traas, 2002) and using the log-logistic distribution according to Aldenberg & Slob (1993) (using the RIVM-program *ETX 1.3a*; Aldenberg, 1993). The PNEC is set at the level of the 5^{th} percentile value (median value, i.e. the 50% confidence level). Furthermore, *ETX 2000_1.407* calculates both the lower and higher 95% confidence limit of the 5^{th} percentile value and *ETX 1.3a* calculates the lower 95% confidence limit of the 5^{th} percentile value: these values will also be

presented, in addition to the median value. The probability distribution of the datasets used for the calculations of the 5th percentile values have been checked with the Anderson-Darling goodness-of-fit test for normality (D'Agostino and Stephens, 1986) and with the Kolmogorov- Smirnov test (which is incorporated in *ETX 1.3a*). The latter method tests the goodness-of-fit for both a log-normal and log-logistic distribution. 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. Since in some cases both a log-normal and log-logistic distribution is statistically rejected, non-parametric estimates of the 5th percentiles values were made in addition. In this method, the 5th percentile value is estimated by inverse linear interpolation from the two data points (log transformed NOEC values) that enclose the 5th percentile value to find non-parametric estimates. Points are plotted at (rank-0.5)/n, named Hazen plotting positions (Cunnane, 1978).

The use of a log-normal frequency distribution of NOEC values for PNEC calculation (in case the database is sufficiently large and meets the taxonomic requirements to apply statistical extrapolation) was recommended by a workshop held in London early in 2001 on the use of statistical extrapolation (see also further below in this section). The *ETX temporary 1.2* program uses extrapolation constants (*k)* for a log-normal distribution that were reported by Aldenberg & Jaworski (2000). The use of previously reported statistical extrapolation methods, either using a log-logistic distribution (e.g. according to Aldenberg & Slob, 1993) using a log-normal distribution with earlier reported *k* values (e.g. according to Wagner & Løkke, 1991), however, results in very similar outcomes since the *k* values of the different methods are very similar, as will also be shown by the different calculations in this report.

It is realised that the assumption that the distribution functions normally used in statistical extrapolation (viz. log-logistic, log-normal or triangular) applies to the species sensitivities for zinc is in principle invalid due to the fact that zinc is an essential element. Hence, when the environmental concentrations become so low that the organisms will not be able to obtain their necessary zinc supply, effects due to deficiency will start to occur and the species sensitivity curve will go up again. Despite this problem we still think that these distribution functions can be used in practice provided that in the selection of the underlying toxicity data care is taken that the organisms do not suffer from effects due to deficiency. Thus, care need to be taken that the organisms were cultured at conditions relevant to the ecosystem. Furthermore it must be noted that in the added risk approach it is assumed that effects due to deficiency do not occur since the natural background concentrations should be able to provide the necessary input of the essential element.

It is further noted that the aforementioned three distribution functions generally lead to PNEC values that are similar or at least not very different from each other. Especially the log-logistic and log-normal distribution functions result in similar results (see the results in this report and further OECD, 1992a and Van Straalen et al., 1999).

There are a number of other concerns related to the use of statistical extrapolation methods in effect assessment (deriving of PNEC values). The statistical concerns include assumptions about the shape of the distribution function and whether or not the group of species tested is a random sample of this distribution. The ecological concerns include assumptions on the appropriateness to extrapolate single-species data to the functioning of complex communities and ecosystems, and on the appropriateness to extrapolate from laboratory to field conditions (e.g. Forbes and Forbes, 1993 and Smith and Cairns, 1993). It is noted that a number of these concerns also apply to other ecotoxicological extrapolation methods that use single-species laboratory toxicity data, including the assessment factor method and, furthermore, that there is also a large uncertainty with respect to the size of assessment factors.

In an attempt to tackle some of the above concerns, Emans et al. (1992, 1993) reviewed a large number of aquatic toxicity studies with organic compounds and metals, both single-species laboratory studies and multiple-species (semi-)field studies, and derived single-species NOEC values and multiple-species NOEC values, respectively. Based on all data, although limited especially with respect to reliable multiple-species toxicity studies, they concluded that there appears to be no difference in sensitivity between laboratory and (semi-)field conditions. In addition, Emans et al. (1992, 1993) compared the multiple-species NOEC values with the results of three ecotoxicological extrapolation methods, namely two statistical extrapolation methods (according to Aldenberg and Slob, 1993 and Wagner and Løkke, 1991, respectively) and the "EPA"-method (that uses assessment factors, as the method described in the TGD) and found the best correlation with statistical extrapolation. Furthermore, Versteeg et al. (1999) recently concluded for a series of substances, including zinc, that laboratory-generated single-species chronic studies can be used to establish concentrations protective of model ecosystems, and likely whole ecosystem, effects. For detailed data on laboratory to field extrapolation, for zinc specifically see section 3.3.2.1.4.

The London workshop on the use of statistical extrapolation for the derivation of PNEC values in case of data-rich substances was held in January 2001 in the framework of the EU Existing Substances program. This workshop was specifically aimed at the use of statistical extrapolation for the derivation of PNEC values for the metals zinc, cadmium and hexavalent chromium, since for these metals large chronic databases are available. The workshop recommended to include statistical extrapolation in the derivation of PNEC values for these metals, provided the chronic database meets certain requirements (EC, 2001).

The major recommendations that were made at the workshop are the following (EC, 2001).

- General requirements for input data (chronic NOEC values): at least 10 values and preferably more than 15 values, for different species.
- Taxonomic requirements for input data for the aquatic (freshwater) database: at least 8 taxonomic groups, using the EPA list of 8 groups required for the derivation of the "final chronic value" (PNEC equivalent, also calculated by means of statistical extrapolation) as a starting point. It is noted that the EPA list may over represent fish species (the phylum Chordata is represented by 3 families of fish or by 2 families of fish and 1 amphibian species) and that primary producers (algae, higher plants) are not included in the list. There is therefore a need to include algae and higher plants.
- A similar approach should be considered for other compartments; no specific proposals were given, however.
- For comparable data on the same toxicological endpoint for a particular species, the geometric mean value should be used as input. In case the toxicity is highly dependent on water or soil characteristics, then in addition the full data set could be used or several calculations could be performed on the basis of grouped data, for example for different pH ranges.
- Distribution function: the log-normal distribution (e.g. the methods of Wagner & Løkke (1991) and Aldenberg & Jaworska (2000) is a pragmatic choice because of its mathematical properties (methods exist that allow for most in-depth analysis of various uncertainties).
- Level of protection: Pragmatically, the 5^{th} percentile value with 50% confidence could be used (median 5^{th} percentile value) .
- Uncertainty considerations: Depending on the database and the confidence limits of the 5^{th} percentile value derived from that database, an assessment factor (AF) should be applied on the 5^{th} percentile value, thus PNEC = 5^{th} percentile value/AF. The assessment factor should be between 5 and 1, to be judged on a case by case basis. Lowering the AF of 5 on

the basis of increased confidence needs to be fully justified. In determining the size of the assessment factor to be applied on the median 5th percentile value, the following points were mentioned as a guide:

- 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);
- The diversity and representativeness of the taxonomic groups covered by the database, including also the variation represented relating to differences in the life forms, feeding strategies and trophic levels of the organisms (see TGD);
- The mode of action of the chemical;
- 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;
- In comparison a non-parametric extrapolation method is used. It must be noted that the non-parametric extrapolation method is under discussion for relatively small sample sets, because in such a case this method does not efficiently use the information on the entire 'tail' but heavily relies on only the few datapoints at the left tail (Van der Hoeven, 2001);
- Comparisons between field and mesocosm studies and the 5th percentile and mesocosm/field studies to evaluate the laboratory to field extrapolation.

In this RAR the chronic databases for zinc (aquatic and terrestrial) have been examined on the basis of the criteria recommended by the workshop (EC, 2001) and included in the current EU Technical Guidance Document on Risk Assessment (EC, 2003) and PNEC values ($PNEC_{add,\ aquatic}$ and $PNEC_{add,\ terrestrial}$) were derived by statistical extrapolation (using different distribution functions, see the previous part of this section). In addition, the use of the assessment factor method is given for comparison. Based on a comparison of the PNEC values derived by the different statistical extrapolation methods with the underlying NOEC values and with the results of (model) ecosystem and field studies, "final" PNEC values (with underlying justification) are proposed for surface water and soil, respectively.

3.3.2 Aquatic compartment

Except for some algal growth tests mentioned in section 3.3.2.1.2 and the three tests in section 3.3.2.4, the tests described in this section are tests in which a soluble zinc salt was used as test compound, although it is noted that in some cases data on the test compound are lacking.

3.3.2.1 Toxicity to aquatic organisms

There is a large database on the aquatic toxicity of soluble zinc, including acute toxicity data (LC50 and EC50 values) and chronic toxicity data (NOEC values) for a variety of aquatic organisms, including the major taxonomic groups, i.e. algae, crustaceans and fish. Note that the aquatic toxicity data on sediment organisms and aquatic microorganism are not included in this section, but in section 3.3.2.2. and section 3.3.2.3, respectively.

In addition to the data in section 3.3.1.1 and 3.3.1.2, the following is noted with respect to the derivation and the selection of the freshwater NOEC values used for $PNEC_{add,\ aquatic}$ derivation.

Reliability

Analysis of exposure concentrations

Analysis of exposure concentrations, recommended in most (OECD) guidelines, is considered to be an important criterion. The major issue, however, is whether or not it is likely that the exposure concentrations will be adequately maintained over the course of the test. Actual exposure concentrations were only determined in a limited number of studies. Although limited, these studies indicate that the exposure concentrations were usually adequately maintained in renewal test systems (see footnotes Table 3.3.2.a; freshwater studies). Data on actual versus nominal concentrations are usually not available for static systems, but because most freshwater tests were conducted in renewal test systems and flow-through test systems (the latter considered to be the most suitable system to maintain exposure levels) the analysis of exposure concentrations has not been used as a selection criterion.

Derivation of NOEC values

The general procedure and order of preference for deriving NOEC values, already descibed in section 3.3.1 has been applied. All NOEC values (including EC10 values) used for $PNEC_{add, aquatic}$ derivation have been checked for reliability on the basis of the range of test concentrations, as follows:

- If the NOEC is <100 µg/l, the separation factor between the NOEC and LOEC should not exceed a factor of 3.2.
- If the EC10 is used as NOEC equivalent, the EC10 should not be more than 3.2-times lower than the lowest concentration used in the test.

It is noted that the results of all tests met these criteria, thus no tests had to be rejected because of the above reliability criteria.

Relevance

$PNEC_{add}$ surface water: freshwater versus saltwater

Based on abiotic factors (physico-chemical water characteristics), including natural background concentrations of essential and other elements, freshwater and saltwater can be regarded as different environments, each with organisms adapted to that environment. Therefore, in the revised TGD (2003) different PNECs are introduced, i.e. one for freshwater and one for marine waters. Derivation of the PNEC for surface waters as well as the risk characterisation of the current risk assessment report, however, mainly followed the 'old' TGD (1996) that only aimed at freshwater and which provided 'only' guidance for deriving a freshwater PNEC. For pragmatic reasons the PNEC for freshwater is used in a few local marine exposure scenarios in the current risk assessment. The saltwater toxicity data that are included in the RAR have not been scrutinised to the extent the freshwater data have, and therefore will only be given for comparison with the freshwater data, but not to derive a PNEC for saltwater.

$PNEC_{add}$ freshwater: water type and abiotic factors as criteria for data selection

In the freshwater environment, abiotic factors that influence the speciation of zinc (and thus may influence the bioavailability and toxicity, see also section 3.3.2.1.3) vary considerably. Hardness is usually considered to be the major factor or one of the main factors influencing the aquatic toxicity, together with pH and alkalinity. Since these factors are interrelated (in natural freshwaters the pH is proportional to alkalinity, and alkalinity -and hence, pH- is proportional to hardness) it is practically impossible to establish the influence of each

individual factor. Even the combined influence of these factors on zinc toxicity is difficult to establish and, moreover, species may respond different to the various abiotic factors. The current data do not allow quantitative relationships between zinc toxicity and these factors and even qualitative relationships are loose (see section 3.3.2.1.1). Nevertheless, abiotic factors of the test waters have been taken into account for freshwater data selection, as follows.

Both natural and artificial test waters are accepted, provided that the major physico-chemical characteristics (in particular pH and hardness) are similar to the ranges that are encountered in natural freshwaters. This is in accordance with the view of the OECD Workshop on Aquatic Toxicity Testing of Sparingly Soluble Metals, Inorganic Metal Compounds and Minerals (CANMET, 1995)[14]. In addition, the background zinc concentration has been taken into account. The risk characterisation is aimed at European waters; therefore, characteristics of European freshwaters should preferably be used as guidance. It is emphasised, however, that it is difficult to give precise ranges of these factors because of the lack of detailed field data, especially on background zinc concentrations. Moreover, the current dataset of aquatic toxicity data and the current OECD guidelines for aquatic toxicity tests do not allow a too stringent data selection based on the above factors. The current OECD guidelines allow the use of both natural and artificial (reconstituted) test waters, and the recommended test waters (e.g. for algae and fish, respectively) are very different.

The following values for pH, hardness and background zinc concentrations have been used for data selection, primarily departing from the current OECD guidelines:

Table 3.73 Data selection for pH, hardness and background zinc concentrations

pH:	minimum value: 6
	maximum value: 9
Hardness:	minimum value: 24 mg/l (as $CaCO_3$)
	maximum value: 250 mg/l (as $CaCO_3$)
Background zinc concentration:	minimum value for soluble zinc: around 1 µg/l.

It is realised that the selected ranges of the three criteria will not cover all European aquatic systems, e.g. various aquatic systems in the Scandinavian countries. In particularly, hardness is much lower in the Scandinavian countries, although also other abiotic parameters differ from the 'mean' situation in European freshwaters. Therefore, a soft water $PNEC_{add, aquatic}$ has been derived, in addition to the generic $PNEC_{add, aquatic}$.

It is noted that the references used for the current aquatic toxicity dataset usually do not contain data on the background concentration of zinc in the test water and in a number of cases also data on pH and/or hardness are lacking. Thus, a stringent application of the above mentioned (minimum and maximum) limits for all three parameters, especially the zinc concentration, would very strongly reduce the dataset, which is not acceptable from a practical point of view. Therefore the following has been decided:

- When there are data reported on these parameters, the above selection criteria will be used.
- When there are no data reported on these parameters:
  - Tests that have been conducted in artificial waters will be excluded when data on pH and/or hardness are lacking.

[14] This recommendation (still) has to be worked out by expert groups to establish the acceptable ranges for the most relevant factors such as hardness, pH and particulate matter, and to be implemented in the OECD guidelines.

- Tests that have been conducted in natural waters will be maintained, unless there are clear indications that the (above) parameters in the water strongly deviate from real environmental conditions. For example, tests in waters that received special treatment to remove zinc (and other cations such as Ca and Mg) will be excluded. On the other hand, tests conducted in untreated natural United States' waters that have been reported to contain a background zinc concentrations which may be considerably below 1 µg/l (depending on natural seasonal variations), such as Lake Superior water, will not be excluded.

With respect to the above relevance criteria it is emphasised that few studies have been rejected solely because the values for pH, hardness and/or background zinc concentration (Cb) were either too low or too high. The majority of the studies that were rejected because of relevance criteria are studies that were conducted in artificial test waters for which no data on pH and/or hardness were reported.

Data on other abiotic factors such as particulate matter or dissolved organic carbon (DOC) are much too scarce, which limits using these abiotic factors as selection criteria.

Note that a further selection criterion was used to select the NOEC values from two new studies in which a large number of chronic toxicity tests with *Daphnia magna* (Heijerick et al., 2003) and with three different species, viz. alga *Pseudokirchneriella subcapitata, Daphnia magna*, and fish *Oncorhynchus mykiss* (De Schamphelaere et al. 2003; study from "Conclusion i" program) were performed in artifial test media in which a number of abiotic factors, especially pH, hardness and DOC concentration, were used in different combinations. The results of these studies clearly indicate that the toxicity of zinc in water is also influenced by the DOC concentration (see section 3.3.2.1.1). Therefore, a DOC concentration of 2 mg/l was selected as upper limit. This value is based on several OECD guidelines, viz. OECD 201-alga growth test (no addition of DOC recommended), OECD 211-Daphnia reproduction test (recommended TOC concentration: <2 mg/l) and OECD 210/212/215-fish tests (recommended TOC concentration: <2 mg/l). A DOC concentration of 2 mg/l is nearly equal to the mean 5^{th}-percentile value for DOC in EU waters (2.2 mg/l: arithmetic mean value calculated from the range of the 5^{th}-percentile values for DOC (1.6-3.3 mg/l) for EU waters in the pH range of 6.5-8.5, reported by Heijerick et al., 2002).

It is emphasised that the upper limit for DOC (2 mg/l) only holds for the tests from Heijerick et al. (2003) and De Schamphelaere et al. (2003) that were performed in artificial waters. The tests by De Schamphelaere et al. (2003) that were performed in natural waters represent natural EU water conditions and therefore these tests are included in the data set that was used for the derivation of the generic $PNEC_{add,\ aquatic}$. This reasoning is in line with the relevance criteria.

With regard to the old studies (i.e all other tests that were used for the derivation of the generic $PNEC_{add,\ aquatic}$), no data are available on DOC concentratons in the test waters (except for the *Daphnia magna* study by Paulauskis & Winner, 1988). Thus this selection criterion was not used for these studies. Regarding the tests performed in artificial test waters it can be stated that the DOC concentration will have been considerably below 2 mg/l, as no DOC was added. In some of the natural, untreated test waters, the DOC concentration may have been higher than 2 mg/l.

Culture and test conditions (related to acclimation/adaptation)

To ensure that test organisms are adapted to the test conditions, the culture and test conditions should preferably be similar. Adaptation to very low or very high zinc concentrations may influence the sensitivity to zinc. Therefore, tests with organisms that have been adapted to very low or very high zinc concentrations should be excluded for PNEC derivation. This issue

has been used as selection criterion, with the comment that in many references no data have been reported on the culture conditions (in those cases it is assumed that culture and test conditions were similar, as is common practice) and that other considerations may overrule this criterion, see also below.

Test species: endemic versus non-endemic species

The culture and test conditions are considered much more relevant than the origin of the species and the OECD guidelines recommend the use of a number of "standard" species which do not have a world-wide distribution. Moreover, using the origin of species as criterion would considerably reduce the dataset and limit the data to only a few species / taxa, which may obscure variation in sensitivity. Therefore, the origin of the test species has not been used as selection criterion.

3.3.2.1.1 Abiotic factors influencing the aquatic toxicity of zinc

Introduction

Conventionally, the environmental risk assessment of a substance in the water would be comparing the estimated concentration (PEC) in the water to the PNEC for water. In that situation both the PEC and the PNEC would be based on the dissolved concentration of zinc in water. The derivation of the $PNEC_{add}$ for zinc in water is described in section 3.3.2.1.5.

Possible effects of some water chemistry characteristics that may affect the bioavailability and toxicity of zinc will be discussed. In addition, a more holistic approach is presented where on the one hand the speciation of zinc in the water and on the other hand the effect it has on aquatic species is taken into account in one multifactorial approach, which is called the Biotic Ligand Model (BLM). Then, the results of a recent research program are described that will be used for deriving water chemistry related corrections for the current soil risk assessment of zinc. Finally, a tiered approach will be discussed that will be used for implementing the bioavailability correction to the PEC in the current risk assessment report of zinc.

Water chemistry characteristics that may affect zinc bioavailability

Physico-chemical water characteristics such as background concentration of zinc, pH, and hardness influence the chemical speciation of zinc and other metals in water and thus may influence the bioavailability and toxicity.

Background concentration of zinc

According to the metalloregion concept as described in section 3.3.1, adaptation to natural background levels and probably also to test conditions may influence the sensitivity to zinc (Muyssen and Janssen, 2000). Muyssen and Janssen (2001a) indeed showed that *Daphnia magna* increased their tolerance towards zinc when cultured in the laboratory under varying zinc concentrations by a factor of 2-3, and found an optimum zinc concentration between 300 and 450 µg/L. Muyssen and Janssen (2001b) also showed that *Raphidocelis subcapitata* and *Chlorella vulgaris* increased their tolerance towards zinc when cultured in the laboratory under zinc concentrations between 1.4 and 65 µg/L by a factor of 2-3. They concluded that background zinc concentrations should be considered in the evaluation of toxicity test results for risk assessment purposes. These latter findings need further study to base a risk assessment on. However, not many studies have looked at the relationship between background concentration of zinc in water and its influence on toxicity. Therefore, an empirical approach was taken. From the studies that were selected to derive the PNEC for the freshwater compartment and those studies that were evaluated and were found reliable but not

relevant (see Section 3.3.2.1 for explanation), all background concentrations were plotted against the NOEC to evaluate a possible relationship between this background zinc concentration and toxicity in figure 3.3.2.1.1. A distinction is made between the data originating from various sources (closed symbols) and the data from a recent research program (open symbols, data from De Schamphelaere et al., 2003).

As can be seen from Figure 3.3.2.1.1, there seems to be no clear trend for the closed symbols. With increasing background concentration, the NOEC does not clearly increase or decrease. It must be noted, however, that these data from various sources are in fact not suitable to examine this potential relationship. Part of the scatter for the closed symbols in this Figure 3.3.2.1.1 will also be caused by other abiotic factors that may influence the toxicity. Whether the sensitivity of organisms is really determined by the natural background concentrations in which they live can only be determined by actual toxicity testing of the same organism taken from different field conditions with different natural background levels but comparable to other abiotic conditions. Since the data from the recent research program (open symbols, data from De Schamphelaere et al., 2003) relate to only a few test waters and a few zinc background concentrations, no clear relationship is found between toxicity and zinc background concentration as well. Based on Figure 3.3.2.1.1 it is concluded that there is a too poor basis to derive background dependent PNEC values for freshwater.

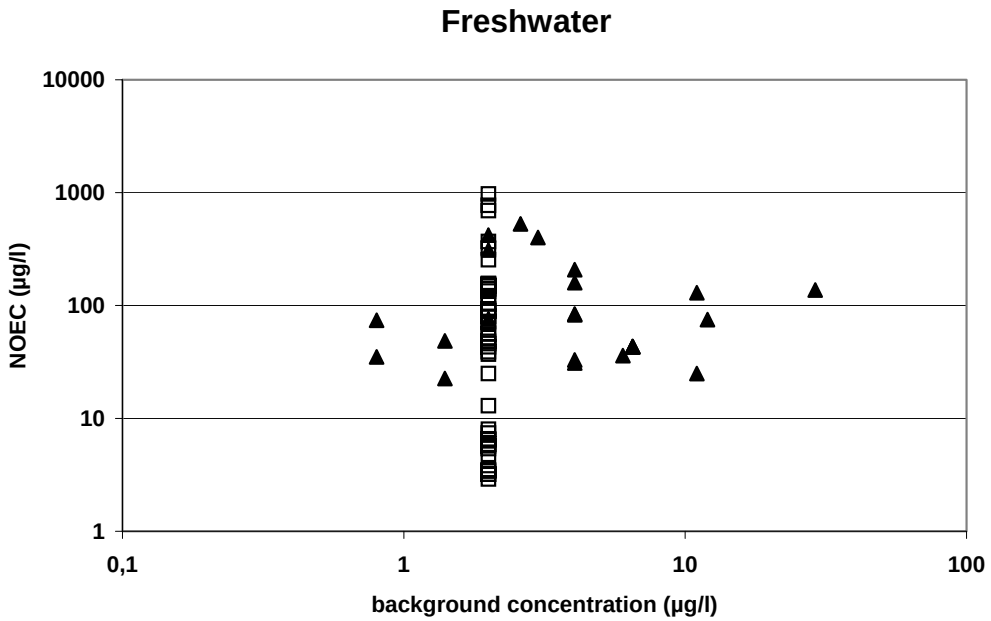


Figure 3.13 Relationship between the background concentration of zinc in water and toxicity, expressed as the No Observed Effect Concentration (NOEC). Data from all studies that were used for deriving the $PNEC_{add, aquatic}$, those that were found reliable but not relevant (closed symbols), and those from the recent research program (De Schamphelaere et al., 2003) (open symbols).

pH

With respect to pH, the database in Annex 3.3.2.A shows two older studies that studied the influence of pH on the aquatic toxicity. Belanger and Chery (1990) studied toxicity of zinc at pH values of 6, 8 and 9. They found no effects or a poor relationship between pH and toxicity with *Ceriodaphnia dubia*. Chapman et al. (1980) studied toxicity of zinc at pH values of 7.5, 7.7 and 8.4. They found that toxicity increased with increasing pH with *Daphnia magna*.

These two contradicting studies with only two species cannot be used as a basis for a pH-normalisation of aquatic toxicity. In addition, a recent research program (De Schamphelaere et al., 2003) provided further information on the relationship between pH and toxicity to *Pseudokirchneriella subcapitata*, *Daphnia magna* and *Oncorhynchus mykiss*. Chronic toxicity to zinc was studied between pH 5.5 and 8.5. The latter study showed that the modifying effect of pH was a factor 2 to 3 for the rainbow trout, a factor 3 to 4 for the *D. magna*, and a factor >20 for the algae. When all data from the three species from the latter study (open symbols in Figure 3.3.2.1.2) are pooled no clear relationship is observed between pH and chronic toxicity. Data originating from various sources (closed symbols) including those from Belanger and Cherry (1990) and Chapman et al. (1980) are enclosed in Figure 3.3.2.1.2 and show no clear relationship between pH and toxicity. Based on Figure 3.3.2.1.2 it is concluded that there is a too poor basis to derive pH dependent PNEC values for freshwater, although pH does seem to affect chronic toxicity of zinc, e.g. to algae.

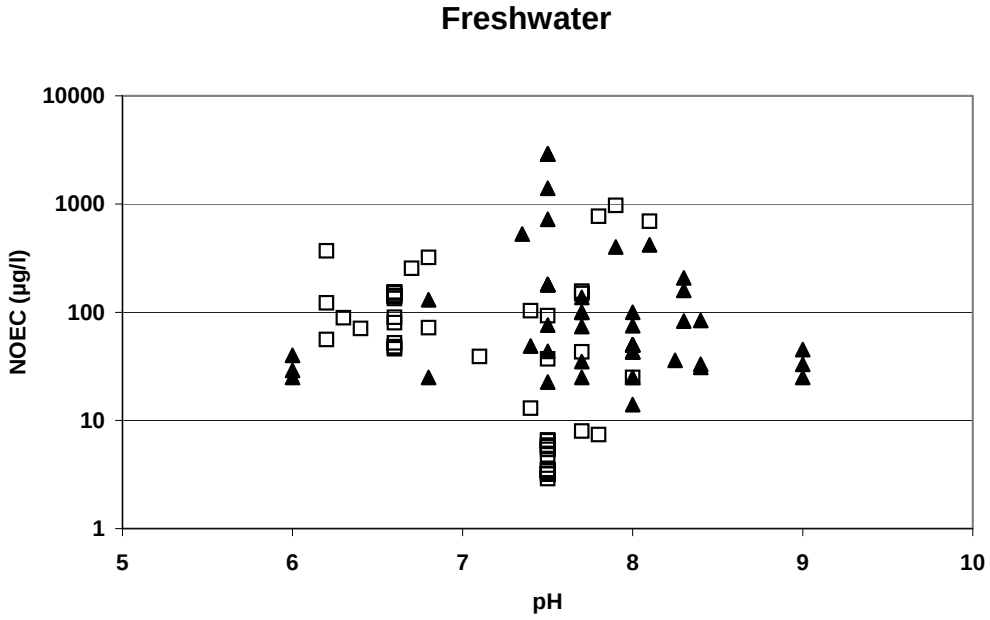


Figure 3.14 Relationship between the pH in water and toxicity, expressed as the No Observed Effect Concentration (NOEC). Data from all studies that were used for deriving the $PNEC_{add, aquatic}$, those that were found reliable but not relevant (closed symbols), and those from the recent research program (De Schamphelaere et al., 2003) (open symbols).

Hardness

In this section the possible effects of hardness on aquatic toxicity will be discussed. With respect to those abiotic factors influencing the toxicity of metals in fresh water, total hardness (determined by the calcium and magnesium content in the water) is usually considered as one or the main factor. The toxicity of metals is generally assumed to be inversely related to hardness. In some countries, for example the United States and Canada, the relevance of this factor is reflected in hardness-related Water Quality Criteria (WQC's) for a number of metals. For zinc, the United States WQC's, both the "final acute value" for short-term exposure and the "final chronic value" for long-term exposure, are hardness related, in contrast to e.g. the Canadian WQC[15] for zinc that is independent of hardness.

[15] In the United States, hardness-related Water Quality Criteria (WQC's) were set for Cd, Cr(III), Cu, Ni, Pb and Zn. For the metals As(V), As(III)), Be, Cr(VI), Hg, Sb, Se and Tl, the WQC's were set independently of hardness (U.S. EPA, 1991, cited in BKH, 1995). In Canada, hardness-related WQC's were set for Cd, Cu, Ni and

The discussion will focus on (1) hardness versus chronic toxicity for zinc, (2) the acute-to-chronic extrapolation for zinc, (3) some general observations on the effect of hardness on the chronic aquatic toxicity of metals, and (4) the results of a recent research program that studied the effects of hardness on the chronic toxicity of zinc to three aquatic organisms.

1. Hardness versus chronic toxicity for zinc.

There are only a few studies on the relationship between water hardness and the chronic aquatic toxicity of zinc from the older literature.

In two studies with the water flea *Daphnia magna* the effect of hardness on chronic zinc toxicity was studied by increasing the hardness of the original soft water by adding both $CaSO_4$ and $MgSO_4$. In this way other factors such as alkalinity are affected as little as possible.

- In one study, increasing the hardness of the test water from 50 to 200 mg/l (as $CaCO_3$) resulted in a 6-fold increase in the NOEC for reproduction (thus, in a decrease in toxicity); the NOEC for survival was less affected (Paulauskis and Winner, 1988, life-cycle studies, see Table 3.3.2.a).
- In the second study, increasing the hardness from 50 to 100 mg/l (as $CaCO_3$) resulted in a significant ($p < 0.05$) decrease in mortality at 125 µg/l, the only zinc concentration tested. The decrease in toxicity was not accompanied by a decrease in zinc bioaccumulation (Winner and Gauss, 1986).

Thus: twice a decrease in toxicity with increasing hardness was shown, and once a poor relationship between hardness and toxicity.

In a study using *Daphnia magna,* the effect of hardness on chronic zinc toxicity was studied by increasing the hardness of the original soft water (with hardness 22-60 mg/l, as $CaCO_3$) by adding $CaSO_4$, $MgCl.{}_2H_2O$, $NaHCO_3$, and $KHCO_3$. Increasing the hardness from 50 to 100 mg/l resulted in a 2-fold decrease in the NOEC for survival and reproduction, i.e., an increase in toxicity. A further increase in hardness from 100 to 200 mg/l did not affect the NOEC (Chapman et al., 1980, life-cycle studies, see Table 3.3.2.a). The result of this study is contradictory to the result of the studies by Winner and Gauss (1986) and Paulauskis and Winner (1988) conducted with the same test organism. According to the latter authors, the higher toxicity in the hard (hardness 200 mg/l) and medium-hard (hardness 100 mg/l) test waters used by Chapman et al. (1980), can be explained by the higher alkalinity in these waters compared to the soft (hardness 50 mg/l) water, as follows. A high alkalinity results in the formation of insoluble zinc complexes with the carbonates determining the alkalinity, increasing the toxicity to daphnids by increasing oral exposure. This effect of alkalinity on toxicity will also be valid for other filter-feeding organisms and other metals. Thus: an initial increase and no further increase in toxicity with increasing hardness was shown.

In life-cycle tests with *Ceriodaphnia dubia*, no consistent effect of hardness and pH (the two variables tested independently) on the chronic toxicity of zinc was found. In this study each pH value (6, 8 and 9) was tested in combination with each hardness value (81, 118, 168 mg.l). The maximum difference between the NOEC values for reproduction was a factor of 3 (Belanger and Cherry, 1990, see Table 3.3.2.a). Thus: no clear relationship between hardness and toxicity was shown.

In a study with *Daphnia magna* the influence of hardness (35, 110, 240, 370 and 445 mg/l; only one test at both the lowest and highest value), pH (6.5, 7.25, 8.0 and 8.5; only one test at

Pb. For the metals Zn, As, Cr, Hg and Se, the WQC's were set independently of hardness (CCME, 1994; cited in BKH, 1995).

both the lowest and highest value), and organic matter (DOC: 2, 10, 21, 32 and 40; only one test at both the lowest and highest concentration) on reproduction were tested independently, but always in combination with the other two factors (Heijerick & Janssen, 1999; Heijerick et al., 2003). A total of 17 tests were performed, but the number of combinations was lower. The results of this study show the following. With respect to the NOEC (endpoint: net reproductive rate), the concentration of DOC was the only parameter that showed a statistically significant relationship ($p = 0.001$) with the chronic NOEC: increasing NOEC with increasing DOC concentration. With respect to the EC10 values for this endpoint there was a significant relationship with both the DOC concentration ($p = 0.0001$) and the pH ($p = 0.023$): NOEC increasing with increasing DOC concentration and pH, as well as for pH*DOC ($p= 0.029$). There was no statistically significant effect of hardness ($p = 0.343$) or hardness^{square} (H*H: $p = 0.057$), although the results show an increase of EC10 with increasing hardness from 50-100 mg/l to higher levels, with a maximum EC10 around a hardness of 250 mg/l. A further increase in hardness, however, did not result in a further increase, but in a decrease in EC10. The NOEC values ranged from 209 µg Zn/l (in one test at pH 7.25, hardness 240 mg/l and DOC 2 mg/l and in a second test at pH 8, hardness 110 mg/l and DOC 10 mg/l) to 1000 µg Zn/l (in one test at pH 7.25, hardness 240 mg/l and DOC 40 mg/l), resulting in a maximum difference with a factor of 5 (Heijerick & Janssen, 1999; Heijerick et al., 2003).[16]

The study thus showed that pH and hardness had some, but small effects on the NOECs, while DOC showed a larger influence on the NOECs. The study provides equations that may be useful to normalise NOEC values for the three different abiotic factors. Thus: only small effects of hardness on toxicity were shown.

2. Acute-to-chronic extrapolation for zinc.

The hardness-related freshwater "final chronic values" for zinc derived by the U.S. EPA are based on a "final chronic equation" that is based on a "final acute equation" and a "final acute-chronic ratio". The "final acute equation" is based on the relationship between hardness and acute toxicity, because of the lack of data on the relationship between hardness and the chronic toxicity of zinc (U.S. EPA, 1987; U.S. EPA, 1980)[17]. In U.S. EPA (1980) it was stated that the available aquatic toxicity data on zinc indicate that hardness effects are much less dramatic for chronic toxicity than for acute toxicity of zinc, and that the slope of the hardness-toxicity regression may be near zero or even negative for some species. Furthermore, U.S. EPA (1980) did derive a hardness-related "final chronic equation", but stated that it would be reasonable to use one freshwater "final chronic value" for all hardness values[18]. Despite these

[16] The NOEC values from this study were rejected for PNEC derivation, as in all but one tests the hardness was >250 mg/l and/or the DOC concentration was >2 mg/l (in all but one tests the DOC concentration was ≥ 10 mg/l), thus the values for hardness and especially DOC were above the upper limit values used in this RAR, see section 3.3.2.1. Furthermore, for each test only the NOEC, EC10 and EC50 were reported, without underlying results per concentration, thus the validity of the individual tests could not be checked. All NOEC values from this study are listed in Table 3.3.2.a - Part II (studies rejected for PNEC derivation).

[17] Based on the "final chronic equation" used in U.S. EPA (1987), being $e^{\{0.8473\ [\ln (\text{hardness})]\ + 0.7614\}}$, the freshwater "final chronic values" are 2 µg/l at a hardness of 0 mg/l (as $CaCO_3$), 60 µg/l at a hardness of 50 mg/l, 110 µg/l at a hardness of 100 mg/l, and 190 µg/l at a hardness of 200 mg/l.
U.S EPA (1991), cited in BKH (1995) most probably used the same equation, because of a "chronic criterion" of 110 µg/l at a hardness of 100 mg/l.
U.S. EPA (1980) used a slightly different "final chronic equation", being $e^{\{0.83\ [\ln (\text{hardness})]\ + 0.85\}}$. According to U.S. EPA (1980), the criterion is for "total recoverable zinc" (being the free ion, plus salts with hydroxide, carbonate and sulphate etc.).

[18] The proposed freshwater "final chronic value" for all hardness values was 47 µg/l, being the lowest "chronic value" (the geometric mean value of the NOEC and the LOEC) for both a sensitive invertebrate in hard water and a medium sensitive fish in soft water.

remarks with respect to the weak and species-dependent relationship between hardness and chronic toxicity, U.S. EPA (1987) derived hardness-related "final chronic values", although no relevant new data compared to U.S. EPA (1980) were presented. Since the chronic hardness related equation lacks a firm basis, and it is questioned if it can be applied to all aquatic species, it does not support introducing hardness related PNECs for water.

3. General observations on the effect of hardness on the chronic aquatic toxicity of metals.

In recent evaluations of aquatic toxicity data on metals, Crommentuijn et al. (1997) and Meyer (1999) draw several relevant conclusions on the relationship between hardness and chronic toxicity of various metals.

The (assumed) inverse relationship between water hardness and aquatic toxicity of various metals is based mainly on the results from acute toxicity tests, conducted with relatively high metal concentrations. Based on the results of the acute tests, the inverse relationship between hardness and toxicity is considered to be unequivocal, although the following comments should be taken into account (Crommentuijn et al., 1997):

- In most acute tests on the relationship between water hardness and toxicity, fish were used as test organisms; data for species from other taxonomic groups, which in part have different uptake mechanisms than fish, are much less available.
- In natural waters, hardness is qualitatively related to a number of other abiotic factors including alkalinity, ionic strength and pH. In most tests on the effects of hardness on metal toxicity, hard waters were diluted with distilled or otherwise de-ionised water to reduce hardness, resulting in a simultaneous reduction in the other factors. These factors can influence the toxicity of metals in two ways: by influencing the chemical speciation of the metal in water (and hence affecting the bioavailability) and by influencing the uptake and binding of available metal by biological tissues. Based on data on chemical speciation, it is expected that the toxicity of metals will be highest in soft, acid waters, i.e. under environmental conditions that favour the presence of the simple (hydrated) metal ion. In this respect, hardness is the best indicator for water conditions influencing metal toxicity rather than the sole influencing factor. Because water hardness is determined by the calcium and magnesium ions, which are divalent, hardness will specifically affect the uptake and binding of other divalent metal ions by biological tissues.
- Based on the above it can be assumed that the chronic toxicity of metals, similarly as acute toxicity, will be affected by water hardness (and related factors). However, based on the results of chronic toxicity tests, conducted with relatively low metal concentrations, it is concluded that the relationship between water hardness and chronic toxicity of metals appears to be much less consistent than that between hardness and acute toxicity. Furthermore, the influence can be relatively small, especially in the range of hardness between around 50 and 200 mg/l (as $CaCO_3$): the available chronic toxicity studies with zinc, cadmium, copper and chromium usually showed a 2- to 3-fold decrease in toxicity with increasing hardness, with a maximum of around 5-fold. Besides this also examples of tests exist in which no effect or even an increase of toxicity with increasing hardness was found.

Meyer (1999) showed that a relationship between hardness and acute toxicity to fish could be mechanistically explained. A combination of (a) competitive binding of transitional-metal cations, hardness cations, and protons to transition-metal-binding sites on fish gills and (b) aqueous complexation of transition-metal cations by carbonate ions explains the relationship. At midrange hardness (between ca. 20 – 200 mg/l as $CaCO_3$) a one to one relationship is expected between hardness and acute toxicity if alkalinity covaries with hardness. If alkalinity is constant while hardness is varied a different relationship is expected. At extremely low

hardness no relationship is expected (Meyer, 1999). This study did not focus on other species than fish or on the relationship between chronic toxicity and hardness.

4. The recent research program on hardness and chronic toxicity of zinc.

The recent study by De Schamphelaere et al. (2003) provided further information on the relationship between hardness and toxicity to *Pseudokirchneriella subcapitata*, *Daphnia magna* and *Oncorhynchus mykiss*. Chronic toxicity to zinc was studied between hardness 25 and 250 mg/l as $CaCO_3$. The study showed that the modifying effect of hardness was a factor 10 for the rainbow trout, a factor 3 to 4 for the *D. magna*, and a factor 2 for the algae. When all data from the three species from this study (open symbols in Figure 3.3.2.1.3) are pooled no clear relationship is observed between hardness and chronic toxicity. Data originating from various sources (closed symbols) are enclosed in Figure 3.3.2.1.3 and also show no clear relationship between hardness and toxicity.

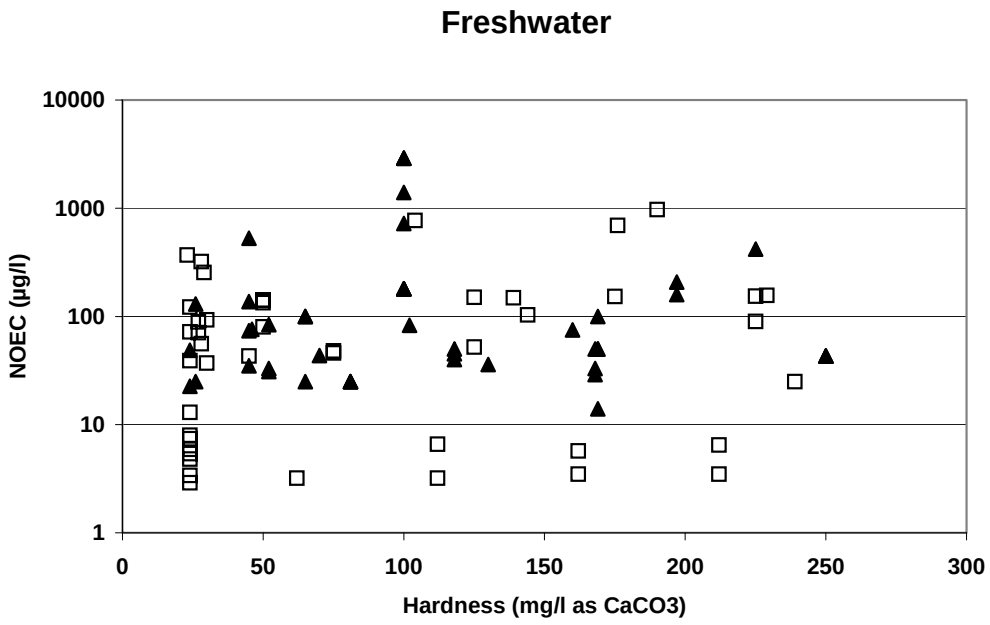


Figure 3.15 Relationship between hardness in water and toxicity, expressed as the No Observed Effect Concentration (NOEC). Data from all studies that were used for deriving the $PNEC_{add, aquatic}$, those that were found reliable but not relevant (closed symbols), and those from the recent research program (De Schamphelaere et al., 2003) (open symbols).

5. Concluding remarks on hardness

Based on Figure 3.3.2.1.3 it is concluded that there is a too poor basis to derive hardness dependent PNEC values for freshwater, although hardness does seem to affect the chronic toxicity to zinc, e.g. to rainbow trout. However, only data have been selected for a 'mean' European situation, where hardness is between 24 and 250 mg/L as $CaCO_3$. Since, in particularly in the Scandinavian Countries, but also on other parts of Europe, hardness may be lower than 24 mg/L as $CaCO_3$, the effect of hardness on toxicity will be separately addressed for soft waters in section 3.3.2.1.5.

Biotic Ligand Model

In this section (1) some general background information is provided on the Biotic Ligand Model, which is followed by (2) a summary of the results of an extensive research program on the development and validation of BLMs for three aquatic species in European surface waters, which in turn is followed by (3) the use of these BLMs in correcting the bioavailability of zinc in water in the current risk assessment report.

General background

During recent years, the Biotic Ligand Model (BLM) has been proposed as a tool to evaluate quantitatively the manner in which water chemistry affects the speciation and biological availability of metals in aquatic systems. This is an important consideration because it is the bioavailability and bioreactivity of metals that control their potential to cause adverse effects. The BLM approach has gained widespread interest amongst the scientific, regulated and regulatory communities because of its potential for use in developing water quality criteria and in performing aquatic risk assessment for metals. The BLM does this in a way that considers the important influences of site-specific water quality (Paquin et al., 2002). Figure 3.16 shows the BLM-concept. Free zinc ions (Zn^{2+}) bind to the biotic ligand of organisms, which may be transport sites and / or toxic action sites. The concentration of Zn bound to the biotic ligand is directly proportional to the toxic effect, and independent of the physicochemical characteristics of the test medium. The binding constant of zinc to the biotic ligand appears to be time-dependent and directly related to metal toxicity, and the number of binding sites at the gills of rainbow trout increased following prior zinc exposure. Therefore, to obtain a stable binding constant several hours of exposure are required (Alsop and Wood, 2000).

The chemical activity of Zn^{2+} is, however, reduced by binding to organic (dissolved organic carbon, DOC) and inorganic ligands that reduce the bioavailability and thus reduce the toxicity. Inorganic ligands include OH^- and CO_3^{2-}. The concentrations of these ligands are increased at increased pH and increased alkalinity of the test medium, respectively. Cations in solution can compete with zinc for the biotic ligand, which also reduces bioavailability tot the biotic ligand and thus reduces toxicity. The speciation of Zn^{2+} is calculated by the WHAM V-model (Tipping, 1994), which is an integral part of the BLM software (Hydroqual, 2002). The interaction between Zn^{2+} and competing cations is estimated in the study by De Schamphelaere et al. (2003).

Van Riemsdijk (2001) recently commented on the use of WHAM (Windermere Humic Acid Model, e.g. Tipping, 1994) with respect to the effect of DOC on bioavailable zinc in freshwater, which is equated with free zinc. The model parameters in WHAM as far as published are based on an extremely limited data set, with respect to the binding of zinc to humic and fulvic acids, which forms the basis for the model (Van Riemsdijk, 2001). In a recent study of Milne (2000), the literature has been very thoroughly screened, and newly determined data (not yet published) have been added to derive generic parameters for zinc binding. The lack of good data in the literature is probably due to the difficulty in measuring the free zinc concentration. Milne (2000) has used new, yet unpublished data for zinc binding to a humic acid, obtained with an improved Donnan membrane technique (Temminghoff et al., 2000; Wang et al., 2001).

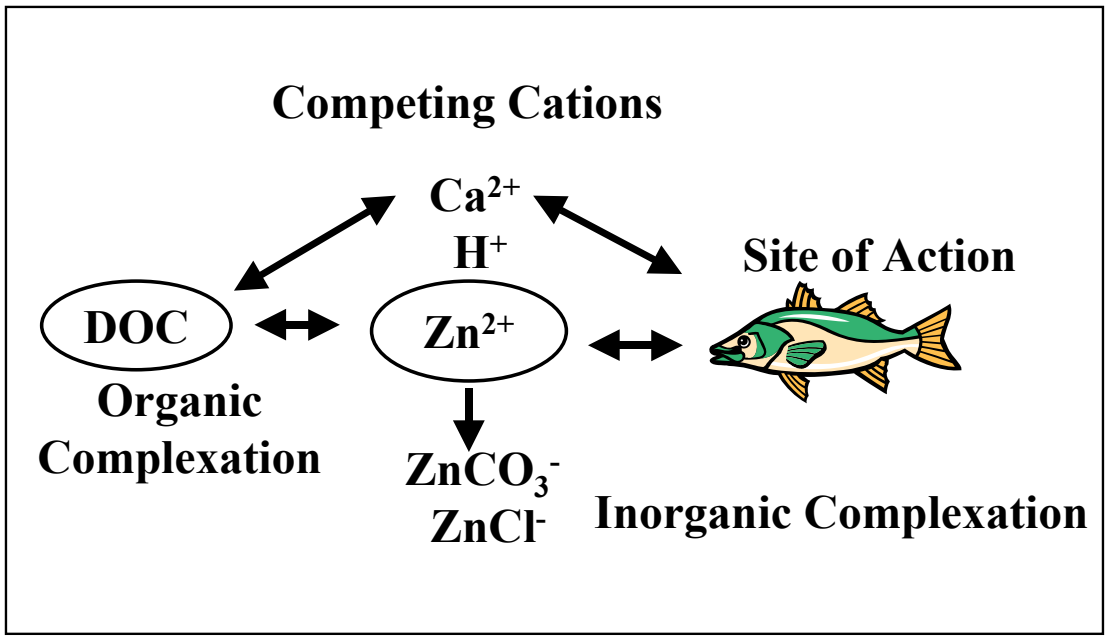


Figure 3.16 Summary of the BLM-concept.

Development and validation of BLMs for European surface waters

The overall objectives of a recent research program (De Schamphelaere et al., 2003, Heijerick et al., 2003) were (1) to develop a Biotic Ligand Model (BLM) predicting chronic zinc-toxicity to three standard test organisms, i.e. the rainbow trout *O. mykiss*, the invertebrate *D. magna*, and the green alga *P. subcapitata*; and (2) to validate the developed BLM with different surface waters which are representative for the observed variation of physico-chemistry in EU surface waters.

The BLM development was based on a series of (uni-variate) chronic toxicity experiments in standard test media in which the major water quality parameters, that are expected to affect zinc toxicity, were varied (i.e. H^+, Ca^{2+}, Mg^{2+}, Na^+). The validation of the developed model (see below) was performed using zinc-spiked natural waters, which were chemically characterised with respect to the BLM input parameters. The BLMs were tested with regard to their potential to predict Zn-speciation, Zn-complexation and chronic zinc toxicity over a relevant range of water chemistry parameters.

The methodology by De Schamphelaere et al. (2003) is followed to develop the BLMs and is based on the assumption that the BLMs can be defined as follows:

$$ECx_{Zn^{2+}} = \frac{f_{ZnBL}^{x\%}}{(1 - f_{ZnBL}^{x\%}) \cdot K_{ZnBL}} \cdot \left\{1 + K_{CaBL} \cdot (Ca^{2+}) + K_{MgBL} \cdot (Mg^{2+}) + K_{NaBL} \cdot (Na^{+}) + K_{HBL} \cdot (H^{+})\right\}$$

where:

ECx_{Zn}^{2+} = the zinc concentration, expressed as free Zn^{2+}-activity, causing x% of effect
$f_{ZnBL}^{x\%}$ = the fraction of binding sites that is occupied by Zn when x% of effect occurs
K_{ZnBL} = the stability constant of zinc binding to the biotic ligand (BL)
K_{CaBL}, K_{MgBL}, K_{NaBL}, K_{HBL} = the stability constants of competing cations for binding to the biotic ligand
(Ca^{2+}), (Mg^{2+}),(Na^{+}),(H^{+}) = the chemical activity of competing cations in the test medium

It was demonstrated that mortality was the most sensitive endpoint for chronic zinc toxicity to juvenile rainbow trout. The developed BLM is thus based on mortality data. The results of this study (De Schamphelaere et al. 2003) clearly illustrated the importance of bioavailability modifying factors for chronic zinc toxicity to juvenile rainbow trout. Observed 30d-EC50, 30d-EC10 and 30d-NOEC values ranged between 108 and 1970 µg Zn/L, 38.4 and 902 µg Zn/L and 31.5 and 885 µg Zn/L, respectively. The difference between the lowest and the highest toxicity thus varied from a factor 18 to a factor 28. In this study the order of importance of toxicity modifying effects was Ca (factor ~ 10) > DOC (factor ~5) > Mg (factor 3 to 4) > pH (H^+, factor 2 to 3) > Na (factor 2). Hence, neither of these factors should be disregarded in evaluating possible risks of chronic zinc exposure to fish species. The developed fish-BLM was able to predict all chronic effect concentrations within a factor 2 of the observed effect concentrations, not only for lab waters but also for natural surface waters. Hence, the variation of a factor 20 observed in all toxicity tests was reduced to a factor 2 by using the BLM, indicating that the fish-BLM accurately describes the mechanistic effects of bioavailability factors on chronic zinc toxicity. The relevant BLM-constants for rainbow trout are shown in Table 3.74.

Table 3.74 BLM-constants for acute and chronic zinc toxicity to juvenile rainbow trout (De Schamphelaere et al., 2003)

	Acute	**Chronic (5th p-BLM)**	**Chronic (50th p-BLM)**	**Chronic (95th p-BLM)**
Log KZnBL (a)	5.31	5.31	5.31	5.31
Log KCaBL	3.76	3.35	3.70	4.01
Log KMgBL	3.51	3.04	3.15	3.31
Log KNaBL	2.88	2.33	2.45	2.61
Log KHBL	6.73	6.24	6.36	6.52
$f_{ZnBL}^{50\%}$ (b)	0.141±0.035	0.189±0.043	0.146±0.028	0.104±0.018
$f_{ZnBL}^{10\%}$ (b)	NA	0.067±0.015	0.049±0.009	0.034±0.006
f_{ZnBL}^{NOEC} (b)	NA	0.100±0.047	0.074±0.029	0.051±0.018

1) log KZnBL set to the same value as reported in Heijerick et al. (2002) for the acute Zn-BLM for *D. magna*

2) mean ± one-sided 95% confidence limit

The results of the ecotoxicity tests with the invertebrate Daphnia magna also clearly illustrated the importance of bioavailability modifying factors for chronic zinc toxicity to this species. Observed 21d-EC50 and 21d-NOEC-values were between 107 and 372 µg Zn/L, and between 47.9 and 168 µg Zn/L, respectively, indicating a factor of 4 difference between the lowest and the highest toxicity observed. In this study the order of importance of competitive effects was Ca2+ (factor 3 to 4) = pH (factor 3 to 4) > Mg2+ (factor 2 to 3) > Na+ (factor 1.5). Five mg DOC/L resulted in a decrease of toxicity with about factor 1.3 to 1.5, which is comparable to the factor 5 decrease observed with rainbow trout in a test with a DOC concentration, which was 4x higher. Thus, a similar importance of DOC for rainbow trout and D. magna can be suggested. Due to technical (equipment) failure during the final model validation, more test results containing natural DOC were presently not available. In general, it can be concluded that the developed daphnid-BLM was able to predict all 21-d EC50s within a factor 2 of the observed effect concentrations. Moreover, the BLM was able to

reproduce well the mechanistic effects observed in the tests, i.e. competition and complexation. The relevant BLM-constants for Daphnia magna are shown in Table 3.75

Table 3.75 BLM-constants for acute (Heijerick et al., 2002) and chronic (De Schamphelaere et al., 2003) zinc toxicity to Daphnia magna

	Acute	**Chronic**
Log KZnBL (a)	5.31	5.31
Log KCaBL	3.34	3.25
Log KMgBL	3.12	2.71
Log KNaBL	2.37	1.92
Log KHBL	-	5.91
$f_{ZnBL}^{50\%}$ *(b)*	0.417	0.117±0.13
f_{ZnBL}^{NOEC} *(b)*	NA	0.077±0.015

5) Data from Heijerick et al. (2002)
6) mean ± one-sided 95% confidence limit

For the green alga *P. subcapitata* it was demonstrated that the observed 72h-ErC50 and 72h-ErC10s were between 25.8 and 1630 µg Zn/L and between 4.8 and 608 µg Zn/L, respectively, indicating a factor of 79 and 117 difference between the lowest and the highest toxicity, respectively. In this study the order of importance of toxicity modifying effects was pH (factor >20) > DOC (factor 14) > Mg (factor 2). With regard to interactions at the biotic ligand, only the pH effect was included in the alga-BLM. The DOC-effect was, similarly as for the other organisms, taken into account by the speciation model WHAM V (Tipping, 1994). The developed model demonstrated a good predictive capacity for the field waters tested: the developed model decreases the variation in toxicity from about a factor of 100 to about a factor of 2, indicating that the BLM can be used for predicting chronic zinc toxicity to algae species. The relevant BLM-constants for the algae are shown in Table 3.76.

Table 3.76 BLM-constants for chronic zinc toxicity to P. subcapitata

	Chronic
Log K_{ZnBL} (a)	0.538 pH +2.25
$f_{ZnBL}^{50\%}$ (b)	0.454±0.038
f_{ZnBL}^{NOEC} (b)	0.143±0.037

Since critical biotic ligand concentrations of zinc for *P. subcapitata*are pH-dependent and covered by the stability constant for ZnBL, 10the constants for the other competing cations were of negligible importance

mean ± one-sided 95% confidence limit

Overall this study consistently illustrates the importance of bioavailability parameters for chronic toxicity of zinc to rainbow trout, Daphnids and algae and demonstrates that changes in zinc bioavailability to aquatic organisms can be quantified and predicted. Quantitative differences were noted with regard to the effect of the individual parameters on chronic toxicity across the three organisms. First, the toxicity differences, caused by bioavailability parameters are highest for algae (factor 100) and lower for fish (factor 20) and Daphnids (factor 4). For algae the pH effect was the most important while the effects of Ca, Mg and Na

were negligible. For Daphnids, hardness and pH seemed to be equally important, whereas for rainbow trout the effect of Ca was more important than the effect of pH. The DOC-effect seemed to be most pronounced for algae and similar for Daphnids and rainbow trout. Despite of these differences, BLMs for all three organisms were able to take into account these differences. The BLMs were able to significantly reduce the variation associated with the effect concentrations, i.e. chronic effect concentrations were generally predicted within a factor two from the observed values for all organisms studied, for both lab waters and field waters.

In summary, the studies where the BLMs were developed, revealed valuable information on binding constants of H^+, Ca^{2+}, Mg^{2+}, Na^+ and Zn^{2+} with the biotic ligands, for each of the studied aquatic organisms. These were all laboratory studies using artificial water. The binding constants were derived at a level where zinc showed chronic toxicity. The information on the binding constants is necessary for running the Biotic Ligand Models. All binding constants were found to be independent on other variables, except the binding constant for zinc with the algae, which appeared to be pH-dependent. The internal validation of the model, i.e. comparing the model output with the experimental output, showed that predictions were within a factor of two of the experimental values, thus showing a very well performance of the model. However, it must be noted that experimental values could vary within a factor of two as well.

In the validation studies, several field waters from several sites from Europe were tested and chronic toxicity of zinc was measured in these waters with the same three organisms. The variability of three main water characteristics of these field waters was as follows and covers a great portion of the European freshwaters (De Schamphelaere et al. 2003): DOC ranged between 4.8 and 27.4 mg/L, pH ranged between 5.2 and 8.4, and hardness ranged between 2.5 and 238 mg/L as $CaCO_3$. The field waters contained varying compositions of the cations, i.e. H^+, Ca^{2+}, Mg^{2+}, Na^+, that were studied in the series of studies that were used for developing the BLMs. In addition, the field waters contained dissolved organic matter. The researchers assumed that the dissolved organic carbon as measured in the field almost completely, i.e. 99.9%, consisted of fulvic acids and took the binding constant of zinc to fulvic acids from the literature. Thus, only 0.1% of the DOC was assumed to consist of humic acids. Again, predictions were within a factor of two of the experimental values for the algae and fish studies, thus showing a very well performance of the model. Koukal et al. (2003) postulated several explanations to account for their observed results where Suwannee River fulvic acids (SWFA) did not affect toxicity of zinc to *Pseudokirchneriella subcapitata*, while the presence of soil and peat humic acids did. They argued that the SWFA complexes with zinc are labile and undergo rapid dissociation or that these fulvic acids coagulated which altered metal complexing behaviour, or that fulvic acids has a lower ability to adsorb on cell membranes at pH > 7. The stronger reduction in toxicity of the humic acids was postulated to be explained by a reduced bioavailability due to the zinc-humic acid complexes and due to adsorption of the humic acid to the algal surfaces, shielding the cells from free zinc ions. Depending on the origin of fulvic acids and the pH of the water a different complexing ability may thus arise.

It is thus concluded that the BLMs developed in this study reduce the variation in toxicity, which is probably due to differences in Zn bioavailability, from up to a factor 100 to a factor of 2. Increasing the DOC, Ca^{2+}, Mg^{2+}, or Na^+ concentrations lead to lower toxicity for all three trophic levels. In the case when acidity (H^+) increases or pH drops, toxicity increases for Daphnia and fish, while it leads to lower toxicity for algae.

Implementation of the BLMs in the current risk assessment report

It is concluded that the BLMs developed in the study by De Schamphelaere et al. (2003) and Heijerick et al. (2003) reduce the toxicity variation (due to differences in Zn bioavailability) from up to a factor 100 to a factor of 2. Therefore, there is a scientific basis that the incorporation of the developed BLMs into the current risk assessment makes them relevant for assessing the possible impact of zinc on the environment.

The following stepwise approach is taken for implementing the BLMs for correcting for the bioavailability of zinc in surface waters for those sites or regions that have a $PEC_{add}/PNEC_{add} > 1$, when no bioavailability correction would be applied (Figure 3.17). If the $PEC_{add}/PNEC_{add} < 1$, conclusion (ii) is reached. The bioavailability correction will be applied to the PEC_{add}, and not to the $PNEC_{add}$. One of the main reasons for correcting the PEC_{add} is that no BLMs are available for each individual organism from the ecotoxicity database.

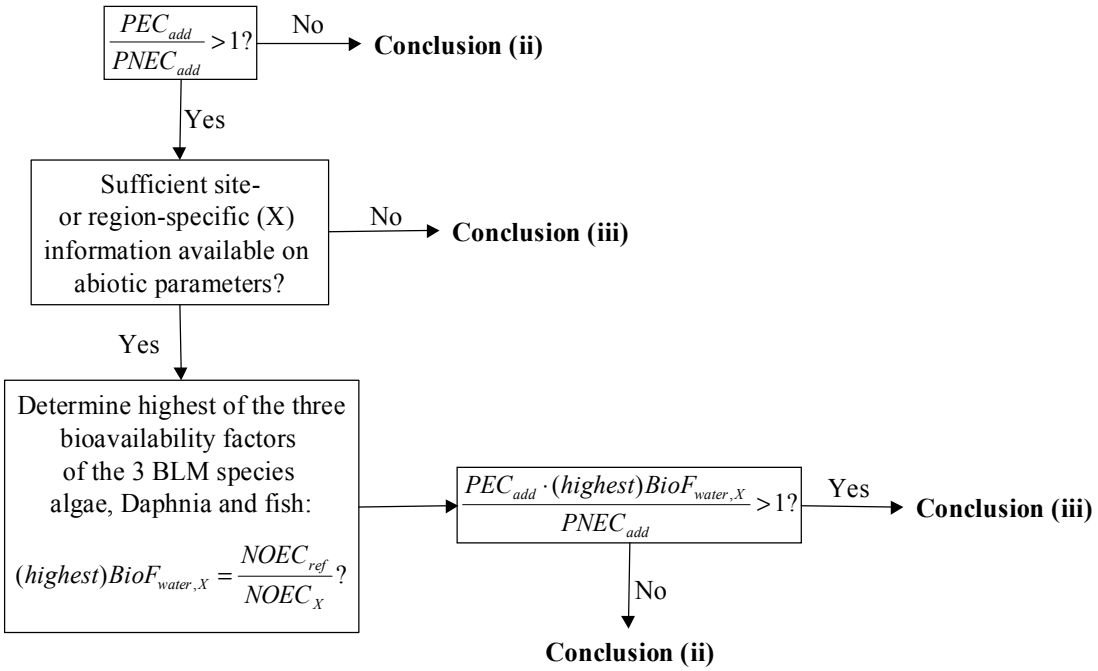


Figure 3.17 Decision tree for correcting the PEC_{add} for reduced bioavailability in water using Biotic Ligand Models (BLMs).

Firstly, the chronic NOEC values for algae, Daphnia and fish (the 3 BLM species) need to be predicted at a site or a region X, using the BLMs for the three aquatic species (De Schamphelaere et al., 2003) under the site-specific conditions or water chemistry of that site or region. This will result in $NOEC_x$ values for that site or region. If no sufficient site or region-specific information on the abiotic parameters is available, no bioavailability correction is possible, and conclusion (iii) will be reached.

The chronic $NOEC_x$ values then need to be compared with a reference NOEC value ($NOEC_{ref}$). This $NOEC_{ref}$ value is calculated using the BLMs under reference water chemistry conditions (see Table 3.77). The reference water chemistry conditions (ref) are taken from the GEMS-B database (see Table 4.11 in Heijerick et al., 2003), and are selected as follows. For all organisms: 10^{th} percentile of DOC. For *D. magna and O. mykiss*: 10^{th} percentile of inorganic parameters (including pH and hardness). For *P. subcapitata*: 90^{th} percentile of inorganic parameters (including pH and hardness).

Table 3.77 Summary of reference NOEC values ($NOEC_{ref}$) for the three aquatic species for which BLMs have been developed under reference water chemistry conditions[19].

Species	$NOEC_{ref}$
O. mykiss	184
D. magna	86
P. subcapitata	21

This $NOEC_{ref}$ is a reasonable worst-case situation that mimics the situation where bioavailability of zinc is very high and thus can be regarded as a reference value for the bioavailability at the site or region X. The NOEC at the site or region X ($NOEC_X$) is then regarded as a surrogate for the actual bioavailable concentration of zinc at that site or region X, and is calculated with the BLM-models for the alga, Daphnid and fish. Furthermore, the BLM models provide sufficiently conservative outcomes that are in good accordance with the generic PNEC. Moreover, the BLM estimates generally overestimate toxicity, i.e. the predicted NOECs are lower than the experimental values, which provides further support that the BLMs result in sufficiently conservative outcomes. Therefore, the BLMs are regarded as being sufficiently validated and sufficiently conservative.

The bioavailability factors (BioF) are then derived for each of the 3 BLM species as follows:

$$BioF_{water,X} = \frac{NOEC_{ref}}{NOEC_x}$$

The highest value of the three $BioF_{water,X}$ values for the three species is selected to ensure that a conservative approach and bioavailability factor (BioF) is taken, i.e. the smallest correction for bioavailability.

Then, the bioavailability correction to the PECadd at the site or region X can be made. If needed the total PEC concentration (PEC_{total}) must be recalculated to the dissolved PEC concentration ($PEC_{dissolved}$) prior to this bioavailability correction to the PECadd:

$$PEC_{dissolved} = \frac{PEC_{total}}{[1 + K_d \cdot Cs \cdot 10^{-6}]}$$

Then, the zinc background concentration needs to be subtracted from the measured zinc monitoring data:

$$PEC_{add} = PEC_{dissolved} - Cb_{dissolved}$$

[19] The reference water chemistry conditions (ref) are taken from the GEMS-B database (see Table 4.11 in Heijerick et al., 2003). The water chemistry conditions are selected as follows. For all organisms: 10th percentile of DOC. For *D. magna and O. mykiss*: 10th percentile of inorganic parameters (including pH and hardness). For *P. subcapitata*: 90th percentile of inorganic parameters (including pH and hardness).

The bioavailable concentration of the added zinc concentration in the water at the site or region X can be calculated from

$$PEC_{add,\ bioavailable} = PEC_{add} \times BioF_{water,X}$$

Subsequently, the risks quotients (RCR) are calculated from:

$$RCR = PEC_{add,bioavailable} / PNEC_{add}$$

It must be noted that the precondition for following the approach as described above is that information on the relevant abiotic factors or water chemistry is available or can be estimated. The following situations may occur:

1. For regional exposure: (a) big rivers with a good description and characterisation of the abiotic factors; these data can be used as input for the bioavailability translator; (b) when no data on the abiotic factors are available, a regional analysis may be used.
2. For local exposure: when data are available, they should be used, otherwise data from regional analyses could be used.

In case no reliable information on the abiotic factors is available, then no bioavailability correction can take place and the PEC_{add} – not corrected for bioavailability - will be compared to the $PNEC_{add}$.

Since there are some uncertainties in the approach presented on using the BLMs in correcting the measured concentrations to take into account bioavailability, various options will be taken forward to the risk characterisation section. The first option will be the non-corrected PEC_{add}, other options will be to include the bioavailable corrected PEC_{add}, using different combinations of abiotic parameters as input.

Conclusions on abiotic factors

It is concluded that there is a too poor basis to correct the PNEC or to correct the PECs based solely on one of the water chemistry properties. In other words a univariate background, a univariate pH dependent, or a univariate hardness dependent PNEC or PEC cannot be used. However, the integrative Biotic Ligand Model that incorporates various mitigating factors will be used to take into account bioavailability of zinc in surface waters, and to correct the PECs, where appropriate.

To further take into account some uncertainty in various parameters as well as to provide some ideas on the sensitivity of the calculations, three scenarios will be used in the risk characterisation when showing the bioavailability corrections:

a) the first scenario will be when no bioavailability correction will be used, i.e. the PECadd will be completely based on the added, dissolved zinc concentration;
b) the second scenario will make use of the Biotic Ligand Models in a conservative way, i.e. by selecting the 90^{th}-percentile value of the added, dissolved zinc concentration in the water. In addition, for the BLM for algae, the 10^{th}-percentile value of the DOC and the 90^{th}-percentile values of all other abiotic parameters will be used, and for the

BLMs for Daphnia[20] and fish, the 10th-percentile value of the DOC and the 10th-percentile values of all other abiotic parameters will be used; and

c) the third scenario will make use of the Biotic Ligand Models in a less conservative way, i.e. by selecting the 90th-percentile value of the added, dissolved zinc concentration in the water, the 50th-percentile value of the DOC and the 50th-percentile values of all other abiotic parameters.

These scenarios are generic scenarios and will not cover e.g. temporal variations, high input of acid and zinc after snowmelt.

3.3.2.1.2 Acute toxicity to aquatic organisms

The soluble test compounds used in both the freshwater and saltwater short-term studies that are summarised below usually were (hydrated) zinc sulphate or zinc chloride. Occasionally other soluble zinc compounds were used.

The wide range of acute LC50 and EC50 values can amongst others be ascribed to the wide range of test waters, and include both nominal (added) and actual (measured) concentrations. For additional data on abiotic factors influencing the acute toxicity of zinc, see e.g. U.S. EPA (1987) and Janus (1993).

Acute toxicity to freshwater organisms

Skidmore and Firth (1983) reviewed the results of acute toxicity tests with invertebrates and fish from the Northern Hemisphere. In this review information on the test compounds which were used is not reported, but it is assumed that the test compounds were soluble zinc salts. For invertebrates (13 tests), 24/96-h LC50 values range from 0.04 to 32 mg/l. For fish these values range from 0.14 to 40 mg/l. Thirteen out of the 52 tests (25%) resulted in LC50 values <1 mg/l; the majority of the tests resulted in values ranging from 1 to 10 mg/l.

The U.S. EPA (1987) reports acute LC50 and EC50 values, which range from 0.032 to 41 mg/l for invertebrates (47 tests), and which range from 0.066 to 41 mg/l for fish (127 tests), two exceptionally high values (up to 300 mg/l) for the guppy excluded (U.S. EPA, 1987). The tests were selected on the basis of U.S. EPA Guidelines. It must be noted that in an earlier U.S. EPA report (U.S. EPA, 1980), acute LC50 and EC50 values >60 mg/l were reported for some invertebrate species (worm and insect species).

The reader is referred back to section 1.3.2 for additional acute toxicity data. This section also contains the data that were actually selected for the current classification and labelling proposals of zinc (and zinc compounds).

Acute toxicity to saltwater organisms

The combined data reported by Mance (1987) and by the U.S. EPA (1987) show 24/96-h LC50 and EC50 values of 0.17 to 950 mg/l for invertebrates. Most of these values range from about 1 to 10 mg/l, but a number of these values is below 0.5 mg/l. Lower LC50 and EC50 values, 0.065 to 0.12 mg/l, have been reported for early life stages of invertebrates (Janus, 1993). Fish generally appear to be less sensitive than invertebrates. The combined data

20 According to the decision tree (Figure 3.3.2.1.5) the highest BioF value of the three BLM calculations is to be taken for the bioavailability correction to the PECadd. However, since the BLM results from the Daphnia will always be in between those from the algae and the fish, the BLM values for the Daphnia will not be shown in the risk characterisation section.

reported by Mance (1987) and by the U.S. EPA (1987) show acute LC50 and EC50 values of 0.19 to 83 mg/l for fish, the majority of the values ranging from 3 to 30 mg/l.

3.3.2.1.3 Chronic toxicity to aquatic organisms

The test compounds used in both the freshwater and saltwater long-term studies that are summarised below were soluble zinc salts, except "insoluble" zinc metal powder (used in one study) and zinc oxide (used in two studies). In a number of references the test compound used in the study was not reported, but it is assumed, and sometimes reported, that a soluble zinc salt was used. All toxicity data underlying this section are listed in the Tables 3.3.2.a to 3.3.2.d in Annex 3.3.2.A.

All tests are single-species laboratory tests with water-only exposure, with the exception of the tests summarised in Table 3.3.2.e (tests in sediment-water systems). The results of two of the chronic tests in sediment-water systems in which detailed data on the actual exposure concentration in the overlying water or pore water were reported, are also included in Part I of Table 3.3.2.a and included in the data set of NOEC values used for PNEC derivation. These tests are one with the amphipod *Hyalella azteca* reported by Borgmann and Norwood (1997) and one with the midge *Chironomus tentans* reported by Sibley et al. (1996). Unless stated otherwise the results refer to the zinc concentration in water (µg/l) or sediment (mg/kg dry weight).

Chronic toxicity to freshwater organisms

Data on chronic toxicity tests resulting in NOEC values for freshwater algae, invertebrates and fish are summarised in Table 3.3.2.a (Annex 3.3.2.A). The "species mean" NOEC values, based on studies that were used for PNEC derivation (freshwater $PNEC_{add, aquatic}$), range from 17 to 660 µg/l, see Table 3.78, Figure 3.3.2.1.5-A, and the underlined values in Part I of Table 3.3.2.a[21].

Based on quality (Q) or relevance (R) criteria a number of studies listed in Table 3.3.2.a (Annex 3.3.3.A) are considered not useful for PNEC derivation (see sections 3.3.1.1 and 3.3.2.1 for general information on the selection criteria used). The rejected studies, all listed in Part II of Table 3.3.2.a, have the annotation Q or R, indicating the reason for not using the study. With respect to the annotation R, the specific relevance criterion or criteria used to reject the study can be derived directly from the reported items in Table 3.3.2.a, for example the lack of data on pH and/or hardness in an artificial test medium, or a hardness value that is below the minimum value used as selection criterion. In some cases the reason(s) for not using a study cannot be derived directly from Table 3.3.2.a, especially in case of the annotation Q. In all cases, specific information on the reason(s) to reject a study can be found in the footnotes of Table 3.3.2.a. When a study is used for PNEC derivation despite it does not meet all Q and/or R criteria, specific information can be found in the footnote as well. If reported by the original study, the footnote also provides additional information, which is important with respect to the quality and relevance criteria, such as the culture and test conditions (including background zinc concentrations).

21 The "species mean" NOEC is the geometric mean value, in case more than one NOEC for the same toxicological endpoint is available for a specific species. In case there is only one test for a specific organism, the "species mean" NOEC simply is the NOEC (for the most sensitive endpoint), derived from that test. With respect to the aquatic toxicity data base, "species mean" NOEC values are used as input in the ecotoxicological extrapolation methods to derive $PNEC_{add}$ values.

The NOEC values listed in Table 3.3.2.a are based on nominal (added) concentrations (Cn), if possible. In a number of studies the NOEC values are based on the actual concentrations; for most of these data, especially for the tests performed in artificial test waters, it is known that the background zinc concentration in the test water was very low compared to the concentrations tested, thus the actual concentrations will have been very similar to the nominal (i.e. added) concentrations. The test compounds were usually (hydrated) zinc sulphate, zinc chloride, and occasionally zinc nitrate. The water solubility of these compounds is orders of magnitude higher than the test concentrations used. In some references the test compound used in the study was not reported, but it is assumed that a soluble zinc salt was used. It is noted that the freshwater chronic data include three tests in which "insoluble" metallic zinc powder or zinc oxide was used. Of these three test, all three with the alga *Pseudokirchneriella subcapitata*, two tests were accepted for $PNEC_{add}$ derivation, see also hereafter.

Below, somewhat more detailed data are given on the "species mean" NOEC values for freshwater algae, invertebrates and fish (Table 3.3.2.a-Part I in Annex 3.3.2.A).

Algae

For freshwater unicellular algae there is only one "species mean" NOEC (17 µg/l, for *Pseudokirchneriella subcapitata*, formerly known as *Selenastrum capricornutum or Raphidocelis subcapitata*). This value is the geometric mean value of 25 NOEC values from different tests (endpoint growth) and is the lowest "species mean" NOEC in the freshwater database. In two of these tests an "insoluble" test compound was used, viz. metallic zinc powder (Van Woensel, 1994a) and zinc oxide (Van Ginneken, 1994a). As the NOEC values derived from these two tests are based on the actual dissolved-zinc concentration in the test solution, these NOEC values are included in the database used for $PNEC_{add,\ aquatic}$ derivation. In the further tests with this algal species, all from De Schamphelaere et al. (2003), $ZnCl_2$ was used as test compound.

For freshwater multicellular algae there is also only one "species mean" NOEC (60 µg/l, for the filamentous alga *Cladophora glomerata*). This "species mean" NOEC is based on only one test result (single NOEC from one test; endpoint growth), from Whitton et al. (1967).

All NOEC values from the algal growth inhibition tests listed in Table 3.3.2.a-Part I are from tests that were conducted in test waters containing a maximum concentration of the chelating agent EDTA of $10x10^{-3}$ mmol/l. This EDTA concentration is ten times higher than the permitted chelator concentration of $1x10^{-3}$ mmol/l, as mentioned in OECD guideline 201 (alga, growth inhibition test). The upper limit for the EDTA concentration ($10x10^{-3}$ mmol/l) used for the selection of the algal tests has been chosen arbitrarily (but considering the results of all algal tests, including the rejected tests, see Table 3.3.2.a-Part II), because high to very high EDTA concentrations can strongly reduce the toxicity of zinc (see also Janus, 1993).

Invertebrates

The "species mean" NOEC values for freshwater invertebrates range from 37 µg/l for the water flea *Ceriodaphnia dubia* (crustacean; geometric mean value of 13 NOEC values from different tests; endpoint reproduction) to 400 µg/l for the zebra mussel *Dreissena polymorpha* (molluscs; single NOEC from one test; endpoint survival). The data on freshwater invertebrates include porifera, mollucs, crustaceans and insects. Most data on freshwater invertebrates are available for the water flea species *Daphnia magna* and *Ceriodaphnia dubia* (crustaceans).

Fish

The "species mean" NOEC values for freshwater fish range from 44 µg/l for the flagfish *Jordanella floridae* (geometric mean value of 2 NOEC values from different tests; endpoint growth) to 660 µg/l for the zebrafish *Brachydanio rerio* (geometric mean value of 9 NOEC values from different tests; reproductive endpoint hatching).

Based on the "species mean" NOEC values for freshwater organisms and the underlying NOEC values from the different tests it is not possible to draw a sound conclusion on possible differences in sensitivity amongst the different taxonomic groups studied, because of the very limited number of test species within some taxonomic groups and the differences in test design, e.g. differences in test waters, or the origin or strain (clone) of the test species. Even in tests with a particular aquatic species from the same culture and tested in a series of (parallel) tests performed in one test laboratory, a (fairly) wide range of NOEC values have been found. For example, the study by De Schamphelaere et al. (2003) resulted in ranges of NOEC values of 25 to 974 µg/l for rainbow trout *Oncorhynchus mykiss* (12 tests), 4.9 to 124 µg/l for alga *Pseudokirchneriella subcapitata* (23 tests) and 48 to 155 µg/l for *Daphnia magna* (11 tests). A second example of a wide range of NOEC values is found in the ring test with the zebrafish *Brachydanio rerio*, in which the NOEC values for hatching ranged from 180 to 2900 µg/l (9 tests) and the NOEC values for survival ranged from 2900 to 11500 µg/l (10 tests).

The available data show a clear effect of abiotic factors on the aquatic toxicity of zinc (see section 3.3.2.1.1 for a comprehensive discussion on this issue), obscuring differences in species sensitivity. However, based on all available data it appears that unicellular algae may be more sensitive than invertebrates and fish.

Chronic toxicity to saltwater organisms

Data on chronic toxicity tests resulting in NOEC values for saltwater algae and invertebrates are summarised in Table 3.3.2.b (from Janus, 1993) in Annex 3.3.2.A. The "species mean" NOEC values used for PNEC derivation (saltwater $PNEC_{add,\ aquatic}$) range from 10 to 2700 µg/l, see Table 3.79, Figure 3.3.2.1.5-B and the underlined values in Part I of Table 3.3.2.b. Most values are based on nominal concentrations (Cn). The test compounds were (hydrated) zinc sulphate, zinc chloride, and in one test zinc nitrate. The water solubility of these compounds is orders of magnitude higher than the test concentrations used. In some references the test compound was not reported, but it is assumed that a soluble zinc salt was used in the study. Some tests that were rejected for PNEC derivation are summarised in Part II of Table 3.3.2.b.

Below, somewhat more detailed data are given on the "species mean" NOEC values for saltwater algae and invertebrates (Table 3.3.2.b–Part I in Annex 3.3.2.A). Useful data for saltwater fish were not available.

Algae

The "species mean" NOEC values for saltwater algae (all but one tests: unicellular algae) range from 10 µg/l for *Schroederella schroederi* (single NOEC from one test) and *Thalassiosira rotula* (single NOEC from one test) to 2700 µg/l for *Phaeodactylum tricornutum* (geometric mean value of 3 NOEC values from different tests).

Invertebrates

The "species mean" NOEC values for saltwater invertebrates range from 10 µg/l for the echinoderm *Arbacia lixula* (single NOEC from one test) to 1000 µg/l for the mollusc *Scrobicularia plana* (single value from one test). The data on saltwater invertebrates include coelenterates, annelids, molluscs, crustaceans and echinoderms.

Based on the "species mean" NOEC values for saltwater organisms and the underlying NOEC values from the different tests it is not possible to draw a sound conclusion on possible differences in sensitivity amongst the different taxonomic groups studied, because of the limited number of test species within some groups and the differences in test design (see also freshwater). As in freshwater there is a wide range of NOEC values. Based on all available data it appears that some species of algae are among the most sensitive organisms.

3.3.2.1.4 Mesocosm and field studies

It is important to relate the results from single-species toxicity data from laboratory tests (see section 3.3.2) with the results of (model) ecosystem studies and field studies. The present section will describe the available literature on model ecosystems and field studies.

In Table 3.3.2.i-Part A (field studies) and Table 3.3.2.i-Part B (laboratory studies) in Annex 3.3.2.B an overview of freshwater (model) ecosystem studies is given. The data include the two zinc studies that are included in Emans et al. (1992, 1993) and the six zinc studies (of which four studies are underlying the data for only one model ecosystem) that are included in Versteeg et al. (1999). These two publications compared for a number of organic compounds and metals (Zn included) the results of single-species laboratory data (NOEC values and the results of several statistical extrapolation methods, including 5th percentile values calculated from the distribution(s) of NOEC values) with the results of (model) ecosystem studies. These two publications resulted in the general conclusion that there appears to be no significant difference in sensitivity in the laboratory compared to the (semi-) field condition and furthermore, that results of statistical extrapolation methods such as median 5th percentile values appear to be sufficiently protective to (model) ecosystems. However, this general conclusion cannot be applied simply to the currently available single-species toxicity data for zinc (including the median 5th percentile value, see section 3.3.2.1.5) and multiple-species toxicity data for zinc (see below), as the databases used by Emans et al. (1992, 1993) and Versteeg et al. (1999) were more limited than the databases in this RAR (see further section 3.3.2.1.5).

The available (model) ecosystem data for zinc are limited to a small number of different (model) ecosystems and usually limited to periphyton (bacteria and or algae); only in two systems invertebrates were included.

Below the main results of the studies in Table 3.3.2.i (Annex 3.3.2.B) are summarised.

- A field study in outdoor artificial streams resulted in a nominal Multi-Species NOEC of 25 µg/l (actual total-Zn concentration: ≤20 µg/l [detection limit]); the nominal LOEC was 50 µg/l (actual total-Zn concentration: 34-87 µg/l). The study was performed in New river water with a pH of 8.1-8.4 and a hardness of 66-89 mg/l. In this model ecosystem, effects on periphyton, zooplankton and macro-invertebrates (clams and snails) were studied in several tests; the results were reported in Belanger et al. (1986), Genter et al. (1987) and Farris et al. (1989, 1994) (Table 3.3.2.i – Part A in Annex 3.3.2.B).
- A field study with phyto- and zooplankton resulted in effects on several endpoints, including quantitative analysis of zooplankton, at an actual total-Zn concentration of 17 µg/l (actual dissolved-Zn concentration: 16 µg/l), the lowest concentration tested (LOEC). The study was performed in Lake Michigan (hardness around 70 mg/l; pH not reported) (Marshall et al., 1983; Table 3.3.2.i – Part A in Annex 3.3.2.B).

- *From this study, Emans et al. (1992, 1993) derived a Multi-Species NOEC of 1.7 µg/l, using NOEC = LOEC/10. Based on the criteria used for NOEC derivation in this RAR (see section 3.3.1.2) this estimated NOEC is considered to be unreliable, due to the too high extrapolation factor of 10.*
- A study in a laboratory flow-through system with periphyton resulted in a NOEC of around 10 µg/l (actual total-Zn concentration) for the most sensitive, biomass-related endpoints: bacterial activity (^{3}H-incorporation), periphyton photosynthetic activity (^{14}C-incorporation) and periphyton dry weight. The study was performend in river Göta Älv water with a pH of 6.1-7.1 and a hardness of around 24 mg/l. The NOEC for algal biomass (chlorophyll *a* content) and species richness (the number of different taxa or groups of taxa) was 27 µg/l) and that for species composition (relative abundance) was 117 µg/l. According to the authors of the study (Paulsson et al., 2000a) the high sensitivity of the biomass-related endpoints is probably due to an indirect effect, i.e. the interaction of zinc and phosphorus, leading to nutrient depletion. This is supported by the lower sensitivity of community structure and also indicated by the much higher NOEC for the PICT (pollution induced community tolerance) response: 630 µg/l (Paulsson et al., 2000a; Table 3.3.2.i – Part B in Annex 3.3.2.B).
- Two further studies in a laboratory flow-through system with periphyton resulted in effects on biomass-related endpoints at actual dissolved-Zn concentrations of 73 µg/l (nominal: 50 µg/l) and 4.2 µg/l (nominal: 3 µg/l), respectively, the lowest concentrations tested (LOEC values). The tests were performed in dechlorinated tap water with a pH of 7.8-8.0 and a hardness of 65-74 mg/l (Niederlehner & Cairns, 1993; Pratt et al., 1987; Table 3.3.2.i – Part B in Annex 3.3.2.B). In the former study, species richness was not significantly affected at 73 µg/l. In the latter study, species richness was lower at 4.2 µg/l than the control value, but no statistics were reported for this endpoint. *From these two studies, Versteeg et al. (1999) derived Multi-Species NOEC values of 73 and 10 µg/l, respectively, probably based on species richness.*

- A laboratory study with phytoplankton resulted in inhibition of photosynthesis at nominal total-Zn concentrations of 27 and 21 µg/l in Lake Alpnach water (pH 7.6-8.7; hardness 280-340 mg/l) and Lake of Lucerne water (pH 7.6-8.7; hardness 170-220 mg/l), respectively (LOEC values) (Gächter, 1976; Table 3.3.2.i – Part B in Annex 3.3.2.B). The LOEC values are the geometric mean EC(20%) values. The estimated NOEC values are 14 and 11 µg/l, respectively (NOEC = LOEC/2).
  From this study, Emans et al. (1992, 1993) derived a Multi-Species NOEC of 4.3 µg/l. It is not clear where this value is based on. In the publication of Gächter (1976) it is stated that phytoplankton photosynthesis was not adversely affected if the concentration increase above the background levels did not exceed 5.10^{-8} mole Zn/l. which is 3.3 µg/l. It may be that Emans and al. (1993) considered this value to be NOEC and made a typing errror or added the lowest background zinc concentration (1.0 µg/l) to the value of 3.3 µg/l.

Data on a study with phytoplankton (Nosov, 1981), and an outdoor study with periphitic communities (bacteria, fungi, algae and ciliate protozoans) (Williams & Mount, 1965) are given as additional information in Annex 3.3.2.i.. These studies are not useful for the derivation of a NOEC or LOEC, amongst others since no quantitative results can be derived from these studies.

In conclusion, the field and mesocosm studies thus show effects of zinc in the low range of 10-20 µg/l, and depending on the endpoint also at higher concentrations. The range of 10-20 µg/l includes measured total-Zn concentrations.

3.3.2.1.5 PNECadd for surface water (PNECadd, aquatic)

As stated in section 3.3.2.1 the freshwater and saltwater data are not combined to derive the $PNEC_{add,\ aquatic}$, but this value will be derived from the freshwater data only. $PNEC_{add}$ values for surface water were derived from the geometric "species mean" NOEC values, using the two different extrapolation methods described in section 3.3.1.3, i.e. the use of an assessment factor and statistical extrapolation, with several calculations for the latter methods, using different frequency distribution functions. For comparison, the $PNEC_{add,\ aquatic}$ values derived from the saltwater data are given.

The underlined "species mean" NOEC values for freshwater organisms (n = 18) listed in Part I of Table 3.3.2.a in Annex 3.3.2.A and for saltwater organisms (n = 28) listed in Part I of Table 3.3.2.b in Annex 3.3.2.A were used in the calculations using statistical extrapolation. These "species mean" NOEC values are also shown in Tables 3.78 and 3.79 for freshwater and marine species, respectively. For the species sensitivity distributions, see Figures 3.3.2.1.5-A (freshwater organisms) and 3.3.2.1.5-B (saltwater organisms).

Table 3.78 "Species mean" NOEC values that are used as input values for deriving the 5th percentile values as a basis for the freshwater $PNEC_{add,\ aquatic}$.

Taxonomic groups	"Species mean" NOEC values (µg/l)
Algae (unicellular)	17
Algae (multicellular)	60
Poriferans	43; 43; 43; 65
Molluscs	75; 400
Crustaceans	37; 42; 88
Insects	137
Fish	44; 50; 78; 189; 530; 660

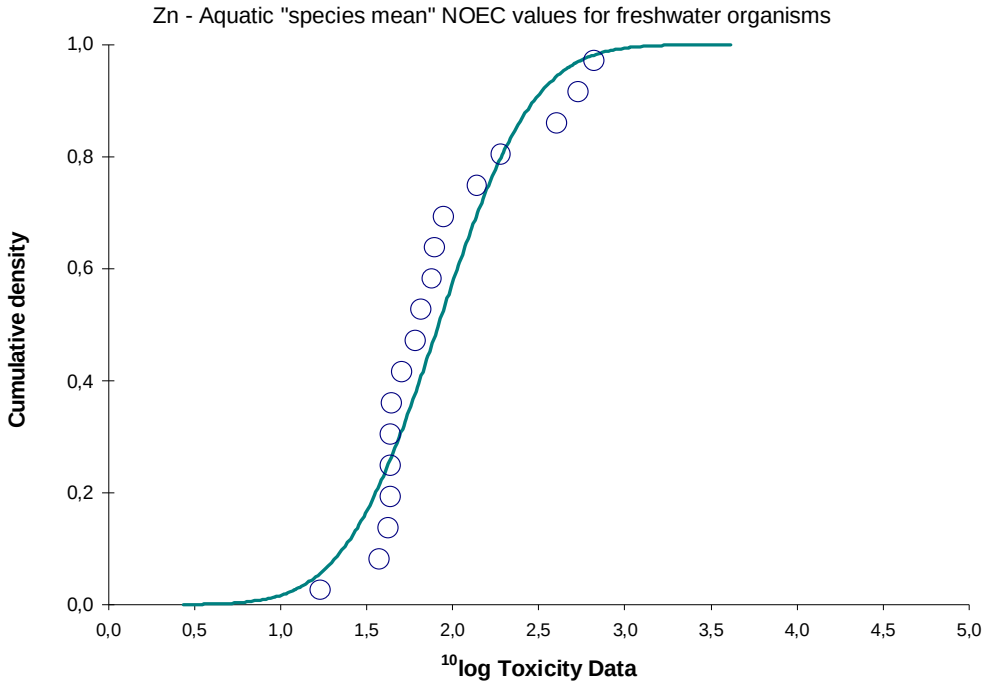


Figure 3.18 Freshwater organisms: species sensitivity distribution based on "species mean" chronic NOEC values.

Table 3.79 "Species mean" NOEC values that are used as input values for deriving the 5th percentile values as a basis for the saltwater $PNEC_{add, aquatic}$.

"Species"	"Species mean" NOEC values (µg/l)
Algae (unicellular)	10; 10; 10, 15; 15; 20; 32; 100; 100; 100; 140; 200; 500; 2700
Algae (multicellular)	100
Coelenterates	300
Annelids	100; 100; 100; 320
Molluscs	19; 50; 50; 1000
Crustaceans	18; 120; 440
Echinoderms	10

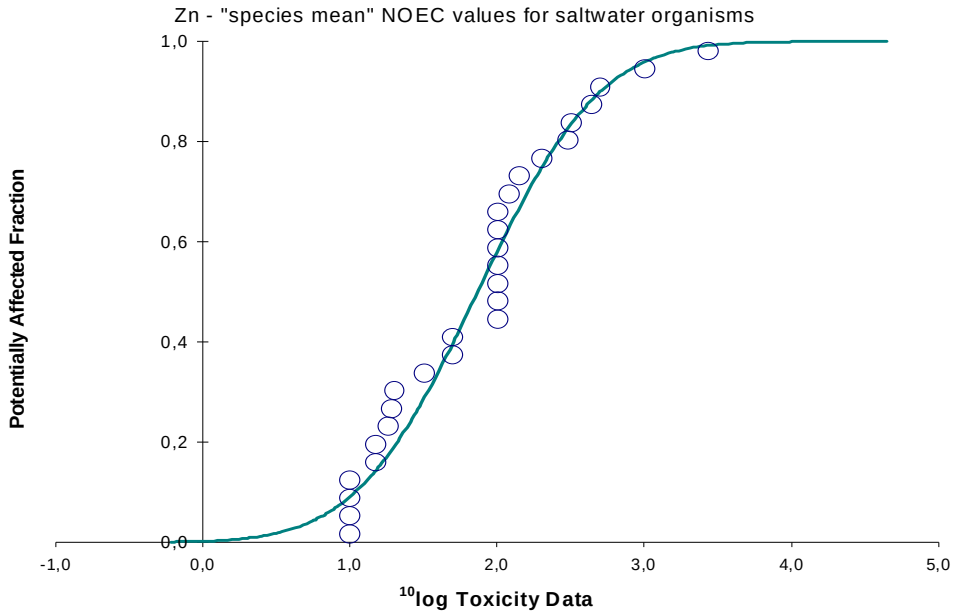


Figure 3.19 Saltwater organisms: species sensitivity distribution based on "species mean" chronic NOEC values.

The results of the different calculations are shown in Table 3.80, and footnotes. The use of an assessment factor of 10 according to the TGD results in a $PNEC_{add,\ aquatic}$ of 1.7 µg/l for freshwater and 1.0 µg/l for saltwater. The use of statistical extrapolation results in median 5th percentile values (and "equivalent" values, see footnotes) ranging from 15 to 21 µg/l and 6 to 10 µg/l for freshwater and saltwater respectively. All results are for dissolved zinc (see section 3.3.1.1).

Table 3.80 Lowest NOEC and 5th percentile values in case of statistical extrapolation. (All values in µg/l, for dissolved-Zn)

	Lowest NOEC	**(Lowest NOEC)/10**	**5th percentile log-normal**	**5th percentile log-logistic**
Freshwater (n =18) [1]	17	1.7	15.6 (median) *7.2 (lower 95% CI)* *26.2 (higher 95% CI)*	15.4 (median) *6.4 (lower 95% CI)*
Saltwater (n = 28) [2]	10	1.0	6.1 (median) *2.6 (lower 95% CI)* *11.6 (higher 95% CI)*	6.1 (median) *2.3 (lower 95% CI)*

[1]: **Freshwater:** Using the Anderson-Darling Goodness-of-Fit test for normality (modified A^2), a log-normal distribution is accepted at a significance level of 1%, indicating that the probability that these data derive from a log-normal distribution is small (1%). Using the Kolmogorov-Smirnov test, both a log-normal and a log-logistic distribution are accepted at significance levels up to 5%, thus accepting both distributions.

Results non-parametric 5th percentile value estimate: Referring to the overall and enlarged probability plots, we can interpolate at the cumulative density of 0.05 to find non-parametric estimates, but not very reliable for this small sample size, i.e. small with respect to the use of non-parametric extrapolation. For the freshwater "species mean" NOEC values (18 data points), the cumulative density of 0.05 is just below the 1th data point (17 µg/l), resulting in an estimated 5th percentile value of around 15 µg/l. Linear interpolation for log-concentrations yields a 5th percentile value of 17.3 µg/l. It must be noted that the non-parametric extrapolation method is under discussion for relatively small sample sets, because in such a case this method does not efficiently use the information on the entire 'tail' but heavily relies on only the few datapoints at the left tail (Van der Hoeven, 2001).

Results triangular distribution (included in ETX 1.3a): "Chronic value" is 18.4 µg/l.

Results "extreme value" distribution: Using this distribution function, EURAS calculated a median 5th percentile value of 20.6 µg/l, with a lower and higher 95% CI of 13.3 and 31.6 µg/l, respectively (EURAS paper "Effects assessment: derivation PNECwater zinc", submitted as additional information to the industry comments of March 2004 on the draft RAR Zn Metal). According to the statistical evaluation performed by EURAS, this distribution function has a better fit than the log-normal distribution function and showes a smaller uncertainty around the 5th percentile value, as is shown by the difference between the median 5th percentile value and lower 95% CI: this difference is a factor of 1.5 when using the "extreme value" distribution and a factor of 2.2 when using the log-normal distribution function.

[2]: **Saltwater:** Using either the Anderson-Darling Goodness-of-Fit test for normality (modified A^2) or the Kolmogorov-Smirnov test, a log-normal distribution is accepted at significance levels up to 10%, indicating that the probability that these data derive from a log-normal distribution is high (10%). Using the Kolmogorov-Smirnov test, a log-logistic distribution is rejected at a significance level of 1%, indicating that the probability that these data derive from a log-logistic distribution is very small (<1%).

Results non-parametric 5th percentile value estimate: Referring to the overall and enlarged probability plots, we can interpolate at the cumulative density of 0.05 to find non-parametric estimates, but not very reliable for this small sample size, i.e. small with respect to the use of non-parametric extrapolation. For the saltwater "species mean" NOEC values (28 data points), the cumulative density of 0.05 is between the the 1th and 2nd data point, which are both 10 µg/l. Linear interpolation for log-concentrations also yields a 5th percentile value of 10 µg/l. It must be noted that the non-parametric extrapolation method is under discussion for relatively small sample sets, because in such a case this method does not efficiently use the information on the entire 'tail' but heavily relies on only the few datapoints at the left tail (Van der Hoeven, 2001).

Results triangular distribution (included in ETX 1.3a): "Chronic value" is 10 µg/l.

Predicted no effect concentration ($PNEC_{add, aquatic}$) for the aquatic compartment

A comparison of the database of freshwater "species mean" NOEC values with the major recommendations made at the January 2001 Workshop on statistical extrapolation (EC, 2001

; see also section 3.3.1.3) shows the following:

- The number of chronic NOEC values (n = 18; "species mean" NOEC values) meets the general requirement for the number of input data (minimum requirement: 10 NOEC values, preferably more than 15 NOEC values).
    - Chronic NOEC values are available for 1 unicellular algal species, 1 multicellular algal species (macro alga), 4 sponge species, 2 mollusc species, 3 crustacean species, 1 insect species and 6 fish species. The database includes all 8 taxonomic groups (families) mentioned in the EPA list that has been taken as a starting point. It was further recommended at the January 2001 Workshop to include primary producers (algae and higher plants) since primary producers are not included in the EPA list. In the database of accepted NOEC values, data for algae are included, but data for higher plants are lacking. However, data for freshwater higher plants are included in the database of rejected NOEC values. The rejected NOEC values for higher plants are from the following studies (see also Table 3.3.2.a–Part II (rejected studies) in Annex 3.3.2.A). A long-term study with four different species of freshwater higher plants *(Elodea nuttallii, Callitrische platycarpa, Spirodela polyrhiza and Lemna gibba)* resulted in unbounded NOEC values of $\geq$ 650 µg/l for all four plant species (endpoints: survival and growth). The plants used in this study were obtained from unpolluted ditches or ponds in the

Netherlands and grown in filtered ditch water with a pH of 8.0 (Van der Werff & Pruyt, 1982). A study with duckweed *Lemna minor* resulted in a NOEC of 160 µg/l (endpoint: growth) at pH 5 and hardness of 310 mg/l in artifial medium (Jenner & Janssen-Mommen, 1993). Tests with duckweed *Lemna pauciscostata* resulted in a NOEC of 5000 µg/l (endpoint: growth) at pH 4 or 5 and hardness of 700 mg/l in an artificial medium and tests in an other artificial medium resulted in about 60-80% growth inhibition at 1000 µg/l at pH 6 or 7 and hardness of 120 mg/l (Nasu & Kugimoto, 1981). From the data for these six plants species it can be concluded that aquatic higher plants do not appear to be very sensitive to zinc toxicity in comparison with algae or animals and thus the lack of useful NOEC values for higher plants is acceptable. Furthermore, the database of accepted NOEC values includes a relatively high NOEC (60 µg/l) for the macro alga *Cladophora glomerata* and macro algae resemble higher plants.

- It is noted that the Anderson-Darling test indicates that there is only goodness-of-fit for the log-normal distribution at a low significance level (1%). The Kolmogorov-Smirnov test, however, accepts both the log-normal and log-logistic distribution at a higher significance level (5%).

Based on the above, the use of statistical extrapolation is preferred for $PNEC_{add}$ derivation rather than the use of an assessment factor on the lowest NOEC. In accordance with the Workshop recommendation the 5th percentile value is set at the 50% confidence level, using a log-normal distribution function, which would result in a value of 15.6 µg/l for dissolved zinc in freshwater.

Based on uncertainty considerations the London workshop recommended to apply an assessment factor on the 50% confidence value of the 5th percentile value (thus PNEC = 5th percentile value/AF), with an AF between 5 and 1, to be judged on a case by case basis. Based on the available data, there are several reasons to use an assessment factor smaller than 5 and higher than 1:

- There is a relative large database, resulting in a relatively high reliability of the 5th percentile value. This is also shown by the small difference between the 50% confidence level and the 95% confidence limits found for the log-normal, log-logistic and "extreme value" distribution functions: in all cases: less than a factor of 2.5. This would support an AF smaller than 5.
- The median 5th percentile values calculated with the log-normal and the log-logistic distribution functions are nearly equal and also nearly equal to the result of the non-parametric distribution function (see Table 3.80 and footnotes), although with regard to the latter method is it noted that this method is under discussion for relatively small sample sets. The results of these three statistical extrapolation methods, being 15-15.6 µg/l, are somewhat lower than the results of two other statistical extrapolation methods used (the triangular and the "extreme values" distribution functions), being 18.4-20.9 µg/l. Based on this, there is no need for an assessment factor.
- The data are from tests in a variety of natural freshwaters, covering a considerable part of the wide range of freshwater types and freshwater characteristics (pH value, hardness and background zinc concentration) that are normally found in (European) freshwaters. Tests in natural freshwaters with characteristics that were not within the boundaries set for pH, hardness and background zinc concentration were excluded from the database. A number

of studies was not conducted in natural waters, but in artificial (reconstituted) freshwaters. Also tests in artificial waters with deviating characteristics were excluded, as well as tests in artificial waters without data on the characteristics. Therefore, the data properly reflect the European aquatic compartments. This would also support an AF smaller than 5.

- There are general indications that the bioavailability of metals under real life conditions can be lower than the bioavailability in the laboratory toxicity tests. On the one hand this is taken into account by comparing the dissolved concentrations at both the PEC and PNEC side. On the other hand, the dissolved fractions under real-life environmental conditions, may contain higher amounts of DOC and other complexing agents than in laboratory tests and the toxicity of zinc is reduced at higher DOC concentrations (see subsection "Biotic Ligand Model" in section 3.3.2.1.1). Based on this there is no need for an assessment factor. It is emphasised that the bioavailability related to DOC and other abiotic factors has been taken into account in the bioavailability factors that are applied on the PEC, see section 3.3.2.1.1.
- The median 5th percentile value of 15.6 µg/l may not be sufficiently protective, as in 15 of the 25 accepted tests with alga *Pseudokirchneriella subcapitata* and in one of the 13 accepted tests with crustacean *Ceriodaphnia dubia* a NOEC below this value was found. This would support an AF higher than 1, although the "species mean" NOEC values (17 µg/l for *P. subcapitata* and 37 µg/l for *C. dubia*) both are above the median 5th percentile value. Furthermore, all 15 tests with *P. subcapitata* that resulted in a NOEC below the median 5th percentile value (all from the study by DeSchamphelaere et al., 2003) were performed in artifial test water with a very low DOC concentration, while DOC was found to be an important mitigating factor for the toxicity of this algal species (see subsection "Biotic Ligand Model" in section 3.3.2.1.1). This would support an AF smaller than 5, but bigger than 1.
- The results of laboratory and field (model) ecosystem studies with zinc show that major effects on ecosystems are unlikely at the above-mentioned 5th percentile level. However, in some ecosystem or field studies, effects were found in the range of 10-20 µg/l (including measured total-Zn concentrations), i.e. effects on biomass-related endpoints. Thus, effects were found at or below the median 5th percentile value (15.6 µg/l). Effects on species richness, i.e. community structure, are less sensitive and were mostly found above the median 5th percentile level (see section 3.3.2.1.4 and Annex 3.3.2.B for an overview of the (model) ecosystem studies). This would support an AF smaller than 5, but bigger than 1.

It is noted that in Emans et al. (1992, 1993) and Versteeg et al. (1999) the databases of single-species NOEC values and ecosystem NOEC values for zinc were more limited than the databases in this RAR, especially regarding the single-species data.

- *Emans et al. (1992, 1993) derived median 5th percentile values of 3.5 and 4.0 µg/l (log-normal and log-logistic distribution, respectively; underlying single-species data not reported), thus 4-times lower than the median 5th percentile value derived in this RAR (15.6 µg/l) and Emans et al. (1992, 1993) included only two ecosystem NOEC values, being 1.7 and 4.3 µg/l. One of these two values is 2-times lower than the median 5th percentile values derived by Emans et al. (1992, 1993), although it is noted that both ecosystem NOEC values now are considered to be unreliable, as the NOEC = LOEC/10 (see section 3.3.2.1.4).*
- *Based on the single-species data used by Versteeg et al. (1999) the median 5th percentile value is about 7 µg/l, thus 2-times lower than the median 5th percentile value derived in this RAR (15.6 µg/l) and Versteeg et al. (1999) included only three ecosystem NOEC values, being 10, 20 and 73 µg/l, thus in this study the three*

ecosystem NOEC values were higher than the median 5th percentile value derived by Versteeg et al. (1999)[22].

Overall conclusion on $PNEC_{add, aquatic}$:

In conclusion, the above procedure results in a median 5th percentile value of 15.6 µg/l and justifies the use of an assessment factor of 2. Arguments for the factor 2 are provided above and result in a $PNEC_{add, aquatic}$ that is sufficiently protective for the most sensitive species and for the field situation. Thus, a **$PNEC_{add, aquatic}$ of 7.8 µg/l for dissolved zinc in freshwater** is proposed. This value is used in the risk assessment, which is aimed at freshwater. For pragmatic reasons it will also be used for a number of local marine scenarios.

In the risk characterisation, the freshwater PNECadd, aquatic will be applied for both freshwater (except for soft waters with a hardness <24 mg/l, see below) and for saltwater, as no saltwater $PNEC_{add, aquatic}$ was derived, see section 3.3.2.1. Although there are sufficient NOEC values available for saltwater organisms to apply statistical extrapolation and a 5th percentile value for saltwater was calculated in this RAR, the 5th percentile value for saltwater is considered to be too unreliable for saltwater $PNEC_{add, aquatic}$ derivation, because the saltwater NOEC values (from Janus, 1993) were not updated and not checked for reliability based on the criteria that have been used in this RAR for the freshwater NOEC values (only unbounded NOEC values from tests with saltwater organisms were rejected, as those for freshwater organisms). Although the comparison of the 5th percentile value based on the freshwater NOEC values and that based on the saltwater NOEC values may suggest that saltwater organisms are more sensitive to zinc than freshwater organisms, a sound comparison between the toxicity of zinc to freshwater and saltwater organisms cannot be made, as the saltwater NOEC values were not checked for reliability. According to the comments made by the UK Competent Authority, the issue of the sensitivity to zinc of saltwater organisms versus that of freshwater organisms may be considered further in the EU Water Framework Directive.

The fraction of zinc that is dissolved in surface water depends on abiotic factors, especially the suspended matter concentration (C_{susp}). Hence, no single value can be given for the $PNEC_{add, aquatic}$ expressed as total zinc. In the TGD a C_{susp} of 15 mg/l is used for "standard" surface water (freshwater). From this C_{susp} and a Kp_{susp} of 110,000 l/kg (median partition coefficient for the distribution between solid particulate matter and water, see section 3.2.1) and the $PNEC_{add, aquatic}$ for dissolved zinc in freshwater (7.8 µg/l), a $PNEC_{add, aquatic}$ of 21 µg/l is calculated for total zinc in freshwater. When a C_{susp} of 30 mg/l is used, a $PNEC_{add, aquatic}$ of 34 µg/l is calculated for total zinc in freshwater.

Predicted no effect concentration ($PNEC_{add, aquatic}$) for soft water

22 Versteeg et al. (1999) reported both the original "single-species" NOEC values and ecosystem NOEC values and the "corrected" values (normalised to a water hardness of 50 mg/l, as $CaCO_3$. using the U.S. EPA normalisation method). For the comparion of the "single-species" data and ecosystem data the normalised NOEC values were used. Actual figures (e.g. median 5th percentile value) were not reported, but the "single-species" NOEC values were plotted as log-logistic distribution function (median and lower 95% confidence interval) of the "species mean" NOEC values and the geometric mean and 95% lower and upper confidence interval of the ecosystem NOEC values. Note that the "single-species" data used by Versteegh are actual "single-genera" data, as genera instead of species were used as the lower taxonomic classification.

Introduction

It is realised that the used ranges of the selection criteria for pH, hardness and background concentration of zinc will not cover all European aquatic systems. Especially for soft waters, defined as waters with a hardness below 24 mg $CaCO_3$/l), the generic $PNEC_{add,\ aquatic}$ of 7.8 µg/l (dissolved Zn) is considered to be not sufficiently protective. Therefore, a soft water $PNEC_{add,\ aquatic}$ has been derived from the generic $PNEC_{add,\ aquatic}$, by dividing the generic $PNEC_{add,\ aquatic}$ by a 'water effect ratio' (WER), thus:

soft-water $PNEC_{add,\ aquatic}$ (dissolved-Zn) = generic $PNEC_{add,\ aquatic}$ (dissolved-Zn)/WER

The 'water effect ratio' (WER)[23] has been derived from the soft water testing programme (Muyssen et al., 2003; Källqvist et al., 2003), in which the toxicity of zinc for alga *Pseudokirchneriella subcapitata,* daphnid *Daphnia longispina*, and brown trout *Salmo trutta* was studied in two natural soft waters, viz. Lake Maridalsvann (mean hardness 8 mg $CaCO_3$/L) and Lake Sandungen (mean hardness 6 mg $CaCO_3$/l). Testing was also done in the same two waters adjusted to a medium hardness of 100 mg $CaCO_3$/l). The soft water testing program was performed in the framework of this risk assessment report and has been described in detail in Annex 3.3.2.C, which also includes an appendix with a summary of the aquatic toxicity studies that have been used for the derivation of the WER. The aquatic toxicity studies are not further described here.

Derivation of water effect ratio (WERs)

Based on the results of the aquatic toxicity tests as described and summarised in the appendix of Annex 3.3.2.C, a 'water effect ratio' (WER), defined as the NOEC (or LOEC) derived from the test performed in the medium hardness water divided by the NOEC (or LOEC) derived in the original soft water, has been calculated for each test. From these WERs, arithmetic and geometric mean WERs were calculated, as follows:

- For each species: mean value of the 2 WERs for the 2 test waters (either based on NOECs or LOECs).
- For each test water: mean value of the 3 WERs for the 3 species (either based on NOECs or LOECs).
- For the combined WERs: mean values of the total of 6 WERs (either based on NOECs or LOECs).

The results of these calculations are listed in Table 3.81.

23 In the United States, the Environmental Protection Agency released a streamlined procedure for determining site-specific values for a Water-Effect Ratio (WER), a criteria adjustment factor accounting for the effect of site-specific water characteristics on pollutant bioavailability and toxicity to aquatic life (see U.S. EPA, *1994: Interim Guidance on Determination and Use of Water-Effect Ratios for Metals* (EPA-823-B-94-001). In the U.S. the Water Effect Ratio is determined as the toxicity observed in the Site water LC (Lethal Concentration) ÷ Lab water LC. In the present study the water from the lakes are taken as the "Site waters". It was recognised that no lab water could be found that could act as a generic European surface water. Therefore, the "Site waters" were adjusted to a hardness of 100 mg/L $CaCO_3$ to mimic a generic European surface water, and are thus used as "Lab waters".

Table 3.81 'Water Effect Ratios' (WERs)

	WER based on NOEC	**WER based on LOEC**
P. subcapitata		
Maridalsvann	1.6	1.6
Sandungen	1.1	1.0
Arithmetic mean (n =2)	1.4	1.3
Geometric mean (n = 2)	1.3	1.3
D. longispina		
Maridalsvann	2.2	2.2
Sandungen	4.4	4.4
Arithmetic mean (n = 2)	3.3	3.3
Geometric mean (n = 2)	3.1	3.1
S. trutta (*)		
Maridalsvann	1.1	1.0
Sandungen	4.5	4.7
Arithmetic mean (n = 2)	2.8	2.9
Geometric mean (n =2)	2.2	2.2
Maridalsvann		
Arithmetic mean (n = 3)	1.6	1.6
Geometric mean (n = 3)	1.6	1.5
Sandungen		
Arithmetic mean (n =3)	3.3	3.4
Geometric mean (n= 3)	2.8	2.7
All Tests		
Arithmetic mean (n = 6)	2.5	2.5
Geometric mean (n = 6)	2.1	2.0

(*) Based on hatching time, the most sensitive endpoint for S. trutta.

Choice of WER

The arithmetic mean WER of 2.5, calculated from the 6 available tests (3 species and 2 test waters) has been used for the derivation of the soft water $PNEC_{add,\ aquatic}$, based on the following considerations:

- The use of a mean WER based on all available test results (thus not the use of only the lowest of all WERs or the lowest 'species mean' WER) is in conformity with the use of all available 'species mean' NOEC values for generic $PNEC_{add, aquatic}$ derivation.
- Based on the low number of tests and the dependency of the NOEC and LOEC values (and thus the resulting WERs) of the separation factor between the concentrations tested, the use of the arithmetic mean WER is considered to be more appropriate than the use of the geometric mean WER, as the use of the somewhat higher arithmetic mean WER results in a somewhat lower (thus more conservative) soft water $PNC_{add, aquatic.}$
- The generic $PNEC_{add, aquatic}$ is based on tests in a variety of test waters, including test waters with a relatively low hardness (starting with a hardness of 24 mg $CaCO_3$/l. Thus the use of the highest WER (4.7) is considered to be too conservative.

Soft water $PNEC_{add, aquatic}$

The use of the arithmetic mean WER of 2.5 and the generic $PNEC_{add, aquatic}$ of 7.8 µg/l results in a soft water $PNEC_{add, aquatic}$ of 3.1 µg/l. Note that both the $PNEC_{add, aquatic}$ and the soft water $PNEC_{add, aquatic}$ are for dissolved zinc.

Alternatively, when the standard assessment factor approach would be used on the results of the soft water testing programme in natural waters (Annex 3.3.2.C-Appendix), this would result in a soft water $PNEC_{add, aquatic}$ of 4.2 µg/l (based on the lowest NOEC of 42 µg/l, for daphnid *Daphnia longispina* and an assessment factor of 10). This indicates that the use of the arithmetic mean WER of 2.5 on the generic $PNEC_{add, aquatic}$ is not likely to underestimate the toxicity in low hardness natural waters24.

Based on all data, preference is given to the soft water $PNEC_{add, aquatic}$ based on the first option. Thus the risk assessment for soft waters is based on a **soft water $PNEC_{add, aquatic}$ of 3.1 µg/l**, for dissolved zinc.

It is emphasised that the soft water $PNEC_{add, aquatic}$ will be applied only to waters with a low hardness, i.e. less than 24 mg/l (as $CaCO_3$) and will not be used as a default value in case data on hardness are lacking; the use of the generic $PNEC_{add, aquatic}$ remains the starting point of the risk assessment. See Annex 3.3.2.C for additional data and guidance on the application of the soft water $PNEC_{add, aquatic}$ in the risk assessment.

3.3.2.2 Toxicity to sediment organisms

3.3.2.2.1 Abiotic factors influencing the sediment toxicity of zinc

Introduction

Conventionally, the environmental risk assessment of a substance in the sediment would be comparing the estimated concentration (PEC) in the sediment to the PNEC for sediment. In that situation both the PEC and the PNEC would be normalised to wet or dry weight concentrations in the sediment with units mg/kg. The derivation of the $PNEC_{add}$ for zinc in sediment is described in section 3.3.2.2.3. In the present section this approach is called wet or dry weight normalised PNEC-approach. For metals that may bind to sulphides in the sediment

24 The standard assessment factor approach was used earlier in a discussion paper prepared by the rapporteur, resulting in a preliminary soft water PNECadd, aquatic of 1.4 µg/l, derived from the lowest 'species mean' NOEC of 14 µg/l, for alga Pseudokirchneriella subcapitata, based on the results of 5 test performed in artificial test waters with a hardness up to 24 mg CaCO3/l (Sijm & Janus, 2002).

and thus be sequestered in the sediment a different approach can be taken in the environmental risk assessment. This second approach is called the AVS-approach in the present section.

First, the wet or dry weight normalised PNEC-approach will be discussed. Second, the AVS-approach. Third, a two-tiered approach will be discussed that will be used for implementing both approaches in the current risk assessment report of zinc.

The wet or dry weight normalised PNEC-approach

The conventional approach as laid down in the TGD for assessing the risk of substances in sediment, would be determining the PNEC, or in the present RAR the $PNEC_{add}$, expressed as the (added) concentration of a substance in the sediment on a dry or wet weight basis.

This conventional approach is similar as the risk assessment of other substances in the sediment. The advantage of this approach is that monitoring data on environmental concentrations in sediments as well as data derived from laboratory ecotoxicity studies can be easily used and compared, provided they can be equally judged with regard to bioavailability, routes of uptake, background concentration, etc. Furthermore, total concentrations of metals in sediments remain fairly constant over prolonged period of times, which may make it better to interpret from a management point of perspective.

However, a major critique of this conventional approach is that metals in general, and zinc in particular, show a variety of occurrences in sediment and a variety in bioavailability, and subsequently may show a variety of toxic effect concentrations. Most measurements, however, do not differentiate between these various occurrences, such as how much of the metal is complexed by (hydro)oxides or organic matter, or in the case of anaerobic sediment, by sulphides.

DiToro et al. (2002) convincingly showed that dry or wet weight normalised or total concentrations are not a good expression for an effect concentration. For example, their analyses of mortality data for the amphipod *Ampelisca abdita* determined in 10-d toxicity tests in field-collected marine sediments (from Hansen, 1996a) showed significant mortality in the entire range of total zinc concentration in the various sediments, ranging between 1 and 1000 µg/g dry weight. The total concentration of a metal in sediment, expressed on a dry or wet weight basis thus will not necessarily express the bioavailability of the metal for *Ampelisca abdita* in that specific sediment. It is noted that the sediment samples used in the study by Hansen et al. (1996a) also contained other metals, but also the normalised total concentrations of several metals in sediments were poorly related to toxic effects, most of the toxicity-related data could not be explained (in some cases, the toxicity appeared to be related to organic pollutants rather than to the metals present). However, after excluding the data for a number of sediment samples that likely contained organic pollutants and combining the mortality data for the remaining sediment samples with mortality data determined in a number of further short-term (mostly 10-d) studies in metal-polluted freshwater and marine sediments (including spiked sediments and fied-polluted sediments; tests with freshwater and marine species), the analysis still showed a poor relationship between mortality and metal concentrations (ERMR-methodology in Table 3.82).

The consequences of using a wet or dry weight normalised PNEC is that it may not adequately be used in the risk assessment of zinc in sediment. This approach may thus overestimate the risk of zinc in the sediment.

The AVS-approach

General background

In the early nineties, the Acid-Volatile Sulphide (AVS) hypothesis, which is based on equilibrium partitioning, was introduced by DiToro and others to predict the toxicity of divalent cations of metals, including Zn^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+}, Hg^{2+}, and Pb^{2+} in sediment. These metals are referred to as Simultaneously Extracted Metals (SEM), i.e. the metals which are liberated from the sediment together with AVS by the cold extraction of sediment in approximately 1 N HCl. In unpolluted sediments, AVS is mainly composed of amorphous FeS and MnS. In sediments polluted with divalent cations of metals that are less soluble than FeS, these metals will bind to the sulphide and replace Fe^{2+}. The binding of SEM to AVS thus results in the formation of highly insoluble metal sulphides that precipitate in the sediment. These metal-sulphides limit the SEM concentration in the porewater (interstitial water) and also possibly the bioavailability and toxicity for benthic organisms. This assumes that exposure via the porewater is the main route of exposure. One mole of AVS can theoretically bind one mole of SEM. This would result in very low concentrations of all SEM metals in the porewater when the molar amount of AVS exceeds that of SEM. Alternatively, when the molar amount of SEM exceeds that of AVS, the metals may partition between the sediment and the porewater. In the latter situation, the concentrations of the SEM metals in the porewater also depend on (i) the total SEM concentration, (ii) the metals present and the relative solubility of their metal-sulphides (Ni>Zn >Cd>Pb>Cu), and (iii) the partitioning of the metals with non-AVS sediment components such as organic matter and iron or manganese oxides (Fe/MnO_x).

The amount of SEM related to the amount of AVS was originally expressed as the molar ratio:

$$\frac{[SEM]}{[AVS]}$$

where:

[SEM] is the molar concentration of divalent metal cations in the sediment (µmol/g dry weight)

[AVS] is the molar concentration of acid-volatile sulphide in the sediment (µmol/g dry weight).

In theory, no effects are expected when the molar amount of SEM is lower than that of AVS, i.e. when the SEM/AVS ratio is <1 (Allen et al., 1993; DiToro et al., 1992; Swartz et al., 1985). Conversely, effects may occur when the SEM/AVS ratio is >1. Especially at a value just above 1, the molar ratio is not a suitable predictor of potential effects, because the ratio gives no information on the absolute amount of SEM present in excess of AVS. For example, at a molar ratio of 1.1, the absolute amount of SEM is 1.1 µmol/g at an AVS concentration of 1 µmol/g and 11 µmol/g at an AVS concentration of 10 µmol/g, the latter SEM concentration more likely to result in effects than the former. Hence, the molar difference, i.e. SEM-AVS, is a more suitable predictor of potential effects:

$$[SEM] - [AVS]$$

At a molar SEM-AVS difference of <0 that corresponds to a molar SEM/AVS ratio <1, no effects are expected, while at a molar SEM-AVS difference of >0 that corresponds to a molar SEM/AVS ratio >1, effects may occur.

Recently, the approach was slightly modified by DiToro et al. (2002) to take into account the binding of excess SEM to organic matter. The following equation for chronic toxicity illustrates the modification and shows when chronic toxicity can be expected according to DiToro et al. (2002):

$$\frac{[SEM]-[AVS]}{f_{oc}} > 100\frac{\mu mol}{g_{oc}}$$

where:

f_{oc} is the fraction organic carbon content of the sediment (g_{oc}/g dry weight).

100 is proposed by DiToro et al. (2002) as a conservative cut-off value, below which it is unlikely that effects may occur (see further in this section for the data underlying this value).

The arguments that are used to derive this equation are that AVS in excess of SEM will bind the metals in the sediment, and will render the metals less bioavailable for many organisms. If SEM is in excess of AVS, the excess metals will partition over (pore)water, organic carbon in the sediment and possibly other binding sites. Empirically it was found that the organic carbon normalisation improves the description of currently available sediment toxicity data, and results in a relatively small window in concentrations where there is uncertainty of whether or not effects may occur (DiToro et al., 2002).

Table 3.82 shows the windows for acute toxicity for various methodologies, i.e. for total metal concentrations, expressed either as ERMR or as SEM, for SEM/f_{oc}, for SEM/AVS, SEM-AVS, and for (SEM-AVS)/f_{oc}. In the case of SEM, DiToro et al. (2002) compared the results for acute toxicity with the area where effects would not be predicted (SEM < 1 µmol/g, 24.8% of the data), the area where effects would be predicted (SEM > 140 µmol/g, 5.6% of the data), and the area of uncertain effects (1 µmol/g < (SEM < 140 µmol/g, 69.6% of the data, see Table 3.3.2.2.1.1). According to this exercise from DiToro et al. (2002), the (SEM-AVS)/f_{oc} model would provide the least uncertainty in predicting either 'non-toxic' or 'toxic' sediments.

Table 3.82 Ranges and percentage[a] of observations classified as non-toxic, toxic or uncertain for the various methodologies for metal toxicity, i.e. mortality, in sediment, both in lab and field studies (DiToro et al., 2002).

Methodology to explain metal toxicity data in sediment	Non-toxic[b] Range [percentage]	Uncertain Range [percentage]	Toxic Range [percentage]
ERMR[c]	<0.1 [11.7%]	0.1-180 [84.5%]	>180 [3.8%]
SEM	<1 µmol/g [24.8%]	1-140 µmol/g [69.6%]	>140 µmol/g [5.6%]
SEM/f_{oc}	<250 µmol/g_{oc} [34.2%]	250-4500 µmol/g_{oc} [50.6%]	>4500 µmol/g_{oc} [15.2%]
SEM/AVS	<2 [63.7%]	2-40 [27.1%]	>40 [9.2%]
SEM-AVS	<1.7 µmol/g [59.9%]	1.7-180 µmol/g [36.7%]	>180 µmol/g [3.4%]
(SEM-AVS)/f_{oc}	<150 µmol/g_{oc} [58.8%]	150-3400 µmol/g_{oc} [25.5%]	>3400 µmol/g_{oc} [15.7%]

A 90th percentile value for correct predictions

B Non-toxic is defined as ≤ 24% mortality (DiToro et al., 2002)

C ERMR is the effects range median ratio, defined as the average of the ratio of the metal concentration divided by the ERM for each individual metal present in the sediment. ERM refers to the effects range-median concentration and is identified as the 50th percentile value from a biological effects database for sediments. The ERM, the effect range median, is an empirical guideline for contaminated sediments based on total concentrations of metals in the sediments (Long and Morgan, 1990; Long et al., 1995; Long et al., 1998). For zinc the ERM, the effect range median, is 410 mg/kg dry weight.

Recently, Shine et al. (2003) have further evaluated zinc toxicity in sediments in the USA. The primary goal of their study was to compare different approaches and models used to estimate the toxicity of metals in sediments. The focus of the evaluation was on the extent to which a method was able to correctly classify a toxic sample as toxic and a non-toxic sample as non-toxic. Acute toxicity data were used from 357 samples chosen from eight sources including freshwater and marine sediments. Species tested were *Hyalella azteca*, *Chironomus riparius*, *Neanthes arenaceodentata*, *Capitella capitata*, *Lumbriculus variegates*, *Helisoma* spp., *Ampelisca abdita* and *Chironomus tentans*. The results on the SEM/AVS model evaluation showed that this approach has a very high sensitivity (96 %), i.e. the probability to which a model correctly classifies a non-toxic sample as non-toxic and is therefore regarded as protective of the environment. The SEM/AVS model provides a low positive predictive power of 55 %. Because the latter is the likelihood that a sample exceeding the theoretical value of SEM/AVS=1 is in fact toxic, it means that in a large number of cases exceeding the SEM/AVS ratio does not result in any observed toxic effects. This is not surprising since both the SEM/AVS threshold of 1 and SEM-AVS threshold of 0 are not intended to predict toxicity but intended to tell something about when absence of toxicity can be expected.

The AVS hypothesis was confirmed in individual single-species acute lethality tests, using different benthic freshwater and saltwater organisms, including amphipods, oligochaetes and snails, and different divalent metals (cadmium, copper, nickel and zinc, as well as metal mixtures) added to the sediment. The results of these tests, all conducted in the laboratory, consistently showed no toxicity when the molar SEM/AVS ratios were $\leq$ 1, and that

sediments having a ratio of >1 were frequently toxic but nearly as frequently non-toxic. The absence of toxicity found in a number of sediments having an SEM/AVS ratio >1 indicates that AVS is not the only binding component of metals in sediment. The studies further showed that the absence or presence of toxicity was coincident with the absence or presence of toxicological relevant metal concentrations in the porewater.

The above mentioned studies have only involved short-term exposures, i.e. a test duration of up to 10 days, in acute lethality tests using homogenised sediments, thus disturbing the normal AVS gradient in sediments.

In the last decade, six long-term studies were conducted to validate the AVS hypothesis with respect to chronic effects of metal-spiked sediments (spiked with zinc, cadmium or a metal mixture). These chronic toxicity studies include two life cycle single-species laboratory studies (DeWitt et al., 1996; Sibley et al., 1996), one laboratory colonisation study (Hansen et al., 1996b) and three field colonisation studies (Boothman et al., 2001; Liber et al., 1996; Hare et al., 1994). The studies were performed in freshwater or saltwater sediment. The three studies that used Zn-spiked sediment (in one study: metal mixture including Zn) are included in Table 3.83 and are indicated by an asterisk (*). The other three studies used Cd-spiked sediments; these studies are not included in Table 3.83. See Annex 3.3.2.D (Table 3.3.2.e and Table 3.3.2.f) for an extensive summary of all six studies, including data on the toxicological endpoints studied.

The results of the abovementioned chronic toxicity studies were used by DiTo et al. (2002) for a comparison of chronic toxicity with the $(SEM\text{-}AVS)/f_{oc}$ area where effects would not be predicted ($(SEM\text{-}AVS)/f_{oc} < 150$ μmol/g_{oc}), the area where effects would be predicted ($(SEM\text{-}AVS)/f_{oc} > 3400$ μmol/g_{oc}), and the area of uncertain effects (150 μmol/g_{oc} < $(SEM\text{-}AVS)/f_{oc}$ < 3400 μmol/g_{oc}). See Table 3.82 for these areas that are based on acute lethal toxicity tests, see earlier. It appeared that of the 19 treatment samples where effects were not expected, i.e. sediment samples with a $SEM\text{-}AVS)/f_{oc}$ value of < 150 μmol/$g_{oc,}$ only one sediment sample had significant effects. This sediment sample (from the field colonisation in Cd-spiked sediment; from Hare et al., 1994) had a $(SEM\text{-}AVS)/f_{oc}$ value of 57 μmol/$g_{oc.}$ Of the 7 treatment samples where effects may or may not have been predicted, effects were observed in 6 of them. Note that the 26 sediment samples are from six different sediments, as from each study all treatments were presented either as concentration without effect or concentration with effect. There were no sediment samples with a $(SEM\text{-}AVS)/f_{oc}$ value of ≥ 3400 μmol/g_{oc}.

The equation does not further explore explicit binding to other sites. A value of 100 μmol/g_{oc} is proposed by DiToro et al. (2002) as a conservative cut-off value, below which it is unlikely that effects may occur. However, a more conservative approach that includes all no observed effects values would possibly be below 57 μmol/g_{oc} to include the one sediment sample that did show effects at this value (see above).

In the four abovementioned colonisation studies, the abundances of major taxa (including classes and families) which colonised the initially defaunted sediments, were studied; three of these studies also included determinations of abundances down to genera and species. All colonisation studies included a variety of organisms differing in morphology and (feeding and burrowing) behaviour and thus expected to differ in exposure and sensitivity. Only in Hare et al. (1994) detailed data on the (feeding and/or burrowing) behaviour of a number of species included in the field colonisation study in Cd-spiked sediment are reported. These data may be indicative for exposure. For example, larvae of the phantom midge *Chaoborus punctipennis* spend the night high in the water column feeding on zooplankton, during the day they remain near the sediment-water interface. The abundance of this species was not affected, as might be expected since they do not live in the sediment nor feed on the sediment.

In contrast, larvae of the large red chironomid *Chironomus (salinarius gp) sp.* burrow deep in the sediment and have their guts filled with sediment. The abundance of this species was significantly decreased at the highest exposure level, as might be expected since they are intimately associated with the sediments. It is noted, however, that data on the behaviour of the organisms are not necessarily predictive of effects, as there are other factors involved, including differences in sensitivity. For example, larvae of the red chironomid *S. coracina*, which show the same burrowing and feeding activity as *Chironomus (salinarius gp) sp.* were not affected. In addition to the fact whether or not direct exposure to metal sulphides in the sediment occurs by the ingestion of sediment, it is relevant whether or not ingested metal sulphides will be bioavailable after ingestion. The evaluated references do not contain data on this issue.

The overview studies from DiToro et al. (2002) and Shine et al. (2003) as well as some individual studies clearly show that in many cases, both in the laboratory and in the field, correcting the metal concentration in the sediment for sulphides provides a much better basis for assessing the risks than when using the wet or dry weight normalised concentrations in the sediment. In particularly, the study by DiToro et al. (2002) was used as a trigger to perform additional validation studies in European freshwater sediments, which will be described in the next section.

Validation of the AVS-approach for European freshwater sediments

In this section briefly the results of a long-term, European field study is described, which is followed by a further validation of the AVS-approach from this and other studies.

Results of a long-term field colonisation study in four different EU freshwater sediments

Recently, a long-term, field study was conducted to validate the concentrations of zinc in European freshwater sediments that are tolerated by benthic macroinvertebrate communities and to determine whether there is a relationship with the AVS-approach (Burton et al., 2003; see also Annex 3.3.2.D (Table 3.3.2.f – Part C)). The study design consisted of spiking sediments with 400 and 1,200 mg/kg dry weight zinc from four differing environments in Europe, including two lake ecosystems (Ankeveen lake and Smallenberg lake; one sampling date at these two sites) and two riverine ecosystems (Pallaza river and Biesbosch river; three sampling dates at these two sites). Spiked sediments were returned to the sampling site to allow recolonisation for 6 – 37 weeks.

In total, 228 cases of data comparison with different statistical means were performed by the researchers and re-analysed by the Rapporteur. The researchers and the Rapporteur agreed in 225 cases on the statistical analyses, and there are only 3 cases where they are not in agreement.

The results of this field study performed at four different test sites indicate that there is a poor relationship between the total zinc concentrations in the sediments and effects on benthic macroinvertebrates. For example, Ankeveen sediment was found to be non-toxic at a total zinc concentration of 913 mg Zn/kg dry weight, while Pallanza sediments were toxic at 175 mg Zn/kg dry weight. Regarding the validity of the AVS-approach the following results were found.

No treatment effects were observed in 6 sediments samples. For these sediment samples, SEM/AVS ratios fell in a range of 0.2 to 2.9, with 4 of the 6 values below 1. Carbon normalised SEM-AVS values ([SEM-AVS/f_{oc}]) of these sediment samples in which no effects were determined were <100 μmol/g_{oc} in 5 samples and 154 μmol/g_{oc} in 1 sample.

Treatment effects were found in 10 sediment samples with SEM/AVS ratios ranging from 0.7 to 43, with 6 ratios between 0.7 and 2.3 and 4 ratios between 8.3 and 43. Carbon normalised SEM-AVS values ([SEM-AVS/f_{oc}]) of these sediment samples in which effects were determined ranged between 0 and 1975 μmol/g_{oc}, with 5 values <100 μmol/g_{oc} and 5 values >100 μmol/g_{oc}.

Based on these data it is concluded that the results of this field study generally support both the SEM/AVS model as the SEM-AVS model, as also stated by Burton et al. (2003). However, no validation was found for the cut-off value of 100 μmol/g_{oc} for the carbon normalised SEM-AVS value ([SEM-AVS/f_{oc}) as proposed by DiToro et al. (2002). The results of this study are also included in the further validation of the AVS-approach below.

Further validation of the AVS-approach

All single-species studies (short and long-term laboratory studies) and colonisation studies (long-term laboratory and field studies, including the field colonisation study in EU freshwater sediments performed by Burton et al. 2003) with sufficient information to express NOEC or LOEC values as SEM/AVS, SEM-AVS and (SEM-AVS)/f_{oc} are shown in Table 3.83 (based on the data from Table 3.3.2.e and Table 3.3.2.f in Annex 3.3.2.D). The studies were all conducted in freshwater sediments spiked with Zn, except the study by Boothman et al. (2001) that used a saltwater sediment spiked with equimolar concentrations of Zn, Cd, Cu and Ni. With respect to the evaluation of SEM/AVS, SEM-AVS and (SEM-AVS)f_{oc} models, one NOEC and (if available) one LOEC is given for each single-species study, each with corresponding SEM/AVS, SEM-AVS and (SEM-AVS)/f_{oc} value. For the colonisation studies, these data are given for each sampling date and concentration tested. The data reported in Table 3.83 are based on the actual SEM, AVS and f_{oc} concentrations measured in the sediment samples. The SEM concentration is either the SEM_{Zn} concentration (when only Zn was measured in the sediment) or $SEM_{total\ metal}$ concentration ($\Sigma SEM_{Zn,\ Cu,Pb,Cd,Hg,,}$ when Zn and other divalent metals were measured in the sediment). The $SEM_{total\ metal}$ concentration is primarily zinc as only zinc was added to the sediments, except in the study by Boothman et al. (2001), that used sediment spiked with equimolar concentrations of Zn, Cd, Cu and Ni.

Table 3.83 includes the three long-term studies in Zn-spiked sediments that were also evaluated by DiToro et al. (2002), viz, the studies by Sibley et al. (1996), Liber et al. (1996) and Boothman et al. (2001), and is supplemented with more recent studies. See Annex 3.3.2.D (Table 3.3.2.e and Table 3.3.2.f) for an extensive summary of all studies listed in Table 3.83.

In general, the results of the studies in Table 3.83 confirm the AVS hypothesis. In most studies the concentrations at which toxic effect or no toxic effect were assessed were higher than expected from the molar SEM/AVS ratio and molar SEM-AVS difference. The 1-yr field colonisation study by Liber et al., (1996) showed "minor" ecosystem effects at a year-average SEM/AVS ratio of 1.1 and SEM-AVS difference of 1.0, thus at the minimum values at which effects are expected. The "minor" ecosystem effects were observed at one sampling time during the study, at a SEM/AVS ratio of 0.6 and 0.7 and a SEM-AVS difference of -3.5 and -3.4, respectively, thus at values where effects would not be predicted (and further at another sampling time at a SEM/AVS ratio of 1.1 and a SEM-AVS difference of 1.1). In the field colonisation study by Burton et al. (2003) effects were found in Lake Ankeveen at a SEM/AVS ratio of 0.7, thus also at a value were effects would not be expected. Finally, in the 3-wk single-species study with *C. tentans* (Farrar & Bridges, 2002, 2003) growth was affected at a SEM/AVS ratio of 0.5 and SEM-AVS difference of -26. Thus, in these 4 cases, effects were found at a molar SEM/AVS ratio of <1 and/or a molar SEM-AVS difference of <0, while in the 15 further cases effects were found as expected at a molar SEM/AVS ratio of >1 and/or a molar SEM-AVS difference of >0,

In addition, in the 17-wk laboratory colonisation study with cadmium-spiked sediments (Hansen et al., 1996b, Table 3.3.2.f – Part D; study not included in Table 3.83), "major" ecosystem effects occurred at a SEM/AVS ratio of 0.9 and SEM-AVS difference of -2.9. This is the only colonisation study in which "major" ecosystem effects were observed, while no or only minor effects would be expected on the basis of the molar SEM/AVS ratio and molar SEM-AVS difference.

Table 3.83 also shows the concentrations at which toxic effects or no toxic effects were determined expressed as (SEM-AVS)/f_{oc} values. The 30 no-effect concentrations include 5 (SEM-AVS)/f_{oc} values that are >100 μmol/g_{oc}, the cut-off value as proposed by DiToro et al. (2002). Thus in these 5 cases, with (SEM-AVS)/f_{oc} values ranging from 118 to 1800 μmol/g_{oc}, no effects where found while effects would be expected. However, the 19 effect concentrations include 9 (SEM-AVS)/f_{oc} values that are <100 μmol/g_{oc}, thus in these 9 cases, effects were found at (SEM-AVS)/f_{oc} values were effects would not be predicted. Of these 9 cases, 5 had (SEM-AVS)/f_{oc} values of 10 to 92 μmol/g_{oc} and 4 had (SEM-AVS)/f_{oc} values that were ≤ 0 μmol/g_{oc}. Since half of the effect concentrations expressed as (SEM-AVS)/f_{oc} fell below the cut-off value of 100 μmol/g_{oc}, this cut-off value should be discussed further.

In addition, in the 17-wk laboratory colonisation study with cadmium-spiked sediments (Hansen et al., 1996b, Table 3.3.2.f – Part D; study not included in Table 3.83), "major" ecosystem effects occurred at a (SEM-AVS)/f_{oc} value <0 μmol/g_{oc}.

The analysis of the recent European field recolonisation study and of some of the literature data dealing with the effects of (AVS-corrected) zinc versus toxicity in laboratory and field studies are further discussed below. The plots are based on Table 3.83.

Table 3.83 All no toxic response- and all toxic response-values from the relevant sediment studies where zinc was used and where the AVS-models could be evaluated.

Reference	Species	Test substance	Sediment	Toxicity expressed as			
				SEM (mmol/kg d.w.)	SEM/AVS	SEM-AVS (mmol/kg d.w.)	(SEM-AVS)/foc ($mmol/kg_{oc}$)
no toxic response-values							
Farrar and Bridges (2003)	Tubifex tubifex adults	ZnCl2	pond	17.5			
Nguyen et al. (2005)	Hyalella azteca (1-w old)	ZnCl2	Stream	7.3	0.9	-1.2	-60
Farrar and Bridges (2002),(2003)	Hyalella azteca 1-2 wk old	ZnCl2	lake	3.5	1.9	1.6	160
Sibley et al. (1996) *	Chironomus tentans P (newly hatch larvae → F1 (life cycle)	ZnCl2	lake	13	1.8	5.9	118
Farrar and Bridges (2002),(2003)	Chironomus tentans, 1-d old	ZnCl2	pond	11.7	0.3	-28	-2800
Farrar and Bridges (2002),(2003)	Chironomus tentans, 2nd-3rd instar	ZnCl2	lake	7	4	5.2	520
Liber et al. (1996)	Hyalella azteca	ZnCl2	pond	11.9	1.1	1.0	9.1
Liber et al. (1996)	Chironomus tentans	ZnCl2	pond	11.9	1.1	1.0	9.1
Burton et al. (2003)	Recolonisation study	ZnCl2	Ankeveen lake	7.2	0.2	-28.3	-316
Burton et al. (2003)	Recolonisation study	ZnCl2	Schmallenberg lake, June	8.7	0.5	-7.6	-83
Burton et al. (2003)	Recolonisation study	ZnCl2	Schmallenberg lake, June	17	1.5	6	61

Burton et al. (2003)	Recolonisation study	ZnCl2	Schmallenberg lake, September	2.1	0.5	-2.3	-43
Burton et al. (2003)	Recolonisation study	ZnCl2	Schmallenberg lake, December	5.2	0.6	-3.8	-44
Burton et al. (2003)	Recolonisation study	ZnCl2	Biesbosch river, September	4	2.9	2.6	154
Boothman et al. (2001) *	Recolonisation study	Mixture (Zn,Ni,Cd,Pb,Cu)	Marine	27	3	18	1800
(to be continued)							
Reference	**Species**	**Test substance**	**Sediment**	**Toxicity expressed as**			
				SEM (mmol/kg d.w.)	SEM/AVS	SEM-AVS (mmol/kg d.w.)	(SEM-AVS)/foc ($mmol/kg_{oc}$)
no toxic response-values *(continued)*							
Liber et al (1996) *	Recolonisation study	ZnCl2	Pond (overall 1-year result)	11.9	1.1	1.0	9.1
Liber et al (1996) *	Recolonisation study	ZnCl2	pond, July 1993	5.3	1.0	-0.2	-1.8
Liber et al (1996) *	Recolonisation study	ZnCl2	pond, July 1993	12.4	1.6	4.5	41.3
Liber et al (1996) *	Recolonisation study	ZnCl2	pond, August 1993	0.7	0.2	-3.3	-30.3
Liber et al (1996) *	Recolonisation study	ZnCl2	pond, August 1993	1.2	0.2	-2.1	-19.3
Liber et al (1996) *	Recolonisation study	ZnCl2	pond, August 1993	2.5	0.4	-2.8	-25.7
Liber et al (1996) *	Recolonisation study	ZnCl2	pond, August 1993	5.5	0.6	-3.3	-30.3
Liber et al (1996) *	Recolonisation study	ZnCl2	pond, August 1993	12.8	1.1	-0.4	-3.7
Liber et al (1996) *	Recolonisation study	ZnCl2	pond, October 1993	0.6	0.2	-4.1	-37.6
Liber et al (1996) *	Recolonisation study	ZnCl2	pond, October 1993	1.2	0.4	-2.1	-19.3
Liber et al (1996) *	Recolonisation study	ZnCl2	pond, October 1993	2.3	0.5	-2.8	-25.7
Liber et al (1996) *	Recolonisation study	ZnCl2	pond, May 1994	1.9	0.4	-2.7	-24.8
Liber et al (1996) *	Recolonisation study	ZnCl2	pond, May 1994	2.4	1.0	-0.3	-2.8

Liber et al (1996) *	Recolonisation study	ZnCl2	pond, May 1994	6.2	0.9	-0.5	-4.6
Liber et al (1996) *	Recolonisation study	ZnCl2	pond, May 1994	14	1.3	3.2	29.4
Liber et al (1996) *	Recolonisation study	ZnCl2	pond, July 1994	4.7	0.7	-2.8	-25.7
toxic response-values							
Farrar and Bridges (2003)	Tubifex tubifex adults	ZnCl2	pond	40.1			
Nguyen et al. (2005)	Hyalella azteca (1-w old)	ZnCl2	stream	12.1	1.4	3.7	185
Farrar and Bridges (2002),(2003)	Hyalella azteca 1-2 wk old	ZnCl2	lake	7.1	4.0	5.3	530
Farrar and Bridges (2002),(2003)	Hyalella azteca 1 wk old	ZnCl2	pond	3.4	12.3	2.8	140
Sibley et al. (1996) *	Chironomus tentans P (newly hatch larvae → F1 (life cycle)	ZnCl2	lake	29	4.3	22	440
(to be continued)							
Reference	**Species**	**Test substance**	**Sediment**	**Toxicity expressed as**			
				SEM (mmol/kg d.w.)	SEM/AVS	SEM-AVS (mmol/kg d.w.)	(SEM-AVS)/foc ($mmol/kg_{oc}$)
toxic response-values *(continued)*							
Farrar and Bridges (2002),(2003)	Chironomus tentans, 1-d old	ZnCl2	pond	22	0.5	-26.2	-2620
Farrar and Bridges	Chironomus tentans,	ZnCl2	lake	14.8	6.4	12.5	1250

(2002),(2003)	2nd-3rd instar						
Burton et al. (2003)	Recolonisation study	ZnCl2	Pallanza river	2.8	43	2.7	1503
Burton et al. (2003)	Recolonisation study	ZnCl2	Pallanza river	4.0	33	3.9	1975
Burton et al. (2003)	Recolonisation study	ZnCl2	Ankeveen lake	33	0.7	0	0
Burton et al. (2003)	Recolonisation study	ZnCl2	Schmallenberg lake, September	9.9	1.8	4.5	92
Burton et al. (2003)	Recolonisation study	ZnCl2	Schmallenberg lake, December	10.2	1.7	4.1	52
Burton et al. (2003)	Recolonisation study	ZnCl2	Biesbosch river, June	3.5	1.6	1.3	14
Burton et al. (2003)	Recolonisation study	ZnCl2	Biesbosch river, June	6.0	1.6	2.2	24
Burton et al. (2003)	Recolonisation study	ZnCl2	Biesbosch river, September	10	8.3	8.8	576
Burton et al. (2003)	Recolonisation study	ZnCl2	Biesbosch river, December	3.9	2.3	2.2	148
Burton et al. (2003)	Recolonisation study	ZnCl2	Biesbosch river, December	10.5	9.8	9.5	793
Liber et al (1996)	Recolonisation study	ZnCl2	pond, October 1993	5.3	0.6	-3.5	-32.1
Liber et al (1996)	Recolonisation study	ZnCl2	pond, October 1993	9.1	0.7	-3.4	-31.2
Liber et al (1996)	Recolonisation study	ZnCl2	pond, July 1994	11	1.1	1.1	10.1

***** These long-term studies are also included in the evaluation of the AVS approach by DiToro et al. (2002).

Figure 3.20 shows the plot where total zinc, expressed as SEM in the sediment is related to toxicity. This figure shows that there are two 'no toxic response' values from Liber et al. (1996) and no single 'toxic response' below the $PNEC_{add}$ for sediment (49 mg/kg dry weight, see section 3.3.2.2.2.). It must be further noted that all these values were reconverted to molar concentrations. The plot thus implies that the $PNEC_{add}$ is sufficiently protective and perhaps too conservative.

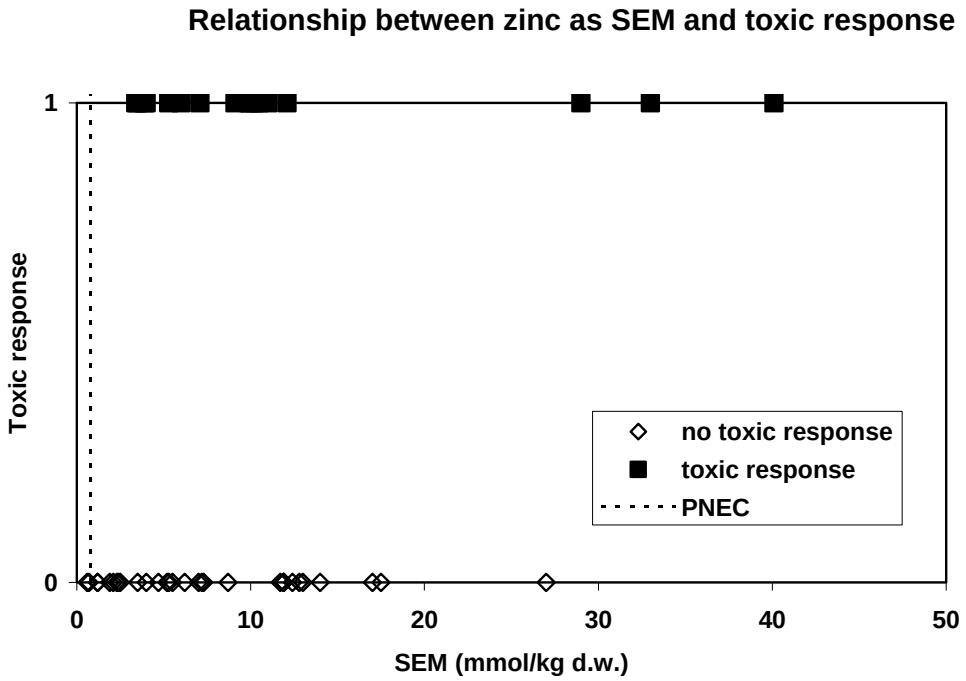


Figure 3.20 Relationship between the toxic response (0 = no toxic response , 1 = toxic response) and the total zinc concentration, expressed as Simultaneous Extracted Metal (SEM). Data from Table 3.85. The PNECadd is included for comparison.

Figure 3.21 provides the relationship between toxicity and SEM/AVS. Figure 3.21 shows that there are several 'no toxic response' values below and above the theoretical value of 1. The theoretical value implies that only 'no toxic response' would be expected at a value below 1, while the plot shows that there are studies with a SEM/AVS value < 1 that show toxicity. Furthermore, there are three 'toxic response' values below the theoretical value of 1, while the remaining 'toxic response' values are above 1. One of these values (of Burton et al., 2003) was subject of discussion between the researchers and the Rapporteur, i.e. the researchers did not conclude there was a 'toxic response', while the Rapporteur did. The two remaining 'toxic response' values originate from Liber et al. (1996). All 'toxic response' values that are below 1 are above 0.5. The plot thus implies that the SEM/AVS model on the one hand is sufficiently protective, since 'no toxic response' values are found when toxicity is expected and most of the 'toxic response' values are indeed found at SEM/AVS-ratios above 1. On the other hand the SEM/AVS model seems not sufficiently protective, since a few 'toxic response' values are found at a SEM/AVS ratio below 1 (and above 0.5).

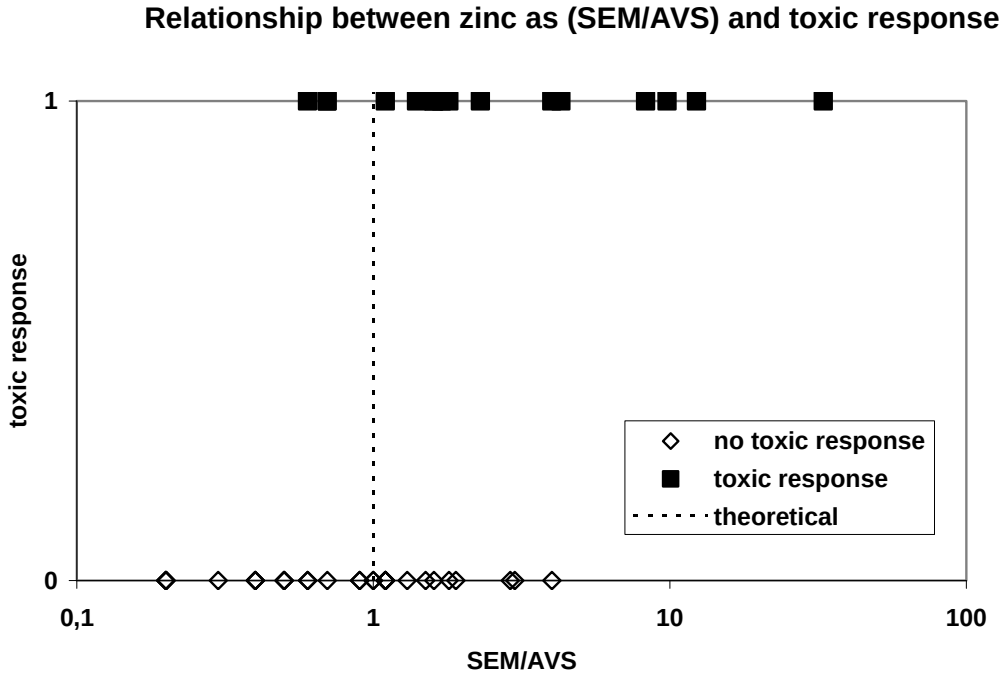


Figure 3.21 Relationship between the toxic response (0 = no toxic response, 1 = toxic response) and the total zinc concentration, expressed as SEM, and corrected for sulphide (AVS) in the sediment. Data from Table 3.85.

Figure 3.22 provides the relationship between toxicity and (SEM-AVS). Figure 3.22 shows that there are several 'no toxic response' values below and above the $PNEC_{add}$. Only 'no toxic response' would be expected at a value below the $PNEC_{add}$, while the plot shows that there are studies with a (SEM-AVS) value < $PNEC_{add}$ that show toxicity. Furthermore, there are three 'toxic response' values below the $PNEC_{add}$, while the remaining 'toxic response' values are above the $PNEC_{add}$. Two of these values originate from Liber et al., 1996, and one originates from Burton et al. (2003). The plot thus implies that the (SEM-AVS) model on the one hand is sufficiently protective, since 'no toxic response' values are found when toxicity is expected and most of the 'toxic response' values are indeed found at (SEM-AVS)-values above the $PNEC_{add}$. On the other hand the (SEM-AVS) model seems not sufficiently protective, since a few 'toxic response' values are found at a (SEM-AVS) values below the $PNEC_{add}$.

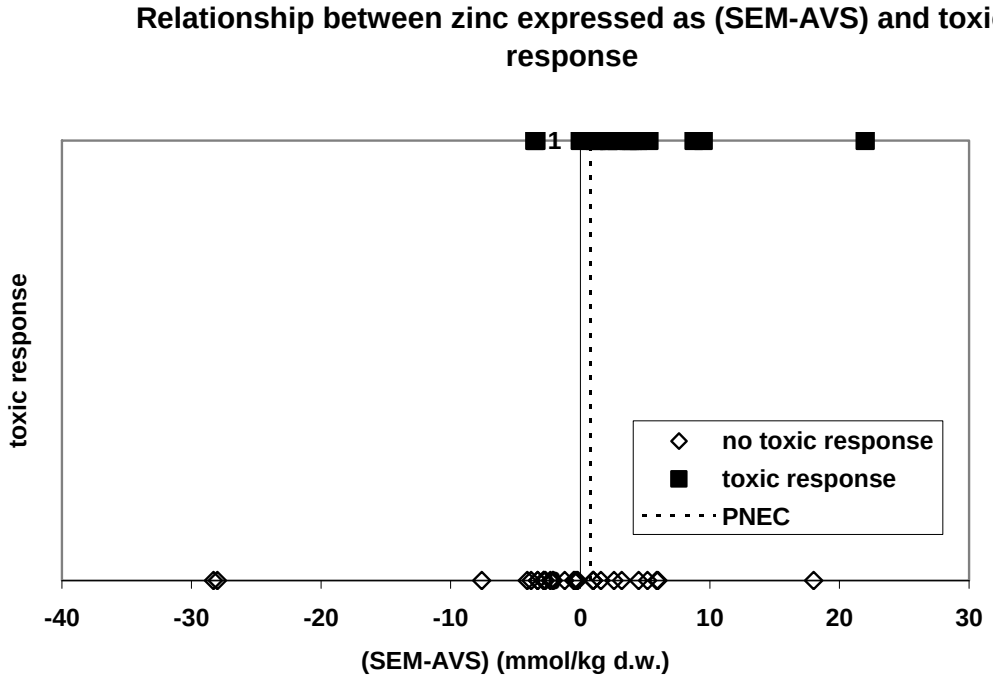


Figure 3.22 Relationship between the toxic response (0 = no toxic response, 1 = toxic response) and the total zinc concentration, expressed as (SEM-AVS) in the sediment. Data from Table 3.85. The $PNEC_{add}$ is included for comparison.

Figure 3.23 provides the relationship between [(SEM-AVS)f_{oc}] and toxic response. Figure 3.23 shows that there are several 'no toxic response' values below and above the theoretical value of 100 μmol/g_{oc}. The theoretical value implies that only 'no toxic response' values would be expected at a value below 100 μmol/g_{oc}, while the plot shows that there are several studies with a [(SEM-AVS)/f_{oc}] value > 100 μmol/g_{oc} that still do not show toxicity. Furthermore, there eight 'toxic response' values below the theoretical value of 100 μmol/g_{oc} while the remaining 'toxic response' values are above 100 μmol/g_{oc}. One of these values (of Burton et al., 2003) was subject of discussion between the researchers and the Rapporteur, i.e. the researchers did not conclude there was a 'toxic response', while the Rapporteur did. The plot thus implies that this [(SEM-AVS)/f_{oc}] model is not sufficiently protective, since several 'toxic response' values are found below the value of 100 μmol/g_{oc}.

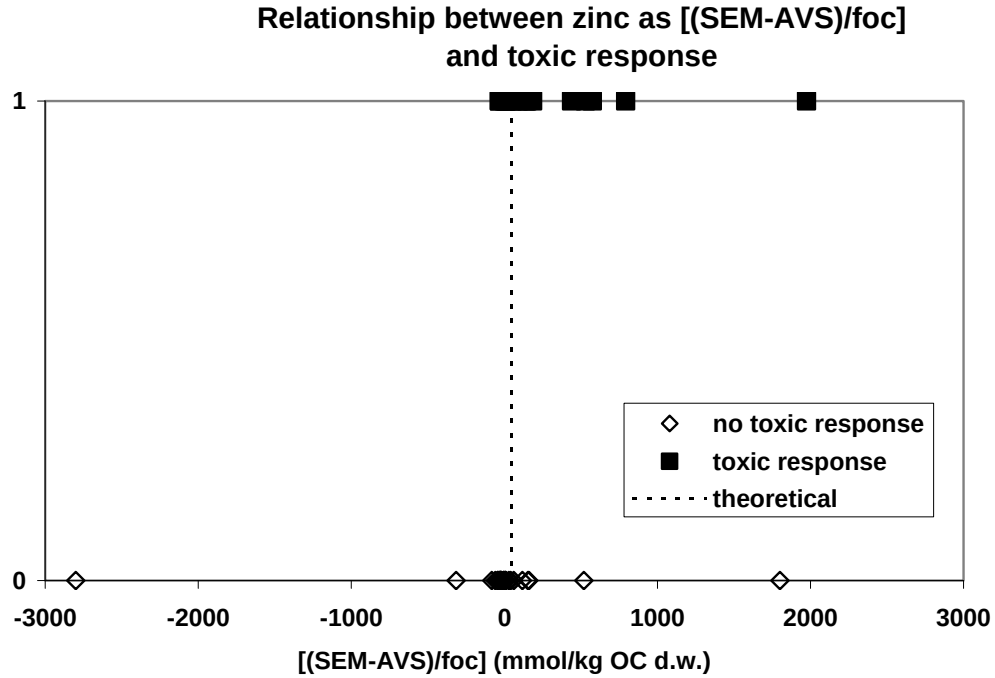


Figure 3.23 Relationship between the toxic response (0 = no toxic response, 1 = toxic response) and the zinc concentration, expressed as SEM, and corrected for sulphide (AVS) and organic carbon (f_{oc}) in the sediment. Data from Table 3.85.

In conclusion, the plots illustrate clearly that the conventional approach where the $PNEC_{add}$ is used and is expressed on a wet or dry weight basis, may be too conservative, and that the $(SEM-AVS)/f_{oc}$ is not sufficiently conservative. It must be noted that the plots only contain the individual laboratory and field studies on zinc toxicity. The plots do not contain the much more extensive analysis by DiToro et al. (2002) and Shine et al. (2003) who also included many field studies where effects of many metals were studied. Furthermore, the simple approach in the plots only distinguish between effects (closed symbols) and no effects (open symbols) but do not reveal the magnitude of the effects. Overall, the AVS-approach, in particularly the (SEM-AVS)-approach seems a more appropriate approach than the wet or dry weight normalised $PNEC_{add}$.

Evaluation of the AVS-approach

Since the AVS-approach is a new methodology many issues were raised in accepting its applicability in the environmental risk assessment of zinc in sediment. In this section some of the major issues will be briefly discussed.

Reducing uncertainty

As clearly shown and explained by DiToro et al. (2002) and Shine et al. (2003) and supported by additional validation studies in Europe the AVS-approach is better able to account for bound and less available metals in the sediment than the conventional wet or dry weight normalised PNEC-approach. It must be noted though that the AVS-approach is merely empirically derived.

The AVS-approach explains better why no effects are observed in studies with relatively high metal concentrations in the sediment by accounting for non- or less available metal in those anaerobic sediments (DiToro et al., 2002; Shine et al., 2003).

The AVS-approach explains better when no toxicity will occur, where toxicity does occur, and leaves a small portion of situations where there is no clear prediction on whether there will be toxicity or not (see percentages under 'Uncertain' in Table 3.82).

Validation of the AVS-approach

There is a growing evidence of studies that support the AVS-approach as explained in the previous section. However, there are still few toxicity studies that have been performed on single metals in sediments, and even less for zinc as the only metal. Furthermore, the few available sediment toxicity studies do not always provide the required information on SEM, AVS, f_{oc} and a NOEC or LOEC.

Several studies (part of the studies described in Burton et al., 2003; Farrar and Bridges, 2003; Hansen et al., 1996; part of the studies described in Liber et al., 1996) that did measure SEM, AVS, and showed effects (and a resulting NOEC), showed a negative value for (SEM-AVS), and does not support the cut-off level of 100 $\mu mol/g_{oc}$).

Overall, the AVS-approach, in particularly the (SEM-AVS)-approach seems a more appropriate approach than the wet or dry weight normalised $PNEC_{add}$.

Dietary route

Questions remain on the contribution of the dietary route to the total amount of metal taken up and to the observed toxicity of metals in the sediment. For example, it remains unclear whether the species that were tested in the various single-species and colonisation studies also represent those species that may take up metals significantly from the sediment. For example, two studies (Lee et al., 2000; Griscom et al., 2000) showed that for various metals, e.g. Cd, Ni, Ag and Zn, there was a much better relationship between the concentrations of the metals in four benthic organisms and SEM in the sediment than between organisms' concentrations and (SEM-AVS) content. The latter studies thus question the assumption that metals are not available at anaerobic conditions in the sediment, where there is excess AVS. The latter studies thus also question the underlying assumption in the AVS-approach, i.e. it is only the free metal ion concentration in the (pore)water that relates to bioavailability and toxicity. Thus, if the underlying studies that were used by DiToro et al. (2002) to develop the AVS-approach, did not include species that are able to somehow significantly extract metals from the sediment in their gastro-intestinal tract, the concept of this AVS-approach is questionable.

The recent study by Van Sprang et al. (2003) showed that in sediment where zinc levels were as high as 8,000 mg/kg dry weight, and where AVS-concentrations were also very high, AVS-corrected zinc concentrations expressed as SEM/AVS and (SEM-AVS) were below 1 and 0, respectively. Biological monitoring, however, did show significant effects to sediment organisms, thus showing that at high zinc concentrations, the AVS-corrected zinc concentration should not be exclusively used in risk assessment. The finding that toxicity was observed even when the AVS-corrected values indicated that there was no excess zinc available for uptake and toxicity, can be explained by a significant contribution of other routes than the (pore) water such as via the dietary route for the uptake of zinc at these very high zinc concentrations, and subsequent toxicological effects. Either AVS thus does not bind zinc effectively at such high concentrations or other stressors were causing the observed toxicity in this study.

Bioaccumulation

With respect to the applicability of the SEM-AVS concept and bioaccumulation, the SEM-AVS concept was originally developed to predict the absence of toxicity and not to predict toxicity or bioaccumulation phenomena. Nevertheless, some publications have investigated

the applicability of the SEM-AVS concept on these issues. Ankley et al. (1996) made a comprehensive review of the available literature on metal bioaccumulation versus sediment metal/AVS relationships to further examine the tenet that AVS controls metal bioavailability. The preponderance of these studies indicated reduced accumulation of metals at sediment metal/AVS ratios of less than 1. However, there were exceptions to this general observation, two of which occurred in short-term laboratory experiments with cadmium- or nickel spiked sediments. In these studies there appeared to be a linear accumulation of cadmium and nickel body burdens with increasing sediment metal concentrations irrespective of the metal/AVS ratio. Unfortunately, some of these studies are confounded by the fact that the organisms were not gut-purged prior to analysis of tissue cadmium. In more recent work cadmium, nickel and zinc bioaccumulation was examined in four types of invertebrates (Lee et al., 2000). Their latter results showed that metal concentrations in animal tissue correlated with metal concentrations extracted from sediments, but not with metal in pore water across a range of reactive sulphide concentrations. However, the relevance of these observations in terms of hazard or risk for an essential element as zinc has not been addressed (Vangheluwe et al., 2003).

The general cut-off value of 100 µmol/g_{oc}

The cut-off value of 100 µmol/g_{oc} in the case (SEM-AVS)/f_{oc} is used to correct metal concentrations in the sediment (DiToro et al., 2002), is proposed for all metals. Following the equation and assuming a condition where the AVS concentration would be zero, this assumption would imply that toxicity is the same for all the metals involved. This seems to be contradicted by the fact that the intrinsic toxicity of e.g. cadmium is much higher than that of e.g. zinc. This seems to limit using the general cut-off as a universal method for all metals in the case when excess metals are available in the sediment.

Assessing a single metal under the AVS-approach

When the AVS-approach would be followed in the risk assessment of a metal, it is not SEM but e.g. zinc or any other single metal that is under discussion. Yet, when using the (SEM-AVS) approach, monitoring studies need to measure the various metals involved. The questions remain how to deal with natural or ambient background concentrations of the metal under consideration and how to deal with the other metals involved in this approach. Most likely, the individual background concentrations of the various metals should be summed for their contribution to SEM.

Seasonal and spatial variability

SEM, AVS and f_{oc} may vary seasonally and spatially in the environment. For example, anaerobic sediment (high AVS-content) may become aerobic (low AVS-content) after storm events or strong currents, in the latter case returning the metals into a more soluble form. The question is thus how to deal with these variations for risk assessment purposes. A worst-case approach may be to identify these variations and assuming a relatively high value for SEM and relatively low values for AVS and f_{oc}. This requires, e.g. seasonally monitoring of these properties, and selecting the most crucial values for actual risk assessment. It must be noted that variations in AVS are higher, e.g. up to tenfold, than variations in SEM or f_{oc}, e.g. within a factor of two (e.g. Van den Berg et al., 1998). In addition, sediment may ultimately be dredged and put to soil with accompanying major changes in redox potential and thus major changes in AVS and bioavailability.

With respect to temporal and spatial changes in AVS and SEM observed in the evaluated laboratory and field studies a few general observations can be made. Temporal changes in the sediments were low for AVS (usually within a factor of 2) and very low for SEM (especially

at higher concentrations). Spatial changes with respect to sediment depth were high for AVS (factor 5 to >20). The AVS concentrations increase with increasing depth, which is related to increasing anoxic conditions. The SEM concentrations also appear to increase with increasing depth, but (much) less than AVS. As the overall result, the SEM-AVS difference and the SEM/AVS ratio in the surface layer of the sediment are considerably higher than in deeper sediment layers, resulting in higher potential toxicity in the surficial sediment. Based on the results of their 1-yr field colonisation study, Hare et al. (1994) concluded the following:

- "The small variation over time of reactive Cd, AVS and dissolved-Cd concentrations measured in the experimental containers suggests that temporal variations in contaminant exposure can be ignored in the interpretation of the biological results." However, they also stated that large seasonal variations in AVS, by as much as two orders of magnitude, were found in some U.S. lakes. The variations have been attributed to fluctuations in either temperature (indirect, by influence on biological activity, namely on primary production and sediment microbial activity) or hypolimnetic oxygen (Leonard et al, 1993; Howard and Evans, 1993). Moreover, the test location in the study by Hare et al. (1994) was at a depth of 15 m, thus the influence of turbulence is expected to be rather low.
- "The depth variations in AVS concentrations are potentially problematic for the characterisation of the SEM/AVS ratio. Ideally, the choice of a depth interval for the calculations of the SEM/AVS ratio should be based on knowledge of the burrowing behaviour of the animals present."
- "In 10-d laboratory tests, 50% mortality of marine amphipods was found at molar Cd/AVS ratios of 1.5-2.2 (DiToro et al., 1990). Similar laboratory results were found for freshwater amphipods (Ankley et al., 1991), oligochaetes and gastropods (Carlson et al., 1991). Yet in our field study there were few detectable toxic effects over a 1-yr period, even at Cd/AVS ratios as high as 10." It must be noted that only one determination of abundances was made, i.e. 1 year after spiking of the sediments. It cannot be excluded, therefore, that temporary effects on benthic organisms may have occurred.
- The overestimation of toxicity in laboratory bioassays may be caused by stressed animals or destroyed vertical gradients of AVS and metals in homogenised sediment. In the field study also homogenised sediments were used, but there was a 1-yr period for the partial re-establishment of the gradient. Moreover, in the field study pH was lower than in the laboratory studies, which may resulted in an increased competition between H^+ and Cd^{2+} ions for uptake sites on animals in the field study. This may have resulted in reduced cadmium uptake and toxicity at low pH (Campbell and Stokes, 1985). However, preliminary results from field and laboratory studies with C. punctipennus and other insects suggest that for pH > 5.5 (the anoxic porewater in our study had a pH near 6), H^+ does not effectively compete with Cd^{2+} (Hare and Tessier, unpublished). Furthermore, the species usually used in laboratory tests (amphipods, gastropods, oligochaetes) have been shown to be more sensitive to Cd exposure than some members of the major field community members, i.e. the Chironomidae. However, this generalisation should be applied with caution to our study species, for most of which no toxicity data appear to be available. Furthermore, the sensitively of closely related species can differ widely.

A general remark on the field studies is that the difference in sensitivity of closely related species causes a general drawback of field studies where the surviving organisms are often juveniles which are not determined up to the species level. The replacement of a sensitive species by a related resistant species can go unnoticed in field studies.

With further respect to spatial and temporal variability (seasonality) in AVS concentrations that may affect zinc bioavailability, several studies reported on the dynamic behaviour of AVS in natural systems. Besides the inherent spatial variations observed between different sampling locations AVS concentrations also differ with depth. Most often the AVS concentration increases with increasing sediment depth and is linked to the redox gradient present in the sediment. This increase may already occur over a small sediment distance (0-10 cm) (Van den Berg et al., 1998; Van den Berg et al., 2001).

In addition to the spatial component AVS concentrations tend to be the higher at the end of the summer and during fall, and lower in winter and spring (Howard and Evans, 1993; Van den Hoop et al., 1997; Grabowski et al., 2001). This seasonal variability is not always being observed. Most of the studies reporting on seasonal variability of AVS have been addressing uncontaminated sediments. For example, the study by Liber et al. (1996) clearly indicated that ZnS is substantially more stable than FeS. The latter was the dominant AVS form in the control sediment, which showed a clear seasonal variation. However, the same level of fluctuation was not observed in zinc spiked sediment, with lowest seasonal fluctuation occurring at the highest zinc levels.

Hence, a proper understanding with respect to the spatial and seasonal variations of AVS and SEM levels is required in order to apply the AVS concept correctly. Three interconnected factors may help to explain the observed patterns reported in the literature with regard to the variation in AVS concentration:

- diagenetic processes variations in temperature, oxygen and organic carbon content influencing the microbiological activity,
- the stability of the metal sulphide complex with respect to oxidation, and
- bioturbation.

In some rivers and lakes stratification[25] due to temperature differences may occur in winter. As a result oxygen levels may be lower at the bottom of a lake due to this phenomenon. If oxygen levels drop, AVS levels will increase but a lot will depend on the microbial activity during the occurrence of the anoxic period. Microbial activity is the lowest in the winter period. After spring turnover oxygen levels will increase and AVS will be more oxidised but on the other hand due to the higher temperatures in spring and summer microbial activity will also increase yielding a higher sulphate reduction rate. The net result is that AVS concentrations tend to be generally higher at the end of the summer and during fall and lower in winter and spring (Table 3.84).

25 Different types of stratification may occur (e.g. temperature, salt). In estuarine environments and river systems influenced by salt water intrusion stratification may occur due to the salt gradient. In these environments oxygen free conditions will be present in the deep water layers during fall and summer and hence sulphide levels will be high during these periods.

Table 3.84 Measured AVS concentrations in European freshwater sediments (EUROECOLE, 2001)

Country	Sampling location	Sampling date	AVS (µmol/g dry weight)
Belgium	Bihain (river)	11-2000	3.0
		04-2001	3.5
Belgium	Somerain (river)	11-2000	40.0
		04-2001	2.5
The Netherlands	Ankeveen (river)	11-2000	25.8
		05-2001	4.3

The sampling strategy used to set up the Flanders database (Vangheluwe et al., 2003) was aimed at sampling sediments when AVS levels are the lowest. Sampling was conducted from March 2002-May 2002 and as such represents in terms of AVS concentrations a worst case scenario (i.e. lowest AVS levels).

The observed vertical gradient in sediment AVS is mainly caused by the oxidation of AVS near the sediment/overlying water interface. A factor that also may contribute to the formation of AVS depth gradients is sediment bioturbation (DeWitt, 1996). Peterson et al. (1996) used different densities of the burrowing oligochaete *Lumbriculus variegatus* in a series of laboratory experiments to evaluate the effect of bioturbation on the oxidation of AVS and subsequent bioavailability of cadmium and zinc spiked into freshwater sediments. They showed that the burrowing activity of oligochaete worms significantly reduced AVS concentrations in surficial sediments in a density related manner and resulted in elevated interstitial water concentrations of Cd but surprisingly not for Zn, for which they have no explanation.

The transient nature of AVS may, however, be overstated relative to predicting the fate of all metal-sulphide complexes in aquatic sediments. Most of the studies evaluating seasonal/spatial variations of AVS have been addressing uncontaminated sediments, hence were looking at the dynamics of FeS that is relatively labile while CdS, CuS, PbS and ZnS are less susceptible to oxidation than iron sulphides (Vangheluwe et al., 2003).

AVS-approach also suitable for oxidised sediment layers?

With respect to the AVS-approach in oxidised environment, however, common criticisms against the AVS approach that the model is only appropriate for use with anoxic sediments appears to be unfounded. First of all, in very oxidised and dynamic sediments AVS levels will be very low and hence the bioavailability will be predicted to be high. Ignoring additional binding phases such as Fe/Mn (oxy)hydroxides, organic carbon, carbonates, makes this approach already conservative. In the cases where both oxic and anoxic sediments are present the SEM-AVS model still successfully predicts the absence of toxicity on a consistent basis. A possible explanation for this observation is the fact that the aerobic layer is in general rather thin, typically on the order of only a few millimeters to a few centimetres in thickness (Carlton and Klug, 1990; Hesslein 1976; Statzner et al., 1988; DiToro et al., 1991) so that the exposure is largely driven by the anaerobic chemistry. Since the oxidation process is often

relatively slow the rate of conversion of insoluble metal sulphide to dissolved metal in the pore water occurs slowly but might still be important in environments that are not very dynamic. At the other hand, any surficial layer metal sulphide that becomes dissolved in the pore water, as a result of metal sulphide oxidation, will not simply build up in the pore water and remain there. Rather it will be subject to diffusion from the pore water into the overlying water as it is produced. Given that the aerobic layer is quite thin, this diffuse flux will tend to offset any increase in pore water metal levels that occur as a result of the oxidation process. Furthermore, pore water metal concentrations will not necessarily be chemically available to benthic organisms, since any metal that is present in the pore water has the potential to form non-bioavailable metal complexes with other pore water ligands, thereby further reducing the potential for toxicity (Vangheluwe et al., 2003).

Future water quality improvements

The impact of future water quality improvements on the generation of AVS is an interesting issue. As such no scientific papers have been found that investigated this phenomenon. However, based on the mechanisms underlying AVS generation some plausible scenario can be developed. AVS production is the result of microbial induced sulphate reduction. Sulphate reduction rates and hence sulphide formation are influenced by the organic carbon content of the sediment. The three main stocks of natural input of organic carbon into the aquatic system are primary production (phytoplankton), dead organic matter and decomposers. If a water body experiences less organic input over time as hypothesised, e.g., due to reduced municipal and industrial wastewater loads, that does not mean that there will not be adequate organic matter (OM) within the system to allow for the generation of new AVS. The OM load to a system, and thus the AVS production, are not only linked to anthropogenic sources, but also to the natural characteristics of the drainage basin.. The benthic ecology literature, e.g. the work of Liber et al. in the Canadian arctic (personal communication Liber) shows that it does not take very much OM in sediment to allow for the establishment of a benthic community. The results of Liber et al. indicate that diamond mine tailings with very low OM content were able to support a benthic community after only one year on the bottom of a small lake (ice-covered for approx. 9-10 months). The surficial tailings layer (0-2 cm) had a TOC content of 1.5%, but the TOC source is unknown, but could have been a combination of both autochthonous and allochthonous inputs. Although the open water season in the arctic is short, the days are very long thus allowing for adequate primary production. Finally, it is noted that any zinc already complexed in the sediment as ZnS should stay as ZnS even with reduced generation of new AVS. Sediment tray studies by e.g. Liber et al. (personal communication) showed that ZnS is relatively stable and unlikely to oxidise as readily as FeS. Thus, the majority of the ZnS present in sediment would stay as such even with a reduction in the generation of new AVS (Vangheluwe et al., 2003).

Furthermore, there may be a concern that when the rate of oxidation processes exceeds that of the formation of sulphides, metals may be mobilised. The transient nature of AVS in this regard may, however, be overstated and is depending on the nature of the metal-sulphide complex. Most of the studies evaluating seasonal/spatial variations of AVS have been addressing uncontaminated sediments, hence were looking at the dynamics of FeS that is relatively labile. The oxidation of iron sulphide in sediments cannot be taken as indicative of the oxidation of other metal sulphide complexes, which are more (e.g. zinc or cadmium sulphide) stable (Peterson et al., 1996). Iron sulphides are more susceptible towards oxygen diffusion from the overlying water than other metal sulphide complexes. DiToro et al. (1996a and 1996b) investigated the kinetics of FeS and CdS oxidation and showed that the oxidation of sediment AVS appears to be biphasic, which may indicate that a more resistant component is present as well as a reactive component similar to the synthetic FeS used in their

experiments. These observations are consistent with the results of recent studies. Simpson et al. (1998) demonstrated that while FeS and MnS are labile and rapidly oxidisable phases, CdS, CuS, PbS and ZnS are kinetically stable for several hours. Sundelin and Eriksson (2001) provide further evidence that can reduce the concern on remobilization for other metal sulphides. They showed that after long term oxygenation of sediment cores (3 to 7 months), Cd, Zn and Cu remain comparatively unavailable. As alluded above these observations can partly be explained by the higher stability of the cadmium copper and zinc sulphides with regard to oxidation than iron sulphides but the long-term stability is suggesting that other ligands in addition to AVS are important for metal bioavailability. Buykx (2000) showed that aeration of a sediment during 3 weeks hardly affected the speciation of Ni, Cu and Pb. Zn and Cd were released as AVS levels decreased but were subsequently bound as carbonates or adsorbed to other binding phases. This is consistent with the findings of Mahony et al. (1996) and DiToro et al. (2002) regarding metal binding to organic carbon in sediments, but also adsorption to several other major other components (e.g. iron and manganese hydrous oxides) present in sediment solid phases can influence the distribution of metals. Zhuang et al. (1994) investigated the effect of aeration on cadmium bioavailability in sediments in a series of lab aeration experiments in batch reactors during periods of approximately one month. During aeration the concentrations of metals associated with AVS and those with pyrite decreased. At the same time there were increases in the concentrations of hydrous iron and manganese oxides and these materials became increasingly more important in the binding of cadmium. Following the aeration more than 50 % of the cadmium was associated with the extractable iron and manganese components and approximately 2 % of the cadmium released during the oxidation of AVS entered in the liquid phase (Vangheluwe et al., 2003).

The impact of dredging

The concern that dredging of sediments could result in a potential increase in dissolved concentrations of metals in the surface water, primarily related to environmental conditions promoting the shift of trace metals from the particulate state to the dissolved state, e.g. by oxidation of reduced phases, has already been largely answered by the arguments presented above under the two previous headings. Since dredging activities are typically intermittent processes in which increased turbidity levels already quickly return to the natural background situation after 30-45 minutes (Van Parys et al., 2001), it is not expected that remobilization of metals will occur to a large extent. Van den Berg et al. (2001b) collected data on remobilization during a large scale experimental dredging project conducted under field conditions. The results showed that dredging activities do not notably influence dissolved concentrations of trace metals in the water column. Burton et al. (1991) provide an overview of similar studies supporting these observations. These observations could be related to a relatively slow oxidation of metal sulphides or a fast re-supply of liberated trace metals over e.g. freshly formed Mn- and Fe-(hydr)oxides that may provide an efficient new sorptive phase for trace metals (Vangheluwe et al., 2003).

Summary on dynamic, bioturbated and oxidising field conditions

There is a preponderance of evidence showing that the SEM/AVS model is applicable in dynamic, bioturbated and oxidising field conditions due to the enhanced stability of sulphide complexes of copper, cadmium, zinc, nickel and lead relative to the stability of the iron and manganese monosulphide complexes. FeS and MnS therefore act as a buffer for the oxidation of the other metal sulphides. When finally the less soluble metal sulphides are oxidised, freshly formed iron and manganese oxides together with the organic carbon coating on sediment particles may act as new reactive surfaces that have high affinity for free metal ions. As such the concern of remobilization under oxidised conditions is minimal.

Sampling protocols

Since AVS varies with depth of the sediment and organisms reside in various parts and depths of the sediment, sampling conditions of AVS should be better defined, e.g. which depth, how many times during a season, etc. Currently, different depths are sampled in various studies that may need harmonisation in the future when the AVS-approach is used on a wider scale.

Summary on the evaluation of the AVS-approach

Overall, taken all other arguments together, there is sufficient scientific evidence to adopt the SEM/AVS or (SEM-AVS) model.

On the one hand, the proposed correction for AVS can be considered a conservative approach since:

1. Adsorption on organic carbon and complexation with carbonates is not taken into account
2. Other bioavailability mediators such as co-precipitation of zinc with e.g. iron/manganese oxyhydroxides are not being considered, and
3. Mitigating effects of pore water composition are being ignored.

On the other hand, some studies show deviations from the SEM/AVS or (SEM-AVS) model (see e.g. Figures 3.21. to 3.23.), while other studies challenge the entire concept (e.g. Ankley et al., 1996; Griscom et al., 2000; Lee et al., 2000).

Overall, the overview studies by DiToro et al. (2002), Shine et al. (2003) and various individual laboratory and field studies (e.g. Burton et al., 2003) provides sufficient evidence that the AVS-approach is appropriate. Since there are, however, some remaining uncertainties, e.g. on the dietary contribution, efficient, the AVS-approach should be used with some conservatism. Furthermore, PEC/PNEC-ratios should always be evaluated in addition to the AVS-corrected zinc concentrations in sediment, which is illustrated by the study by Van Sprang (2003). He showed that in sediment where zinc levels were as high as 8,000 mg/kg dry weight, and where AVS-concentrations were also very high, AVS-corrected zinc concentrations expressed as SEM/AVS and (SEM-AVS) were below 1 and 0, respectively. Biological monitoring, however, did show significant effects to sediment organisms, thus showing that at high zinc concentrations, the AVS-corrected zinc concentration should not be exclusively used in risk assessment. The finding that toxicity was observed even when the AVS-corrected values indicated that there was no excess zinc available for uptake and toxicity, can be explained by a significant contribution of other routes than the (pore) water such as via the dietary route for the uptake of zinc at these very high zinc concentrations, and subsequent toxicological effects. Either AVS thus does not bind zinc effectively at such high concentrations or other stressors were causing the observed toxicity in this study.

Implementation of the AVS-approach in risk assessment

Since both approaches, i.e. the wet or dry weight normalised PNEC-approach and the AVS-approach, seem to have merits as well as limitations, the following two-tiered approach (Figure 3.24) will be used in the risk assessment of zinc in sediment:

Tier 1: Assess the region or site-specific risk of zinc in the sediment, based on the ratio of the PEC_{add} and the $PNEC_{add}$.

1) If the ratio is less than 1, no potential risk can be assumed (conclusion (ii)).
2) If the ratio is higher than 1 then go to Tier 2.

Tier 2: Assess the region or site-specific risk taking into account AVS, by measuring (SEM-AVS), or include a generic bioavailability correction of 0.5. Calculate the excess zinc concentration, according to the equation in Figure 3.3.2.2.1.5. Then, characterise the risk, following Figure 3.3.2.2.1.5.

It must be noted that the applicability of the generic bioavailability correction ($BioF_{sediment, generic}$) may be limited and a reservation is made for oligotrophic, shallow sediments.

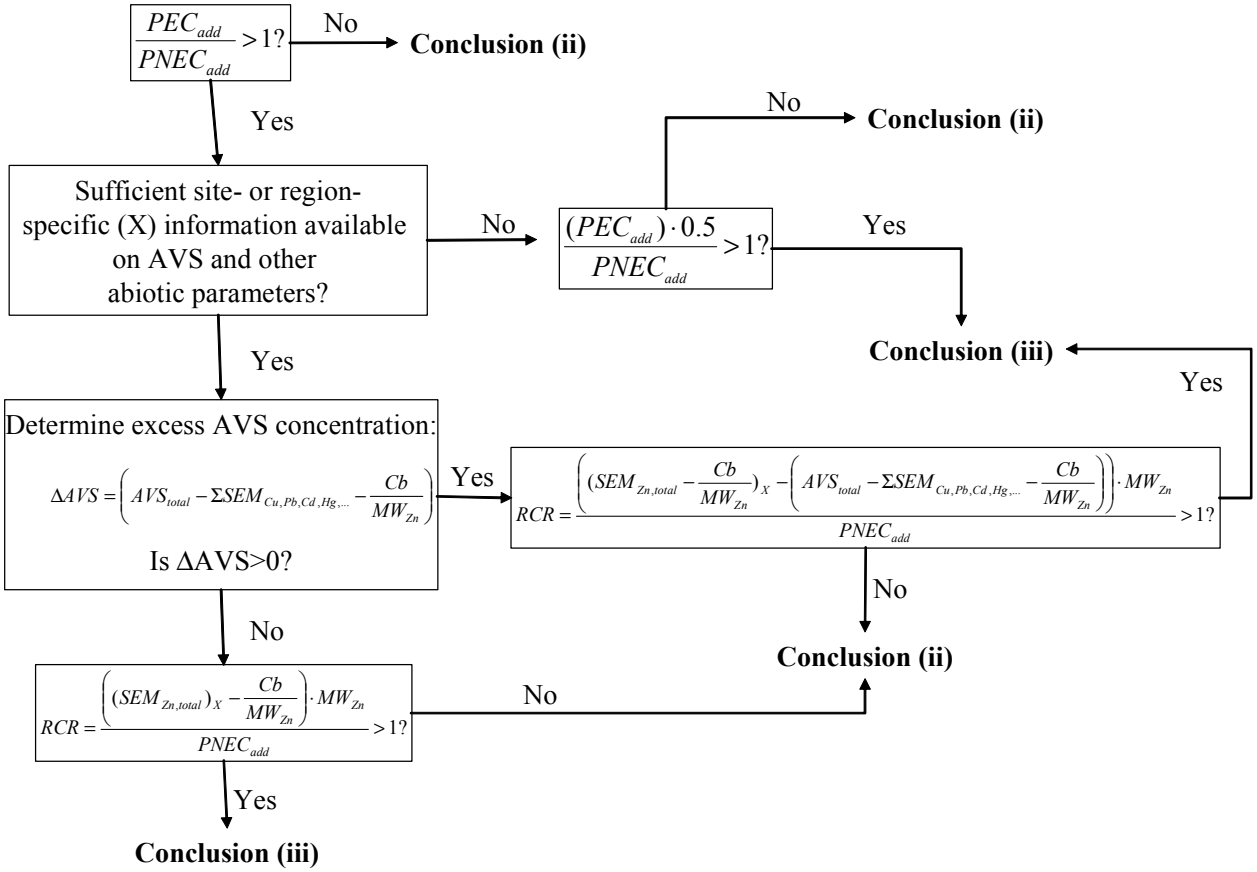


Figure 3.24 Decision tree for correcting the PEC_{add} for reduced bioavailability in sediment using an AVS-correction model. A generic bioavailability correction of 0.5 is applied in case no sufficient information on AVS is available.

Under Tier 2, the following stepwise approach is proposed to integrate AVS and thus to integrate bioavailability in calculating the PEC.

Since the aim of the RCR is to provide an equation where the denominator only contains the $PNEC_{add}$, and where the numerator adequately corrects for the background concentration and for (excess) AVS), the following equation is proposed, where first ΔAVS is calculated

$$\Delta AVS = \left(AVS_{total} - \Sigma SEM_{Cu,Pb,Cd,Hg,...} - \frac{Cb}{MW_{Zn}} \right)$$

MW_{Zn} = molecular weight of zinc (=65.38 g/mol)
Cb = background concentration of zinc in the sediment (mg/kg)

ΔAVS = excess of AVS, i.e. AVS_{total} corrected for metals that bind stronger than zinc to AVS (mol/kg) and corrected for the background zinc concentration, which is assumed to bind to AVS as well

if ΔAVS would become <0, then insufficient sulphide would be available to sequester the anthropogenic zinc in the sediment. The risk characterisation ratio would then become:

$$RCR = \frac{\left((SEM_{Zn,total})_X - \frac{Cb}{MW_{Zn}} \right) \cdot MW_{Zn}}{PNEC_{add}}$$

if ΔAVS would become >0, then there is sufficient sulphide available to sequester the anthropogenic zinc in the sediment

$$RCR = \frac{\left((SEM_{Zn,total} - \frac{Cb}{MW_{Zn}})_X - \left(AVS_{total} - \Sigma SEM_{Cu,Pb,Cd,Hg,\ldots} - \frac{Cb}{MW_{Zn}} \right) \right) \cdot MW_{Zn}}{PNEC_{add}}$$

The $SEM_{Zn,total,X}$ is the sum of the anthropogenic part and the background concentration of zinc on a molar basis. The bioavailable added zinc concentration is determined by first subtracting the background zinc concentration, and then by subtracting the excess AVS. The latter is defined as the total AVS minus the AVS that is bound by metals that are more strongly bound to AVS than zinc ($\Sigma SEM_{Cu,Pb,Cd,Hg,\ldots}$), minus the background zinc concentration. Following this approach it is assumed that this remaining AVS binds to total zinc concentration, i.e. to the anthropogenic part and the background zinc in the sediment. Effectively, part of this remaining AVS sequesters the background zinc concentration, and thus corrects the $SEM_{total,\ Zn,\ X}$ for the background concentration of zinc. The more AVS is present the more it can reduce the $SEM_{total,\ Zn,\ X,}$ and it does thus adequately correct for AVS.

The added risk approach assumes that the organisms are only affected by added zinc - so the background zinc has no effect on organisms. The background zinc can however bind to AVS, so it needs to be taken into account when determining how much AVS is available to bind to the added zinc. One can then assume and be reasonably certain that the background bound in this way is unavailable as well as having no effect.

Regarding the binding of AVS to the background zinc the following must be noted. If no other metals than zinc were present in sediment this would make sense, i.e. in the absence of an anthropogenic zinc source the natural background zinc will have the first opportunity to bind with AVS. Then if zinc is added in surplus this anthropogenic zinc would only be able to bind with the amount of AVS not yet bound to the natural zinc background. However, first of all sediments are multi-metallic of nature. Hence, in presence of metals with a higher affinity than zinc the natural background zinc will be displaced from its Zn-sulfide complex joining as such the pool of anthropogenic zinc that will bind subsequently to newly formed AVS without having the further distinction between natural zinc of anthropogenic zinc. Secondly, the natural background is continuously replenished in the water column due to leaching of minerals and as such this natural background cannot be distinguished from the anthropogenic part. Therefore, the assumption that AVS binds first to the background may be overestimated in reality. Since no further information is available, the approach as described above is used.

If no sufficient site or region-specific information on the abiotic parameters is available, only a generic bioavailability correction is possible, and then either conclusion (ii) or conclusion (iii) will be reached.

It must be noted that even when there is no excess zinc it may be that equimolar amounts or more of AVS sequesters very high zinc concentrations in the sediment. Whether or not this situation is still without risks to benthic organisms should be further investigated.

It must be further noted that in most Dutch sediments, zinc comprises 80-90% of SEM (Van den Hoop et al., 1997). Furthermore, the total zinc concentration as reported in some monitoring campaigns may be different from the zinc concentration when determined in combination with AVS-measurements, due to different analytical extraction methods that may have been used. However, since EURAS (Vangheluwe et al., 2003) showed that the SEM_{Zn} concentration represents 87.8 % (= median value, 10P value = 60%) of the total zinc content of the sediment, it is assumed that the different analytical methods do show result in the same concentrations. Furthermore, the correction for different analytical methods is not used while it is currently not known which analytical methods have been used for all the monitored data and because the difference would be academic if it would concern modelled data.

With respect to the choice of the x^{th}-percentile value for the abiotic parameters in the case where many temporal and spatial varying data are available for a site or a region, different options may be used that may allow for sufficient realism as well as conservatism. This will be further discussed in the risk characterisation section.

In addition, the following situations may occur:

- For regional exposure: (a) big rivers with a good description and characterisation of the abiotic factors; these data can be used as input for the equations; (b) when no data on the abiotic factors are available, a regional analysis may be used.
- For local exposure: when data are available, they should be used, otherwise data from regional analyses could be used.

A generic regional bioavailability factor

Vangheluwe et al. (2003) has proposed using a generic regional bioavailability factor ($BioF_{sediment}$) value of 0.13, 0.16 or 0.17 that is based on a Flemish database and data from The Netherlands on AVS, SEM and total zinc measurements. In this EURAS report, detailed information is presented on the Flanders database. The report also provide two approaches on how to derive a generic bioavailability factor, and further proposes that Flanders case can be used as a regional scenario.

The overall aggregated database for Europe consisted of 226 data points for which a complete set of SEM-AVS data is available. Most data available are from sampling stations in the Flemish region of Belgium and the Netherlands. Data on SEM/AVS concentrations in other European countries is limited.

The database was used to elaborate frequency distributions of ambient AVS, organic carbon and SEM_{Zn} exposure concentrations in the sediments in order to derive the different percentiles for the investigated parameters for the sediment compartment. This analysis yielded for the overall freshwater data set available for Europe (n = 226) a median (50^{th} percentile) AVS concentration of 8.1 µmol/g dry wt. and a 90^{th} percentile of 75.5 µmol/g dry wt. The 50^{th} and 90^{th} percentile for the fraction of organic carbon was calculated to be 0.012 and 0.038 %. In applying the SEM-AVS model for a specific metal, such as zinc, it has to be taken into consideration that SEM represents the sum of different metals acting in a

competitive manner when binding to AVS. In general, zinc represented 41 to 96 % of the total SEM. Based on the overall freshwater data set (n = 226) available for Europe a median SEM-Zinc concentration of 2.1 µmol/g dry wt. and a 90th percentile of 8.3 µmol/g dry wt (with corresponds to 137 mg/kg dry wt and 543 mg/kg dry wt, respectively) was obtained. For the Flemish database a median SEM-Zinc concentration of 2.0 µmol/g dry wt. and a 90th percentile of 7.8 µmol/g dry wt (with corresponds to 131 mg/kg dry wt and 510 mg/kg dry wt, respectively).

The main purpose of the study, however, was to assess the probability of the occurrence of a SEM-AVS difference larger than zero and a (SEM-AVS)foc larger than 100 µmol/g OC for zinc. The latter will not be summarised here, since it will not be used in the current risk assessment report. In this regard two approaches have been followed. At first the coupled SEM-AVS data for each sampling station was used to derive the cumulative frequency distribution. Secondly, since it was not feasible to sample all possible sets of conditions encountered in sediments in Europe a probabilistic approach was adapted in order to develop a representative statistical characterisation of site specific characteristics across Europe. Therefore from the aggregated database, values of the different distributions were randomly selected for each variable with the use of Monte Carlo analysis and used to generate the cumulative distribution function of the excess SEM_{Zn}.

Applying a Monte Carlo analysis, values (2,000 iterations) from the elaborated cumulative distribution functions of AVS, SEM_{zn} and foc were generated and used to generate the SEM-AVS distributions. From these it is possible to quantify the likelihood that the excess SEM zinc is smaller or equal to zero for European sediments. This approach allowed quantifying the likelihood that the excess SEM is smaller or equal to zero for European sediments, hence allowing the bioavailability to be assessed and predicted. For zinc the probability of obtaining an excess Zn (SEM_{Zn} - ΔAVS > 0 or SEM_{Zn}/ΔAVS >1) is 28.1 % indicating that this is the chance that a fraction of Zn present in sediments may be potentially bioavailable depending on the presence of organic carbon and other binding substrates (Fe/Mn oxides) in sediments and sediment pore water that may reduce further bioavailability. The chance that no excess SEM_{Zn} is present, and consequently, no toxicity is predicted in sediments is 72 %.

For the database of really measured coupled SEM-AVS data the probability that SEM_{Zn} - Δ AVS (= excess SEM_{Zn}) is larger than 0 in Europe is 23.7 % while in 76.3 % of the sediments no excess SEM_{Zn} is observed (Figure 3.25).

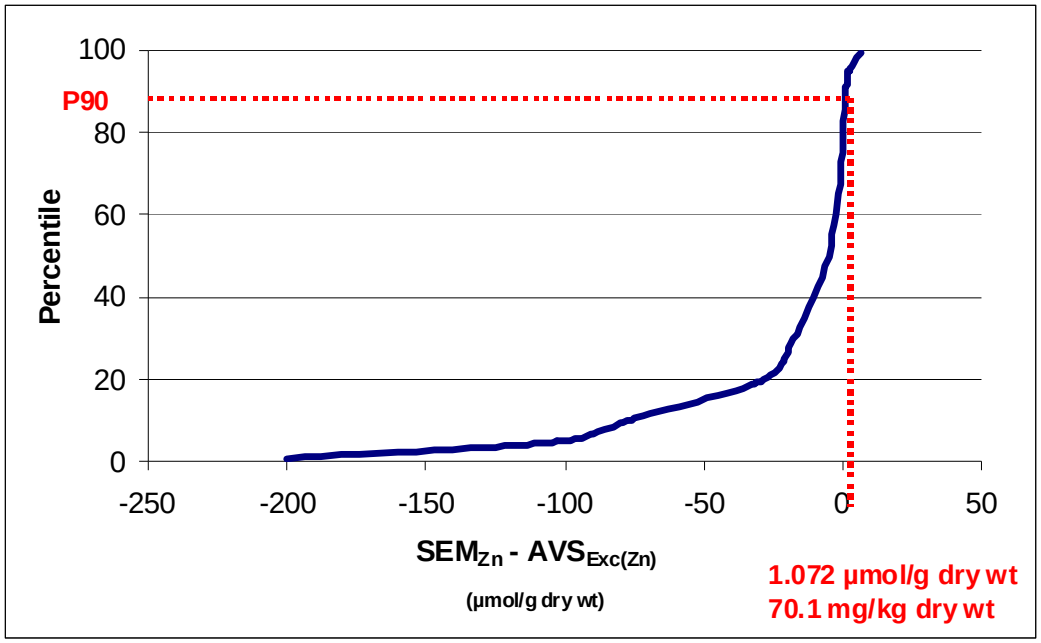


Figure 3.25 Cumulative distribution of excess zinc, expressed as (SEM_{Zn} – $AVS_{Exc(Zn)}$), coupled data of the Flanders database, with an indication of the 90th percentile.

Further analysis of the data suggests that a coupling (covariance) of SEM_{Zn} and ΔAVS_{Zn} occurs. Figure 3.26. illustrates the observed trend between and SEM_{Zn} for those data points where $\Delta AVS_{Zn} > SEM_{Zn}$. Sediments where $SEM_{Zn} > \Delta AVS_{Zn}$, were excluded from the analysis since in that case part of the measured SEM_{Zn} was not bound to AVS and can therefore affect the identification of a possible relationship between both parameters.

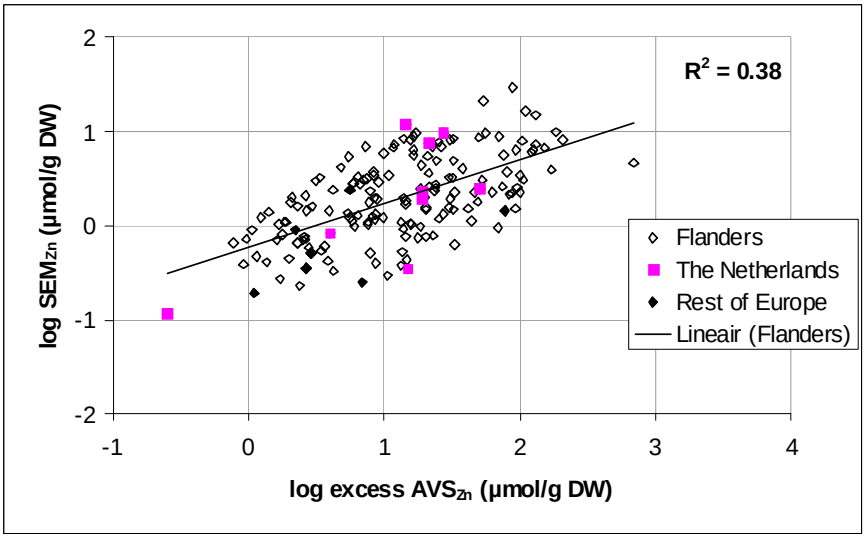


Figure 3.26 Covariance between AVS_{Zn} and SEM_{Zn} in European sediments.

Figure 3.26 clearly shows a trend indicating that SEM_{Zn} increases with increasing ΔAVS_{Zn}. Covariance between SEM_{Zn} and AVS has been suggested in literature and has been explained by the fact that Zn-sulfides are more stable than Fe-sulfides (Liber et al., 1996). Based on these findings it can be concluded that measured coupled $\Delta AVS_{Zn}/SEM_{Zn}$ combinations should be preferentially being used (Vangheluwe et al., 2003).

A cumulative frequency analysis of AVS-SEM combinations, using data from the Flemish and Dutch datasets (226 sediments) clearly shows that low AVS (i.e. 10P) and high SEM (i.e. 90P) combinations are not observed.

Vangheluwe et al. (2003) conclude that the Flanders case is considered appropriate for other European regions. However, most of the data from The Netherlands seem to be in the upper distribution of that of Flanders with respect to both AVS and SEM. In addition, the report proposes to derive the generic $BioF_{sediment}$ value following two approaches. The first approach results in a $BioF_{sediment}$ of 0.13 that is the ratio of the 90th-percentile of the bioavailable zinc (90th P $SEM_{Zn,\ bioavailable}$) to the 90th-percentile of the total zinc (90th P Zn_{total}) from the Flemish database. The second approach results in the average value of the 10th-, 50th- and 90th-percentile values of the ratios using the coupled data of total zinc and bioavailable zinc. Since the individual values are 0, 0 and 0.48, the average value is 0.16. Furthermore, based on 16 data from The Netherlands the values of the 10th-, 50th- and 90th-percentiles of the ratios are 0. 0 and 0.50, which results in an average value of 0.17.

The representativeness of the Flemish database seems not to fully cover the data on AVS and SEM outside Flanders. Furthermore, taking the average of the 10th-, 50th- and 90th-percentile values is not a conservative approach for deriving a generic $BioF_{sediment}$ value. There are many data above these proposed average generic values of between 0.13-0.17.

Furthermore, the analysis by Vangheluwe et al. (2003) of 16 Dutch sediments showed $BioF_{sediment}$ values ranging from 0 to 0.59 (Table 3.85). $BioF_{sediment}$ values greater than 0 were found for example for the Meuse river and the lake Ketelmeer.

Table 3.85 Overview of the individual $BioF_{sediment}$ values calculated for several locations in The Netherlands. Total zinc concentrations are only given when bioavailable zinc was predicted to occur (Vangheluwe et al., 2003).

Location	SEM Zn - $\triangle AVS_{Zn}$ (µmol/g dry wt.)	Total Zinc (µg/g dry wt.)	$BioF_{sediment}$
Schoonrewoerdse Wiel	- 48.8		0
Oostvaarders Plassen	- 17.6		0
Nieuwersluis	-17		0
Ketelmeer	- 2.7		0
Ketelmeer A	5.8	736	0.51
Ketelmeer B	2.1	396	0.34
Ketelmeer C	4.2	739	0.37
Ketelmeer D	3.3	742	0.29
Leeghwaterplas	- 14.7		0
Botlek	- 13.7		0
Meuse C	9.4	1,044	0.59
Meuse A	0.9	692	0.08
Meuse B	- 17.9	699	0
Meuse D	7.2	994	0.48
Ankeveen	-3.2		0
Marken (lake)	-0.1		0

However, since most values do not exceed 0.50, this latter value is taken as the generic regional $BioF_{sediment}$ value for those regions in Europe for which no region-specific data on SEM and AVS are available. The PECs from those regions ($PECregion_{total}$) will thus be multiplied by this $BioF_{sediment}$ prior to comparing them to the $PNEC_{total}$ for sediment in the risk characterisation. The value of 0.50 may on the one hand be too conservative, following the Flemish database exercise, and on the other hand a more realistic worst-case value (see earlier).

$$PECregion_{total,bioavailable} = PECregion_{total} \times BioF_{sediment,\ generic}$$

It must be noted that the applicability of the $BioF_{sediment,\ generic}$ may be limited and a reservation is made for oligotrophic, shallow sediments. It must be further noted that here again, the PEC_{total} (including zinc background concentration) will be used and then compared to the $PNEC_{total}$ (including the zinc background concentration):

$$RCR = PECregion_{total,bioavailable} / PNEC_{total,generic}$$

If the SEM/AVS approach is going to be used more extensive in e.g. (future) monitoring campaigns, the practical issues regarding sampling technique, depth, interval, representativeness of different habitats in one recipient etc. must be justified and harmonised.

Conclusions on abiotic factors

It is concluded that there is a scientific basis to correct the PECs on the sediment chemistry, i.e. correcting for bioavailability using the AVS-concept.

To further take into account some uncertainty in various parameters as well as to provide some ideas on the sensitivity of the calculations, three scenarios will be used in the risk characterisation when showing the bioavailability corrections:

- the first scenario will be when no bioavailability correction will be used, i.e. the PECadd will be completely based on the added zinc concentration;
- the second scenario will make use of the AVS-concept in a conservative way, i.e. by selecting the 90th-percentile value of the added zinc concentration in the sediment, the 10th-percentile value of the AVS and the 10th-percentile values of all other abiotic parameters; and
- the third scenario will make use of the AVS-concept in a less conservative way, i.e. by selecting the 90th-percentile value of the added zinc concentration in the sediment, the 50th-percentile value of the AVS and the 50th-percentile values of all other abiotic parameters.

3.3.2.2.2 Toxicity of zinc in freshwater sediments

Toxicity data are available for freshwater benthic organisms and for microbe-mediated processes, which will be separately discussed.

Sediment toxicity data for freshwater benthic organisms

Single-species studies (laboratory studies)

There is a limited database of single-species tests performed in freshwater water-sediment systems with Zn-spiked sediments. The available data are for benthic invertebrates (sediment-dwelling organisms) and include both short-term tests (up to 10 days exposure) and long-term tests (3 to 8 weeks exposure), which are summarised in Table 3.3.2.e in Annex 3.3.2.D)[26]. With the exception of one test with the worm *Tubifex tubifex* (oligochaete), the tests used either the amphipod *Hyalella azteca* (crustacean) or the midge *Chironomus tentans* (insect). The latter two species (together with the worm *Lumbricus variegatus)* are the most frequently utilised freshwater benthic species for assessing the toxicity of substances in spiked freshwater sediments or in field-collected freshwater sediments and for which species-specific standard sediment test protocols have been developed by the ASTM (1995), U.S. EPA (2000) and OECD (2000).

The four chronic toxicity tests that are considered to be useful for PNEC derivation ($PNEC_{add, sediment}$) are summarised in Part I of Table 3.3.2.e in Annex 3.3.2.D. The four tests were performed in unpolluted sediments with a background Zn concentration (Cb) of 22 to 55 mg/kg d.w. In addition to survival at least one other endpoint (growth and/or reproduction) was studied in each test. The 4-w test with *T. tubifex* (Farrar & Bridges, 2003) resulted in a NOEC of 1101 mg/kg d.w. The 3-w and 8-w tests with *C. tentans* (Farrar & Bridges, 2002, 2003; Sibley et al., 1996) resulted in NOEC values of 609 and 795 mg/kg d.w., respectively. The 6-w test with *H. azteca* (Nguyen et al., 2005) resulted in a NOEC of 488 mg/kg d.w. This is the lowest chronic NOEC for benthic invertebrates in the current database of the studies that are considered to be useful for PNEC derivation. Note that the above NOEC values from all four studies are based on the added concentration (Cn), in this case being the actual concentration measured minus Cb, the background concentration.

The toxicity tests that are considered to be not useful for PNEC derivation are summarised in Part II of Table 3.3.2.e in Annex 3.3.2.D. These rejected tests include two chronic toxicity tests with *H. azteca* (Borgmann & Norwood , 1997; Farrar & Bridges, 2001, 2002, 2003). The first chronic toxicity test with *H. azteca* (Borgmann & Norwood, 1997) was performed in a harbour sediment that was strongly polluted with Zn (background Zn concentration: 1500 mg/kg d.w) and therefore considered to be not useful (relevance criterion). In the second chronic toxicity test with *H. azteca* (Farrar & Bridges, 2001, 2002, 2003), growth was significantly ($p<0.05$) reduced by 25% at an added-Zn concentration of 221 mg/kg d.w., the lowest concentration tested (LOEC), and dose-related further reduced at the higher test concentrations. Although a NOEC can be estimated from this study (i.e. using $NOEC^e$ = LOEC/3), this study has been rejected, as both the accepted chronic toxicity test with *H. azteca* (Nguyen et al., 2005, Table 3.3.2.e – Part I) and the rejected chronic toxicity test with *H. azteca* (Borgmann & Norwood, 1997; Table 3.3.2.e-Part II) do not indicate that growth is the most sensitive endpoint for *H. azteca* exposed to zinc. Moreover, the accepted study by Nguyen et al. (2005), that included both growth and survival as toxicity endpoints, resulted in a "real" NOEC. The further rejected tests, with *H. azteca* or *C. tentans*, are too limited with respect to exposure time to derive chronic NOEC values.

Colonisation studies (laboratory and field studies)

In the last decade, two long-term field colonisation studies have been performed in sediments spiked with zinc (Liber et al., 1996; Burton et al., 2003), using in total five different

26 It is noted that no effort has been made to retrieve all available data regarding the acute lethality of Zn in sediments.

freshwater sediments. In addition, one long-term field colonisation study has been performed in a marine sediment spiked with a mixture of metals, including Zn (Boothman et al., 2001). These studies were conducted to validate the AVS hypothesis with respect to chronic effects of Zn-spiked or metal-spiked sediments and have been discussed earlier in section 3.3.2.2.1.

See Annex 3.3.2.D (Table 3.3.2.f – Part C) for an extensive summary of these studies, including data on the toxicological endpoints studied. Table 3.3.2.f (Part D) also summarises two colonisation studies in Cd-spiked sediments (Hare et al., 1994; Hansen et al., 1996b) that also have been discussed in section 3.3.2.2.1.

Sediment toxicity data for microbe-mediated processes in freshwater

Data on microbial toxicity tests conducted in anaerobic sediment-water systems are summarised in Table 3.3.2.g in Annex 3.3.2.D. The data (from Van Beelen and co-workers) include different microbe-mediated processes, viz. methane production (which is the last stage of the anaerobic degradation of organic matter) and the mineralisation of specific organic substrates of either natural or anthropogenic origin. The results include EC10, EC50, IC10 and IC50 values. The microbial toxicity tests do not include single-species (growth) tests. All tests were conducted in sediment samples of the river Rhine estuary and used zinc chloride as test compound. The sediment samples contained a high background concentration of zinc (Cb: 800 mg/kg d.w.). Therefore, these tests are considered to be not useful for $PNEC_{add,\ sediment}$ derivation (relevance criterion) and are not further described here.

3.3.2.2.3 Predicted no effect concentration for sediment (PNECadd, sediment)

According to the TGD, the PNEC for sediment ($PNEC_{add,\ sediment}$) can be derived from sediment toxicity data for benthic organisms (sediment-dwelling organisms), although the selection of test species and test methods are still being discussed, as well as the assessment factors to be used to derive the PNEC. In the absence of toxicity data for benthic organisms, the PNEC for sediment may provisionally be calculated using the equilibrium partitioning (EP) method. Chronic sediment toxicity data from single-species tests in Zn-spiked freshwater sediments are available (although limited to three freshwater species, viz. the worm *Tubifex tubifex,* the midge *Chironomus tentans* and the amphipod *Hyalella azteca*; these species are among the benthic organisms most frequently used for assessing sediment toxicity) and thus have been used to derive the $PNEC_{add}$ for freshwater sediment. In addition, the EP method as described in the TGD has been used for comparison.

$PNEC_{add,\ sediment}$ using the sediment toxicity data for benthic organisms

For benthic invertebrates there are only four useful chronic NOEC values, viz. one for the oligochaete *Tubifex tubifex* (1101 mg/kg d.w.), two for the insect *Chironomus tentans* (609 and 795 mg/kg d.w.) and one for the crustacean *Hyalella azteca* (488 mg/kg d.w.), see section 3.3.2.2.2 and Annex 3.3.2.D – Table 3.3.2.e (Part I). These NOEC values are expressed as the added-Zn concentration (Cn, being actual-Cb). Both with respect to the number of chronic NOEC values and the number of different species, these data are too limited to apply statistical extrapolation (see section 3.3.1.3). Thus, the $PNEC_{add,\ sediment}$ has been derived from the lowest chronic NOEC, i.e. the NOEC of 488 mg/kg d.w for *H. azteca* (from Nguyen et al., 2005)[27]. The above benthic species (*H. azteca, C. tentans* and *T. tubifex*)

27 In the December 2004 draft version of this RAR, an estimated NOECge of 84 mg/kg dw (actual total-Zn

represent three taxonomic groups of invertebrates with different living and feeding conditions, thus according to the TGD an assessment factor of 10 should be used on the lowest chronic NOEC (488 mg/kg d.w., for *H. azteca)* which results in a $PNEC_{add, sediment}$ of 49 mg/kg d.w.

As for soil (see section 3.3.3.1.5) the use of the lowest "species mean" NOEC is considered less appropriate, because sediments are less homogeneous than surface water with respect to abiotic characteristics.

The following issues have been taken into consideration to apply an assessment factor of 10 to the lowest NOEC (488 mg/kg d.w):

<u>The available database of freshwater chronic NOEC values derived from laboratory tests with benthic species and the relative sensitivity of these benthic species for zinc</u>

The database of chronic NOEC values that are considered to be useful for PNEC derivation is limited to 4 values, for 3 different species. This supports the use of an assessment factor of 10 according to the TGD.

The data below show that *H. azteca* may be a sensitive species among the benthic species studied for zinc and for some other metals (which might allow an assessment factor of <10), but based on the results of the field study by Burton et al. (2003, 2005) in which a toxic response was found at zinc concentrations below the lowest NOEC from laboratory studies, see also issue 2 below) there is uncertainty whether or not this species is indeed the most benthic sensitive species towards metals (which supports the use of an assessment factor of 10).

The result of the short-term (10-d) tests with H. azteca and C. tentans performed in sediment from the same lake (Farrar & Bridges, 2002; see Table 3.3.2.e – Part II) and further short-term (10-d) tests with water only exposure (Phipps et al., 1995) indicate that H. azteca is more sensitive to zinc than C. tentans, especially regarding endpoint survival. However, the data in Part I of Table 3.3.2.e show that the lowest chronic NOEC for H. azteca (488 mg/kg d.w., based on endpoint survival) is only slightly lower than that for C. tentans (609 mg/kg d.w., based on endpoint growth). The same holds when comparing the "species-mean" chronic values for these species: 665 mg.kg d.w. for H. azteca (based on endpoint survival) and 696 mg/kg d.w. for C. tentans (based on endpoint growth), also expressed as the added-Zn concentration[28]*. The lowest chronic NOEC for T. tubifex (1101 mg/kg d.w.; Table 3.3.2.e –*

concentration) was derived from the LOECg of 252 mg/kg dw (NOECge = LOECg/3 = 252/3 = 84 mg/kg dw), equivalent to a $NOEC_g^e$ of 74 mg/kg dw for added Zn ($NOEC_g^e$ = $LOEC_g$/3 = 221/3 = 74 mg/kg dw). The $NOEC_g^e$ of 74 mg/kg dw for added Zn was used as key study for $PNEC_{add, sediment}$ derivation, i.e $PNEC_{add, sediment}$ was $NOEC_g^e$/2 = 74/2 = 37 mg/kg dw. Although the derivation of an estimated NOEC from a LOEC at which 20-30% inhibition is found (as is the case in this study) is in conformity with the criteria for NOEC derivation used in this RAR, the estimated $NOEC_g^e$ from this study now has been rejected, as a new valid long-term study with *Hyalella azteca* has been performed; this new study (Nguyen et al., 2005) does not indicate that growth is the most sensitive endpoint for *H. azteca* exposed to zinc. Also the rejected long-term *Hyalella azteca* study by Borgmann & Norwood, 1997 (see Table 3.3.2.e-Part II) does not indicate that growth is the most sensitive endpoint for *H. azteca* exposed to zinc.

28 The "species mean" chronic NOECs for the two species *H. azteca* and *C. tentans* are given for illustrative purposes only (the use of the "species mean" NOEC for PNECadd,sediment derivation is considered less appropriate, as the tests were performed in different sediments). The "species mean" chronic NOEC for *H. azteca* (665 mg/kg d.w) is the geometric mean value of 488 mg/kg d.w. (Table 3.3.2.e – Part I, from Nguyen et al., 2005, Table 3.3.2.e – Part I) and 905 mg/kg d.w. (Table 3.3.2.e- Part II, from Farrar & Bridges, 2001, 2002, 2003), based on endpoint survival. The latter study was rejected as the study did not result in an overall NOEC, as growth was affected at the lowest concentration tested, see earlier footnote.
The "species mean" chronic NOEC for *C. tentans* (696 mg/kg d.w) is the geometric mean value of 609 and 800 mg/kg d.w. (Table 3.3.2.e – Part I, from Farrar & Bridges, 2002,2003 and Sibley et al., 1996, respectively), based on endpoint growth.

Part I) is around 2-times higher than that for H. azteca and C. tentans. All aforementioned NOEC values are expressed as the added-Zn concentrations.

The relatively high sensitivity of Hyalella azteca and Chironomus spp. to metals compared to the sensitivity of Tubifex tubifex is confirmed by short-term to long-term tests in which four benthic invertebrates (Hyalella azteca, Chironomus riparius, Tubifex tubifex and the mayfly Hexagonia spp.) were exposed to Cd, Cu or Ni in spiked-sediment (Milani et al., 2001). The tests with C. riparius were short-term tests (10-d exposures), while those with the other three species were long-term tests (21-d exposures of H. spp. and 28-d exposures of H. azteca and T. tubifex). The endpoints studied were survival (LC25 and LC50 values) of all four species, growth of H. azteca, C. riparius and H. spp. and reproduction of T. tubifex. The results for growth and reproduction were reported as IC25 (inhibitory concentration, equivalent to EC25 values). The relative sensitivity of the four species depended both on endpoint and metal studied. Growth and reproduction were more sensitive endpoints than survival, but in some tests the LC25 and the IC25 values were very similar. The following results are based on the most sensitive endpoint (growth or reproduction). For nickel, H. azteca was the most sensitive species (IC25 40 mg/kg d.w. which is $\geq$ 2-times lower than that for the other three species: 83, 146 and 408 mg/kg d.w. for H. spp., C. riparius and T. tubifex, respectively). For cadmium, H. azteca, H. spp. and C. riparius were nearly equally sensitive (IC25 values of 10, 14 and 16 mg/kg d.w., all three values considerably lower than that of 301 mg/kg d.w for T. tubifex). For copper, H. spp. was the most sensitive species (IC25 38 mg/kg d.w., which is $\geq$ 2-times lower than that for the other three species: 76, 78 and 181 mg/kg d.w. for H. azteca, C. riparius and T. tubifex, respectively). The study by Milani et al. (2001) also included 96-h lethal toxicity test (water-only exposures) with the aforementioned four species and three metals. In these 96-h water-only exposures, H. azteca was found the most sensitive species for Cd and Ni and C. riparius was found to be the most sensitive species for Cu. The above results further show that the relative sensitivity of the species exposed for 10-28 days to the metals in spiked-sediment are not accurately predicted from the 96-h water-only exposures.

Results of field studies

Liber et al. (1996)

In the 1-year study by Liber et al. (1996), performed at one site (with five consecutive sampling dates), "minor" effects were observed at added (being actual-Cb) SEM_{Zn} concentrations of 310 mg/kg d.w. (on the third sampling date) and at 725 mg/kg d.w. (on the third and fifth sampling date), the highest two concentrations tested. Overall, the highest concentration tested was considered to be the overall $NOEC_{ecosystem}$.

Burton et al. (2003, 2005)

In the study by Burton et al. (2003, 2005), performed at four European sites (with one to three consecutive sampling dates per site; exposure time 6 up to 37 weeks), only two Zn concentrations, nominal 400 and 1200 mg/kg d.w., were tested along with the control, thus reliable $NOEC_{ecosystem}$ values could not be derived from these study. The range of the actual Zn concentrations in Burton et al. (2003, 2005) are:

- 175-358 mg/kg d.w. at the low-Zn treatment, and
- 270-913 mg/kg d.w. at the high-Zn treatment.

The nominal low-Zn treatment was 400 mg/kg d.w.; at this treatment level the added (being actual-Cb) concentrations were 75-528 mg/kg d.w. for SEM_{Zn} and 119-255 mg/kg d.w. for added Zn. The nominal high-Zn treatment was 1200 mg/kg d.w.; at this treatment level the added (being actual-Cb) concentrations were 244-2030 mg/kg d.w. for SEM_{Zn} and 214-782 mg/kg d.w. for added Zn. The high-Zn treatment resulted in "major" effects in all four sites,

except at the first sampling date in Smallenberg river. The low-Zn treatment did not result in "major" effects in Ankeveen lake and Smallenberg lake. At the low-Zn treatment the concentrations in Ankeveen lake (only one sampling date) were 345 mg/kg d.w. for added SEM_{Zn} and 165 mg/kg d.w. for added Zn and in Smallenberg lake (three sampling dates) 75-528 mg/kg d.w. for added SEM_{Zn} and 205-226 mg/kg d.w. for added Zn.

In the Pallanza river (only one sampling date available) and Biesbosch river (on two out of three sampling dates) "major" effects were found at the low-Zn treatment, with the most severe effects in Pallanza river. At the low-Zn treatment, the toxic response concentrations in Pallanza river were 162 mg/kg d.w for added SEM_{Zn} and 119 mg/kg d.w. for added Zn and the toxic response concentrations in Biesbosch river were 132 or 209 mg/kg d.w for added SEM_{Zn} and 255 or 178 mg/kg d.w. for added Zn. Although the field study by Burton et al. (2003, 2005) was conducted to validate the AVS hypothesis with respect to chronic effects of Zn in sediments and not to derive NOEC and LOEC values (only two zinc treatmens per sediment were used), the results of this study clearly show adverse effects at added SEM_{Zn} and added Zn concentrations in the range of around 100-200 mg/kg d.w. in sediments of two out of four study sites. Due to the limited number of field studies, this supports an assessment factor of 10.

The implementation of the AVS-approach in the risk assessment

When the $PEC_{add}/PNEC_{add}$ ratio is >1 (tier 1, with no bioavailability correction on either the PEC_{add} or $PNEC_{add}$), the PEC_{add} is corrected for bioavailability by using the AVS-approach (specifically the SEM-AVS approach) in tier 2,while the generic $PNEC_{add}$ is not corrected for bioavailability in tier 2 (see earlier in Section 3.3.2.2.1 for further explanation of the two-tiered approach used in the risk assessment for sediments). Hence, the actual $PEC_{add}/PNEC_{add}$ ratio is underestimated, as only a part of the NOEC underlying the $PNEC_{add}$ will have been bioavailable (i.e. not bound to AVS) and a part will not have been bioavailable (i.e. bound to AVS). Furthermore, the results of Table 3.83 in Section 3.3.2.2.1 show that some of the toxic response values expressed as SEM-AVS are <0, indicating a toxic response in the presence of an excess amount of AVS compared to $SEM_{(Zn)}$. Overall, this issue supports the use of an assessment factor of 10 (or even higher, as the default factor of 10 according to the TGD assumes that the bioavailablity of the PEC is equal to that of the PNEC).

The results of the Equilibrium Partition (EP) method

The EP method (see also below) in which the $PNEC_{add.\ sediment}$ has been estimated from the $PNEC_{add,\ aquatic}$, results in a $PNEC_{add,\ sediment}$ of 860 mg/kg d.w., which is nearly 2-times higher than the lowest NOEC for benthic species (488 mg/kg d.w.). This would support an assessment factor of <10. It is emphasised, however, that the EP-method has limitations for the derivation of a reliable $PNEC_{add,\ sediment}$, especially for metals, because of the uncertainties (assumptions) that are mentioned in the section below.

Conclusion

Based on the above data, an assessment factor of 10 on the lowest chronic NOEC for the benthic species *H. azteca* (488 mg/kg d.w, for added Zn; based on single-species laboratory studies) is considered to be justified, leading to a $PNEC_{add,\ sediment}$ of 49 mg/kg dry weight. This value is equivalent to a $PNEC_{add,\ sediment}$ of 11 mg/kg wet weight[29].

29 For the dry to wet weight normalisation of the PNECadd, sediment it is assumed that the sediment contains 10% solids (density 2500 kg/m^3) and 90% water (density 1000 kg/m^3) by volume, i.e. 22% solids by weight. These properties are set equal to those of suspended matter, thus the $PNEC_{add,\ suspended\ matter}$ equals the $PNEC_{add,\ sediment}$ (according to the TGD). See also subsection "$PNEC_{add,\ sediment}$ using the equilibrium partitioning (EP) method".

$PNEC_{add,\ sediment}$ using the equilibrium partitioning (EP) method

In conformity with the calculation of the PEC_{add} for sediment (see Chapter 2), the properties of suspended matter are used to calculate the $PNEC_{add}$ for sediment, i.e., $PNEC_{add,\ sediment}$ = $PNEC_{add,\ suspended\ matter}$. This results in a $PNEC_{add,\ sediment}$ of 187 mg/kg <u>wet</u> sediment, as follows (according to the TGD; the Kp_{susp} is from section 3.2.2.1 of this risk assessment report).

1. $K_{susp\text{-}water}$: $Fwater_{susp} + (Fsolid_{susp} \times Kp_{susp} \times RHO_{solid}) =$
$0.9\ m^3/m^3 + (0.1\ m^3/m^3 \times 110\ m^3/kg \times 2{,}500\ kg/m^3) =$
$0.9\ m^3/m^3 + 27{,}500\ m^3/m^3 =$
$27{,}501\ m^3/m^3$

2. $PNEC_{add,\ sed} = PNEC_{add,\ susp} : (K_{susp\text{-}water} / RHO_{susp}) \times PNEC_{add,\ aquatic} =$
$(27{,}501\ m^3/m^3 / 1{,}150\ kg/m^3) \times 7.8\ mg/m^3 =$
187 mg/kg <u>wet</u> sediment

Where:
$K_{susp\text{-}water}$ = volumetric suspended matter / water partition coefficient (m^3/m^3)
$Fwater_{susp}$ = volume fraction water in suspended matter (m^3/m^3)
$Fsolid_{susp}$ = volume fraction solids in suspended matter (m^3/m^3)
Kp_{susp} = suspended matter / water partition coefficient (m^3/kg)
RHO_{solid} = density of the solid fraction (kg/m^3)
$PNEC_{add,\ sed}$ = Predicted No Effect Concentration in sediment (mg/kg <u>wet</u> sediment)
$PNEC_{add,\ susp}$ = Predicted No Effect Concentration in suspended matter (mg/kg <u>wet</u> suspended matter)
RHO_{susp} = bulk density of wet suspended matter (kg/m^3)
$PNEC_{add,\ aquatic}$ = Predicted No Effect Concentration in water (mg/m^3)

The above $PNEC_{add,\ sediment}$ of 187 mg/kg <u>wet</u> sediment (22% solids by weight) is equivalent to a $PNEC_{add,\ sediment}$ of 860 mg/kg <u>dry</u> sediment.

It is emphasised that the EP-method in which the PNEC for sediment is derived from that for water has limitations for the derivation of a reliable $PNEC_{add,\ sediment}$, because of the uncertainties (assumptions) that are discussed below.

The equilibrium partitioning method was originally proposed by Pavlou and Weston (1984) to develop sediment quality criteria for organic substances, and is further described elsewhere (Shea, 1988; DiToro et al., 1991; OECD, 1992b; Van der Kooy et al., 1991). Three important assumptions are made when applying this method. Firstly, it is assumed that bioavailability, bioaccumulation and toxicity are closely related to the pore water concentration. Secondly, it is assumed that equilibrium exists between the chemical sorbed to the particulate sediment and the pore water and that these concentrations are related by a partition coefficient. Thirdly, it is assumed that the sensitivity distributions for aquatic and benthic organisms are equal.

Overall conclusion on PNEC $_{add, sediment}$:

Based on all data, preference is given to the $PNEC_{add, sediment}$ based on the sediment toxicity data for benthic organisms. Thus the risk characterisation for sediments is based on a *$PNEC_{add, sediment}$ of 49 mg/kg dry weight*, equivalent to a $PNEC_{add, sediment}$ of 11 mg/kg wet weight.

In the risk characterisation, the above $PNEC_{add, sediment}$ which is based on sediment toxicity data for freshwater benthic organisms will be applied for both the freshwater and saltwater environment, as no $PNEC_{add, sediment}$ could be derived for the saltwater environment. For saltwater benthic organisms no chronic toxicity data for Zn-spiked sediments are available.

3.3.2.3 Toxicity to aquatic microorganisms (bacteria and protozoa)

Data on toxicity tests with bacterial and protozoan species, resulting in different toxicity values (LC50, EC10, EC50 and NOEC values) are summarised in Table 3.3.2.c (Annex 3.3.2.A). Tests with aquatic microorganisms are used to derive the PNEC for effluent ($PNEC_{add, microorganisms}$). Zinc was added either as zinc chloride or zinc sulphate in both the bacterial and protozoan tests. Most data were based on nominal concentrations, and all the tests were static tests.

The bacterial studies, including single-species tests and mixed-population tests (e.g. activated sludge, respiration inhibition tests), resulted in EC50 values ranging from 0.74 mg/l (test with bacteria in a natural seawater sample) to 900 mg/l (activated sludge test). EC10 values of 1.8 and 0.3 mg/l, respectively, were found in two tests with bacterium *Pseudomonas putida*. An activated sludge test resulted in a NOEC of 15 mg/l.

The protozoan tests resulted in LC50 values ranging from 0.25 mg/l (test with *Drepanomonas revoluta)* to 50 mg/l (test with *Euplotes patella*). Protozoan tests with *Euglena viridis* and *Tetrahymena pyriformis* resulted in NOEC values of 4.2 and 1.33 mg/l, respectively.

3.3.2.3.1 Predicted no effect concentration for STP effluent (PNECadd, microorganisms)

It must be noted that a number of the tests in Table 3.3.2.c cannot be used to derive a $PNEC_{add, microorganisms}$ or is considered less relevant in this respect, because of the current guidance in the TGD (Chapter 3 - § 3.4). For example, tests with saltwater organisms, such as the MICROTOX-test with the marine bacterium *Vibrio fisheri* (formerly known as *Photobacterium phosphoreum*), are excluded.

The activated sludge respiration inhibition test reported by Dutka et al. (1983) showed the lowest useful EC50 value (for bacteria), with a 3-h EC50 of 5,200 µg/l. In this test, activated sludge originating from a STP receiving mainly domestic sewage was inoculated in vessels containing synthetic medium (referring to OECD, 1976; not available) with different $Zn_2SO_4.7H_2O$ concentrations. The vessels were continuously aerated and respiratory activity (dissolved O_2 concentration) was measured after 30 minutes and 3 hours of incubation. Using an assessment factor of 100 according to the TGD (Chapter 3 - § 3.4) on the EC50 of 5,200 µg/l would result in a $PNEC_{add, microorganisms}$ of 52 µg/l**.** Using an assessment factor of 1 on the lowest EC10 of 300 µg/l in the *Pseudomonas putida* test reported by Van Beelen and Fleuren-Kemilä (1997) would give a $PNEC_{add, microorganisms}$ of 300 µg/l. Using the protozoan toxicity data would lead to a $PNEC_{add, microorganisms}$ of 25 µg/l, applying a factor 10 on the lowest LC50 of 250 µg/l, for *Drepanomonas revoluta,* reported by Madoni et al. (1994). The last

mentioned would give the lowest $PNEC_{add.\ microorganisms}$. Although this protozoan species is known to occur in sewage treatment plants and according to a recent TGD amendment protozoa results should be used for deriving a $PNEC_{STP}$, preference is given in this case to the 'classical' activated sludge test with a **$PNEC_{add,\ microorganisms}$ of 52 µg/l for dissolved zinc in effluent**. This value has been used in the risk characterisation. It is noted that there are insufficient useful data for aquatic microorganisms to apply statistical extrapolation.

3.3.2.4 Toxicity of zinc from metallic zinc powder to freshwater organisms

The three tests (algae, daphnids and fish) in which zinc powder was used as test compound are summarised in Table 3.3.2.d (Annex 3.3.2.A). The tests were conducted with the same lot of zinc powder, having a median diameter of 13.4 µm and a purity of 98.4%. For more data on the design of the studies, including the dissolution protocol, see the footnotes under Table 3.3.2.d. The results of these studies, see below, are expressed as the actual dissolved-Zn concentration.

Aquatic toxicity - algae

A growth test with the alga *Pseudokierchneriella subcapitata* (formerly known as *Selenastrum capricornutum*) resulted in a 72-h EC50 for dissolved zinc of 150 µg/l (endpoint: specific growth rate), and a 72-h NOEC for dissolved zinc of 50 µg/l (endpoints: specific growth rate and biomass) (Van Woensel, 1994a). It is noted that similar growth tests have been conducted with the same algal species, using a soluble zinc compound or using "insoluble" ZnO as test compound, see Table 3.3.2.a in Annex 3.3.2.A). These tests, all using soft to very soft artificial test media, resulted in comparable NOEC values if expressed as dissolved zinc, i.e. NOEC values for dissolved zinc in the range of 5-50 µg/l, regardless whether the soluble or the "insoluble" test compound was used.

Aquatic toxicity - invertebrates

A short-term *Daphnia magna* immobilisation test resulted in a 48-h NOEC for dissolved zinc of 150 µg/l. An EC50 could not be derived from the test results (Vos, 1994). The 48-h NOEC from this short-term test is very similar or within a factor of 2 of a large number of NOEC values (endpoints: survival and/or reproduction) derived in long-term *D. magna* tests in which a soluble zinc salt was used as test compound (see Table 3.3.2.a in Annex 3.3.2.A).

Aquatic toxicity - fish

In a 96-h acute toxicity test with the fish *Brachydanio rerio,* no effect was found for dispersed zinc powder at 100 mg/l (limit test). The actual dissolved-zinc concentration in this zinc powder dispersion was 2,360 µg/l (Van Ginneken, 1994c).

The data from these tests, although very limited with respect to the number of studies, indicate that zinc (ion) may be dissolved from zinc powder dispersions to a level that results in toxic effects to aquatic organisms. In addition, the test results -expressed as dissolved zinc- are similar to those from tests with soluble zinc salts.

3.3.3 Terrestrial compartment

3.3.3.1 Toxicity to terrestrial organisms

For soil, toxicity data on terrestrial species (invertebrates and plants) as well as for microorganisms are available. The toxicity data on invertebrates and plants are from single-species tests that study the common ecotoxicological parameters survival, growth and/or reproduction. The toxicity data on microorganisms are from tests in which microbe-mediated soil processes, including C-mineralization and N-mineralization (the major, intertwined soil processes that are involved in the degradation of organic matter), were studied. This kind of microbial toxicity tests are in fact multiple-species tests, because these microbe-mediated processes (functional parameters; also called sumparameters) reflect the action of many species in the microbial community of a soil. The microbial toxicity data do not include single-species microbial tests with soil microorganisms, because data on these tests are scarce and usually relate to studies that are conducted in aqueous media (see also section 3.3.2.3) which hampers an effect assessment for soil. Moreover, it can be argued that effects on soil processes are more relevant to terrestrial ecosystems than effects on single microbial species or effects on microbial species diversity, because soil processes such as C-mineralization can be performed by a variety of microorganisms.

The sorption of substances in soil depends on soil characteristics such as organic matter content, clay content and pH (see also section 3.2.3.1). Hence, the bioavailability and toxicity to soil organisms, may also depend on soil characteristics. Strictly spoken this means that the results of tests conducted in different soils, with different characteristics, cannot be compared as such (at least, when the results are expressed as total concentration in soil; either nominal or actual), but should be normalised to standard conditions. For non-ionic organic compounds the data should be normalised on the basis of the organic matter content, because it is assumed that the bioavailability for non-ionic substances is determined by the organic matter content only (TGD, Chapter 3 - § 3.6)[30].

For metals and other inorganic substances, however, the organic matter content is not the only factor influencing the bioavailability; for these substances, factors including the clay content and pH of the soil are also involved and the influence of these factors on the sorption and bioavailability can be similar or higher than that of the organic matter content. In the framework of setting environmental quality objectives (PNEC derivation) the following normalisation methods were used or proposed:

- In the framework of The Netherlands' environmental policy, terrestrial toxicity data for metals were normalised on the basis of clay and/or organic matter content, using metal-specific equations based on so-called "reference lines". These reference lines are based on correlations between these two soil factors and the ambient background concentration of zinc in unpolluted soils. For zinc both factors, thus clay and organic matter content, are included in the reference line (see also 3.3.3.1.4). The use of these reference lines is also mentioned in appendix VIII of the TGD.
- In the framework of the industry comments on drafts of this Risk Assessment Report, McLaughlin (1998, 1999) normalised the terrestrial toxicity data for zinc on the basis of pH (see also 3.3.3.1.4).

30 Standard soil according to the TGD: organic matter content of 3.4%.

It is emphasised, however, that the relationship between soil characteristics, sorption and bioavailability of metals is very complex. Moreover, because bioavailability is not only influenced by abiotic factors, but also by biotic factors. This means that the influence of soil factors on sorption and bioavailability is not necessarily the same. For example, a study on the partitioning of metals in 20 Dutch soils shows a strong relationship between the soil / water partition coefficient (Kp_{soil}) for zinc, varying from 6 to 7,000 l/kg, and the pH (Janssen et al., 1997a). A parallel bioaccumulation study with earthworms in the same soils, however, shows only a weak relationship between the bioconcentration factor for zinc and the pH. In addition to pH, clay content and aluminiumoxide content were found to influence the bioconcentration factor (Janssen et al., 1997b). Moreover, differences with respect to uptake mechanisms most probably result in differences with respect to the influence of soil factors on bioavailability and toxicity.

Based on the above and on further data on this issue in section 3.3.3.1.4, it was earlier concluded that normalisation methods lack sufficient scientific validity to use for metals. However, based on the results of a recent, integrative research program, quantitative regressions are available to correct the PEC for abiotic parameters, i.e. to correct for bioavailability as discussed and explained in section 3.3.3.1.1.

In addition to the data in section 3.3.1.1 and 3.3.1.2, the following is noted with respect to the derivation and the selection of the terrestrial NOEC values used for $PNEC_{add,\ terrestrial}$ derivation.

Reliability

Derivation of NOEC values

The general procedure and order of preference for deriving NOEC values, already descibed in section 3.3.1, is also used for the terrestrial data. However, in addition to the "original" NOEC values derived in conformity with the general procedure (i.e. "real" NOEC values or NOEC values that were either set equal to the EC10 or were derived from LOEC values), an "alternative" NOEC was derived from a number of microbial studies (Table 3.3.3.a-Part I in Annex 3.3.3.A) and plant studies (Table 3.3.3.d in Annex 3.3.3.A) and used for PNEC derivation instead of the "original" NOEC values, because of reliability considerations.

An alternative NOEC from a test was derived if the NOEC was relatively low (below 100 mg/kg d.w.) and if:

a) The real NOEC was derived from a test in which a high separation factor (higher than 3.2) was used between the NOEC and LOEC. In a relatively large number of microbial studies a maximum of 3 concentrations was tested, with a separation factor of 10, thus it is not possible to derive the NOEC with great accuracy. For example, the NOEC of 10 mg/kg d.w. derived from a test range of 0-10-100 mg/kg d.w. (nitrification test in the loamy sand Leefield, reported by Wilson, 1977).
b) *The NOEC was set at the EC10 level and this value is more than 3.2 times lower than the lowest test concentration (thus extrapolated relatively far outside the actual test range)*[31]*. For example, the NOEC = EC10 of 12 mg/kg d.w., from a test range of 0-50-100-200 mg/kg d.w. (respiration test reported by Chang & Broadbent, 1981).*

In case of point (a), the alternative NOEC is preferably set at the EC10 level (in conformity with the preference used in the general procedure). If calculation of the EC10 is not possible,

31 The factor of 3.2 used in point (a) and point (b) is based on a geometric series at a factor of √10 which is often used in ecotoxicity studies.

the alternative NOEC is derived from the LOEC using an assessment factor, provided the percentage inhibition at the LOEC was ≤ 30% (also according to the general procedure). If an alternative NOEC could be derived, both the original NOEC and the alternative NOEC (the latter value used for PNEC derivation) are given in Table 3.3.3.a (microbial studies) and Table 3.3.3.d (plant studies) in Annex 3.3.3.A. However, the alternative NOEC will be used for PNEC derivation. In case no alternative NOEC could be derived (neither by NOEC = EC10, nor by NOEC = LOEC / AF), the original NOEC values concerned were not used for PNEC derivation.

With respect to a number of the microbial studies by Tabatabai and co-workers it is noted that only one concentration (resulting in effect, thus considered as LOEC) was used next to the control. Although it was not possible in those cases to check the concentration-effect relationship, the results of these tests were used to derive the NOEC from the LOEC, provided the percentage inhibition at the LOEC was ≤ 30%.

Calculation of EC10 values (microbial toxicity studies)

As stated in section 3.3.1, a number of EC10 values, used as NOEC equivalent, was calculated by the rapporteur, in case a NOEC could not be derived and the EC10 was not reported. The EC10 values were derived from a logistic, sigmoidal dose response model according to Haanstra et al. (1985):

$$Y = c / \{1+\exp [b.(X – a)]\}$$

where: Y = response
c = control response (set at 1, i.e. top of the curve fixed at control value)
b = slope parameter
a = logarithm of the EC50
X = logarithm of the exposure concentration

Graphpad Prism Software was used for fitting the model and calculation of the EC10.

Along with the control the calculation of the EC10 requires at least two concentrations that result in effects. Further prerequisites used:

- If only two effect concentrations are available, the percentage effect at the highest concentration must differ 15% or more compared to the lowest concentration, for example 20% inhibition at the lowest concentration and 35% inhibition at the highest concentration.
- If only two effect concentrations are available, the percentage effect at the highest concentration must be lower than 70%.

It is noted that the aforementioned prerequisites of 15% difference in inhibition and 70% maximum inhibition, respectively, are based on the rapporteurs' practice with the use of the logistic model and thus are not absolutely "fixed" values which are inherent to this or other (logistic) models used for EC10 calculation. Thus it may be "around 15%" and "around 70%" as well. However, the greater the deviations from these boundaries the lower the reliability of the EC10 calculation will be. The "15%-rule" does not apply to effect concentrations that are around the EC10, e.g. 8% and 12%, respectively. In that case the NOEC is chosen as the concentration resulting in <10% inhibition, see section 3.3.1.

Relevance

Soil type and abiotic soil factors as criteria for data selection

In soils, abiotic factors that influence the speciation of zinc (and thus may influence the bioavailability and toxicity) may differ considerably. The clay content, the organic matter content and the pH value are considered (the) major factors influencing the terrestrial toxicity. Further discussion on the influence of each or of the combined influence of these factors can be found in section 3.3.3.1.1. The soil type and the major soil characteristics (pH, organic matter content, clay content) have not been used in a stringent matter for data selection. Nevertheless, the soil type and major soil characteristics have been taken into account, see *conclusion* of this section.

The background zinc concentration (Cb) has not been used for data selection, because of the lack of data on background zinc concentrations in most of the test soils (Cb not reported in the majority of the studies, especially for the soils used in the microbial studies).

Background information on soil characteristics

Table 3.86 shows the ranges of pH, organic matter content, clay content, and background zinc concentration (Cb) of the EU soils (soils sampled in European countries) used in the terrestrial toxicity studies. The table shows the ranges of these characteristics for the studies in EU soils that have been used for PNEC derivation, on the basis of the grouped data for microbe-mediated processes (from Table 3.3.3a – Part I), invertebrates (Table 3.3.3.b. – Part I) and plants (from Table 3.3.3.d –Part I), see Annex 3.3.3.A. In addition, the last column in Table 3.86 shows the total ranges of these characteristics, based on the data for all EU soils used in the studies (including the rejected studies). It is noted that most of the rejected studies were performed in soils that are already included in the database of studies used for PNEC derivation. It is further noted that around half of the invertebrate toxicity tests were performed in artificial OECD soil (see further below for the characteristics of this artificial soil).

As far as reported, all soil samples were topsoil samples. The data for two EU subsoil samples used in the acetate mineralization study by Van Beelen et al. (1994b) (Table 3.3.3.a – Part II in Annex 3.3.3.A) are not included in Table 3.86. In one of these subsoil samples the organic matter content (0.3%) was somewhat lower than the lowest value in the EU topsoil samples (0.5%) and in the other subsoil sample the pH value (8.2) was somewhat higher than the highest value in EU topsoil samples (7.7).

The reported characteristics are for untreated soils (except in some plants tests; see the study by MacLean, 1974). Data on relevant changes in soil characteristics, especially on pH changes due to treatment, are given in the footnotes of Tables 3.3.3.a to 3.3.3.d in Annex 3.3.3.A.

Table 3.86 Soil characteristics of studies using EU soils (topsoil samples). Data from Annex 3.3.3.A - Tables 3.3.3.a to 3.3.3.d.

	Studies on microbe-mediated processes*	Studies on invertebrates*	Studies on plants*	All studies using EU soils**
pH	3.0 – 7.7	3.0 - 7.5	5.0 – 5.6	2.8 – 7.7
Organic matter content (%)	1 – 40	1- 40	2 – 8	1 – 40
Clay content (%)	1 – 60	5 – 51	4 – 40	0.5 – 60
Background concentration of zinc (mg/kg d.w.)	7 – 226	7 – 191	8 – 57	6 – 226

* Data from studies used for PNEC derivation..

** Data from studies used for PNEC derivation and studies that were rejected on the basis of criteria other than soil characteristics, for example tests with insoluble test compounds.

Table 3.87 shows the ranges of pH, organic matter content, clay content, and background zinc concentration (Cb) of the non-EU soils (soils sampled outside Europe) used in the terrestrial toxicity studies.

Table 3.87 Soil characteristics of studies using non-EU soils (topsoil samples).Data from Annex 3.3.3.A - Tables 3.3.3.a to 3.3.3.d.

	Studies on microbe-mediated processes*	Studies on invertebrates*	Studies on plants*	All studies using non-EU soils**
pH	4.6 – 8.2	7.1	4.9 – 8.3	4.6-8.3
Organic matter content (%)	1 – 64 (1 – 9) ***	22	0.5 –10	0.5 – 71
Clay content (%)	2 – 51	-	13 – 23	2 – 51
Background concentration of zinc (mg/kg d.w.)	7 – 136	-	51 – 106	7 – 136

* Data from studies used for PNEC derivation. The invertebrate toxicity data used for PNEC derivation only include one study in non-EU soil (Khalil et al., 1996)

** Data from studies used for PNEC derivation and studies that were rejected rejected on the basis of criteria other than soil characteristics, for example tests with insoluble test compounds.

*** Organic matter content: 71% in one soil (two microbial tests: both rejected) and 64% in one soil (one microbial test: used). In all other soils used in the microbial toxicity tests, the organic matter content was 9% or lower.

The aforementioned ranges for pH, organic matter content, clay content, and background zinc concentration (Cb) do not necessarily represent the full ranges for EU soils and non-EU soils, respectively. The data are based only on the terrestrial toxicity studies that were evaluated in this report. The data show, however, already a wide range of values for each characteristic, in both EU soils (Table 3.86) and non-EU soils (Table 3.87), as could be expected on additional data on these characteristics in the varying textural soil classes. Likewise, the ranges for EU soils and non-EU soils are (very) similar, with the exception of the very high organic matter content in two non-EU soils.

The lowest background zinc concentrations (Cb) listed in Table 3.86 (EU soils) and Table 3.87 (non-EU soils) are 6 mg/kg d.w. and 7 mg/kg d.w, respectively. The former value was measured in the topsoil sample of a humic sand collected in "De Peel" (a natural reserve in the South of the Netherlands) and used in the acetate mineralization test by Van Beelen et al.

(1994b) that was rejected because of an unbounded NOEC. Of the tests that were used for $PNEC_{add}$ derivations, a total of 11 tests (viz 6 microbial tests, 3 invertebrate tests and 2 plant tests) were performed in soils with a Cb of <10 mg/kg d.w, i.e 7 or 8 mg/kg d.w. These soils are four different sandy soils and one silty loam, see Annex 3.3.3.A. These and further data on background zinc concentrations (Cb) in natural soils indicate that the Cb is related to soil type. Sandy soils show the lowest Cb, with minimum values in the range of 1 to 5 mg/kg d.w. (see also data in section 3.2).

Around half of the invertebrate toxicity tests were not conducted in natural soils, but in artificial OECD soil. This artificial soil is normally composed of 10% sphagnum peat, 20% kaolin clay and 70% sand (on a dry weight basis), resulting in an organic matter content of around 10% and a clay content of 20%. The pH of this soil is normally adjusted to pH 6.0 $\pm$ 0.5 by the addition of calcium carbonate (OECD guideline 207: Earthworm, Acute Toxicity Test). In some invertebrate tests, the pH and/or organic matter content were adapted (to study the effect of these characteristics on zinc toxicity), resulting in a total range of 4.0 to 6.3 for the pH value and 5% to 15% for the organic matter content. Due to variations in the components, the organic matter and clay content will also show some variations: the clay content in the different studies ranged from 8% to 20% (see Table 3.3.3.b in Annex 3.3.3.A). The aforementioned ranges for pH, organic matter content and clay content in the artificial soils used in the tests all are within the ranges listed in Table 3.86 and Table 3.87 for natural soils.

For artificial OECD soil containing 10% organic matter and 14% clay, a background zinc concentration of 2 mg/kg and 14 mg/kg was reported by Smit & van Gestel (1998) and Van Gestel & Hensbergen (1997), respectively (see Table 3.3.3.b in Annex 3.3.3.A). It is noted that the background zinc concentration in OECD soil depends on the composition of the soil.

Conclusion

Based on the above the following has been decided on the use of soil type and the major soil characteristics (pH, organic matter content and clay content).

Natural soils

1) EU soils (Table 3.86)

All tests in EU soils have been accepted, regardless whether or not there are data on the soil type and the major soil characteristics (and/or Cb). In case there would be indications that one or more of these parameters strongly deviate from real environmental conditions (see Table 3.3.3.1-A), for example by pH adjustment to an extreme value, a test in EU soil should be excluded. This is not the case, however, for any of the studies in the database currently used for PNEC derivation.

2) Non-EU soils (Table 3.87)
   a) If there are data reported on soil characteristics, the values for these parameters in non-EU soils have to be within or similar to the boundaries of the ranges for these parameters in EU soils. In the database currently used for PNEC derivation, there are no relevant deviations with respect to these parameters, with the exception of one test in the respiration study by Lighthart et al. (1993). The study was conducted in a non-EU soil with an organic matter content of 64%, but is used for PNEC derivation. Thus, no studies were rejected on the basis of this criterion. The Cb range in the accepted tests in non-EU soils (7-136 mg/kg d.w.) is within that in EU soils (3-226 mg/kg d.w.).
   b) If there are no data reported on soil characteristics and Cb, the relevance of the test has been judged on a case by case basis; data on soil type and treatment are

decisive. In the database currently used for PNEC derivation, there are ten plant toxicity tests in non-EU soils (all from the study by Boawn & Rasmussen, 1971) for which the soil type was reported (silty loam) and further only the pH value (7.0). These tests are accepted since silty loam is one of the soil types present in the EU region. In addition, the database includes one microbial toxicity test in non-EU soil (Rodgers & Li, 1985: dehydrogenase activity) and one plant toxicity test in non-EU soil (Kalyanaraman & Sivagurunnathan, 1993; *Vigna mungo*) for which there are no data on the soil type used and only data on one of the characteristics (2% OM and pH 6.2, respectively, thus within the boundaries for EU soils). These tests are also accepted, since there are no indications for a special treatment of the soils. Thus, no studies were rejected on the basis of this criterion.

Artificial soils

- If there are data reported on soil characteristics and Cb, the values for these parameters have to be within the boundaries of the ranges for these parameters in EU soils.
- If there are no data reported on soil characteristics, the test will not be used for PNEC derivation, unless the artificial soil is "standard" OECD soil, without specific adaptations.

Note: no studies were rejected on the basis of this criterion.

Other criteria for data selection

In section 3.3.1 it is already stated that only the results of tests, in which the organisms were exposed to zinc alone, added as soluble zinc salt, are used for PNEC derivation, thus excluding tests with metal mixtures. For the terrestrial database these criteria also exclude tests in soils that contain a metal mixture due to the addition of substrates such as sewage sludge or due to deposition, such as soils contaminated by emissions from smelter works (e.g. Spurgeon & Hopkin, 1996a).

Only tests that expose the organism(s) through the soil have been used, thus excluding microbial toxicity tests that measure the microbial activity in litter (e.g. the respiration tests by Chaney et al., 1978 and Spalding, 1979) and excluding tests in which invertebrates are exposed through their feed (e.g. Marigomez et al., 1986: gastropod *Arion ater*). Based on this, also tests, in which invertebrates are exposed to soluble zinc, which was added to manure or sludge, are excluded (e.g. Neuhauser et al., 1984 and Hartenstein et al., 1981: earthworm *Eisenia fetida.)*

Only tests that were performed in more or less freshly-spiked soils, i.e. soils in which the test was started within some weeks after spiking and ended within 6 months after spiking, as tests in aged soils may underestimate the toxicity, see section 3.3.3.1.1. Based on this, especially the results of a number of microbial tests have been rejected, see further section 3.3.3.1.2.

3.3.3.1.1 Abiotic factors influencing the terrestrial toxicity of zinc

Introduction

Conventionally, the environmental risk assessment of a substance in the soil would be comparing the estimated concentration (PEC) in the soil to the PNEC for soil. In that situation both the PEC and the PNEC would be normalised to wet or dry weight concentrations in the soil with units mg/kg. The derivation of the $PNEC_{add}$ for zinc in soil is described in section 3.3.3.1.5. For zinc that resided in the soil for some time, however, a correction need to take place because it is not the total concentration of zinc in this aged soils that is bioavailable. In

addition, depending on soil type a differentiation is needed since some soils bind zinc stronger than other soils, which additionally affects bioavailability.

Physico-chemical soil characteristics thus may influence the sorption of metals in soil and thus may influence the bioavailability and toxicity of zinc and other metals in soil. According to the data in section 3.2.3.1, zinc is strongly adsorbed to oxides and hydroxides, silica, calcium carbonate, clay particles and organic matter and the sorption tends to increase with increasing pH. In general, it is assumed that bioavailability and toxicity of zinc are inversely related to sorption, thus bioavailability and toxicity of zinc are assumed to be highest in relatively acid soils with a low clay and organic matter content. Strictly speaking this means that the results of tests conducted in different soils, with different characteristics, cannot be compared or used for PNEC derivation as such (at least, when the results are expressed as total concentration in soil) but should be normalised to standard conditions.

Firstly, the following parameters that may be important for terrestrial toxicity of zinc (and other metals) are discussed: background concentration of zinc, pH, normalisation based on organic matter and clay content, porewater concentration, and CEC. Secondly, the results of a recent research program are described that will be used for deriving soil-properties and ageing related corrections for the current soil risk assessment of zinc. Third, a tiered approach will be discussed that will be used for implementing ageing and soil-properties related bioavailability corrections to the PEC in the current risk assessment report of zinc.

Soil characteristics that may affect zinc bioavailability

Background concentration of zinc

According to the metalloregion concept, adaptation to natural background levels and probably also test conditions may influence the sensitivity to zinc. However, not many studies have explicitly examined the relationship between background concentration of zinc in soil and its influence on toxicity. Therefore, an empirical approach was taken. From the studies that were selected to derive the PNEC for the soil compartment and those studies that were evaluated and were found reliable but not relevant (see Section 3.3.3.1 for explanation), all background concentrations were plotted against the NOEC to evaluate a possible relationship between this background zinc concentration and toxicity in Figures 3.27, 3.28 and 3.29 for microbe-mediated processes, invertebrates and plants, respectively. A distinction is made in these figures between the data originating from various sources (closed symbols) and the data from a recent research program (open symbols, data from Lock et al., 2003 and Smolders et al., 2003).

As can be seen from those Figures, there seems to be no clear trend for the closed symbols. With increasing background concentration, the NOEC does not clearly increase or decrease. It must be noted, however, that these data from various sources are in fact not suitable to examine this potential relationship. It is not always clear whether the organisms are in fact adapted to the concentration in which they are cultured or whether they are adapted to the concentration in the blanks of the test. Although it is assumed that background concentration in the blank and in which they are cultured are the same this information is often lacking. In addition, part of the scatter for the closed symbols in these Figures will also be caused by other abiotic factors that may influence the toxicity. Whether the sensitivity of organisms is really determined by the natural background concentrations in which they live can only be determined by actual toxicity testing of the same organism taken from different field conditions with different natural background levels but comparable other abiotic conditions. The data from the recent research program (open symbols, data from Lock et al., 2003 and Smolders et al., 2003) relate to 15 various European soils and a range of zinc background concentrations (7-191 mg/kg). No clear relationship is found between toxicity (NOECs) and

zinc background concentration for microbe-mediated processes, invertebrates and plants (open symbols in the Figures). However, the results from the recent research program actually comprise two different sets of studies. One set is based on Substrate Induced Respiration (SIR), where glucose is used as substrate. The other set is based on Potential Nitrification Rate (PNR), which is the nitrification at unlimited substrate (NH_4^+). The regressions for the individual sets of microbe-mediated processes versus toxicity, expressed not as NOEC but as EC50, are as follows:

SIR: log (EC50) = 1.7 + 0.76 (0.19-1.33) x log background Zn
(R^2=0.42, Q^2=0.13, n=14, $p<0.05$)

PNR: log (EC50) = 1.2 + 0.76 (0.30-1.22) x log background Zn
(R^2=0.55, Q^2=0.42, n=13, $p<0.01$)

Where:

EC50 is expressed as added zinc in mg/kg
Background zinc is expressed as mg/kg
The values in parentheses provide the 95% confidence interval.

Also McLaughlin and Smolders (2001) concluded that background zinc concentrations in soil affect the zinc sensitivity of soil microbial processes, but noted that considerable scatter exists in the relationships obtained.

Conclusion

Based on these latter data it is concluded that there is a weak but statistical basis to relate background zinc concentration in soil to microbe-mediated processes.

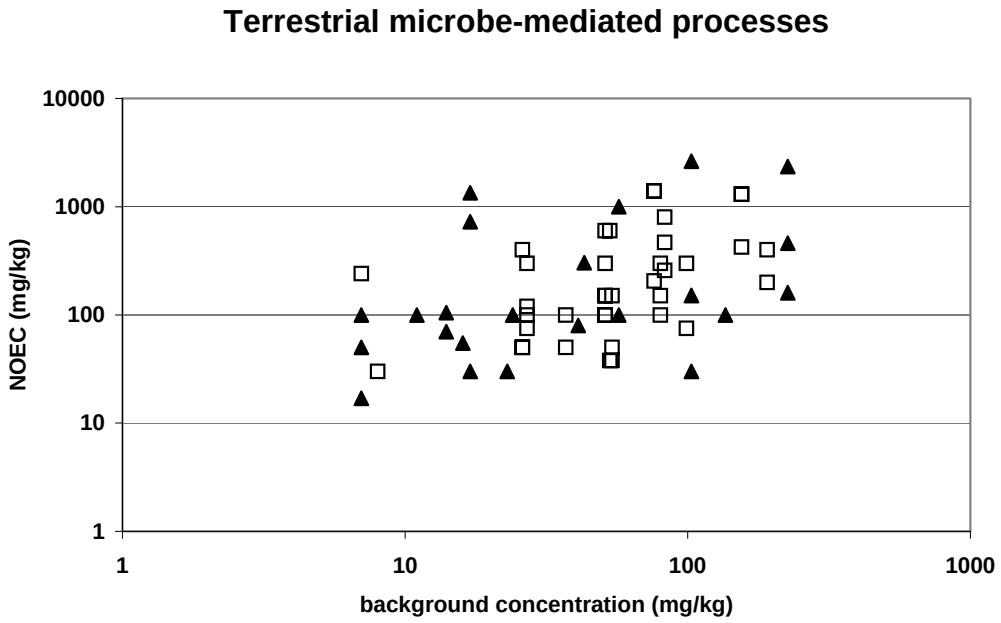


Figure 3.27 Relationship between the background concentration of zinc in soil and toxicity, expressed as the No Observed Effect Concentration (NOEC) for terrestrial microbe-mediated processes. Data from all studies that were used for deriving the $PNEC_{add,terrestrial}$, those that were found reliable but not relevant (closed symbols), and those from the recent research program (Smolders et al., 2003) (open symbols).

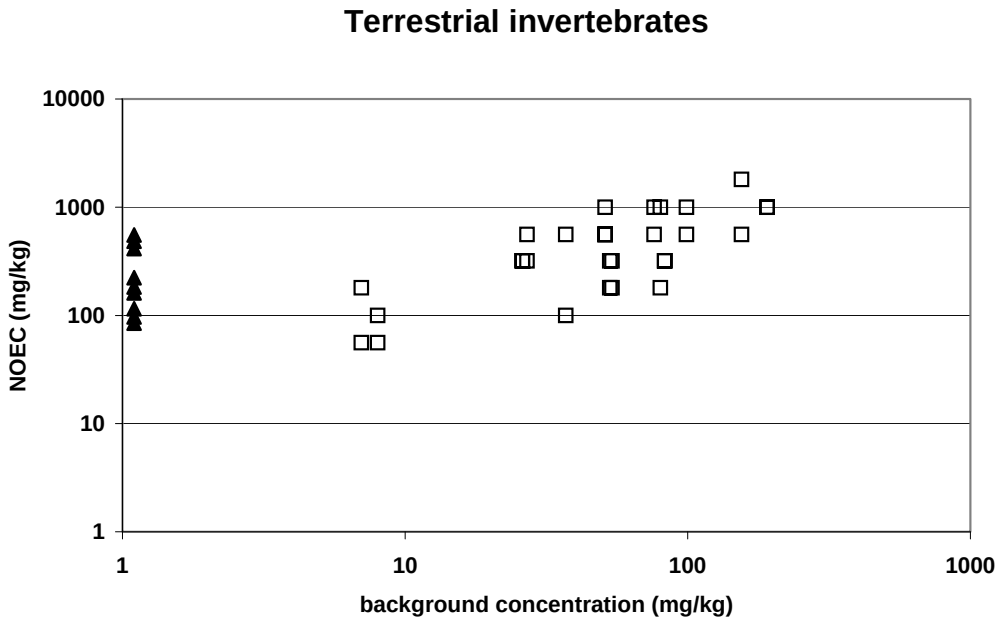


Figure 3.28 Relationship between the background concentration of zinc in soil and toxicity, expressed as the No Observed Effect Concentration (NOEC) for terrestrial invertebrates. Data from all studies that were used for deriving the $PNEC_{add,terrestrial}$, those that were found reliable but not relevant (closed symbols), and those from the recent research program (Lock et al., 2003) (open symbols).

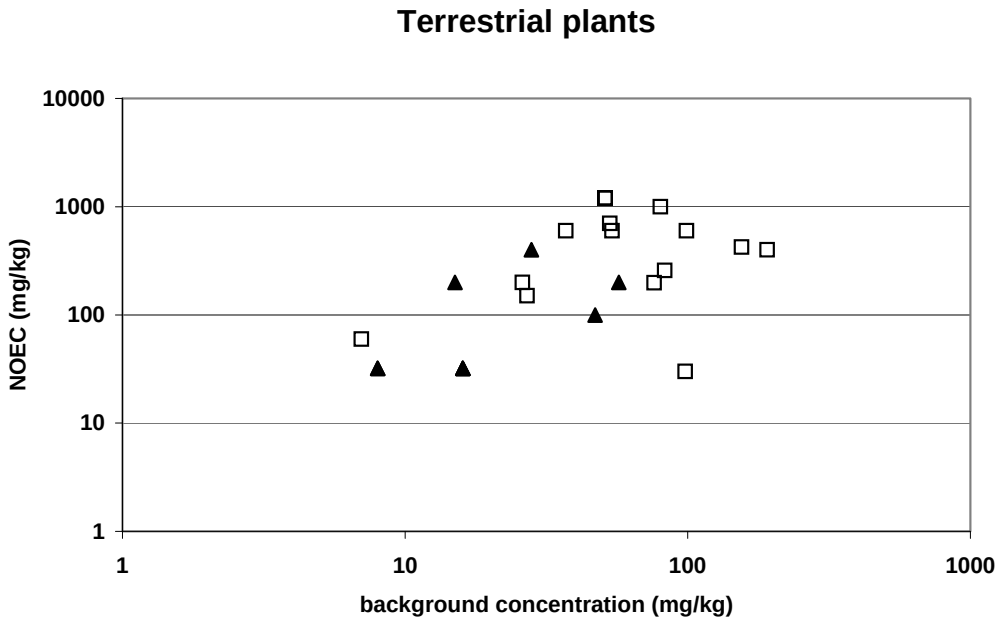


Figure 3.29 Relationship between the background concentration of zinc in soil and toxicity, expressed as the No Observed Effect Concentration (NOEC) for terrestrial plants. Data from all studies that were used for deriving the $PNEC_{add,terrestrial}$, those that were found reliable but not relevant (closed symbols), and those from the recent research program (Smolders et al., 2003) (open symbols).

pH

Two approaches are discussed that help to conclude whether or not pH is a mitigating factor for zinc toxicity in the terrestrial compartment. Firstly, the suggestion by McLaughlin is discussed who proposes to normalise effect concentrations in the terrestrial compartment, using pH. Secondly, an empirical relationship between pH and effect concentrations in the terrestrial environment is discussed.

McLaughlin studies

McLaughlin has presented two studies in which he normalised terrestrial toxicity data on zinc on the basis of pH effects on zinc retention by soil. This normalisation method was applied to NOEC values for microbe-mediated processes (McLaughlin, 1998) and to NOEC and EC50 values for soil invertebrates (McLaughlin, 1999). In his method the toxicity values in soil (in mg/kg) are converted to pore water concentrations (in mg/l). The basic, physical-chemical, idea of this pH-normalisation is that the resulting pore water concentrations would form a better basis for interpreting the ecotoxicity data. This, since pH determines the pore water concentration of zinc (e.g. Janssen et al., 1997a). For the pH-normalisation, he used the equation: [pore water endpoint (mg/l)] = [(soil endpoint (mg/kg) / $10^{-3.16+0.89(soil\ pH)}$]. The rationale for taking the equation was that it was based on an extensive database of data derived in the laboratory (added Zn to the soils studied), i.e. based on the study of Anderson and Christensen (1988). The equations of two other studies for pH-normalisation were not taken, because these studies included less extensive databases and contained data that were obtained from both laboratory studies (Zn added to the soils) and field soils.

The most important part of the equation is the exponent before the pH, which is 0.89 in the study of Anderson and Christensen (1988), and is significantly different in the two other studies, i.e. 0.61 and 0.44 for Janssen et al. (1997a) and Buchter et al. (1989), respectively. The choice of the equation and the pH-exponent will affect both the resulting pore water concentrations, as well as the variability in the pore water concentrations.

McLaughlin (1999) found that the variability in the population of NOEC and EC50 values for invertebrates was significantly reduced after pH-normalisation. This was explained by grouping of outliers at high pH around the mean pH-normalised value. McLaughlin (1999) suggested that outliers after pH-normalisation could be attributed to interactions between zinc and other more toxic ions, such as aluminium and manganese, at low pH. This suggestion is not further validated. However, the relative standard deviations after and before pH-normalisation were not reduced (NOEC values) or were even increased (EC50 values). Furthermore, the pH-normalisation of the soil ecotoxicity data may have a physical-chemical basis, but there is yet no evidence for a biological basis. A pH-normalisation as proposed by McLaughlin can only be applied for species for which direct uptake via the pore water is the dominant uptake route. At present, evidence for pore water related uptake is present only for a limited number of plant species and for microorganisms. Other soil dwelling organisms (like invertebrates) are probably exposed via a combination of uptake routes, such as pore water, food, and direct ingestion of soil (Lock and Janssen, 2001b). Only limited information is available that shows that the contribution of additional uptake routes can also be quantified on the basis of metal concentrations in the pore water. In addition, it should be noted that some plant species are capable of modifying their local environment, e.g. the pH, which will also limit the possibility of describing metal uptake on the basis of pore water concentrations.

The suggested pH normalisation of the equilibrium between sorbed and dissolved zinc is only a part of the picture. The uptake of zinc from the pore water to organisms can also be pH dependent. Even with simple microorganisms under defined conditions there is a strong effect of the pH, which is in the opposite direction. Apparently, the uptake and toxicity of dissolved zinc is decreased at low pH (Van Beelen and Fleuren-Kemilä, 1997: Plette, 1996; Plette et al., 1999). While in some cases there is an increasing toxicity of a metal with decreasing pH, in other cases there is a decreasing toxicity with decreasing pH. Plette et al. (1999) explain these apparently contradictory observations by considering the interaction between an organism and metal ions present in soil to be the result of a competition for that metal ion by all components, including the organism, present in the system. They thus describe a pH-dependent metal binding to the biotic surface and a pH-dependent binding to soil. Whereas the binding of the metal ion to the soil decreases with decreasing pH, the binding of the metal ion to the biotic surface when present in the soil can either increase or decrease with pH.

Empirical relationship between pH and NOEC

Not many studies have explicitly examined the relationship between pH in soil and its influence on the toxicity of zinc. Therefore, an empirical approach was taken. From the studies that were selected to derive the PNEC for the soil compartment and those studies that were evaluated and were found reliable but not relevant, the pH was plotted against the NOEC to evaluate a possible relationship between pH and toxicity in Figures 3.30, 3.31 and 3.32 for microbe-mediated processes, invertebrates and plants, respectively. A distinction is made in these figures between the data originating from various sources (closed symbols) and the data from a recent research program (open symbols, data from Lock et al., 2003 and Smolders et al., 2003), where 15 European soils were used that varied in pH from 3.0-7.5.

As can be seen from those Figures, there seems to be no clear trend for the closed symbols. With increasing pH, the NOEC does not clearly increase or decrease. It must be noted, however, that these data from various sources are in fact not suitable to examine this potential relationship. It is not always clear whether the organisms are in fact adapted to the pH in which they are cultured or whether they are adapted to the pH in the blanks of the test. Although it is assumed that the pH in the blank and the culture are the same this information is often lacking. In addition, part of the scatter for the closed symbols in these Figures will also be caused by other abiotic factors that may influence the toxicity. Whether the sensitivity

of organisms is really determined by pH in which they live can only be determined by actual toxicity testing of the same organism taken from different field conditions with different pH but comparable other abiotic conditions. The data from the recent research program (open symbols, data from Lock et al., 2003 and Smolders et al., 2003) relate to various soils and a whole range of pH. No clear relationship is found, however, between toxicity (NOECs) and pH for microbe-mediated processes, invertebrates and plants.

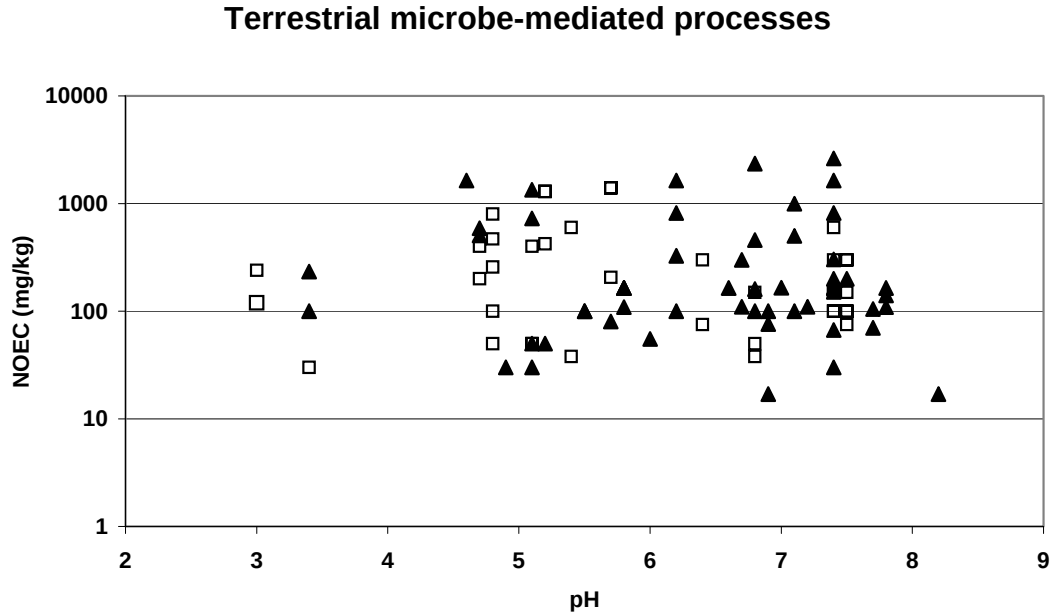


Figure 3.30 Relationship between pH in soil and toxicity to terrestrial microbe-mediated processes, expressed as the No Observed Effect Concentration (NOEC). Data from all studies that were used for deriving the PNECadd,terrestrial, those that were found reliable but not relevant and those from the recent research program (Smolders et al., 2003) (open symbols).

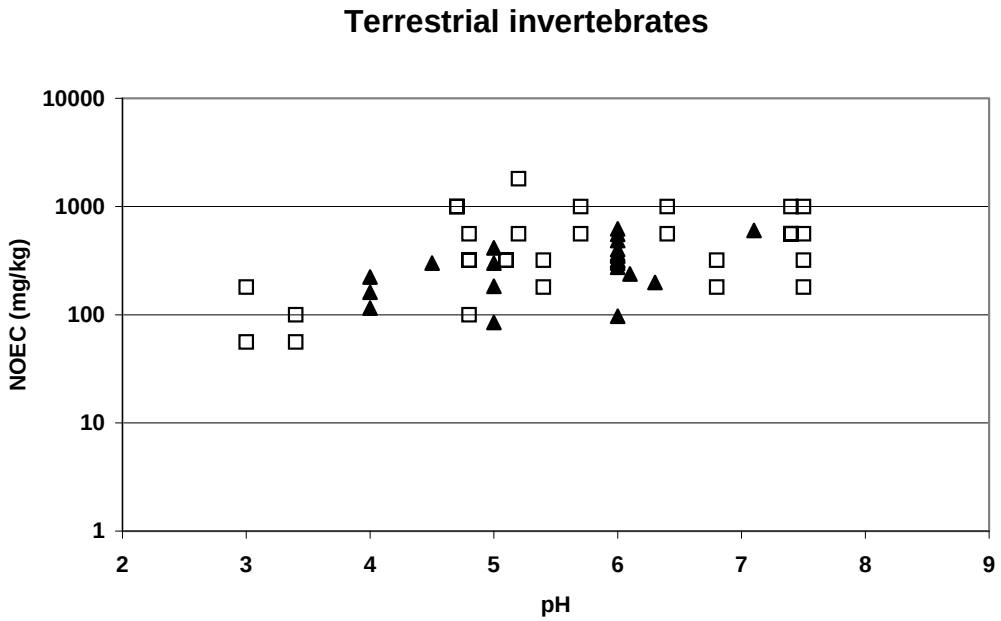


Figure 3.31 Relationship between pH in soil and toxicity to terrestrial invertebrates, expressed as the No Observed Effect Concentration (NOEC). Data from all studies that were used for deriving the $PNEC_{add,terrestrial}$, those that were found reliable but not relevant (closed symbols), and those from the recent research program (Lock et al., 2003) (open symbols).

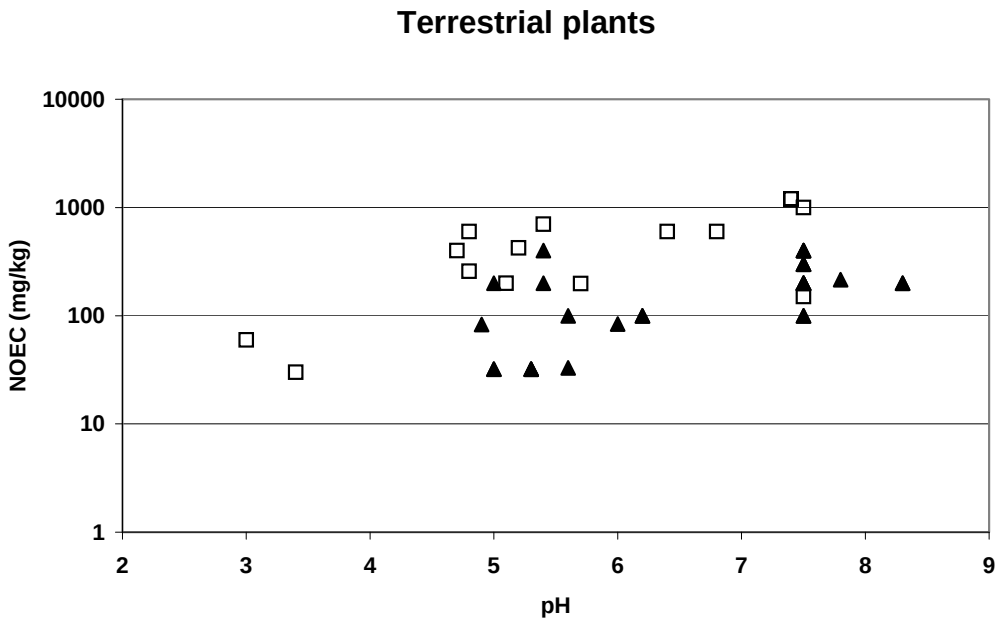


Figure 3.32 Relationship between pH in soil and toxicity to terrestrial plants, expressed as the No Observed Effect Concentration (NOEC). Data from all studies that were used for deriving the $PNEC_{add,terrestrial}$, those that were found reliable but not relevant (closed symbols), and those from the recent research program (Smolders et al., 2003) (open symbols).

Although it is realised that data from studies performed under a variety of conditions are included in the plots, it can still be concluded that there is a too poor basis to derive pH dependent PNEC values.

Since all pH and NOEC combinations are plotted in the same figures, individual studies that have examined the role of pH on toxicity cannot be seen. The results of these individual studies are briefly summarised below. Various studies using different plant species by McLean (1974) show toxicity at low pH and no observed toxicity at higher pH, but these studies show no clear relationship. Spurgeon and Hopkin (1996b) also found no clear relationship between pH and toxicity with *Eisenia fetida*. Sandifer and Hopkin (1997) found increasing toxicity with decreasing pH with *Folsomia candida*, while De Haan et al. (1985) found an opposite effect with *Avena sativa*, i.e. decreasing toxicity with decreasing pH.

Conclusion

From this latter information and that from the Figures 3.30, 3.31 and 3.32, no consistent conclusion can be drawn that pH is a single modulating factor for terrestrial species and microbe-mediated processes. Uptake by soil organisms and bioavailability from the soil is not or only poorly related to pH for zinc. Thus, pH-normalisation may have a physical-chemical basis, but is not supported by a biological basis.

Clay and organic matter content

In The Netherlands, so-called "reference lines" for zinc and other metals have been derived by correlating ambient background concentrations of metals in the soil (measured in a series of remote rural areas in The Netherlands) to the clay and organic matter content. To this end, the 90^{th} percentiles of the ambient background concentrations were used. For zinc, this resulted in the following reference line: [Zn] = {50 + 1.5 x (2 x %clay + %OM)} mg/kg dry weight. For zinc in a standard soil (defined as a soil containing 25% clay and 10% OM), this results in a

"natural" background concentration of 140 mg/kg dry weight (see also section 3.2 of this report and Appendix VIII of the TGD).

It is noted that this Dutch method is purely based on the correlation between the relatively low background concentrations of zinc in soil and the clay and organic matter content, and not related to toxicity that usually occurs at relatively high zinc concentrations. Moreover, a difference in bioavailability between the natural part of the total zinc concentration in soil (which may partly strongly be embedded in the soil minerals) and the added, anthropogenic part of the total zinc concentration in soil (which is considered to be more available, see also section 3.1) is not taken into account in this method.

Not many studies have explicitly examined the relationship between organic mater content or clay content in soil and its influence on the toxicity of zinc. Therefore, an empirical approach was taken. From the studies that were selected to derive the PNEC for the soil compartment and those studies that were evaluated and were found reliable but not relevant, either the soil organic matter content (% OM) or the soil clay content (% Clay) was plotted against the NOEC to evaluate a possible relationship in Figures 3.33 to 3.38 for microbe-mediated processes, invertebrates and plants. A distinction is made in these figures between the data originating from various sources (closed symbols) and the data from a recent research program (open symbols, data from Lock et al., 2003 and Smolders et al., 2003).

As can be seen from those Figures, there seems to be no clear trend for the closed symbols. With increasing % OM or % Clay, the NOEC does not clearly increase or decrease. It must be noted, however, that these data from various sources are in fact not suitable to examine this potential relationship. It is not always clear whether the organisms are in fact adapted to the % OM or % Clay in which they are cultured or whether they are adapted to the % Om or % Clay in the blanks of the test. Although it is assumed that they are the same this information is often lacking. In addition, part of the scatter for the closed symbols in these Figures will also be caused by other abiotic factors that may influence the toxicity. Whether the sensitivity of organisms is really determined by the % OM or % Clay they live in can only be determined by actual toxicity testing of the same organism taken from different field conditions with different % OM or % Clay but comparable other abiotic conditions. The data from the recent research program (open symbols, data from Lock et al., 2003 and Smolders et al., 2003) relate to various soils and a range of % OM (1-40) and % Clay (5-51). No clear relationship is found, however, between toxicity (NOECs) and % OM and % Clay for microbe-mediated processes, invertebrates and plants.

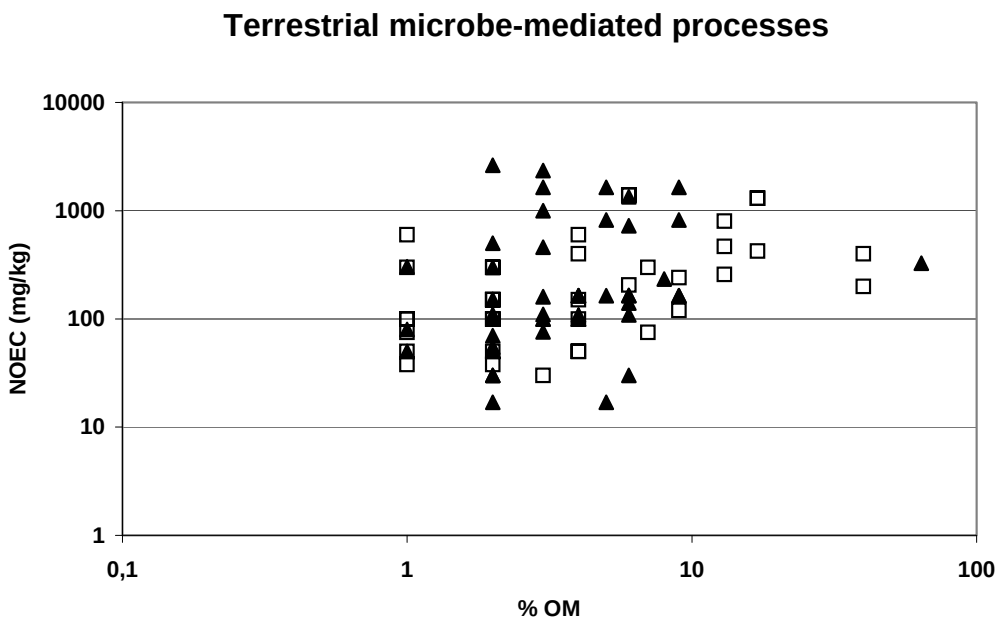


Figure 3.33 Relationship between organic matter (% OM) content in soil and toxicity to terrestrial microbe-mediated processes, expressed as the No Observed Effect Concentration (NOEC). Data from all studies that were used for deriving the $PNEC_{add,terrestrial}$, those that were found reliable but not relevant (closed symbols), and those from the recent research program (Smolders et al., 2003) (open symbols).

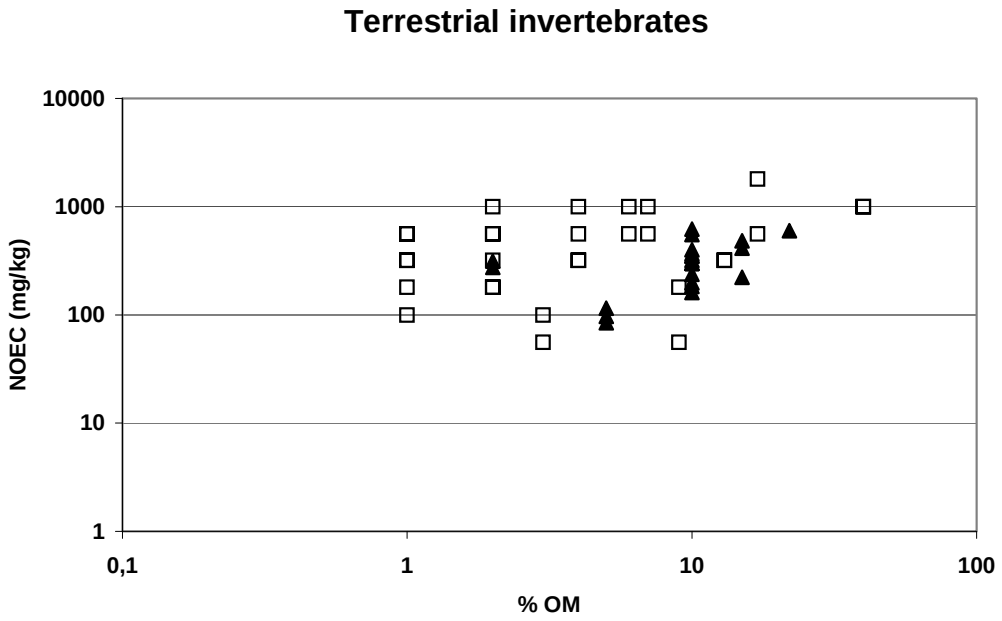


Figure 3.34 Relationship between organic matter (% OM) content in soil and toxicity to terrestrial invertebrates, expressed as the No Observed Effect Concentration (NOEC). Data from all studies that were used for deriving the PNECadd,terrestrial, those that were found reliable but not relevant (closed symbols), and those from the recent research program (Lock et al., 2003) (open symbols).

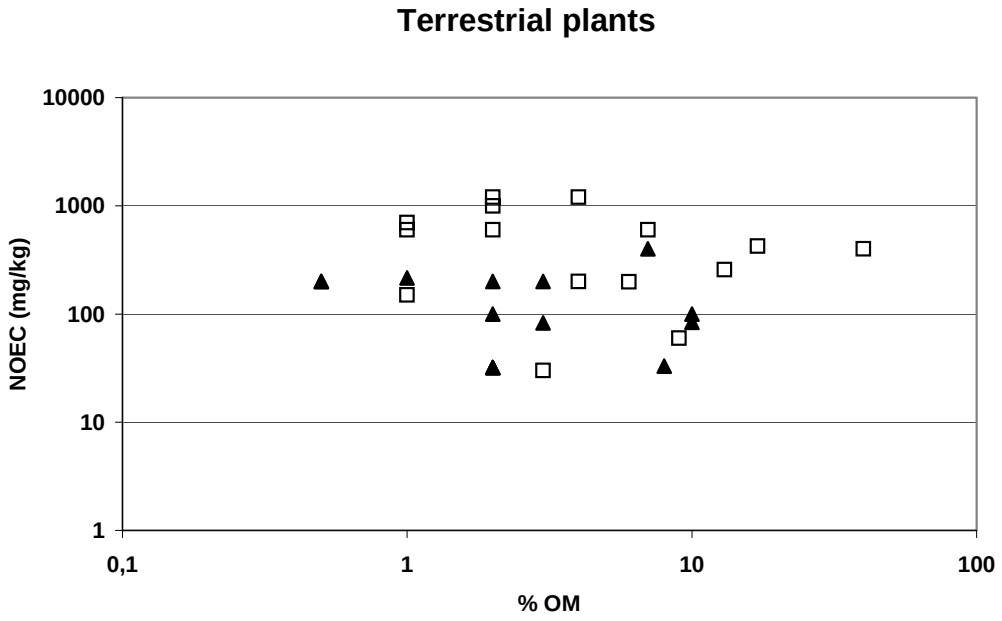


Figure 3.35 Relationship between organic matter (% OM) content in soil and toxicity to terrestrial plants, expressed as the No Observed Effect Concentration (NOEC). Data from all studies that were used for deriving the $PNEC_{add,terrestrial}$, those that were found reliable but not relevant (closed symbols), and those from the recent research program (Smolders et al., 2003) (open symbols).

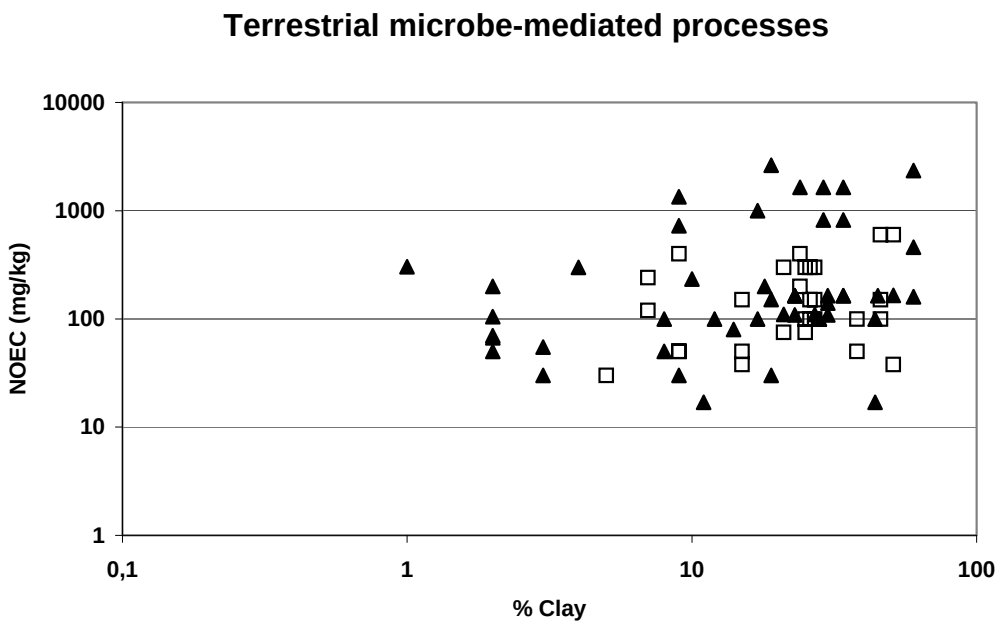


Figure 3.36 Relationship between clay content (% Clay) in soil and toxicity to terrestrial microbe-mediated processes, expressed as the No Observed Effect Concentration (NOEC). Data from all studies that were used for deriving the $PNEC_{add,terrestrial}$, those that were found reliable but not relevant (closed symbols), and those from the recent research program (Smolders et al., 2003) (open symbols).

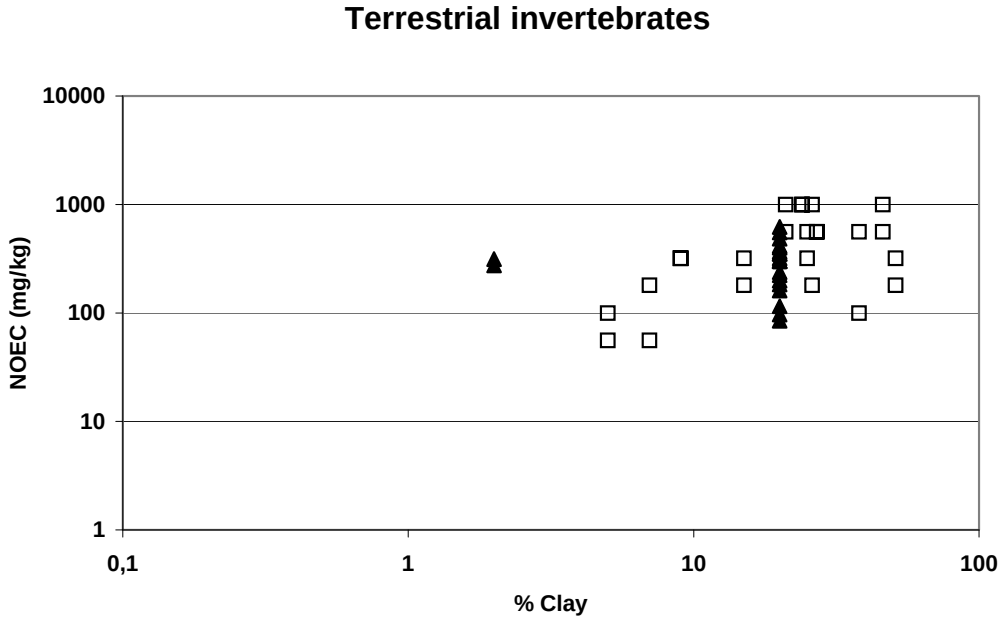


Figure 3.37 Relationship between clay content (% Clay) in soil and toxicity to terrestrial invertebrates, expressed as the No Observed Effect Concentration (NOEC). Data from all studies that were used for deriving the $PNEC_{add,terrestrial}$, those that were found reliable but not relevant (closed symbols), and those from the recent research program (Lock et al., 2003) (open symbols).

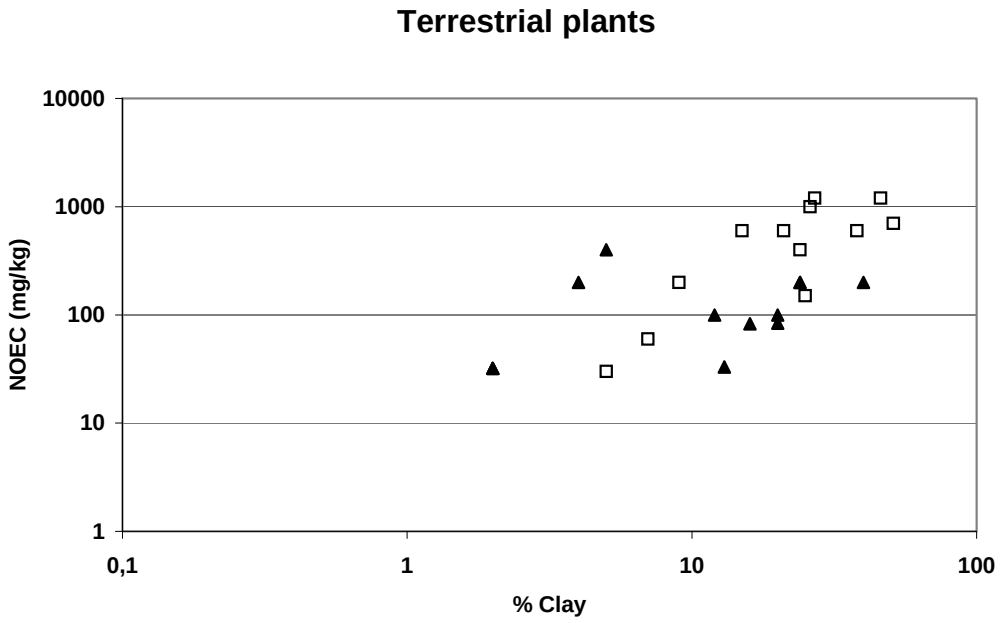


Figure 3.38 Relationship between clay content (% Clay) in soil and toxicity to terrestrial plants, expressed as the No Observed Effect Concentration (NOEC). Data from all studies that were used for deriving the $PNEC_{add,terrestrial}$, those that were found reliable but not relevant (closed symbols), and those from the recent research program (Smolders et al., 2003) (open symbols).

With respect to microbe-mediated processes, Van Beelen and Doelman (1997) state that the effect of pollutants on these processes depends both on abiotic factors (soil characteristics that influence sorption, precipitation or complexation of substances) and biotic factors (intrinsic sensitivity of the microbial community). Based on this they conclude that the above normalisation still faces a number of complicating factors and that more research is needed before soil correction factors can be applied for microbial toxicity tests. Moreover, a study on the effect of zinc on N-mineralization (respiration) in different soil types showed that the Fe content was the main abiotic factor related to the effect of zinc, followed by the clay content; the other abiotic factors studied were pH, CEC, organic matter, lime and Mn (Doelman and Haanstra, 1984).

Conclusion

Toxicity and thus probably also uptake by soil organisms and bioavailability from the soil is not or only poorly related to soil organic matter or clay content.

Pore water concentration

Biological availability is often thought to be comparable to chemical availability, for example in the case of uptake of metals by plants (Römkens and Groenenberg, 2001). There is still considerable debate as to whether even for plant uptake there is more than just the solution fraction relevant for uptake, or the pore water concentration or the free metal ion activity (Parker and Pedler, 1997). In general, it has been shown that the uptake of various elements can be described quite well with either the free metal activity (e.g. for Cu, Temminghoff, 1998) or the amounts of metals extracted by $CaCl_2$ (such as Cd and Zn uptake by lettuce). Lock and Janssen (2001b) reported that the porewater related zinc concentrations are not the only bioavailable zinc fractions and that dietary metal exposure might also be an important route of uptake under environmentally relevant conditions for the invertebrate *Folsomia candida*. Aging and dietary uptake should be studied urgently in order to be able to perform effect-based risk assessments of metal contaminated soils. Similar conclusions were drawn by

Lock and Janssen (2003a) when studying the comparative toxicity of a zinc salt, zinc powder and zinc oxide to *Eisenia fetida*, *Enchytraeus albidus* and *Folsomia candida*, and by Lock and Janssen (2003b), when studying the effect of new soil metal immobilising agents on metal toxicity to *Eisenia fetida* and *Folsomia candida*.

However, various conditions can be mentioned where either the binding capacity or the chemical conditions change to such an extent that the equilibrium between solid and solution phase will be changed completely. For example, the study by Oste et al. (2001) shows that addition of reactive like Beringite and lime had similar immobilising effects on zinc and cadmium. This study also showed that by reducing the actual bioavailability as measured by a dilute salt extraction, the uptake by plants was greatly reduced, but uptake by earthworms was not. The explanation is that earthworms eat soil, and impose their own 'chemical climate' on the soil as it passes the animals intestines. For those organisms whose uptake is directly related to the availability in the soil solution (e.g. plants and soil micro-organisms like bacteria), changes in the concentration that result from changes in soil pH (addition of lime, Beringite etc.) will result in an immediate reduction in exposure. For soil-consuming organisms, however, the exposure is hardly affected since the amount they ingest by eating soil is much larger than that from the soil solution. As yet it is unknown to what degree the changes in bioavailability are reversible. Considering that, it means that changes in bioavailability as measured by changes in the $CaCl_2$ extractable metal pool, which has been considered as the basis for ageing, do not reflect changes in bioavailability for organisms that accumulate metals other than the ones from the soil solution alone. Thus, concentrations of metals in the pore water of the soil or the metal activity in the soil solution lack sufficient basis for risk assessment.

CEC

The Cation Exchange Capacity (CEC) of a soil is a measure of how much cations, including heavy metals can be kept from the soil solution. CEC and pH often are collinear, i.e. when pH increases, so does CEC and vice versa. However, not many studies have explicitly examined the relationship between CEC of a soil and its influence on zinc toxicity. Only from the studies from the recent research program (Lock et al., 2003; Smolders et al., 2003) information on the relationships between CEC (and pH) for invertebrates and plants could be retrieved. The data from the recent research program relate to 15 European soils and a range of CEC (1.9-36.3 cmol/kg) and pH (3.0-7.5). The regressions for the zinc toxicity to plant (wheat) and two invertebrates (F. candida and E. fetida) versus CEC (and pH), where toxicity is expressed as EC50, are as follows:

Wheat (T. aestivum):

$$\log (EC50) = 1.1 + 0.87\ (0.45\text{-}1.29) \times CEC + 0.12\ (0.02\text{-}0.22) \times pH$$
(R^2=0.84, Q^2=0.74, n=14, $p<0.001$ (CEC), $p<0.05$(pH))

F. candida:

$$\log EC50 = 1.4 + 1.14 \log CEC \text{ (F. candida)}$$
(R^2=0.84, Q^2=0.78, n=15, $p<0.001$)

E. fetida:

$$\log EC50 = 1.9 + 0.79 \log CEC \text{ (E. fetida)}$$
(R^2=0.77, Q^2=0.70, n=14, $p<0.001$)

Where:

EC50 is expressed as added zinc in mg/kg
The values in parentheses provide the 95% confidence interval.

Boyd and Williams (2003) also found a strong relationship (R^2=0.99, full relationship not provided) between CEC and the toxicity of zinc to the nematode *Caenorhabditis elegans* in three soils with varying soil properties (% Sand: 74-98, % Silt: 0-16, % Clay: 2-10, % OM: 1.4-5.1, pH: 5.7-7.8, CEC: 2.4-28.4). This indicates that CEC affects the bioavailability of zinc in soil to a nematode besides that to the invertebrates and the plant.

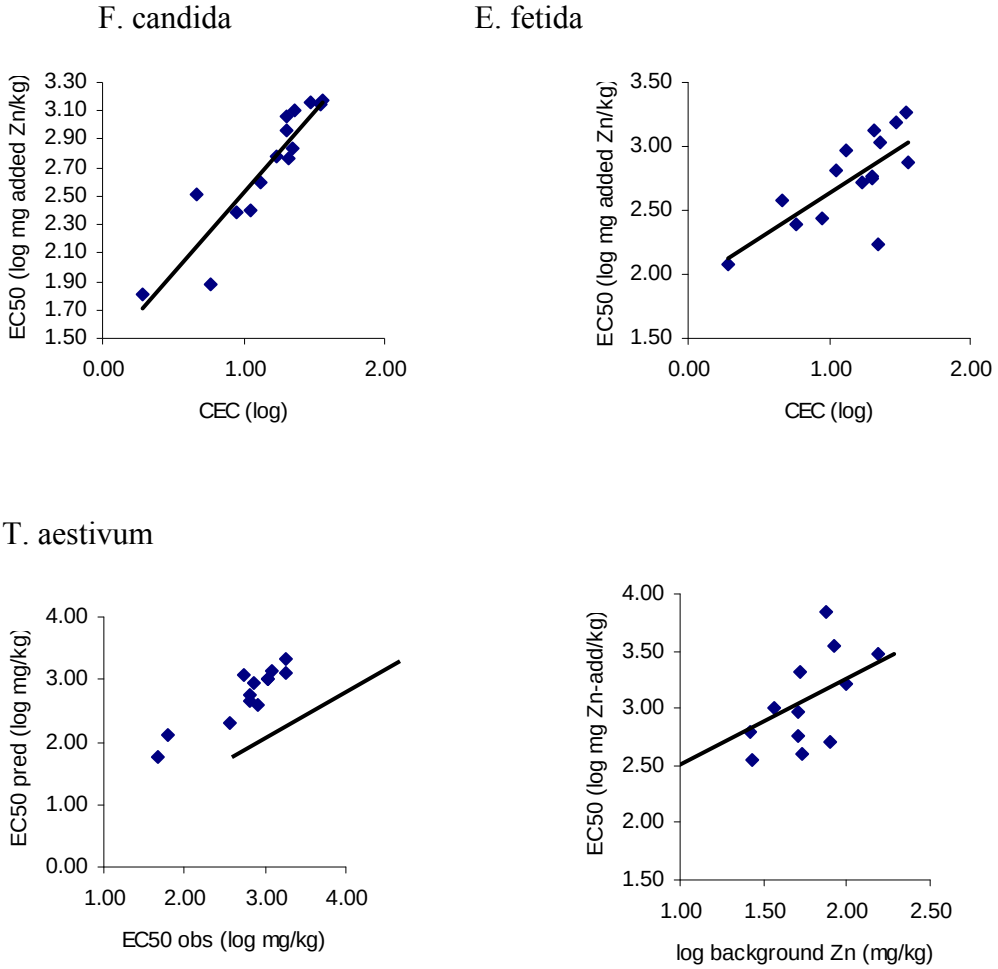


Figure 3.39 Relationship between CEC in soil and toxicity to the terrestrial invertebrates F. candida and E. fetida, and to the plant T. aestivum, expressed as the EC50 (Lock et al., 2003; Smolders et al., 2003).

Conclusion

Based on these latter data it is concluded that there is a strong and statistical basis to relate CEC (and pH) in soil to zinc toxicity to plants and invertebrates. This indicates that the storage capacity of a soil for cations does have an important mitigating effect on the bioavailability and toxicity of zinc.

Studies on and validation of the influence of mitigating factors to bioavailability for European soils

A conservative approach assumes that all of the zinc in the terrestrial (soil) compartment, measured as total zinc per unit of dry or wet weight, is completely bioavailable. The aims of a recent research program on bioavailability for the terrestrial compartment (Davis et al., 2003; Lock et al., 2003; Smolders et al., 2003) were to demonstrate that the total zinc concentration in the soil is not completely bioavailable, but depends on the composition of abiotic components of the soil, and that there were significant lab-to-field differences. The program aimed to obtain information on

- the role abiotic soil factors have on the chronic toxicity of zinc towards various types of biological endpoints, i.e. plant, soft-bodied and hard-bodied invertebrates, and various microbial endpoints, and
- lab-to-field differences, among them ageing, shock and acclimation effects.

The results from Smolders et al. (2003) on the effects of field and laboratory zinc contamination on soil microbial processes and plant growth are summarised as follows. Toxicity thresholds of zinc (Zn) in soil vary several orders of magnitude among species, soil types, and age or source of Zn contamination. This project was designed to identify (i) the role of soil properties on Zn toxicity in soils freshly spiked with $ZnCl_2$ in the laboratory and (ii) the difference in Zn toxicity between freshly spiked soils and soils contaminated in the field. The toxicity was tested with plant growth (pot trial with wheat seedlings) and with 3 microbial processes (respiration in soils amended with either glucose or a plant residue and nitrification potential).

Fifteen uncontaminated topsoils (pH 3.0-7.5, 0.4-23% organic carbon, 5-51% clay content, CEC 1.9-36.3 cmol/kg, zinc background 7-191 mg/kg) were collected throughout Europe. Soil samples were taken in Belgium (3x), France, Germany, Greece (2x), Italy, The Netherlands (3x), Spain, Sweden, and the United Kingdom (2x). These soils were spiked in the laboratory with $ZnCl_2$ and toxicity tests started within 3 days after spiking. In addition, soils were sampled in 3 transects towards galvanised electricity transmission towers (pylons). The soil total Zn concentrations gradually increased in each transect from background values (76-155 mg Zn/kg) to elevated Zn concentrations near the pylon (2100-3740 mg Zn/kg). Soil samples taken at the furthest distance from the Zn source were spiked with $ZnCl_2$ to a range of total Zn concentrations similar to those in the transect. The 4 toxicity tests performed in 15 spiked soils yielded 53 significant dose-response relationships. The no observed effect dose (NOEC, added Zn) of each test varied between 30-1400 mg Zn/kg. The range in NOEC (added) values was generally larger among soils (for each test) than among tests (for each soil), confirming that soil properties have a major role in the Zn toxicity. Soil solution Zn concentrations do not explain the variability in total Zn thresholds (e.g. EC50 values) *among* spiked soils. The total Zn thresholds (e.g. EC50 values) are mainly explained by CEC and pH (plant growth) and background Zn (2 microbial processes). Regression models with these properties explain 42-85% of the variability of the EC50 for plants, nitrification and glucose respiration. The respiration assay in plant residue amended soils did not show significant correlations with background Zn. However, this test proved to be rather insensitive (% inhibition at largest dose smaller than 50% in all soils), hence the observed EC50 values are

all extrapolated values and are inaccurate. Field transect soils, contaminated with Zn over time by corrosion of galvanised electricity transmission towers, showed no clear evidence of Zn toxicity in any of the three microbial assays conducted and in the plant growth assay, with one exception (plant test in one transect). In contrast, spiking these soils always yielded significant dose-response curves and total Zn EC50 values were 0.3-7.7 (mean: 2.1) -fold lower than the largest total Zn in the transect. There was a consistently larger soil solution Zn concentration in spiked soils than in field contaminated soils at corresponding total Zn. Soil solution Zn concentration is a better lab to field translator than total Zn to explain the lack of pronounced toxicity (e.g. elimination of plant growth or nitrification) in *corresponding* field contaminated soils. It is concluded that soil properties have a major role on Zn toxicity threshold in freshly spiked soils and that Zn toxicity in soils contaminated gradually over time is found at much larger total Zn concentrations than in corresponding freshly spiked soils. Soil solution Zn concentrations in field contaminated soils are not large enough to predict pronounced Zn toxicity as based on effects in the corresponding spiked soils.

The results from Lock et al. (2003) on the laboratory zinc ecotoxicity testing for soil invertebrates are summarised as follows. To study the effect of abiotic factors on the toxicity of zinc to terrestrial invertebrates, toxicity tests with *Eisenia fetida* and *Folsomia candida* were performed in 15 field soils spiked with different zinc concentrations. These soils were chosen in such a way that soil characteristics affecting bioavailability, such as pH and cation exchange capacity, represented the range of values that can be found in European soils. For *E. fetida*, the 28d EC50s, 28d EC10s and 28d NOECs ranged from 120 to 1820 mg Zn/kg dry wt, 20.5 to 1150 mg Zn/kg dry wt and 100 to 1000 mg Zn/kg dry wt, respectively. For *F. candida*, the 28d EC50s, 28d EC10s and 28d NOECs ranged from 64.0 to 1500 mg Zn/kg dry wt, 10.8 to 1210 mg Zn/kg dry wt and 56 to 1800 mg Zn/kg dry wt, respectively. For both species, it was found that 28d EC50s expressed as added zinc concentrations in the soil could be normalised on the basis on the cation exchange capacity (measured at soil pH). The 28d NOECs of both species could also be normalised on the basis of the cation exchange capacity. However, using the 28d EC10s, only for *F. candida* could a significant model be obtained, i.e. normalisation of 28d EC10s on the basis of the cation exchange capacity can be performed for only one of the two test species. Using these models normalising zinc toxicity data, the uncertainty in the zinc toxicity outcome decreased from over an order of magnitude to approximately a factor two. Additionally, the developed models can be used to normalise toxicity data taken from literature, indicating that the proposed models can be used to account for differences in bioavailability in existing data sets.

The range in 28d EC50s, 28d EC10s and 28d NOECs was much higher when the toxicity data were expressed in total pore water zinc concentrations compared to toxicity data expressed in added or total zinc concentrations in the soil. A significant relationship was found between toxicity values expressed as pore water zinc concentrations and pH, with zinc toxicity increasing with increasing pH. Possible explanations are proton competition at a lower pH, alternatively organisms may be mainly exposed through pore water zinc at low pH while at a higher pH, organisms may be more exposed through oral uptake.

Both test organisms seem to be affected by the same zinc toxicity modifying factor. On basis of the results of this study it can be suggested that there does not seem to be a significant difference in the way zinc affects hard-bodied and soft-bodied terrestrial organisms. To assess the effect of ageing, three transects of zinc contaminated field soils were sampled and their toxicity was compared with that of the control soils of the transect that was spiked with different zinc concentrations. Zinc toxicity to *E. fetida* and *F. candida* was in almost all cases higher in the spiked soils compared to the zinc contaminated transect soils, which can be

explained by the higher total pore water concentrations in the spiked soils. The difference in zinc bioavailability can be chemically explained (see Smolders et al., 2003).

The results from Davis et al. (2003) on the effects of zinc contamination on soil microbial processes and on the elucidation of shock and adaptation effects are summarised as follows. The study was aimed to assess the importance of shock and adaptation on the toxicity of Zn to the microbial community of soils and the potential effects on developing laboratory based risk assessments for Zn in soils.

Two complimentary approaches were used. Shock effects were examined in four contrasting soils selected to provide a representative range of different soil parameters. Three different spiking methods were adopted: (i) spiking of 100% of pre-determined EC50 dose immediately, (ii) the addition of the same Zn dose over 22 weeks and (iii) addition of the whole Zn dose immediately before microbial assessment. Shock was further investigated by the addition of an equivalent salt (Cl-) dose to that provided by spiking with $ZnCl_2$.

Community adaptation in the field was investigated by the re-inoculation of two Zn spiked sterilised soils with a microbial extract from the same soils, either not contaminated (low Zn; control) or highly contaminated. The microbial activity of the high-Zn and low-Zn inocula was compared with an unsterilised soil spiked with Zn, and with the corresponding sterilised field transect soils re-inoculated with a microbial extract from a low Zn containing soil. The experiment was sampled after re-inoculation and again 3 months later to determine potential adaptation / tolerance within the community.

The soils in both experiments were examined using a range of soil microbial bioassays, including potential nitrification rate (PNR), dehydrogenase activity, microbial biomass content, substrate induced respiration (SIR). Potential shock effects were further investigated by the Pollution-Induced Community Tolerance (PICT) method and measurement of basal respiration.

Concentrations of Zn in soil solutions increased with the Zn dose added to the soil. Concentrations of Zn in soil solution decreased with incubation time in two neutral soils, but this did not occur in the two acid soils. The neutral pH soils showed that increased Zn concentration decreased potential nitrification rate and substrate utilisation. The inhibitory effect of Zn spiking was not greater when Zn was spiked just before bioassays than Zn spiking at the start of the experiment or weekly additions, indicating no shock effects on the microbial community. Furthermore, the addition of an equivalent amount of $CaCl_2$ to that of $ZnCl_2$ indicated that there was no shock effect of salt or Cl^-. Results of dehydrogenase activity, SIR and microbial biomass showed that no adaptation or tolerance was evident when comparing the soils re-inoculated with microbial extracts from high and low field Zn contaminated soils. PNR could not be determined reliably in the two acid soils because of accumulation of large concentrations of nitrate in the soils before the bioassay.

The findings of this series of experiments indicate that shock and adaptation effects may not need to be accounted for when developing risk assessments using laboratory experimental data. In the field transects there was a lack of inhibition of microbial assays up to very large total Zn concentrations in soils. These results show that soil solution Zn was very much higher in laboratory spiking studies than at equivalent total Zn concentrations in well established Zn gradients in the field. This means that lab to field extrapolation needs to account for these differences in solubility in longer term.

Evaluation of lab-to-field differences and soil-properties related bioavailability corrections

Available normalisation methods implicitly or explicitly assume that the metal concentration or activity in the pore water or soil solution can be related to uptake, and normalisation would

be relevant for risk assessment. This assumption, however, lacked sufficient scientific basis for the risk assessment of zinc (and other metals) in soil when data from various literature sources were compiled. Only recently, a thorough investigation that systematically studied the effects on zinc to various microbial processes, plants, and terrestrial invertebrates in a series of fifteen European soils, could be used to normalise the terrestrial toxicity data on zinc. Furthermore, this recent study and some other literature studies now provide sufficient evidence to take into account lab-to-field differences and the soil properties that affect the bioavailability of zinc in soil. Firstly, the rationale for taking into account lab-to-field differences will be explained. Secondly, the rationale for taking into account the soil properties that affect the bioavailability of zinc in soil will be explained. Then, the approach that is taken to take into account one or both corrections, where appropriate, for correcting the PEC will be explained.

Evaluation of lab-to-field differences for correcting the PEC

Several transect studies in the research program on bioavailability for the terrestrial compartment (Davis et al., 2003; Lock et al., 2003; Smolders et al., 2003) evaluated the differences between the laboratory and the field in order to come up with a lab-to-field correction. Invertebrate testing was used as well as plant testing and three different microbial tests. In summary, these results showed the following (see also Table 3.88):

- The lab-to-field ratios of the transect studies depend on and vary among the different tests (invertebrates, plant, microbial tests) as well as among the various levels of the chronic endpoints (EC50, EC10, NOEC, and LOEC);
- More reliable data were obtained for EC50 values and less reliable data were obtained for NOEC and LOEC values, however, for some endpoints, in particular for the microbial tests, no EC50 values could be derived;
- More reliable data were obtained for the dry weight based concentrations than for the pore water based concentrations. Furthermore, all studies reported by Smolders et al. (2003) and Lock et al. (2003) showed that pore water based concentrations show much more variety than dry weight based concentrations with respect to zinc toxicity. In addition, since the $PNEC_{add}$ for soil is also dry weight based, the final discussions on and the conclusions of lab-to-field translators are mainly based on the dry weight based concentrations;
- Most of the ratios are higher than 1, while a few are lower than 1, and another number of ratios could not be determined, but are probably higher than 1. A ratio higher than 1 indicates that toxicity observed in the field is less than in the laboratory at a similar concentration of zinc;
- The EC10 values should be used mainly, not the NOEC, LOEC or EC50 values. This is because the NOEC and LOEC are determined by the variability among replicates and this variability is not the same in spiked and field-contaminated soils, i.e. NOEC or LOEC ratios are affected by differences in "noise" rather than real toxicity differences. The preferred point of comparison is the EC50, however several microbial tests did not reach the EC50 in even the spiked soils. This means that information of these tests would be excluded from the analysis based on EC50s.
- Microbial assays are clearly less sensitive than the plant and invertebrate tests, because in every case the under field conditions the microbial assays gave unbounded NOECs.
- The rationale for this lab-to-field difference is still mainly empirical, while some speculations indicate that it can relate to ageing processes, i.e. the longer the zinc is in the soil the more it will be encaptured in a less bioavailable form in the soil;

- In many cases no toxicity was observed in the field at concentrations higher than 470 mg/kg dry weight or much higher, i.e. levels that are more than 1-2 orders of magnitude higher than the $PNEC_{add}$ for soil.

Furthermore, additional data from the literature support the observation that toxicity observed in the field is at much higher levels than those observed in the laboratory. For example, Smolders et al. (2003) found in seven soil/endpoint combinations that the ratios field-to-lab are significantly higher than 1, and found no effects at levels between >175 and >433 mg/kg of zinc in the field situations. Also Smit and van Gestel (1998) showed this observation and found no effects at concentrations >1528 mg/kg of zinc in the field.

Table 3.88 The ratios of EC10 values (based on dry weight normalised zinc concentrations) in field contaminated soil to that in corresponding spiked soil from Smolders et al. (2003) and Lock et al. (2003). Mean values per test soil added.

Test	Rhydtalog soil	De Meern soil	Zeveren soil
Eisenia fetida	1.3	>2.8*	1.0
Folsomia candida	>12.3*	>5.5*	3.1
T. aestivum	>11*	>6.1*	0.8
Transect mean for species	>8.3	>4.8	1.6
OVERALL MEAN FOR SPECIES	>4.9		
Nitrification	>3.5*	>4.3*	>13*
Maize residue mineralization	>8.6*	>2.6*	>3.3*
Glucose mineralization	>2.4*	>1.8*	>12.3*
Transect mean for processes	>4.8	>2.9	>9.6
Overall mean for microbial processes	>5.8		
Overall mean per test soil *(combined data for species and processes)*	>6.5	>3.9	>5.6

* No or no reliable EC10 could be derived for the field contaminated soil. For the calculation of the ratio, the highest concentration measured in the field contaminated soil has been used as unbounded EC10 (viz. Rhydtalog 2100 mg/kg, De Meern 2520 mg/kg and Zeveren 3740 mg/kg).

In Tables 3.89 and 3.90, the lab-to-field ratios derived from a number of literature tests are presented. Table 3.89 shows the ratios based on EC50 values, while Table 3.90 shows the ratios based on NOECs. The tests were performed in the framework of the Dutch project 'Validation of toxicity data and risk limits for soils' (Posthuma et al. (1998), see further section 3.3.3.1.4.). In this latter Dutch project the toxicity of Zn was determined in freshly-spiked sand soil (PANH) and compared with that in the same soil that after spiking with zinc was placed in uncovered outdoor plots for up to nearly 2 years (PANH-aged).

Table 3.89 The ratios of EC50 values (based on dry weight normalised zinc concentrations) in aged PANH soil to that in corresponding spiked soil.

Test	Aged 2y	Aged 1y	Aged 4 mo	Aged 2 mo	Aged 1 mo
Glutamic acid mineralisation		1.1			0.8
F. candida	8.3				
E. crpyticus	3.4				
T. pratense	8.7	5.6[a]	1.8[b]	1.6[c]	
Overall mean	3.9				
OVERALL MEAN AFTER 1 OR 2 Y OF AGEING	5.4				

A the lowest of two ratios (5.6 and 5.9) for two endpoints is reported here (endpoint root yield).

B the lowest of two ratios (1.8 and 2.2) for two endpoints is reported here (endpoint shoot yield).

C the lowest of two ratios (1.6 and 1.9) for two endpoints is reported here (endpoint shoot yield).

Table 3.90 The ratios of NOEC values (based on dry weight normalised zinc concentrations) in aged PANH soil to that in corresponding spiked soil.

Test	Aged 2y	Aged 1y	Aged 4 mo	Aged 2 mo	Aged 1 mo
Glutamic acid mineralisation		2.2			1.3
F. candida	2.4[a]				
T. pratense	3.1[b]	2.9	1.2	1.2	
Overall Mean	2.0				
OVERALL MEAN AFTER 1 OR 2 Y OF AGEING	2.7				

A two endpoints have a NOEC ratio of 2.4 (reproduction and growth).

B the lowest of two ratios (3.1 and 6.7) for two endpoints is reported here (endpoint germination).

C the lowest of three ratios (2.9, 4.0 and 6.7) for three endpoints is reported here (endpoint germination).

The data presented in Tables 3.88 - 3.90 were used to compile Table 3.91, which presents an overall, integrated picture of the available information. This table shows the EC10/EC10 ratios for Rhydtalog, De Meern and Zeveren soil and the NOEC/NOEC ratios for PANH soil. Please note that for the PANH only the ratios from tests in soil samples that were aged for 1 year and 2 years have been used in the calculations.

Table 3.91 Mean of EC10/EC10 ratios for Rhydtalog, De Meern and Zeveren soils and NOEC/NOEC ratios for PANH soil.

Test soil	Organism/process	Lab-to-field ratio
Rhydtalog	E fetida	1.3
De Meern	E fetida	>2.8
Zeveren	E fetida	1.0
Rhydtalog	F. candida	>12
De Meern	F. candida	>5.5
Zeveren	F. candida	3.1
PANH	F. candida (2 year aged soil)	2.4
Rhydtalog	T. aestivum	>11
De Meern	T. aestivum	>6.1
Zeveren	T. aestivum	0.8
PANH	T. pratense (2 year aged soil)	3.1
PANH	T. pratense (1 year aged soil)	2.9
Rhydtalog	Nitrification	>3.5
De Meern	Nitrification	>4.3
Zeveren	Nitrification	>13
Rhydtalog	Maize residue mineralisation	>8.6
De Meern	Maize residue mineralisation	>2.6
Zeveren	Maize residue mineralisation	>3.3
Rhydtalog	Glucose mineralisation	>2.4
De Meern	Glucose mineralisation	>1.8
Zeveren	Glucose mineralisation	>12
PANH	Glutamic acid mineralisation (1 year aged soil)	2.2
	Overall mean	4.8

Mean value is the arithmetic mean.

From Table 3.91 the following summary remarks can be made:

- The results from the Dutch 'validation' project (PANH soil) are in good agreement with those of Smolders et al. (2003) and Lock et al. (2003), both usually showing less zinc toxicity under field conditions than under laboratory conditions.
- The mean ratios per test soil for species and for processes are similar, i.e. within a factor of 2 (except in Zeveren soil: 1.6 for species *versus* >9.6 for processes).
- The majority of the ratios, i.e. 19 out of 22 values are clearly higher than 1, varying from 2 to >13. In 15 out of 22 values the ratio was higher than ≈ 3. Moreover, many of the available ratios are unbounded; in those cases no effect was found up to the highest concentration measured in the field-polluted soil. Only 3 out of the 22 values are around 1 (0.8-1.3).
- The mean ratios per test soil, based on the combined data for species and processes, are >6.5 for Rhydtalog soil, >3.9 for De Meern soil, >5.6 for Zeveren soil and 2.7 for PANH

soil. Note that the lowest value is for aged PANH soil (a soil that after spiking with zinc was placed in uncovered outdoor plots for up to nearly 2 years, while the other three soils were more gradually polluted with zinc over a period of around 10 to 50 years due to corrosion of galvanised electricity transmission towers.

Furthermore, Stevens et al. (2003) assessed the cationic metal toxicity and associated anionic salts. They observed that when soils were contaminated with a metal salt toxicity decreased when the soil was leached. For zinc that was added as zinc nitrate to five soils, leaching increased the EC50 for *Lactuva sativa* by 1.4 to 3.7-fold. The shift in EC50 was not a direct result of toxicity of the nitrate ion but was an indirect effect of the salinity increasing metal concentrations in soil solution and increasing its bioavailability for a given total metal concentration.

Given these latter observations, it is concluded that there is sufficient justification to assume that toxicity under field conditions is less than under laboratory conditions. Therefore a lab-to-field factor of 3 is proposed for all soils. However, only in cases when ageing has lasted more than a year. A ratio of 2 should only be used in cases where a rapid increase in zinc soil concentration could occur, e.g. due to the melting of snow, when ageing has occurred for less than one year. This lab-to-field factor needs to be applied to the total zinc concentration, since it is based on total zinc concentrations.

Soil-properties related corrections for the PEC

From the studies within the framework of the recent research program on the bioavailability of zinc in soil, a number of regression equations between on the one hand toxicity data for plant, invertebrates, and microbial processes, and on the other hand a number of soil properties were reported by Smolders et al. (2003) and Lock et al. (2003). A crucial question was whether the equations fulfil the general criteria that are commonly accepted for allowance of regression equations for regulatory purposes. Criteria for the regulatory acceptance were recently recommended by a group of experts of various disciplines and various background during a workshop organised by ECETOC (Setubal, Portugal, March 4-6, 2002). Following up on previous recommendations of Hermens et al. (2003) in the framework of an EU-sponsored Research Project, values of performance parameters in terms of number of data points versus number of descriptors (to avoid overfitting of the experimental data), critical values of Q^2 (indication of the predictive capability of the regression equation reported on the basis of internal validation) and R^2_{adj} (=R^2 adjusted for the number of degrees of freedom) were recommended. These criteria are developed on top of the basic criteria that apply to all (regression) models in which significance of the model equations at for instance a pre-specified p-level is assessed. The latter criteria are, however, not indicative of the predictive power of the model.

Experts participating in the Setubal workshop recommended that validation of models is essential and it was agreed that in addition to traditional measures of goodness-of-fit (R^2), assessment of predictive power (Q^2) is essential, and must be reported (Cronin et al., 2003a; Cronin et al., 2003b; Eriksson et al., 2003; Jaworska et al., 2003). The following criteria are of relevance in this respect:

The value of R^2 should be at least 0.7 (i.e.: > 70 % of the variance in the data is to be explained by the model).
The value of Q^2 should be at least 0.5 (i.e.: > 50 % of the variance in the data is to be explained by the model).
There should be at least 5 data points per descriptor.

If one of these criteria is not met, then it is recommended not to use the regression equation for regulatory purposes.

Based on the regressions between zinc toxicity for microbial processes (versus background zinc concentration), for plants (versus CEC and pH), and for invertebrates (versus CEC) from Smolders et al. (2003) and Lock et al. (2003) and data from the literature an overview of regressions is provided in Table 3.92 that will assist in further interpreting these regressions.

Table 3.92 Overview table on conclusion (i) and literature regressions relating to bioavailability of zinc in soil.

Organism	Statistics	X-variable	Y-variable	Slope (95% confidence intervals)	Source
INVERTEBRATES					
F. candida	R^2 = 0.84 Q^2 = 0.78 N=15	Log EC50 (added zinc in mg/kg)	Log CEC	1.14 (0.84-1.42) p<0.001	Lock et al. (2003)
F. candida	R^2 = 0.89 N=9	Log NOEC	Log CEC	1.19	Lock and Jansen (2001a)
E. fetida	R^2 = 0.77 Q^2 = 0.70 N=14	Log EC50 (added zinc in mg/kg)	Log CEC	0.79 (0.52-1.06) p<0.001	Lock et al. (2003)
E. fetida	R^2 = 0.80 N=9	Log NOEC	Log CEC	1.18±0.22	Spurgeon and Hopkin (1996b)
A. caluginosa	R^2 = 0.77 N=5	Log LC50	Log CEC	0.50	RIVM (un-published)
E. albidus	R^2 = 0.91 N=11	Log LC50	Log CEC	1.25	Lock et al. (2000)
Invertebrates (3 species, 11 studies, 23 NOECs)	NS	Log NOEC	Log CEC	-	RAR
PLANTS					
T. aestivum	R^2 = 0.84 Q^2 = 0.74 N=14	Log EC50 (added zinc in mg/kg)	Log CEC PH	0.87 (0.45-1.29) p<0.001 0.12 (0.02-0.22) p<0.05	Smolders et al. (2003)
A. sativa	R^2 = 0.68 N=6	Log NOEC	Log CEC	1.65±0.56	De Haan et al. (1985)
MICROBIAL PROCESSES					
PNR	R^2 = 0.55 Q^2 = 0.42 N=13	Log EC50 (added zinc in mg/kg)	Log background zinc (mg/kg)	0.76 (0.30-1.22) p<0.01	Smolders et al. (2003)
SIR	R^2 = 0.42 Q^2 = 0.13 N=14	Log EC50 (added zinc in mg/kg)	Log background zinc (mg/kg)	0.76 (0.19-1.33) p<0.05	Smolders et al. (2003)
Microbial processes	R^2 = 0.31 N=28	Log NOEC (added zinc in mg/kg)	Log background zinc (mg/kg)	0.72 p<0.01	RAR
Respiration	NS N=5	Log EC50 (added zinc in mg/kg)	Log background zinc (mg/kg)	-	Doelman and Haanstra
Arylsulphatase	NS N=4	Log EC50 (added zinc in mg/kg)	Log background zinc (mg/kg)	-	Doelman and Haanstra
Urease	NS N=4	Log EC50 (added zinc in mg/kg)	Log background zinc (mg/kg)	-	Doelman and Haanstra

NS = not significant

With respect to the regressions on plants and invertebrates, the Table shows that the regressions of the recent research program all have R^2-values > 0.70 and Q^2-values > 0.50 and therefore meet the recommended criteria from the Setubal workshop. One outlier in the *E.*

fetida regression is recognised, since it refers to exclusion of a soil with exceptional properties, i.e. heavy clay. It is also recognised that for those regressions the slopes for NOECs and EC50 are similar (Table 3.92):

- for *F. candida* the observed slope for log CEC for the NOEC is 1.19 and that for the EC50 is 1.14 (0.84-1.42)
- for *E. fetida* the observed slope for log CEC for the NOEC is 1.18±0.22 and that for the EC50 is 0.79 (0.52-1.06)
- for *T. aestivum* the relevant observed slopes for the EC50 are 0.87 (0.45-1.29, for log CEC) and 0.12 (0.02-0.22, for pH) that cannot be simply compared to NOEC data.

Using the equations from the recent research program on the plant *T. aestivum* (Smolders et al., 2003), the study with the plant *Lactuca sativa* (Stevens et al., 2003) is used for external validation purposes, i.e. to check the validity of the equations, although the two studies did not use the same plant species. The results are shown in Table 3.93.

Table 3.93 Results of the experimentally determined EC50 values for zinc toxicity to lettuce (L. sativa) in five different soils (Stevens et al., 2003) and the corresponding predicted EC50 values based on the equation for T. aestivum (Smolders et al., 2003).

Soil #	pH	CEC	EC50 (mg/kg)		Factor (predicted ÷ experimental)
			Experimental for *L. sativa*	Predicted from the equation for *T. aestivum*	
1	5.5	17.6	94	698	7.4
2	6.2	3.3	3	197	65.8
3	8.5	6.4	266	663	2.5
4	6.8	9.5	289	584	2.0
5	5.7	16.3	75	690	9.2

This external validation exercise shows that the equation from the recent research program systematically underestimates toxicity to *L. sativa,* in one soil even up to a factor of 66. However, it is still concluded that the regressions for plants and invertebrates are statistically sound and sufficiently take into account the variance in the chronic endpoints, but should be used with some caution to avoid underestimating toxicity.

With respect to the regressions on microbial processes they have a weak predictive power, i.e. all R^2-values are < 0.70 and all Q^2-values are < 0.50: R^2=0.55 and Q^2=0.42 for PNR; R^2=0.42 and Q^2=0.13 for SIR. This means that when one would have to apply these regressions to a soil, with known zinc background concentration, but not tested in the recent research program, other unknown parameters may be more important in affecting the toxicity of zinc than background concentration. However, it is recognised that the slopes of the various regressions for microbial processes (two regressions from the conclusion (i) project and one from the meta analysis from the RAR, see Table 3.92) come up with similar slopes, i.e. 0.76, 0.76 and 0.72, respectively. In general, lower R^2 values on the microbial datasets can be expected, due to the higher inherent variability of these tests. Although the soil microbial processes yield lower R^2 values, the models are statistically significant. Furthermore, three other individual studies from Doelman & Haanstra (see Table 3.92) failed to produce significant relationships.

In summary, in addition to the lab-to-field correction another bioavailability correction is justified that takes into account the soil properties. Based on the results of the recent research program soil properties corrections are produced for background zinc concentration (for microbial processes), CEC (soil invertebrates and plants) and pH (plants). Since terrestrial microbial processes as well as terrestrial plants and invertebrates need to be protected, the approach to correct for bioavailability taking soil properties into account, should be performed in a conservative way. That means that the smallest correction from either the microbial related equations or the plants and invertebrates related equations should be used to correct for bioavailability of zinc in the soil.

The bioavailability correction thus should (a) make use of the available knowledge of soil-type dependent bioavailability of zinc and (b) take into account all remaining uncertainties by using the 'lowest CI of slope' (see below).

The available knowledge on the soil-type dependent bioavailability results in the following equations that are proposed for correcting bioavailability in soil:

$$LogEC50=1.4+1.14logCEC \text{ (F. candida)}$$

$$LogEC50=1.9+0.79logCEC \text{ (E. fetida)}$$

$$LogEC50=1.1+0.87logCEC + 0.12\ pH \text{ (wheat)}$$

$$LogEC50=1.2+0.76logZn_{BG} \text{ (nitrification)}$$

$$LogEC50=1.7+0.76logZn_{BG} \text{ (respiration)}$$

Where

- EC50 is the 50% effect concentration
- CEC is cation exchange capacity
- Zn_{BG} is the zinc background concentration in soil.
- Further information on the mean slopes and their upper and lower values of the 95% confidence limits has been given earlier. Note that background concentrations refer to ambient concentrations, not natural background. The soils used in the experiments were sampled from agricultural areas (not all), i.e. the tests and the assessments are made on soils that may already contain zinc from diffuse sources.

The remaining uncertainties include the uncertainties

- on the average values taken for CEC and Zn_{BG} for some soil scenarios when calculating the average slope, while in reality individual soils have lower CEC and lower Zn_{BG} values and would result in lower bioavailability corrections
- on the zinc background concentration, since ambient zinc concentrations are used for the background and where it is unclear which part of this concentration is natural and which part is of anthropogenic nature
- on the fact that zinc background concentration is used as a modifier for bioavailability for zinc, which may mean that with increasing pollution more zinc is accepted in soil, may be due to adaptation or acclimation of the soil microbial populations, but which is an unwanted situation. Furthermore, Davis et al. (2003) did not show the gradual adaptation or acclimation to zinc in soil.
- on the regressions for microbial processes since they have poor predictability which means that even when the confidence intervals of the slopes are provided, there is remaining and non-quantifiable uncertainty (that may be inherent to soil microbial populations or may be due to yet unknown soil properties or to other yet undefined properties)

- on the representativity of the soil tests for the entire soil ecosystems. It was agreed that these two invertebrates, one plant, and two microbial processes were to be used and would represent the most important soil flora and fauna when starting the recent research program. However, the outcomes should be evaluated following the second question of that program, i.e. "Will the protection goals be met; i.e. to sufficiently protect the various types of species within a compartment, e.g. are relevant species of various feeding behaviour tested?" Since the regressions for the different species and processes are different, uncertainty remains on whether the various types of species and processes within the soil compartment will be sufficiently protected when using the equations for those five soil species and processes.

The 'slopes' of the models for EC50s are used to normalise the NOEC values in the database for differences in soil properties, i.e. CEC, pH and background Zn. These slopes are uncertain which means that the normalisation can both overestimate as well as underestimate the effect of soil properties on the HC5. In this section, HC5s are calculated from the database normalised to reference soil properties using the mean slopes and their upper and lower values of the 95% confidence limits. The slopes of the model are given in the Table 3.94 below. This approach uses the following steps:

- Sort the NOEC values of the existing database[32] in 4 different groups, i.e. plants, invertebrates (2 groups: worms and springtails; there are no other invertebrates), and microbial processes.
- Link these data with the soil properties (CEC, pH and background Zn) of the soils in which the test was performed. If CEC is unknown, it is estimated from % clay, % OM and pH[33]. If these soil properties are unknown, these data will not be used for normalisation. However, these data are still used for the generic HC5 estimation.
- The NOECs are normalised using the corresponding slopes (Table 3.94 provides the mean, lower and upper confidence intervals of the slopes) to 'reference' soil properties (the abiotic factors: CEC, background or pH), i.e. the abiotic factor of the soil for which the bioavailability corrections are calculated. The normalisation equation is

$$NOEC_{ref} = NOEC\left[\frac{abiotic\_factor_{ref}}{abiotic\_factor}\right]^{slope}$$

- The $NOEC_{ref}$ is the normalised NOEC value. The *Eisenia* slope was applied to all worm NOECs, the *Folsomia* slope to all *Folsomia* NOECs.
- The HC_5 of the normalised data are calculated with ETX (log logistic model; Aldenberg, 1993; Aldenberg & Slob, 1993) and the HC_5/HC_5generic is the $BioF_{soil}$. The HC_5generic is the HC_5 of not normalised data for which abiotic factors exist. There are two $BioF_{soil}$ values, one based on plants and invertebrates and the other on the microbial processes.

32 The NOEC values of the plant data of the recent research program were not included as agreed at TMIII 2003; all NOEC values of microbial functions and invertebrates were included. The *Eisenia* model was applied to all worms, the *Folsomia* model for all *Folsomia* NOECs.

33 CEC at soil pH is usually not reported but can be predicted from %clay, %OM and soil pH based on an existing multivariate model (CEC=(30.4+4.4*pH)*%clay/100+(-35+30*pH)*%OM/100; Helling et al., 1964) that is calibrated on natural soils; however, for OECD soils, it is assumed that the clay has no CEC contribution since this clay mineral has no permanent charge and it's variable charge is inferior to that of the organic matter which is at least 5% in these studies. This assumption yields CEC=17 cmol/kg at pH 7 whereas measurements of CEC of that soil yield 14.9 (Lock et al., 2000; Lock and Janssen, 2001a; Lock and Janssen, 2001b).

For illustration purposes, 'reference' soil properties are calculated for 3 soil types in Table 3.95. These soil properties and three soil types may reflect the range of soil properties encountered in Europe. This analysis shows that the uncertainty on the normalised HC5 values is large when applied to extreme soil conditions, but not at average soil conditions. This is logical considering that the large uncertainty band at the extremes of the conditions on which the regression models were based. The upper and lower limits of the HC5 values of the 3 soil groups do not overlap, illustrating that the HC5 differs significantly among soils.

Table 3.94 The slopes that are used to calculate the soil-property related availability factors and their uncertainty (lower and upper 95% confidence values).

Species	**Slope**		
	Lower 95% confidence value (smallest effect of soil properties)	Mean	Upper 95% confidence value (largest effect of soil properties)
Eisenia sp.	0.52	0.79	1.06
Folsomia candida	0.84	1.13	1.42
Plants	0.45 (CEC) 0.02 (pH)	0.87 (CEC) 0.12 (pH)	1.29 (CEC) 0.22 (pH)
Microbial processes	0.19	0.76	1.33

Table 3.95 The HC5 of normalised NOEC values for which the normalisation equations have included the uncertainties of the slopes.

	HC5 with smallest slope	**HC5 with mean slope**	**HC5 with largest slope**
Plants and invertebrates: generic HC5=49 mg/kg dwt			
Acid forest soil: *pH 3.7; CEC 5 cmol/kg*	38	19	8.5
Arable sandy soil *pH 6.0; CEC 16.5 cmol/kg*	73	83	82
Peat *pH 5.9; CEC 33 cmol/kg*	100	148	186
Soil microbial processes: generic HC5=26 mg/kg dwt			
Soil with background Zn 10 mg/kg	22	10	3.1
Soil with background Zn 50 mg/kg	30	36	26
Soil with background Zn 124 mg/kg	36	71	87

The mean and smallest slopes of the models are used to normalise the toxicity database to properties of 8 soils as used in the RAR using the procedure outlined before. The bioavailability factors ($BioF_{soil}=HC_5/HC_5$ generic) are shown in Tables 3.96, 3.97 and Figure 3.41 and seem to correlate between the approaches based on microbial processes and plants/invertebrates. This may be due to the fact that the soils with larger CEC (large clay content and OM content) tend to contain larger zinc concentrations. In the 15 soils collected for the recent research program, there was a positive correlation between these two

parameters (R^2=0.67, see Figure 3.40). The observation that availability factors are similar for both plants/invertebrates as for soil microbial processes facilitates the risk assessment because it suggests that the HC_5 tends to vary in a similar fashion among soil types.

Depending on the soil type, the slope with the lowest confidence interval does not always result in the most conservative bioavailability factor. In Figure 3.41 this is illustrated by the crossing of the various lines following the calculations for the different slopes. Therefore, not in all cases the most conservative value is taken.

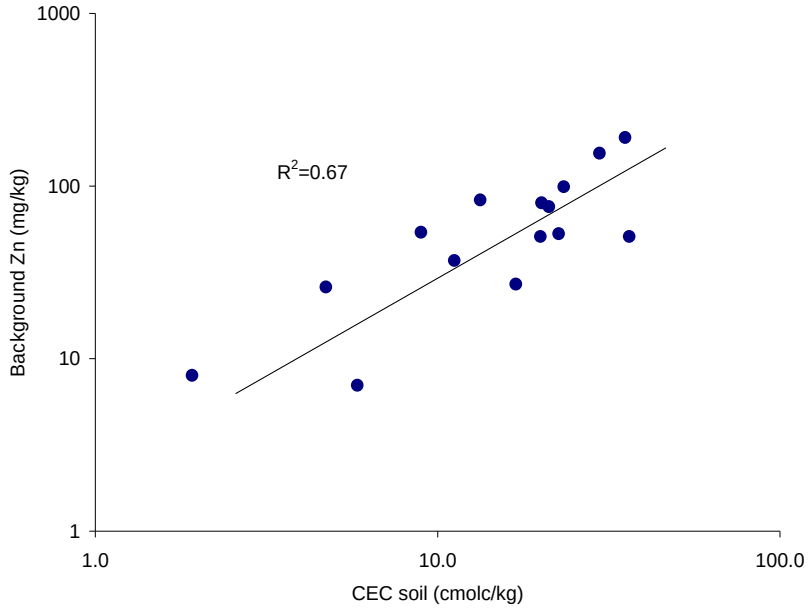


Figure 3.40 The relationship between CEC and background Zn for 15 uncontaminated soils (Davis et al., 2003; Lock et al., 2003; Smolders et al., 2003): $LogZn_{BG}$=-0.1+0.72logCEC (p<0.001)

Table 3.96 The bioavailability factors ($BioF_{soil}$) for 8 typical soils used in the RAR based on either soil microbial processes or on the plant and invertebrate dataset, using the lowest confidence intervals of slopes. The bold value represents the conservative approach.

Scenario	pH (water)	CEC at soil pH ($cmol_c/kg$)	Soil total Zn (mg/kg)	microbial processes		plants/invertebrates	
				HC5	$BioF_{soil}$ $	HC5	$BioF_{soil}$ $
Generic HC_5				26		49	
Generic HC_5: selected data for which abiotic factors exist				26		49	
Cattle farms, sandy soil (extensive) (1993)	5.83	10.97	28	27.0	1.1	59.8	1.2
Cattle farms, sandy soil (intensive) (1993)	5.94	6.98	32	27.7	1.1	47.9	1.0
Cattle farms, sandy soil (1994)	5.9	7.7	31	27.5	1.1	50.3	1.0
Forest, sandy soil (1994)	3.7	5.03	10.1	22.3	0.86	37.8	0.77
Arable farm, sandy soil (1995)	5.96	16.53	31	27.5	1.1	73.1	1.5
Cattle farm, peaty soil (1995)	5.93	33.04	124	35.8	1.4	100.1	2.0
Arable farms – marine clay soil (1996)	8.09	14.42	68	32.0	1.2	87.0	1.8
Cattle farms – river clay soils (1996)	6.59	28.88	172	38.1	1.5	96.3	2.0

$AF = HC_5/HC_5 generic

Table 3.97 The bioavailability factors ($BioF_{soil}$) for 8 typical soils used in the RAR based on either soil microbial processes or on the plant and invertebrate dataset, using the mean slopes. The bold value represents the conservative approach.

Scenario	pH (water)	CEC at soil pH ($cmol_c/kg$)	Soil total Zn (mg/kg)	microbial processes		plants/invertebrates	
				HC5	$BioF_{soil}$ $	HC5	$BioF_{soil}$ $
Generic HC_5				26		49	
Generic HC_5: selected data for which abiotic factors exist				26		49	
Cattle farms, sandy soil (extensive) (1993)	5.83	10.97	28	22.9	0.9	57.0	1.2
Cattle farms, sandy soil (intensive) (1993)	5.94	6.98	32	25.5	1.0	39.5	0.8
Cattle farms, sandy soil (1994)	5.9	7.7	31	24.8	1.0	42.7	0.9
Forest, sandy soil (1994)	3.7	5.03	10.1	10.6	0.4	19.0	0.4
Arable farm, sandy soil (1995)	5.96	16.53	31	24.8	1.0	83.0	1.7
Cattle farm, peaty soil (1995)	5.93	33.04	124	71.2	2.8	147.0	3.0
Arable farms – marine clay soil (1996)	8.09	14.42	68	45.2	1.7	108.2	2.2
Cattle farms – river clay soils (1996)	6.59	28.88	172	91.3	3.5	148.0	3.0

$ $BioF_{soil} = HC_5/HC_5$ generic

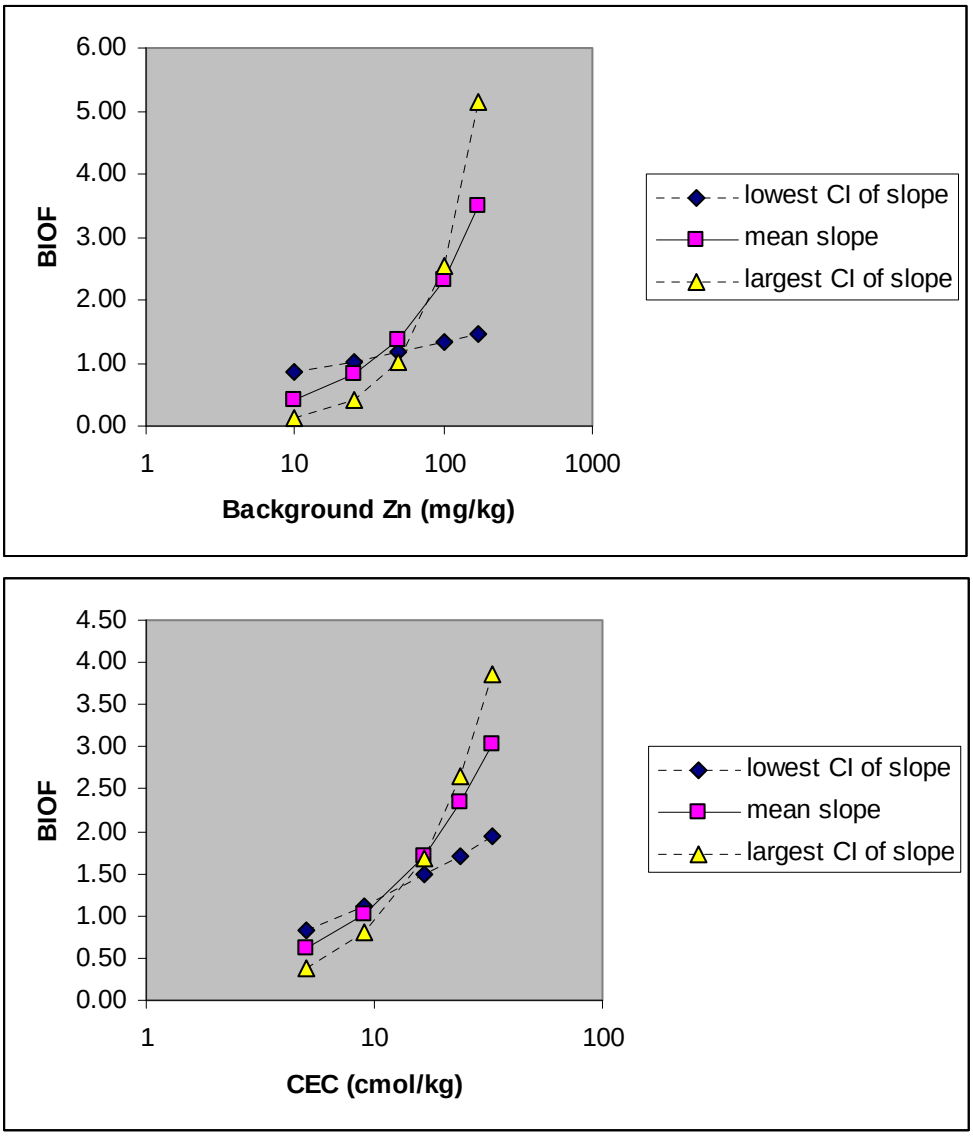


Figure 3.41 Availability factors (BIOF=$BioF_{soil}$) predicted from the mean and 95% confidence intervals (CI) of the slopes. Predictions for microbial processes (top) and plants and invertebrates (bottom). Ignoring bioavailability corrections effectively leads to a constant BIOF of 1.0 throughout.

Implementation of lab-to-field differences and soil-properties related bioavailability corrections to the PEC in the current risk assessment report

It is concluded that there is sufficient scientific basis to correct the PECs in soil for lab-to-field differences and for soil-type dependent factors. The following stepwise approach is taken for implementing the bioavailability correction of zinc in soil for those sites or regions that have a $PEC_{add}/PNEC_{add} > 1$, when no bioavailability correction would be applied (Figure 3.42). If the $PEC_{add}/PNEC_{add} < 1$, conclusion (ii) is reached. The bioavailability correction will be applied to the PEC_{add}, and not to the $PNEC_{add}$. One of the main reasons for correcting the PEC_{add} is that insufficient information is available for each individual organism from the ecotoxicity database.

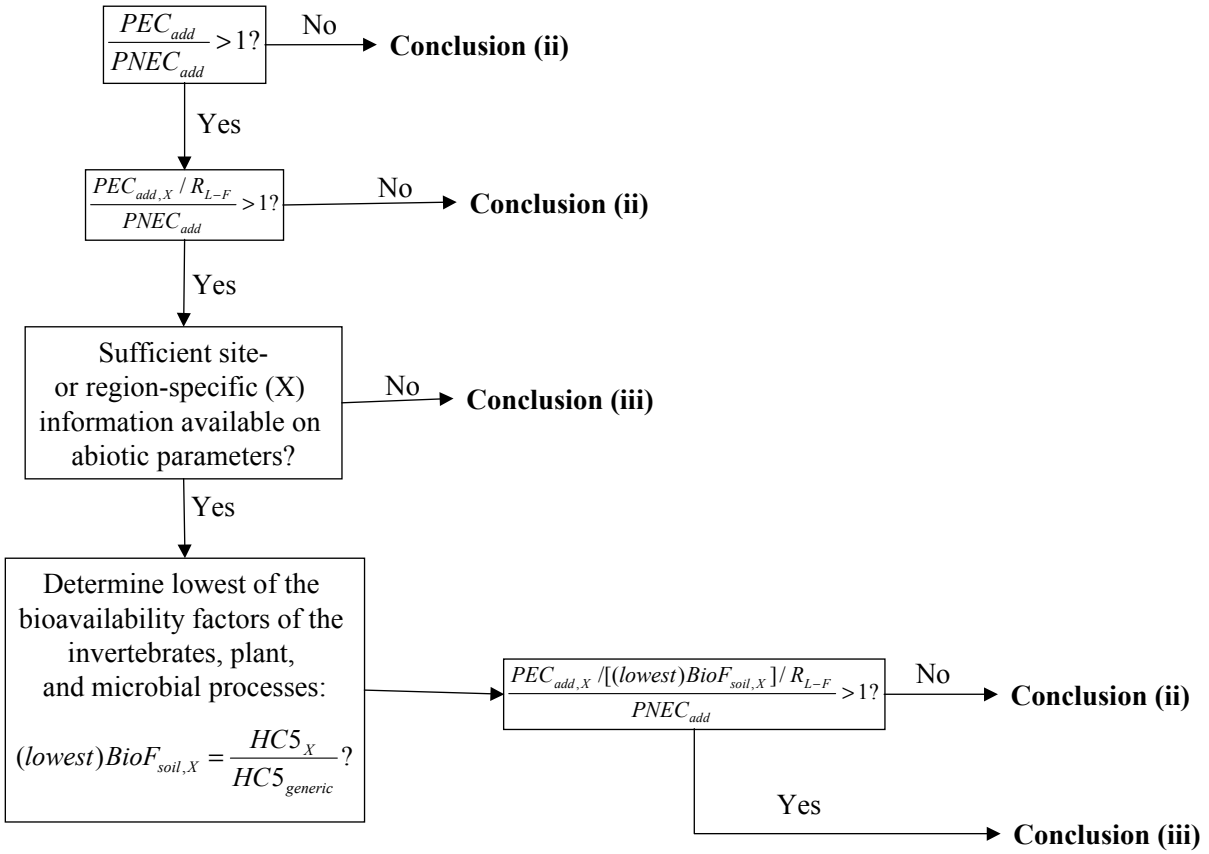


Figure 3.42 Decision tree for correcting the PEC_{add} for reduced bioavailability in soil using soil-type dependent correction factors ($BioF_{soil,X}$) and the lab-to-field correction (R_{L-F}).

In this approach a pragmatic decision has been followed to first start with the PEC correction for lab-to-field differences and then, where appropriate, the soil-type dependent correction factors. The main reason for this pragmatic approach is that the soil-type dependent correction is a laborious exercise since it requires recalculation of all NOECs in the database. Therefore, if the lab-to-field correction results in conclusion (ii), this laborious exercise does not need to be performed.

Firstly, the approach thus starts with the correction for the lab-to-field differences to the PECadd. Although the lab-to-field differences were derived from total concentrations, i.e. including the site-specific or region-specific background concentration (Cb), most of those total concentrations were much higher than the Cb. The contribution of the Cb is thus much less and it is then justified to apply the lab-to-field correction to the added zinc concentration, i.e. the PECadd. The PECadd should thus be divided by the generic lab-to-field ratio R_{L-F} , which is 3 for the generic soil if ageing has occurred for one year or longer. A ratio of 2 should only be used in cases where a rapid increase in zinc soil concentration could occur, e.g. due to the melting of snow, when ageing has occurred for less than one year. If data on the site-specific background concentration are not available, the generic background concentration should be used.

This lab-to-field correction is thus applied to the PECadd .

Subsequently, the risk ratio (RCR) for the lab-to-field corrected PECadd at the site or region X can be determined as follows:

$$RCR = \left(\frac{PEC_{add}}{PNEC_{add}} \right)_{lab-field-corrected,X} = \frac{(PEC_{add})/R_{L-F}}{PNEC_{add}}$$

There will be no risk (conclusion ii) if this ratio ≤ 1, and there will be a potential risk (conclusion iii) if this ratio > 1. In the latter case, the soil-properties corrected PEC should be determined to further evaluate the potential risk.

Example 1

A hypothetical soil has a PECadd = 60 mg/kg dwt.

The $PNEC_{add}$ = 26 mg/kg dwt.

The uncorrected risk ratio would be 2.3 [= 60/26].

The lab-to-field correction is to be applied to the added concentration, and this would lead to a lab-to-field corrected risk ratio of 0.77 [=(60/3)/26].

In this case, the risk ratio is < 1, conclusion (ii) would be drawn, and no further soil-type correction would have to take place.

If the soil-property is applied there must be sufficient information on the abiotic factors of the soil. If no sufficient site or region-specific information on the abiotic parameters is available, no bioavailability correction is possible, and conclusion (iii) will be reached. If there is sufficient information on the abiotic factors, the bioavailability factors ($BioF_{soil,X}$), as explained in the previous section and Tables 3.96 and 3.97 need to be determined for the invertebrate species, the plant, and the microbial processes. The PECadd then needs to be divided by the lowest $BioF_{soil,X}$. Since the regressions that are used have been derived for the PECadd, the resulting $BioF_{soil,X}$ should be applied to the PECadd, and not to the PECtotal.

Then, similar to the approach when the lab-to-field correction would be applied to the PECadd, this lab-to-field correction will be applied to the soil-type corrected PECadd to derive the RCR:

$$RCR = \left(\frac{PEC_{add}}{PNEC_{add}} \right)_{soil-typecorrected\&lab-to-fieldcorrected} = \frac{(PEC_{add}/[(lowest)BioF_{soil,X}])_X/R_{L-F}}{PNEC_{add}} > 1?$$

There will be no risk (conclusion ii) if this ratio ≤ 1, and there will be a potential risk (conclusion iii) if this ratio > 1. In the latter case, the soil-properties corrected PEC should be determined to further evaluate the potential risk.

Example 2

Two hypothetical soils, one forest, sandy soil and one river clay soil at a cattle farm, both have PECadd = 90 mg/kg dwt.

The $PNEC_{add}$ = 26 mg/kg dwt.

The uncorrected risk ratios for both soils would be 3.5 [= 90/26].

The lab-to-field correction is to be applied to the added concentration, and this would lead to a lab-to-field corrected risk ratio of 1.15 [=(90/3)/26].

In this case, the risk ratio is > 1 and the soil-type correction is to take place, provided sufficient information would be available on the soil type.

From table 3.96, the lowest BioF values are 0.77 and 1.5 for the forest, sandy soil and the river clay soil at a cattle farm, respectively.

The soil-type correction would thus lead to PEC_{add} (soil-type corrected) of 117 and 60 mg/kg dwt for the forest, sandy soil and the river clay soil at a cattle farm, respectively.

The further lab-to-field correction then leads to a risk ratio of 1.5 [=(117/3)/26] and 0.77 [=(60/3)/26] for the forest, sandy soil and the river clay soil at a cattle farm, respectively.

In this case, conclusion (iii) would be drawn for the forest, sandy soil, and conclusion (ii) would be drawn for the river clay soil at a cattle farm.

Although it may seem that the PNEC is divided out in the equations above, this is not the case, i.e. the toxicity data are not divided out. There are actually three HC_5 values included in the equation as shown below:

$$RCR = \frac{PEC_{add,X} \cdot HC_{5\,generic(plants,invertebrates)}}{\frac{HC_{5(plants,invertebrates)}}{2} \cdot R_{L-F} \cdot HC_{5(plants,invertebrates),X}}$$

- $HC_{5\ (plants,\ invertebrates)}$ in the equation is the HC_5 from the total dataset, with no normalisation. (it could be thought of as $HC_{5,\ generic,\ total}$)
- $HC_{5\ generic\ (plants,\ invertebrates)}$ is the HC_5 from the sub-set of data for which normalisation is possible, but using the data without normalisation.
- $HC_{5\ (plants,\ invertebrates),\ x}$ is the HC_5 from the sub-set of data, this time with normalisation.

In the situation where two of these are the same, then the third remains and so there will always be a value derived from the toxicity data present in the calculation.

In the approach shown in Figure 3.42 the first two of these values are very similar:

$$RCR \approx \frac{PEC_{add,X} \cdot}{\frac{1}{2} \cdot R_{L-F} \cdot HC_{5(plants,invertebrates),X}}$$

In the extreme or ideal case, all of the data could be normalised, in which case the first two HC_5 values would be identical and the equivalence above would be exact. This equation then shows that the PNEC for this specific case (soil type or location) is derived from the full data set normalised to the abiotic factors of the soil type or location - in effect a dataset specific to the soil type – with the assessment factor of 2. This is effectively a PNEC for the specific soil type or location.

Conclusions on abiotic factors

It is concluded that there is a scientific basis to correct the PECs for lab-to-field differences as well as for soil properties to take into account the bioavailability of zinc in soil.

To further take into account some uncertainty in various parameters as well as to provide some ideas on the sensitivity of the calculations, three scenarios will be used in the risk characterisation when showing the bioavailability corrections:

- the first scenario will be when no bioavailability correction will be used, i.e. the PECadd will be completely based on the added zinc concentration;

- the second scenario will make use of the lab-to-field correction and the soil properties correction in a conservative way, i.e. by selecting the 90th-percentile value of the added zinc concentration in the soil, the smallest slope of the regressions and the 10th-percentile values of all other abiotic parameters; and
- the third scenario will make use of the lab-to-field correction and the soil properties correction in a conservative way, i.e. by selecting the 90th-percentile value of the added zinc concentration in the soil, the mean slope of the regressions and the 50th-percentile values of all other abiotic parameters.

3.3.3.1.2 Toxicity to soil microbe-mediated processes

Data on microbial toxicity tests are summarised in Table 3.3.3.a (Annex 3.3.3.A). The data include major microbe-mediated processes, i.e. C-mineralization (respiration, usually measured by CO_2 production), including C-mineralization of specific substrates, N-mineralization (measured by ammonification and/or nitrification), and enzyme activities. These tests are multi-species tests, in which the native soil microbial community is exposed and in which a functional parameter such as soil respiration is measured. These tests thus do not indicate which microbial species or taxa are affected.

The test compounds were usually (hydrated) zinc sulphate or zinc chloride, which have a high water solubility. Occasionally "insoluble" zinc oxide or zinc carbonate were used. For general information on microbial toxicity tests, especially on tests for microbe-mediated processes, and the ecotoxicological relevance thereof, see Van Beelen & Doelman (1997).

The exposure times of the microbial tests ranged from some hours to 1.5 years. In many tests for enzyme activities, the activity is measured within hours after the addition of zinc to the soil (e.g., the studies by Tabatabai and co-workers), since differences in microbial activities between control and exposed soils can be measured within a very short time. In tests that measure the mineralization of a specific substrate after the addition of a minute amount of a pure organic substrate to the soil (e.g. the acetate mineralization tests by Van Beelen et al., 1994b), the exposure time can also be limited to a short time (days). On the other hand, mineralization processes in soils amended with organic substrates such as sludge or alfalfa (e.g., the respiration study by Chang & Broadbent, 1981) and especially in unamended soils are much slower and thus a longer exposure time is needed. Thus, in these microbial tests there is no clear difference between short- and long-term tests (as in the single-species tests) and exposure time is not used for data selection, with the exception of the rejection of tests performed in aged soil (general criterion, see section 3.3.3.1). In the Doelman & Haanstra studies, both the 6-w EC10 vlaues and 1.5-yr EC10 values were reported for the enzyme activities (arylsulphatase, phosphatase and urease) measured in the different test soils. Based on the above criterion, only the 6-w results were used for PNEC derivation, whether the 6-w EC10 was lower than the 1.5-yr EC10 or not. This selection is in accordance with the selection made by Van Beelen & Doelman (1997). It should be noted, however, that in the Doelman & Haanstra studies no consistent relationship between exposure time and EC10 was found, neither between exposure time and EC50.

The test results in Table 3.3.3.a (Annex 3.3.3.A) include NOEC values and effect concentrations based on inhibition of the process studied. In a relatively large number of microbial toxicity studies, a NOEC could not be derived directly from the data reported, because often all test concentrations resulted in an effect, due to the limited number of (relatively high) test concentrations[34]. In those cases, NOEC values have been estimated by

34 In many microbial tests, a maximum of three concentrations was tested, differing by a factor of 10.

the rapporteur (see section 3.3.1 for the general procedure and Table 3.3.3.a for study-specific data).

The test results are based on the reported nominal zinc concentration (Cn) in soil, but if possible the test results were expressed both as the nominal zinc concentration and the total zinc concentration in soil, the latter derived by adding the nominal concentration to the background concentration that was measured in the control test soil. Actual measurements in soil after the addition of the test compound were only reported in a few studies.

The nominal NOEC values (Cn) for microbe-mediated processes, based on studies that were used for PNEC derivation, range from 17 to 2623 mg/kg dry weight (d.w.), see Table 3.98 and Figure 3.3.3.1.1 (based on the underlined values in Part I of Table 3.3.3.a in Annex 3.3.3A). These values are all from tests with soluble zinc salts and include a relatively large number of estimated NOEC values, including the "alternative" NOEC values as explained in section 3.3.3.1. The lowest NOEC (17 mg/kg d.w.) was derived from C-mineralization (respiration) tests in two different soils (Chang & Broadbent, 1981; Lighthart et al., 1983) and the highest NOEC (2623 mg/kg d.w) was derived from a test for phoshatase activity (Doelman & Haanstra, 1989). When comparing the data for the different major processes, the lowest NOEC values are similar: 17 mg/kg d.w. for C-mineralization (including C-mineralization of specific substrates), 38 mg/kg d.w. for N-mineralization and 30 mg/kg d.w. for enzyme activities. The highest NOEC values for these processes are also similar: 1400, 1000 and 2623 mg/kg d.w., respectively. Based on this, it is assumed that there is no significant difference in sensitivy between the different microbe-mediated processes.

The wide range of NOEC values and effect concentrations, even for the same endpoint such as soil respiration, can amongst others be ascribed to differences in soil characteristics and composition of the microbial communities. Different soils have different microbial communities, the species diversity depending on the soil characteristics. Microbe-mediated soil processes such as respiration are performed by different microbial species, all contributing to the soil process. Under toxic stress, sensitive species performing a specific process can disappear, but due to a shift to less sensitive species the process may continue, thus obscuring toxicity (Van Beelen and Doelman, 1997).

Based on the criteria used in this report, a number of studies listed in Table 3.3.3.a (Annex 3.3.3.A) are considered not used for NOEC derivation and a number of studies for which a NOEC could be derived were not used for PNEC derivation (see section 3.3.1 and section 3.3.3.1 for data selection); these studies are listed in Part II of Table 3.3.3.a (Annex 3.3.3.A).

3.3.3.1.3 Toxicity to terrestrial invertebrates and plants

Data on chronic single-species toxicity tests resulting in NOEC values for soil invertebrates and plants are summarised in Table 3.3.3.b and Table 3.3.3.d, respectively (Annex 3.3.3.A). In a number of the invertebrate studies, EC50 and/or LC50 values were also reported; these and additional EC50 and LC50 values are listed in Table 3.3.3.c (Annex 3.3.3.A).

The test compounds were usually (hydrated) zinc sulphate, zinc chloride, zinc nitrate or other zinc salts such as zinc acetate, which have a high water solubility. In the tests with soil invertebrates, occasionally "insoluble" zinc oxide or zinc carbonate were used.

Almost all test results for invertebrates and all test results for plants are based on the reported nominal zinc concentration (Cn) in soil, but if possible the test results were expressed both as the nominal zinc concentration and the total zinc concentration in soil, the latter derived by adding the nominal concentration to the background concentration that was measured in the

test soil. Actual measurements in soil after the addition of the test compound were only reported in a few studies.

NOEC values for invertebrates

The nominal NOEC values (Cn) for invertebrate species, based on studies that were used for PNEC derivation, range from 32 mg/kg d.w. for the insect *Folsomia candida* to 1000 mg/kg d.w. for both *F. candida* and the earthworm *Eisenia fetida,* see Table 3.99 and Figure 3.28 (based on the underlined values in Part I of Table 3.3.3.b in Annex 3.3.3.A). These values (of which both the lowest and highest NOEC value were from the study by Lock et al, 2003) are based on tests with soluble test compounds. The "species mean" NOEC values calculated from these data range from 280 mg/kg d.w. for the earthworm *E. fetida* (based on 25 NOEC values for this species) to 600 mg/kg d.w. for the earthworm *Aporrectodea caliginosa* (being the only NOEC value for this species), see Table 3.3.3.b-Part I (Annex 3.3.3.A).

It is noted that although there is a relatively large number of recent, high quality studies, the useful invertebrate data are limited to only four different species (three earthworm and one insect species). Based on the criteria used in this report, a number of the studies listed in Table 3.3.3.b are considered not useful for PNEC derivation (see section 3.3.1 and section 3.3.3.1 for data selection); these studies are listed in Part II of Table 3.3.3.b (Annex 3.3.3.A).

NOEC values for plants

The nominal NOEC values (Cn) for plant species, based on studies that were used for PNEC derivation, range from 32 mg/kg d.w. for two species (*Trifolium pratense* (red clover) and *Vicia* sativa (vetch)) to 400 mg/kg d.w. for four species (*Lactuca sativa* (lettuce)*, Avena sativa* (oat)*, Pisum sativum* (Alaska pea) and *Lycopersicon esculentum* (tomato)), see Table 3.99 and Figure 3.28 (based on the underlined values in Part I in Table 3.3.3.d in Annex 3.3.3.A). These values are based on tests with soluble test compounds. The "species mean" NOEC values calculated from these data also range from 32 to 400 mg/kg d.w., with the lowest value for *V. sativa* and the highest value for *L. sativa, P.sativum* and *L. esculentum.* It is noted that for each of these four species only one NOEC value is available.

Based on the criteria used in this report, a number of the studies listed in Table 3.3.3.d are considered not useful for PNEC derivation (see section 3.3.1 and section 3.3.3.1 for data selection). It is noted that a large number of tests were rejected because they resulted in an unbounded NOEC; this concerns especially the study by McLean (1974) in which 250 mg/kg d.w. was the highest test concentration. Some other tests from this study, resulting in a low NOEC (below 100 mg/kg d.w.) were also rejected based on quality criteria: these values were derived from tests in which a (too) high separation was used, thus the reliability of these values is low. With respect to the latter tests, no alternative NOEC could be derived. The rejected studies are listed in Part II of Table 3.3.3.b (Annex 3.3.3.A).

Comparison of sensitivity of invertebrates and plants

The total range of selected NOEC values for invertebrates (32 to 1000 mg/kg d.w.) and plants (32 to 400 mg/kg d.w.) and the underlying data suggest that (some) plant species may be more sensitive than invertebrate species. However, the data also show that for both invertebrates and plants the toxicity of zinc may strongly vary with the soil type and soil characteristics. For example, for the insect *Folsomia candida* the NOEC values based on tests in different soils range from 32 to 1000 mg/kg d.w. (Lock et al., 2003 and other studies; all values selected for $PNEC_{add}$ derivation), for the plant *Lactuca sativa* (lettuce) from 10 mg/kg d.w. to $\geq$ 250 mg/kg d.w. (McLean, 1974; all tests rejected) and for the plant *Avena sativa* (oat) from100 mg/kg d.w. to $\geq$ 800 mg/kg d.w. (De Haan et al., 1985; unbounded NOEC values rejected), thus obscuring real differences in sensitivy between species or between the two groups of species (invertebrates *versus* plants). Furthermore, the invertebrate database is limited to only four different species (three earthworm and one insect species). Based on this, it is assumed that there is no significant difference in sensitivy between invertebrates and plants.

3.3.3.1.4 Field studies and laboratory to field extrapolation

This section summarises the results of field studies on zinc that have been carried out in the framework of a large Dutch research project entitled "Validation of toxicity data and risk limits for soils" (Posthuma et al., 1998, which is the final summarising report of the whole project). Results of this project have been summarised earlier in section 3.3.3.1.1 and, together with the results of laboratory studies in which the toxicity of Zn in field-contaminated soils was compared with that in the corresponding Zn-spiked soils (Smolders et al., 2003; Lock et al., 2003), used to derived lab-to-field ratios (see section 3.3.3.1.1).

In the Dutch "validation" project, the toxicity of zinc to different terrestrial species (invertebrates, plants and micro-organisms) was studied in both laboratory and field tests to study the (ecological) relevance of both laboratory toxicity data and generic risk limits derived from these data. Among the environmental risk limits for zinc that were evaluated in this project is the median 5th percentile (equivalent to the $PNEC_{add,\ terrestrial}$ as derived in this report, see section 3.3.3.2), derived with statistical extrapolation using a log-logistic frequency distribution according to Aldenberg and Slob (1993). The 5th percentile value leading to a generic environmental risk limit is derived by Crommentuijn et al. (1997) and is based on "species" and "processes" data. The generic risk limit was compared with the results of the field data.

Three types of studies were performed in the project:

- Laboratory tests in which standard test organisms (earthworms, potworms, springtails, plants and micro-organisms) were exposed to soils from a field gradient in the vicinity of a former zinc smelter in Budel (The Netherlands) and to different kinds of experimentally contaminated soils under controlled conditions.
- Outdoor experiments in which standard test organisms (earthworms, potworms, springtails and plants) were exposed to zinc in an experimentally contaminated field plot. The soil used in the field plot site was collected near Heel (Limburg, NL) and indicated as PANH soil.
- Observations on indigenous communities of micro-organisms, nematodes and enchytraeids (potworms) in the field gradient and in the experimentally contaminated field plot.

Results from the studies were included in the respective tables (Annex 3.3.3.A) and used for PNEC derivation in the present RAR, when the studies met the criteria. Additional results of the project on ageing related effects are briefly presented below.

- Average EC10 values for the effect of zinc on cocoon production of *E. andrei* were 306, 781, 642, and 930 mg/kg in 1995, 1995, 1996, and 1996, respectively. Average EC50 values for the effect of zinc on cocoon production of *E. andrei* were 1200, 1320, 1597, and 1676 mg/kg in 1995, 1995, 1996, and 1996, respectively. In all cases pH varied between 6.4 and 7.3. Thus, where EC10 values increased during time, EC50 values remained relatively constant.
- Average NOEC values for the effect of zinc on juvenile production of *F. candida* were 879, 889, and 1367 mg/kg in 1994, 1995, and 1996, respectively. Average EC50 values for the effect of zinc on juvenile production of *F. candida* were 940, 1491, and 1749 mg/kg in 1994, 1995, and 1996, respectively. The pH in the 1994 study was between 5.6-6.0, while the pH in the later studies was 6.4-7.2.
- Average NOEC values for the effect of zinc on seed germination of *T. pratense* were 705, >829, and >885 mg/kg in 1994, 1995, and 1996, respectively. Average NOEC values for the effect of zinc on shoot growth of *T. pratense* were 71, 320, and 125 mg/kg in 1994, 1995, and 1996, respectively. Average EC50 values for the effect of zinc on shoot growth of *T. pratense* were 117, 340-546, and 526 mg/kg in 1994, 1995, and 1996, respectively. The pH in the 1994 study was between 5.6-6.0, while the pH in the later studies was 6.4-7.2.
- Average NOEC values for the effect of zinc on the number of nematodes were 495, 829, and 1367 mg/kg in 1994, 1995, and 1996, respectively. Average NOEC values for the effect of zinc on the number of nematodes' taxa were 295, 190, and 316 mg/kg in 1994, 1995, and 1996, respectively. Average NOEC values for the effect of zinc on the species diversity of nematodes were 495, 190, and 316 mg/kg in 1994, 1995, and 1996, respectively. Average NOEC values for the effect of zinc on the nematodes community Principal Response Curves were 75, 115, and 125 mg/kg in 1994, 1995, and 1996, respectively. The pH in the 1994 study was between 5.6-6.0, while the pH in the later studies was 6.4-7.2.

The main conclusions of the project are the following (largely based on Posthuma et al., 1998):

- Differences in sensitivity between laboratory and field vary and depend on the species. There is no reason to assume that laboratory species are consistently and decisively more sensitive or insensitive than species in the field, so that it may be assumed that the results of standardised laboratory tests can be used in this respect for the derivation of generic risk limits. However, for long-term risk assessment, it is recommended to improve the design of tests with respect to ecologically relevant parameters such as population development.
- Only for two species, the invertebrate *Folsomia candida* and the plant *Trifolium pratense*, the effect of ageing seemed to take place. This was shown by comparing toxic effects in the laboratory studies between freshly contaminated experimental field plot soil (PANH), and soil taken from the plot after ageing under outdoor conditions. The EC50 values in the aged soil were about 10 times higher than those in freshly contaminated soil. For *F. candida,* this finding seems to be confirmed by the results of laboratory tests in freshly contaminated Budel reference soil and aged Budel soil. The Budel soil was aged in the laboratory, after experimental treatment. For *T. pratense* sensitivity between freshly contaminated Budel reference soil and aged Budel soil differed only by a factor of 2. The

microbial data (glutamate mineralization) tested in freshly contaminated and aged PANH soil only showed a difference of a factor of 1.5 (based on both EC50 and NOEC values). The field tests with *F. candida* and *T. pratense* in the experimental PANH plot also showed an increase in EC50 values with time. It is noted that the "ageing" effect in PANH soil is not purely caused by an ageing process, but also by initial leaching of zinc which occurred shortly after the addition of the $ZnCl_2$ solutions to the soil, as indicated by zinc measurements at different time intervals. Furthermore, the pH of the experimental plot increased approximately one to two units within 6 months after the zinc treatment, which also may have contributed to the differences in zinc toxicity between freshly contaminated and aged PANH soil (Van Riemsdijk, 2001). Based on all data it is concluded that ageing may play a role in the decrease of zinc toxicity with time, but that other factors (leaching, pH and species differences) also are involved.

- Several endpoints, however, did not show an effect of ageing, e.g. the effect of zinc on the number of nematodes' taxa and on the species diversity of nematodes.
- Based on the laboratory studies with the invertebrates *F. candida, E. andrei, E. Crypticus,* the plant *T. pratense,* and bacterium *Pseudomonas putida,* five (geometric species mean) NOEC values were available from tests in freshly contaminated PANH soil and aged PANH soil. For the freshly contaminated soil, this resulted in a median 5^{th} percentile value of 36 mg/kg d.w. For the PANH-aged soil this resulted in a median 5^{th} percentile value of 224 mg/kg d.w. These 5^{th} percentile values are based on the soil characteristics of PANH and PANH-aged soil and recalculated from the values reported by Posthuma et al., 1998 for standard soil, containing 25% clay and 10% OM. The recalculation is based on 2% clay and 2% OM in both the PANH and PANH-aged soil. For microbe-mediated processes no project-specific values could be calculated since the minimum required number of NOEC values were not available for this soil. The median 5^{th} percentile value that was derived in the Netherlands' is 7 mg/kg d.w., based on data for microbe-mediated processes. The Dutch value based on species is higher, i.e. 57 mg/kg d.w.. These latter values are also based on the soil characteristics of PANH and PANH-aged soil. All these values are for the "maximum permissible addition", i.e., do not include the background zinc concentration in soil and thus equivalent to the $PNEC_{add,\ terrestrial}$ values derived in section 3.3.3.2.
- Field observations on indigenous communities of microorganisms and nematodes in the experimental field plot showed that no significant differences from the reference situation were observed at the 5^{th} percentile values. The functioning of the microbial community and species diversity of nematodes, however, were affected at concentrations exceeding the 50^{th} percentile values. The 50^{th} percentile values could be obtained from a similar probability density plot as e.g. shown in Figures 3.3.3.1.1 and 3.3.3.1.1. Field observations in the polluted Budel gradient, that include zinc concentrations far above the 50^{th} percentile value, showed no clear relationship between zinc concentrations and the abundance and species diversity of nematodes and enchytraeids. This can be explained by the fact that the Budel gradient is rather heterogeneous, with large variations in abiotic factors such as organic matter content and moisture content that contribute significantly to the observed biotic variation. In the experimental plot the variation is much lower since this soil was homogenised.
- With respect to the microbial "Pollution Induced Community Tolerance (PICT)" it was found that, after a 100 years exposure history, zinc tolerance along the Budel gradient increased with a factor of 100. Average EC50 values of approximately 10 and 1000 mg Zn/l, experimental exposure concentrations, for the reference Budel soil and the most polluted Budel soil, respectively, were found. In the experimental field plot zinc tolerance increased by a factor of 4 only. An increased zinc tolerance is indicative for effects in the

microbial community. At the Budel sites PICT occurred at soil concentrations above 35 mg/kg d.w., whereas it occurred above 206 mg/kg d.w. at the field test site (Rutgers & Breure, 1999). The acetate mineralization method showed PICT above 124 mg/kg d,.w. The results further showed that 27 to 40% of the microbial community was inhibited at 334 mg/kg at the field test site (Van Beelen et al., 2001).

- Based on all data for zinc it was concluded that no or only minor effects were observed at the 5th percentile value for zinc, while considerable effects were found near and beyond the 50th percentile value. Thus, the median 5th percentile value is generally in good agreement with actual no-effect levels in the field.

3.3.3.1.5 Predicted no effect concentration for the terrestrial compartment (PNECadd, terrestrial)

Both the tests on terrestrial species (plants and invertebrates) as well as the tests on microbe-mediated processes can be used to derive the PNEC for the terrestrial compartment. It is proposed to treat them separately in this calculation process, for the following reasons. Tests on microbe-mediated processes usually pertain to multiple-species tests, whereas the statistical extrapolation method in its original form pertains to single-species test results only.

Separate use of the single-species data and the multiple-species microbial data is made because of the possible differences in sensitivity between species-specific parameters such as growth and reproduction (that are measured in single-species toxicity tests such as the invertebrate and plant tests) and functional parameters such as soil respiration (that are measured in multiple-species microbial toxicity tests). The multiple-species microbial tests focus on functions of the indigenous communities in substrates (soil or litter) from various origins rather than at sensitivities of species. Each multiple-species (function) test can be considered to yield a result as if it were a single-species test, namely they yield a single NOEC for each test. Each tested community is unique, like each species in the structure-based approach. So, a range of such tests yields a range of sensitivities of communities, especially regarding functions, that can be treated in statistical extrapolation methods to obtain a PNEC, that protects against functional loss across a range of ecosystems. Although not original, this concept is theoretically fully in line with the very basis of the extrapolation method, namely that the collection of tested sensitivities can be statistically treated as representative for a whole system, either structurally or functionally.

It is noted that the above distinction between the two datasets of NOEC values is not necessary in case assessment factors are used to derive the PNEC, because in that case only the lowest NOEC is used, regardless of the kind of test ("processes" versus "species").

$PNEC_{add}$ values for soil were derived from the ecotoxicological data, using the two different extrapolation methods described in section 3.3.1.3 i.e. the use of assessment factors and statistical extrapolation, with several calculations for the latter method, using different frequency distribution functions.

With respect to abiotic characteristics it is noted that soil is less homogeneous than surface water. Based on this and because of the wide range of NOEC values that have been found for microbe-mediated processes tested in different soils and to a lesser extent for species tested in different soils, the use of geometric mean NOEC values for either microbe-mediated processes or species (as used for aquatic species when deriving the $PNEC_{add,\ aquatic}$) is considered less appropriate. Thus, preference is given to the use of the individual NOEC values from the different tests. With respect to the species data, the results of the calculations based on the "species mean" NOEC values will be given as well, for comparison. With

respect to the data for microbe-mediated processes an additional argument not to use a mean value for a specific process is that different soils have different microbial communities (see also section 3.3.3.1.2).

In case several microbe-mediated processes were studied in one soil (see the studies by Doelman and Haanstra and those by Tabatabai and co-workers), the question arises whether all data should be used for PNEC derivation. Thus, should one NOEC for each endpoint studied, resulting in several NOEC values for a specific soil, be used, or should only the lowest value be used. In section 3.3.3.1 it is stated that effects on soil processes may be more relevant than effects on single microbial species or on soil microbial species diversity, which is an argument to use only the lowest value for each soil. On the other hand, different processes reflect the action of different microbial species or communities, which is an argument to use all NOEC values. Moreover (probably related to the aforementioned) the available data do not indicate that a specific process or some processes are more sensitive than others, see for example the studies by Doelman and Haanstra. Finally, when different species have been tested in a specific soil, also one NOEC for each species is used for PNEC derivation. Based on this it was decided to use all data, i.e. one NOEC for each process studied in a specific soil.

The NOEC values for microbe-mediated processes (Table 3.3.3.a-Part I in Annex 3.3.3.A: underlined values; n = 97), and the combined NOEC values (n = 74) for invertebrates (Table 3.3.3.b-Part I in Annex 3.3.3.A: underlined values; n = 45) and plants (Table 3.3.3.d-Part I in Annex 3.3.3.A: underlined values; n = 29) were used in the calculations using statistical extrapolation (Tables 3.98 and 3.99). For species, calculations were also made on the basis of the combined "species mean" values (n = 20, of which 4 values for invertebrates and 16 for plants, Table 3.99).

Table 3.98 NOEC values for soil microbial processes that are used as input values for deriving the 5th percentile values as a basis for the soil $PNEC_{add,\ terrestrial}$.

Microbe-mediated processes	NOEC values (Cn, in mg/kg d.w.) (n=97)
C-mineralization (respiration), including mineralization of specific substrates * (n=39)	17; 17; 30; 30; 38; 50; 50; 50; 55; 80; 100; 100; 100; 100; 100; 100; 100; 110; 110; 120; 150; 150; 165; 200; 240; 300; 300; 300; 303; 327; 400; 469; 600; 600; 800; 1300; 1300; 1400; 1400
N-mineralization (n=26)	38; 50; 50; 50; 75; 75; 100; 100; 100; 100; 100; 109; 150; 150; 164; 164; 164; 164; 206; 233; 257; 300; 300; 400; 424; 1000
Enzyme activities (n=32)	30; 30; 48; 52; 64; 67; 70; 76; 105; 109; 140; 145; 151; 160; 164; 164; 164; 200; 200; 460; 500; 508; 590; 728; 820; 820; 1341; 1640; 1640; 1640; 2353; 2623

* C-Mineralization of specific substrates (e.g. acetate or plant residu): also referred to as "substrate induced respiration" (SIR).

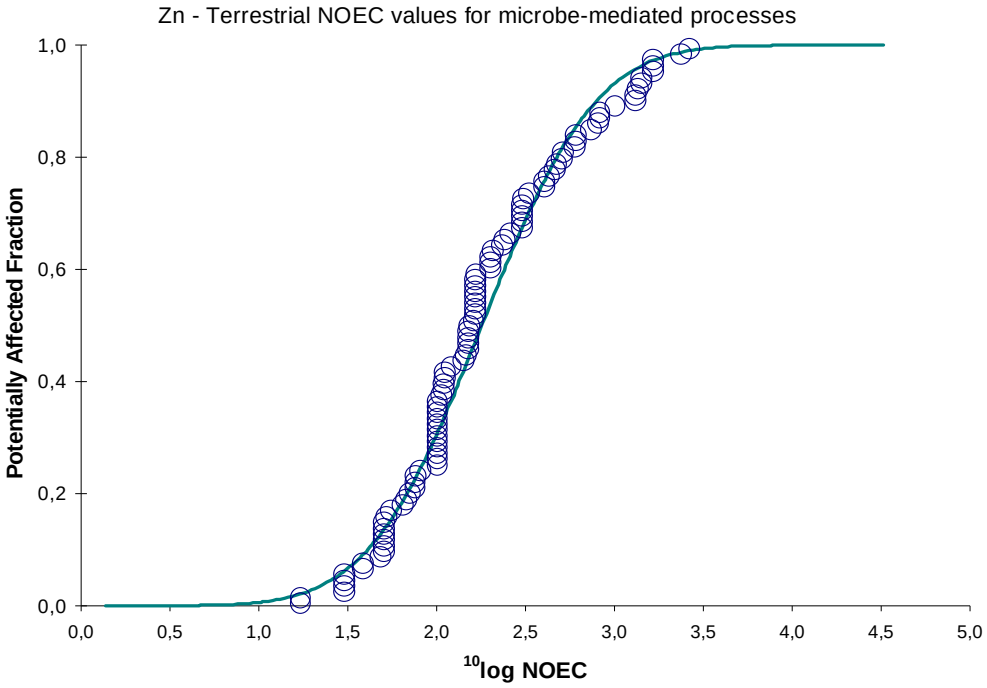


Figure 3.43 Microbe-mediated processes: sensitivity distribution based on NOEC values.

Table 3.99 Individual and "species mean" NOEC values for plants and invertebrates that are used as input values for deriving the 5th percentile values as a basis for the soil $PNEC_{add,\ terrestrial}$.

Taxonomic groups	**Individual NOECs (Cn, in mg/kg d.w.) (n=74)**	**"Species mean" NOECs (Cn, in mg/kg d.w.) (n=20)**
Oligochaetes * (n=27)	85; 97; 100; 115; 161; 180; 180; 180; 183; 199; 223; 237; 320; 320; 320; 350; 350; 350; 414; 484; 553; 560; 560; 560; 600; 1000; 1000	280; 320; 600
Insects ** (n=18)	32; 100; 275; 300; 300; 300; 300; 314; 320; 320; 320; 320; 366; 399; 560; 620; 1000; 1000	320
Plants *** (n=29)	32; 32; 32; 32; 32; 33; 83; 84; 100; 100; 100; 100; 100; 200; 200; 200; 200; 200; 200; 200; 200; 215; 300; 300; 300; 400; 400; 400; 400	32; 45; 89; 100; 140; 170; 200; 200; 200; 200; 200; 300; 300; 400; 400; 400

* 3 species of Oligochaetes; ** 1 insect species; *** 16 plant species

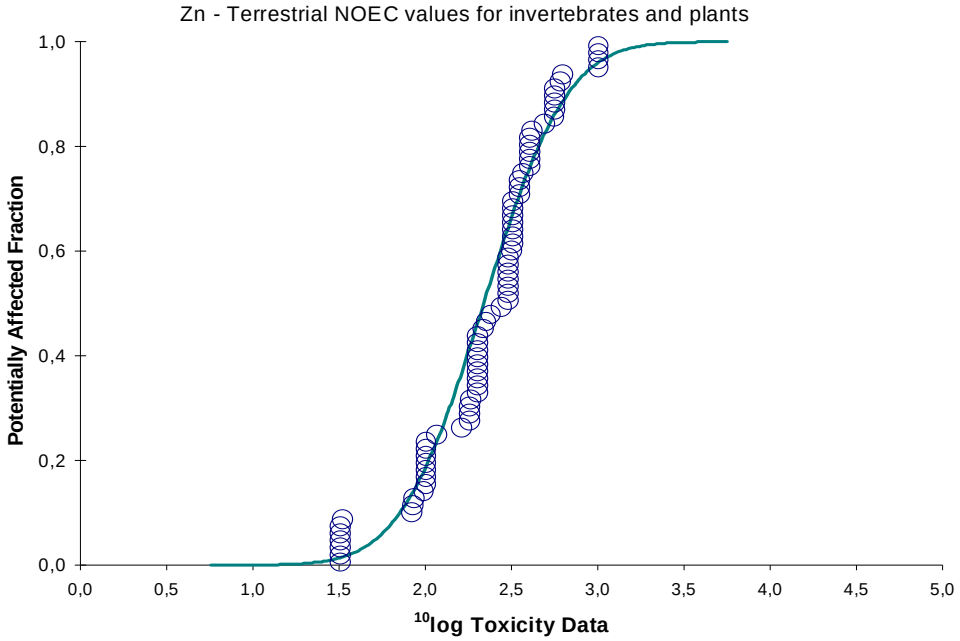


Figure 3.44 Invertebrates and plants: Species sensitivity distribution based on chronic NOEC values.

The results of the different calculations are shown in Table 3.98 (microbe-mediated processes) and Table 3.99 (plants and invertebrates), and footnotes. The use of an assessment factor of 10 according to the TGD results in a $PNEC_{add,\ terrestrial}$ of 1.7 mg/kg d.w. based on the lowest NOEC for microbe-mediated processes and 3.2 mg/kg d.w. based on the lowest NOEC for species. The use of statistical extrapolation results in median 5th percentile values (and "equivalent" values, see footnotes) ranging from 27 to 38 mg/kg d.w. and 31 to 52 mg/kg d.w., based on the individual NOEC values for microbe-mediated processes and species, respectively.

Table 3.100 Lowest NOEC and 5th percentile values of microbe-mediated processes for $PNEC_{add,\ terrestrial}$ derivation. All values in mg/kg d.w.

	Lowest NOEC	**(Lowest NOEC)/10**	**5th percentile log-normal [1]**	**5th percentile log-logistic [1]**
Microbe-mediated processes (n=97)	17	1.7	27 (median) *19 (lower 95% CI)* *35 (higher 95% CI)*	27 (median) *19 (lower 95% CI)*

[1]: Using either the Anderson-Darling Goodness-of-Fit test for normality (modified A^2) or the Kolmogorov-Smirnov test, a log-normal distribution is rejected at a significance level of 1%, indicating that the probability that these data derive from a log-normal distribution is very small (<1%). Using the Kolmogorov-Smirnov test, a log-logistic distribution is also rejected at a significance level of 1%.

Results non-parametric 5th percentile value estimate: Referring to the overall and enlarged probability plots, we can interpolate at the cumulative density of 0.05 to find non-parametric estimates. For the NOEC values for microbe-mediated processes (97 data points), the cumulative density of 0.05 is very near the 5th data point, which is 30 mg/kg d.w. Linear interpolation for log-concentrations also yields a 5th percentile value of 30 mg/kg d.w.

Results triangular distribution (included in ETX 1.3a): "Chronic value" is 38 mg/kg d.w.

Table 3.101 Lowest NOEC and 5th percentile values of plants and invertebrates for $PNEC_{add, terrestrial}$ derivation. All values in mg/kg d.w.

	Lowest NOEC	**(Lowest NOEC)/10**	**5th percentile log-normal**	**5th percentile log-logistic**
Invertebra-tes and plants (n=74, all individual values) [1]	32	3.2	52 (median) *39 (lower 95% CI)* *65 (higher 95% CI)*	52 (median) *37 (lower 95% CI)*
Invertebra-tes and plants (n=20, geometric mean values) [2]	32	3.2	58 (median) *34 (lower 95% CI)* *84 (higher 95% CI)*	57 (median) *32 (lower 95% CI)*

[1]: Using either the Anderson-Darling Goodness-of-Fit test for normality (modified A^2) or the Kolmogorov-Smirnov test, a log-normal distribution is rejected at a significance level of 1%, indicating that the probability that these data derive from a normal distribution is very small (<1%). Using the Kolmogorov-Smirnov test, , a log-logistic distribution is also rejected at a significance level of 1%.

Results non-parametric 5th percentile value estimate: Referring to the overall and enlarged probability plots, we can interpolate at the cumulative density of 0.05 to find non-parametric estimates. For the individual NOEC values for plants and invertebrates (74 data point), the cumulative density of 0.05 is between the 3d and 4th data point, which are both 32 mg/kg d.w. Linear interpolation for log-concentrations also yields a 5th percentile value of 32 mg/kg d.w.

Results triangular distribution (included in ETX 1.3a): "Chronic value" is 32 mg/kg d.w.

[2]: Using either the Anderson-Darling Goodness-of-Fit test for normality (modified A^2) or the Kolmogorov-Smirnoff test, a log-normal distribution is accepted at significance levels up to 2.5% and rejected at a significance level of 5%, indicating that the probability that these data derive from a normal distribution is rather small (between 1% and 2.5%). Using the Kolmogorov-Smirnov test, a log-logistic distribution is accepted at significance levels of 1%-10%, indicating that the probability for a logistic distribution is considerably higher than that for a normal distribution.

Results non-parametric 5th percentile value estimate: Referring to the overall and enlarged probability plots, we can interpolate at the cumulative density of 0.05 to find non-parametric estimates, but not very reliable for this small sample size, i.e. small with respect to the use of non-parametric extrapolation. For the "species mean" NOEC values for plants and invertebrates (20 data points), the cumulative density of 0.05 is at the 1th data point which is 32 mg/kg dw. Linear interpolation for log-concentrations also yields a 5th percentile value of 32 mg/kg dw. It must be noted that the non-parametric extrapolation method is under discussion for relatively small sample sets, because in such a case this method does not efficiently use the information on the entire 'tail' but heavily relies on only the few data points at the left tail (Van der Hoeven, 2001).

Results triangular distribution (included in ETX 1.3a): "Chronic value" is 31 mg/kg d.w.

Predicted no effect concentration for the terrestrial compartment ($PNEC_{add, terrestrial}$)

Based on microbe-mediated processes

A comparison of the microbial database of NOEC values with the major recommendations made at the London Workshop on statistical extrapolation (EC, 2001; see also section 3.3.1.3) shows that:

- The number of NOEC values (n = 97) meets the general requirement for the number of input data (minimum requirement: 10 NOEC values; preferably more than 15 NOEC values).
- NOEC values are available for the two major soil microbe-mediated soil processes, i.e. C-mineralization (respiration, including C-mineralization of specific substrates) and N-

mineralization (including ammonification and nitrification), and for a number of enzyme activities. Data on microbe-mediated processes are considered to be more relevant than data on single microbial soil species, since soil-mediated processes (which are performed by a variety of microbial species) are important for soil functions such as the mineralization of litter. No recommendations were given by the Workshop for the required diversity of microbial data (neither for microbe-mediated processes nor for microbial species), but based on the recommendations for the freshwater compartment to include at least 8 taxonomic groups (families), the microbial toxicity database is considered to be sufficiently large to meet the "taxonomic" requirement (although based on these data, diversity in microbial species must be interpreted as diversity in microbe-mediated processes).

- It is noted that the goodness-of-fit for both a log-logistic and a log-normal distribution are rejected at a significance level of 1%. The use of these distributions results in the same median 5th percentile value and this value is close to the results of the non-parametric distribution and the triangular distribution, see Table 3.100.

Based on the above, the use of statistical extrapolation is preferred for $PNEC_{add}$ derivation rather than the use of an assessment factor on the lowest NOEC. In accordance with the Workshop recommendation the 5th percentile value is set at the 50% confidence level, using a log-normal distribution function, which would result in a value of 27 mg/kg d.w.

Based on uncertainty considerations the Workshop recommended to apply an assessment factor on the 50% confidence value of the 5th percentile value (thus $PNEC_{add}$ = median 5th percentile value/AF), with an AF between 1 and 5, to be judged on a case by case basis. Based on the available data, there are several reasons to use an assessment factor smaller than 5.

- There is a large microbial database, resulting in a relatively high reliability of the median 5th percentile value; this is also shown by the small difference between the 50% confidence level and the 95% confidence limits found for both the log-normal and log-logistic calculation. In both cases less than a factor of 2. This supports an AF smaller than 5.
- In most microbial tests the exposure time ranged from some weeks to some months and in a number of tests, e.g. for enzyme activities, the effect was measured 30 minutes after the addition of zinc, added as soluble zinc salt. This may overestimate the risk since the exposure time may have been too short for adaptation of the microbial communities and for reduced bioavailability. This also supports an AF smaller than 5.
- The microbial data origin from tests in a variety of soils, both EU soils and non-EU soil, covering the wide range of soil types and soils characteristics (pH value, clay content, organic matter content and background zinc concentration) that are normally found in European soils. This also supports an AF smaller than 5.
- Each tested community is unique, similar as each species in a structure-based approach. So, the range of tests yields a range of sensitivities of communities, especially regarding functions. The results from these tests can be treated in statistical extrapolation methods to obtain a PNEC that protects against functional loss across a range of ecosystems. This also supports an AF smaller than 5.
- The median 5th percentile value of 27 mg/kg d.w. may not be sufficiently protective, as a NOEC of 17 mg/kg d.w. was found in 2 of the 97 tests that were used for $PNEC_{add}$ derivation, i.e. the respiration test by Chang and Broadbent (1981) and one of the respiration tests by Ligthart et al. (1983), see Table 3.3.3..a in Annex 3.3.3.A. Since the median 5th percentile value is higher than all remaining 95 NOEC values, an AF smaller than 5 and slightly higher than 1 should be used.

- The use of the log-logistic and log-normal distribution results in the same median 5th percentile value and this value is close to the results of the non-parametric distribution and the triangular distribution, see Table 3.100. Based on this, there is no need for an assessment factor.
- With respect to laboratory to field extrapolation there is no need for an assessment factor, see sections 3.3.3.1.1 and 3.3.3.1.4. Actually: AF <1, but the lower toxicity in the field is taken into account in the lab-to-field factor that is applied to the PEC, see section 3.3.3.1.1.

In conclusion, the above procedure results in a median 5th percentile value of 27 mg/kg d.w. and justifies the use of an assessment factor of 1, based on the data for microbe-mediated processes. Arguments for the factor 1 are provided above and result in a $PNEC_{add, terrestrial}$ of 27 mg/kg d.w., that is sufficiently protective for most of the sensitive microbial species and processes and for the field situation.

Based on invertebrates and plants

A comparison of the species database of NOEC values for invertebrates and plants, combined) with the major recommendations made at the London Workshop on statistical extrapolation (EC, 2001; see also section 3.3.1.3) shows that:

- The number of chronic NOEC values (n = 74, for a total of 20 different species) meets the general requirement for the number of input data (minimum requirement: 10 NOEC values; preferably more than 15 NOEC values).
- Chronic NOEC values are available for 4 invertebrate species (3 earthworm species *(Oligochaetes)* and 1 insect species (springtail *Folsomia candida, Collembola)*) and 16 plant species. The invertebrates database is limited to 4 species of 2 families and does not include data on two other major taxa, namely *Gastropoda* (snails) and C*rustacea* (e.g. woodlice). However, snails and woodlice are living more on the soil than in the soil and are feeding especially on plants and litter/organic detritus, respectively. The plants database is considerably larger, including 16 species of 7 families. Furthermore, unbounded NOEC values ($\geq$500 mg/kg d.w.) are available for 2 plant species not included in the selected database. No recommendations were given by the Workshop for the required diversity of terrestrial species, but based on the recommendations for the freshwater compartment to include at least 8 taxonomic groups (families), the combined invertebrates and plants database is considered to be sufficiently large to meet the "taxonomic" requirement (a total of 9 families is represented in the combined invertebrates and plants database)**.**
- Furthermore, the species data for plants are from tests in a variety of soils, covering a considerable part of the wide range of soil types and soils characteristics (pH value, clay content, organic matter content and background zinc concentration) that are normally found in European soils (although most plant studies were performed in non-EU soils). A relatively large number of the tests with invertebrates were not conducted in natural soils but in artificial (OECD) soils. However, the characteristics of the artificial soils were within the ranges of those found in EU soils.
- It is noted that there is no goodness-of-fit for both the log-normal and the log-logistic distribution at a significance level of 1% (based on the distributions for the individual NOEC values). The use of these distributions results in the same median 5th percentile value; this value is 1.6-times higher than the results of the non-parametric extrapolation and the triangular distribution, see Table 3.101.

Based on the above, the use of statistical extrapolation is also preferred for $PNEC_{add}$ derivation when using the combined invertebrates and plants dataset. Combined with the earlier mentioned preference for the use of the individual NOEC values this would result in a 5th percentile value of 52 mg/kg d.w., when set at the 50% confidence value of the log-normal distribution.

Based on uncertainty considerations the Workshop recommended to apply an assessment factor on the 50% confidence value of the 5th percentile value (thus PNEC = median 5th percentile value/AF), with an AF between 1 and 5, to be judged on a case by case basis. Based on the available data, there are several reasons to use an assessment factor larger than 1 and smaller than 5.

- The limited number of data for invertebrates, being a very large and important taxonomic group, would support an AF greater than 1.
- The fact that in the plant studies reproduction was not included as toxicological endpoint, with the exception of the study with *Avena sativa* (oat) in which grain yield was studied, would also support an AF greater than 1.
- It is noted that there is no goodness-of-fit for both the log-normal and the log-logistic distribution at a significance level of 1% (based on the distributions for the individual NOEC values). The use of these distributions results in a median 5th percentile value that is 1.6-times higher than the results of the non-parametric extrapolation and the triangular distribution. This supports an AF greater than 1.
- The median 5th percentile value of 52 mg/kg d.w. may not be sufficiently protective, as a lower NOEC was found in 7 of the 74 tests that were used for $PNEC_{add}$ derivation, i.e. a NOEC of 32 mg/kg d.w. in one of the tests with invertebrate *Folsomia candida* (Lock et al., 2003), a NOEC of 32 mg/kg d.w. in four of the tests with plant *Trifolium pratense* (Van den Hoeven and Henzen, 1994b,c; Hooftman and Henzen, 1996), a NOEC of 32 mg/kg d.w. in the test with plant *Vicia sativa* and one of the tests with plant *Hordeum vulgare* (Luo and Rimmer, 1995), see Annex 3.3.3.A. Since the median 5th percentile value is lower than all remaining 67 NOEC values , an AF smaller than 5 and higher than 1 should be used.
- With respect to laboratory to field extrapolation there is no need for an assessment factor, see section 3.3.3.1.1. and 3.3.3.1.4. Actually: AF <1, but the lower toxicity in the field is taken into account in the lab-to-field factor that is applied to the PEC, see section 3.3.3.1.1.

In conclusion, the above procedure results in a median 5th percentile value of 52 mg/kg d.w. and justifies the use of an assessment factor of 2, based on the data for species. Arguments for the factor 2 are provided above and result in a $PNEC_{add,\ terrestrial}$ of 26 mg/kg d.w. that is sufficiently protective for the most sensitive species and for the field situation.

For comparison, using the "species mean" NOEC values for invertebrates and plants would result in a median 5th percentile value of 58 mg/kg d.w. and, using an assessment factor of 2, a $PNEC_{add,\ terrestrial}$ of 29 mg/kg d.w.

Overall conclusion on $PNEC_{add,\ terrestrial}$:

In conclusion, the above procedures results in a **$PNEC_{add,\ terrestrial}$ of 26 mg/kg dry soil**, derived from the median 5th percentile value (52 mg/kg d.w.) for species and applying an assessment factor of 2. This $PNEC_{add,\ terrestrial}$ is just below (but nearly equal to) the value derived from the data for microbe-mediated processes (27 mg/kg d.w, being the median 5th percentile value; assessment factor of 1) and therefore selected as $PNEC_{add,\ terrestrial}$.

For comparison, using all terrestrial NOEC values (n = 171) i.e. those for microbe-mediated processes (n = 97) and those for invertebrates and plants (n = 74) combined in one data set, this would result in a median 5th percentile value of 35 mg/kg d.w.

In wet soil containing 60% solids (density 2,500 kg/m^3), 20% water and 20% air by volume, i.e. 88% solids by weight, the above $PNEC_{add, terrestrial}$ of 26 mg/kg dry soil is equivalent to a $PNEC_{add, terrestrial}$ of 23 mg/kg wet soil.

It is realised that the used criteria (see section 3.3.3.1) may not cover all European terrestrial systems. However, the resulting terrestrial toxicity database and $PNEC_{add, terrestrial}$ may serve as a starting point for other types of soil as well, but further caution should be taken in e.g. using the $PNEC_{add, terrestrial}$ for other types of soil.

3.3.4 Non-compartment specific effects relevant to the food chain (secondary poisoning)

Based on the ICDZ data (Cleven et al., 1993) on bioaccumulation of zinc in animals and on biomagnification (i.e. accumulation and transfer through the food chain), it is concluded that secondary poisoning is considered to be not relevant in the effect assessment of zinc. Major decision points for this conclusion are the following. The accumulation of zinc, an essential element, is regulated in animals of several taxonomic groups, for example in molluscs, crustaceans, fish and mammals. In mammals, one of the two target species for secondary poisoning, both the absorption of zinc from the diet and the excretion of zinc, are regulated. This allows mammals, within certain limits, to maintain their total body zinc level (whole body homeostasis) and to maintain physiologically required levels of zinc in their various tissues, both at low and high dietary zinc intakes. The results of field studies, in which relatively small differences were found in the zinc levels of small mammals from control and polluted sites, are in accordance with the homeostatic mechanism. These data indicate that the bioaccumulation potential of zinc in both herbivorous and carnivorous mammals will be low.

Based on the above data, secondary poisoning and the related issues bioaccumulation and biomagnification are not further discussed in this report.

3.4 RISK CHARACTERISATION

3.4.1 General

The use of the added risk approach implies that in the risk characterisation the added Predicted Environmental Concentrations (PEC_{add}'s) in the various environmental compartments are compared with the corresponding added Predicted No Effect Concentrations ($PNEC_{add}$'s). In section 3.2 local concentrations are calculated for STP, soil, water, sediment and air. Except for the PEC_{STP}, these local concentrations have to be corrected for the regional background (PEC_{add} regional), according to the TGD equation $ECloca l_{add} = Cloca l_{add} + PECregiona l_{add}$. The regional exposure assessment, including regional monitoring data is described in section 3.2.5.3. In case measured environmental concentrations are used in the risk characterisation, either the natural background concentration has to be subtracted from the measured environmental concentration (resulting in a traditional "PEC_{add} / $PNEC_{add}$" ratio) or the natural background concentration has to be added to the $PNEC_{add}$ (resulting in a traditional "PEC / PNEC" ratio). Finally, a correction for bioavailability is carried out in the risk characterisation stage. For those scenarios where the

uncorrected PEC values would yield a PEC/PNEC ratio above 1, a (possible) bioavailability correction is made for surface water, sediment and soil (see decision trees in sections 3.3.2.1.1, 3.3.2.2.1 and 3.3.3.1.1 of Zinc Metal RAR). Final conclusions of the risk assessment are based on the corresponding 'corrected' PEC/PNEC ratios.

The reader is referred back to section 3.1 General introduction for more background information on the use of the added risk approach.

For air, the average measured concentration in the Netherlands of 0.04 μg/m^3 is chosen as regional background. (The natural background component in the value of 0.04 μg/m^3 is assumed to be negligible). Preference is given to this measured value as it is the result of a valid, representative monitoring programme. Besides, this figure is within the same order of magnitude as the calculated PEC_{add}'s at regional scale (0.006 μg/m^3 for the NL-region and 0.01 for the EU-region). For soil, following the TGD, the PEC regional in natural soil has to be added as background to the local concentration. The calculated value of 0.5 mg/kg wwt is used as regional background in the current risk assessment. For water PEC_{add}'s regional (dissolved) of 6.7 μg/l or 8.8 μg/l could be chosen as background values. These concentrations are derived from the measured average 90th percentile value of 41 μg/l [35](total) for regional waters in the Netherlands in 1997, corrected for, respectively, 3 and 12 μg/l natural background. Preference is given to these measured values as they are the result of valid, representative monitoring programmes. The figure for the Netherlands is supported by data from the large EU-survey (Denzer et al., 1999) in which a average 90-percentile value of 59.2 μg/l (total) is reported for the EU during the period 1994-1998. (Shortcomings of the Denzer et al. database are discussed in section 3.2.5.3.4. Although only considered as 'indicative' in the current risk assessment, the 90P value for total zinc from Denzer et al. does give some overall EU picture that is useful for comparison purposes as described above). For comparison: the calculated $PEC_{regional,\ add}$ values (dissolved) amounts to 4.5 μg/l (12.2 μg/l total) for the NL-region and 6.2 μg/l (16.8 μg/l total) for the EU-region. The PECs sediment are calculated from the PEC water ($PEClocal_{add}$ = $Clocal_{add}$ + $PECregional_{add}$) via the equilibrium partitioning method.

For water and sediment, in the current local risk characterisation initially only the $Clocal_{add}$ values (thus without the regional PEC_{add}) will be compared with the $PNEC_{add}$. At first the local aquatic risk characterisation thus focuses on the contribution of point sources to the potential risks, thereby neglecting the contribution of diffuse sources. If the regional PEC_{add} would have been added for sediment, all local scenarios would have resulted in $PEC_{add}/PNEC_{add}$ ratios larger than 1. This because the regional PEC_{add} already exceeds the $PNEC_{add}$ of 11 mg/kg wwt. This holds for both calculated and measured sediment concentrations. For this reason for sediment all scenarios with a $Clocal_{add}/PNEC_{add}$ ratio between 0 and 1 a **conclusion iii*)** will be drawn, indicating that due to (possibly) high added regional background concentrations a risk for sediment at local scale cannot be excluded. It has to be noted that this conclusion would not be influenced by applying the generic sediment bioavailability correction factor of 0.5 (see section 3.3.2.2.1).

The situation is somewhat less pronounced for the surface water compartment. With a $PNEC_{add}$ of 7.8 μg/l the regional $PEC_{add}/PNEC_{add}$ would lie between 0.8 (PEC_{add} of 6.7 μg/l) and 1.1 (PEC_{add} of 8.8 μg/l). When using an (arbitrary) average bioavailability correction factor of 0.6[36] these ratios would become, respectively 0.5 and 0.7. As a result of this, it is

35 Natural background value of 3 and 12 μg/l are subtracted from this value and, subsequently, the total figures are re-calculated to a dissolved zinc concentration (41-3 = 38 μg/l divided by 4.3 results in 8.8 μg/l; 41-12 = 29 μg/l divided by 4.3 results in 6.7 μg/l)

36 See Table 3. in RAR on Zinc Metal. Average of realistic worst case and average BioF for average NL data.

decided that for $Clocal_{add}/PNEC_{add}$ ratios between 0.5[37] and 1 a **conclusion iii*)** will be drawn, indicating that due to (possibly) high (added) regional background concentrations a local risk for water cannot be excluded. For scenarios with a surface water $Clocal_{add}/PNEC_{add}$ ratio < 0.5 the local contribution to the (added) regional background is assumed to be negligible (**conclusion ii**).

For those scenarios in which the involved process type does intrinsically not result in water emissions a **conclusion ii)** is drawn for water and sediment.

It is important to note that the above-mentioned distinction between a (normal) conclusion iii) and a conclusion iii*) is not only made because of transparancy, but also because the regional background is due to a variety of zinc compounds (and thus not only the zinc compound specifically addressed in the local risk characterisation).

In section 3.4.2 a general reflection is given on the uncertainties in the zinc risk assessments.

3.4.2 Uncertainties in environmental risk assessment

The current risk assessment on zinc metal and zinc compounds has been conducted according to the TGD following a deterministic approach, i.e. 'single' PEC/PNEC ratios were estimated for the various protection goals. The RA conclusions are subsequently based on these PEC/PNEC ratios. Although overall a deterministic approach is followed, some elements of a probabilistic approach/sensitivity analysis were already incorporated within the current RA. The advantage of such probabilistic approach/sensitivity analysis elements is that more insight is provided in the uncertainties of the RA. Information about those RA uncertainties should ideally play a role in determining the need and/or magnitude of risk reduction steps.

Below, several aspects of the uncertainties in the current zinc RA(s) are discussed. It is attempted to focus on the uncertainties around the most important parameters in the RA. This to ultimately gain more insight into the overall uncertainty of the RA. No comprehensive, quantitative uncertainty analysis throughout the entire zinc risk assessment is performed. Many data are lacking for such a quantitative evaluation. On top of that, there is also no scientific consensus yet on how to carry out an uncertainty analysis within the ESR program in such a way that it can adequately assists policy makers in decision making (CSTEE, 2004). The below-described exercise can therefore 'only' be characterised as a limited, qualitative uncertainty analysis.

37 A $Clocal_{add}/PNEC_{add}$ of between 0.5 and 1 should theoretically also be corrected for bioavailability. This would give ratios between 0.3 and 0.6 when using the correction factor of 0.6. Such ratios could just raise the overall $PEC_{add}/PNEC_{add}$ ratio, thus including the regional background, to levels above one.

Added risk approach

A first remark could be made on the added risk approach that is generally applied in the zinc RA. Uncertainties when using the added risk approach vary from conceptual aspects to practical implementation issues. Now it is assumed that the $PNEC_{add}$ can be used in a rather universal way, i.e., independent of the height of the natural background. For example the $PNEC_{add}$ water of 7.8 µg/l is assumed to be applicable both in an EU region with a natural zinc background of e.g., 5 µg/l and 20 µg/l. One could argue that more than doubling the amount of zinc, in case of the 5 µg/l background, may have a fundamentally different impact on water organisms than in an ecosystem where the allowable addition is relatively lower (20 µg/l). This is an important 'conceptual uncertainty' of the added risk approach, but it is very difficult to make a statement about the magnitude of the uncertainty. The selection of the appropriate natural background value could be brought forward as an example of a practical implementation point. This uncertainty is probably lower than the one for the conceptual aspects (if comparable at all). Furthermore, by applying a range (rather than a single value) of natural background concentrations (3-12 µg/l) for correcting EU surface water monitoring data the influence of this parameter on the overall outcomes was made transparent in the RA (sensitivity analysis).

Default exposure scenarios

The local exposure assessment is hampered in a number of cases by a lack of site-specific exposure data. This particularly holds for the downstream use scenarios for the zinc compounds other than zinc metal, for each of the environmental compartments, water, sediment and soil. In those cases the TGD default exposure scenarios were applied. Although the most suitable Industry and Use Categories were selected, the TGD defaults mostly represent rather worst case emissions to the environment. The uncertainty around these default emissions is expected to be (very) high. Jager (1998) evaluated/validated the EUSES model and concluded that the default release estimation from the TGD largely contributes to the overall uncertainty of the environmental risk assessment. The possible deviation between defaults and actual measured emission values ranges from 1-1000. It is emphasised that Jager (1998) performed a generic evaluation, mostly based on organic substances, so it is definitely not related to zinc or metals specifically. The subsequent distribution modelling from emissions towards a generic PEC according to the TGD is founded on a mixture of average and realistic worst case (e.g. dilution factor of 10) assumptions. These uncertainties are expected to be lower than those around the default release estimation (see above).

Bioavailability

A thorough research programme on bioavailability correction was conducted for the RA of zinc and zinc compounds. For surface water no zinc bioavailability correction was performed on the PECs from the above-mentioned generic scenarios, whereas for soil and sediment a generic bioavailability correction is used, with a factor 3 (ageing only) and 2, respectively. The application of these bioavailability factors is undoubtedly a reduction of uncertainty in the zinc RA. If monitoring data were available for local sites they were mostly limited to a low number of measurements. In most cases no 90^{th} P-value could be extracted from the data, so only single measurements (if shown to be valid) were used in the local risk characterisation. Single measurements implicitly encompass a higher uncertainty than 90^{th} P-values. Site-specific bioavailability factors (surface waters) or site-specific SEM-AVS measurements (sediment) further reduced uncertainties for the local site-specific exposure assessment. It is stressed that site-specific SEM-AVS data were only available in a very few cases. Site-specific bioavailability factors for soil (soil type dependent correction factors in addition to generic soil correction factor for ageing) were even not applied in any of the local

scenarios due to lacking data. The site-specific bioavailability correction for surface water is done for both an average and a realistic worst case setting depending on the physico-chemical characteristics of the particular water. In practice the bioavailability correction factors applied in the zinc RA for surface water ranged between 0.2 and 1 (see sections 3.4.3 and 3.4.4).

Regional exposure assessment

The regional exposure assessment consists of both a modelled approach (SimpleBox/EUSES) based on regional emission data and actual zinc monitoring data in the environment. The regional PECs were calculated based on three varying Kp-values showing the impact – sensitivity - of this parameter on the estimations. Jager (1998) generally characterised the regional distribution module in EUSES as 'optimistic' with respect to degree of conservatism. In his survey (again mainly based on organic chemicals) the possible deviation between modelled and measured regional values ranges from 0.001-10. In the regional zinc risk characterisation most emphasis is laid on the measured data as they were considered to be most useful (lowest uncertainty). For both surface water and sediment 90th P-values were available for many regions from long-term monitoring networks throughout the EU. The use of these 90th P-values implicitly takes into account the variability of the regional data, but it represents of course a conservative estimate. Similar to the local exposure assessment the use of bioavailability factors additionally reduced uncertainties at the regional scale. Here, SEM-AVS information that could overrule the total PEC/PNEC sediment approach was only available for one region (Flanders). A number of other important uncertainties around the regional measured data for water and sediment are separately discussed in section 3.4.4.1 (e.g. possible influence of point sources, historical contamination etc.). For potential zinc accumulation in agricultural soil the final conclusions are founded on the probabilistically based Alterra study (De Vries et al. ,2004). This study makes explicit the uncertainty/variability in the various steps of the assessment, including the time scale estimates for reaching the critical zinc concentrations in soil.

PNEC values

When discussing uncertainties in the effects assessment of zinc a distinction should be made between the PNEC derivation of surface water and soil on the one hand and sediment and STP on the other. The $PNEC_{add}$ values for surface water and soil are based on a statistical extrapolation method, whereas the $PNEC_{add}$ values for sediment and STP effluent are based on the traditional approach, i.e. the lowest NOEC divided by a certain assessment factor. For water and soil the statistical extrapolation method could be used as both data sets met the criteria for using it. A great advantage of the statistical extrapolation method is that it addresses the variability of the data set. The 5th percentile of the species sensitivity distribution is of course an arbitrary choice for the protection level and, moreover, the probabilistic element of the method is partly counterbalanced by additionally using the AFs on the 5th percentile. Additionally, intrinsic uncertainties when using NOEC values rather than statistically more underpinned EC_x values in either the statistical extrapolation method or the traditional approach should be mentioned as well. In most cases only NOEC values were available for zinc, but this is true for most other ESR chemicals.

The current zinc $PNEC_{add}$ for surface water amounts to 7.8 µg/l (5th percentile of 15.6 µg/l divided by an AF of 2), whereas the 5th percentile 95% confidence limit ranges from 7.2 µg/l (lower limit) to 26.2 µg/l (upper limit). The $PNEC_{add}$ water is therefore only slightly above the lower C.I. limit value, so it can be considered as a conservative value. On the other hand the uncertainty around the $PNEC_{add}$ water is expected to be rather limited.

The data set for soil is split into plants/invertebrates and soil micro-organisms. Arguments for this distinction are extensively elaborated in section 3.3.3.1.5. The 5th percentile for

plants/invertebrates was estimated to be 52 mg/kg dwt and with an additional AF of 2 a $PNEC_{add}$ of 26 mg/kg dwt was derived. This value is outside the range of the 95% confidence limits around the 5th percentile: 39 mg/kg dwt (lower limit) and 65 mg/kg dwt (upper limit). The $PNEC_{add}$ soil just based on plants/invertebrates should therefore be considered as (very) conservative. The value is supported, however, by the $PNEC_{add}$ for soil micro-organisms. Here a 5th percentile of 27 mg/kg dwt is estimated and an AF of 1 was considered appropriate. The 95% confidence interval for the 5th percentile for micro-organisms amounts to 19 mg/kg dwt (lower limit) and 35 mg/kg dwt (upper limit). The match between the $PNEC_{add}$ vlaues for plants/invertebrates and soil micro-organisms reduces the uncertainty for the overall $PNEC_{add}$ soil (26 mg/kg dwt being the lowest of the two).

The $PNEC_{add}$ sediment of 49 mg/kg dwt is derived from a *Hyallela* NOEC of 488 mg/kg dwt and an assessment factor of 10. Insufficient data were available to apply the statistical extrapolation method for sediment.. The uncertainty around the $PNEC_{add}$ sediment is assumed to be higher than that for water and soil. The same is true for the $PNEC_{add}$ STP (not further discussed here).

The $PNEC_{add}$ for surface water (and sediment) is used for both freshwater and marine environments. Section 3.3.2.1.5 refers to the uncertainties of the followed approach on this issue. Those local exposure scenarios in the current RAR with emissions to sea implicitly include this uncertainty.

Relative uncertainties

In the risk characterisation the outcomes of the exposure assessment are combined with those from the effect assessment. It is obvious that uncertainties in the individual building blocks of the risk assessment could be greater when integrated in the risk characterisation part. As may be clear from the previous sections, a quantitative estimation of the uncertainties of the various parameters is difficult, if not impossible. Therefore, only some thoughts can be provided on the *relative* uncertainties for different scenarios. It may be understood that a PEC/PNEC ratio for a local scenario based on generic TGD assumptions is most probably surrounded by a larger uncertainty than a PEC/PNEC ratio based on a valid 90th P-value from a long-term regional monitoring network (assuming the same PNEC). The PEC/PNEC ratios for either surface water or soil, additionally, contain less uncertainty than those for sediment (assuming the same PEC). Consequently, a PEC/PNEC ratio based on a generic local exposure scenario for the sediment compartment plausibly contains a (much) larger uncertainty than a PEC/PNEC ratio based on a representative 90th P-value for surface water. Any further quantitative pronouncements on the uncertainties in the risk assessment on zinc and zinc compounds would be speculative. However, above-mentioned aspects could be important in the risk management phase.

Table 3.102 The local (PE)C_{add} values and (PE)C_{add} / $PNEC_{add}$ ratios used in the local risk characterisation of zinc metal. The (PE)C_{add} values and hence the (PE)C_{add} / $PNEC_{add}$ values are not corrected for bioavailability.

Company	PEC effluent STP (dissolved)	C_{add} water (dissolved)	C_{add} sediment	PEC_{add} agricultural soil	PEC/ PNEC STP	C_{add} / $PNEC_{add}$ water	Cadd / $PNEC_{add}$ sediment	PEC_{add} / $PNEC_{add}$ agr. soil
	(µg/l)	(µg/l)	(mg/kgwwt)	(mg/kgwwt)				
Production companies:[1]								
Company 1	624	101	2,423	3.44	12	13	233	0.14
Company 1: measured concentrations		113 [6]				13.7 / 14.1 [7]		
Company 3	375	1.92	45.9	14.5	7.2	0.25	4.3	0.60
Company 3: measured concentrations		9 [6]				0.6 / 1.0 [7]		
Company 4	407	165	3,949	2.78	7.8	21	380	0.12
Company 4: measured concentrations		38 [6]	41 [4] / 193 [5] [6]			4.2 / 4.6 [7]	0.9 [4] / 15 [5] [8]	
Company 8	1,215	197	4,714	12.2	23	25	453	0.51
Company 8: measured concentrations		4-19 [6]				1.8 / 2.2 [7]		
Company 9	63	10.3	246	1.00	1.2	1.3	24	0.042
Company 12	0	0	0	0.85	0	0	0	0.035
Company 15	177	7.9	189	8.53	3.4	1.0	18	0.36
Company 15: measured concentrations		131 [6]	1289-1911 [6]			9.0 / 9.5 [7]	120-180 [8]	
Company 16	7	$2.64.10^{-4}$	$6.31.10^{-3}$	0.636	0.13	0.000034	0.0006	0.027
Company 18	60	$5.43.10^{-3}$	0.13	3.6	1.2	0.00070	0.012	0.15
Company 20 (1995)	1,517	154	3,679	9.95	not appl. [9]	20	354	0.41
Company 20 (2002)	1,248	127	3,027	4.22	not appl. [9]	16.2	290	0.18
Company 20: measured concentrations		3-6 [6]	370 [6]			0-0.2 / 0.2-0.6 [7]	32 [8]	

Company	PEC effluent STP (dissolved)	C_{add} water (dissolved)	C_{add} sediment	PEC_{add} agricultural soil	PEC/ PNEC STP	C_{add} / $PNEC_{add}$ water	Cadd / $PNEC_{add}$ sediment	PEC_{add} / $PNEC_{add}$ agr. soil
	(µg/l)	(µg/l)	(mg/kgwwt)	(mg/kgwwt)				
Company 21 (1998)	133	21.5	514	8.97	2.5	2.8	49	0.37
Company 21 (2002)	10	1.61	38.5	6.17	0.19	0.21	4	0.26
Company 21: measured concentrations		0.4-16 [6]	22-109 [6]			0-1.5 / 0-1.9 [7]	0-7.5 [8]	
Company 22	140	22.7	542	0.568	2.7	2.9	52	0.024
Company 23	229	0.109	2.62	1.69	4.4	0.014	0.3	0.070
Company 24	0	0	0	0.96	0	0	0	0.040
Company 26	0	0	0	1.14	0	0	0	0.048
Company 27 [2]	90	3.37	81	5.98	1.7	0.43	7.7	0.25
Company 27 total [3]	516	19.4	464	6.62	9.9	2.5	45	0.276
Company 27: measured concentrations		151-377 [6]				18.5-47 / 18.9-47.5 [7]		
Company 28	1,316	20.6	492	0.793	25	2.6	47	0.033
Company 28: measured concentrations		91	348-1900 [6]			6.5 / 7.0 [7]	30-178 [8]	
GALVANISING:								
GHDG: aqueous discharges from run-off, reported waste water concentrations for 20 plants in the Netherlands	0.084-1.78	0.014-0.289	6.92	0.5	0.0016-0.034	0.0018-0.037	0.03-0.66	0
Continuous Hot Dip Galvanising (CHDG): additional assessment	1,787	290	6,935	888	34	37	667	37
CHDG Company A	590	95.7	2,288	0.5	11	12	220	0.021
CHDG Company B	8.3	1.34	32	0.5	0.16	0.17	3	0.021
CHDG Company B: measured concentrations		5.7 [6]				0.1 / 0.6 [7]		
CHDG Company C	25.9	$3.57.10^{-3}$	$8.53.10^{-2}$	0.5	0.50	0.00046	0.008	0.021
CHDG Company E1	0	0	0	0.5	0	0	0	0.021

Company	PEC effluent STP (dissolved)	C_{add} water (dissolved)	C_{add} sediment	PEC_{add} agricultural soil	PEC/ PNEC STP	C_{add} / $PNEC_{add}$ water	Cadd / $PNEC_{add}$ sediment	PEC_{add} / $PNEC_{add}$ agr. soil
	(µg/l)	(µg/l)	(mg/kgwwt)	(mg/kgwwt)				
CHDG Company E2 (new data; year unknown)	5.0	0.811	19.4	2.98	0.10	0.10	1.8	0.12
CHDG Company G1 and G2	54.2	8.78	210	27.4	1.0	1.1	20	1.1
CHDG Company H	32.6	5.29	126	16.7	0.63	0.68	12.3	0.69
CHDG Company H: measured concentrations		9.4 [6)]				0.6 / 1.0 [7)]		
CHDG Company I	1.95	0.317	7.58	1.47	0.038	0.041	0.7	0.061
CHDG Company J1	0	0	0	0.5	0	0	0	0.021
CHDG Company K1	46.0	0.0957	2.28	0.500	0.89	0.012	0.22	0.021
CHDG Company K3	143	0.208	4.98	0.514	2.7	0.027	0.47	0.021
CHDG Company L1	292	3.14	75.1	0.508	5.6	0.40	7.2	0.021
CHDG Company L2	24.4	0.131	3.14	0.500	0.47	0.017	0.3	0.021
CHDG Company M1	0	0	0	0.5	0	0	0	0.021
CHDG Company M3	0	0	0	0.5	0	0	0	0.021
CHDG Company M4	0	0	0	0.5	0	0	0	0.021
CHDG Company M5	0	0	0	0.5	0	0	0	0.021
CHDG Company M6	$3.88.10^{-3}$	$6.29.10^{-4}$	$1.50.10^{-2}$	0.502	0.000075	0.000081	0.0014	0.021
CHDG Company M7	2.17	0.352	8.42	1.58	0.042	0.045	0.8	0.066
CHDG Company O	1.16	0.188	4.51	1.08	0.022	0.024	0.4	0.045
CHDG Company P	207	16.1	386	104	4.0	2.1	37	4.3
CHDG Company Q	123	20	477	60.5	2.4	2.6	46	2.6
CHDG Company R	0	0	0	0.500	0	0	0	0.021
CHDG Company T	115	$2.32.10^{-5}$	$5.54.10^{-4}$	57.4	2.2	$2.97.10^{-6}$	$5.3.10^{-5}$	2.4

Company	PEC effluent STP (dissolved)	C_{add} water (dissolved)	C_{add} sediment	PEC_{add} agricultural soil	PEC/ PNEC STP	C_{add} / $PNEC_{add}$ water	Cadd / $PNEC_{add}$ sediment	PEC_{add} / $PNEC_{add}$ agr. soil
	(µg/l)	(µg/l)	(mg/kgwwt)	(mg/kgwwt)				
CHDG Company U	30.0	0.606	14.5	15.5	0.58	0.078	1.4	0.65
CHDG Company V	17.6	2.85	68.1	9.22	0.34	0.37	6.5	0.38
CHDG Company W	no data	no data	no data	no data	no data	no data	no data	no data
CHDG Company X	107	17.3	415	53.6	2.1	2.2	40	2.2
CHDG Company Y1 and Y2	3.83	0.621	14.9	2.40	0.074	0.080	1.5	0.10
CHDG France	119 (66.3-222)	19.2 (10.8-36.0)	460 (257-862)	59.4 (33.4-111)	2.4 (0-2.3)	2.5 (1.4-4.6)	45 (24-83)	2.5 (1.4-4.6)
Electro Galvanizing (EG): additional assessment	1,210	196	4,695	602	23	25	451	25
EG Company D	416	0.0304	0.727	0.5	8.0	0.0039	0.07	0.021
EG Company F	81.6	2.52	60.4	41.0	1.6	0.32	5.8	1.7
EG Company G3	90.9	14.7	353	45.6	1.7	1.9	34	1.9
EG Company G3: measured concentrations		3.8-19 [6)]				1.8 / 2.2 [7)]		
EG Company J2	174	28.3	677	87.1	3.4	3.6	65	3.6
EG Company K2	306	2.63	63	0.5	5.9	0.34	6	0.02
EG Company K2: measured concentrations		3.8 [6)]				0 / 0.3 [7)]		
EG Company K4	191	31.1	743	0.5	3.7	4.0	72	0.02
EG Company M1	93.8	15.2	364	47.1	1.8	2.0	35	2.0
EG Company M2	no data	no data	no data	no data	no data	no data	no data	no data
EG Company M4	17.4	2.83	67.7	no data	0.34	0.36	6.5	no data
EG Company M6	17.4	2.83	67.7	9.16	0.34	0.36	6.5	0.38
EG Company N	766	0.458	10.9	381	15	0.059	1.1	16
EG Company W+X	107	17.3	415	53.6	2.1	2.2	40	2.2

Company	PEC effluent STP (dissolved)	C_{add} water (dissolved)	C_{add} sediment	PEC_{add} agricultural soil	PEC/ PNEC STP	C_{add} / $PNEC_{add}$ water	Cadd / $PNEC_{add}$ sediment	PEC_{add} / $PNEC_{add}$ agr. soil
	(µg/l)	(µg/l)	(mg/kgwwt)	(mg/kgwwt)				
BRASS:								
Brass company 1	0	0	0	0.500	0	0	0	0.021
Brass company 2	37.6	6.10	146	0.789	0.72	0.78	14	0.033
Brass company 3	19.0	3.08	73.7	0.682	0.37	0.40	7	0.028
Brass company 4	9.70	1.57	37.6	0.581	0.19	0.20	3.6	0.024
Brass company 5	24.0	3.87	92.6	1.37	0.46	0.50	9	0.057
Brass company 6	310	50.3	1,203	0.657	6.0	6.4	115	0.027
Brass company 7	22.4	3.64	87.1	0.531	0.43	0.47	8	0.022
Brass company 8	194	31.4	752	1.17	3.7	4.0	72	0.049
Brass company 9	9.05	1.47	35.1	0.959	0.17	0.19	3	0.040
Brass company 10	116	18.9	451	0.584	2.2	2.4	43	0.024
Brass company 11	8.53	1.38	33.1	-	0.16	0.18	3	-
Brass company 12	1,312	213	5,090	1.09	25	27	489	0.045
ALLOY AND DIE CASTING:								
Alloy production: company 1	413	67	1,602	0.572	7.9	8.6	153	0.024
Alloy production: company 3	0	0	0	0.5	0	0	0	0.021
Alloy production: company 4	0.017	$3.64.10^{-5}$	0.00087	0.605	$3.24.10^{-4}$	$4.66.10^{-6}$	$8.16.10^{-5}$	0.025
Alloy production: company 4: measured concentrations			28 [4] / 24 [5] [6]					
Alloy production: company 5	0	0	0	0.509	0	0	0	0.021
Alloy production: company 6	0	0	0	0.505	0	0	0	0.021
Alloy production: company 7	48.4	7.88	188	0.508	0.93	1.0	18	0.021
Die casting: UK data (4 sites) water emissions	0.465-18.6	7.55E-02 – 3.02	1.80-72.2	not appl.	0.009-0.36	0.010-0.39	0.18-7	not appl.

Company	PEC effluent STP (dissolved)	C_{add} water (dissolved)	C_{add} sediment	PEC_{add} agricultural soil	PEC/ PNEC STP	C_{add} / $PNEC_{add}$ water	Cadd / $PNEC_{add}$ sediment	PEC_{add} / $PNEC_{add}$ agr. soil
	(µg/l)	(µg/l)	(mg/kgwwt)	(mg/kgwwt)				
Die casting: UK data	847	137	3,285	2.71	16	18	316	0.11
Die casting: German data	1.48	0.240	5.75	0.517	0.028	0.031	0.6	0.022
Die casting: France data	28.8	4.67	112	0.506	0.55	0.60	11	0.021
ROLLED/WROUGHT ZINC:								
Rolled/wrought zinc: company 1	24.2	3.93	94	0.503	0.47	0.50	9	0.021
Rolled/wrought zinc: company 1: measured concentrations		6 [6]	12 [4] / 15 [5] [6]			0.2 / 0.6 [7]	0 / 0 [8]	
Rolled/wrought zinc: company 2	0	0	0	0.504	0	0	0	0.021
Rolled/wrought zinc: company 3	0	0	0	0.514	0	0	0	0.021
Rolled/wrought zinc: company 4	0.017	$3.64.10^{-5}$	0.00087	0.605	$3.24.10^{-4}$	$4.66.10^{-6}$	$8.16.10^{-5}$	0.025
Rolled/wrought zinc: company 4: measured concentrations			28 [4] / 24 [5] [6]					
Rolled/wrought zinc: company 5	0.814	0.132	3.16	0.544	0.016	0.017	0.3	0.023
ZINC POWDER/DUST:								
Zinc powder/dust: companies 27 and A min an max emission air	not appl.	not appl.	not appl.	0.503-2.31	not appl.	not appl.	not appl.	0.021-0.10
Zinc powder/dust: companies 11 and 27 min and max emission water	5.81-19.8	0.943-3.21	22.6-76.7	not appl.	0.11-0.38	0.12-0.41	2.1-7.4	not appl.
Zinc powder/dust: remaining two companies with unknown emissions	4.53	0.74	17.6	0.655	0.087	0.095	1.7	0.027

Some production companies (numbers 2, 5, 6, 7, 10, 11 13, 14, 17, 19 and 25) finally indicated not to be a zinc metal producer and therefore no information is presented for these companies;

Only zinc metal production separated from the other activities at this site;

Total emission values and concentrations of this zinc metal production site, including those at the production of zinc alloys, zinc calots (semis) and zinc powders;

Downstream;

Upstream;

Measured concentration is a PEC value (total Zn) in stead of a C_{add} value;

PEC/$PNEC_{add}$ value based on the measured PEC value minus the natural background concentration of 3 and 12 µg/l (total Zn) and a $PNEC_{add}$ of 21 µg/l (total Zn);

PEC/$PNEC_{add}$ value based on the measured PEC value minus the natural background concentration of 140 mg/kg dwt and a $PNEC_{add}$ of 49 mg/kg dwt;

No STP; emission waters are discharged directly to surface water.

not appl Not applicable

3.4.3 Local risk characterisation

The local C_{add} and PEC_{add} values for zinc metal and the corresponding $(PE)C_{add}$ /$PNEC_{add}$ ratios are listed in Table 3.102. It is emphasised that these C_{add} and PEC_{add} values and the $(PE)C_{add}$ /$PNEC_{add}$ ratios are not corrected for bioavailability (first step in bioavailability decison trees in sections 3.3.2.1.1, 3.3.2.2.1 and 3.3.3.1.1). Subsequent corrections for the bioavailability of zinc in water, sediment and soil (if allowed) are discussed in the sections below.

Table 3.105 finally presents the overall results of the local risk characterisation after the various bioavailability correction steps (if relevant). Bioavailability correction is only carried out in case the uncorrected $(PE)C_{add}$ / $PNEC_{add}$ ratio exceeds one. In addition, no bioavailability correction is done for the PEC STP.

3.4.3.1 Aquatic compartment

3.4.3.1.1 STP effluent and sludge

STP effluent

The PECs STP (total), as calculated in paragraph 3.2.5.2 for the various scenarios have been re-calculated to dissolved values. This because the $PNEC_{add}$ of 52 µg/l for microorganisms is expressed as a dissolved zinc concentration.

Production

The PEC_{STP} for the production sites of zinc metal exceeds the $PNEC_{add}$ for microorganisms in a number of cases **(conclusion iii)**. The highest $PEC_{STP}/PNEC_{add}$ ratio is 23 for site no. 8. All PEC_{STP} values for production sites refer to an industrial WWTP and are based on site-specific emission data in combination with a site-specific effluent flow rate. In addition, in most cases also a location specific zinc removal efficiency in the WWTP is given. For all other production scenarios a **conclusion ii)** is drawn for the STP.

Use categories

The PEC_{STP} for the processing sites of zinc metal exceeds the $PNEC_{add}$ for microorganisms in a number of scenarios: 'CHDG' (a number of individual sites and the additional generic assessment), 'EG' (a number of individual sites and the additional generic assessment), 'brass' (a number of individual sites), 'alloy and die casting' (a number of individual sites) and 'die casting' (UK data) (**conclusion iii**). In contrast with the production scenarios (see above), also additional generic scenarios have been used for the processing of zinc. This due to a lack of (sufficient) site-specific data for some use categories.

The PEC_{STP} / $PNEC_{add}$ ratio is <1 for the remaining use categories **(conclusion ii).**

Sludge

In the Netherlands the maximum zinc concentration in sludge intended for use on agricultural soils is 300 mg/kg dwt (BOOM2 Decision). In section 3.1 some very high zinc concentrations in sludge are mentioned which clearly exceed this limit value. It is noted, however, that the production companies indicated that the sludge of their sites is either re-used into the process or disposed off in controlled landfills (see 3.2.5.2) and thus not applied on agricultural soils. The rapporteur further realises that the maximum sludge content for soil application is not an official TGD endpoint.

Table 3.103 Characteristics (DOC, hardness and pH) of local waters for which the local (PE)C_{add} / $PNEC_{add}$ ratio for surface water exceeds one (without correction for bioavailability (see Table 3.102) (production and use of zinc metal). Corresponding bioavaialability factors ($BioF_{water}$) are calculated with Biotic Ligand Model for algae and fish. $BioF_{water}$s in bold represent the values that will be used in the risk characterisation for, respectively, average (50P DOC and 50P inorganics) and realistic worst case (algae 10P DOC and 90P inorganics and fish: 10P DOC and 10P inorganics) conditions. Both the uncorrected local (PE)C_{add} / $PNEC_{add}$ ratio and the corrected local (PE)C_{add} / $PNEC_{add}$ ratio (realistic worst case and average) for surface water are presented. No bioavailability correction is performed for discharges to sea.

	Remark	DOC (mg/l)		pH			Hardness (CaCO3 mg/l)			BioF algae	BioF algae	BioF fish	BioF fish	PEC water 3)	(PE)C_{add} / $PNEC_{add}$	(PE)C_{add} / $PNEC_{add}$	(PE)C_{add} / $PNEC_{add}$
		10P	50P	10P	50P	90P	10P	50P	90P	10-90	50-50	10-10	50-50	(µg/l)	uncorrected	r.w.c.	avg.
Production 1	Sea														13 6)	no correction	
	M Sea													300	14 2)	no correction	
Production 4		9.7 1)	15.3-18.2	6.5 1)	7.21-7.32	7.5 1)	46.7 1)	154-181	343	0.3	0.1	**0.6**	**0.2**		21 6)	13 7)	4.2 7)
	M	9.7 1)	15.3-18.2	6.5 1)	7.21-7.32	7.5 1)	46.7 1)	154-181	343	0.3	0.1	**0.6**	**0.2**	100	4.2 2)	2.5 4)	0.8 4)
Production 8	Sea														25 6)	no correction	
	M Sea													50	1.8 2)	no correction	
Production 9	Sea														1.3 6)	no correction	
Production 15	M	3.0 1)	5.2	7.0 1)	7.8	8.1 1)	44.7 1)	160.8	328	0.8	**0.4**	**1**	0.4	100	4.2 2)	4.2 4)	1.7 4)
Production 20	Sea														16 6)	no correction	
Production 21	M Sea													43	1.5 2)	no correction	
Production 22		4.4 1)	7.69	6.1 1)	6.84	7.1 1)	106.9	257	1400	0.5	0.6	**1**	**0.5**		2.9 6)	2.9 7)	1.5 7)
Production 27 t	Calc.	9.7 1)	15.3-18.2	6.5 1)	7.21-7.32	7.5 1)	46.7 1)	154-181	343 1)	0.3	0.1	**0.6**	**0.2**		2.5 6)	1.5 7)	0.5 7)
Production 27	M	9.7 1)	15.3-18.3	6.5 1)	7.21-7.33	7.5 1)	46.7 1)	154-182	343 1)	0.3	0.1	**0.6**	**0.2**	400-1000	18-47 2)	11-28 4)	3.7-9.4 4)
Production 28	Calc.	3.3 1)	5.7	7.1 1)	7.9	8.2 1)	42.8 1)	154	314 1)	0.7	**0.4**	**1**	0.4		2.6 6)	2.6 7)	1.0 7)
	M	3.3 1)	5.7	7.1 1)	7.9	8.2 1)	42.8 1)	154	314 1)	0.7	**0.4**	**1**	0.4	149	6.5 2)	6.5 4)	2.6 4)
CHDG: additional ass.															37 6)	no correction	

	Remark	DOC (mg/l)		pH			Hardness (CaCO3 mg/l)			BioF algae	BioF algae	BioF fish	BioF fish	PEC water [3]	(PE)C_{add} / $PNEC_{add}$	(PE)C_{add} / $PNEC_{add}$	(PE)C_{add} / $PNEC_{add}$
		10P	50P	10P	50P	90P	10P	50P	90P	10-90	50-50	10-10	50-50	(µg/l)	uncorrected	r.w.c.	avg.
CHDG A (Fin.)		5.8 [1]	>10 [5]	6.4	7.1	7.4	12.8	46	94	0.3	0.2	**0.9**	**0.6**		12[6]	11[7]	7.4[7]
CHDG G1 & G2		2.02	2.81	7.9	8	8.1	197	223	250	1	**0.7**	**1**	0.4		1.1[6]	1.1[7]	0.8[7]
CHDG P		1.71	2.88	7.1 [1]	7.9	8.2 [1]	37	111	229	1	**0.7**	**1**	0.5		2.1[6]	2.1[7]	1.5[7]
CHDG Q	Sea														2.6[6]	no correction	
CHDG X	Sea														2.2[6]	no correction	
CHDG France															2.5[6] (1.4-4.6) [6]	no correction	
EG: additional. assessment															25[6]	no correction	
EG G3	Calc.	2.02	2.81	7.9	8	8.1	197	223	250	1	**0.7**	**1**	0.4		1.9[6]	1.9[7]	1.3[7]
	M	2.02	2.81	7.9	8	8.1	197	223	250	1	**0.7**	**1**	0.4	50	1.8 [2]	1.8 [4]	1.3 [4]
EG J2	Sea														3.6[6]	no correction	
EG K4	Sea														4.0[6]	no correction	
EG M1		1.6 [1]	2.7	7.2 [1]	8	8.3 [1]	168	248	312	1	**0.7**	**1**	0.4		2.0[6]	2.0[7]	1.4[7]
EG W+X	Sea														2.2[6]	no correction	
Brass 2		2.09	2.7	7.5	7.7	7.8	195	257	292	1	**0.7**	**1**	0.5		1.6[6]	1.6[7]	1.1[7]
Brass 3		1.14	1.9	8	8.1	8.4	151	169	265	1	**0.9**	**1**	0.5		1.3[6]	1.3[7]	1.2[7]
Brass 6 (UK)		-	-	-	-	-	-	-	-						6.4[6]	no correction	
Brass 8		5.3	7.6	8.3	8.6	8.9	160	220	280	**0.5**	**0.4**	0.4	0.2		4.0[6]	2[7]	1.6[7]
Brass 10		-	-	-	-	-	-	-	-						2.4[6]	no correction	
Brass 12		-	-	-	-	-	-	-	-						27[6]	no correction	
Alloy 1		9.7 [1]	15.3-18.2	6.5 [1]	7.21-7.32	7.5 [1]	46.7 [1]	154-181	343 [1]	0.3	0.1	**0.6**	**0.2**		8.6[6]	5.2[7]	1.7[7]

	Remark	DOC (mg/l)		pH			Hardness (CaCO3 mg/l)			BioF algae	BioF algae	BioF fish	BioF fish	PEC water 3)	(PE)C_{add} / $PNEC_{add}$	(PE)C_{add} / $PNEC_{add}$	(PE)C_{add} / $PNEC_{add}$
		10P	50P	10P	50P	90P	10P	50P	90P	10-90	50-50	10-10	50-50	(µg/l)	uncorrected	r.w.c.	avg.
Die casting UK		-	-	-	-	-	-	-	-						18[6]	no correction	

M Based on measured concentration in surface water.

Calc Based on calculated concentration in surface water.

1) No specific data available: values are calculated on the bases of the GEMS-A database.

1) Local PEC_{add} / $PNEC_{add}$ value based on the measured PEC value (previous column, being the measured total zinc concentration) minus the natural background concentration of 12 µg/l (total Zn) and a $PNEC_{add}$ of 21 µg/l (total Zn), see also footnote 3.

2) Local PEC water: Local measured total zinc concentration in surface water.

3) Realistic worst case (r.w.c.) and average (avg.) local PEC_{add} / $PNEC_{add}$ value based on the measured PEC value minus the natural background concentration of 12 µg/l (total Zn) and a $PNEC_{add}$ of 21 µg/l (total Zn), see also foornote 2. According to information from company alloy 4 their contribution to the significant zinc elevation between upstream and downstream zinc levels is negligible.

4) BioF is calculated with a DOC of 10.

5) Local C_{add} / $PNEC_{add}$ value based on the calculated local C_{add} (dissolved Zn) and a $PNEC_{add}$ of 7.8 µg/l (dissolved Zn), see also footnote 7.

6) Realistic worst case (r.w.c.) and average (avg) local C_{add} / $PNEC_{add}$ value based on the calculated local C_{add} (dissolved Zn) and a $PNEC_{add}$ of 7.8 µg/l (dissolved Zn), see also footnote 6).

Table 3.104 Site-specific information on sediment SEM/AVS for a number of production and processing sites.

		AVS_{total} µmol/gDW	**SEM Zn** µmol/gDW	**SEM Zn, bioav.** µmol/g.DW	**AVS_{total}-Cb**	**RCR**
Production 4 [1)]		2.6-23.7	2.98-14.2	-9.5-0.38	1.57-22.7	-12.7 - 0.51
Production 15		226	90.4	-136	225	-181
Production 28		179	81.2	-98	178	-130
Alloy 1 [1)]		2.6-23.7	2.98-14.2	-9.5-0.38	1.57-22.7	-12.7 - 0.51
Alloy 4 [2)]		0.28-0.3	1.47-2.65	1.19-2.35	-0.72 - -0.74	0.59 - 2.2
Rolled Zinc 1		0.13-0.76	1.01-1.08	0.32-0.88	-0.26 - -0.89	-0.020 - 0.074
Rolled Zinc 4 [2)]		0.28-0.3	1.47-2.65	1.19-2.35	-0.72 - -0.74	0.59 - 2.2

1. Same site.
2. Same site.

3.4.3.1.2 Surface water (incl. sediment)

Production

Surface water. For a number of production sites the calculated local C_{add} (dissolved Zn) in water is greater than the $PNEC_{add}$ in surface water of 7.8 µg/l (dissolved Zn). The exposure assessments for all production companies are based on site-specific emission data. For several production companies, also site-specific measured zinc concentrations (total Zn) were reported for the receiving surface water (see section 3.2.5.2); these measured concentrations are indicated as "PEC" water in Table 3.103. Measured data are generally preferred above calculated values and as such further used in this risk characterisation. After correction of these measured concentrations for a natural background concentration range of 3-12 µg/l, these values (local "PEC_{add}" values) can be compared with the $PNEC_{add}$ water (total Zn: 21 µg/l). It is clear that for a number of sites the (natural background corrected) monitoring data exceed the $PNEC_{add}$. The same is true for a number of the calculated local C_{add} values for which no accompanying monitoring data are available.The above-mentioned considerations refer to a comparison of the measured data (local "PEC_{add}" values) and/or local C_{add} values with the $PNEC_{add}$, without any additional correction for the bioavailability of zinc in the receiving surface water. The subsequent step is that for those scenarios for which a local $(PE)C_{add}$ / $PNEC_{add}$ ratio above 1 is identified without correction, a correction for bioavaialability will be carried out. This will be done based on the BLM models for algae and fish (see section 3.3.2.1.1). However, prerequisite for applying these BLM models is that reliable and site/region-specific information on the key parameters for using the BLM model is available. For all production sites with an uncorrected local $(PE)C_{add}$ / $PNEC_{add}$ ratio above 1 such data were submitted. Bioavailability factors are being derived for two scenarios of abiotic conditions. One scenario refers to an average setting and the second one to a 'realistic worst case' setting. The highest bioavailability factor ($BioF_{water}$) is subsequently used in the risk characterisation by multiplying the original $(PE)C_{add}$ with this $BioF_{water}$. The data are presented in Table 3.103. Because the $BioF_{water}$ for either fish or algae dominated the bioavailibility correction, the $BioF_{water}$ values for Daphnia are not presented in Table 3.103. If a site has a discharge to seawater, no bioavailability correction is performed, as the BLM models were developed for freshwaters.

Table 3.103 shows that for none of the production sites the 'realistic worst case' bioavailability correction would bring the local (PE)C_{add} / $PNEC_{add}$ ratios to values below 1. As a result a **conclusion iii**) should be drawn for those production sites. The rapporteur realises that the measured concentrations also include an ambient (regional) component. This may explain the fact that in some cases (sites no. 27 and 28) the local risk characterisation based on measured data points to a potential risk, whereas the risk characterisation for the same sites based on calculated data is less conclusive (especially when using 'average' corrected local C_{add} / $PNEC_{add}$ ratios).

For all remaining production sites i.e. those not included in Table 3.103 a **conclusion ii)** is drawn, as the local C_{add} / $PNEC_{add}$ ratio is < 0.5 and the PEC_{add} / $PNEC_{add}$ ratio (available only for site no. 3) is < 1.

Sediment. For a number of production sites the calculated local C_{add} in sediment exceeds the $PNEC_{add}$ sediment of 11 mg/kg wwt (Table 3.102). The same would be true when using the measured sediment data that are available for some sites (indicated as local "PEC" and, after correction for the natural background concentration [140 mg/kg dwt, equivalent to 49 mg/kg wwt] as local "PEC_{add}"). These considerations refer, however, to a comparison of the local (PE)C_{add} without any correction for the bioavailability of zinc in the sediment. The subsequent step is that for those scenarios for which a local (PE)C_{add} / $PNEC_{add}$ ratio above 1 is identified without correction, a correction for sediment bioavaialability will be carried out. This will be done based on the SEM/AVS method (see section 3.3.2.2.1). However, prerequisite for applying the SEM/AVS method is that reliable and site-specific information on the key parameters for using the SEM/AVS method is available. For three production sites (no. 4, 15 and 28) with an uncorrected local (PE)C_{add} / $PNEC_{add}$ ratio above 1 such data were submitted. The needed SEM/AVS data are presented in Table 3.104. Similar to water both an 'average' and a 'realistic worst case' setting should be covered when applying the SEM/AVS method. However, insufficient data on the variability of AVS levels were provided to allow such comparison. If a production site has a discharge to sea, no bioavailability correction is performed. For the three above-mentioned production sites (no. 4, 15 and 28) the SEM/AVS method reduces the PEC_{add} / $PNEC_{add}$ ratio to a value below 0 **(conclusion ii)**.

For the remaining sites with an uncorrected local (PE)C_{add} / $PNEC_{add}$ ratio above 1, no site-specific data were submitted on SEM/AVS contents. Therefore only the generic sediment bioavailability correction factor of 0.5 can be applied. This implies that the original sediment (PE)C_{add} from Table 3.102 are multiplied with a factor 0.5. After this correction the (PE)C_{add} / $PNEC_{add}$ ratio remains above 1 for these scenarios (**conclusion iii**). For the remaining production sites the ratio is < 1, but due to (possibly) high regional background concentrations a local risk cannot be excluded (**conclusion iii***).

Use categories

Surface water. The local (PE)C_{add} in water for the processing sites of zinc metal exceeds the $PNEC_{add}$ for surface water in a number of scenarios, being 'CHDG' (a number of individual sites and the additional generic assessment), 'EG' (a number of individual sites and the additional generic assessment), 'brass' (a number of individual sites), 'alloy and die casting' (one individual site) and 'die casting' (UK data). In contrast with the production scenarios (see above), also additional generic scenarios have been used for some of the processing scenarios of zinc. This due to a lack of (sufficient) site-specific data for those use categories. The above-mentioned considerations refer to a comparison of the local (PE)C_{add} and $PNEC_{add}$ without any correction for the bioavailability of zinc in the receiving surface water. The subsequent step is that for those scenario for which a local (PE)C_{add} / $PNEC_{add}$ ratio above 1 is identified without correction, a correction for bioavaialability will be carried out. This will be

done based on the BLM models (see above for production sites). Note that no suitable site-specific data on water characteristics needed to use the BLM models implies no bioavailability correction. For some processing sites with an uncorrected local (PE)C_{add} / $PNEC_{add}$ ratio above 1, reliable and site/region-specific information on the key parameters for using the BLM model was submitted. The data are presented in Table 3.103. If a processing site has a discharge to sea, no bioavailability correction is performed.

Table 3.103 shows that for none of the processing sites the 'realistic worst case' bioavailability correction would bring the local (PE)C_{add} / $PNEC_{add}$ ratios to values below 1, thus a **conclusion iii** should be drawn for these sites. The same implicitly holds for processing sites for which no suitable correction data were submitted.

For a number of scenarios the (PE)C_{add} / $PNEC_{add}$ ratio is between 0.5 and 1. For those scenarios a potential risk at local scale cannot be excluded due to (possibly) high regional background concentrations (**conclusion iii***). The (PE)C_{add} / $PNEC_{add}$ ratio is <0.5 for the remaining processing scenarios **(conclusion ii).**

Sediment. For sediment the local (PE)C_{add} / $PNEC_{add}$ ratio is larger than 1 for a number of scenarios, being 'CHDG' (a number of individual sites and the additional generic assessment), 'EG' (a number of individual sites and the additional generic assessment), 'brass' (a number of individual sites), 'alloy and die casting' (a number of individual sites), 'die casting' (UK and France data), 'rolled/wrought zinc' (site no. 1) and 'zinc powder/dust' (sites no. 11 and 27 and remaining ones). These considerations refer, however, to a comparison of the local (PE)C_{add} and $PNEC_{add}$ without any correction for the bioavailability of zinc in the sediment. The subsequent step is that for those scenarios for which a (PE)C_{add} / $PNEC_{add}$ ratio above 1 is identified without correction, a correction for sediment bioavaialability will be carried out. This will be done based on the SEM/AVS method (see above for production sites). For three processing sites reliable and site-specific information on the key parameters for using the SEM/AVS method data was submitted. The needed SEM/AVS data are presented in Table 3.104. For processing sites 'alloy no. 1' and 'rolled zinc 1' the SEM/AVS method reduces the (PE)C_{add} / $PNEC_{add}$ ratio to a value below 1 **(conclusion ii)**. When using the SEM/AVS method the (PE)C_{add} / $PNEC_{add}$ ratio is still above 1 for processing site 'alloy no.4/rolled zinc 4' (**conclusion iii**). For the remaining sites with an uncorrected local (PE)C_{add} / $PNEC_{add}$ ratio above 1, no site-specific data were submitted on SEM/AVS contents. Therefore only the generic sediment bioavailability correction factor of 0.5 can be applied. This implies that the original sediment (PE)C_{add} from Table 3.102 are multiplied with a factor 0.5. After this correction the (PE)C_{add} / $PNEC_{add}$ ratio remains above 1 for these scenarios (**conclusion iii**), except for some 'CHDG' sites and 'zinc powder/dust remaining scenarios', see Table 3.105.

The (corrected) local C_{add} / $PNEC_{add}$ ratio is <1 for the remaining use category scenarios, but due to (possibly) high regional background concentrations a potential risk at local scale cannot be excluded **(conclusion iii*).**

3.4.3.2 Terrestrial compartment

Production

For all production sites, the local PEC_{add} for soil (agricultural soil) is below the $PNEC_{add}$ (**conclusion ii**).

Use categories

Some use category scenarios, i.c. both generic and site-specific ones for 'CHDG' and 'EG', resulted in local PEC_{add} / $PNEC_{add}$ ratios >1 (see Table 3.102). As relevant data are lacking to perform a site-specific

correction for bioavailability in soil (soil type characteristics), only the generic soil correction factor of 3 (R_{L-F}: ageing aspects) can be applied. This implies that the original terrestrial PEC_{add} values from Table 3.102 are divided by a factor 3. After this correction the PEC_{add} / $PNEC_{add}$ ratio for soil remains above 1 for six scenarios **(conclusion iii),** see Table 3.105. For the remaining (corrected) scenarios the PEC_{add} / $PNEC_{add}$ ratios are all < 1 (**conclusion ii**).

3.4.3.3 Atmospheric compartment

A quantitative risk characterisation for exposure of organisms to airborne zinc is not possible. This because there are no useful data on the effects of airborne zinc on environmental organisms and thus no PNEC for air could be derived. The PECs in air will be used for the risk assessment of man indirectly exposed via the environment (Chapter 4).

3.4.3.4 Secondary poisoning

Not relevant.

Table 3.105 Summary of the uncorrected and corrected local (PE)C_{add} / $PNEC_{add}$ ratios used in the local risk characterisation of zinc metal.

	Uncorrected				Corrected			
Company	PEC /PNEC STP	C_{add} / $PNEC_{add}$ water	C_{add} / $PNEC_{add}$ sediment	PEC_{add} / $PNEC_{add}$ agr. soil	C_{add} / $PNEC_{add}$ water r.w.c. [3]	C_{add} / $PNEC_{add}$ water avg. [3]	C_{add} / $PNEC_{add}$ sediment	PEC_{add} / $PNEC_{add}$ agr. soil
PRODUCTION COMPANIES:								
Company 1	12	13	233	0.14	13 [9]		117	
Company 1: measured concentrations		13.7 / 14.1 [7]			13.7 / 14.1 [7] [9]			
Company 3	7.2	0.25	4.3	0.60			2.2	
Company 3: measured concentrations		0.6 / 1.0 [7]						
Company 4	7.8	21	380	0.12	13	4.2	-12.7 - 0.51 [12]	
Company 4: measured concentrations		4.2 / 4.6 [7]	0.9 [4] / 15 [5] [8]		2.5	0.8	-12.7 - 0.51 [12]	
Company 8	23	25	453	0.51	25 [9]		227	
Company 8: measured concentrations		1.8 / 2.2 [7]			1.8 / 2.2 [7] [9]			
Company 9	1.2	1.3	24	0.042	1.3 [9]		12	
Company 12	0	0	0	0.035				
Company 15	3.4	1.0	18	0.36			-181 [12]	
Company 15: measured concentrations		9.0 / 9.5 [7]	120-180 [8]		4.2	1.7	-181 [12]	
Company 16	0.13	0.000034	0.0006	0.027				
Company 18	1.2	0.00070	0.012	0.15				
Company 20 (1995)	not appl.	20	354	0.41	20 [9]		177	
Company 20 (2002)	not appl.	16.2	290	0.18	16.2 [9]		145	
Company 20: measured concentrations		0-0.2 / 0.2-0.6 [7]	32 [8]				16	
Company 21 (1998)	2.5	2.8	49	0.37	2.8 [9]		25	

	Uncorrected				Corrected			
Company	PEC /PNEC STP	C_{add} / $PNEC_{add}$ water	C_{add} / $PNEC_{add}$ sediment	PEC_{add} / $PNEC_{add}$ agr. soil	C_{add} / $PNEC_{add}$ water r.w.c. [3]	C_{add} / $PNEC_{add}$ water avg. [3]	C_{add} / $PNEC_{add}$ sediment	PEC_{add} / $PNEC_{add}$ agr. soil
Company 21 (2002)	0.19	0.21	4	0.26			1.8	
Company 21: measured concentrations		1.5 / 1.9 [7]	0-7.5 [8]		1.5 / 1.9 [7] [9]		0-3.7	
Company 22	2.7	2.9	52	0.024	2.9	1.5	26	
Company 23	4.4	0.014	0.3	0.070				
Company 24	0	0	0	0.040				
Company 26	0	0	0	0.048				
Company 27 [1]	1.7	0.43	7.7	0.25			3.8	
Company 27 total [2]	9.9	2.5	45	0.276	1.5	0.5	22	
Company 27: measured concentrations		18.5-47 / 18.9-47.5 [7]			11-28	3.7-9.4		
Company 28	25	2.6	47	0.033	2.6	1	-130 [12]	
Company 28: measured concentrations		6.5 / 7.0 [7]	30-178 [8]		6.5	2.6	-130 [12]	
GALVANISING:								
General Hot Dip Galvanising (GHDG): aqueous discharges from run-off, waste water conc. for 20 NL plants	0.0016-0.034	0.0018-0.037	0.03-0.66	0				
Continuous Hot Dip Galvanising (CHDG): additional assessment	34	37	667	37	37 [10]		333	12
CHDG Company A	11	12	220	0.021	11	7.4	110	
CHDG Company B	0.16	0.17	3	0.021			1.5	
CHDG Company B: measured concentrations		0.1 / 0.6 [7]						
CHDG Company C	0.50	0.00046	0.008	0.021				
CHDG Company E1	0	0	0	0.021				
CHDG Company E2 (new data; year unknown)	0.10	0.10	1.8	0.12			0.9	

	Uncorrected				Corrected			
Company	PEC /PNEC STP	C_{add} / $PNEC_{add}$ water	C_{add} / $PNEC_{add}$ sediment	PEC_{add} / $PNEC_{add}$ agr. soil	C_{add} / $PNEC_{add}$ water r.w.c. [3)]	C_{add} / $PNEC_{add}$ water avg. [3)]	C_{add} / $PNEC_{add}$ sediment	PEC_{add} / $PNEC_{add}$ agr. soil
CHDG Company G1 and G2	1.0	1.1	20	1.1	1.1	0.8	10	0.37
CHDG Company H	0.63	0.68	12.3	0.69			6.2	
CHDG Company H: measured concentrations		0.6 / 1.0 [7)]						
CHDG Company I	0.038	0.041	0.7	0.061				
CHDG Company J1	0	0	0	0.021				
CHDG Company K1	0.89	0.012	0.22	0.021				
CHDG Company K3	2.7	0.027	0.47	0.021				
CHDG Company L1	5.6	0.40	7.2	0.021			3.6	
CHDG Company L2	0.47	0.017	0.3	0.021				
CHDG Company M1	0	0	0	0.021				
CHDG Company M3	0	0	0	0.021				
CHDG Company M4	0	0	0	0.021				
CHDG Company M5	0	0	0	0.021				
CHDG Company M6	0.000075	0.000081	0.0014	0.021				
CHDG Company M7	0.042	0.045	0.8	0.066			0.4	
CHDG Company O	0.022	0.024	0.4	0.045				
CHDG Company P	4.0	2.1	37	4.3	2.1	1.5	18	1.4
CHDG Company Q	2.4	2.6	46	2.6	2.6 [9)]		24	0.87
CHDG Company R	0	0	0	0.021				
CHDG Company T	2.2	$2.97.10^{-6}$	$5.3.10^{-5}$	2.4				0.8
CHDG Company U	0.58	0.078	1.4	0.65			0.7	

	Uncorrected				Corrected			
Company	PEC /PNEC STP	C_{add} / $PNEC_{add}$ water	C_{add} / $PNEC_{add}$ sediment	PEC_{add} / $PNEC_{add}$ agr. soil	C_{add} / $PNEC_{add}$ water r.w.c. [3]	C_{add} / $PNEC_{add}$ water avg. [3]	C_{add} / $PNEC_{add}$ sediment	PEC_{add} / $PNEC_{add}$ agr. soil
CHDG Company V	0.34	0.37	6.5	0.38			3.2	
CHDG Company W	no data	no data	no data	no data			no data	no data
CHDG Company X	2.1	2.2	40	2.2	2.2 [9]		20	0.7
CHDG Company Y1 and Y2	0.074	0.080	1.5	0.10			0.7	
CHDG France	2.4 (0-2.3)	2.5 (1.4-4.6)	45 (24-83)	2.5 (1.4-4.6)	2.5 (1.4-4.6) [10]		22 (12-42)	0.83 (0.47-1.5)
Electro Galvanizing (EG): additional assessment	23	25	451	25	25 [10]		226	8.3
EG Company D	8.0	0.0039	0.07	0.021				
EG Company F	1.6	0.32	5.8	1.7			2.9	0.57
EG Company G3	1.7	1.9	34	1.9	1.9	1.3	17	0.63
EG Company G3: measured concentrations		1.8 / 2.2 [7]			1.8	1.3		
EG Company J2	3.4	3.6	65	3.6	3.6 [9]		33	1.2
EG Company K2	5.9	0.34	6	0.02			3	
EG Company K2: measured concentrations		0 / 0.3 [7]						
EG Company K4	3.7	4.0	72	0.02	4.0 [4]		36	
EG Company M1	1.8	2.0	35	2.0	2	1.4	17	0.67
EG Company M2	no data	no data	no data	no data			no data	no data
EG Company M4	0.34	0.36	6.5	no data			3.3	no data
EG Company M6	0.34	0.36	6.5	0.38			3.3	
EG Company N	15	0.059	1.1	16			0.5	5.3
EG Company W+X	2.1	2.2	40	2.2	2.2 [9]		20	0.73
BRASS:								

	Uncorrected				Corrected			
Company	PEC /PNEC STP	C_{add} / $PNEC_{add}$ water	C_{add} / $PNEC_{add}$ sediment	PEC_{add} / $PNEC_{add}$ agr. soil	C_{add} / $PNEC_{add}$ water r.w.c. [3]	C_{add} / $PNEC_{add}$ water avg. [3]	C_{add} / $PNEC_{add}$ sediment	PEC_{add} / $PNEC_{add}$ agr. soil
Brass company 1	0	0	0	0.021				
Brass company 2	0.72	0.78	14	0.033			7	
Brass company 3	0.37	0.40	7	0.028			3.5	
Brass company 4	0.19	0.20	3.6	0.024			1.8	
Brass company 5	0.46	0.50	9	0.057			5	
Brass company 6	6.0	6.4	115	0.027	6.4 [11]		58	
Brass company 7	0.43	0.47	8	0.022			4	
Brass company 8	3.7	4.0	72	0.049	2	1.6	36	
Brass company 9	0.17	0.19	3	0.040			1.6	
Brass company 10	2.2	2.4	43	0.024	2.4 [10]		22	
Brass company 11	0.16	0.18	3	-			1.6	-
Brass company 12	25	27	489	0.045	27 [10]		245	
ALLOY AND DIE CASTING:								
Alloy production: company 1	7.9	8.6	153	0.024	5.2	1.7	-12.7 - 0.51 [12]	
Alloy production: company 3	0	0	0	0.021				
Alloy production: company 4	$3.24.10^{-4}$	$4.66.10^{-6}$	$8.16.10^{-5}$	0.025				
Alloy production: company 4: measured concentrations							0.59 - 2.2 [12]	
Alloy production: company 5	0	0	0	0.021				
Alloy production: company 6	0	0	0	0.021				
Alloy production: company 7	0.93	1.0	18	0.021			9	
Die casting: UK data (4 sites) water emissions	0.009-0.36	0.010-0.39	0.18-7	not appl.			0.08-3.5	not appl.
Die casting: UK data	16	18	316	0.11	18 [11]		159	

	Uncorrected				Corrected			
Company	PEC /PNEC STP	C_{add} / $PNEC_{add}$ water	C_{add} / $PNEC_{add}$ sediment	PEC_{add} / $PNEC_{add}$ agr. soil	C_{add} / $PNEC_{add}$ water r.w.c. 3)	C_{add} / $PNEC_{add}$ water avg. 3)	C_{add} / $PNEC_{add}$ sediment	PEC_{add} / $PNEC_{add}$ agr. soil
Die casting: German data	0.028	0.031	0.6	0.022				
Die casting: France data	0.55	0.60	11	0.021			5	
ROLLED/WROUGHT ZINC:								
Rolled/wrought zinc: company 1	0.47	0.50	9	0.021			-0.020 - 0.074 12)	
Rolled/wrought zinc: company 1: measured concentrations		0.2 / 0.6 7)	0 / 0 8)					
Rolled/wrought zinc: company 2	0	0	0	0.021				
Rolled/wrought zinc: company 3	0	0	0	0.021				
Rolled/wrought zinc: company 4	$3.24.10^{-4}$	$4.66.10^{-6}$	$8.16.10^{-5}$	0.025				
Rolled/wrought zinc: company 4: measured concentrations							0.59 - 2.2 12)	
Rolled/wrought zinc: company 5	0.016	0.017	0.3	0.023				
ZINC POWDER/DUST:								
Zinc powder/dust: companies 27 and A min an max emission air	not appl.	not appl.	not appl.	0.021-0.10			not appl.	
Zinc powder/dust: companies 11 and 27 min and max emission water	0.11-0.38	0.12-0.41	2.1-7.4	not appl.			1.1 – 3.6	not appl.
Zinc powder/dust: remaining two companies with unknown emissions	0.087	0.095	1.7	0.027			0.8	

1) Only zinc metal production separated from the other activities at this site;

2) Total emission values and concentrations of this zinc metal production site, including those at the production of zinc alloys, zinc calots (semis) and zinc powders;

3) Bioavailability correction for realistic worst case (r.w.c.) and average (avg.) PEC/PNEC value (see Table 3.103);

4) Downstream;

5) Upstream;

6) Measured concentration is a PEC value instead of a C_{add} value;

7) PEC/$PNEC_{add}$ value based on the measured PEC value minus the natural background concentration of 3 and 12 µg/l (total Zn) and a $PNEC_{add}$ of 21 µg/l (total Zn);

8) PEC/$PNEC_{add}$ value based on the measured PEC value minus the natural background concentration of 140 mg/kg dwt and a $PNEC_{add}$ of 49 mg/kg dwt;

9) No bioavailability correction is performed for discharges to sea;

10) No data available to perform a bioavailability correction;

11) No bioavailability correction is performed for the UK;

12) Cadd/$PNEC_{add}$ sediment is based on site specific SEM/AVS data (see Table 3.104).

not appl Not applicable

Industry Annex 3.4.3 contains recent local exposure information for a number of zinc producers and users. These data were not used in the current risk assessment, but can be useful for the risk reduction phase. **(Disclaimer: Industry annex 3.4.3 was found by the Rapporteur to be useful to risk management because it sheds further light on the recent local exposure data. Annex 3.4.3 has not been formally approved by either the Rapporteur or TC NES, but this Annex is included in the RAR Zn Metal-Environment Annexes report)**

3.4.4 Regional risk characterisation (including line sources)

3.4.4.1 Aquatic compartment (incl. sediment)

Surface water

Both calculated and measured regional zinc concentrations in surface water have been reported in section 3.2.5.3. The calculated PEC_{add}s regional amount to 12.2 and 16.8 µg/l (total) for, respectively, the NL-region and the EU-region. Using the $PNEC_{add}$ surface water of 21 µg/l (total; default of 15 mg/l suspended matter) results in PEC/PNEC ratios of 0.6 (NL-region) and 0.8 (EU-region). These PEC/PNEC ratios refer to values without an additional correction for bioavailability in surface water. At this stage of the risk assessment for zinc it is unnecessary to figure out which of those two scenarios reflects the real world situation at best. This because a great amount of Dutch and EU monitoring data are available for zinc in surface waters. In the regional risk characterisation preference is given to these monitoring data, as they are from prolonged monitoring programmes and are considered to be representative and valid. It should be mentioned, however, that both the calculated PECs fall within the range of monitoring data (after adding natural background concentration). In the risk characterisation only measured data for the period after 1995 will be used from Table 3.62, as they are considered to be most representative for the current risk assessment.

A number of areas in e.g. the Nordic countries (Sweden, Norway and Finland) and parts of Spain are characterised by 'soft water' conditions (hardness < 24 mg/l, as $CaCO_3$) for which the PNEC soft water should be applied (see section 3.3.2.1.5 and Annex 3.3.2). However, based on the available information it was difficult to assign distinct European areas where softwater conditions prevail. It was therefore decided that the entire risk characterisation for the soft water regions, had to be left out of account in the present generic risk assessment. This may be dealt with on a national/regional level during risk management (for guidance on application of the soft water PNEC, see Annex 3.3.2.C).

The average 90th-percentile values in major Dutch rivers and smaller regional waters have earlier been presented in Figure 3.4. A compilation of the data from this figure is shown in Table 3.106, in combination with the corresponding $PEC_{add}/PNEC_{add}$ ratios. Natural background concentration of 3 (or 6 for Meuse) and 12 µg/l have been subtracted from the monitoring data and, as it concerns Netherlands surface water (higher suspended matter concentration) data, the $PNEC_{add}$ of 33 µg/l (total; 30 mg/l suspended matter) has been used. Table 3.106 illustrates that in all monitored waters there is a decreasing trend in zinc concentrations during the period 1985-1998. In 1985, for all waters a $PEC_{add}/PNEC_{add}$ ratio larger than 1 is found. The Meuse river was found to have the highest ratios, 6.2-6.4. Based on data from the recent years 1997 and 1998 the $PEC_{add}/PNEC_{add}$ratio is still >1 in the Meuse (1.7-1.9). This conclusion is supported by data on dissolved zinc levels in the Meuse (22.6 µg/l in 1997 and 15.1 µg/l in 1998). In the Rhine and Scheldt the 1997-1998 ratios have all

become < 1. For Dutch regional and state waters the PEC/PNEC ratios vary around 1 (0.8-1.2) for the years 1997-1998.

Table 3.106 Measured PEC_{add} values and $PEC_{add}/PNEC_{add}$ ratios (without correction for bioavailability) for surface waters in the Netherlands during the period 1985-1998. For further explanation: see text.

	1985	**1995**	**1996**	**1997**	**1998**
Rhine, average 90th P-value (μg/l)	69	39	40	28	26
Corrected values (resp. 3 and 12 μg/l)	66-57	36-27	37-28	25-16	23-14
Rhine $PEC_{add}/PNEC_{add}$	2-1.7	1.1-0.8	1.1-0.8	0.8-0.5	0.7-0.4
Meuse, average 90th P-value (μg/l)	217	152	158	102	68
Corrected values (resp. 6 and 12 μg/l)	211-205	146-140	152-146	96-90	62-56
Meuse $PEC_{add}/PNEC_{add}$	6.4-6.2	4.4-4.2	4.6-4.4	2.9-2.7	1.9-1.7
Scheldt, average 90th P-value (μg/l)	134	32	27	29	24
Corrected values (3 and 12 μg/l)	131-122	29-20	24-15	26-17	21-12
Scheldt $PEC_{add}/PNEC_{add}$	3.9-3.7	0.9-0.6	0.7-0.4	0.8-0.5	0.6-0.4
Regional, average 90th P-value (μg/l)*	96	53	46	41	n.a.
Corrected values (3 and 12 μg/l)	93-84	50-41	43-34	38-29	-
Regional $PEC_{add}/PNEC_{add}$	2.8-2.5	1.5-1.2	1.3-1.0	1.2-0.9	-
State waters, average 90th P-value (μg/l)*	76	74	77	44	40
Corrected values (3 and 12 μg/l)	73-64	71-62	74-65	41-32	37-28
State waters $PEC_{add}/PNEC_{add}$	2.2-1.9	2.2-1.9	2.2-2.0	1.2-1.0	1.1-0.8

* Regional waters are small waters spread over the Netherlands. Sampling points differ per year.

** State waters are large surface waters in the Netherlands minus Rhine, Meuse and Scheldt.

Data from France and Germany (Table 3.62) show that for a number of waters the PEC/PNEC would exceed 1. For example the 90 P value for the Rhin-Meuse area in North east France amounts to 99 μg/l, resulting in PEC/PNEC ratios of 3 (3 μg/l natural background) and 2.6 (12 μg/l natural background) using the PNEC of 33 μg/l.

The 90P values of 146 μg/l (1999) and 110 μg/l (2000) from the monitoring network in Flanders (Belgium) clearly exceed the current PNECs for surface water. PEC/PNEC ratios for 1999 would amount to 4.1-4.3 (resp. natural background correction of 12 and 3 μg/l) with the $PNEC_{add}$ of 33 μg/l. For 2000 the PEC/PNEC ratios would be 3-3.2. In 41% of the Flanders sampling points, out of a total of 670, the zinc water concentration is found to be above 100 μg/l. This means that the corresponding PEC/PNEC ratio is above 2.7-2.9 ($PNEC_{add}$ of 33 μg/l). Similar PEC/PNEC ratios are found in 27% of the sampling points (total of 806 sampling points) in 2000. In 15% (1999) and 8% (2000) of the sampling points the zinc

surface water level is above 200 µg/l. The corresponding PEC/PNEC is then above 5.7-6 ($PNEC_{add}$ of 33 µg/l).

Recent data (2001) for the Walloon Region (Belgium) indicate that 23% of the sampling points (total of 179) have PEC/PNEC ratios above 1.2-1.4 ($PNEC_{add}$ of 33 µg/l) or 1.8-2.2 ($PNEC_{add}$ of 21 µg/l). This refers to data points with a zinc concentration above 50 µg/l. In 10% of the cases the concentration exceeds 100 µg/l. The PEC/PNEC ratios then exceed 2.7-2.9 ($PNEC_{add}$ of 33 µg/l) or 4.1-4.6 ($PNEC_{add}$ of 21 µg/l). The few points with a zinc concentration above 200 µg/l have a PEC/PNEC ratio greater than 5. It should be noted that the total zinc data from Walloon Region are based on a somewhat different extraction method (‘’zinc extractible’) which may account for maximally about 30% underestimation of ‘real’total zinc levels.

In Figure 3.5 (section 3.2.5.3.4) the average 90P surface water data are given for various sampling locations across the Meuse river (period 1996-2000). Zinc levels in this Meuse transect are found to range from 29 µg/l (Dave) to 129 µg/l (Engis). High levels are also measured in Liege and Kinrooi (both 106 µg/l). Using the PNEC of 33 µg/l and subtracting both 6 and 12 µg/l (Meuse) the PEC/PNEC ratios for these Meuse points become: Dave 0.5-0.6; Engis: 3.5-3.7; Liege and Kinrooi: 2.8-3.0.

It can be stated that zinc concentrations in Netherlands (and probably also in several other EU countries) surface waters have decreased clearly during the period 1985-1998. However, recent monitoring concentrations in the Netherlands (Meuse) and the EU indicate that the PNEC is still being exceeded in a number of surface waters. The above-mentioned conclusion refers to the monitoring data without correction for bioavailability in surface water. In Table 3.107 those regional waters are presented that result in PEC/PNEC ratios above 1 for surface water without correction (as discussed above). Additionally, the table gives the typical abiotic parameters for those regions/waters that are necessary to apply the various BLM models for performing the bioavailability corrections. The corresponding bioavailability factors for two scenarios of abiotic conditions are presented as well. The scenarios refer to an average setting and a ‘realistic worst case’ setting (see local risk characterisation).

An overall view on Table 3.107 results shows that the average $BioF_{water}$ for the various EU waters ranges from 0.3 to 0.9 and the realistic worst case $BioF_{water}$ from 0.6 to 1.0. When linking the uncorrected PEC/PNEC ratios for the various regional waters with the corresponding bioavailability factors (multiplication of original PEC_{add} with BioF), the following conclusion can be drawn: irrespective whether the average factor or the realistic worst case factor is taken the PEC/PNEC ratios will in most cases remain substantially above 1 for the various EU waters. This is especially the case for the Meuse (The Netherlands and Belgium), Flanders, Walloon Provences, various German rivers and the French region. The PEC/PNEC ratios for the regional and state waters in the Netherlands (Table 3.104) will become just below 1 after correction with the BioF for ‘NL average’ in Table 3.105 The Flanders PEC/PNEC ratios for surface water remain above one, even if the 90P DOC levels from the GEMS-A (substitute for Flanders) would be used (correction factors of 0.3 and 0.6). Table 3.108 gives a summary of the risk characterisation for the regional measured surface water data. Overall, a **conclusion iii)** is drawn for the regional scale as in a number of EU areas the measured surface water concentration of zinc exceeds the PNEC. This conclusion includes the correction step for the bioavailability of zinc in surface water.

Further considerations on conclusion iii) for regional surface water: influence of point sources, historical emissions etc..

The influence of point sources will of course be great in highly populated and industrialised areas like the Ruhr area in Germany or Flanders, Belgium. On the other hand it is felt that this is no reason to discard these data from the database. In those areas the large number of point sources in fact constitute the high regional background of zinc and every sampling station would lie somewhere in the vicinity of a point source. Data from sampling stations in the very near vicinity of the discharge point of the point source, i.e. the 'official' TGD defined PEC_{local}, may even point to higher zinc concentrations in those areas. For several regions in the Elbe river basin the LAWA report "Zielvorgaben zum Schutz oberirdischer Binnengewässer, Band II (1998) identifies former mining activities as one of the main reasons to high zinc levels. For some other German regions, the LAWA report mentions that former mining activities or contaminated sediments are an additional emission source. However, the report also underlines several times that it was not possible to quantify the shares of specific sources at that time. In addition, emissions from (former) mining areas may still contribute to present zinc levels in those waters at a scale larger than the immediate vicinity of the mine. For that reason it is felt that these data should not be discarded from the exposure assessment. Another aspect being relevant, especially for Flanders, is the low STP connection rate in that region. Untreated wastewater from many point sources may be responsible for considerable zinc input in Flanders. This because household sewage emissions are shown to constitute to a significant part of the zinc wastewater input in The Netherlands (see section 3.2.5.3.1), a region being comparable to Flanders in population density.

It can be concluded that several explanations can be given why the zinc concentration in those areas exceeds the levels in the 'model' region The Netherlands. This has to do with a.o. more intense and condensed industrial activities, the presence of (former) mining activities and, possibly, a lower STP connection rate. The geographical scale of those EU areas with high zinc levels go beyond the local scale as defined in the TGD. **However, when deciding about (possible) emission reduction measures, the available information on potential zinc emission sources in that particular area has to be carefully taken into account**.

The zinc industry executed a further analysis on the available regional monitoring data. Besides more technical correction steps, e.g. an outlier analysis, industry also made a selection of data that are assumed to be influenced by point sources and historical industrial activities (mining). The impact of other point sources than presently covered in RAR (a.o. from the EPER 2004 database) is also brought forward in industry's analysis. As an example the impact of a large zinc emitting company at the Meuse river was identified via the EPER database. It refers to a large fertiliser producer (unintentional zinc emissions) at the Meuse river that is not specified in the zinc metal RAR. The contribution of this company to current zinc levels in Meuse surface water could be significant.

The analysis conducted by industry is presented in Annex 3.2.5. After a thorough validity check by the corresponding regional water quality managers the analysis in Annex 3.2.5 may be very useful for risk management purposes. (Disclaimer: The Industry annex 3.2.5. was found by the Rapporteur to be useful to risk management because it sheds further light on the possible sources of zinc and zinc compounds that contribute to regional concentrations from monitoring studies. Annex 3.2.5. has not been formally approved by either the Rapporteur or TC NES.

Trends based on recent surface water monitoring data

The risk assessment is generally based on emission and monitoring data until 1999. Below more recent surface water monitoring data are discussed and it is indicated to what extent these new data would influence the overall conclusions for surface water.

The regional zinc water concentrations for the Netherlands are 53 and 54 µg/l for, respectively, 1999 and 2000 (CBS/RIVM Milieucompendium, 2004). These values are higher than the 1997 value of 41 µg/l as given in Table 3.104. The concentration of 41 µg/l is used in the RAR as the regional background, because it is considered to be representative for an ambient regional background. The main reason is that sampling points in this regional Dutch dataset are not directly linked with obvious point sources. The value of 41 µg/l yielded a PEC/PNEC ratio below one, including bioavailability correction. When using the more recent figures of 53 and 54 µg/l the upper limit of the PEC/PNEC range would become (slightly) above one: the PEC/PNEC range is 0.4-1.2 when using a natural background correction of 3 and 12 µg/l, bioavailability factors of 0.8 (r.w.c.) and 0.3 (average), and a PNEC of 33 µg/l. In general it can be stated that the regional concentrations in The Netherlands clearly decreased until 1990, but afterwards they seem to remain at a rather constant level of around 50 µg/l. An explanation for this phenomenon may be the diffuse zinc emissions from agriculture (manure application). This emission source seems to be a major diffuse zinc input nowadays in the Netherlands and it was shown to remain more or less constant during this period (see section 3.2.5.3.1). Recent data for the Rhine and 'state waters' in the Netherlands follow the decreasing trend in zinc levels as observed earlier (PEC/PNECs below one). For the B-NL border point on the Scheldt river a 90P value of 64 µg/l is measured in 1999 (CBS/RIVM Milieucompendium 2004) which is higher than the previous period (see Table 3.106). Values for the Scheldt in 2000 and 2001 are lower again, but for the year 2002 the zinc concentration amounts to 90 µg/l zinc (total). The latter value for total zinc is accompanied by 15 µg/l dissolved zinc. Zinc levels in the Scheldt river apparantly fluctuate during the recent years. The corresponding PEC/PNEC ratios for the Scheldt go from clearly below one to values that are at or above unity for the years 1999 and 2002.

For the Meuse river at the Dutch border (Eijsden) data for 1999, 2000 and 2001 point to a further decrease in zinc concentrations compared to the data used in Table 3.106. The corresponding PEC/PNEC would become just above one or slightly below. For the year 2002, however, a 90P value of 101 µg/l total zinc is reported, accompanied by a 90P value of 14 µg/l for dissolved zinc. Both 2002 Meuse values would give PEC/PNEC ratios above one after natural background and bioavailability correction: 2.2-2.9 (total) and 1.3-1.7 (dissolved).

From the very recent data from the Netherlands it can be concluded that the zinc levels in the Meuse may still exceed the PNEC. The **conclusion iii** for the Meuse therefore still holds. There may be uncertainty about the conclusion ii) earlier drawn for the Scheldt river based on recent data. Although recent regional background levels (diffuse sources) point to a high degree of zinc 'saturation' in the Netherlands, a conclusion ii) is still felt to be most appropriate.

More recent figures for France (2000-2002) point to a lower value for the Rhin-Meuse area (90P of 24 µg/l). Rather low values are also reported for Rhone Mediterranee Corse (8.2 µg/l) and Seine Normandie (6 µg/l). For the region Artoie Picardie a 90P value of 214 µg/l is found, including a possibly doubtful figure of 420 µg/l at one sampling station. It should also be noted that about 50% of the measurements (total of 185 measurements at 7 sampling stations) had a zinc level below the detection limit of 50 µg/l. These measurements were discarded from the 90P calculation, which may have caused a bias towards a higher 90P

value. Excluding the single value of 420 µg/l (and still also excluding measurements below the detection limit of 50 µg/l) would give a 90P value of 80 µg/l for Artoie Picardie. In the region Adour Garonne a 90P value of 174 µg/l was calculated based on 538 measurements above the detection limit, but excluding all data below the detection limit. This may again give a bias towards a high overall 90P value. A 90P value of 90 µg/l is calculated for the same region when only the data with a high detection limit (50 µg/l) were excluded. This gives a less biased value for that region. For France it can be concluded that the use of more recent data would result in a different conclusion for the Rhin-Meuse area (from conclusion iii) to conclusion ii), but data from 2000-2002 for other regions, in particular Artoie Picardie and Adour Garonne, show PEC/PNEC ratios above one (including appropriate bioavailability correction; see Table 3.108).

A recent survey in the UK (Environmental Agency report 6848, 2005) based on (dissolved) zinc monitoring data in different UK river catchment areas points to 'a relatively unfavourable regional zinc risk characterisation for surface waters in England'. The PEC/PNEC ratios of approximately 2 to 4 from the UK are typical of the estimates produced for other European regional surface waters with elevated zinc levels. The UK survey included a bioavailability correction similar to the one applied in the current zinc risk assessment (only average BioF!). The zinc industry commented on the original EA study, and, subsequently, the EA reacted on this. The EA response was that the report will need minor revision, but this will not change the overall conclusions.

Table 3.107 Characteristics (DOC, hardness and pH) of EU regional waters for which PEC/PNEC surface water exceeds one (without correction for bioavailability). Corresponding bioavailability factors ($BioF_{water}$) are calculated with Biotic Ligand Models for algae and fish. $BioF_{water}$ in bold represent the values that will be used in the risk characterisation for, respectively, average (50P DOC and 50P inorganics) and realistic worst case (algae 10P DOC and 90P inorganics and fish: 10P DOC and 10P inorganics) conditions.

	DOC (mg/l)		**pH**			**Hardness ($CaCO_3$ mg/l)**			**BioF algae 10-90**	**BioF algae 50-50**	**BioF fish 10-10**	**BioF fish 50-50**
	10P	50P	10P	50P	90P	10P	50P	90P				
Meuse B/NL	1.9	2.6	7.4	7.6	7.9	143	205	244	1.0	0.8	0.7	0.5
German rivers [2]												
Aller	4.5	5.5	7.6	7.7	7.9	161	179	189	0.6	0.5	0.4	0.3
Elbe	4.8	5.5	7.5	7.8	8.3	308	415	512	0.6	0.5	0.3	0.2
Ems	6.7	7.1	7.3	7.5	7.8	223	253	297	0.4	0.3	0.7	0.4
Main	2.5	3.2	7.7	7.9	8.3	238	287	314	0.9	0.7	0.5	0.3
Mosel	2.4	3.2	7.6	7.8	8.1	325	409	510	0.9	0.7	0.3	0.2
Mulde	3.4	4.2	7.0	7.3	7.6	145	179	205	0.7	0.5	0.7	0.5
Oder	4.8	5.6	7.5	7.8	7.9	216	229	240	0.5	0.4	0.6	0.4
Saale	4.5	5.5	7.7	7.9	8.1	511	775	1042	0.6	0.5	0.2	0.1
Saar	3.4	4.0	7.6	7.8	8.0	228	277	322	0.7	0.6	0.5	0.3
Weser	3.8	5.0	7.6	7.8	8.1	490	750	1012	0.8	0.6	0.2	0.1
Flanders [1]	2.4	4.1	7.0	7.8	8.1	42	151	308	0.9	0.6	1.0	0.4
Flanders, add. DOC	9.3 (90P)		7.0	7.8	8.1	42	151	308	0.3 (90-90)	0.3 (90-50)	0.6 (90-10)	0.3 (90-50)
Walloon Region	1.7	2.9	7.1	7.8	8.3	31	134	341	1.0	0.7	1.0	0.5
NL large lakes	5,3	7,6	8,3	8,6	8,9	160	220	280	0.5	0.3	0.4	0.1
NL small lakes	4,1	9,9	7,6	7,8	8	160	220	280	0.6	0.3	0.8	0.3

NL streams/brooks	13,9	18,2	7,3	7,4	7,5	118	220	322	0.3	0.2	0.6	0.2
NL ditches	15,3	27,5	6,1	6,9	7,7	260	350	440	0.2	0.1	0.6	0.2
NL sandy spring	1,2	2,2	6,6	6,7	6,8	76	79	82	0.7	0.6	1.0	1.0
NL Rhine	2,1	2,8	7,7	7,8	7,9	201	220	233	0.9	0.8	1.0	0.4
NL Meuse	2,3	3,3	7,5	7,7	7,9	165	220	275	0.9	0.7	1.0	0.4
NL 'average'	6.3	10.2	7.3	7.6	7.8	163	218	273	0.4	0.2	0.8	0.3
France Rhin Meuse	2.0	3.4	7.5	7.8	8.0	168	248	312	1.0	0.6	1.0	0.5

1. No specific data available. GEMS-A database is taken as substitute.
2. For a number of other German rivers with an 'uncorrected' PEC/PNEC > 1 no information on abiotic conditions is available. For those water the BioF based on GEMS-A will be used as default substitute (see Table cc).

Table 3.108 Summary table of regional risk characterisation for surface water, including the bioavailability correction. The PECs are the 90P values based on monitoring data for various EU regions. In those cases where no data on prevailing abiotic conditions are available, the $BioF_{water}$ based on the GEMS-A database are used as default estimate (respectively 1 (rwc) and 0.6 average).

	PEC	**PEC_{add} 0**		**BioF**		**Corrected PEC_{add}**				**PEC_{add} / $PNEC_{add}$**			
				rwc	aver	rwc	rwc	aver	aver	rwc	rwc	aver	aver
Meuse NL													
1997	102	96	90	1	0.8	96	90	76.8	72	**2.9**	**2.7**	**2.3**	**2.2**
1998	68	62	56	1	0.8	62	56	49.6	44.8	**1.9**	**1.7**	**1.5**	**1.4**
1997 (dissolved)	22.6	21.6	19.6	1	0.8	21.6	19.6	17.28	15.68	**2.8**	**2.5**	**2.2**	**2.0**
1998 (dissolved)	15.1	14.1	12.1	1	0.8	14.1	12.1	11.28	9.68	**1.8**	**1.6**	**1.4**	**1.2**
Flanders													
1999	146	143	134	1	0.6	143	134	85.8	80.4	**4.3**	**4.1**	**2.6**	**2.4**
2000	110	107	98	1	0.6	107	98	64.2	58.8	**3.2**	**3.0**	**1.9**	**1.8**
1999 (DOC ↑)	146	143	134	0.6	0.3	85.8	80.4	42.9	40.2	**2.6**	**2.4**	**1.3**	**1.2**
2000 (DOC ↑)	110	107	98	0.6	0.3	64.2	58.8	32.1	29.4	**1.9**	**1.8**	1.0	0.9
Meuse B													
Engis	129	123	117	1.0	0.8	123	117	98	94	**3.7**	**3.5**	**3.0**	**2.9**
Liege	106	100	94	1.0	0.8	100	94	80	75	**3.0**	**2.8**	**2.4**	**2.3**
Kinrooi	106	100	94	1.0	0.8	100	94	80	75	**3.0**	**2.8**	**2.4**	**2.3**
Walloon Prov													
Range 50-100	50	47	38	1	0.7	47	38	32.9	26.6	**> 1.4**	**> 1.2**	> 1.0	> 0.8
Range 100-200	100	97	88	1	0.7	97	88	67.9	61.6	**> 2.9**	**> 2.7**	**> 2.1**	**> 1.9**
Range > 200	200	197	188	1	0.7	197	188	137.9	131.6	**> 6.0**	**> 5.7**	**> 4.2**	**> 4.0**
Germany [1]													
Aller	169	166	157	0.6	0.5	99.6	94.2	83	78.5	**3.0**	**2.9**	**2.5**	**2.4**
Elbe	72	69	60	0.6	0.5	41.4	36	34.5	30	**1.3**	**1.1**	1.0	0.9
Ems	88	85	76	0.7	0.4	60	53	34	30	**1.8**	**1.6**	1.0	0.9
Erft	66	63	54	1	0.6	63	54	37.8	32.4	**1.9**	**1.6**	**1.1**	1.0
Freib. Mulde	140	137	128	1	0.6	137	128	82.2	76.8	**4.2**	**3.9**	**2.5**	**2.3**
Hunte	104	101	92	1	0.6	101	92	60.6	55.2	**3.1**	**2.8**	**1.8**	**1.7**
Ilm	103	100	91	1	0.6	100	91	60	54.6	**3.0**	**2.8**	**1.8**	**1.7**
Inn	60	57	48	1	0.6	57	48	34.2	28.8	**1.7**	**1.5**	1.0	0.9
Lausitz. Neisse	62	59	50	1	0.6	59	50	35.4	30	**1.8**	**1.5**	**1.1**	0.9
Lenne	90	87	78	1	0.6	87	78	52.2	46.8	**2.6**	**2.4**	**1.6**	**1.4**
Main	53	50	41	0.9	0.7	45	36.9	35	28.7	**1.4**	**1.1**	**1.1**	0.9
Mosel	52	49	40	0.9	0.7	44.1	36	34.3	28	**1.3**	**1.1**	1.0	0.8
Mulde	114	111	102	0.7	0.5	77.7	71.4	55.5	51	**2.4**	**2.2**	**1.7**	**1.5**
Nidda	70	67	58	1	0.6	67	58	40.2	34.8	**2.0**	**1.8**	**1.2**	**1.1**
Oder	60	57	48	0.6	0.4	34	29	23	19	**1.0**	**0.9**	0.7	0.6
Ruhr	61	58	49	1	0.6	58	49	34.8	29.4	**1.8**	**1.5**	**1.1**	0.9
Rur	179	176	167	1	0.6	176	167	105.6	100.2	**5.3**	**5.1**	**3.2**	**3.0**
Saale	135	132	123	0.6	0.6	79.2	73.8	79.2	73.8	**2.4**	**2.2**	**2.4**	**2.2**
Saar	68	65	56	0.7	0.6	45.5	39.2	39	33.6	**1.4**	**1.2**	**1.2**	1.0
Sachs. Saale	60	57	48	1	0.6	57	48	34.2	28.8	**1.7**	**1.5**	1.0	0.9

	PEC	PEC_{add} [0]		BioF		Corrected PEC_{add}				PEC_{add} / $PNEC_{add}$			
Schwarzenb.	60	57	48	1	0.6	57	48	34.2	28.8	**1.7**	**1.5**	1.0	0.9
Sieg	166	163	154	1	0.6	163	154	97.8	92.4	**4.9**	**4.7**	**3.0**	**2.8**
Stever	82	79	70	1	0.6	79	70	47.4	42	**2.4**	**2.1**	**1.4**	**1.3**
Unstrut	73	70	61	1	0.6	70	61	42	36.6	**2.1**	**1.8**	**1.3**	1.1
Ver. Mulde	291	288	279	1	0.6	288	279	172.8	167.4	**8.7**	**8.5**	**5.2**	**5.1**
Weisse Elster	90	87	78	1	0.6	87	78	52.2	46.8	**2.6**	**2.4**	**1.6**	**1.4**
Weser	181	178	169	0.8	0.6	142.4	135.2	106.8	101.4	**4.3**	**4.1**	**3.2**	**3.1**
Wupper	51	48	39	1	0.6	48	39	28.8	23.4	**1.5**	**1.2**	0.9	0.7
Zwick. Mulde	86	83	74	1	0.6	83	74	49.8	44.4	**2.5**	**2.2**	**1.5**	**1.3**
France													
Rhin-Meuse	99	96	87	1	0.6	96	87	58	52	**3.0**	**2.6**	**1.8**	**1.6**
Artoie Picardie 2000-2002 [2]	*214*	*211*	*202*	*1*	*0.6*	*211*	*202*	*126.6*	*121.2*	***6.4***	***6.1***	***3.8***	***3.7***
Artoie Picardie 2000-2002 [3]	*80*	*77*	*68*	*1*	*0.6*	*77*	*68*	*46.2*	*40.8*	***2.3***	***2.1***	***1.4***	***1.2***
Adour Garonne 2000-2002 [4]	*174*	*171*	*162*	*1*	*0.7*	*171*	*162*	*120*	*113*	***5.2***	***4.9***	***3.6***	***3.4***
Adour Garonne 2000-2002 [5]	*90*	*87*	*78*	*1*	*0.7*	*87*	*78*	*61*	*55*	***2.6***	***2.4***	***1.8***	***1.7***

[0] Subtraction of natural background of, respectively, lower limit 3 μg/l (6 μg/l in case of Meuse river) and upper limit 12 μg/l for <u>total</u> zinc. For dissolved zinc and natural background of 1-3(4) μg/l is used.

[1] In case measurements from more than one sampling station is available for a particular river, the highest 90P value is used is taken forward in the risk characterisation.

[2] 90P value not including the measurements below the detection limit of 50 μg/l.

[3] 90P value not including the measurements below the detection limit of 50 μg/l and excluding the possibly doubtful value from one sampling station.

[4] 90 P value based on data above the detection limit.

[5] 90 P value based on all data and using a value of detection limit/2 for values below detection limit (= 10 μg/l).

<u>Sediment</u>

The calculated regional concentrations (PEC_{add}) of zinc in sediment are 504 mg/kg dwt for the NL-region and 696 mg/kg dwt for the EU-region, excluding a natural background level of 140 mg/kg dwt. Monitoring data of sediments in the Netherlands are found in the same order of magnitude or higher (e.g. average/maximum values of 1001/2089 mg/kg and 293/4003 mg/kg dwt in Hollandsch Diep for freshly deposited layers). The latter values refer to freshly deposited layers, which means that the contribution of historical pollution is less relevant.. Recent sediment monitoring data from France and Germany and (Table 3.64) point to zinc concentrations (90P values) from 300-2500 mg/kg dwt. Swedish sediment concentrations (50P values; unaffected by point sources) amount to 150 and 240 mg/kg dwt for, respectively, Northern and Southern Sweden. Sediments in Norwegian lakes were found to have zinc levels (90P) of 361 and 195 mg/kg dwt, respectively at surface and at 30-50 cm depth. Some recent, individual data are available for zinc sediment concentrations in the Meuse (Belgium, Walloon Region). Values for 1999-2000 range from 319 to 907 mg/kg dwt, measured at three different locations in the Meuse. A much larger set of sediment is available from the Belgium Flanders monitoring network (VMM, 2003). Importantly, zinc levels in another Flanders sediment database are accompanied by SEM/AVS measurements).

In addition to the sediment monitoring data there are also measured suspended matter zinc concentrations in major Dutch rivers (see Figure 3.7). These data are compiled from a large monitoring programme in the Netherlands (CIW/RIZA). Similar to surface water the zinc suspended matter concentrations have decreased during the period 1988-1998. Recent figures (1998) for the Meuse are 2600 mg/kg dwt, for the Rhine 817 mg/kg dwt and for the Scheldt 552 mg/kg dwt.

Regional PEC/PNEC ratios based on both calculated and measured data would point to a potential risk to sediment-dwelling organisms. Except for Northern Sweden, all above-mentioned data (after subtraction of natural background concentration of 140 mg/kg dwt from measured data) are (much) higher than the $PNEC_{add}$ sediment of 49 mg/kg dwt. This conclusion is based, however, on no correction for the bioavailability of zinc in sediment (SEM/AVS method; see section 3.3.2.2.1). However, the SEM/AVS method for bioavailability correction can at present only be applied on a regional data set for Flanders (Belgium), because only for this particular set of zinc sediment measurements the individual, corresponding SEM/AVS data are available. For the remaining regional sediment data, specific information on prevailing SEM/AVS levels is lacking. Therefore 'only' the default correction factor of 0.5 for the PEC could be applied in those cases. However, even with this additional factor of 0.5, for nearly all data the PEC/PNEC ratios remain substantially above 1 **(conclusion iii).**. It is emphasised that for the Netherlands this conclusion is based on freshly deposited sediment and suspended matter ('future sediment'). The **conclusion iii)** also holds for the calculated PEC_{add} regional.

Table 3.109 gives an overview of the regional sediment data and the corresponding PEC/PNEC ratios, both uncorrected and corrected for bioavaialability.

Table 3.109 Regional EU measured zinc sediment concentrations (PEC) and corresponding $PEC_{add}/PNEC_{add}$ ratios with and without bioavaialiblity correction (generic sediment bioavailability correction factor of 0.5).

	PEC (mg/kg dwt)	**PEC_{add} (PEC-140 mg/kg dwt)**	**PEC_{add}/ $PNEC_{add}$ (uncorr.)**	**PEC_{add} corrected (factor 0.5)**	**PEC_{add}/ $PNEC_{add}$ (corrected)**
Rhine (Lobith, NL) 90P value	770	630	13	315	7
Germany, various regions					
Aller	1500	1360	28	680	14
Elbe	1696	1556	32	778	16
Ems	480	340	7	170	4
Lausitzer Neisse	680	540	12	270	5
Main	403	263	5	132	3
Mosel	1029	889	18	445	9
Mulde	3230	3090	65	1545	32
Nahe	398	258	5	129	2
Neckar	452	312	6	156	3
Rhein	546	406	8	203	4
Saale	2519	2379	49	1189	25
Saar	593	453	9	227	5
Swarzbach	1557	1417	29	708	15
Schwarze Elster	1033	893	18	447	9
Spree	1010	870	18	435	9
Vereinig. Mulde	1600	1460	30	730	15
Warnow	465	325	7	163	3
Weser	879	739	15	370	8
Sweden					
Northern Sweden (median)					
Southern Sweden (median)	150	10	0.2	5	0.1
	240	100	2	50	1
France					
Artoie Picardie	1200	1060	22	530	11
Rhin Meuse	1908	1768	37	884	18
Seine Normandie	463	323	7	162	3
Loire Bretagne	989	849	18	425	8
Adour Garonne	340	200	4	100	2
Rhone Mediterranee Corse:	372	232	5	116	2
*Belgium, Flanders (90P value)**	604	464	10	232	5

	PEC (mg/kg dwt)	PEC_{add} (PEC-140 mg/kg dwt)	PEC_{add}/ $PNEC_{add}$ (uncorr.)	PEC_{add} corrected (factor 0.5)	PEC_{add}/ $PNEC_{add}$ (corrected)
Norwegian lakes (90P)					
surface layer	361	221	5	110	2
30-50 cm depth	195	55	1.2	27	0.6
Meuse Belgium					
Dave	334	194	4	97	2
Andenne	697	557	12	279	6
Vise	907	767	16	384	8
NL Hollandsch Diep East	1001 (av) 2089 (max)	861	18	431	9
NL Hollandsch Diep West	293 (av) 4003 (max)	153 3863	3 80	77 1931	2 40
NL Dordtsche Biesbosch clay	1131 (av) 2802 (max)	991 2662	21 55	495 1331	10 28
NL Dordtsche Boesbosch sand	663 (av) 1904 (max)	523 1764	11 37	262 882	5 18

* For Flanders data the SEM/AVS method will eventually be used for drawing conclusions. The SEM/AVS data originate from another dataset in Flanders than the one on which this 90P value is based.

For the Flanders data the following conclusion can be drawn: in 41 % of the cases (77 out of 190 sampling points) the PEC/PNEC would exceed 1 without any correction. In 9% of the cases the PEC/PNEC is greater than 1 based on the SEM/AVS method. Thus for 9% of the sampling stations in the Flanders data base (out of total number of 190 points) potential risks to sediment-dwelling organisms cannot be excluded (**conclusion iii**).

Further considerations on conclusion iii) for regional sediment: influence of point sources, historical emissions etc.

In essence the same kind of remarks can be made on the risk characterisation for sediment as for water (see above). High zinc sediment levels are being found in highly populated and industrialized EU regions. Numerous and/or major point sources rather than diffuse emission sources may well explain a number of these high regional, or at least 'beyond local scale', zinc concentrations. It has to be noted that especially for sediment, being a sink for zinc, historical pollution may (partly) explain the high zinc concentration in several EU areas. **When deciding about (possible) emission reduction measures, the available information on potential zinc emission sources in that particular area has to be carefully taken into account. Reference is made to Annex 3.2.5 (see regional water).**

Line source emissions: road borders

Section 3.2.5.3.4 provides some information on measured zinc concentrations in motorway run-off streams and in sediments alongside motorway waters. High zinc concentrations (18-1500 µg/l) in motor-way run-off have been measured in the Netherlands and for the UK a maximum figure of 489 µg/l is available. Although the amount of data on zinc concentrations in aquatic ecosystems alongside motorways is limited, it can be expected that comparable

concentrations can be found at much more locations within the EU. Using either the PNEC of 33 µg/l or 21 µg/l and applying a dilution factor of 10 on the measured data would result in PEC/PNEC ratios for surface waters near motorways far above 1. Further correction for bioavailability (BLM) would not reduce such PEC/PNEC ratios to levels below 1 (maximum PEC correction factor is about 0.2). The same would be true if one compares the difference between upstream and downstream runoff discharge point in UK (Maltby study) sediment concentrations (137 and 338 mg/kg dwt, respectively) and the current $PNEC_{add}$ for sediment (PNEC = 49 mg/kg dwt). Applying a generic sediment bioavailability correction factor of 0.5 would still yield a PEC/PNEC ratio above 1. The maximum accumulation of around 60 mg/kg dwt from the HA/EA study (Moy et al., 2002) is also higher than the PNEC add of 49 mg/kg dwt, but the additional correction factor of 0.5 would bring it to a PEC/PNEC ratio below 1. The HA/EA study should be used with caution, however, due to the great variation between sampling points. When using the average sediment concentration of 720 mg/kg dwt in untreated runoff from the HA/EA study as worst case situation for EU water systems receiving untreated motorway runoff, the sediment PEC/PNEC ratio would clearly exceed one.

Some aspects (fate and effects) need to be addressed before a final conclusion can be drawn on the potential risk of zinc in suface waters near motorways.

Section 3.3 of the RAR on ZnO presents the results of a dissolution test with tyre debris (LISEC, 2000). It is felt that these studies on transformation/dissolution of tyre debris do not provide evidence that the measured data in motorway run-off and sediments cannot be used in the current risk characterisation. One reason is that the time scale of the tests, i.e. 7 days, is considered not to be relevant for the risk characterisation of aquatic ecosystems near road borders. This because the zinc from tyre debris will be present in the aquatic ecosystems for much longer time periods. It can be assumed that a considerable part of the tyre debris will initially deposit onto sediment from which afterwards resuspension to the water phase may occur. Both biotic and abiotic degradation of the matrix may occur in real world situations during such longer periods. It should be borne in mind, however, that after 7 days already 35 % of the available Zn in the tyre debris was released into the test medium in the LISEC study. Another reason is that tyre debris is not the only source of zinc in motorway run-off streams. Corrosion from traffic furniture (crash barriers, lampposts etc.), oil leakage, road surface wearing and exhaust gasses will also contribute to zinc levels in motorway runoff streams. The aquatic fate of these zinc sources is off course different from that of zinc oxide in tyre debris. A third reason is that the impact of the broad spectrum of 'real world' environmental conditions (e.g. pH) rather than simply using the ISO medium, on the dissolution of tyre debris is unknown.

Maltby et al. performed three studies on the impact of motorway runoff on freshwater ecosystems (Maltby et al., 1995a; Maltby et al. , 1995b and Boxall and Maltby, 1997).

In the first study (Maltby et al., 1995a) the biotic diversity was investigated in seven streams alongside the M1 motorway in the UK. Upstream biodiversity was compared to the diversity downstream from motorway runoff discharge points. Chemical analysis showed that concentrations of PAHs and heavy metals (mainly zinc, cadmium, chromium and lead) were elevated in the downstream sampling points. Most pronounced effects on biotics were found on the macroinvertebrate diversity. For 57% of the streams surveyed, the macroinvertebrate assemblages at the station receiving motorway runoff was less diverse and contained fewer pollution-sensitive taxa than the assemblages at the uncontaminated station. The most affected species were Gammarus pulex (Amphipoda), Potamopyrgus jenkisi (Mollusca), and Sphaeriidae (Mollusca) (significant reduction). The number of chironomid larvae and

tubificid worms was found to increase in the contaminated sites. No effects on either the diversity or the abundance of epilithic algae was found.

In the second study (Maltby et al., 1995b) further laboratory testing was carried out with Gammarus pulex, being one of the most severely affected species in the field study. Via a number of short term (17 d.) toxicity experiments with spiked media it was demonstrated that it is most probably the PAH fraction rather than metals, including zinc, that caused the observed effects in the laboratory tests with Gammarus.

Boxall and Maltby (1997) tried to investigate whether PAHs were indeed the major toxicants in the sediment extracts. The results of the additional studies with Gammarus pulex indicated that three PAHs, i.c. phenanthrene, fluoranthene and pyrene, accounted for a considerable amount of the observed toxicity.

Despite the elegancy of the three Maltby studies it cannot be excluded that at present no effects due to zinc will occur in aquatic ecosystems alongside motorways. This because:

- of the affected species (amphipods and molluscs) in the field, only Gammarus was further tested in the laboratory by Maltby et al. Implicitly, there is no information on the sensitivity of e.g. the molluscs.
- only acute tests (17 days) were carried out in the laboratory follow-up tests. The authors stated themselves: “the linking of observations from acute toxicty experiments to the observed differences in upstream/downstream populations of G.pulex and more general community composition is obviously problematic”.
- much higher runoff levels (up to 1500 µg/l) have been reported, whereas the maximum average concentration in the Malthby study amounts to 489 µg/l. Much higher zinc sediment concentrations (may therefore) occur alongside motorways with unknown effects, but in any case largely exceeding the PNEC sediment.
- the seven streams alongside one motorway (M1) in the UK investigated by Maltby et al. only represent a very small fraction of the wide variety of sediment ecosystems (different abiotic conditions and different organisms) within the EU.

The UK HA/EA study (Moy et al., 2002) also investigated potential effects of motorway runoff on sediment biota (see section 3.2.5.3.4). Macro-invertebrate communities below discharges of untreated runoff were found to be marginally affected, but changes were considered too small to draw firm conclusions. It was not possible to eliminate the possibility of confounding effects in this (limited) field study. Nevertheless, the authors concluded that “….highway drainage from these five sites appears not to have adversely affected the macro-invertebrate communities in the receiving waters”. One may dispute the overall conclusion from the authors based on the available data, but is should be realised that even if statistically effects would have been found in the HA/EA study, it would remain speculative what the causing pollutant(s) might have been. Although the HA/EA study comprises more sites than the Maltby study, still only a very limited and non EU/UK representative numer of sites has been taken into account. Furthermore, most of the HA/EA sites referred to locations where one form of runoff treatment occured before discharge to the aquatic ecosystem. This implicitly does not reflect realistic worst case situations, which are known to occur at a large scale throughout the EU, where no treatment of road runoff takes place.

Conclusion on roadborders

The Rapporteur is aware that the number of monitoring data on zinc concentrations in aquatic ecosystems alongside motorways is limited. This in great contrast to the large set of monitoring data for soils near roads. Additionally, the instrumentarium is currently lacking to estimate concentrations via exposure modelling. However, the available monitoring data are considered to be reliable and indicative, pointing to rather high zinc concentrations in both motorway run-off and sediment. Comparable levels are expected to be found throughout the EU. The available measured data for surface water and sediment do (clearly) exceed the corresponding PNECs, even after a generic correction for bioavailability. The available information from the short-term fate of tyre debris in ecotoxicity medium and from both the Maltby et al. and HA/EA studies on potential 'field' effects is considered not sufficient to exclude potential risks.

Balancing the available data and uncertainties a **conclusion i)** is considered most appropriate for aquatic ecosystems, including sediments, alongside motorways in the EU. Further work is needed to investigate the impact of zinc from traffic at a broader scale. Details of this conclusion i) program for water will be elaborated and will be linked with ongoing activities on this issue within the EU.

3.4.4.2 Terrestrial compartment

Non-agricultural soils

In the Netherlands and other EU Member States there are a number of areas which are highly contaminated with zinc (and other heavy metals) due to former industrial activities. Levels up to 1750 mg/kg dwt have for example been measured in Budel, the Netherlands. The contaminations are mostly due to historical emissions from zinc smelters etc. It is evident that in these areas the $PNEC_{add}$ for soil (26 mg/kg dwt) is exceeded by far. A great number of studies have indeed reported on occurring effects (e.g. disappearance of plant species) on terrestrial ecosystems in these areas. In most cases such areas are mapped out properly and local land development plans or sanitation measures have been (or should be) designed accordingly. In the present risk assessment it was therefore decided not to pay further attention to the regions affected by historical pollution.

High zinc levels in soil that strongly exceed the PEC_{add} were also measured around electricity pylons (data for the Netherlands: 200 to 650 mg/kg dwt in the topsoil; no further EU data). It should be stated, however, that for the Netherlands the observed high levels are most probably due to historical emissions. This because nowadays these galvanised steel pylons are coated in the Netherlands, which prevents zinc emissions via atmospheric corrosion. The situation in other EU Member States is unknown.

It is noted that the data in this section on "non-agricultural" soils also include data on agricultural soils, but that the zinc concentrations in these agricultural soils are mainly increased by industrial activities or corrosion and not by agricultural use (see below for data on agricultural soils in which the zinc concentrations are mainly increased by agricultural use).

Agricultural soils

In paragraph 3.2.5.3 regional PEC_{add}s in agricultural soil have been calculated. These estimations were based on the diffuse zinc emissions to soil. Manure application is by far the major contributor of these soil emissions. The PEC_{add} for agricultural soil is 64 mg/kg dwt both for the NL-region and the EU-region. Comparing these PEC_{add} with the $PNEC_{add}$ for soil

based on the micro-organisms (26 mg/kg dwt) results in a $PEC_{add}/PNEC_{add}$ ratio of 2.5. Applying the bioavailability correction factor for soil, i.e. a factor 3[38] for ageing (R_{L-F}) on the PEC_{add}, results in a $PEC_{add}/PNEC_{add}$ ratio of 0.8. As it concerns a generic scenario no soil-type correction has been carried out, which is of course a theoretical case. Availability factors for various types of agricultural soil range between 1.0 (sandy soil) and 1.5 (river clay) (Table 3.96), so the impact on the $PEC_{add}/PNEC_{add}$ ratio would have been rather low.

One should realise that the regional PEC_{add}s are calculated with the multi-media fate model SimpleBox (level III Mackay-type). This model predicts the concentration in soil after it has reached a steady state concentration. Figure 3.19 gives the relationship between time and the concentration as the percentage of such steady state situation. The figure demonstrates that it would take almost 400 years before the agricultural soil concentration has reached 95% of its steady state.

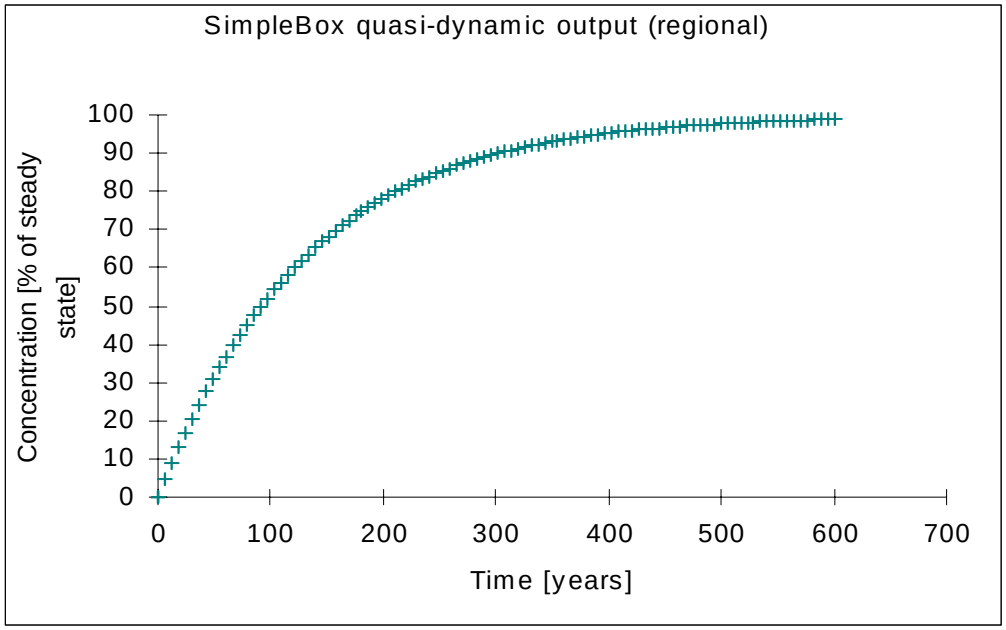


Figure 3.45 Relationship between time and the agricultural soil concentration (upper 20 cm) of zinc as the percentage of the steady state concentration (SimpleBox2 calculation). Input parameters for NL regional exposure scenario were used.

In paragraph 3.2.5.3.3 an alternative approach is discussed for the estimation of zinc levels in agricultural soil. This approach focuses on the actual balances between input and output of zinc in agricultural soils Table 3.56 showed the net accumulation (in g/ha/year) for various soil types and land uses in The Netherlands. The data come from the Alterra study (De Vries et al., 2004)., which can be considered the most recent and suitable study on this topic. Furthermore, the data for The Netherlands from the Alterra report were shown to be representative for other North-Western Europe having similar intensive agriculture activities. Whereas zinc via manure inputs in The Netherlands may be among the highest in Europe, the total inputs do not deviate as much from other European Countries most likely due to the fact that inputs from sludge are much higher in most other countries. On the basis of the zinc flux rates the course of zinc concentrations in time were estimated by De Vries et al. (2004). This was conducted via a dynamic model. The present and future (steady-state) zinc concentrations

38 The generic factor of 3 (R_{L-F}) should theoretically only be applied on total zinc concentrations, thus including the natural background. If data refer to added levels, strictly speaking a somewhat lower factor should be used. For pragmatic reasons, however, a factor of 3 is taken, which may be a (slight) overestimation in some cases.

are subsequently compared with the critical zinc limit in soil. This critical Zn limit in soil in the Alterra report is based on the current $PNEC_{add}$ of 26 mg/kg dwt and the bioavailability corrections of the calculated PEC_{add} values (using the generic, ageing-related lab-to-field factor of 3 and the soil-type specific bioavailability corrections). As background concentration the Alterra report uses three different options, all derived from a representative soil monitoring data set for The Netherlands: 1) 'standard approach': top soil (0-30 cm), excluding obvious contaminated sites, 2) 'subsoil content': zinc levels at depth of 60-100 cm, reflecting the lowest anthropogenic influence and 3) 'present content': top soil (0-30 cm), including obvious contaminated sites. Following this method at present in 0.43 % of the total agricultural plots in The Netherlands (n= app. 5000) the zinc concentration in soil exceeds the critical zinc concentration. Most of these present exceedings are being found in peat soils (2.4% of the peat plots). These plots only include historically polluted sites, such as the 'Toemaakdekken' (historical compost) or floodplain soils. Table 3.110 presents the percentage of plots at which the steady-state concentration exceeds the critical zinc concentration. For grassland the standard approach results in 100% of the plots exceeding the critical limit for the soil types calcareous sand and clay. High percentages are also estimated for grassland for the soil types clay (80%) and peat (72%), whereas relatively low exceedings will occur in sand (1.9%) and loess (20%). Based on all plots for grassland the percentage of plots exceeding the critical level amounts to 51%. For arable land the overall value is 55% and, similar to grassland, differences occur between the various types of soil. Sand and peat soils show the lowest percentages for arable land. The impact of other background concentrations than the standard approach on these precentages is rather low for both grassland and arable land (Table 3.110). The differences between the percentages of plots for present and subsoil hardly differ from the standard approach, with the exception of the combinations 'grassland on calcareous sand', 'grassland on loess' and 'arable land on loess'.

The time period it will take before the critical zinc limit is reached in The Netherlands is indicated in Table 3.111. It shows that the average time periods to reach critical levels for grassland range from 161 years for peat soil to 589 years for clay soil. For arable land longer time periods are estimated: 444 years (sand) to 1704 years (loess). When using the subsoil zinc content (60-100 cm) as alternative background value in most cases shorter time periods are being estimated, e.g. 81 years for peat, 125 years for clay calcareous and 163 years for sand calcareous (all grass land).

Table 3.110 Percentage of plots at which the steady-state Zn concentration exceeds the critical Zn concentration for a present, subsoil and standard background Zn concentration (De Vries et al., 2004). See text for further explanation.

Soil type	% of plots exceeding critical Zn limit					
	Grass land			Arable land		
	Present Zn content	Subsoil Zn content	Standard approach	Present Zn content	Subsoil Zn content	Standard approach
Sand	1.3	3.3	1.9	1.7	6.6	3.1
Sand calcareous	0	100	100	57	94	63
Clay	70	93	80	61	79	66
Clay calcareous	99	100	100	96	99	98
Loess	6.2	96	20	0.57	95	76
Peat	45	81	72	20	30	23
All	42	56	51	52	59	55

Table 3.111 Averages of time periods (yr) to reach critical limits for Zn on the considered land use types and soil types where exceedance did occur in the course of time for the standard and alternative approaches to calculate Zn background concentrations concentration (De Vries et al., 2004). See text for further explanation.

Soil type	Average time period to reach critical Zn limits (yr)					
	Grass land			Arable land		
	Present Zn content	Subsoil Zn content	Standard approach	Present Zn content	Subsoil Zn content	Standard approach
Sand	580	545	291	550	372	444
Sand calcareous	-	163	373	1108	600	577
Clay	641	389	589	1205	924	885
Clay calcareous	395	125	262	937	315	594
Loess	275	266	435	897	450	1704
Peat	348	81	161	937	440	773
All	418	171	268	948	369	643

Whereas Table 3.111 presents the average time periods for reaching critical levels, the confidence limits (5% and 95%) around these average values are given in Table 3.112 for the standard approach. For the overall time period in grassland the 5th and 95th percentiles amount to, respectively, 34 and 506 years (average of 268 years). For arable land the values are 194 years (5th P) and 1747 (95th P) with an average time period of 643 years before the critical zinc limit is reached.

Table 3.112 Averages of time periods to reach the critical limits for Zn on those types of land use soil type where exceedance did occur in the course of time. Standard approach is taken as background concentration (De Vries et al., 2004). Values in brackets give the range between 5% and 95% (90 percentile ranges).

Soil type	Time period to reach critical Zn limits (yr)					
	Grass land		Arable land			
Sand	291	(26-495)	444	(81-1180)		
Sand calcareous	373	(372-372)	577	(67-1108)		
Clay	589	(92-2741)	885	(204-2978)		
Clay calcareous	262	(92-457)	594	(205-1352)		
Loess	435	(80-681)	1704	(848-1960)		
Peat	161	(29-391)	773	(111-2509)		
All	268	(34-506)	643	(194-1747)		

The Alterra report contains a (limited) uncertainty analysis showing the impact of varying a number of parameters. One of such parameters is already discussed above, i.e. the influence of different background input values. Other parameters that were used in the uncertainty analysis were soil properties and their impact on the soil sensitivity factor, and zinc uptake rates (soil-plant relationships). Generally the impact of varying these parameters is found to be rather limited and the general picture of reaching the critical zinc limit within approximately 100 to 500 years in grassland soils and 300 to 900 years in arable land soils remains unchanged. The same is true for the percentage of plots where exceedance of the critical limit will occur.

The input of zinc via manure in the Alterra report is based on actual (year 2000) Dutch nitrogen (N) application rates and the Zn/N ratio in manure. This input is also assumed to remain constant over time in the standard approach that is followed. However, alternative scenarios were run based on the situation that animal manure is applied on land strictly meeting the current EU standards for N input, i.e. 170 and 250 kg/ha/y (170 kg N ha^{-1} yr^{-1} is the current level of maximum levels of nitrogen that can be added to soils (Nitrates Directive 91/676/EEC) and 250 kg ha^{-1} yr^{-1} is the level of the derogation request from the Netherlands specifically for grassland). Table 3.113 shows that the percentage of plots at which the steady-state zinc concentration exceeds the critical level remains unaffected for arable land (55%). For grassland the exceedance of plots decreases from 51% to 40% when using a Zn input related to a maximum N input of 250 kg/ha/yr and it further reduces to 30% with the N target of 170 kg/ha/yr. Complying with current EU standards for N application would thus result in a huge increase in the time periods for reaching critical levels in grassland. Table 3.114 indicates that time periods increase from an overall average of 268 years when the Zn input for the year 2000 is used to 2515 years with the target of 170 kg/ha/yr. The impact on arable land is negligible: 643 years (standard) versus 706 years (170 kg N/ha/yr).

Table 3.113 Percentage of plots at which the steady-state Zn content exceeds the critical Zn content for two alternative inputs (Zn input related to an N input of 250 kg.ha^{-1}.yr^{-1} for grassland and 170 kg.ha^{-1}.yr^{-1} for arable land or 170 kg.ha^{-1}.yr^{-1} for both land use types) and the standard input, using data for the year 2000 (De Vries et al., 2004).

Soil type	% of plots exceeding critical Zn limit				
	Grass land			Arable land	
	N input 170	N input 250	Standard input	N input 170	Standard input
Sand	0.81	1.3	1.9	2.9	3.1
Sand calcareous	0	0	100	63	63
Clay	50	62	80	66	66
Clay calcareous	98	99	100	97	98
Loess	2.1	9.0	20	76	76
Peat	12	40	72	22	23
All	30	40	51	55	55

The authors of the Alterra report emphasise that the focus of their study is on zinc accumulation in the soil compartment. The increase of zinc in ground water (leaching) and surface water (leaching and/or run-off) due to agricultural activities is beyond the scope of the study. It should be borne in mind, however, that zinc run-off from agricultural soil was indicated to be an important input source for surface water in The Netherlands (see section 3.2.5.3.1).

Table 3.114 Averages of time periods to reach critical Zn limits for two alternative inputs (Zn input related to an N input of 250 kg.ha^{-1}.yr^{-1} for grassland and 170 kg.ha^{-1}.yr^{-1} for arable land or 170 kg.ha^{-1}.yr^{-1} for both land use types) and the standard input, using data for the year 2000 (De Vries et al., 2004).

Soil type	Average time period to reach critical Zn limits (yr)				
	Grass land			Arable land	
	N input 170	N input 250	Standard input	N input 170	Standard input
Sand	3740	2530	291	456	444
Sand calcareous	-	-	373	577	577
Clay	2706	1124	589	1300	885
Clay calcareous	2792	587	262	647	594
Loess	371	235	435	1704	1704
Peat	714	341	161	800	773
All	2515	613	268	706	643

Nicholson et al (2003) made an inventory of heavy metal inputs to agricultural soil in England and Wales for the year 2000 (see section 3.2.5.3.3). They estimated the time required to raise soil metal concentrations from background to limit concentrations after heavy metal addition from several sources. For the limit value they used a value of 200 mg/kg dwt and as background 88 mg/kg dwt. Nicholson et al concluded: "*Soil zinc would be raised to the limit value (200 mg Zn/kg dry soil) after approximately 80 years of sewage sludge applications*

compared with 130-164 years if pig or laying hen manures were applied at rates of 250 kg/ha total N. However, these times would be decreased if soil Zn concentrations were already elevated above background values, if more than one material was applied to a field each year or if application rates or Zn concentrations were higher than those assumed here.

When interpreting the Nicholson conclusions one has to keep in mind that a different limit value (added value of 200-88=112 mg/kg dwt!) was used than in the above-mentioned estimations for The Netherlands. Furthermore it refers to a simple linear extrapolation model in which zinc removal routes like leaching and uptake by crops were not addressed. Nicholson et al. also did not account for any bioavailability correction, but they clearly state that this is an important aspect. This all in contrast to the Alterra study in which a dynamic model was used incorporating all relevant parameters. The validity and practicability of the Nicholson study is therefore limited for the risk characterisation, but this UK study supports the conclusions of the NL study regarding the ongoing accumulation of zinc in agricultural soils.

Conclusion on agricultural soils

Diffuse zinc emissions to agricultural soil result in net accumulation rates in several EU areas with intensive agricultural activities. On the basis of the outcomes of the Alterra study (De Vries et al., 2004) it can be concluded that current animal manure application rates on land will ultimately result in an exceedance of the critical zinc concentrations in soil. This is expected to occur at a relatively large scale, i.e. in about 50% of the agricultural soils. However, the time period for reaching these critical zinc concentrations in agricultural soils is estimated to be (relatively) long. On average, depending on the type of soil, it will take 100 to 500 years for grassland and 300 to 900 years for arable land. Complying with the EU standard for N-application on agricultural land would significantly enhance these time scales. The Alterra study is initially based on the situation for The Netherlands, but it is adequately substantiated that this scenario is representative (realistic worst case) for regions with a comparable, intensive agriculture in the European Union. It has to be recognised that substantial differences occur in manure, fertilizer, compost and sludge application rates between EU regions.

The CA meeting concluded that there is at present no need to implement risk reduction measures beyond those which are already in place (11th Joint Meeting June 2005). A **conclusion ii)** is therefore drawn for agricultural soil at regional scale. The CAs concluded that there are no existing risks from zincs in agricultural soils. They also considered that existing legislation relating to sludge and manure management (86/278/EEC; 91/676/EEC; and 1831/2003) provide an adequate framework to address and prevent any future risks relating to zinc accumulation. It is, however, expected that the Commission will take the information provided in the risk assessment on zinc accumulation into account in future policy proposals relating to soil.

Line sources: road borders

Very high zinc concentrations (up to 1500 mg/kg dwt) have been measured in road borders alongside motorways in the Netherlands and other EU member states. A compilation of these data is given in Figure 3.9 and 10 (section 3.2.5.3.4). The overall picture shows a clear accumulation of zinc in a rather thin top soil layer and a exponentially decreasing concentration over the distance from the curb of the road. Moreover, zinc levels are found to decrease with decreasing road intensity. Such levels largely exceed the $PNEC_{aded}$ terrestrial of 26 mg/kg dwt, irrespective of which natural background concentration is chosen. In a number of cases the PEC/PNEC ratios would also remain above 1 after the corresponding correction for bioavaialability (ageing and soil-type). Very recently, however, a EU policy agreement was reached (CA decision 2003) about the formal distinction between the road technosphere

and ecosystem. The agreed borderline of the road technosphere is dependant on the Average Daily Traffic Intensity (ADTI) and is definied as follows: motorways (ADTI > 60,000): 5-6 meters; regional roads (ADTI > 14,000): 3-4 m and urban roads (ADTI > 1,000): 1-2 meters. When applying these ranges on the available data set for zinc levels in soil road borders, it becomes clear that the observed zinc accumulation in road borders is mostly related to the technosphere. The data sets have been investigated at the level of individual data and in those cases where the zinc concentration in the 'ecosystem area' alongside roads is elevated compared to the prevailing natural background, the PNEC, included a correction for bioavailability, and would not be exceeded.

Conclusion on road borders

The Rapporteur considers the available data set on monitoring data on zinc concentrations in soils alongside motorways as sufficiently large and representative to draw conclusions. The data point to high zinc concentrations in the vicinity of the road at levels clearly exceeding the PNEC even after correction for bioavailability. However, based on the recently agreed distinction of technosphere versus ecosystem, those sampling points with PEC/PNEC ratios above 1 are found to lie within the technosphere. For this reason, based on the currently available data set a **conclusion ii)** is considered most appropriate for the terrestrial ecosystem area alongside EU roads.

Sludge

The rapporteur realises that STP sludge is not an official endpoint according to the TGD. Nevertheless some attention will be paid to the quality of sludge in comparison with current quality criteria for sludge for application as fertiliser on soil. Figure 3.8 clearly demonstrates the pattern of zinc concentration in sludge from communal STPs in the Netherlands during the period 1981-1997. In 1981 most sludge had a zinc concentration of more than 1500 mg/kg dwt, whereas in 1997 the majority falls within the class: >500-1000 mg/kg dwt. Zinc sludge levels from several other EU countries show more or less the same trend.

Despite this decrease, however, an important conclusion is that current sludge zinc concentrations from communal STPs still exceed the present-day operative Dutch quality criterion of 300 mg/kg dwt (BOOM2 decision).

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RISK ASSESSMENT

ZINC METAL

CAS-No.: 7440-66-6

EINECS-No.: 231-175-3

Final report, May 2008

Environment

ANNEXES

Rapporteur for the risk evaluation of zinc metal is the Ministry of Housing, Spatial Planning and the Environment (VROM) in consultation with the Ministry of Social Affairs and Employment (SZW) and the Ministry of Public Health, Welfare and Sport (VWS). Responsible for the risk evaluation and subsequently for the contents of this report is the rapporteur.

The scientific work on this report has been prepared by the Netherlands Organization for Applied Scientific Research (TNO) and the National Institute of Public Health and Environment (RIVM), by order of the rapporteur.

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PREFACE

For zinc metal (CAS No. 7440-66-6), zinc distearate (CAS No. 557-05-1 / 91051-01-3), zinc oxide (CAS No.1314-13-2), zinc chloride (CAS No.7646-85-7), zinc sulphate (CAS No.7733-02-0) and trizinc bis(orthophosphate) (CAS No.7779-90-0) risk assessments were carried out within the framework of EU Existing Chemicals Regulation 793/93. For each compound a separate report has been prepared. It should be noted, however, that the risk assessment on zinc metal contains specific sections (as well in the exposure part as in the effect part) that are relevant for the other zinc compounds as well. For these aspects, the reader is referred to the risk assessment report on zinc.

INTRODUCTION

This document includes most of the Annexes of the Risk Assessment Report on Zinc metal (RAR Zn metal). The numbers of the Annexes refer to the section numbers in the RAR Zn metal. The Annexes were prepared by the rapporteur, with the exception of Annex 3.4.3 which was prepared by Industry; this Annex is included at the end of this report.

It is emphasised that Industry also prepared a comprehensive Annex on the regional aquatic compartment (i.e. Annex 3.2.5: Refinement of the exposure assessment and risk characterisation – regional aquatic compartment). This annex includes data for water and sediment. Annex 3.2.5 is not included in this report, but available as a separate document. In the RAR Zinc metal the reader is also referred to this Annex.

Annex A Summarised description of test parameters in LISEC tests of 1997 with Zinc (LISEC, 1997a) and indication of major deviations from the recommended method of the EU.

Annex 1.3.1.A. Summarised description of test parameters in LISEC tests of 1997 with Zinc (LISEC, 1997a) and indication of major deviations from the recommended method of the EU.

	LISEC tests	**LISEC tests**	**LISEC tests**	**LISEC tests**	**recommended (ECB)**
Test parameter	zinc powder 1 (medium size)[6]	very fine zinc powder[7]	zinc powder 2 (medium size)[8]	coarse zinc powder[9]	metal as powder metal in massive form
Particle size S_v surface/volume ratio S_m,surface/mass ratio Purity	469.37 μm 0.0278 m^2/cm^3 38.93 cm^2/g[(12)] 99.98%	6.85 μm 1.33 m^2/cm^3 1861.25 cm^2/g 98.5%[10]	206.90 μm - 47.33 cm^2/g > 99.5%	< 75 μm : 3%; < 2mm [11] - 4.2 cm^2/g[(13)] > 99.9%	-smallest available on the market (powders) -1 mm default (massive)
Loading rates[14]	1) 1.0, 3.1, 10.2, 30.3 and 100.5 mg Zn/l 2) 10.498, 10.908, and 10.007 mg Zn/l [16] 3) 10.704, 10.040, and 10.710 mg Zn/l [16]	1) 1.1, 10.6, and 100.3 mg/l	1) 10.5 mg Zn/l	1) 3.0, 10.5, 31.2 and 89.6 mg Zn/l	100 mg/l 10 1
Definition of water medium	1) sterilized algal medium (OECD 201)[2] 2) daphnia medium (OECD 202) 3) natural surface water[3]	1) sterilized algal medium (OECD 201)[2]	1) sterilized algal medium (OECD 201)[2]	1) sterilized algal medium (OECD 201)[2]	based on ISO 6341 medium modified for pH, hardness and buffering (see below)
PH	1) 7.7-8.2[4] 2) 8.4[5] 3) 7.3	1) 7.7-8.2 [4]	1) 7.7-8.2 [4]	1) 7.7-8.2 [4]	single pH from range 6.0-8.5 to optimise the dissolution process for the 24 hour, 7 and 28 day test.

Alkalinity	-set by medium	-set by medium	-set by medium	-set by medium	-set by medium
Water hardness	1) 40 mg $CaCO_3$/l 2) 215 mg $CaCO_3$/l 3) 110 mg $CaCO_3$/l	1) 40 mg $CaCO_3$/l	1) 40 mg $CaCO_3$/l	1) 40 mg $CaCO_3$/l	$CaCO_3$: 50 mg/l
Buffer system	not reported	not reported	not reported	not reported	reference to Canada programme (carbonate/bicarbonate buffer)[1]
Oxygen concentration	not reported	not reported	not reported	not reported	level above 70% of saturation
Mixing	850 rpm	850 rpm	850 rpm	850 rpm	mild orbital shaking (e.g. 100 rpm)
Test temperature (C)	probably according to the protocol: 20 ± 2 °C, not reported	probably according to the protocol: 20 ± 2 °C, not reported	probably according to the protocol: 20 ± 2 °C, not reported	probably according to the protocol: 20 ± 2° C, not reported	20-25 °C
Test apparatus	1) - 2,3) wrapped in aluminum foil	1) wrapped in aluminum foil	1) wrapped in aluminum foil	1) wrapped in aluminum foil	-in dark -avoid biological contamination and evaporation
Separation	1, 2, 3) centrifugation 1) occurrence of adsorption was evaluated [18]	1) centrifugation	1) centrifugation	1) centrifugation	-centrifugation -if not possible, filtration[4] -eliminate losses due to adsorption
Analysis	-analytical method: AAS for zinc ion -detection limit: 0.010 mg/l -time intervals: 1, 2, 3) 0, 2, 4, 8, 24 hours and 4, 8, 12, and 16 days[17]	-analytical method: AAS for zinc ion -detection limit: 0.010 mg/l -time intervals: 1) 0, 2, 4, 8, 24 hours and 4, 8, 12, and 16 days[17]	-analytical method: AAS for zinc ion -detection limit: 0.010 mg/l -time intervals: 1) 0, 2, 4, 8, 24 hours and 4, 8, 12, and 16 days [17]	-analytical method: AAS for zinc ion -detection limit: 0.010 mg/l -time intervals: 1) 0, 2, 4, 8, 24 hours and 4, 8, 12, and 16 days[17]	-analysis of supernatant at time point: 0h, 4h, 8h, 1d, 2d, 4d, 7 d and if 28 days test at 14d and 28 d. -atomic absorption spectrophotometry (AAS) for metal ion[5]
Duration	16 days	16 days	16 days	16 days	-step two or full test for 7 days -extended test for 28 days

1: the limit for reporting is 0.016 mg Zn/l

2: without EDTA, as suggested at the OECD meeting in Ottawa (1995)

3: water was filtered on a 0.45 μm filter and stored in the freezer

4: pH at the start of the tests varied

5: pH after equilibration with air

6: mainly used for galvanising purposes

7: mainly used as zinc dust

8: used for battery powder

9: used as Zn shot

10: due to natural oxidation

11: to stimulate the massive form

12: calculated by LISEC from the S_v and density

13: calculated by LISEC from the mean diameter (1mm) and density

14: 1 l vessels are used, three vessels per loading rate

15: pH was measured at 0, 2, 4, 8, 24 hours and 4, 8, 12, and 16 days in duplicate

16: mean mass loading was 10.47, and 10.48 mg Zn/l for medium 2 and 3, respectively

17: 10 ml samples were taken at different time intervals, were centrifuged for 15 min (9.000 g), 8 ml supernatant was acidified (1% HNO3) and analyzed for dissolved Zn conc.

18: dissolution was followed as a function of time using different volume/surface area; 150 ml bottles with 100 ml medium and mean mass loadings of 103.8 and 1027.9 mg/l; 10 ml samples were taken after 24 hours, 4 and 16 days and analyzed for Zn conc.; results were compared with those obtained for 10 and 100 mg Zn/l in 1000 ml medium

Annex 1.3.1.B. Measured dissolved zinc concentration (mg Zn/l) after 24 hours, 8 and 16 days for four different zinc powders.

test parameter	**LISEC tests**											
	zinc powder 1 (medium size)			very fine zinc powder*			zinc powder 2 (medium size)			coarse zinc powder*		
Particle size	469.37 µm			6.85 µm			206.90 µm			< 75 µm : 3%; < 2mm		
	Results dissolved mg Zn/l											
Loading (mg/l)[1]	24 h	8 days	16 days	24 h	8 days	16 days	24 h	8 days	16 days	24 h[2,3]	8 days	16 days
1) 1 mg/l	0.036	0.148	0.122	0.261	0.442	0.599	-	-	-	0.002	0.026	0.040
10	0.113	0.327	0.259	0.910	0.949	1.040	0.308	0.664	0.792	0.006	0.061	0.090
100	0.185	0.605	0.762	1.048	2.137	1.248	-	-	-	0.120	0.432	0.621
2) 10 mg/l	0.194	0.299	0.335	-	-	-	-	-	-	-	-	-
3) 10 mg/l	0.177	1.003	1.626	-	-	-	-	-	-	-	-	-

1) sterilized algal medium (OECD 201)

2) daphnia medium (OECD 202)

3) natural surface water

* : Underlined types of zinc powders were used for classification and provisional classification for zinc as a powder and zinc in massive form, respectively.

1 : Results at loading rates of 3 and 30 mg/l are not presented in the table.

2 : Loading rate was 3.06 instead of 1 mg/l.

3 : Calculated dissolved zinc concentration after 8 days were comparable.

Annex 1.3.1.C. Summarised description of test parameters + measured concentrations in LISEC tests of 2002 (LISEC, 2002)

Study Nr	**WE-14-024**	**WE-14-024**	**WE-14-029**	**WE-14-031**
Test substance	Zinc granulates	Zinc granulates	Zinc granulates	Zinc granulates
Particle size Purity	75 µm 3% < 2 mm	75 µm 3% < 2 mm	1 - 2 mm	1 - 2 mm
Loading rates	**1)** 3, 10, 30 and 90 mg Zn/l **2)** 10 mg Zn/l	3 mg Zn/l	3, 10, 30 and 90 mg Zn/l	3 mg Zn/l
Definition of water medium	sterilized medium, according ISO 6341	sterilized medium, according ISO 6341	sterilized medium, according ISO 6341	sterilized medium, according ISO 6341
pH	**1)** 5.9 – 6.2 **2)** 7.8 – 8.0	5.8 – 5.9	5.9 – 6.1	5.6 – 5.7
Water hardness	65 mg $CaCO_3$/l	46 mg $CaCO_3$/l	6.5 mg $CaCO_3$/l	3.3 mg $CaCO_3$/l
Buffer system	5% CO_2	5% CO_2	0.5% CO_2	0.5% CO_2
Oxygen concentration	7.9 – 8.9	8.1 – 8.4	8.6 – 9.2	8.8 – 11.5
Mixing (rpm)	100	100	100	100
Test temperature (°C)	19.3 – 22.6	20.9 – 23.8	20.0 – 22.1	18.8 – 20.9
Test apparatus	Pre-cleaned and acid rinsed closed glass bottles	Pre-cleaned and acid rinsed closed glass bottles	Pre-cleaned and acid rinsed closed glass bottles	Pre-cleaned and acid rinsed closed glass bottles
Separation	0.2 µm filter	0.2 µm filter	0.2 µm filter	0.2 µm filter
Analysis	Acidification (1% HNO_3) and analysis by Inductive coupled Axial Plasma spectrometry (ICAP)	Acidification (1% HNO_3) and analysis by Inductive coupled Axial Plasma spectrometry (ICAP)	Acidification (1% HNO_3) and analysis by Inductive coupled Axial Plasma spectrometry (ICAP)	Acidification (1% HNO_3) and analysis by Inductive coupled Axial Plasma spectrometry (ICAP)
Sampling time	2 and 6 h, 1, 4 and 7 days	6 h, 1, 4, 7, 15 and 28 days	1, 2, 5 and 7 days	1, 4, 7, 15 and 28 days

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K. van der Kerken (LISEC, 2002). Transformation/dissolution tests with Zn shots in ecotox medium, Study nr: WE-14-024, Part 2. Chronic transformation/dissolution tests

K. van den Branden (LISEC, 2002). Transformation/dissolution tests with Zn shots in ecotox medium, Study nr: WE-14-029, Part 1. Acute transformation/dissolution tests

K. van den Branden (LISEC, 2002). Transformation/dissolution tests with Zn shots in ecotox medium, Study nr: WE-14-031, Chronic transformation/dissolution tests

Annex 1.3.1.D. Measured dissolved zinc concentration (mg Zn/l) after 7 and 28 days for four different zinc powders.

test parameter		LISEC tests											
		zinc granulates (5% CO_2 buffer)			zinc granulates (5% CO_2 buffer)			zinc granulates (0.5% CO_2 buffer)			zinc granulates (0.5% CO_2 buffer)		
Particle size		75 μm			75 μm			1 -2 mm			1 -2 mm		
		Results mean dissolved mg Zn/l											
Loading (mg/l)	pH	24 h	4 days	7 days	24 h	7 days	28 days	24 h	5 days	7 days	24 h	7 days	28 days
3	6	0.042	0.120	0.233	0.010	0.128	0.652	0.035	0.076	0.103	0.028	0.162	0.487
10	6	0.050	0.264	0.552				0.098	0.233	0.323			
30	6	0.122	0.626	1.30				0.113	0.441	0.690			
90	6	0.367	2.07	4.34				0.655	2.290	3.090			
10	8	0.023	0.046	0.066									

ANNEX 1.3.2a

TABLE 1: SHORT-TERM AQUATIC TOXICITY OF ZINC TO ALGAE, CRUSTACEANS AND FISH (accepted studies)

Species	anal.	Test type	test subst[1]	Purity[2]	pH	hardness	test water	exp. time	Criterion	value (mg/l)[3]	reference [4]
Algae											
Selenastrum capricornutum	Y	S	ZnO**	99.4%	7.5	24	am	72 h	E_rC_g50	0.136	Van Ginneken, 1994a
Selenastrum capricornutum	Y	S	Zn Powder	98.4%	7.4	24	am	72 h	E_rC_g50	0.150	Van Woensel, 1994a
Crustacea											
Daphnia magna	Y	F		-	6.95	130	dt	48 h 96 h	LC50 LC50	0.80 0.068	Attar & Maly, 1982
Daphnia magna	Y	S		rg	7.7	45.3	-	48 h	LC50	0.10	Biesinger & Christensen, 1972
Daphnia magna	Y	S s		rg	7.55	45	dt	48 h	LC50	0.28	Cairns et al., 1978
Daphnia magna	Y	S s	ZnBr2	ag	8.5	180-200	-	48 h	LC50	0.86	Magliette et al., 1995
Daphnia magna	N	S s		-	7.2-7.4	45	nw	48 h	LC50	0.068	Mount & Norberg, 1984
Daphnia magna	N	-		rg	8.4	52	-	72 h	LC50	0.14	Paulauskis & Winner, 1988
Daphnia magna	N	-		rg	8.3	102	-	72 h	LC50	0.21	Paulauskis & Winner, 1988
Daphnia magna	N	-		rg	8.3	197	-	72 h	LC50	0.34	Paulauskis & Winner, 1988
Daphnia magna	Y	S s	Zn powder	98.4%	7.7	262	-	48 h	EC50	0.15-0.5	Vos, 1994
Daphnia pulex	Y	S s			7.55	45	dt	48 h	LC50	0.50	Cairns et al., 1978

Species	anal.	Test type	test subst[1]	Purity[2]	pH	hardness	test water	exp. time	Criterion	value (mg/l)[3]	reference [4]
Daphnia pulex	N	S s			7.2-7.4	45	nw	48 h	LC50	0.107	Mount & Norberg, 1984
Ceriodaphnia reticulata	N	S			7.2-7.4	45	nw	48 h	LC50	0.076 b	Mount & Norberg, 1984
Ceriodaphnia dubia	N	S			6-6.5	280-300	rw	48 h	LC50	> 0.530 b	Schubauer-Berigan et al., 1993
Ceriodaphnia dubia	N	S			7-7.5	280-300	rw	48 h	LC50	0.360 b	Schubauer-Berigan et al., 1993
Ceriodaphnia dubia	N	S			8-8.5	280-300	rw	48 h	LC50	0.095 b	Schubauer-Berigan et al., 1993
Pisces											
Cyprinus carpio	Y	S			8.0	55	-	96 h	LC50	7.8	WHO, 1996
Oncorhynchys kisutch, 0.47 g	N	S		rg	7.1-8.0	41	rw	96 h	LC50	0.82	Buhl & Hamilton, 1990
Oncorhynchys kisutch, 0.63 g	N	S		rg	7.1-8.0	41	rw	96 h	LC50	1.81	Buhl & Hamilton, 1990
Oncorhynchys kisutch, 0 94 g	N	S		rg	7.1-8.0	41	rw	96 h	LC50	1.65	Buhl & Hamilton, 1990
Oncorhynchus mykiss, 0.6 g	N	S		rg	7.1-8.0	41	rw	96 h	LC50	0.17	Buhl & Hamilton, 1990
Oncorhynchus mykiss, juvenile	N	F			7.1	23	-	96 h	LC50	0.136	WHO, 1996
Oncorhynchus mykiss, juvenile	N	F			6.8	26	-	96 h	LC50	0.43	WHO, 1996
Oncorhynchus mykiss 25-70 g,	Y	F			7.3	137	-	96 h	LC50	2.6	WHO, 1996
Oncorhynchus mykiss, 160-290 g	Y	F			7.1	143	-	96 h	LC50	2.4	WHO, 1996
Pimephales promelas	N	S			6-6.5	280-300	rw	96 h	LC50	0.780	Schubauer-Berigan & Dierkes, 1993
Pimephales promelas	N	S			7-7.5	280-300	rw	96 h	LC50	0.330	Schubauer-Berigan & Dierkes, 1993
Pimephales promelas	N	S			8-8.5	280-300	rw	96 h	LC50	0.500	Schubauer-Berigan & Dierkes, 1993

Species	anal.	Test type	test subst[1]	Purity[2]	pH	hardness	test water	exp. time	Criterion	value (mg/l)[3]	reference [4]
Pimephales promelas, 0.0 8 g	N	F			7.8	220	-	96 h	LC50	2.61	WHO, 1996
Thymallus arcticus, 0.20 g	N	S			7.1-8.0	41	rw	96 h	LC50	0.14	Buhl & Hamilton, 1990
Thymallus arcticus, 0.85 g	N	S			7.1-8.0	41	rw	96 h	LC50	0.17	Buhl & Hamilton, 1990

1. If not indicated otherwise the tests were performed with either zinc chloride or zinc sulphate.

2. Purity is not checked for all studies.

3. The L(E)C50s are based on dissolved zinc.

4. References are listed in Annex 1.3.2 b

* Red seal grade

** EPM-grade

a: temperature 10 °C, Daphnia species originating from alpine lake

b: Assumed that dissolved zinc concentration in culture conditions were similar to test conditions.

ag: analytical grade;

rg: reagent grade

nw: natural water

rw: reconstituted water

am: artificial medium

dt: dechlorinated tap water

g: growth (r= growth rate; b = biomassa)

s: conducted according to standard test method, i.e EPA or OECD

S: static test

F: flow through test

Y: yes

N: no

TABLE 2: SHORT-TERM AQUATIC TOXICITY TO CRUSTACEANS [5] (rejected studies)

Species	anal.	test type	Test Subst	Purity[1]	pH	hardness[2]	Test water	exp. time	criterion	value (mg/l)[3]	reference (RI)[4,5]
Algae											
Ankistrodesmus falcatus	N	S		ag	-	-	am	96 h	EC50	2.4	Magdaleno et al., 1997
Chlorella vulgaris	N	S		-	-	-	-	96 h	EC50	2.4	WHO, 1996
Selenastrum capricornutum	N	S		-	6.0-6.3	-	-	96 h	LC50	0.03	Chiaudani & Vighi, 1978
Selenastrum capricornutum	N	S	ZnO*	99.8%	8.5	24	am	72 h	E_rC_g50 E_rC_b50	0.135 0.034	Lisec, 1997b
Crustacea											
Daphnia magna	N	S s		>99%	6.5	-	rw	48 h	LC50	0.151	Oikari et al., 1992
Daphnia magna	N	-		-	-	-	-	48 h	IC50	1.857	Arambasic et al.,1995
Daphnia magna	-	-	Zn 2+	-	6.94	-	-	48 h	IC50	4.40	Belabed et al., 1994
Daphnia magna	Y	S		rg	7.7	45.3	-	48 h	LC50	0.28 a	Biesingen & Christensen, 1972
Daphnia magna	-	-		-	-	-	-	72 h	LD50	0.57 b	Braginsky & Shcherban, 1979
Daphnia magna	-	-		-	-	-	-	72 h	LD50	1.01 c	Braginsky & Shcherban, 1979
Daphnia magna	-	-		-	-	-	-	72 h	LD50	0.014 d	Braginsky & Shcherban, 1979
Daphnia magna	-	-		-	-	-	-	72 h	LD50	0.005 e	Braginsky & Shcherban, 1979
Daphnia magna	Y	-		ag	7.6	54	Rw	48 h	LC50	0.33	Chapman et al., 1980
Daphnia magna	Y	-		ag	8.1	105	Rw	48 h	LC50	0.53	Chapman et al., 1980
Daphnia magna	Y	-		ag	8.2	196	Rw	48 h	LC50	0.66	Chapman et al., 1980
Daphnia magna	Y N	S s	ZnO	-	8.5-8.8	-	-	48 h	LC50	0.098 24.6	Gale et al., 1992
Daphnia magna	Y	S s		-	8.5-8.8	-	-	48 h	LC50	0.21	Gale et al., 1992

Species	anal.	test type	Test Subst	Purity[1]	pH	hardness[2]	Test water	exp. time	criterion	value (mg/l)[3]	reference (RI)[4,5]
	N									47.7	
Daphnia magna	Y N	S s		-	8.5-8.8	-	-	48 h	LC50	0.62 29.4	Gale et al., 1992
Daphnia magna	-	- s		-	-	-	rw	48 h	LC50	0.92	Hall et al., 1986
Daphnia magna	N	- s		rg	-	-	-	48 h	EC50	2.1	Janssen & Persoone, 1993
Daphnia magna	N	S		rg	7.6	240	-	48 h	LC50	0.69	Khangarot et al., 1987
Daphnia magna	N	S s		rg	7.6	240	nw	48 h	LC50	0.56	Khangarot & Ray., 1987
Daphnia magna	N	-		-	6.0	-	-	48 h	LC50	0.24	LeBlanc, 1982
Daphnia similis	N	-		-	-	-	-	96 h	LC50	0.25	Soundrapandian &Venkataraman, 1990
Daphnia sp	N	S s		-	8.5	114	-	48 h	LC50	3.2	Quereshi, et al., 1980
Daphnia hyalina	N	S			7.2	-	nw	48 h	LC50	0.04 a,b	Baudouin & Scoppa, 1984
From reviews:											
Daphnia magna	N	S			6.5	-	-	48 h	LC50	0.244	WHO, 1996
Daphnia magna	N	S			-	-	-	48 h	LC50	0.75	WHO, 1996
Daphnia magna	-	-			-	45	-	48 h	LC50	0.56	U.S. EPA, 1980
Daphnia magna	N	S			7.4-8.2	44-53	-	48 h	EC50	0.1	WHO, 1996
Daphnia magna	N	S			7.4-8.2	44-53	-	48 h	EC50	0.28	WHO, 1996
Pisces											
Carassius auratus, 1-2 g	N	S			7.5	20	-	96 h	LC50	6.44	WHO, 1996
Cyprinus carpio, 3.2 cm	N	S			7.1	-	-	96 h	LC50	0.45-1.34	WHO, 1996
Cyprinus carpio, 6.0 cm	N	S			7.1	-	-	96 h	LC50	1.64-2.25	WHO, 1996
Cyprinus carpio, 47-62 mm	N	S			6.3	19	-	96 h	LC50	3.12	WHO, 1996

Species	anal.	test type	Test Subst	Purity[1]	pH	hardness[2]	Test water	exp. time	criterion	value (mg/l)[3]	reference (RI)[4,5]
Lepomis macrochirus, 1-2 g	N	S			7.5	20	-	96 h	LC50	4.85-5.82	WHO, 1996
Lepomis macrochirus, 1-2 g	N	S			8.2	360	-	96 h	LC50	40.9	WHO, 1996
Lepomis macrochirus, 1-2 g	N	S			7.5	20	-	96 h	LC50	6.44	WHO, 1996
Lepomis macrochirus	-	-			-	45	-	96 h	LC50	2.4	U.S. EPA, 1980
Oncorhynchus mykiss	-	-			-	-	-	96 h	LC50	0.55	U.S EPA, 1980
Oncorhynchus mykiss, juvenile	Y	F			6.4-8.3	-	-	96 h	LC50	0.55	WHO, 1996
Oncorhynchus mykiss, juvenile	N	F			7.8	333	-	96 h	LC50	7.21	WHO, 1996
Pimephales promelas, 1.2 g	N	S			7.5	20	-	96 h	LC50	0.77-0.96	WHO, 1996
Pimephales promelas, 1.2 g	N	S			8.2	360	-	96 h	LC50	33.4	WHO, 1996
Pimephales promelas, 1.2 g	N	S			7.5	20	-	96 h	LC50	0.88	WHO, 1996
Pimephales promelas, 1.2 g	N	S			7.5	20	-	96 h	LC50	2.33	WHO, 1996
Poecilia reticulata, 0.1-0.2 g	N	S			7.5	20	-	96 h	LC50	1.27	WHO, 1996

1. If not indicated otherwise the tests were performed with either zinc chloride or zinc sulphate.
2. Purity is not checked for all studies.
3. The L(E)C50s are based on dissolved zinc.
4. References are listed in Annex 1.3.2 b
5. Studies with a reliability index III (not reliable) or IV (unknown reliability)

a: animals were fed
b: juveniles, temperature 20 ° C
c: females, temperature 20 ° C
d: juveniles, temperature 30 ° C
e: females, temperature 30 ° C
ag: analytical grade;
rg: reagent grade
nw: natural water
rw: reconstituted water
s: conducted according to standard test method, i.e EPA or OECD
S: static test
F: flow through test
Y: yes
N: no

ANNEX 1.3.2b REFERENCES SHORT-TERM TOXICITY TESTS

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ANNEX 1.3.2c

Use of critical surface approach

Short description:

To use the critical surface approach transformation data at a given time, at different loadings, and with several metal particles size, are needed. The transformation data i.e. dissolved metal concentrations with different particle specific areas (mm^2/g) could be plotted against the surface loading (mm^2/l). From these data, it is possible to determine the critical surface loading (mm^2/l) which is capable of releasing the metal ion concentration corresponding with the lowest available L(E)C50. For a full explanation of the critical surface approach reference will be made to an OECD-document which is still under preparation (OECD, 1998).

Use of critical surface approach for zinc:

The industry followed such an approach and obtained for zinc a classification scheme based on cut-off values for critical diameters (mm). The medium sized powders would be classified as N R51-53. However, it should be noted that this classification scheme would probably be changed when transformation data are obtained at a lower pH i.e. 6. The medium sized zinc powders are likely to be classified as N R50-53.

Annex 3.2.A Zinc consumption in agriculture in the EU

Zinc consumption in agriculture in the EU

Country	Feedgrade				Fertilisers and pesticides (t)	Total Zinc output to soil (t)	Utilized agricultural area (1000 ha)	kg zinc/ha
	Zinc in ZnO (t)	Zinc in $ZnSO_4$ (t)	Total Zn input (t)	Zinc output (t)				
Belgium/Lux.	800	0	800	680	0	680	1502	0,453
Denmark	875	30	905	769	0	769	2721	0,283
Germany	3900	50	3950	3358	10	3368	17335	0,194
Greece	290	0	290	247	0	247	5163	0,048
Spain	1460	0	1460	1241	0	1241	29649	0,042
France	3285	600	3885	3302	2300	5602	30215	0,185
Ireland	365	0	365	310	0	310	4530	0,068
Italy	1825	0	1825	1551	0	1551	15701	0,099
The Netherlands	1025	500	1525	1296	70	1366	1969	0,694
Austria	145	0	145	123	0	123	3412	0,036
Portugal	180	0	180	153	0	153	3967	0,039
Finland	290	0	290	247	40	287	2150	0,133
Sweden	365	0	365	310	0	310	3177	0,098
United Kingdom	1170	20	1190	1012	30	1042	15858	0,066
Total	15975	1200	17175	14599	2450	17049	137349	0,124

Sources : ZOPA data/FEFAC/EUROSTAT 1998
ZOPA TC/AC/14 june 1999

Annex 3.2B. Zinc emissions in the Netherlands in 1998 (in t/y) (slightly different from 1999 data). Data based on Dutch Emissie Registratie.

Sector	Source	Water-Direct	Water-Indirect	Water-Total	Water load	Soil	Air
Waste treatment	Waste treatment		0,3	0,3			
	Waste treatment companies[1]	0,2	0,7	0,9	0,2		
	Waste disposal sites	0,4	2,1	2,5	0,4	2,3	
	Ground water treatment	0,2		0,2	0,2		
Waste treatment	Total	0,8	3,1	3,9	0,8	2,3	0,17
Building and construction		0,3	0,0	0,4	0,3		
Building and construction	Total	0,3	0,0	0,4	0,3		0,02
Chemical industry	Inorganic chemicals bulk production		0,1	0,1			
	Chemical industry[1]	22,8	3,2	26,0			
	Dyes and colours		0,7	0,7			
	Other chemical products		0,0	0,0			
	Production of man-made fibres and plastics		0,5	0,5			
	Paint production		0,0	0,0			
	Detergents industry		0,0	0,0			
Chemical industry	Total	22,8	4,6	27,4	22,8		5,06
Consumers	Boults and screws	0,2	3,0	3,2	0,2	0,1	
	Discharging domestic waste water	1,1	113,4	114,5	1,1	0,5	
	Other applications	1,5	19,9	21,4	1,5	0,7	
	Roofs and gutters, housing	5,5	70,7	76,2	5,5	2,4	
Consumers	Total	8,4	207,0	215,3	8,4	3,7	4,67
Water companies		0,0	0,0	0,0	0,0		
Water companies	Total	0,0	0,0	0,0	0,0		0,001
Energy sector		0,0	0,1	0,1	0,0		

Energy sector	Total	0,0	0,1	0,1	0,0		0,89
Trade, Services and Government	Constructions		27,0	27,0			
	Trade, Services and Government[1]	0,5	0,8	1,3	0,5		
	Roofs and gutters utility buildings	1,9	9,2	11,1	1,9		
Trade, Services and Government	Total	2,4	37,0	39,4	2,4		0,02
Agriculture	Corrosion greenhouse	1,5		1,5	1,5	4,6	
	Shot	2,9		2,9	2,9	16,2	
	Agriculture[1]	0,0	0,0	0,0	0,0	2220,0	
Agriculture	Total	4,4	0,0	4,4	4,4	2240,8	0,04
Other industries	Automotive industry		0,0	0,0			
	Cacao industry		0,1	0,1			
	Beverage and drinks industry		0,0	0,0			
	Glass industry		0,0	0,0			
	Vegetables and fruit processing		0,4	0,4			
	Historical contamination	2,1		2,1	2,1		
	Iron works		0,0	0,0			
	Cardboard industry		1,5	1,5			
	Plastic goods industry		0,1	0,1			
	Leather tanning		0,1	0,1			
	Metal and electrotechnical industry		3,5	3,5			
	Non-ferro		0,3	0,3			
	Surface treament and other metalworking		3,0	3,0			
	Other industries[1]	9,2	8,6	17,7	9,2		
	Other foods industries		0,4	0,4			
	Fats and vegetable oil production		0,3	0,3			
	Rubber industry		0,2	0,2			
	Meat works and meat products		1,2	1,2			

	Textile finishing		0,5	0,5			
	Manufacture of bicyles and motorbikes		0,1	0,1			
	Manufacture of metal products		5,8	5,8			
	Textile processing		2,2	2,2			
	Dairy industry		0,1	0,1			
Other industries	Total	11,2	28,4	39,6	11,2		58,2
Reffineries		0,2	0,0	0,2	0,2		
Reffineries	Total	0,2	0,0	0,2	0,2		0,001
Traffic and transport	Tire wearing	29,7	49,1	78,7	29,7	118,6	7,69
	Corrosion crash barriers	2,7		2,7	2,7	24,0	
	Corrosion lampposts	0,0	0,2	0,2	0,0	0,01	
	Corrosion zinc anodes, ships	23,9		23,9	23,9		
	Corrosion zinc anodes, lock gates	27,7		27,7	27,7		
	Exhaust gas	0,0	0,0	0,0	0,0	0,02	0,24
	Vehicle use	0,6	1,2	1,8	0,6	2,5	13,06
	Motor oil leakage	0,1	0,2	0,4	0,1	0,58	
	Brake lining wearing	0,0	0,0	0,0	0,0	0,04	0,24
	Transports	0,1	1,9	2,0	0,1	0,06	
	Exhaust gas, recreational shipping	0,0		0,0	0,0		0,0002
	Road surface wearing	0,1	0,2	0,3	0,1	0,33	0,02
Traffic and transport	Total	85,0	52,8	137,7	85,0	146,1	21,24
Nature	Deposition				8,1	90,0	
Nature	Total				8,1	90,0	
Seawage water treatment plants	Effluents STP				123,4		
	Untreated sewage water				0,0		
	Overflow				16,0		
	Overflow, deposition				0,5		

	Rainwater sewer				32,0		
	Rainwater sewer, deposition				1,5		
	Composting sewage sludge					43,8	
	Other reuse of sewage sludge					26,8	
	Sewage sludge incineration					167,7	
Seawage water treatment plants	Total				173,4	238,3	
All sectors	Total	135,5	332,9	468	317	2721	90,3
1) No subdivision into sources, for many sectors this source contains the individually registered companies							

INDUSTRY ANNEX 3.2.5
Refinement of the exposure assessment and risk characterisation – regional aquatic compartment

See section Industry Annexes at the end of this report.

Annex 3.2.5a

Spanish monitoring data

Heavy metals concentrations, organic matter contents and other parameters in agricultural and grassland Spanish soils.

Source: LÓPEZ ARIAS, M. & GRAU CORBÍ, J.M., 2004. Metales pesados, materia orgánica y otros parameters de la capa superficial de los suelos agrícolas y de pastos de la España Peninsular.

A research-project was carried out to determine heavy metal concentrations, organic matter contents and some edaphic characteristics in agricultural topsoils in Spain. Covering the entire Spanish peninsula, from 2001 to 2003, a plot was selected from each 64 ha of arable land area or from every 128 ha of grassland area, extracting a compound sample of 19 or 21 subsamples from each plot. The following seven heavy metals (Cd, Cr, Cu, Hg, Ni, Pb and Zn) were determined in each sample extracted by *aqua regia* digestion, and oxidized organic carbon, pH, electrical conductivity, carbonates and granulometric fraction were also determined. On the agricultural and grassland soils, 3669 sampling plots were selected and 2713 of these were analyzed.

Annex 3.2.5a Summary of zinc concentrations in Spanish soils (in mg/kg) (extracted from *LÓPEZ ARIAS, M. & GRAU CORBÍ, J.M., 2004*)

	Sandy soil	Loamy soil	Balanced soil	Clay soil	High clay content soil
Textural Class	1	2	3	4	5
minimum	5	8	7	16	26
average	47	55	56	67	79
median	36	44	49	56	70
maximum	1264	549	484	1254	162
90th P	87	98	91	93	119
No. parcels	650	712	1015	225	41

ANNEX 3.3. AQUATIC AND TERRESTRIAL TOXICITY DATA BASE

ANNEX 3.3.2.A. AQUATIC TOXICITY DATA BASE
ANNEX 3.3.2.B. FRESHWATER (MODEL) ECOSYSTEM STUDIES
ANNEX 3.3.2.C. DERIVATION OF SOFT WATER $PNEC_{add, aquatic}$
ANNEX 3.3.2.D. SEDIMENT TOXICITY DATA BASE
ANNEX 3.3.3.A . TERRESTRIAL TOXICITY DATA BASE

INTRODUCTION TO ANNEX 3.3

The ecotoxicological data summarised in the Tables in Annex 3.3 include all data that have been used to derive $PNEC_{add}$ values for surface water, STP-effluent, sediment and soil (see Risk Assessment Report Zinc Metal, section 3.3: Effects assessment). As mentioned in RAR Zinc Metal section 3.1 (General introduction) and section 3.3.1 (General introduction to the Effects assessment), the "added risk approach" has been used in this risk assessment report on zinc, both in the exposure assessment and effects assessment. With respect to the effects assessment the added risk approach implies that the PNEC is derived from toxicity data that are based on the added zinc concentration in the tests. This results in an "*added* Predicted No Effect Concentration" ($PNEC_{add}$).

All aquatic and terrestrial toxicity data in the RAR Zinc Metal and the Annexes are expressed as zinc, not as the test compound, because zinc itself is considered to be the causative factor for toxicity.

The results of the aquatic toxicity studies are expressed as either the actual (measured) concentration or, usually, as the nominal (added) concentration (Cn). The actual concentrations include the background concentration (Cb) of zinc. Because of the "added risk approach", the results based on actual concentrations have been corrected for background, if possible. This correction for background is based on the assumption that only the added concentration of zinc is relevant for toxicity. In case both actual and nominal concentrations were reported, the results are expressed as nominal concentrations, provided the actual concentrations were within 20% of the nominal concentrations.

The results of almost all terrestrial toxicity studies are expressed as the nominal concentration (Cn) in soil; actual concentrations were only reported in a few studies. In a number of studies the background concentration (Cb) in the test soil was reported in addition to the nominal test concentrations.

Sources and selection of ecotoxicological data

See RAR Zinc Metal section 3.3.1.1 (Sources and selection of ecotoxicological data) for a comprehensive overview of:

(i) The sources of the ecotoxicological studies (from reviews, especially Janus (1993)[40] and WHO, (1996)[41], extensive literature searches performed by the

40 Janus, J.A. (1993)
Integrated Criteria Document Zinc: Ecotoxicity, Appendix to RIVM report No. 710401028 (Cleven, R.M.F.J., Janus, J.A. Annema, J.A. and Slooff , W. Eds., 1993), National Institute of Public Health and the Environment, Bilthoven, The Netherlands.
(Originally published in 1992, as Appendix to RIVM-report 710401019)

41 WHO (1996)
Environmental Health Criteria for Zinc, Draft, summary, evaluation, conclusions and recommendations of the

rapporteur and the industry, and new studies performed in the framework of the RAR Zinc Metal). It is noted that the data included in the effects asessment focus on chronic toxicity studies (long-term studies) that used soluble, inorganic zinc salts as test compound and from which No Observed Effect Concentrations (NOEC values) for relevant toxicological endpoints (in particular survival, growth and reproduction) could be derived, as chronic NOEC values are used rather than acute LC50 or EC50 values to derive PNEC values. It is noted that Annex 3.3.2.A (Aquatic toxicity data base), which includes data for freshwater and saltwater organisms, does not include short-term tests resulting in acute LC50 or EC50 values, with the exception of Table 3.3.2.c (Toxicity to aquatic microorganisms) and Table 3.3.2.d (Toxicity of zinc metal powder to freshwater organisms), the latter summarising the base-set data for zinc metal. See Annex 1.3.2a for acute LC50 and EC50 values for freshwater organisms; the data in Annex 1.3.2.a have been used for classification and labelling, see also Chapter 1 of RAR Zinc Metal.

(ii) The criteria for the selection of the toxicity data that were used to derive $PNEC_{sdd}$ values. The selection of the toxicity data is based on reliability (quality) criteria (mainly derived from internationally accepted guidelines for toxicity tests, such as the OECD guidelines and relevance criteria. Examples of the relevance criteria used for the data selection of all toxicity data (aquatic and terrestrial) are the exclusion of tests that were performed in media containing high to very zinc background concentrations (thus only tests in relatively unpolluted test media were accepted), the exclusion of tests with mixed-metal exposure (thus only tests in which the organisms were exposed only to zinc were accepted) and the exclusion of tests with "insoluble" zinc salts such as zinc oxide and zinc carbonate (thus only tests with soluble zinc salts such as zinc chloride and zinc sulphate were accepted).

In addition to the selection criteria mentioned in RAR Zinc Metal section 3.3.1.1 (Sources and selection of ecotoxicological data), there are additional reliability and especially relevance criteria, which are specific for tests in either the aquatic or terrestrial compartment. The additional relevance criteria are based on the water and soil characteristics (abiotic factors). See RAR Zinc Metal section 3.3.2.1 (Toxicity to aquatic organisms) and section 3.3.3.1 (Toxicity to terrestrial organisms) for the additional selection criteria for tests in water and soil, respectively.

Derivation of NOEC values (methods)

The methods that have been used for the derivation of NOEC values (No Observed Effect Concentrations), being "real" NOEC values or NOEC values derived from effect concentrations, are essentially the same as outlined in the EU Technical Guidance Document on Risk Assessment (EC, 2003)[42].

If possible, "real" NOEC values were derived from the data reported, i.e. the NOEC is one of the concentrations actually used in the test. In order of preference:

IPCS task group.
Final report published in 2001 (Environmental Health Criteria Series 221: Zinc,
International Programme on Chemical Safety, World Health Organization, Geneva

42 EC (2003)
Technical Guidance document on Risk Asessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances, Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances, and Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. European Commission, Joint Research Center, Institute for Health and Consumer Protection, European Chemicals Bureau, Ispra (VA), Italy

1) Statistical analysis: the NOEC is the highest concentration (in a series of test concentrations) showing no statistical significant effect (inhibition) compared to the control. Significance level: p = 0.05 (optional: the p = 0.01 level if reported instead of the p = 0.05 level).
2) If no statistical analysis has been applied: the NOEC is the highest concentration that results in < 10% inhibition compared to the control.

In both cases there must be a consistent concentration-effect relationship, i.e the LOEC is the concentration at which and above which statistical significant toxicity is found (1) or, when no statistical analysis has been applied (2), >10% inhibition is found.

If the "real" NOEC could not be derived from the data reported, the following procedure was used to derive the NOEC. In order of preference:

1) The NOEC is set at the EC10 level.
   a) Especially in more recent references on terrestrial toxicity data there is increasing preference for the benchmark dose approach. Hence, a benchmark dose (usually the EC10) was reported in a number of references instead of the NOEC. The EC10, which is calculated from the concentration-effect relationship, is used as NOEC equivalent, unless the "real" NOEC was also reported or could be derived from the data reported. The reported EC10 values mostly refer to terrestrial studies.
   b) Furthermore, a number of EC10 values was calculated by the rapporteur; most of these calculations refer to microbial toxicity studies (see section 3.3.3 for further explanation on the method and prerequisites used by the rapporteur for the calculation of EC10 values).
2) The NOEC is derived from the LOEC
   If the EC10 was not reported and could not be calculated, the NOEC was derived from the LOEC using the following "extrapolation" factors:
      a) NOEC = LOEC/2, in case inhibition is >10% but ≤20%, e.g. LOEC = EC(15%).
      b) NOEC = LOEC/3, in case inhibition is >20% but ≤30% e.g. LOEC = EC(25%).
   If the percentage inhibition at the LOEC is >30% or in case the percentage inhibition at the LOEC is unknown, no NOEC is derived.

With respect to "rule 2b" it is noted that the EU Technical Guidance Document on Risk Assessment (EC, 2003) does not mention the derivation of a NOEC from a LOEC in case inhibition at the LOEC is >20%, while in the RAR Zinc Metal the derivation of a NOEC from a LOEC up to 30% effect has been used in some aquatic toxicity studies and especially in terrestrial microbial toxicity studies. The use of the higher effect level is justified by the use of a higher extrapolation factor. Regarding the microbial data the use of NOEC = LOEC/3 allows the calculation of a number of "alternative" NOEC values from tests that resulted in a "real" NOEC that is considered to be unreliable. For further explanation see RAR Zn Metal section 3.3.3.1 (Toxicity to terrestrial organisms).

References

All references of the ecotoxicological studies summarised in Annex 3.3 are included in the List of References at the end of Annex 3.3. In some cases the references are also included in the separate Annexes, viz. in Annex 3.3.2.B, Annex 3.3.2.C and Annex 3.3.2.D.

ANNEX 3.3.2.A. AQUATIC TOXICITY DATA BASE

Table 3.3.2.a. Chronic toxicity of zinc to freshwater organisms: NOEC values
Part I: Studies useful for freshwater $PNEC_{add, aquatic}$ derivation
Part II: Studies not useful for freshwater $PNEC_{add, aquatic}$ derivation

Table 3.3.2.b. Chronic toxicity of zinc to saltwater organisms: NOEC values
Part I: Studies useful for saltwater $PNEC_{add, aquatic}$ derivation
Part II: Studies not useful for saltwater $PNEC_{add, aquatic}$ derivation

Table 3.3.2.c. Toxicity of zinc to (aquatic) microorganisms: NOEC and EC values

Table 3.3.2.d. Toxicity of zinc metal powder to freshwater organisms: NOEC and EC values

Table 3.3.2.a. Chronic toxicity of zinc to freshwater organisms: NOEC values
Part I: Studies useful for freshwater $PNEC_{add,\ aquatic}$ derivation

Organism & life stage	A	Test-type	Test-comp.	Test-water	pH	Hard-ness	Exp.-time	Criterion	Result (µg Zn/l)
Algae (unicellular)									
Pseudokirchneriella subcapitata	+	S	Zn powder	art. (OECD; no EDTA)	7.4	24	3-d	$NOEC_g$ Van Woensel '94a [56]	**50** (actual)
Pseudokirchneriella subcapitata	+	S	ZnO (EPM-grade)	art. (OECD; no EDTA)	7.5	24	3-d	$NOEC_g$ Van Ginneken '94a [57b,58,59]	**24** (actual)
Pseudokirchneriella subcapitata (code: Na-2.7 nM)	+	S	$ZnCl_2$	art. (OECD; no EDTA)	7.5	24	3-d	$NOEC_g^e$ De Schamphelaere et al.,'03 [64]	**5.4** (actual) [64a]
Pseudokirchneriella subcapitata (code: Ca-1.0 mM)	+	S	$ZnCl_2$	art. (OECD; no EDTA)	7.5	112	3-d	$NOEC_g^e$ De Schamphelaere et al.,'03 [64]	**5.2** (actual) [64b]
Pseudokirchneriella subcapitata (code: Ca-1.5 mM)	+	S	$ZnCl_2$	art. (OECD; no EDTA)	7.5	162	3-d	$NOEC_g^e$ De Schamphelaere et al.,'03 [64]	**5.5** (actual) [64c]
Pseudokirchneriella subcapitata (code: Ca-2.0 mM)	+	S	$ZnCl_2$ (OECD;	art. no EDTA)	7.5	212	3-d	$NOEC_g^e$ De Schamphelaere et al.,'03 [64]	**5.5** (actual) [64d]
Pseudokirchneriella subcapitata (code: Mg-0.5 mM)	+	S	$ZnCl_2$	art. (OECD; no EDTA)	7.5	62	3-d	$NOEC_g^e$ De Schamphelaere et al.,'03 [64]	**5.2** (actual) [64e]
Pseudokirchneriella subcapitata (code: Mg-1.0 mM)	+	S	$ZnCl_2$	art. (OECD; no EDTA)	7.5	112	3-d	$NOEC_g^e$ De Schamphelaere et al.,'03 [64]	**8.6**(actual) [64f]
Pseudokirchneriella subcapitata (code: Mg-1.5 mM)	+	S	$ZnCl_2$	art. (OECD; no EDTA)	7.5	162	3-d	$NOEC_g^e$ De Schamphelaere et al.,'03 [64]	**7.7** (actual) [64g]
Pseudokirchneriella subcapitata (code: Mg-2.0 mM)	+	S	$ZnCl_2$	art. (OECD; no EDTA)	7.5	212	3-d	$NOEC_g$ De Schamphelaere et al.,'03 [64]	**8.5**(actual) [64h]
Pseudokirchneriella subcapitata (fcode: Na-3.2 mM)	+	S	$ZnCl_2$	art. (OECD; no EDTA)	7.5	24	3-d	$NOEC_g^e$ De Schamphelaere et al.,'03 [64]	**6.8**(actual) [64i]
Pseudokirchneriella subcapitata (code: Na-3.7 mM)	+	S	$ZnCl_2$	art. (OECD; no EDTA)	7.5	24	3-d	$NOEC_g^e$ De Schamphelaere et al.,'03 [64]	**7.9**(actual) [[64j]
Pseudokirchneriella subcapitata (code: Na-4.7 mM)	+	S	$ZnCl_2$	art. (OECD; no EDTA)	7.5	24	3-d	$NOEC_g$ De Schamphelaere et al.,'03 [64]	**7.4**(actual) [64k]
Pseudokirchneriella subcapitata (code: Na-7.2 mM)	+	S	$ZnCl_2$	art. (OECD; no EDTA)	7.5	24	3-d	$NOEC_g^e$ De Schamphelaere et al.,'03 [64]	**4.9**(actual) [64l]
Pseudokirchneriella subcapitata (code: pH-6.2)	+	S	$ZnCl_2$	art. (OECD; no EDTA)	6.2	24	3-d	$NOEC_g$ De Schamphelaere et al.,'03 [64]	**124** (actual) [64m]

(to be continued)

Table 3.3.2.a. Chronic toxicity of zinc to freshwater organisms: NOEC values

(continued) Part I: Studies useful for freshwater $PNEC_{add, aquatic}$ derivation

Organism & life stage	A	Test-type	Test-comp.	Test-water	pH	Hard-ness	Exp.-time	Criterion	Result (µg Zn/l)
Algae (unicellular) (continued)									
Pseudokirchneriella subcapitata (code: pH-6.8)	+	S	$ZnCl_2$	art. (OECD; no EDTA)	6.8	24	3-d	$NOEC_g$ De Schamphelaere et al.,'03 [64]	**74** (actual) [64n]
Pseudokirchneriella subcapitata (code: pH-7.1)	+	S	$ZnCl_2$	art. (OECD; no EDTA)	7.1	24	3-d	$NOEC_g$ De Schamphelaere et al.,'03 [64]	**41** (actual) [64o]
Pseudokirchneriella subcapitata (code: pH-7.4)	+	S	$ZnCl_2$	art. (OECD; no EDTA)	7.4	24	3-d	$NOEC_g$[e] De Schamphelaere et al.,'03 [64]	**15** (actual) [64p]
Pseudokirchneriella subcapitata (code: pH-7.7)	+	S	$ZnCl_2$	art. (OECD; no EDTA)	7.7	24	3-d	$NOEC_g$ De Schamphelaere et al.,'03 [64]	**10** (actual) [64q]
Pseudokirchneriella subcapitata (code: pH 7.8)	+	S	$ZnCl_2$	art. (OECD; no EDTA)	7.8	24	3-d	$NOEC_g$ De Schamphelaere et al.,'03 [64]	**9.4**(actual) [64r]
Pseudokirchneriella subcapitata (code: Brisy-R)	+	S	$ZnCl_2$	river (DOC: 2.9 mg/l)	6.2	28	3-d	$NOEC_g$ De Schamphelaere et al.,'03 [64]	**58** (actual) [64s]
Pseudokirchneriella subcapitata (code: Brisy-N)	+	S	$ZnCl_2$	river (DOC: 2.5 mg/l)	6.3	27	3-d	$NOEC_g$ De Schamphelaere et al.,'03 [64]	**91** (actual) [64t]
Pseudokirchneriella subcapitata (code: Voyon-R)	+	S	$ZnCl_2$	river (DOC: 3.7 mg/l)	6.4	27	3-d	$NOEC_g$ De Schamphelaere et al.,'03 [64]	**73** (actual) [64u]
Pseudokirchneriella subcapitata (code: Markermeer-R)	+	S	$ZnCl_2$	lake (DOC: 5.9 mg/l)	8.0	239	3-d	$NOEC_g$[e] De Schamphelaere et al.,'03 [64]	**27** (actual) [64v]
Pseudokirchneriella subcapitata (code: Ankeveen-R)	+	S	$ZnCl_2$	ditch (DOC: 22 mg/l)	7.4	144	3-d	$NOEC_g$ De Schamphelaere et al.,'03 [64]	**105** (actual) [64w]
Pseudokirchneriella subcapitata				(n =25)		geometric mean		$NOEC_g$	**17** (actual)
Algae (multicellular)									
Cladophora glomerata 1 cm fragments	-	S	-	art.	8.4	>35	3-d	$NOEC_g$ Whitton '67 [13]	**60** (Cn)
Poriferans									
Ephydatia fluviatilis cells → sponges	-	S	$ZnCl_2$	art. (M4)	8	250	7-d	$NOEC_d$ Van de Vyver, '01 [61]	**43** (Cn)
Ephydatia muelleri cells → sponges	-	S	$ZnCl_2$	art. (M4)	8	250	7-d	$NOEC_d$ Van de Vyver, '01 [61]	**43** (Cn)
Spongilla lacustris cells → sponges	-	S	$ZnCl_2$	art. (M4)	8	250	7-d	$NOEC_d$ Van de Vyver, '01 [61]	**65** (Cn)
Eunapius fragilis cells → sponges	-	S	$ZnCl_2$	art. (M4)	8	250	7-d	$NOEC_d$ Van de Vyver, '01 [61]	**43** (Cn)

(to be continued)

Table 3.3.2.a. Chronic toxicity of zinc to freshwater organisms: NOEC values

(continued) Part I: Studies useful for freshwater $PNEC_{add,\ aquatic}$ derivation

Organism & life stage	A	Test-type	Test-comp.	Test-water	pH	Hard-ness	Exp.-time	Criterion	Result (µg Zn/l)
Molluscs									
Dreissena polymorpha length 1.6-2.2 cm	+	R	$ZnCl_2$	lake	7.9	270 (Ca)	10-w	$NOEC_f$	100 (Cn) (actual: 101)
								$NOEC_s$	**400** (Cn) (actual: 382)
								$NOEC_g$	≥1,400 (Cn) (actual: 1,266)
								Kraak et al' 94 [48]	
Potamopyrgus jenkinsi juveniles (length 1.7 ± 0.1 cm)	+	R	$ZnCl_2$	lake	8.0	160 (Ca)	16-w	$NOEC_g$	**75** (Cn) (actual: 72)
								Dorgelo et al '95 [49]	
Crustaceans									
Ceriodaphnia dubia P < 1 d → F_1 [lc]	+	R	-	river (N)	6	81	1-w	$NOEC_r$	**25** (Cn)
								Belanger & Cherry '90 [22]	
Ceriodaphnia dubia P < 1 d → F_1 [lc]	+	R	-	river (N)	8	81	1-w	$NOEC_r^e$	**25** (Cn) [17]
								Belanger & Cherry '90 [22]	
Ceriodaphnia dubia P < 1 d → F_1 [lc]	+	R	-	river (N)	9	81	1-w	$NOEC_r$	**25** (Cn)
								Belanger & Cherry '90 [22]	
Ceriodaphnia dubia P < 1 d → F_1 [lc]	+	R	-	river (A)	6	118	1-w	$NOEC_r^e$	**40** (Cn) [18]
								Belanger & Cherry '90 [22]	
Ceriodaphnia dubia P < 1 d → F_1 [lc]	+	R	-	river (A)	8	118	1-w	$NOEC_r$	**50** (Cn)
								Belanger & Cherry '90 [22]	
Ceriodaphnia dubia P < 1 d → F_1 [lc]	+	R	-	river (A)	9	118	1-w	$NOEC_r^e$	**45** (Cn) [19]
								Belanger & Cherry '90 [22]	
Ceriodaphnia dubia P < 1 d → F_1 [lc]	+	R	-	river (C)	6	168	1-w	$NOEC_r^e$	**29** (Cn) [20]
								Belanger & Cherry '90 [22]	
Ceriodaphnia dubia P < 1 d → F_1 [lc]	+	R	-	river (C)	8	168	1-w	$NOEC_r$	**50** (Cn)
								Belanger & Cherry '90 [22]	
Ceriodaphnia dubia P < 1 d → F_1 [lc]	+	R	-	river (C)	9	168	1-w	$NOEC_r^e$	**33** (Cn) [21]
								Belanger & Cherry '90 [22]	
Ceriodaphnia dubia P 3 d → F_1 [lc]	-	R	$ZnCl_2$	river	8.0	169	4-d	$NOEC_r^e$	**50** (Cn)
								$NOEC_s^e$	50 (Cn)
								Masters et al., 1991 [51]	
Ceriodaphnia dubia P 3 d → F_1 [lc]	-	R	$ZnCl_2$	river	8.0	169	4-d	$NOEC_r^e$	**14** (Cn)
								$NOEC_s^e$	50 (Cn)
								Masters et al., 1991 [51]	
Ceriodaphnia dubia P < 1 d → F_1 [lc]	-	R	$ZnCl_2$	river	8.0	169	7-d	$NOEC_r^e$	**50** (Cn)
								$NOEC_s^e$	29 (Cn)
								Masters et al., 1991 [51]	
Ceriodaphnia dubia P < 1 d → F_1 [lc]	-	R	$ZnCl_2$	river	8.0	169	7-d	$NOEC_r^e$	**100** (Cn)
								$NOEC_s^e$	100 (Cn)
								Masters et al., 1991 [51]	
Ceriodapnia dubia					(n = 13)	geometric mean		$NOEC_r$	**37** (Cn)

(to be continued)

Table 3.3.2.a. Chronic toxicity of zinc to freshwater organisms: NOEC values

(continued) Part I: Studies useful for freshwater $PNEC_{add, aquatic}$ derivation

Organism & life stage	A	Test-type	Test-comp.	Test-water	pH	Hard-ness	Exp.-time	Criterion	Result (µg Zn/l)
Crustaceans (continued)									
Daphnia magna P < 1 d → F_1 (lc)	+	R	$ZnCl_2$	well	7.5	52	21-d	$NOEC_{r,s}$	**97** (actual) Chapman et al. '80
Daphnia magna P < 1 d → F_1 (lc)	+	R	$ZnCl_2$	well	7.7	104	21-d	$NOEC_{r,s}$	**43** (actual) Chapman et al. '80 [52]
Daphnia magna P < 1 d → F_1 (lc)	+	R	$ZnCl_2$	well	8.4	211	21-d	$NOEC_{r,s}$	**42** (actual) Chapman et al. '80 [52]
Daphnia magna P < 1 d → F_1 [lc]	-	R	$ZnSO_4$.$7H_2O$	pond	8.4	52	7-w	$NOEC_r$[e]	**31** (Cn) Paulauskis & Winner '88 [23]
Daphnia magna P < 1 d → F_1 [lc]	-	R	$ZnSO_4$.$7H_2O$	pond (+ DOC: 0.75 mg /l)	8.4	52	7-w	$NOEC_r$[e]	**33** (Cn) Paulauskis & Winner '88 [23]
Daphnia magna P < 1 d → F_1 [lc]	-	R	$ZnSO_4$.$7H_2O$	pond (+ DOC: 1.5 mg /l)	8.4	52	7-w	$NOEC_r$	**84** (Cn) Paulauskis & Winner '88 [23]
Daphnia magna P < 1 d → F_1 [lc]	-	R	$ZnSO_4$.$7H_2O$	pond	8.3	102	7-w	$NOEC_r$	**83** (Cn) Paulauskis & Winner '88 [23]
Daphnia magna P < 1 d → F_1 [lc]	-	R	$ZnSO_4$. .$7H_2O$	pond	8.3	197	7-w	$NOEC_r$	**159** (Cn) Paulauskis & Winner '88 [23]
Daphnia magna P < 1 d → F_1 [lc]	-	R	$ZnSO_4$. .$7H_2O$	pond (+ DOC: 1.5 mg/l)	8.3	197	7-w	$NOEC_r$	**208** (Cn) Paulauskis & Winner '88 [23]
Daphnia magna P < 1 d → F_1 [lc]	+	R	$ZnCl_2$	lake	7.7	45	3-w	$NOEC_r$[e]	**35** (Cn) Biesinger & Christensen '72 [24]
Daphnia magna P < 1 d → F_1 [lc]	+	R	$ZnCl_2$	lake	7.7	45	3-w	$NOEC_r$	**74** (actual) Biesinger et al. '86 [25]
Daphnia magna P < 1 d → F_1 [lc]	+	R	$ZnCl_2$	lake	8.1	225	3-w	$NOEC_g$ $NOEC_{s,r}$	37 (Cn) [26] **310** (Cn) [26] Enserink et al.'91 [28]
Daphnia magna P + F	+	F	$ZnCl_2$	lake	8.1	225	17-d	$NOEC_{s,r}$[e]	**420** (Cn) [27] Enserink et al.'91 [28]
Daphnia magna P < 2 d → F_1 [lc] (population "I")	+	R	-	lake	7.7	65	3-w	$NOEC_{s,r}$	**100** (Cn) Münzinger & Monicelli '91 [30]
Daphnia magna P < 2 d → F_1 [lc] (population L")	+	R	-	lake	7.7	65	3-w	$NOEC_{s,r}$	**100** (Cn) Münzinger & Monicelli '91 [30]
Daphnia magna P < 2 d → F_1 [lc] (population "P")	+	R	-	lake	7.7	65	3-w	$NOEC_r$[e] $NOEC_s$	**25** (Cn) [29] 100 (Cn) Münzinger & Monicelli '91 [30]

(to be continued)

Table 3.3.2.a. Chronic toxicity of zinc to freshwater organisms: NOEC values

(continued) Part I: Studies useful for freshwater $PNEC_{add, aquatic}$ derivation

Organism & life stage	A	Test-type	Test-comp.	Test-water	pH	Hard-ness	Exp.-time	Criterion	Result (µg Zn/l)
Crustaceans (continued)									
Daphnia magna P < 1 d → F_1 [lc] (code: CA-0.25; MG-0.25; NA-2)	+	R	$ZnCl_2$	art.	6.6	50	3-w	$NOEC_{r,s}$	**82** (actual) [65a] De Schamphelaere et al.,'03 [65]
Daphnia magna P < 1 d → F_1 [lc] (code: CA-05)	+	R	$ZnCl_2$	art.	6.6	75	3-w	$NOEC_{r,s}$	**50** (actual) [65b] De Schamphelaere et al.,'03 [65]
Daphnia magna P < 1 d → F_1 [lc] (code: CA-1)	+	R	$ZnCl_2$	art.	6.6	125	3-w	$NOEC_{r,s}$	**54** (actual) [65c] De Schamphelaere et al.,'03 [65]
Daphnia magna P < 1 d → F_1 [lc] (code: CA-2)	+	R	$ZnCl_2$	art.	6.6	225	3-w	$NOEC_{r,s}$	**92** (actual) [65d] De Schamphelaere et al.,'03 [65]
Daphnia magna P < 1 d → F_1 [lc] (code: MG-05)	+	R	$ZnCl_2$	art.	6.6	75	3-w	$NOEC_{r,s}$	**48** (actual) [65e] De Schamphelaere et al.,'03 [65]
Daphnia magna P < 1 d → F_1 [lc] (code: MG-1)	+	R	$ZnCl_2$	art.	6.6	125	3-w	$NOEC_{r,s}$	**152** (actual) [65f] De Schamphelaere et al.,'03 [65]
Daphnia magna P < 1 d → F_1 [lc] (code: MG-1.5)	+	R	$ZnCl_2$	art.	6.6	175	3-w	$NOEC_{r,s}$	**155** (actual) [65g] De Schamphelaere et al.,'03 [65]
Daphnia magna P < 1 d → F_1 [lc] (code: MG-2)	+	R	$ZnCl_2$	art.	6.6	225	3-w	$NOEC_{r,s}$	**156**(actual) [65h] De Schamphelaere et al.,'03 [65]
Daphnia magna P < 1 d → F_1 [lc] (code: NA-6)	+	R	$ZnCl_2$	art.	6.6	50	3-w	$NOEC_{r,s}$	**143** (actual) [65i] De Schamphelaere et al.,'03 [65]
Daphnia magna P < 1 d → F_1 [lc] (code: NA-9)	+	R	$ZnCl_2$	art.	6.6	50	3-w	$NOEC_{r,s}$	**136** (actual) [65j] De Schamphelaere et al.,'03 [65]
Daphnia magna P < 1 d → F_1 [lc] (code: NA-12)	+	R	$ZnCl_2$	art.	6.6	50	3-w	$NOEC_{r,s}$	**143** (actual) [65k] De Schamphelaere et al.,'03 [65]
Daphnia magna					(n = 27)	geometric mean		$NOEC_r$	**88**
Hyalella azteca P < 1 w → F_1 [lc]	+	R	-	tap	7.9-8.6	130	10-w	$NOEC_{r,s}$ $NOEC_g$	**42** (actual) (Cn: 100) ≥316 (actual) (Cn: 560) Borgmann et al. '93 [50]
Hyalella azteca < 1 w	+	S	$ZnCl_2$	tap	7.9-8.6	130	4-w	$NOEC_s$ $NOEC_g$	166 (actual) 49 (actual-Cb) ≥208 (actual) ≥91 (actual-Cb) Borgmann & Norwood 97 [55]
Hyalella azteca								$NOEC_r$	**42** (actual)

(to be continued)

Table 3.3.2.a. Chronic toxicity of zinc to freshwater organisms: NOEC values

(continued) Part I: Studies useful for freshwater $PNEC_{add, aquatic}$ derivation

Organism & life stage	A	Test-type	Test-comp.	Test-water	pH	Hard-ness	Exp.-time	Criterion	Result (µg Zn/l)	
Insects										
Chironomus tentans P (newly hatched larvae) → F_1 [lc]	+	R	$ZnCl_2$	lake	7.7	45	8-w	$NOEC_{s,g,e,r}$	166 **137**(actual-Cb) Sibley et al. '96 [54]	(actual)
Fish										
Brachydanio rerio eggs < 4 hr → larvae	-	R	$ZnSO_4$.$7H_2O$	art.	7.5	100	2-w	$NOEC_h$ $NOEC_s$ Dave et al. '87 [32]	**2,900** 5,800	(Cn) (Cn)
Brachydanio rerio eggs < 4 hr→ larvae	-	R	$ZnSO_4$.$7H_2O$	art.	7.5	100	2-w	$NOEC_h$ $NOEC_s$ Dave et al. '87 [32]	**180** 5,800	(Cn) (Cn)
Brachydanio rerio eggs < 4 hr → larvae	-	R	$ZnSO_4$.$7H_2O$	art.	7.5	100	2-w	$NOEC_h$ $NOEC_s$ Dave et al. '87 [32]	**720** 5,800	(Cn) (Cn)
Brachydanio rerio eggs < 4 hr → larvae	-	R	$ZnSO_4$.$7H_2O$	art.	7.5	100	2-w	$NOEC_h$ $NOEC_s$ Dave et al. '87 [32]	**180** 5,800	(Cn) (Cn)
Brachydanio rerio eggs < 4 hr → larvae	-	R	$ZnSO_4$.$7H_2O$	art.	7.5	100	2-w	$NOEC_h$ $NOEC_s$ Dave et al. '87 [32]	**180** 2,900	(Cn) (Cn)
Brachydanio rerio eggs < 4 hr → larvae	-	R	$ZnSO_4$.$7H_2O$	art.	7.5	100	2-w	$NOEC_h$ $NOEC_s$ Dave et al. '87 [32]	**180** 5,800	(Cn) (Cn)
Brachydanio rerio eggs < 4 hr → larvae	-	R	$ZnSO_4$.$7H_2O$	art.	7.5	100	2-w	$NOEC_h$ $NOEC_s$ Dave et al. '87 [32]	**2,900** 2,900	(Cn) (Cn)
Brachydanio rerio eggs < 4 hr → larvae	-	R	$ZnSO_4$.$7H_2O$	art.	7.5	100	2-w	$NOEC_h$ $NOEC_s$ Dave et al. '87 [32]	< 720 5,800	(Cn) (Cn)
Brachydanio rerio eggs < 4 hr → larvae	-	R	$ZnSO_4$.$7H_2O$	art.	7.5	100	2-w	$NOEC_h$ $NOEC_s$ Dave et al. '87 [32]	**2,900** 11,500	(Cn) (Cn)
Brachydanio rerio eggs < 4 hr → larvae	-	R	$ZnSO_4$.$7H_2O$	art.	7.5	100	2-w	$NOEC_h$ $NOEC_s$ Dave et al. '87 [32]	**1,400** 11,500	(Cn) (Cn)
Brachidanio rerio					(n = 9)	geometric mean		$NOEC_h$	**660**	(Cn)
Jordanella floridae P (larvae) → F_1 (larvae) [lc] 1-d (from unexposed eggs)	+	F	$ZnSO_4$.$7H_2O$	lake	7.5	44	14-w	$NOEC_g$ $NOEC_s$ $NOEC_{r,h}$ Spehar, '76 [34]	**26** 51 ≥85	(actual) [33a] (actual) (actual)
Jordanella floridae P (larvae) → F_1 (larvae) [lc] 1-d (from exposed eggs)	+	F	$ZnSO_4$.$7H_2O$	lake	7.5	44	14-w	$NOEC_{g,r}$ $NOEC_s$ $NOEC_h$ Spehar, '76 [34]	**75** 139 ≥139	(actual) [33b] (actual) (actual)
Jordanella floridae					(n = 2)	geometric mean		$NOEC_g$	**44**	

(to be continued)

Table 3.3.2.a. Chronic toxicity of zinc to freshwater organisms: NOEC values

(continued) Part I: Studies useful for freshwater $PNEC_{add,\ aquatic}$ derivation

Organism & life stage	A	Test-type	Test-comp.	Test-water	pH	Hard-ness	Exp.-time	Criterion	Result (µg Zn/l)
Fish (continued)									
Oncorhynchus mykiss eyed eggs → fish untill sexual maturity	+	F	$ZnSO_4$	tap	6.8	26	± 2-yr?	$NOEC_s$ $NOEC_g$	**130** (actual - Cb) ≥535 (actual - Cb) Sinley et al. '74 [40]
Oncorhynchus mykiss "fish" (unexposed as eggs)	+	F	$ZnSO_4$	tap	6.8	26	25-d	$NOEC_s$	**25** (actual - Cb) Sinley et al. '74 [41]
Oncorhynchus mykiss eggs → early juveniles	+	F	$ZnCl_2$	well	7.0	27	72-d	$NOEC_s$	**440** (actual) Cairns and Garton '82 [42]
Oncorhynchus mykiss Early juveniles (5-6 w) (code: RF-B; MG-B)	+	F	$ZnCl_2$	art.	7.5	30	30-d	$NOEC_s$	**39** (actual) [66a] De Schamphelaere et al.,'03 [66]
Oncorhynchus mykiss Early juveniles (5-6 w) (code: RF-NA5)	+	F	$ZnCl_2$	art.	7.5	30	30-d	$NOEC_s$	**95** (actual) [66b] De Schamphelaere et al.,'03 [66]
Oncorhynchus mykiss Early juveniles (5-6 w) (code: MG-0.2)	+	F	$ZnCl_2$	art.	7.7	45	30-d	$NOEC_s$	**45** (actual) [66c] De Schamphelaere et al.,'03 [66]
Oncorhynchus mykiss Early juveniles (5-6 w) (code: MG-1)	+	F	$ZnCl_2$	art.	7.7	139	30-d	$NOEC_s$	**151** (actual) [66d] De Schamphelaere et al.,'03 [66]
Oncorhynchus mykiss Early juveniles (5-6 w) (code-MG-2)	+	F	$ZnCl_2$	art.	7.7	229	30-d	$NOEC_s$	**159** (actual) [66e] De Schamphelaere et al.,'03 [66]
Oncorhynchus mykiss Early juveniles (5-6 w) (code: PH-6.5)	+	F	$ZnCl_2$	art.	6.7	29	30-d	$NOEC_s$	**256** (actual) [66f] De Schamphelaere et al.,'03 [66]
Oncorhynchus mykiss Early juveniles (5-6 w) (code: PH-7.5)	+	F	$ZnCl_2$	art.	7.6	28	30-d	$NOEC_s$	**157** (actual) [66g] De Schamphelaere et al.,'03 [66]
Oncorhynchus mykiss Early juveniles (5-6 w) (code: CA-2)	+	F	$ZnCl_2$	art.	7.9	190	30-d	$NOEC_s$	**974** (actual) [66h] De Schamphelaere et al.,'03 [66]
Oncorhynchus mykiss Early juveniles (5-6 w) (code: ANK)	+	F	$ZnCl_2$	ditch (DOC: 23 mg/l)	7.8	104	30-d	$NOEC_s$	**771** (actual) [66i] De Schamphelaere et al.,'03 [66]
Oncorhynchus mykiss Early juveniles (5-6 w) (code: MAR)	+	F	$ZnCl_2$	lake (DOC: 6.2 mg/l)	8.1	176	30-d	$NOEC_s$	**696** (actual) [66j] De Schamphelaere et al.,'03 [66]
Oncorhynchus mykiss Early juveniles (5-6 w) (code: VOY)	+	F	$ZnCl_2$	river (DOC: 3.9 mg/l)	6.8	28	30-d	$NOEC_s$	**324** (actual) [66k] De Schamphelaere et al.,'03 [66]
Oncorhynchus mykiss Early juveniles (5-6 w) (code: BIH)	+	F	$ZnCl_2$	river (DOC: 4.3 mg/l)	6.2	23	30-d	$NOEC_s$	**370** (actual) [66l] De Schamphelaere et al.,'03 [66]
Oncorhynchus mykiss					(n = 15)	geometric mean		$NOEC_s$	**189**

(to be continued)

Table 3.3.2.a. Chronic toxicity of zinc to freshwater organisms: NOEC values

(continued) Part I: Studies useful for freshwater $PNEC_{add,\ aquatic}$ derivation

Organism & life stage	A	Test-type	Test-comp.	Test-water	pH	Hard-ness	Exp.-time	Criterion	Result (µg Zn/l)
Fish (continued)									
Phoxinus phoxinus mature	+	F	$ZnNO_3$. .$4H_2O$	tap	7.5	70	5-m	$NOEC_{s,g}$ Bengtsson '74 [45]	130 (actual)
Phoxinus phoxinus yearlings	+	F	$ZnNO_3$. .$4H_2O$	tap	7.5	70	5-m	$NOEC_{s,g}$ Bengtsson '74 [45]	**50** (actual)
Pimephales promelas eggs < 1d → larvae [els]	+	F	$ZnSO_4$.$7H_2O$	lake	7.7	47	32-d	$NOEC_s$ $NOEC_g$ Norberg-King '89 [46]	129 (actual) ≥129
Pimephales promelas newly hatched larvae	+	R	$ZnSO_4$.$7H_2O$	lake	7.7	47	7-d	$NOEC_g$ $NOEC_s$ Norberg-King '89 [46]	128 (actual) ≥128
Pimephales promelas newly hatched larvae	+	R	$ZnSO_4$.$7H_2O$	lake	7.7	47	7-d	$NOEC_{s,g}$ Norberg-King '89 [46]	117 (actual)
Pimephales promelas newly hatched larvae	+	F	$ZnSO_4$.$7H_2O$	lake	7.7	47	7-d	$NOEC_g$ $NOEC_s$ Norberg-King '89 [46]	129 (actual) ≥129
Pimephales promelas newly hatched larvae	+	F	$ZnSO_4$.$7H_2O$	lake	7.7	47	7-d	$NOEC_{s,g}$ Norberg-King '89 [46]	277 (actual)
Pimephales promelas newly hatched larvae	+	F	$ZnSO_4$.$7H_2O$	lake	7.7	47	7-d	$NOEC_{s,g}$ Norberg-King '89 [46]	291 (actual)
Pimephales promelas newly hatched larvae	+	R	$ZnSO_4$.$7H_2O$	lake	7.7	47	5-d	$NOEC_g$ $NOEC_s$ Norberg-King '89 [46]	128 (actual) ≥128
Pimephales promelas newly hatched larvae	+	R	$ZnSO_4$.$7H_2O$	lake	7.7	47	5-d	$NOEC_s$ $NOEC_g$ Norberg-King '89 [46]	117 (actual) ≥117
Pimephales promelas newly hatched larvae (< 1 d)	+	R	-	lake	7.5	48	7-d	$NOEC_s$ $NOEC_g$ Norberg & Mount '85 [36]	85 (actual) 184 (actual)
Pimephales promelas embryos (gastrula) → larvae	+	R	$ZnSO_4$.$7H_2O$	art.	7.0	100	6-d	$NOEC_d$ Dawson et al.'88 [35]	120 **(**Cn)
Pimephales promelas P → F_1 [lc] (eggs < 1 d) → (larvae 2 m)	+	F	$ZnSO_4$.$7H_2O$	lake	7-8	46	± 8-m	$NOEC_r$ $NOEC_{s,h,,d}$ $NOEC_m$ $NOEC_g$ Benoit & Holcombe '78 [37]	**78** (actual) 145 (actual) 295 (actual) ≥575 (actual)
Pimephales promelas								$NOEC_r$	**78** (actual)

(to be continued)

Table 3.3.2.a. Chronic toxicity of zinc to freshwater organisms: NOEC values

(continued) Part I: Studies useful for freshwater $PNEC_{add, aquatic}$ derivation

Organism & life stage	A	Test-type	Test-comp.	Test-water	pH	Hard-ness	Exp.-time	Criterion	Result (µg Zn/l)
Fish (continued)									
Salvelinus fontinalis P → F_2 [lc] [3-gen.] (yearlings) → (F_2 larvae 12 w)	+	F	$ZnSO_4$.$7H_2O$	lake	7.0-7.7	45	3-yr	$NOEC_h$ $NOEC_{s,g,r}$ Holcombe et al.'79 [43]	**530**(actual) ≥1360(actual)
Salvelinus fontinalis eggs 6 hr → larvae 12 w [els]	+	F	$ZnSO_4$.$7H_2O$	lake	7.2-7.9	45	>12 -w	$NOEC_s$ $NOEC_g$ Holcombe et al.'79 [44]	720 (actual) ≥2,060 (actual)
Salvelinus fontinalis newly hatched larvae → larvae 12 w (from exposed eggs)	+	F	$ZnSO_4$.$7H_2O$	lake	7.2-7.9	45	12-w	$NOEC_s$ $NOEC_g$ Holcombe et al.'79 [44]	720(actual) ≥2,060 (actual)
Salvelinus fontinalis newly hatched larvae → larvae 12 w (from unexposed eggs)	+	F	$ZnSO_4$.$7H_2O$	lake	7.2-7.9	45	>12 -w	$NOEC_s$ $NOEC_g$ Holcombe et al.'79 [44]	1,370 (actual) ≥2,060 (actual)
Salvelinus fontinalis larvae 4 w → larvae 12 -w (from unexposed eggs)	+	F	$ZnSO_4$.$7H_2O$	lake	7.2-7.9	45	8-w	$NOEC_s$ $NOEC_g$ Holcombe et al.'79 [44]	720(actual) ≥2,060 (actual)
Salvelinus fontinalis								$NOEC_h$	**530**

Table 3.3.2.a: To be continued in Part II: Studies not useful for freshwater $PNEC_{add, aquatic}$ derivation

Table 3.3.2.a. Chronic toxicity of zinc to freshwater organisms: NOEC values

Part II: Studies not useful for freshwater $PNEC_{add, aquatic}$ derivation

Organism & life stage	A	Test-type	Test-comp.	Test-water	pH	Hard-ness	Exp.-time	Criterion	Result (µg Zn/l)
Algae (unicellular)									
Chlorella vulgaris	-	S	$ZnSO_4$	art.	-	150	2-w	$NOEC_g$ Ahluwalia & Kaur '88 [1] Not useful: R	400 (Cn)
Chlorella vulgaris	-	S	$ZnCl_2$	art	7-9	55	5-w	$NOEC_g$ Rosko & Rachlin '77 [2] Not useful: Q	560 (Cn)
Chroococcus paris	-	S	$ZnSO_4$ ($.7H_2O$)	art. (BG-11)	7.8	54	10-d	$NOEC_g$ Les & Walker '84 [3] Not useful: Q, R	400 (Cn)
Hormidium rivulare	-	S	$ZnSO_4$ or $ZnCl_2$	art.	6	35-500	1-w	$NOEC_g$ Hargreaves & Whitton '76a [14] Not useful: R, Q	1,000 (Cn)
Kirchneriella subcapitata	-	-	$Zn(NO_3)_2$	art.	-	-	2-w	$NOEC_g^e$ Dragos et al.'88 [5] Not useful: R	95 [4] (Cn)
Monoraphidium contortum	-	-	$Zn(NO_3)_2$	art.	-	-	2-w	$NOEC_g$ Dragos et al.'88 [5] Not useful: R	190 [6] (Cn)
Navicula incerta	-	S	$ZnCl_2$	art.	8.5	-	4-d	$NOEC_g$ Rachlin et al. '83 [7] Not useful: R	1,000 (Cn)
Pseudokirchneriella subcapitata	-	S	$ZnCl_2$	art.	7	15	4-d	$NOEC_g^e$ Bartlett et al. '74 [10] Not useful: R	10 (Cn)
Pseudokirchneriella subcapitata	-	S	-	art.	-	-	2-w	$NOEC_g$ Kuwabara '85 [11] Not useful: R	5 (Cn)
Pseudokirchneriella subcapitata	-	S	ZnO (Read seal)	art.	8.5	12	3-d	$NOEC_g$ LISEC '97 [57a, 59] Not useful: R	8 (Cn)
Pseudokirchneriella subcapitata	+	S	$ZnSO_4$ ($.7H_2O$)	art. (OECD; no EDTA)	7.8	24	3-d	$NOEC_g$ LISEC, 1998 [62, 62-R1] Not useful: Q	8 (Cn)
Pseudokirchneriella subcapitata	+	S	$ZnSO_4$ ($.7H_2O$)	art. (OECD; no EDTA)	7.6	24	3-d	$NOEC_g^e$ LISEC, 1998 [62, 62-R2] Not useful: R	2 (Cn)
Pseudokirchneriella subcapitata	+	S	$ZnSO_4$ ($.7H_2O$)	art. (OECD; no EDTA)	8.4	24	3-d	$NOEC_g$ LISEC, 1998 [62, 62-R3] Not useful: Q	100 (Cn)
Pseudokirchneriella subcapitata	+	S	$ZnSO_4$ ($.7H_2O$)	art. (OECD; no EDTA)	8.4	24	3-d	$NOEC_g^e$ LISEC, 1998 [62, 62-R4] Not useful: R	6 (Cn)

(to be continued)

Table 3.3.2.a. Chronic toxicity of zinc to freshwater organisms: NOEC values

(continued) Part II: Studies not useful for freshwater $PNEC_{add, aquatic}$ derivation

Organism & life stage	A	Test-type	Test-comp.	Test-water	pH	Hard-ness	Exp.-time	Criterion	Result (µg Zn/l)
Algae (unicellular) (continued)									
Pseudokirchneriella subcapitata (code: MG-2.5 mM)	+	S	$ZnCl_2$ (OECD;	art. no EDTA)	7.5	262	3-d	$NOEC_g$[e]	8.0 (actual) De Schamphelaere et al., '03 [64, 64-R1] Not useful: R
Pseudokirchneriella subcapitata (code: pH 5.6)	+	S	$ZnCl_2$ (OECD;	art. no EDTA)	5.6	24	3-d	$NOEC_g$	131 (actual) De Schamphelaere et al., '03 [64, 64-R2] Not useful: R
Pseudokirchneriella subcapitata (code: Bihain-R)	+	S	$ZnCl_2$	river (DOC: 6.3 mg/l)	5.7	16	3-d	$NOEC_g$	358 (actual) De Schamphelaere et al., '03 [64, 64-R3] Not useful: R
Pseudokirchneriella subcapitata (code: Bihain-N)	+	S	$ZnCl_2$	river (DOC: 6.3 mg/l)	5.7	14	3-d	$NOEC_g$	228 (actual) De Schamphelaere et al., '03 [64, 64-R4] Not useful: R
Pseudokirchneriella subcapitata (code: Ossenkolk-R)	+	S	$ZnCl_2$	river (DOC: 6.7 mg/l)	5.8	7	3-d	$NOEC_g$	186 (actual) De Schamphelaere et al., '03 [64, 64-R5] Not useful: R
Scenedesmus quadricauda	-	S	$ZnSO_4$	art.	6.5	-	2-w	$NOEC_g$	100 (Cn)
	-	S	$ZnSO_4$	art.	8.5	-	2-w	$NOEC_g$	230 (Cn) Starodub et al. '87 [8] Not useful: R
Scenedesmus quadricauda	-	S	$ZnSO_4$.$7H_2O$	river	7.5	200	4-d	$NOEC_g$	1,200 (Cn) Bringmann & Kühn '59a,b [9] Not useful: R
Synechococcus (strain 6301)	-	S	$ZnSO_4$	art. (BG-11)	7.8	54	14-d	$NOEC_g$	390 (Cn) Mohanty '89 [12] Not useful: Q, R
Macrophytes									
Callitriche platycarpa	-	R	$ZnSO_4$	ditch	8.0	-	10-w	$NOEC_{s,g}$	≥650 (Cn) Van der Werff & Pruyt '82 [68] Not useful: Q
Elodea nuttallii	-	R	$ZnSO_4$	ditch	8.0	-	10-w	$NOEC_{s,g}$	≥650 (Cn) Van der Werff & Pruyt '82 [68] Not useful: Q
Lemna gibba	-	R	$ZnSO_4$	ditch	8.0	-	10-w	$NOEC_{s,g}$	≥650 (Cn) Van der Werff & Pruyt '82 [68] Not useful: Q
Lemna minor	-	S	-	art.	5	310	≥2-w	$NOEC_g$	160 (Cn) Jenner & Janssen-Mommen '93 [69] Not useful: R
Lemna paucicostata strain 6746	-	S	$ZnSO_4$.$7H_2O$	art.	4/5	700	1-w	$NOEC_g$	5,000 (Cn) Nasu & Kugimoto '81 [67]
Spirodela polyrhiza	-	R	$ZnSO_4$	ditch	8.0	-	10-w	$NOEC_{s,g}$	≥650 (Cn) Van der Werff & Pruyt '82 [68] Not useful: Q

(to be continued)

Table 3.3.2.a. Chronic toxicity of zinc to freshwater organisms: NOEC values

(continued) Part II: Studies not useful for freshwater $PNEC_{add, aquatic}$ derivation

Organism & life stage	A	Test-type	Test-comp.	Test-water	pH	Hard-ness	Exp.-time	Criterion	Result (µg Zn/l)
Poriferans									
Ephydatia fluviatilis	-	F	$ZnCl_2$	art.	7	150	10-d	$NOEC_{t-d}$ $NOEC_g$ Francis & Harrison '88 [15] Not useful: R	3.3 (Cn) 33 (Cn)
Ephydatia fluviatilis cells → sponges	-	S	-	art. (M)	-	300	10-d	$NOEC_{d(*)}$ Richelle et al. '95 [60] Not useful: R	33 (Cn)
Ephydatia muelleri cells → sponges	-	S	-	art. (M)	-	300	10-d	$NOEC_{d(*)}$ Richelle et al. '95 [60] Not useful: R	33 (Cn)
Spongilla lacustris cells → sponges	-	S	-	art. (M)	-	300	10-d	$NOEC_d$ Richelle et al. '95 [60] Not useful: R	65 (Cn)
Ephydatia fluviatilis cells → sponges	-	S	$ZnCl_2$	art. (M)	-	300	7-d	$NOEC_d$ Van de Vyver, '01 [61] Not useful: R	65 (Cn)
Ephydatia muelleri cells → sponges	-	S	$ZnCl_2$	art. (M)	-	300	7-d	$NOEC_d$ Van de Vyver, '01 [61] Not useful: R	65 (Cn)
Spongilla lacustris cells → sponges	-	S	$ZnCl_2$	art. (M)	-	300	7-d	$NOEC_d$ Van de Vyver, '01 [61] Not useful: R	65 (Cn)
Eunapius fragilis cells → sponges	-	S	$ZnCl_2$	art. (M)	-	300	7-d	$NOEC_d$ Van de Vyver, '01 [61] Not useful: R	43 (Cn)
Crustaceans									
Daphnia magna P < 1 d → F_1 [lc] (codeL CA-3)	+	R	$ZnCl_2$	art.	6.6	325	3-w	$NOEC_{r,s}$ De Schamphelaere et al., '03 [65, 65-R1] Not useful: R	158(actual)
Daphnia magna P < 1 d → F_1 [lc] (code: CA-4)	+	R	$ZnCl_2$	art.	6.6	425	3-w	$NOEC_{r,s}$ De Schamphelaere et al., '03 [65, 65-R2] Not useful: R	98 (actual)
Daphnia magna P < 1 d → F_1 [lc (code: MG-4)	+	R	$ZnCl_2$	art.	6.6	425	3-w	$NOEC_{r,s}$ De Schamphelaere et al., '03 [65, 65-R3] Not useful: R	161 (actual)
Daphnia magna P < 1 d → F_1 [lc] (code: PH-5.5)	+	R	$ZnCl_2$	art. (DOC: 5 mg/l)	5.5	50	3-w	$NOEC_{r,s}$ De Schamphelaere et al., '03 [65, 65-R4] Not useful: R	161 (actual)
Daphnia magna P < 1 d → F_1 [lc] (code: PH-6)	+	R	$ZnCl_2$	art. (DOC: 5 mg/l)	6	50	3-w	$NOEC_{r,s}$ De Schamphelaere et al., '03 [65, 65-R5] Not useful: R	168 (actual)
Daphnia magna P < 1 d → F_1 [lc] (code: PH-6.5)	+	R	$ZnCl_2$	art. (DOC: 5 mg/l)	6.5	50	3-w	$NOEC_{r,s}$ De Schamphelaere et al., '03 [65, 65-R6] Not useful: R	161 (actual)

(to be continued)

Table 3.3.2.a. Chronic toxicity of zinc to freshwater organisms: NOEC values (continued) Part II: Studies not useful for freshwater $PNEC_{add, aquatic}$ derivation

Organism & life stage	A	Test-type	Test-comp.	Test-water	pH	Hard-ness	Exp.-time	Criterion	Result (µg Zn/l)
Crustaceans (continued)									
Daphnia magna P < 1 d → F_1 [lc] (code: PH-7)	+	R	$ZnCl_2$	art. (DOC: 5 mg/l)	7	50	3-w	$NOEC_{r,s}$	154 (actual) De Schamphelaere et al., '03 [65, 65-R7] Not useful: R
Daphnia magna P < 1 d → F_1 [lc] (code: PH-7.5)	+	R	$ZnCl_2$	art. (DOC: 5 mg/l)	7.5	50	3-w	$NOEC_{r,s}$	133 (actual) De Schamphelaere et al., '03 [65, 65-R8] Not useful: R
Daphnia magna P < 1 d → F_1 [lc] (code: PH-8)	+	R	$ZnCl_2$	art. (DOC: 5 mg/l)	8	50	3-w	$NOEC_{r,s}$	117 (actual) De Schamphelaere et al., '03 [65, 65-R9] Not useful: R
Daphnia magna P → F_1 [lc] (code: K1)	+	R	$ZnCl_2$	art. (DOC: 10 mg/l)	8.0	370	3-w	$NOEC_{r,s}$	320 (actual) Heijerick et al.,'03 [63] Not useful: R, (Q)
Daphnia magna P → F_1 [lc] (code: K2)	+	R	$ZnCl_2$	art. (DOC: 10 mg/l)	6.5	370	3-w	$NOEC_{r,s}$	320 (actual) Heijerick et al.,'03 [63] Not useful: R, (Q)
Daphnia magna P → F_1 [lc] (code: K3)	+	R	$ZnCl_2$	art. (DOC: 32 mg/l)	8.0	110	3-w	$NOEC_{r,s}$	630 (actual) Heijerick et al.,'03 [63] Not useful: R, (Q)
Daphnia magna P → F_1 [lc] (code: K4)	+	R	$ZnCl_2$	art. (DOC: 32 mg/l)	6.5	110	3-w	$NOEC_{r,s}$	445 (actual) Heijerick et al.,'03 [63] Not useful: R, (Q)
Daphnia magna P → F_1 [lc] (code: K5)	+	R	$ZnCl_2$	art. (DOC: 10 mg/l)	8.0	110	3-w	$NOEC_{r,s}$	209 (atual) Heijerick et al.,'03 [63] Not useful: R, (Q)
Daphnia magna P → F_1 [lc] (code: K6)	+	R	$ZnCl_2$	art. (DOC: 10 mg/l)	6.5	110	3-w	$NOEC_{r,s}$	320 (actual) Heijerick et al.,'03 [63] Not useful: R, (Q)
Daphnia magna P → F_1 [lc] (code: K7)	+	R	$ZnCl_2$	art. (DOC: 32 mg/l)	6.5	370	3-w	$NOEC_{r,s}$	630 (actual) Heijerick et al.,'03 [63] Not useful: R, (Q)
Daphnia magna P → F_1 [lc] (code: K8)	+	R	$ZnCl_2$	art. (DOC: 32 mg/l)	8.0	370	3-w	$NOEC_{r,s}$	630 (actual) Heijerick et al.,'03 [63] Not useful: R, (Q)
Daphnia magna P → F_1 [lc] (code: C1)	+	R	$ZnCl_2$	art. (DOC: 21 mg/l)	7.3	240	3-w	$NOEC_{r,s}$	320 (actual) Heijerick et al.,'03 [63] Not useful: R, (Q)
Daphnia magna P → F_1 [lc] (code: C2)	+	R	$ZnCl_2$	art. (DOC: 21 mg/l)	7.3	240	3-w	$NOEC_{r,s}$	320 (actual) Heijerick et al.,'03 [63] Not useful: R, (Q)
Daphnia magna P → F_1 [lc] (code: C3)	+	R	$ZnCl_2$	art. (DOC: 21 mg/l)	7.3	240	3-w	$NOEC_{r,s}$	575 (actual) Heijerick et al.,'03 [63] Not useful: R, (Q)
Daphnia magna P → F_1 [lc] (code: S1)	+	R	$ZnCl_2$	art. (DOC: 21 mg/l)	6.0	240	3-w	$NOEC_{r,s}$	425 (actual) Heijerick et al.,'03 [63] Not useful: R, (Q)
Daphnia magna P → F_1 [lc] (code; S2)	+	R	$ZnCl_2$	art. (DOC: 21 mg/l)	7.3	35	3-w	$NOEC_{r,s}$	445 (actual) Heijerick et al.,'03 [63] Not useful: R, (Q)

continued)

Table 3.3.2.a. Chronic toxicity of zinc to freshwater organisms: NOEC values (continued) Part II: Studies not useful for freshwater PNECadd, aquatic derivation

Organism & life stage	A	Test-type	Test-comp.	Test-water	pH	Hard-ness	Exp.-time	Criterion	Result (µg Zn/l)
Crustaceans (continued)									
Daphnia magna P → F_1 [lc] (code: S3)	+	R	$ZnCl_2$	art. (DOC: 40 mg/l)	7.3	240	3-w	$NOEC_{r,s}$ Heijerick et al.,'03 [63] Not useful: R, (Q)	1,000 (actual)
Daphnia magna P → F_1 [lc] (code: S4)	+	R	$ZnCl_2$	art. (DOC: 2 mg/l)	7.3	240	3-w	$NOEC_{r,s}$ Heijerick et al.,'03 [63] Not useful: (Q)	209 (actual)
Daphnia magna P → F_1 [lc] code: S5)	+	R	$ZnCl_2$	art. (DOC: 21 mg/l)	7.3	445	3-w	$NOEC_{r,s}$ Heijerick et al.,'03 [63] Not useful: R, (Q)	575 (actual)
Daphnia magna P → F_1 [lc] code: S6)	+	R	$ZnCl_2$	art. (DOC: 21 mg/l)	8.5	240	3-w	$NOEC_{r,s}$ Heijerick et al.,'03 [63] Not useful: R, (Q)	630 (actual)
Hyalella azteca 4 to 5 w	+	S	$ZnCl_2$	tap	7.9-8.6	130	1-w	$NOEC_s$ Borgmann & Norwood 97 [55] Not useful: Q [55a]	208 (actual) 91 (actual-Cb)
Moina macrocopa P < 1 d → F_1 [lc]	-	R	$ZnSO_4$.$7H_2O$	art ?6.5-7.0	-		16-d	$NOEC_s$ $NOEC_r$ Wong '93 [47] Not useful: R	250 (Cn) 500 (Cn)
Insects									
Epeorus latifolium larvae, length 6 mm	-	F	$ZnSO_4$	ground	7.9	83	4-w	$NOEC_{s,e}$ $NOEC_g$ Hatakeyama '89 [31] Not useful: Q	3 (Cn) 30 (Cn)
Ephoron virgo larvae, newborn	+	S	$ZnCl_2$	river (Rhine)	7.8	200	10-d	$NOEC_s^e$ Van der Geest et al., '01 [16] Not useful: Q	720 (actual) 718 (actual-Cb)
Ephoron virgo larvae, newborn	+	S	$ZnCl_2$	art. (M7)	8.0	250	10-d	$NOEC_s^e$ Van der Geest et al., '01 [16] Not useful: Q	1,730 (actual) 1,724 (actual-Cb)
Fish									
Oncorhynchus mykiss P → F_1 (2 g fingerlings)→ 2-yr-old fish through sex. mat.	+	F	$ZnSO_4$	well	7.8	330	2-yr	$NOEC_s$ $NOEC_g$ Sinley et al. '74 [39] Not useful: R	290 (actual - Cb) ≥ 2,170 (actual - Cb)
Oncorhynchus mykiss Early juveniles (5-6 w) (code: RF-MG3)	+	F	$ZnCl_2$	art.	7.4	332	30-d	$NOEC_s$ De Schamphelaere et al.,'03 [66, 66-R1] Not useful: R	90 (actual)
Oncorhynchus mykiss Early juveniles (5-6 w) (code: MG-3)	+	F	$ZnCl_2$	art.	7.8	333	30-d	$NOEC_s$ De Schamphelaere et al.,'03 [66, 66-R2] Not useful: R	165 (actual)
Oncorhynchus mykiss Early juveniles (5-6 w) (code:PH-5.5)	+	F	$ZnCl_2$	art.	5.7	29	30-d	$NOEC_s$ De Schamphelaere et al.,'03 [66, 66-R3] Not useful: R	401 (actual)

(to be continued)

Table 3.3.2.a. Chronic toxicity of zinc to freshwater organisms: NOEC values

(continued) Part II: Studies not useful for freshwater $PNEC_{add, aquatic}$ derivation

Organism & life stage	A	Test-type	Test-comp.	Test-water	pH	Hard-ness	Exp.-time	Criterion	Result (µg Zn/l)
Fish (continued)									
Pimephales promelas larvae 1 d → fry	+	F	$ZnSO_4$.$7H_2O$	well	-	220	4-w	$NOEC_g$ $NOEC_s$ Broderius & Smith jr. '79 [38] Not useful: Q	± 300 (Cn) ± 700 (Cn)

For footnotes Table 3.3.2.a (Part I and II) see next pages; for further information see the "list of abbreviations Table 3.3.2.a to 3.3.2.d"

Abbreviations and footnotes Table 3.3.2.a

d = developmental effects (deformities; malformations; teratogenic effects);

d(*) = developments effects in sponges (measured by cell aggregation, settlement and formation of functional sponges);

e = emergence;

f = filtration rate;

g = growth;

h = hatchability;

m = maturation (sexual development);

r = reproduction;

s = survival;

t-d = tissue-deterioration (interior tissue)

lc = life cycle test;

els = early-life stage test (egg-larval test)

[1] Ahluwalia & Kaur (1988): Alga (unicellular) *Chlorella vulgaris*

Test medium according to Allen and Arnon (1955); the medium contained 4 mg/l EDTA-complex ($10x10^{-3}$ mMol/l), and macro- and micro-elements including 50 µg Zn/l. According to the authors, growth was significantly reduced at 4,000 mg/l, but statistical data were not reported**. Test rejected, based on Relevance criteria (No data on pH and/or hardness values in the artificial test water used).**

[2] Rosko & Rachlin (1977): Alga (unicellular) *Chlorella vulgaris*

No statistics reported. Test medium: sterile Bristol's medium containing macro- and micro-elements (assumed to be chelator free, see footnote 7). Chlorophyll a content per cell was reduced 15% at 560 mg/l, but the number of cells was increased 10%. At 2,400 µg/l both parameters were reduced at least 20%. In this study, growth parameters were measured after about 30 days, which is very long compared to the 3 days mentioned in OECD 201 (Alga, Growth inhibition Test for unicellular algae, with Chlorella vulgaris as one of the recommended species). Test rejected, based on Quality criterion (From the data reported it is not clear whether or not the algae still were exponentially growing and thus the validity of the test is questionable) .

[3] Les & Walker (1984): Alga (unicellular) *Chroococcus paris*

No statistics reported**.** Culture and test medium BG-11 medium, referring to Allen (1968); the medium contained EDTA (1 mg/l, which is around $3x10^{-3}$ mMol/l), and macro- and micro-elements including 0.05 mg Zn/l (*). Nominal test concentrations: 0-100-200-400-1,000-2,000-5,000 µg/l. The 10-d $NOEC_g$ listed in the table is based on the 10-d average specific growth rate, derived from a graph showing for each treatment the optical density at different time intervals from day 1 to dag 10). During the 10-d exposure period, the control culture maintained exponential growth, with the highest growth rate during days 8-10. However, the 72-h NOEC for specific growth rate as used in OECD 201 can not be derived from the graph with any accuracy (the graph only includes data for days 0, 2, 5, 8 and 10). The 10-d $NOEC_g$ for biomass was 200 mg/l. The initial cell concentration was 15 mg d.w./liter. The NOEC for

biomass was 200 µg/l. The NOEC values were derived from a graph showing the optical density at different time intervals.

(*) In Allen (1968), BG-11 medium is not mentioned specifically, but only "modified medium of Hughes et al. (Hughes et al., 1958; not checked). The data on pH, hardness, background zinc concentration, and EDTA concentraion are for the modified Hughes as specified in Allen (1968). See also footnote [12] for corresponding data on (modified) BG-11 medium.

Test rejected, based on both Quality criterion (No NOEC can be derived using a 72-h exposure period according to OECD 201 and Relevance criterion (The background zinc concentration in the artificial culture and test water used was very high: 65 µg/l).

[4] Dragos et al. (1988): Alga (unicellular) *Kirchneriella subcapitata*

Growth parameter: generation time. The NOEC was estimated from the lowest effect concentration (15% increase in generation time at 190 µg/l) using a factor of 2.

[5] Dragos et al. (1988): Algae (unicellular) *Kirchneriella subcapitata* and *Monoraphidium contortum*

No statistics reported. Test medium: modified *Zehnder et al. (1960)* no. 11 medium *(Zehnder et al. (1960) not available);* EDTA omitted. Test concentrations reported: 0.187, 0.750, 1.5, 3 and 4 mg/l, without data on analysis. **Tests rejected, based on Relevance criteria (No data on pH and/or hardness values in the artificial test water used).**

[6] Dragos et al. (1988): Alga (unicellular) *Monoraphidium contortum*

Growth parameters: cell number, chlorophyll a content (in medium and per cell), optical density and generation time. Chlorophyll content per cell increased with increasing zinc concentration.

[7] Rachlin et al (1983): Alga (unicellular) *Navicula incerta*

No statistics reported. Culture and test medium: sterile, chelator free LDM medium, referring to Starr (1978). According to the data reported in Starr (1978), LDM medium contains 100 ml Bristol's solution (containing micro- and macro-element and 900 ml seawater per 1,000 ml of medium. Growth parameter: number of cells. **Test rejected, based on Relevance criterion (Freshwater algae cultured and tested in artificial seawater).**

[8] Starodub et al. (1987): Alga (unicellular) *Scenedesmus quadricaudata*

Statistics: p = 0.05. Test medium: CHU-10 medium; this EDTA-free medium represents a relatively unpolluted lake water (Wong et al., 1982, 1978; Chu, 1942). Growth rate (increase in optical density) measured by spectrometry. **Test rejected, based on Relevance criteria (No data on hardness value the artificial test water used).**

[9] Bringmann & Kühn (1959): Alga (unicellular) *Scenedesmus quadricaudata*

The NOEC (1,200 µg/l) is the reported "Toxicity threshold" for growth (number of cells); the value was reported as 1-1.4 mg/l in Bringmann & Kühn (1959a) and as 1.2 mg/l in Bringmann & Kühn 1959b) The "Toxicity Threshold" (TT) is defined in other publications of Bringmann & Kühn as the concentration at which 3-5% inhibition occurs (the limit of 3% or 5% depends on the organism tested). According to the current (RIVM/CSR '96) guidelines used, the NOEC is set equal to the TT. Test water: filtered river water. Culture conditions: The algae were cultured in city sewage water enriched with macro-elements including $CaSO_4$ (200 mg/l) and $MgSO_4$ (90 mg/l), resulting in a calculated hardness of 220 mg/l, to be added to the unknown parent hardness of the culture medium to achieve the total hardness of this

medium. The algae may have been adapted to high hardness and possibly also to high concentrations of zinc and other (metal) cations present in the sewage water; this may have reduced the sensitivity of the algae to zinc. **Test rejected, based on Relevance criteria (see above).**

[10] Barlett et al. (1974): Alga (unicellular) *Pseudokirchneriella subcapitata*

Test medium: AAPBT-medium *(referring to EPA, 1971; not checked)* containing 300 µg/l Na -EDTA (equivalent to $0.9x10^{-3}$ mMol/l). The NOEC was estimated from the lowest effect concentration (20% decrease in dry weight at 30 µg/l; this percentage decrease was derived from a graph) using a factor of 3. Zinc was completely inhibitory and algicidal at 100 and 700 µg/l, respectively. Test rejected, based on Relevance criterion (The hardness value is below 24 mg/, the minimum value used as criterion for hardness).

[11] Kuwabara (1985): Alga (unicellular) *Pseudokirchneriella subcapitata*

No statistics reported. Test medium: nutrient medium eluted through Chelex-100 to remove cationic impurities. Test media (S-3) were finally filter sterilized (0.45 µm). Computed free Zn-ion concentrations were equal to total-zinc concentrations. Growth parameters: lag phase, growth rate, and stationary phase cell density. Test rejected, based on Relevance criteria (No data on pH and/or hardness values in the artificial test water used. Moreover, the treatment of the test water will have resulted in a very low hardness and a very low zinc concentration, expected to be (far) below the minimum values used as selection criteria).

[12] Mohanty (1989): Alga (unicellular) *Synechococcus*

No statistics reported. Culture and test medium: Modified BG-11 medium, containing 1 µg/ml Fe-EDTA (around $3x10^{-3}$ mMol/l), and macro- and micro-elements including 65 µg Zn/l. Nominal test concentrations: 65 (control), 390 and 590 µg/l.The test was performed by inoculating a known volume of 10-days-old algal samples to the test medium (initial cell concentration: around 6500 cells/ml); the algae were then allowed to grow for 14 days after which a known quantity of algal cells were transferred to fresh medium for repeated culture for 7 to 10 times at 65 and 390 µg/l, respectively. During each transfer, growth parameters (including cell numbers) were measured. The 14-d $NOEC_g$ indicated in the table (390 µg/l) is based on the average specific growth rate over the total period in which growth occured in each of the subsequent exposures, using the average growth data (cell numbers) from a graph representing the average of 10 readings (the graph shows for the control and 390 µg/l concentration the cell numbers/ml medium at different time intervals from day 1 to dag 14). However, at 390 µg/l the increase in cell number was marginal untill the end of day 7, after which a sharp increase up to day 14 was seen, while in control cultures (65 µg/l) the lag phase was about 2 days followed by a progressive increase in the number of cells up to day 11. The 72-h average specific growth rate as used in OECD 201 appeared to be strongly affected at 390 µg/l (based on the data for day 2 and day 4; data for day 3 were not included in the graph), but the derivation of a 72-h NOEC for specific growth rate is not possible due to the limited number of test concentrations. It was further reported that growth was totally inhibited at a concentraton of 590 µg/l. In addition to growth (numbers of cells) the parameters chlorophyll a content, phycocyanin content and protein content were studied as well as the CO_2-fixation capacity and the 8-d total dry mass of algae. Test rejected based on both Quality criterion (No NOEC can be derived using a 72-h exposure period according to OECD 201) and Relevance criteria (The background zinc concentration in the artificial culture and test water used was very high (65 µg/l) and the results are based on 7-10 subsequent exposures which may have resulted in further adaption of the algae in both the control and exposure group).

[13] Whitton (1967): Alga (multicellular) *Cladophora glomerata*

No statistics reported. Growth parameter: weight. Culture medium: Modified No. 10 medium of Chu (1942), containing Fe.EDTA and other micro- and macro-elements. Test medium: EDTA-free culture medium, enriched with 10% membrane-filtered river water from which the alga were collected. Hardness (35 mg/l, as $CaCO_3$) was calculated from the data on the modified No. 10 medium of Chu reported in Hargraves and Whitton (1976b); the total hardness of the test medium will be somewhat higher than 35 mg/l due to the addition of 10% river water. The results for zinc in this EDTA-free medium were reported as "no obvious inhibition at 60 µg/l, "obvious inhibition"at 80 µg/l and "killed" at 100 µg/l. The results for zinc in the same medium containing 3.2 mg/l Na.EDTA ($10x10^{-3}$ mMol/l, equal to the upper limit of EDTA in test medium used in this RAR as selection criterion) were reported as "no obvious inhibiton at 300 µg/l, "obvious inhibition" at 400 µg/l and "killed" at 500 µg/l). Test species ("which appears to be the most abundant filamentous alga in streams around the world") originated from a moderately polluted stream. According to Whitton '67, large numbers of replicates were needed for the tests as marked variation was found between sister flasks (each containing 3 alga fragments which were weighted individually), but there were no data reported on the number of replicates used in the test with zinc (or in the tests with the other metals tested), nor other test specific data, with the exception of the test results. Despite that variation the results of the tests with zinc and other metals show a ("Mendel-like") regularity. The test is accepted with reservation. Although the reported data do not allow a reliable evaluation of the validity of the study, the study is accepted because the test species represents a taxonomic group for which no other zinc toxicity data are available.

[14] Hargraves & Whitton (1976a): Alga (unicellular) *Hormidium rivulare*

No statistics reported. Test medium according to Hargreaves and Whitton (1976b); the medium contained $6x10^{-3}$ mMol/l EDTA and micro- and macro-elements. A series of tests at different pH values (range 2.5 to 7) were conducted in the standard test medium (containing 0.25 mM Ca and 0.1 mM Mg, resulting in a hardness of 35 mg/l, as $CaCO_3$). A further series of tests were conducted at different pH values (range 2.75 to 6) and different hardness (range 35 to 500 mg/l, as $CaCO_3$; hardness increased by the addition of Ca). All tests were performed with a population isolated from an acid mine drainage containing a low pH (3.1) and a very high background zinc concentration (1,000 µg/l). In the standard medium, the toxicity of zinc increased markedly at higher pH values. In all but one tests at pH 6, growth was not reduced at a nominal zinc concentration of 1,000 µg/l (the background zinc concentration in the medium was not reported), provided the minimum Ca concentration was 10 mg/l (0.25 mM, combined with the 0.1 mM Mg resulting in a minimum hardness of 35 mg/l, as $CaCO_3$). Of the two tests at pH 6 and hardness of 35 mg/l, one resulted in no effect at a zinc concentration of 1,000 µg/l, while in the other test growth was reduced about 20%. Test rejected, based on Relevance criteria (Test species originating and adapted to a very high zinc concentration and low pH value) and Quality criteria (poorly reported study and contradictory results).

[15] Francis & Harrison (1988): Poriferan *Ephydatia fluviatilis*

Statistics: only applied for growth rate estimates (based on at least 8 sponges per test concentration). Gemmules of *E. fluviatilis* were collected from lake Pontchartrain, Louisiana and stored untill needed. Each sponge used in the tests was grown from one gemmule and trimmed to the same initila area (about 2 mm^2). Culture and test medium: artificial medium (representing the characteristics of Lake Pontchartrain in Louisiana) containing 6 mg/l Na EDTA.$2H_20$ (16x10 mMol/l) and macro- and microelements, including 0.65 µg Zn/l ($1x10^{-8}$

M, added as $ZnCl_2$: 1.4 µg/l). Culture and test temperature: 25 ^oC. Nominal test concentrations: 0–$5x10^{-8}$-$1x10^{-7}$-$2x10^{-7}$-$3x10^{-7}$-$4x10^{-7}$, $5x10^{-7}$-$9x10^{-7}$.

Effects:

At a zinc concentration of $1x10^{-7}$ Mol/l (6.5 µg/l; nominal) and higher concentrations, a progressive deterioration of interior tissues was observed outwardly (on photomicrographs), although at concentrations up to $5x10^{-7}$ Mol/l (33 µg/l) this was not revealed in the growth rate data (measured by area increase). A concentration of $4x10^{-7}$ Mol/l (26 µg/l) led to sponge dead in approximately three weeks and a concentration of $9x10^{-7}$ Mol/l (59 µg/l) led to death within a few days. Deterioration of interior tissues was not observed at the nominal concentrations of $5x10^{-8}$ Mol/l (3.3 µg Zn/l). Although there were no mortality data reported on sponges exposed to the lowest concentration that resulted in tissue deterioration ($1x10^{-7}$ Mol/l), nor on sponges exposed to concentrations of $2x10^{-7}$ or $3x10^{-7}$ Mol/l), this effect on interior tissues is considered severe enough to set the NOEC at the lowest concentration resulting in this effect.

Background concentration of zinc in the test medium: 0.65 µg/l (1.4 µg $ZnCl_2$/l). According to Francis and Harrison '88, a minimum zinc concentration of $1x10^{-9}$ Mol/l (0.065 µg/l) is sufficient for E. fluviatilis growth and normal growth requires a zinc concentration of $1x10^{-8}$ Mol Zn/l (0.65 µg/l). From this it is concluded that the zinc concentration in the culture and test medium was sufficient for normal growth. It is noted however, that the sponges were adapted to a low background zinc concentration which is at the minimum level used as selection criterion. Furthermore, the study authors reported that E. fluviatilis does not develop tolerance to zinc, thus minimal concentrations associated with toxicity are true threshold concentrations.**Test rejected, based on relevance criteria (The background zinc concentration (0.65 µg/l) in the culture and test medium is below the minimum value used as criterion for Cb (around 1 µg/l). Furthermore, additional studies with this and other sponge species are now available (Richelle et al., 1995; Van de Vijver, 2001), the latter study study performed in Elendt M4 medium; this fully defined medium (see e.g. OECD Guideline 211: Daphnia magna reproduction test) meets all the relevance criteria as used in the present RAR).**

[16] Van der Geest et al. (2003): Insect *Ephoron virgo*

No statistics reported with respect to NOEC derivation. *E. virgo* eggs were collected from the River Waal (a major branch of the River Rhine) and kept at 20 ^0C in either field collected Rhine water or artificial Dutch Standard Water (DSW); after the embryos entered diapause the eggs were kept at 4 ^0C for a minimum of 3 months, after which the diapauze was deactivated by transferring the eggs to a temperature of 20 ^0C, resulting in hatching after 4-6 days. Newly hatched larvae were then used in the tests, witch were performed in either Rhine water or aritificial test water (Elendt M7). The test vessels contained a sediment layer of combusted and washed quatz sand with a grain size of 100-400 µm, as *E. virgo* larvae live in and on the sediment.

In Rhine water (1.2 µm filtered before use) two tests were performed at the following nominal test concentrations: Test 1: 0-3,000-6,000-10,000-16,000-20,000-25,000 µg/l; Test 2: 0-3,000- 6,000-10,000-13,000-16,000-20,000 µg/l. In Elendt M7 three tests were performed at the following nominal test concentrations: Test 1: 0-10,000-20,000-30,000-40,000-50,000 µg/l; Test 2: 0-3,000-6,000-10,000-13,000-16,000-20,000-25,000-30,000-40,000 µg/l; Test 3: 0-5,000-10,000-15,000-30,000-40,000 µg/l. The NOEC values for survival listed in the table are LC10 values (calculated by Van der Geest et al. (2001) from the combined results for Rhine water and Elendt M7, respectively, using the logistic response model from Haanstra et al. (1985). Growth (only determined in the tests in Elendt M7) was a much less sensitive

endpoint than survival, with less than 20% inhibition at the highest concentrations tested. The 10-d LC50 values were 1,840 µg/l in Rhine water and 4,360 µg/l in M7 (based on actual concentrations); these values are statistically significant different at p = 0.01. An acute toxicity test with newborn larvae in Rhine water resulted in a 96-h LC50 of 20,700 µg/l.

The exposure concentrations decreased with 30% to 70% during the 10-d tests, thus the results are based on the average actual concentrations calculated from the day 0 and day 10 measurements (assuming an exponential decrease in time). The actual Zn conconcentrations in the controls (10-20 µg/l) were higher than the reported background concentratons (2 µg/l for filtered Rhine water and 6 µg/l for Elendt M7); this is problably due to the addition of the feeding solution.

Further note: Elendt M7 contains EDTA (1.7 µMol/l; added as Fe-EDTA) and is because of the presence of this chelating agent not recommended in OECD 211 (D. magna reproduction test) for toxicity testing of metals. However, the EDTA concentration is within a factor of two of the maximum chelator concentration of 1 µMol/l as recommended in OECD 201 (Algal growth test) and 6 times lower than the maximum value of 10 µMol/l that is used in this RAR as selection criterion for algal studies (see section 3.3.2.1.2). Furthermore, data for the cladocean *Chydorus sphaericus* (data abovementioned research laboratory, including Boivin , 2000)) and *Daphnia magna* show poor control performance in Elendt M7 without EDTA. For that reason the test in artificial medium was performed in standard Elendt M7.

Tests rejected based on Quality criterion (The study as such is valid, but the exposure time of the larvae was limited to 10 days, which is about 10-times shorter than the larval-stage period of 3 months, thus this is considered to be a short-term study which cannot be used to derive chronic NOEC values).

As mentioned by Industry, the test protocol including the 10-d exposure time in the 'long-term' study was approved by the rapporteur. However, at that time it was not known that this exposure time is about 10-times shorter than the larval-stage period of 3 months. The scientific value of the study (resulting in NOEC (EC10) values of 720 µg/l in Rhine water and 1730 µg/l in artificial medium M7) is that it shows that mayflies are not a taxonomic group that are very sensitive to zinc, as suggested by the rejected 4-w NOEC of 3 µg/l for the mayfly *Epeorus latifolium* (Hatekyama, 1989, see footnote [31]). Both studies with mayflies (Van der Geeest et al., 2003 and Hatekyama, 1989) show poor control survival of the larvae at longer-time exposure in the laboratory.

[17] Belanger & Cherry (1990): Crustacean *Ceriodaphnia dubia* (see also footnote [22])

NOEC = LOEC/2 (19% inhibition at 50 µg/l). An EC10 could not be calculated, as 28% was found at the lower concentration tested (25 µg/). Further concentrations were not tested.

[18] Belanger & Cherry (1990): Crustacean *Ceriodaphnia dubia* (see also footnote [22])

NOEC = EC10, calculated from the two effect concentrations (16% and 49% inhibition at 50 and 100 µg/l, respectively). EC10 calculated by the rapporteur, using the logistic dose-response model according to Haanstra et al. (1985).

[19] Belanger & Cherry (1990): Crustacean *Ceriodaphnia dubia* (see also footnote [22])

NOEC = EC10, calculated from the two effect concentrations (13% and 53% inhibition at 50 and 100 µg/l, respectively). EC10 calculated by the rapporteur, using the logistic dose-response model according to Haanstra et al. (1985).

[20] Belanger & Cherry (1990): Crustacean *Ceriodaphnia dubia* (see also footnote [22])

NOEC = EC10, calculated from the two effect concentrations (21% and 44% inhibition at 50 and 100 µg/l, respectively). EC10 calculated by the rapporteur, using the logistic dose-response model according to Haanstra et al. (1985).

[21] Belanger & Cherry (1990): Crustacean *Ceriodaphnia dubia* (see also footnote [22])

NOEC = LOEC/3 (26% inhibition at 100 µg/l). An EC10 could not be derived, as 30% inhibition was found at the lower concentration tested (50 µg/l). Further concentrations were not tested.

[22] Belanger & Cherry (1990): Crustacean *Ceriodaphnia dubia*

Test with reference to the US EPA method 1002.0 for testing chronic survival and reproduction of Ceriodapnia dubia (US. EPA, 1985; cited in Belanger & Cherry, '90). Reproductive parameter: number of young per female. The NOEC values listed in Table 3.3.2.a sometimes differ from the NOEC values reported by Belanger and Cherry (1990), because in their statistical analysis of the reproduction data, the pH=8 and 0 µg/l Zn treatment in each test water (3 different rivers: New river (Virginia), Amy Bayou river (Louisiana) and Clinch river (Virginia), water 11-µm filtered before use) was considered to be the control value. The NOEC values in Table 3.3.2.a are based on comparisons (per test water) with the 0 µg/l Zn control at corresponding pH. Data on survival reported incompletely, but it appear that survival was not affected at the test concentrations used (nominal: 0, 25 and 50 µg/l in New river water; 0, 50 and 100 µg/l in Amy Bayou and Clinch river water). The parent (ambient) pH of the test waters was 8.1-8.3; the parent hardness of N, A, and C river water was 98, 114 and 182 mg/l (as $CaCO_3$), respectively; the tests were performed with pH 8 acclimated daphnids, cultured in New river or Clinch river water. Measured zinc concentrations not reported separately for each test, but according to the authors measured zinc concentrations were ± 15% of nominal concentrations. Background zinc concentration in all three water were less than 20 µg/l (detection limit; Zn measured as acid soluble metal). According to IND (referring to Shiller & Boyle, 1985), the natural dissolved-Zn concentrations in these rivers, at least in New river, is expected to be very low: in the order of <0.2 µg/l, based on very detailed analysis of similar small rivers in the same area. However, based on measurements in some rivers in Virginia and Louisiana, Shiller & Boyle report zinc concentrations of 0.3-3 µg/l; there is no reference to New river, Amy Bayou or Clinch river specifically.

[23] Paulauskis & Winner (1988): Crustacean Daphnia magna

Statistics (p = 0.05) used for NOEC derivation by Paulauskis & Winner (1988). For both survival and reproduction (brood size) the results of each test were reported as "NEC" ("no-effect-concentration"), defined as the arithmetic mean between the NOEC and the LOEC. As in each test medium 2 or 3 tests were performed (sometimes at different concentrations for the same medium) and in some tests an effect on reproduction was found at the lowest concentration tested, an EC10 for reproduction was calculated by the rapporteur from the combined data of the 2 or 3 tests performed in a specific medium, using the logistic dose-response model according to Haanstra et al. (1985), thus NOEC = EC10. Survival usually was less sensitive than reproduction (only in hard water survival was equally sensitive than reproduction or slighly more sensitive).

Soft test water (hardness 52 mg/l) was prepared by diluting pond water with distilled, deionized, carbon-filtered, Organex-Q-filtered water; this dilution of water contained essentially no trace organic compounds. Medium-hard test waters (hardness 102 mg/l) and hard test waters (hardness 197 mg/l) were prepared from soft water by adding $CaSO_4$ and $MgSO_4$ in quantities that would maintain the approximate 2:1 ratio of calcium to magnesium

in the pond water. Background total zinc concentration in the pond water: 3.5-4.6 µg/l. In the tests of the DOC series, DOC was added as artificial humic acid (sodium salt).

Nominal Zn concentrations in soft water: 0-25-50-75-100-125 µg/l.

Nominal Zn concentrations in soft water plus DOC (0.75 mg/l): 0-(50)-75-100-125-150 µg/l.

Nominal Zn concentrations in soft water plus DOC (1.5 mg/l): 0-(25)-(50)-(75)-100-125-150-175 µg/l (nominal).

Nominal Zn concentrations in medium hardness water: 0-75-100-125-150-(175) µg/l.

Nominal Zn concentrations in hard water: 0-125-150-175-200-(225)-(250) µg/l.

Nominal Zn concentrations in hard water plus DOC (1.5 mg/l): 0-(150)-175-200-225-250-(275) µg/l.

[24] Biesinger & Christensen (1972): Crustacean *Daphnia magna*

Culture and test medium: Lake Superior water, strained through # 20 bolting cloth. Reproductive parameter: total number of young. A 16% reproductive impairment concentration representing "the minimal reproducible value below which the variability in reproduction could not be detected from controls" was reported at 70 µg/l (LOEC). The NOEC (35 µg/l) was estimated from this LOEC using a factor of 2 (i.e. NOEC = LOEC/2). Based on the 3-w LC50 of 158 µg/l for the parent animals (no further data on survival reported) and the 3-w EC50 of 102 µg/l for reproduction, survival was less sensitive to Zn than reproduction. Growth (body weight) of the parent animals after 3-w of exposure was also less sensitive than reproduction, with 28% weight reduction at 175 µg/l (growth was studied at 12 Zn concentrations but only the result at 175 µg/l was reported.

Background zinc concentration in Lake Superior water: 0.8 µg/l (mean), with a range of 1 to 2.7 µg/l *(Lowest Zn level reported should be 0.1 µg/l? See also below: data from Nriagu et al., 1996)*; pH: 7.7 (mean), with a range of 7.4 to 8.2; total hardness 45 mg/l (mean), with a range of 44 to 53 mg/l; alkalinity 42 mg/l (mean), with a range of 41 to 50 mg/l. These water characteristics are not study specific but based on general data on Lake Superior water characteristics mentioned in Biesinger and Christensen (1972).

In Nriagu et al. (1996): Env. Sci. Technol. 30, 178-187, additional information on the background concentration of zinc and other metals in the Great Lakes is reported. In Lake Superior the average dissolved Zn concentration was 0.28 µg/l, with a range of 0.14-0.87 µg/l, varying with sampling point and water depth. The water samples were collected in 1991. Prior to analysis the water samples were filtered through a 0.4 µm pore size Nuclepore filter; details of the filtering system used have been described by Nriagu et al. (1993): J. Great Lakes Res. 19, 175-182

[25] Biesinger et al. (1986): Crustacean *Daphnia magna*

Statistics: p = 0.05. Test water: Lake Superior water, strained through # 20 bolting cloth. In one out of two test performed, reproduction was considerably reduced (40-50%) at 74 µg/l (actual concentration), but this effect was not statistically significant. Tests were conducted at sublethal concentrations (see also Biesinger & Christensen, 1972). Reproductive parameter: total number of young.

[26] Enserink et al (1991): Crustacean *Daphnia magna* (see also footnote [28])

Statistics: p = 0.01. Because only the lowest effect concentrations with respect to growth (120 µg/l) and survival and reproduction (1,000 µg/l) were reported, the NOEC values were derived from these concentrations using a factor of 3.2, i.e. the ratio used between test

concentrations. Thus, the NOEC values listed in the table are real NOEC values. Growth parameter: carapace length of surviving adults (P-generation).

[27] Enserink et al. (1991): Crustacean *Daphnia magna* (see also footnote [28])

Tests were started with exponentially growing populations. The NOEC is the EC10 for yield (mean maximum number of daphnids) reported by Enserink et al. (1991).

[28] Enserink et al. (1991) Crustacean *Daphnia magna*

Test water: Lake IJssel water filtered through a 25 µm mesh and UV-treated. Lake IJssel is part of the River Rhine system**.**

[29] Münzinger & Monicelli (1991): Crustacean *Daphnia magna* (see also footnote [30])

NOEC = EC10, calculated by the rapporteur.

[30] Münzinger & Monicelli (1991): Crustacean *Daphnia magna*

No statistics reported (except for the additional test, see further). Culture and test medium: Lago Maggiore (Italy) water filtered though a 40 µm mesh. Background zinc concentration <6 µg/l. Three different populations were tested separately. Reproductive parameter: number of young. In additional 3-w tests in metal-free water, brood size (eggs/animal) and body length of primiparous animals of all three populations were significantly (p = 0.05) affected at 150 µg/l, the only test concentration used in these additional tests.

[31] Hatekyama (1989): Insect *Epeorus latifolium*

Statistics (p = 0.05) reported on growth data only. Reported hardness of test medium (83 µg/l, as $CaCO_3$) assumed to be 83 mg/l. Qualitatively poor study with a rather low number of test animals (12 per concentration; no replicates), high control mortality (25%), no statistical analyses of survival data, no analyses of the test concentrations (of which the lowest is three times lower than the background zinc concentration in test water: 9 µg/l). According to Hatakyama '89, the high control mortality may be related to the relatively short adaptation time to a lower ambient water temperature (the animals were collected from water at 22 ^{o}C and tested within one week in water at 15 ^{o}C): similar tests with Cu showed a higher than 25% control mortality when the difference between ambient water and test conditions was even higher (23 ^{o}C versus 12.5 ^{o}C) and less than 25% control mortality when the ambient and test temperatures were similar. In the test with zinc, there was a clear concentration dependent effect on survival (3, 4, 6, 9 12 and 12 larvae died at the nominal zinc concentrations of 0, 3, 10, 30, 100 and 300 µg/l, respectively), which is an argument to accept the test. It must be noted, however, that the validity of the NOEC (which has been set at the lowest concentration tested, resulting in a mortality rate of 4/12 compared to 3/12 in the control) can be questioned. The organisms were collected from a river free of any heavy metal pollution (no further data). Except values for pH, total hardness and electric conductivity no further data were reported on the groundwater used in the test. **Test rejected, based on Quality criteria (The validity of the test and the NOEC derived from the study are questionable).**

[32] Dave et al. (1987): Fish *Brachydanio rerio*

Ring test (n = 10; each of the 5 laboratories performed 2 tests) according to a draft ISO 1983 protocol; this protocol is similar to OECD 212: Fish, Short-term Toxicity Test on Embryo and Sac-fry stages, but growth was not studied. For each study, a NOEC, LOEC and MATC (geometric mean value of NOEC and LOEC) was reported for hatching (time) and survival (time), respectively. Parental fish were adopted to the test medium and other test conditions for two weeks.

[33a] Spehar (1976): Fish *Jordanella floridae* (see also footnote [34])

Statistics: p = 0.05. At 51 µg/l, growth of female fish was significantly reduced. Reproductive parameters (mean spawnings per female and embryo production appeared to be reduced at 85 µg/l, although not statistically significant (due to the high variation among all groups).

Test medium: Untreated Lake Superior water; background zinc concentration <1. Measured zinc concentrations: <1 (control)-26-51-85-139-267 µg/l (nominal concentrations not reported).

[33b] Spehar (1976): Fish *Jordanella floridae* (see also footnote [34])

Statistics: p = 0.05. At 139 µg/l, growth of male fish was significantly reduced. Reproductive parameters (mean spawnings per female and embryo production appeared to be reduced at 139 µg/l, although not statistically significant (due to the high variation among all groups).

Test medium: Untreated Lake Superior water. Measured zinc concentrations: 10 (control)-28-47-75-139-267 µg/l (nominal concentrations not reported). It is assumed that the zinc concentration in the control was due to the addition of zinc to the normal background concentration (< 1 µg/l; see 33a) and that the larvae were from eggs exposed to the elevated concentration of 10 µg/l; the reported data are not clear in this respect.

[34] Spehar (1976): Fish *Jordanella floridae* (see also footnote [34])

To control fungus, all embryos were treated with metal-free malachite green (4 mg/l) for 10 min during the first 3 days of incubation. The malachite green concentration is just below 5 mg/l, the concentration that has been reported to increase the zinc permeabiliyy of the vitelline membrane of embryos. Although it can not be excluded that the malachite green treatment may have increased the zinc uptake to some extent, the tests are accepted.

[35] Dawson et al. (1988): Fish *Pimephales promelas*

Statistics (p = 0.05) on growth data only. Concentrations were analysed in stock solutions, not in test waters. Survival and possibly growth were affected at higher concentrations than that resulting in larval deformities. The NOEC for larval deformities was mentioned as such in the reference , but could not be checked based on the data reported. NOEC values for survival and growth were not reported and could not be derived from the data reported. Test medium: FETAX solution (MFS), containing macro-elements (data on micro-elements were not reported).

[36] Norberg & Mount (1985): Fish *Pimephales promelas*

Statistics: p = 0.05. Test medium: Lake Superior water; pH based on Lake Superior water characteristics reported by Spehar (1976). No data on pretreatment of Lake Superior test water. No data on nominal concentrations.

[37] Benoit & Holcombe (1978): Fish *Pimephales promelas*

Statistics: p = 0.05. Test medium: Lake Superior water, passed through an ultraviolet sterilizer; background zinc concentration 2 µg/l (mean of duplicate tanks). Reproductive parameters: the total number of spawnings found on substrates, the total number of eggs adhering to spawning substrates and the percentage of chorions ruptured during removal from substrate were affected at 145 µg/l. According to Benoit and Holcombe '78, these effects were not related to exposure of parental fish, which apparently developed normally at 145 and 295 µg/l. Fish exposed to 295 µg/l and producing abnormal eggs produced normal eggs within a few days after they were transferred to control water. Conversely, mature fish from control water produced abnormal eggs within a few days after they were transferred to 295 µg/l and further investigation revealed that effects on eggs adhesiveness and fragility occurred

before water hardening. Thus, the eggs themselves (and not maturation of the fish) was affected at concentrations up to 295 µg/l. The effects on the eggs were considered to be relevant enough by Benoit and Holcombe to derive from this study a MATC between 78 µg/l and 145 µg/l, being the NOEC and LOEC for these effects. Survival of fish: Eight-week larval survival was determined for i) first-generation fish exposed as egg, ii) first-generation fish not exposed as egg and iii) second-generation fish. In all three cases the NOEC for survival was 145 µg/l. Eight-week larval growth of both first- and second-generation fish was not affected up to the highest test concentration (575 µg/l). Hatchability of first-generation eggs was not affected at 575 µg/l, while hatchability of offspring was affected at 295 µg/l. No data on nominal concentrations reported.

[38] Broderius & Smith jr. (1979): Fish *Pimephales promelas*

No statistics reported. NOEC for growth (dry weight) derived from figure. NOEC for survival estimated from the test concentrations and the statements that survival at 760 and 840 µg/l was about 65% of that in the controls. Four-week larval control survival was low, about 60% (below OECD 210 validity criterion (70%) and far below eight-week larval control survival in the life cycle study by Benoit and Holcombe '78 (>90%). **Test rejected based on Quality criteria (Poorly reported study, the NOEC could not be derived with certainty, too high control mortality).**

[39] Sinley et al. (1974): Fish *Oncorhynchus mykiss*

Statistics (p = 0.05) reported on growth data only. Mortality 6.4% at 640 µg/l versus 0% at concentrations up to 320 µg/l (actual concentrations). Although survival was reduced less than 10% at 640 µg/l, this concentration is considered to be the LOEC and 320 µg/l the NOEC, in accordance with the view of Sinley et al. '74. The test did not yield valid reproduction data, but male and female fertility appeared to be unaffected up to the highest concentration tested (2,200 µg/l). Test medium: well water; background zinc concentration 30 µg/l. No data on nominal concentrations reported. Test rejected, based on Relevance criterion (The hardness value is above 250 mg/l, the maximum value used as criterion for hardness).

[40] Sinley et al. (1974): Fish *Oncorhynchus mykiss*

Statistics (p = 0.05) reported on growth data only. NOEC survival: based on i) mortality of eggs, ii) mortality of yolk-sac fry and iii) mortality of feeding fry and fish. Mortality in feeding fry and fish was 6.9% at 260 µg/l versus 1.4%-2.6% (latter value: control value) at actual concentrations up to 140 µg/l. Eggs and yolk-sac fry were less sensitive. The NOECsurvival (140 µg/l) is in accordance with the view of Sinley et al., 74. Test medium: Dechlorinated tap water; background zinc concentration 11 µg/l. No data on nominal concentrations reported.

[41] Sinley et al. (1974): Fish *Oncorhynchus mykiss*

Mortality 8% at 71 µg/l versus 1% at 36 µg/l and 0% in the control group, respectively. Although survival was reduced less than 10% at 71 µg/l, this concentration is considered to be the LOEC and 36 µg/l the NOEC, in accordance with the view of Sinley et al. '74. Test medium: Dechlorinated tap water; background zinc concentration 11 µg/l. No data on nominal concentrations reported.

[42] Cairns & Garton (1982): Fish *Oncorhynchus mykiss*

Statistics applied. Test medium: UV-sterilized well water, diluted with water treated by reverse osmosis to reduce hardness. Background zinc concentration in test medium <5 µg/l. Before incubation eggs were disinfected by dipping in Wescodyne disinfectant. No data on nominal concentrations reported.

[43] Holcombe et al. (1979): Fish Salvelinus fontinalis

Statistics: p = 0.05. Three-generation test, with i) 5-months exposure of the parental generation (70 g yearlings through adult spawning), ii) 26-month exposure of the second generation (eggs through adult spawning), and iii) 5-months exposure of the third generation (eggs through the early juvenile stage). Survival: determined for the parental generation and for the second and third generation (12-w post-hatch larvae). Reproductive parameters (spawnings/female and viable eggs/female) and hatching: determined for the first and second generation. The parental generation was acclimated to the test conditions for 4 weeks. Test medium: UV-sterilized Lake Superior water. Exposure concentrations: 2.6-39-69-144-266-534-1,360 µg/l (actual concentrations; no data on nominal concentrations reported); the highest concentration was not used for the third-generation exposure. Egg fragility (force required to rupture egg chorions) was significantly reduced at 266 µg/l and higher concentrations, but according to Holcombe et al., '79, only 1,360 µg/l appeared to reduce chorion strength drastically enough to cause possible serious problems during natural spawning in loose gravel. Therefore, they derived a MATC between 530 µg/l (NOEC) and 1,360 µg/l (LOEC), based on hatching.

[44] Holcombe et al. (1979): Fish Salvelinus fontinalis

Statistics: p = 0.05. Test medium: Lake Superior water, passed through an ultraviolet sterilizer. Exposure concentrations: 2.6 (control 1), 3.2 (control 2)-266- 353-534-720-1,360-2,060-4,350 µg/l (no data on nominal concentrations). Some of the exposures were in the larval growth chambers also used in the 3-generation study, see above footnote.

[45] Bengtson (1974): Fish *Phoxinus phoxinus*

Statistics (p = 0.05) reported on growth data only. Test medium: Dechlorinated tap water; background zinc concentration 1 - 12 µg/l (total range). No data on nominal concentrations.

[46] Norberg-King (1989): Fish *Pimephales promelas*

Statistics applied, but no details per test reported. Eight tests with zinc (as $ZnSO_4.7H_2O$) as test compound: one full Early Life Stage Toxicity Test (according to OECD 210), and seven 5-7 days "subchronic" larval tests, to validate the latter short-term alternative for the full ELS-test. For each test a NOEC, LOEC and chronic value (CV or MATC, the geometric mean value of the NOEC and LOEC) were reported for the "sensitive" endpoint(s) survival and growth (as weight). In case only one sensitive endpoint was mentioned, i.e. survival or growth, it has as been assumed that the other endpoint was less sensitive in the test. Toxicity values for endpoints other than survival or growth were not reported, but it was stated that no abnormal development during the embryo stages was observed, nor was egg hatchability affected by expose to zinc. Hereby, the embryo exposure was the least sensitive stage. Test water: Sand and carbon filtered UV-sterilized Lake Superior water; hardness in tests: 44-49 mg/l; pH (mean value) based on the data in Biesinger and Christensen (1972); see also footnote 24. No data on nominal versus measured zinc concentrations; concentrations were measured in test waters and assumed to be actual.

[47] Wong (1993): Crustacean *Moina macroscopa*

Statistics (p = 0.05) reported on survival data only. Test medium: filtered aquarium water. It has been assumed that this test water is artifical. Hence: **Test rejected, based on Relevance criteria (No data on pH and/or hardness values in the artificial test water used)**.

[48] Kraak et al (1994): Mollusc *Dreissenia polymorpha*

Statistics: p = 0.05. Culture and test medium: Sieved (25 µm) and filtered (through sand) Lake Markermeer water. The hardness of this lake water (270 mg/l, as $CaCO_3$, based on the reported hardness of 150 mg/l, as CaO) is somewhat higher than the upper limit of 250 mg/l (as $CaCO_3$) used as selection criterion; the test has been selected however, because Lake Markermeer is part of the river Rhine system. Measured zinc concentrations (3-38-101-382-1,266-2,739 µg/l) within 10% of nominal zinc concentrations (0-40-100-400-1,400-3,000 µg/l) in exposure groups. Growth (dry weight of soft tissues) was not affected at concentrations up to 1,400 µg/l (in the two 3000 µg/l groups this could not be studied, since only one mussel survived at this concentration).

[49] Dorgelo et al. (1995): Mollusc *Potamopyrgus jenkinsi*

Statistics: p = 0.01. Culture and test medium: 0.45 µm filtered Lake Maarsseveen water. Measured zinc concentrations (12-72-115-189-387 µg/l) within 15% of nominal zinc concentrations (0-75-100-200-400 µg/l) in exposure groups.

Results from preliminary tests (not reported in detail) showed an almost complete suppression of growth at 200 and 400 µg/l. Hardness based on reported Ca level (64 mg/l).

[50] Borgmann et al. (1993): Crustacean *Hyalella azteca*

Statistics (p = 0.01) reported on survival data only. Relatively high mortality in the control group (25% and 37% by week 6 and 10, respectively), but test accepted because of high number of test animals (4 replicates of 20 animals/concentration) and non-standard test. Test medium: dechlorinated tap water, originating from Lake Ontario. Measured zinc concentrations (6-13-21-42-108-185-316 µg/l) were only 40-60% of nominal zinc concentrations (0-32-56-100-180-320-560 µg/l) due to sorption. Renewal of test water was only once a week, while sorption to the glass, gauze and/or food and detritus in the exposure flask appears to happen within a few hours (based on Pb measurements in another test).

[51] Masters et al. (1991): Crustacean *Ceriodaphnia dubia*

Statistics: p = 0.05. Tests with reference to the US EPA and ASTM guidelines for testing chronic survival and reproduction of Ceriodapnia. The 7-d exposure is standard; the 4-d exposure was tested to validate a shorter alternative. Culture and test medium: 60 µm filtered Little Miami River water; three generations of *C. dubia* were acclimated in the river water before testing. The results were reported as Chronic Value (CV), being the geometric mean value of the NOEC and LOEC. The NOEC was estimated from the CV by dividing the latter by a factor of $\sqrt{2}$, according to the TGD). In (US) references on aquatic ecotoxicity, the term MATC (Maximum Acceptable Toxicant Concentration) is often used instead of the term CV.

[52] Chapman et al. (1980): Crustacean *Daphnia magna*

Data from US EPA status report. No statistics reported. Parameters survival and reproduction, but only one NOEC (and LOEC and MATC) was reported for each test. Culture and test medium: well water with parent (ambient) hardness of 22-60 mg/l (as $CaCO_3$), adjusted to nominal hardness of 100 and 200 by adding $CaSO_4$, $MgCl_2.2H_2O$, $NaHCO_3$, and $KHCO_3$, to achieve medium-hard and hard water with an average ionic composition as medium hard and hard types of natural (North American) waters. Separate cultures were maintained at each water hardness, so it appears that the animals were acclimated to the hardness of the water before testing.

[53] Cancelled. Pierson (1981): A NOEC could not be derived from this long-term study with fish *Poecilia reticulata.* Study not included in Table 3.3.2.a.

[54] Sibley et al. (1996): Insect C*hironomus tentans*

Sediment-water toxicity study with Zn-spiked sediment. See also Table 3.3.2.e in Annex 3.3.2.D for further data on this study.

Statistics: p = 0.05. The test was conducted in a sediment-water renewal system containing zinc-spiked lake sediment and overlying water that was renewed twice daily with Lake Superior water. Stabilisation of the spiked sediments was determined by monitoring the concentration of zinc in the pore water over a 2-wk period; after this period the sediments were prepared and introduced in the test system on the day prior to test initiating by adding the test organisms. Exposure concentrations in the pore water: 29 (control)-31-56-166-4,200 and 10,000 µg/l (arithmetic mean value of measurements in the H1 = 0-1 cm depth surficial horizon on day 20 and 56 and in the H2 = 1-2 cm depth surficial horizon on day 20 and 56, thus each value represents the mean value of 4 measurements per exposure concentration. At the highest three test concentrations, pore water measurements on day 0 showed zinc concentrations of 38,000, 480,000 and 950,000 µg/l. According to Sibley et al. '96, these very high concentrations on day 0 are probably due to non-equilibrium between zinc in sediment and water and thus not representative for the true exposure received by the organisms; therefore the results of 20- and 56-day measurements were used for effect assessment. Exposure concentrations in the sediment: 55 (control)-135-230-850-1,900-2,650 mg/kg dry weight (arithmetic mean value of day 0, 20 and 56 measurements, which very similar for each exposure concentration.). It is noted that dissolved oxygen (DO) levels in the overlying water declined steadily in all treatments up to the time of emergence (day 24), resulting in levels as low as 1.1 mg/l at concentrations up to 170 µg/l and as low as 0.5 mg/l at 4,200 and 10,000 µg/l. Following initiation of emergence, DO levels increased to 3-4 mg/l, but remained consistently low at the highest two concentrations. The low DO levels at the highest two concentrations may be related to the lack of bioturbation, because little or no larvae survived at these concentrations. No data reported on treatment or characteristics of Lake Superior water used in the test; the pH and hardness of the water is based on Biesinger and Christensen '72.

See also Table 3.3.2.e in Annex 3.3.2.D for the NOEC in sediment derived from this study.

[55] Borgmann & Norwood (1997): Crustacean *Hyalella azteca*

Sediment-water toxicity study with Zn-spiked sediment. See also Table 3.3.2.e in Annex 3.3.2.D for further data on this study.

Statistics: no data. The test was conducted in a sediment-water static system containing zinc-spiked harbour sediment and overlying water (dechlorinated tap water originating from Lake Ontario; pH 7.9-8.6, hardness 130 mg/l; background zinc concentration 6 µg/l). The amount of sediment and water per test beaker was 40 and 160 mg/l, respectively. Test beakers were covered with petri dishes and gently aerated thoughout the tests; the water was not changed, but evaporated water was replaced with double-distilled water. The sediment was spiked by adding zinc chloride in experimental water, in an attempt to keep major ion concentrations as constant as possible. After spiking the sediment was allowed to settle and mixed with control sediment to achieve the lower test concentrations. Both 1-w and 4-w tests were conducted twice, the second half a year later than the first. The results of both tests were the same with respect to the NOEC for survival, but 4-w survival at the LOEC was 65% in the first test and 0% in the second test.

Actual total-Zn concentrations in the sediment: 1,500 (control)-2,400-2,700-4,600-6,400-8,400 mg/kg dry weight. Actual total-Zn concentrations in the overlying water, determined at the end of the exposure period: 117 (sediment-water control)-139-166-208-525-1,763 µg/l. The control Zn concentration in the overlying water in the sediment-water control (117 µg/l) is 20-times higher than the native background Zn concentration (6 µg/l).

See also Table 3.3.2.e in Annex 3.3.2.D for the NOEC values in sediment derived from this study.

[55a] **Test rejected based on Quality criterion (The test as such is valid, but for H. azteca this is a short-term test which cannot be used to derive a chronic NOEC value). In addition, only endpoint survival was studied.**

[56] Van Woensel (1994a): Alga (unicellular) *Pseudokirchneriella subcapitata*

No statistics reported. Test conducted according to OECD-guideline 201 and under GLP. $NOEC_{growth}$ based on the 72-h average specific growth rate (μ). Culture medium: Bold's Basal Medium**.** Test medium according to OECD-guideline No. 201 (nominal background zinc concentration: 1.4 μg/l; hardness 24 mg/l (as $CaCO_3$)), but EDTA was omitted. Test compound: zinc powder (median diameter 13.4 μm; 0.5% residue on 45 μm filter). Growth parameter: cell number (specific growth rate and biomass). The actual background concentration of zinc in the test medium was ≤ 10 μg/l. In the test, a control, a filtrate of a 100 mg Zn/l dispersion of the metallic zinc powder and a series of four dilutions of the filtrate were tested. The filtrate was prepared by filtering the 100 mg Zn/l dispersion of zinc powder, after 24 hour stirring, over a 0.45 μm membrane filter (Millipore). If the concentration of the test substance in the filtrate is expressed as 100% then the following concentrations expressed in % were tested: 0%, 0.95%, 3.05%, 9.76%, 31.25% and 100%; the actual zinc concentrations were ≤ 10, 50, 50, 90, 230 and 760 μg/l, respectively, based on 72-h measurements. The nominal 72-h EC50 for growth rate was 18.78% of the filtrate; the actual value (interpolation from dissolved-Zn measurements in the test solutions) was 150 μg/l (see also Table 3.3.2.d). The nominal 72-h NOEC for both growth rate and biomass was 3.05% of the filtrate (actual dissolved-Zn concentration: 50 μg/l); at the next higher concentration (9.76% of the fittrate; actual dissolved-Zn concentration 90 μg/l), growth rate and biomass were reduced 27% and 69%, respectively. This test is also included in Table 3.3.2.d.

Note that acording to the data reported in Coleman et al. (1971), Bold's Basal Medium contains a background zinc concentration of 1,880 μg/l (see also footnote [6] in Table 3.3.2.c), which is 1300-times higher than that in OECD medium used in the test. There were no data reported on acclimation to the OECD medium prior to the test. Nevertheless, the test resulted in a relatively low NOEC of 50 μg/l and the test is accepted for PNEC derivation.

[57a] LISEC (1993): Alga (unicellular) *Pseudokirchneriella subcapitata*

Statistics: applied to derive EC50 and NOEC values. Test compound: Red Seal-grade ZnO; diameter (d50): 0.57 μm. Test conducted according to OECD-guideline 201 and under GLP. Culture medium: no data. Test medium according to OECD-guideline No. 201 (nominal background zinc concentration: 1.4 μg/l; hardness 24 mg/l (as $CaCO_3$)), but EDTA was omitted. Nominal test concentrations: 0-3.7-8-18-40-87-192 μg dissolved-Zn/l, using a dilution factor of 2.2. The dilutions were prepared as follows: a filtrate (0.1 μm filter) of a 100 mg ZnO/l dispersion in demineralized water was diluted with demineralized water up to 2-times the required test concentration and further 1:1 diluted with the algal suspension, thus reducing the background zinc concentration and hardness of the test medium to about 0.8 μg/l and 12 mg/l, respectively. Reported nominal dissolved-zinc concentrations in test water: based on analyses of zinc in the 0.1 μm filtered stock solution. The algal preculture, used for the inoculation of the test medium, was incubated under the conditions of the test for 3 days.

Toxicological endpoint: growth (biomass) and average specific growth rate; cell numbers measured spectrophotometrically (optical density). At the LOEC for biomass (dissolved-Zn concentration 0.018 mg/l, equivalent to 0.023 mg ZnO/l), 30% inhibition of this endpoint was observed (NOEC <0.004 mg/l; as dissolved Zn). At the LOEC for growth rate (dissolved-Zn

concentration 0.040 mg/l, equivalent to 0.050 mg ZnO/l), 18% inhibition of this endpoint was observed. (NOEC: 0.008 mg/l, as dissolved Zn). Results reported as nominal dissolved-Zn concentration, calculated from the dissolved-Zn concentration measured in the 0.1 µm filtered stock solution. The nominal test concentrations have been confirmed by actual zinc analyses in the test waters. At nominal dissolved-Zn concentrations of 0 (control), 0.004, 0.008 and 0.018 mg Zn/l, the measured dissolved-Zn concentrations were below 0.008 mg/l (detection limit), both at start and end of the test, regardless of the presence of algae in the water (except for the 0.0018 mg/l concentration which decreased from 0.013 mg/l at start to < 0.008 mg/l at end). In the highest three concentrations, the dissolved-Zn concentrations measured at the end of the test were lower in the test waters with algae than in the test waters without algae (reference test waters) due to uptake/adsorption of zinc by algae. The dissolved-Zn concentrations in the reference test waters remained, however, within 80% of the initial concentration throughout the duration of the test.

Dissolution procedure for preparing the stock solution (100 mg ZnO/l dispersion): stirring on a magnetic stirrer for 3 days at room temperature. .

If using NOEC = the lowest test concentration that resulted in less than 10% effect (as applied in the test with Selenastrum capricornutum by Van Ginneken, 1994a), the NOEC for growth rate would be 0.018 mg Zn/l and the NOEC for biomass would be 0.008 mg Zn/l.

Test rejected, based on Relevance criteria (The hardness value is below 24 mg/l, the minimum value used as criterion for hardness, and the background zinc concentration in the artificial test water used is below 1 µg/l, the minimum value for Cb).

[57b] Van Ginneken (1994a): Alga (unicellular) *Pseudokirchneriella subcapitata*

No **s**tatistics reported. Test conducted according to OECD-guideline 201 and under GLP. $NOEC_{growth}$ based on the 72-h average specific growth rate (µ); cell numbers determined with a counting chamber. Culture medium: No data. Test medium according to OECD-guideline No. 201 (nominal background zinc concentration: 1.4 µg/l; hardness 24 mg/l (as $CaCO_3$)), but EDTA was omitted. Test medium sterile-filtered (0.45 µm filter) before use in test. In the test, a control, a filtrate (0.45 µm filter) of a 100 mg ZnO/l dispersion and a series of four dilutions of the filtrate were tested, using a dilution factor of 3.2. If the concentration of the test substance in the filtrate is expressed as 100%, then the following dilutions were tested: 31.25%, 9.76%, 3.05% and 0.95%. Toxicological endpoint: specific growth rate (measured by cell density). Based on the aforementioned "nominal" concentrations, the 72-h EC50, 72-h LOEC and 72-h NOEC were 19.69%, 9.76% and 3.05% of the concentration in the filtrate, respectively. At the LOEC (actual concentration 0.08 mg Zn/l, equivalent to 0.1 mg ZnO/l), 22% inhibition of the specific growth rate was observed. Reported measured dissolved-zinc concentrations in test water: based on analyses of zinc in 0.45 µm filtered test waters.

Actual dissolved background zinc concentration in test medium after 72 hours: 0.024 mg Zn/l (equivalent to 0.03 mg ZnO/l). It is noted that after 72 hours, the 0.95% en 3.05% dissolution of the filtrate (the latter value being the NOEC) contained the same actual dissolved zinc concentration as the control medium. Also the actual dissolved concentrations averaged over the 72-h exposure period (average of 0-h and 72-h measurement) were practically the same in these three groups, varying from 0.016 to 0.024 mg Zn/l (0.02 to 0.03 mg ZnO/l). Actual dissolved concentrations: based on measurements of dissolved zinc (0.45 µm filter); the values listed in Table 3.2.1 are based on measurements after 72 hours.

Dissolution procedure for preparing the stock solution (100 mg ZnO/l dispersion): no data. Culture medium: no data.

[58] Van Ginneken (1994a): Alga (unicellular) *Pseudokirchneriella subcapitata*

Test compound: EPM-grade ZnO ("direct oxide")(batch 193031). Purity 99.37%; Impurities include 0.25% water soluble zinc salts which are dissolved over time, in addition to a rapid dissolution of pure ZnO which takes place up to the concentration of the solubility product of ZnO (Jahn, 1997).

[59] Van Ginneken (1994a): Alga (unicellular) *Pseudokirchneriella subcapitata;* LISEC (1997): Alga (unicellular) *Pseudokirchneriella subcapitata*

According to Jahn (1997), the EPM-grade ZnO is not representative of the most common type of zinc oxide produced: more than 70% of the total ZOPA (Zinc Oxide Producers Association) production is Red Seal-grade ZnO ("indirect oxide"). Red seal-grade ZnO contains virtually no soluble salts.

Jahn (1997) includes an abstract of the draft report "Transformation/dissolution of zinc oxide powders in ecotox media", with the results of a 4-d dissolution study with Red Seal-grade ZnO and a 16-d dissolution study with EPM-grade ZnO, both in "modified algal medium" (background dissolved zinc concentration up to 0.008 mg/l):

The data for Red Seal-grade ZnO show that nominal concentrations of 1 to 500 mg ZnO/l "modified algal medium" resulted in dissolved (0.2 µm filter) zinc concentrations of 0.3 to 0.4 mg Zn/l in 4 days. The 4-d dissolution curves for Red Seal-grade ZnO show an initial rapid increase in dissolved zinc concentrations (especially in the first hours) and almost equilibrium in 4 days, regardless of the nominal concentration.

The data for EPM-grade ZnO show that nominal concentrations of 1 to 500 mg ZnO/l "modified algal medium" resulted in dissolved zinc concentrations of 0.4 to 0.9 mg Zn/l in 4 days and dissolved zinc concentrations of 0.7 to 1.8 mg Zn/l in 16 days. The 16-d dissolution curves for EPM-grade ZnO also show a rapid initial increase in dissolved zinc concentrations, but at the higher concentrations (100 and 500 mg ZnO/l) a slow but steady further increase after day 4.

[60] Richelle et al. (1995): Poriferans *Ephydatia fluviatilis, Ephydatia muelleri,* and *Spongilla lacustris*

No statistics applied. Batches of laboratory cultured sponges grown from 10 gemmules were raised in the culture medium. After 7 days, the sponges were harvested with a spatula and mechanically dissociated by pipetting. The dissociated cells were centrifugated and resuspended in the culture medium (controls) or in the same medium containing zinc. They were then dispensed to multiwell plates and kept for 10 days. All experiments were carried out in triplicate with the same strains which were used for sponge cultures. No data on the number of sponges used per test concentration. Culture and test medium: "M" medium, an inorganic medium according to Rasmont (1961). This medium, prepared from distilled water, contains macro-elements including 2 mM Ca and 1 mg Mg (resulting in a hardness of 300 mg/l); no date on the addition or background concentration of zinc or other micro-elements are given in Rasmont (1961). According to additional information from the zinc industry, no zinc or other trace elements are added to this medium and the measured zinc concentration in this medium is <1 µg/l (May 1st 2001 comments file.doc). Culture and test temperature: 20 °C. Nominal test concentrations: $0\text{-}10^{-7}\text{-}2.5x10^{-7}\text{-}5x10^{-7}\text{-}7.5x10^{-7}\text{-}10^{-6}\text{-}2.5x10^{-6}$, $5x10^{-6}\text{-}10^{-5}\text{-}10^{-4}$ Mol/l, corresponding to 0-6.5-33-49-65-163-325-650-6,500 µg Zn/l

Effects:

In the controls, the cell suspension aggregated into small spherules which fused together, settled on the bottom of the wells and adhered, within 24 h. After 2-3 days, these settled

aggregates reconstituted complete functional sponges characterized by a functional aquiferous system and oscula. They remained in that state during the whole observation period.

A (zinc) concentration was considered by the study authors as:

Non toxic (-) when normal cell aggregation, settlement and development occurred (used in this RAR as LOEC).

Weakly toxic (+) when aggregation, settlement and adherence occurred normally, oscula were formed but degeneration took place rapidly, within 3 or 4 days (used in this RAR as LOEC).

Moderately toxic (++) when there was aggregation, settlement and adherence, but development stopped at that point, no functional sponges were formed.

Toxic (+++) when there was aggregation but no settlement, the aggregates degenerated rapidly.

Highly toxic (++++) when there was no aggregation at all and the cells died within 24 h.

Test rejected, based on relevance criteria (The background zinc concentration in the artificial culture and test medium (<1 µg/l; no zinc added to the artificial medium prepared from distilled water) is at or below the minimum value used as criterion for Cb (around 1 µg/l), the hardness value (300 mg/l) is higher than the maximum value used as criterion for hardness (250 mg/l) and the pH value is not reported. Furthermore, additional tests with these sponge species are now available (Van de Vyver, 2001; see footnote 61), performed in Elendt M4 medium; this fully defined medium (see e.g. OECD guideline 211: Daphnia magna reproduction test) meets all the relevancy criteria as used in the present RAR).

[61] Van de Vyver (2001): Poriferans *Ephydatia fluviatilis, Ephydatia muelleri, Spongilla lacustris,* and *Eunapius fragilis.*

The test method is the same as described in Richelle et al. '95 (see footnote 60). One additional toxicity category was added in Van de Vijver (2001), namely ±: "normal cell aggregation, settlement and development but sponges present a different aspect than controls". Although no further data were reported on the effects seen in this category, the lowest concentration in this category is considered as LOEC, since in most tests the next higher concentration resulted in degeneration of the sponges after the development to sponges (category +, see also Richelle et al. '95). Thus the NOEC was set at the highest concentration of category – (non toxic, see also Richelle et al. '95).

Nominal test concentrations in first set of tests: $0\text{-}3.3\times10^{-7}\text{-}6.6\times10^{-7}\text{-}10^{-6}\text{-}3.3\times10^{-6}\text{-}6.6\times10^{-6}\text{-}10^{-5}\text{-}10^{-4}$ Mol/l, corresponding to 0-21-43-65-215-430-650-6,500 µg Zn/l (range-finding, based on results from the study by Richelle et al. '95).

Nominal test concentrations in second set of tests: $0\text{-}3.3\times10^{-7}\text{-}6.6\times10^{-7}\text{-}10^{-6}\text{-}3.3\times10^{-6}\text{-}5\times10^{-6}\text{-}6.6\times10^{-6}\text{-}10^{-5}\text{-}10^{-4}\text{-}$

10^{-3} Mol/l, corresponding to 0-21-43-65-215-325-430-650-6,500-65,000 µg Zn/l.

All tests were performed in two different artifical media:

I): In Elendt M4 medium (a fully defined medium containing micro-and macro-elements (see e.g. OECD Guideline 211: Daphnia magna reproduction test) that meets all the relevancy criteria as used in the present RAR. The background zinc concentration in control Elendt M4 is 6.5 µg/l (added as $ZnCl_2$: 13 µg/l). The pH is not given in the test report or in OECD 211, but based on that of similar Elendt M7 medium (Van der Geest et al, 2001). No data on acclimation of the sponges to Elendt M4 medium prior to testing.

II): In "M" medium as also used by Richelle et al. '95 (see Table 3.3.2.a-Part II and Footnote [60]); this medium is used as culture medium in the laboratory that performed the tests. The background zinc concentration in this medium normally is <1 µg/l (no zinc added to the artificial medium prepared from distilled water; see footnote [60]; therefore in the study by Van de Vyver (2001) 6.5 µg Zn/l was added to "M" medium to give the same control background zinc concentration as in Elendt M4 medium.

Both series of tests in a particular medium (i.e. Elendt M4 or "M") resulted for each sponge species in identitical NOEC values. The NOEC values given in Table 3.3.2.a - Part I (accepted studies) are based on the tests performed in Elendt M4 medium. **The tests in "M" medium were rejected (see Table 3.3.2.a-Part II) because this medium does not meet the relevance criteria: the hardness value (300 mg/l) is higher than the maximum value used as criterion for hardness (250 mg/l) and the pH value is not reported.** It is noted, however, that the results of the tests in Elendt M4 medium and "M" medium were idential or very similar in this study, see Table 3.3.2.a-Part I and Table 3.3.2.a-Part II. Furthermore, the NOEC values derived in this study were also very similar to those derived in the study by Richelle et al., '95 in "M" medium.

Note: Elendt M4 contains EDTA (6.8 µMol/l) and is because of the presence of this chelating agent not recommended in OECD 211 for toxicity testing of metals. However, the EDTA concentration is below the maximum value (10 µMol/l) used in this RAR as selection criterion for algal studies (see section 3.3.2.1.2) and, moreover, the results of the sponges tests in Elendt M4 (with EDTA) and "M" medium (without EDTA) are identical or very similar (see above), with for two of the sponge species the lowest NOEC in Elendt M4. Thus, in these tests with sponges, the EDTA concentration in Elendt M4 did not affect the zinc toxicity.

[62] LISEC (1998): Alga (unicellular) *Pseudokirchneriella subcapitata* (*)

Draft report, but containing all data needed for evaluation, including the raw data on growth.

Statistics: p = 0.05. Test conducted according to OECD-guideline 201. Test medium according to OECD-guideline No. 201 (nominal background zinc concentration: 1.4 µg/l; hardness 24 mg/l, as as $CaCO_3$), but EDTA was omitted. Nominal test concentrations: 0-3.8-8-18-40-89-200 µg/l (factor 2.2) or 0-12.5-25-50-100-200 µg/l (factor 2). Dissolved-Zn (0.45 µm filtered) concentration measured at the start and at the end of the tests were very close to the nominal concentrations, at least in the reference solutions without algae. The NOEC values listed in Table 3.3.2.a are based on growth rate.

Four test were performed; all four tests were rejected:

[62-R1] Test with LISEC culture without adaptation (cultured on agar medium with 25 µg Zn/l). NOEC (8 µg/l) based on growth rate; the NOEC for biomass is 4 µg/l (both at p = 0.05). **Test rejected, based on Quality criterion (pH range in test too high, see below for further explanation).**

[62-R2] Test with Swedish culture without adaptation (cultured on liquid medium with 0.23 µg Zn/l, which is considerably lower than the minimum value for Cb (around 1 µg/l) used in this RAR). Both biomass (minus 13 %) and growth rate (minus 8 %) inhibited at the lowest test concentration of 4 µg/l (both at p = 0.05). $NOEC^e$ (2 µg/l) = LOEC/2 (no EC10 was derived, as the test was rejected). **Test rejected, based on Relevance criterion (Very low background Zn concentration in the culture medium).**

[62-R3] Test with Swedish culture that was gradually adapted (over a 5-w period) to a Zn concentration in the culture medium of 1.4 µg/l (level in standard OECD medium), starting with a level of ≤0.3 µg/l (adaptation step 1). NOEC (100 µg/l) is both for growth rate and

biomass (both at p = 0.05). **Test rejected, based on Quality criterion (pH range in test too high, see below for further explanation).**

[62-R4]. Test with Swedish culture that after the gradual adaptation from ≤0.3 to 1.4 µg Zn/l (adaptation step 1) was drastically adapted by further cultivation at 18 µg Zn/l (adaptation step 2). Both biomass (minus 14 %) and growth rate (minus 4 %) were inhibited at the lowest test concentration of 12.5 µg/l (both at p = 0.05). $NOEC^e$ (6 µg/l) = LOEC/2 (no EC10 was derived, as the test was rejected). **Test rejected, based on Relevance criterion (Drastical adaptation of the algae to a relatively high Zn concentration).**

[62-R1 and 62-R] Quality criteron pH range:

The total range of pH values in both tests differed nearly 2 pH units (7.8-9.7 and 8.4-10.2) (no values for each test concentration were given). This variation in pH is (too) high and not observed in the other algal tests. For example, in the *P. subcapitata* test with test compound ZnO, performed by the same testing facility (LISEC, 1997), the total range of pH values differed 1 pH unit and the maximum difference between the 0-h and 72-h pH values per concentration was 0.7 pH units, while the highest Zn concentration was very similar. Moreover, the four tests within the LISEC (1998) study, two of which were already rejected earlier, resulted in strongly variable NOEC values, which may be been influenced by variations in pH, background Zn levels in the different culture media and/or adaptation.

(*) In test report named *Raphidocelis subcapitata.*

[63] Heijerick, Janssen and De Coen (2003); Heijerick & Janssen (1999): Crustacean *Daphnia magna*

Tests conducted according to OECD 202. Culture medium: Elendt M4 (hardness 250 mg/l, as $CaCO_3$, pH 7.5-8.5, background Zn concentration 6 µg/l, see OECD 211). Test medium (EEG-medium) prepared from deionized water by adding 65 mg/l $NaHCO_3$, 5.75 mg/l KCl and an amount of concentrated 'hardness solution' (2940 mg/l $CaCl_2.2H_2O$ and 1230 mg/l $MgSO_4.7H_2O$ needed to obtain the required hardness. DOC added from a 0.45 µm-filtered solution of artificial humic acid, added as sodium salt). Actual background Zn concentration: not reported (but no Zn was added to the artificial medium). Test concentrations: not reported (it was reported that in each test a control and 5 Zn concentrations, spanning two log-units, were used. Based on a general statement in Heijerick et al. (2003) on control performance for parent survival and reproduction the tests as such appear to be valid, but the raw data of the tests and data on statistics for NOEC derivation were not reported, thus the validity of the tests and toxicity values (NOEC, EC10 and EC 50 values) could not be checked. Moreover, in all but one of the 17 tests that were performed within this study, the values for hardness (>250 mg/l) and/or DOC concentration (10 to 40 mg/l) are outside the boundaries used in this RAR. All 17 tests rejected, based on Relevance (and Quality) criteria (see above).

[64] De Schamphelaere et al. (2003)(*): Alga (unicellular) *Pseudokirchneriella subcapitata* (BLM study)

Statistics: p = 0.05. Test conducted according to OECD 201. Culture water: drinking water enriched with nutrients: pH 7.5, hardness 90 mg/l (as $CaCO_3$), background Zn concentration 15-20 µg/l. Before testing the algae were pre-acclimated for 5 days under the conditions of standard OECD medium (pH 7.5, hardness 25 mg/l (as $CaCO_3$), background Zn concentration 1.4 µg/l (nominal; measured Zn concentrations <3 µg/l). Standard test medium prepared from deionised water and including 0.12 mM Ca, 0.12 mM Mg and 2.7 mM Na, according to OECD 201. EDTA was omitted from the medium (replaced by artificial humic acid at a concentration of 0.03 mg/l). No zinc was added to the artificial test medium used in the tests, but according to additional data submitted by De Schamphelaere and co-workers, the

background Zn concentration in the artificial test medium was 1-3 µg/l (fulfilling the criterion for the mimimum Zn concentration in artificial media). The composition of the standard test medium was reported to be according to OECD 201 (1984). However, the medium contained 2.7 mM Na (62 mg Na/l), while the standard OECD medium contains 0.6 mM Na (13.7 mg Na/l, from 50 mg $NaHCO_3$/l). Additional calcium, magnesium or sodium was added as chloride salt. Each test included a control and 4 or 5 test concentrations, selected on the basis of the physico-chemical properties of the test water. The results of the tests are based on the dissolved-Zn concentrations measured at the start of the tests. All results in the report are based on endpoint growth rate (when possible reported by De Schamphelaere et al. (2003) as 48-h E_rC50, 48-h E_rC10, 72-h E_rC50, 72-h E_rC10 and 72-h NOE_rC values. The 72-h $NOEC_g$[e] values listed in Table 3.3.2.a (Part I and II) are 72-h E_rC10 values that were derived by De Schamphelaere et al. (2003) when there was a statistically significant effect at the lowest concentration tested. The EC10 values were calculated with the log-logistic response model by Haanstra et al. (1985).

For results on biomass, expressed as 72-h EC50 values, see Heijerick et al. (2002).

Water samples of the natural test waters were concentrated *in-situ* by reverse osmosis; in the laboratory the 50-fold concentrated water samples were diluted with deionised water to yield the original DOC concentration and the Ca and Mg concentrations were adjusted to the concentrations as originally present.. In addition, essential micro-elements (but no Zn) were added. These 'reconstituted' natural waters are Brisy-R, Bihain-R, Voyon-R, Markermeer-R, Ankeveen-R and Ossenkolk-R. The background dissolved-Zn concentrations in these 'reconstituted' natural waters was <5 µg/l (detection limit). The values for pH, hardness and DOC are those measured in these 'reconstitued' natural waters during the toxicity tests and may somewhat deviate from those measured in the original natural waters. Two of the original natural test waters (Brisy-N and Bihain-N) were also included in the test series in natural waters; the background Zn concentrations in these original natural waters were 5 and 32 µg/l, respectively.

(*) Further information provided by the authors of the study in addition to the study report (De Schamphelaere et al., 2003) has been included in the evaluation of the study. The further information included the purity of the test compound ($ZnCl_2$, purity 98%) and the raw data for each test, i.e. the measured dissolved-Zn concentrations (in 0.45 µm filtered water) and the results for the growth rate. The validity criterion for control growth (>16-fold increase in the number of cells) were met in almost all tests. In some tests the control growth was slighly lower, but within 80% of the validity criterion. The authors of the study noted that the tests were performed at a relatively low light intensity and low temperature to prevent too high algal growth that would have resulted in uncontrolled pH, carbon limitation and non-exponential growth. Under the test conditions used, algal growth was exponential thoughout the whole test period and the validity criterion for pH (the pH of the test solution should not normally deviate by more than one pH unit) was met in each test..

Accepted tests

[64a] Test in 'standard' OECD 201 medium (code: Na-2.7 mM)

[64b] Test in 'standard' OECD 201 medium plus 0.88 mM Ca (code: Ca-1.0 mM)

[64c] Test in 'standard' OECD 201 medium plus 1.38 mM Ca (code: Ca 1.5 mM)

[64d] Test in 'standard' OECD 201 medium plus 1.88 mM Ca (code: Ca-2.0 mM)

[64e] Test in 'standard' OECD 201 medium plus 0.38 mM Mg (code: Mg-0.5 mM)

[64f] Test in 'standard' OECD 201 medium plus 0.88 mM Mg (code: Mg-1.0 mM)

[64g] Test in 'standard' OECD 201 medium plus 0.138 mM Mg (code: Mg-1.5 mM)

[64h] Test in 'standard' OECD 201 medium plus 1.88 mM Mg (code: Mg-2.0 mM)

[64i] Test in 'standard' OECD 201 medium plus 0.5 mM Na (code: Na-3.2 mM)

[64j] Test in 'standard' OECD 201 medium plus 1 mM Na (code: Na-3.7 mM)

[64k] Test in 'standard' OECD 201 medium plus 2 mM Na (code: Na-4.7 mM))

[64l] Test in 'standard' OECD 201 medium plus 4.5 mM Na (code: Na-7.2 mM)

[64m]Test in 'standard' OECD 201 medium adjusted to pH 6.2 (code: pH-6.2)

[64n] Test in 'standard' OECD'medium adjusted to pH 6.8 (code: pH-6.8)

[64o] Test in 'standard' OECD 201 medium adjusted to pH 7.1 (code: pH 7.1)

[64p] Test in 'standard' OECD 201 medium adjusted to pH 7.4 (code: pH-7.4)

[64q] Test in 'standard' OECD 201 medium adjusted to pH 7.7 (code: pH 7.7)

[64r] Test in 'standard' OECD 201 medium adjusted to pH 7.8 (code: pH 7.8)

[64s] Test in 'reconstituted' stream Brisy water (code: Brisy-R)

[64t] Test in natural stream Brisy water (code: Brisy-N)

[64u] Test in 'reconstituted' stream Le Voyon water (code: Voyon-R)

[64v] Test in 'reconstiuted' Lake Markermeer water, which is a part of Lake IJssel (code: Markermeer-R)

[64w] Test in 'reconstituted' ditch water of Lake Ankeveen (code: Ankeveen-R)

Rejected tests (Relevance criteria: value(s) for pH, hardness and/or DOC beyond the boundaries selected in this RAR)

[64-R1] Test in 'standard' OECD 201 medium plus 2.38 mM Mg (code: Mg-2.5 mM)

[64-R2] Test in 'standard' OECD 201 medium adjusted to pH 5.6 (code: pH-5.6)

[64-R3] Test in 'reconstituted' creek Bihain water (code: Bihain-R)

[64-R4] Test in natural creek Bihain water (code: Bihain-N)

[64-R5] Test in 'reconstituted' lake Ossenkolk water (code: Ossenkolk-R)

[65] De Schamphelaere et al. (2003)(*): Crustacean *Daphnia magna* (BLM study)

Statistics: p = 0.05. Test conducted according to OECD 211. Culture medium: Elendt M4 ((hardness 250 mg/l, as $CaCO_3$, pH 7.5-8.5, background Zn concentration 6 µg/l, see OECD 211). Standard test medium containing 0.25 mM $CaCl_2$, 0.25 mM $MgSO_4$, 2.078 mM $NaHCO_3$ and 0.078 mM KCl; actual background dissolved-Zn concentration <5 µg/l (detection limit). No zinc was added to the artificial test medium used in the tests, but according to additional data submitted by De Schamphelaere and co-workers, the background Zn concentration in the artificial test medium was 1-3 µg/l (fulfilling the criterion for the mimimum Zn concentration in artificial media). As the standard test medium was prepared from carbon-filted and deionised water, the DOC concentration was assumed to be 0.3 mg/l, as in the fish *O. mykiss* BLM study (see footnote [66]). Additional calcium, magnesium or sodium was added as chloride salt. In all tests of the pH series, a DOC concentration of 5 mg/l (natural DOC, from Lake Ankeveen water, see fish *O. mykiss* BLM study) was added to the test water to control the pH value. Each test included a control and 5 test concentrations, selected on the basis of the physico-chemical properties of the test water The results of the

tests are based on the dissolved-Zn concentrations measured before and after each renewal (renewal: every other day). Toxicologial endpoint: net reproduction rate, expressed as $l_x * m_x$, in which l_x is the age-specific survival and m_x is the number of offspring. When possible the results were reported by De Schamphelaere et al. (2003) as 21-d EC50, 21-d EC10 and 21-d NOEC values.

The test series included tests in natural waters, but due to technical problems these tests were invalid and the results were not reported in De Schamphelaere et al. (2003).

(*) Further information provided by the authors of the study in addition to the study report (De Schamphelaere et al., 2003) has been included in the evaluation of the study. The further information included the purity of the test compound ($ZnCl_2$, purity 98%) and the raw data for each test, i.e. the measured dissolved-Zn concentrations (in 0.45 µm filtered water) and the results for the net reproduction rate. The validity criterion for control survival of the parent aninimals (<20% mortality) was met in all tests and the validity criterion for control reproduction (>60 live offspring per female surviving at the end of the test) was met in all tests, except in the tests from the pH series, in with the control reproductive performance was slighly lower (46-56 live offspring per female). It is noted that the tests from the pH series were rejected based on the relevance criterion for DOC concentration in artificial test water (see Table 3.3.2.a – Part II)..

Accepted tests

[65a] Test in standard medium

Note: The NOEC of 82 µg/l listed in Table 3.3.2.a is the geometric mean NOEC in standard test medium, based on the tests with code CA-0.2 (control of the Ca series), code MG-0.25 (control of the Mg series) and code NA-2 (control of the Na series), resulting in NOEC values of 84 µg/l, 84 µg/l and 79 µg/l, respectively.

[65b] Test in standard medium plus 0.25 mM $CaCl_2$ (code: CA-0.5)

[65c] Test in standard medium plus 0.75 mM $CaCl_2$ (code: CA-1)

[65d] Test in standard medium plus 1.75 mM $CaCl_2$ (code: CA-2)

[65e] Test in standard medium plus 0.25 mM $MgCl_2$ (code: MG-0.5)

[65f] Test in standard medium plus 0.75 mM $MgCl_2$ (code: MG-1)

[65g] Test in standard medium plus 1.25 mM $MgCl_2$ (code: MG-1.5)

[65h] Test in standard medium plus 1.75 mM $MgCl_2$ (code: MG-2)

[65i] Test in standard medium plus 4 mM $NaHCO_3$ (cosde: NA-6)

[65j] Test in standard medium plus 7 mM $NaHCO_3$ (code: NA-9)

[65k] Test in standard medium plus 10 mM $NaHCO_3$ (code: NA-12)

<u>Rejected tests (Relevance criteria: value(s) for pH, hardness and/or DOC beyond the boundaries selected in this RAR</u>)

[65-R1] Test in standard medium plus 2.75 mM $CaCl_2$ (code: CA-3)

[65-R2] Test in standard medium plus 3.75 mM $CaCl_2$ (code: CA-4)

[65-R3] Test in standard medium plus 3.75 mM $MgCl_2$ (code: MG-4)

[65-R4] Test in standard medium adjusted to pH 5.5; DOC (5 mg/l) added to control pH (code: PH-5.5)

[65-R5] Test in standard medium adjusted to pH 6; DOC (5 mg/l) added to control pH (code: PH-6)

[65-R6] Test in standard medium adjusted to pH 6.5; DOC (5 mg/l) added to control pH (code: PH-6.5)

[65-R7] Test in standard medium adjusted to pH 7; DOC (5 mg/l) added to control pH (code: PH-7)

[65-R8] Test in standard medium adjusted to pH 7.5; DOC (5 mg/l) added to control pH (code: PH-7.5)

[65-R9] Test in standard medium adjusted to pH 8; DOC (5 mg/l) added to control pH (code: PH-8)

[66] De Schamphelaere et al. (2003)(*): Fish *Oncorhynchus mykiss* (BLM study)

Statistics: p = 0.05. Test conducted according to OECD 215. Culture water: pH 7.5, hardness 50-70 mg/l (as $CaCO_3$), background Zn concentration 5 µg/l. Before testing the fish were acclimated for 1 week to the standard test medium without zinc. Standard test medium ISO 6341-1982, containing 0.2 mM $CaCl_2$, 0.05 mM $MgSO_4$, 0.078 mM $NaHCO_3$ and 0.01 mM KCl; actual background dissolved-Zn concentration <5 µg/l (detection limit); DOC concentration 0.3 mg/l. The artificial test medium was prepared from deionised water. No zinc was added to the artificial test medium used in the tests, but according to additional data submitted by De Schamphelaere and co-workers, the background Zn concentration in the artificial test medium was 1-3 µg/l (fulfilling the criterion for the mimimum Zn concentration in artificial media). Additional calcium, magnesium or sodium was added as chloride salt. Each test included a control and 4 or 5 test concentrations, selected on the basis of the physico-chemical properties of the test water. The results of the tests are based on the dissolved-Zn (0.45 µm filtered) concentrations measured at 3-d intervals during the tests. Toxicological endpoints: survival (when possible reported by De Schamphelaere et al. (2003) as 96-h LC50, 30-d LC50, 30-d LC10 and 30-d NOEC) and growth rate (30-d results, based on fish weights). In most tests, the growth rate was not affected and ECx values for growth could not be derived. In the four tests in which growth was affected, the effect on growth always occurred at Zn concentrations that also affected survival. Based on this, the NOEC values listed in Table 3.3.2.a (Part I or Part II) are for survival, but also protective for growth. In addition to the tests listed in Table 3.3.2.a (Part I or Part II), a test was performed in the standard medium at pH 8.5. After 1 week no mortality was observed up to the highest nominal zinc concentration of 4,400 µg/l, which is clearly above the water solubility limit of zinc of around 1,000 µg/l at pH 8.5, as shown by the cloudiness of the test solution and the low (<860 µg/l) and variable dissolved-Zn concentrations. This test was stopped after 1 week.

Water samples of the natural test waters were concentrated *in-situ* by reverse osmosis; in the laboratory the 50-fold concentrated water samples were diluted with deionised water to yield the original DOC concentration and the Ca and Mg concentrations were adjusted to the concentrations as originally present. These 'reconstituted' natural waters are BIH (Bihain-R), VOY (Voyon-R), MAR (Markermeer-R) and ANK (Ankeveen-R). The background dissolved-Zn concentrations in these 'reconstituted' natural water was <5 µg/l (detection limit). . The values for pH, hardness and DOC are those measured in the 'reconstitued' waters during the toxicity tests and may somewhat deviate from those measured in the original waters.

(*) Further information provided by the authors of the study in addition to the study report (De Schamphelaere et al., 2003) has been included in the evaluation of the study. The further information included the purity of the test compound ($ZnCl_2$, purity 98%) and the raw data for

each test, i.e. the measured total-Zn and dissolved-Zn (in 0.45 µm filtered water) concentrations, the results for mortallity and growth and the results of the statistical analysis for both endpoints. The validity criteria for control survival (<10% mortality) and control growth (>50% weight increase) were met in all tests.

Accepted tests

[66a] Test in standard ISO 6341-1982 medium

Note: The NOEC of 39 µg/l listed in Table 3.3.2.a is the geometric mean NOEC in standard test medium, based on the tests with code RF-B (range-finding test in standard medium) and code MG-B (control of the Mg series), resulting in NOEC values of 32 µg/l and 48 µg/l, respectively.

[66b] Test in standard ISO 6341-1982 medium plus 5mM NaCl (code: RF-NA5)

[66c] Test in in standard ISO 6341-1982 medium plus 0.2 mM $MgCl_2$ (code: MG-0.2)

[66d] Test in standard ISO 6341-1982 medium plus 1 mM $MgCl_2$ (code: MG-1)

[66e] Test in standard ISO 6341-1982 medium plus 2 mM $MgCl_2$ (code: MG-2)

[66f] Test in standard ISO 6341-1982 medium adjusted to pH 6.5 (code:PH-6.5)

In the tests of the pH-series, the Na concentration was 104-112 mg/l, which is 5-6 times higher than that in standard medium (18 mg/l).

[66g] Test in standard ISO 6341-1982 medium (code: pH-7.5)

In the tests of the pH-series, the Na concentration was 104-112 mg/l, which is 5-6 times higher than that in standard medium (18 mg/l).

Based on this, the result of the above test is not combined with the tests in standard medium (code RF-B and MG-B, see [66a] having the same pH value and other abiotic characteristics (except Na content).

[66h] Test in standard ISO 6341-1982 medium plus 2 mM $CaCl_2$ (code: CA-2)

[66i] Test in 'reconstituted' ditch water of Lake Ankeveen (code: ANK)

[66j] Test in 'reconstiuted' Lake Markermeer water which is a part of Lake IJssel (code: MAR)

[66k] Test in 'reconstituted' stream Le Voyon water (code: VOY)

[66l] Test in 'reconstituted' creak Bihain water (code: BIH)

Rejected tests (Relevance criteria: value(s) for pH, hardness and/or DOC beyond the boundaries selected in this RAR)

[66-R1) Test in standard ISO 6341-1982 medium plus 3 mM $MgCl_2$ (code: RF-MG3)

[66-R2] Test standard ISO 6341-1982 medium plus 3 mM $MgCl_2$ (code: MG-3)

[66-R3] Test in standard ISO 6341-1982 medium adjusted to pH 5.5 (code:PH-5.5)

Ad [64], [65], [66]: De Schamphelaere et al. (2003): BLM sudy

This study with alga *Pseudokirchneriella subcapitata,* daphnid *Daphnia magna,* and fish *Oncorhynchus mykiss* was performed to develop 'Biotic Ligand Models' (BLMs) for these three standard freshwater test organisms and to validate the BLMs in different natural freshwaters that are representative for the variation in water chemistry in EU waters. The development of the BLMs was based on series of (uni-variate) chronic toxicity tests in

standard artificial test media in which the major physico-chemical characteristics, that are expected to affect zinc toxicity, were varied, i.e. H^+ (pH), Ca^{2+}, Mg^{2+}, and Na^{2+}. The BLMs predict the chronic toxicity of zinc on the basis of these physico-chemical water characteristics. The validation of the developed BLMs was based on series of chronic toxicity tests in natural waters.

See further RAR section 3.3.2.1.1 for the BLMs derived from this study.

[67] Nasu & Kugimoto (1981): Macrophyte *Lemna paucicostata*

Tests performed in Hoagland type M medium (containing micro- and macro-elements) with a laboratory culture of *Lemna paucicostata*, at pH values of 4 and 5, respectively. Endpoint growth: frond multiplication. **Test rejected based on Relevance criteria (The values for pH (4 or 5) and hardness (700 mg/l) are outside the boundaries used in this RAR. In addition, the test medium contained a high background zinc concentration of 50 µg/l).**

In this study the toxicity of zinc was also determined in Bonner-Devirian's medium (at pH 6 or 7, respectively, hardness 120 mg/l and a background zinc concentration of 230 µg/l. In this test medium the addition of 1000 µg/ l resulted in about 60% and 80% growth inhibition at pH 6 and 7, respectively.

Both test media did not contain EDTA.

[68] Van der Werff & Pruyt (1982): Macrophytes Callitriche platycarpa, Elodea nuttallii, Lemna gibba, and Spirodela polyrhiza

The plants were obtained from uncontaminated ditches or ponds in the Netherlands. Tests performed in filtered ditch water from the Netherlands. Test concentrations: 0-65-650 µg/l. No effect on survival or growth (biomass) were found up to the highest concentration tested. Tests rejected, based on Quality criterion (unbounded NOEC values).

[69] Jenner & Janssen-Mommen (1993): Macrophyte *Lemna minor*

Tests performed in Rombach (1976) medium (containing micro- and macro-elements and $2.5x10^{-3}$ mmol EDTA) with a laboratory culture of *Lemna minor*. Endpoints growth: multiplication rate (number of fronts) and percentage of total surface covering of the petri dishes. The NOEC was 160 µg/l for both endpoints. The EC50 values were 290 µg/l for surface coverage and 5600 µg/l for multiplicatin rate. **Tests rejected, based on Relevance criteria (The values for pH (5) and hardness (310 mg/l) are outside the boundaries used in this RAR. In addition, the test medium contained a high background zinc concentration of 50 µg/l).**

Table 3.3.2.b. Chronic toxicity of zinc to saltwater organisms: NOEC values

Part I: Studies useful for saltwater $PNEC_{add, aquatic}$ derivation

Organism & life stage	A	Test type	Test-comp	Test water	Salinity °/oo	Exp.-time	Criterion	Result (µg Zn/l)	
Algae (unicellular)									
Amphidinium carteri	-	S	$ZnSO_4$	asw	-	9-d	$NOEC_g$ Braek et al.'76	**100**	(Cn) [1]
Asterionella japonica clone AST N1.1	-	S	$ZnSO_4$	nsw (BS)	35	3-d	$NOEC_g$ Fisher & Jones '81	**30**	(Cn) [2]
Asterionella japonica clone AST C2 or N1.1	-	S	$ZnSO_4$	nsw (BS)	35	3-d	$NOEC_g^e$	**7**	(Cn) [2,3]
Asterionella japonica clone AST C2 or N1.1	-	S	$ZnSO_4$	nsw (CB)	35	3-d	$NOEC_g^e$	**20**	(Cn) [2,4]
Asterionella japonica clone AST N1.1	-	S	$ZnSO_4$	nsw (BS)	35	3-d	$NOEC_g^e$	**7**	(Cn) [5,6]
Asterionella japonica clone AST N1.1	-	S	$ZnSO_4$	nsw (CB)	35	3-d	$NOEC_g^e$	**7**	(Cn) [5,7]
Asterionella japonica clone AST C4	-	S	$ZnSO_4$	nsw (BS)	35	3-d	$NOEC_g$	**20**	(Cn) [2]
Asterionella japonica clone AST C4	-	S	$ZnSO_4$	nsw (CB)	35	3-d	$NOEC_g$ Fisher & Frood '80	**40**	(Cn [2]
Asterionella japonica							$NOEC_g$	**15**	geom. mean
Chaetoceros compressum clone Chaet C2	-	S	$ZnSO_4$	nsw (BS)	35	3-d	$NOEC_g^e$ Fisher & Frood '80	**10**	(Cn) [2,8]
Gymnodinium splendens	-	S	$ZnSO_4$	nsw	32	5-w	$NOEC_g$ Kayser '77 [10]	**500**	(Cn) [9]
Nitzschia closterium clone Nitz C.1	-	S	$ZnSO_4$	nsw (BS)	35	3-d	$NOEC_g$	**40**	(Cn) [2]
Nitzschia closterium clone Flag 8.4	-	S	$ZnSO_4$	nsw (BS)	35	3-d	$NOEC_g^e$ Fisher & Frood '80	**10**	(Cn) [2,11]
Nitzchia closterium							$NOEC_g$	**20**	geom. mean
Phaeodactylum tricornutum	+	F	$ZnCl_2$	nsw	-	2-w	$NOEC_g$ Jensen et al.'74 [12]	**10,000**	(Cn)
Phaeodactylum tricornutum	-	S	$ZnSO_4$	asw	-	10-d	$NOEC_g$	**4,000**	(Cn) [13]
Phaeodactylum tricornutum	-	S	$ZnSO_4$	asw	-	10-d	$NOEC_g$ Braek et al.'76 [1]	**500**	(Cn) [14]
Phaeodactylum tricornutum							$NOEC_g$	**2,700**	geom. mean
Prorocentrum micans	-	S	$ZnSO_4.2H_2O$	nsw	32	5-w	$NOEC_g$ Kayser '77 [10]	**100**	(Cn)
Rhizosolenia spp.	+	S	-	nsw	-	12/24-h	$NOEC_g$ Davies & Sleep '79 [15]	**15**	(Cn)
Schroederella schroederi	-	S	$ZnSO_4.2H_2O$	nsw	32	11-d	$NOEC_g$ Kayser '77 [10]	**10**	(Cn) [9]

(to be continued)

Table 3.3.2.b. Chronic toxicity of zinc to saltwater organisms: NOEC values

Part I: Studies useful for saltwater $PNEC_{add, aquatic}$ derivation

Organism & life stage	A	Test type	Test-comp	Test water	Salinity °/oo	Exp.-time	Criterion	Result (µg Zn/l)
Algae (unicellular) (continued)								
Scrippsiella faeroense	-	S	$ZnSO_4.7H_2O$	nsw	32	7-w	$NOEC_g$	**100** (Cn) [9] Kayser '77 [10]
Skeletonema costatum clone Skel-5	+	F	$ZnCl_2$	nsw	-	2-w	$NOEC_g$	**25** (Cn) [9] Jensen et al.'74 [12]
Skeletonema costatum Clone Skel-5	-	S	$ZnSO_4$	asw	-	10-d	$NOEC_g$	**50** (Cn)
Skeletonema costatum clone Skel-0	-	S	$ZnSO_4$	asw	-	10-d	$NOEC_g$	**100** (Cn) Break et al.'76 [1]
Skeletonema costatum clone Skel C7	-	S	$ZnSO_4$	nsw (BS)	35	3-d	$NOEC_g$	**20** (Cn) [2]
Skeletonema costatum clone Skel C7	-	S	$ZnSO_4$	nsw (BS)	35	3-d	$NOEC_g$[e]	**7** (Cn) [5,16]
Skeletonema costatum clone Skel C7	-	S	$ZnSO_4$	nsw (CB)	35	3-d	$NOEC_g$[e]	**7** (Cn) [5,17]
Skeletonema costatum clone Skel C6	-	S	$ZnSO_4$	nsw (BS)	35	3-d	$NOEC_g$[e]	**30** (Cn) [2,18] Fisher & Frood '80
Skeletonema costatum	-	-	-	-	-	10/14-d	$NOEC_g$	**200** (Cu) MARITOX 9761 [19]
Skeletonema costatum	-	-	-	-	-	10/14-d	$NOEC_g$	**50** (Cu) MARITOX 9761 [19]
Skeletonema costatum							$NOEC_g$	**32** geom. mean
Thalassiosira pseudonana	+	F	$ZnCl_2$	nsw	-	14-d	$NOEC_g$	**100** (Cn) [9] Jensen et al.'74 [12]
Thalassiosira pseudonana	-	S	$ZnSO_4$	asw	-	9-d	$NOEC_g$	**200** (Cn) Break et al.'76 [1]
Thalassiosira pseudonana							$NOEC_g$	**140** geom. mean
Thalassiosira rotula	-	S	$ZnSO_4.7H_2O$	nsw	32	14-d	$NOEC_g$	**10** (Cn) [20] Kayser '77 [10]
Thalassiosira guillardii	-	-	-	-	-	10/14-d	$NOEC_g$	**200** (Cu) MARITOX 9761 [19]
Algae (multicellular)								
Laminaria hyperborea zoospores --> sporophytes	-	R	$ZnSO_4$	nsw	-	4-w	$NOEC_g$	**100** (Cn) Hopkins & Kain '71 [21]
Coelenterates								
Eirene viridula	-	R	$ZnSO_4$	nsw	30	3-m	$NOEC_{mc}$	**300** (Cn) Karbe '72 [22]

(to be continued)

Table 3.3.2.b. Chronic toxicity of zinc to saltwater organisms: NOEC values

Part I: Studies useful for saltwater $PNEC_{add, aquatic}$ derivation

Organism & life stage	A	Test type	Test-comp	Test water	Salinity °/oo	Exp.-time	Criterion	Result (µg Zn/l)
Annelids								
Capitella capitata	-	-	-	-	-	25/40-d ?	$NOEC_r$	**320** (Cu) MARITOX 51618 [19]
Ctenodrilus serratus P --> F [lc]	-	S	$ZnSO_4.7H_2O$	nsw	-	3-w	$NOEC_{s,r}$	**100** (Cn) Reish & Carr '78 [23]
Ctenodrilus serratus	-	-	-	-	-	28/31-d	$NOEC_r$	**100** (Cu) MARITOX 51618 [19]
Ctenodrilus serratus							$NOEC_r$	**100** geom. mean
Nereis arenaceodentata	-	-	-	-	-	4-m?	$NOEC_r$	**100** (Cu) MARITOX 51618 [19]
Ophryotrocha diadema P --> F [lc]	-	S	$ZnSO_4.7H_2O$	nsw	-	3-w	$NOEC_{s,r}$	**100** (Cn) Reish & Carr '78 [23]
Ophryotrocha diadema	-	-	-	- -	-	4-w	$NOEC_r$	**100** (Cu) MARITOX 51618 [19]
Ophryotrocha diadema							$NOEC_r$	**100** geom. mean
Molluscs								
Crassostrea gigas eggs --> larvae	+	R	$ZnSO_4$	nsw	29	5-d	$NOEC_{d,g}$	**50** (Cn) Brereton et al.'73 [24]
Haliotis refescens	-	-	-	-	-	9-d	$NOEC_r$	**19** (Cu) MARITOX 50173 [19]
Mercenaria mercenaria 2-d old larvae	-	R	$ZnCl_2$	nsw	24	8-d	$NOEC_{s,g}$	**50** (Cn) Calabrese et al. '77 [25]
Scrobicularia plana length 4-5 cm	+	R	$Zn(NO_3)_2$	nsw	31	14-d	$NOEC_s$	**1,000** (Cn) Akberali et al '81 [26]
Crustaceans								
Callianassa australiensis	-	-	-	-	-	14-d	$NOEC_s$	**440** (Cu) MARITOX 15338 [19]
Holmesimysis costata 9-d old juveniles	+	R	$ZnSO_4.7H_2O$	nsw	35	7-w	$NOEC_{s,g}$	**18** (actual) Martin et al., '89 [27]
Mysidopsis bahia	-	-	-	-	-	-	$NOEC_r$	**120** (Cu) MARITOX 51549[19] (U.S EPA study)
Echinoderms								
Arbacia lixula	-	-	-	-	-	4-d	$NOEC_r$	**10** (Cu)
	-	-	-	-	-	20-d	$NOEC_s$	1000 (Cu) MARITOX 51385 [19]

Table 3.3.2.b: To be continued in Part II: Studies not useful for saltwater PECadd, aqautic derivation

Table 3.3.2.b. Chronic toxicity of zinc to saltwater organisms: NOEC values

Part II: Studies not useful for saltwater $PNEC_{add, aquatic}$ derivation

Organism & life stage	A	Test type	Test-comp	Test water	Salinity °/oo	Exp.-time	Criterion	Result (µg Zn/l)
Algae (unicellular)								
Chaetoceros compressum clone Chaet C2	-	S	$ZnSO_4$	nsw (CB)	35	3-d	$NOEC_g$ Fisher & Frood '80 Not useful: Q	≥60 (Cn) [2]
Nitzschia closterium clone Nitz C.1	-	S	$ZnSO_4$	nsw (CB)	35	3-d	$NOEC_g$ Fisher & Frood '80 Not useful: Q	≥60 (Cn) [2]
Nitzschia closterium clone Flag 8.4	-	S	$ZnSO_4$	nsw (CB)	35	3-d	$NOEC_g$ Fisher & Frood '80 Not useful: Q	≥60 (Cn [2]
Skeletonema costatum clone Skel C7	-	S	$ZnSO_4$	nsw (CB)	35	3-d	$NOEC_g$ Fisher & Frood '80 Not useful: Q	≥60 (Cn) [2]

All four tests: Rejected, based on Quality criterion (unbounded NOEC values)

For footnotes Table 3.3.2.b (Part I and II): see next pages; for further information see the "list of abbreviations Table 3.3.2a to 3.3.2.d".

Abbreviations and footnotes Table 3.3.2.b

d = developmental effects (deformities);
g = growth;
mc = morphological changes;
r = reproduction;
s = survival
lc: life cycle test.

[1] No statistics reported. Test medium sterilized either by autoclaving or by filter sterilization (0.2 µm filter); no further data on test medium reported. Growth parameter: maximum growth rate (divisions/day), calculated on the basis of cell counts.

[2] No statistics reported. Test water (seawater enriched with nutrients minus Cu, Zn or EDTA) was sterilized by 0.22 µm filter. BS and CB: seawater from 2 different locations. BS usually contained somewhat less dissolved organic carbon and lower background levels of Zn than CB. The background total-zinc concentrations in the test waters were 2 µg/l in BS and 5 µg/l in CB. Growth parameter: relative growth rate (divisions/day) during log-linear growth phase.

[3] The NOEC was estimated from the lowest effect concentration (35% inhibition at 20 µg/l) using a factor of 3.

[4] The NOEC was estimated from the lowest effect concentration (18% inhibition at 40 µg/l) using a factor of 2.

[5] No statistics reported. Test water sterilized by uv-irradiation. BS and CB: seawater from 2 different locations (see [2]); the seawater was not enriched with nutrients. Growth parameter: relative growth rate (divisions/day) during log-linear growth phase.

[6] The NOEC was estimated from the lowest effect concentration (26% inhibition at 20 µg/l) using a factor of 3.

[7] The NOEC was estimated from the lowest effect concentration (21% inhibition at 20 µg/l) using a factor of 3.

[8] The NOEC was estimated from the lowest effect concentration (19% inhibition at 20 µg/l) using a factor of 2.

[9] At the NOEC indicated, growth rate was not (or hardly) affected in the exponential growth phase, but the maximum and/or final cell densities were lower than control values when the test was continued beyond this phase.

[10] No statistics reported. Test water: seawater, enriched with nitrate, phosphate and silicate; test water sterilized by 0.22 µm filter. Growth parameter: number of cells.

[11] The NOEC was estimated from the lowest effect concentration (14% inhibition at 20 µg/l) using a factor of 2.

[12] No statistics reported. Only stock solutions and highest test concentration were analysed for zinc. Growth parameter: relative growth rate, calculated on the basis of cell counts.

[13] No statistics reported. Test medium sterilized either by autoclaving or by filter sterilization (0.2 µm filter); EDTA-free. Growth parameter: maximum growth rate (divisions/day), calculated on the basis of cell counts.

[14] No statistics reported. Test medium sterilized either by autoclaving or by filter sterilization (0.2 µm filter); test medium contained 2.3 µM EDTA (which can chelate 76 µg Zn/l) and a trace mineral mixture. Growth parameter: maximum growth rate (divisions/day), calculated on the basis of cell counts.

[15] No statistics reported. Growth parameter: carbon fixation rate (µg C/l/h) Test population: natural phytoplankton, being almost exclusively diatoms of the genus *Rhizosolenia.*

[16] The NOEC was estimated from the lowest effect concentration (32% inhibition at 20 µg/l) using a factor of 3.

[17] The NOEC was estimated from the lowest effect concentration (23% inhibition at 20 µg/l) using a factor of 3.

[18] The NOEC was estimated from the lowest effect concentration (20% inhibition at 60 µg/l) using a factor of 2.

[19] MARITOX: TNO/DGW ecotoxicological data base on marine organisms; the NOEC values indicated in Table 3.3.2.b have been reported by Scholten et al. (1991). The number following MARITOX refers to the TNO literature system. Data in MARITOX have been evaluated for reliability, in accordance with the system used in AQUIRE; all NOEC values indicated in the table were considered to be reliable.

[20] During the exponential growth phase, growth rate was reduced at 50 µg/l, but the maximum and final cell numbers were not adversely affected at this concentration.

[21] No statistics reported. Growth parameter: number of sporophytes.

[22] No statistics reported. Parameter: macroscopic morphological changes.

[23] Statistics ($p = 0.05$) reported for reproduction. Reproductive parameter: number of worms. Based on the number of initial worms that survived the first 4 days of exposure, reproduction was affected at much lower concentrations than survival.

[24] No statistics reported. Test medium: UV-sterilized seawater. The 5-d exposure period was followed by a 5-d depuration period; growth was measured up to day 10.

[25] The NOEC indicated is the LC5 reported by Calabrese et al. (1977). The estimated percent growth at this concentration was 100%. Background zinc concentration in seawater: 18 µg/l.

[26] No statistics reported.

[27] Statistics: $p = 0.05$. Growth parameter: carapace length. Abotts correction used for control mortality (22%).

Table 3.3.2.c Toxicity of zinc to aquatic microorganisms: NOEC and EC values

Test species / inoculum	A	Test compound	Test medium	Exp. time	Criterion	Result (mg Zn/l)			
Bacteria									
Pseudomonas fluorescens	-	$ZnCl_2$	nut. broth + sterile sewage	10 h	$EC50_g$	50.2			
	-	$ZnCl_2$	nutrient agar +	24 h	$EC50_{cf}$	51.7			
	-	$ZnCl_2$	sterile sewage	2 h	$EC50_{ir}$	228.6			
	-	$ZnCl_2$	distilled water	2 h	$EC50_{ir}$	74.3			
				Codina *et al*., 1993					[15]
Pseudomonas putida	+	$ZnCl_2$	Tris/HCl pH 6.0	½ h	$EC10_{am}$	1.8			
DSM 50026		$ZnCl_2$	BisTris/HCl pH 7.9	½ h	$EC10_{am}$	0.3			
				Van Beelen and Fleuren-Kemilä, 1997					[2]
Vibrio fisheri	-	$ZnSO_4.7H_2O$	sewage + growth medium and 30 ‰ NaCl	16 h	$EC50_g$	>160			
				Gellert and Stommel, 1994					[12]
Spirillum volutans	-	$ZnSO_4.7H_2O$	-	2 h	$EC90_{im}$	11.6			
				Dutka *et al*., 1983					[14]
Vibrio fisheri	-	$ZnSO_4.7H_2O$	standard	½ h	$EC50_{il}$	9.2			
				Gellert and Stommel, 1994					[7]
Vibrio fisheri	-	$ZnSO_4.7H_2O$	standard	-	$EC50_{il}$	26-32			
	-	$ZnSO_4.7H_2O$	20 ‰ NaCl	-	$EC50_{il}$	0.81			
				Steinhauser, 1992					[8]
Vibrio fisheri	-	$ZnSO_4.7H_2O$	23 ‰ NaCl	5 min.	$EC50_{il}$	108			
				McFeters *et al*., 1983					[9]
Vibrio fisheri	-	$ZnSO_4.7H_2O$	-	¼ h	$EC50_{il}$	3.5			
				Dutka *et al*., 1983					[10]
Vibrio fisheri	-	$ZnCl_2$	standard	¼ h	$EC50_{il}$	5.6			
				Codina *et al*., 1993					[16]
Vibrio fisheri	-	$ZnCl_2$	standard sewage	¼ h	$EC50_{il}$	14.5			
				Codina *et al*., 1993					[17]
Activated sludge, industrial	-	$ZnCl_2$	artificial	-	EC50	~45			
					NOEC	15			
				BASF AG, 1980					[3]
Activated sludge, domestic	-	$ZnSO_4.7H_2O$	artificial	3 h	**$EC50_{ir}$**	**5.2**			
				Dutka *et al*., 1983					[4]
Activated sludge, domestic	-	$ZnSO_4$	sewage + glucose	-	$EC50_{ir}$	900			
				Miksch and Schürmann, 1988					[5]
Natural seawater sample	-	$ZnSO_4$	seawater + yeast +	-	$EC50_g$	0.74			
		$ZnSO_4$	'acide nalidixique'	-	$EC50_g$	0.94			
				Delesmont and Delattre, 1983					[11]

Test species / inoculum	A	Test compound	Test medium	Exp. time	Criterion	Result (mg Zn/l)			
(to be continued)									
Protozoa									
Euglena viridis	+	$ZnSO_4.7H_2O$	artificial	21 d	$NOEC_g$	4.2			
				Coleman *et al*., 1971					[6]
Tetrahymena pyriformis	-	$ZnSO_4$	distilled water	96 h	$NOEC_s$	1.33			
				Carter and Cameron, 1973					[13]
Aspidisca cicada	-	$ZnCl_2$	Evian water	24 h	$LC50_s$	2.40			
Blepharisma americanum	-	$ZnCl_2$	Evian water	24 h	$LC50_s$	1.05			
Dexiostoma campyla	-	$ZnCl_2$	Evian water	24 h	$LC50_s$	1.85			
Euplotes affinis	-	$ZnCl_2$	Evian water	24 h	$LC50_s$	3.10			
Euplotes patella	-	$ZnCl_2$	Evian water	24 h	$LC50_s$	50.0			
Paramecium caudatum	-	$ZnCl_2$	Evian water	24 h	$LC50_s$	2.50			
Uronema nigricans	-	$ZnCl_2$	Evian water	24 h	$LC50_s$	2.90			
				Madoni *et al.*, 1992					[1]
Drepanomonas revoluta	-	$ZnCl_2$	Evian water	24 h	$LC50_s$	0.25			
Spirostomum teres	-	$ZnCl_2$	Evian water	24 h	$LC50_s$	0.67			
				Madoni *et al.*, 1994					[1]

Abbreviation and footnotes Table 3.3.2.c; see further the "list of abbreviations Tables 3.3.2.a to 3.3.2.d"

am = acetate-mineralization;

cf = inhibition of colony formation;

g = growth;

il = inhibition of light production;

ir = inhibition of respiration;

mo = mobility;

s = survival

Nut. broth = nutrient broth

General remarks:

- All reported tests were static.

- Hardness was specified only in the *Tetrahymena pyriformis* test of Carter and Cameron (1973).

- The marine bacterium *Vibrio fisheri,* formerly known as *Photobacterium phosphoreum*, is the test species used in the Microtox-test.

[1] All protozoa are ciliates and were isolated from activated sludge from a STP treating domestic waste. Evian natural water was used as a medium in the toxicity tests. pH was adjusted to 7.3 with 0.1 N NaOH. Mortality was scored microscopically.

[2] The test strain was isolated from a channel and is used in water toxicity testing. The EC10 is the concentration inhibiting the $^{14}CO_2$ production from ^{14}C-acetate by 10%. Note that toxicity increased with increasing pH, the two EC10 values are significantly different (p 0.05).

[3] EC50: mean value of EC(25%) and EC(75%), being 30 and 60 mg Zn/l, respectively.

[4] Medium is synthetic, according to *OECD, 1976 (unspecified; not available*). Inoculate originated from a predominantly domestic sewage treatment plant. In the final mixture, the concentration of suspended solids was ca. 1.5 g/l. Samples were kept aerobic by means of aeration. The reported conditions of the test follow OECD guideline 209.

[5] The inoculate from a domestic STP was adapted to sewage water. Glucose and inorganic nutrients were added in the 'medium'. The authors report the EC50 value as 900 mg Zn/l and as 270 mg Zn/g TS (TS = 'Mikroorganismentrockenmasse' = bacterial dry weight). They report a concentration TS of 2.01 g/l on average. This means that 270*2.01 = 543 mg Zn is sorbed per liter sewage water. From the given EC50 of 900 mg Zn/l, the amount of Zn not sorbed to TS is 900-543 = 357 mg/l. It is unclear what the sorption capacity of the rest of the medium is.

[6] Medium is "Bold's Basal Medium". The medium contained a background zinc concentration of 1880 µg/l. Four replicates were tested per concentration. Cell dry weight was measured after 3 weeks. The concentration of 4.2 mg/l (includes the background Zn) was the lowest concentration tested, giving a stimulation of growth of 136%. All higher concentrations inhibited growth.

[7] The test was carried out in standard medium for the DIN 38412, part 341 test with the exception that 30 g/l NaCl was added instead of 20 g/l. The test result is the average of 4 individual tests. Concentrations and EC50 (40.5 mg/l) were expressed as $ZnSO_4.7H_2O$, resulting in a EC50 of 9.2 mg Zn/l.

[8] Test protocol was DIN 38412, part 34 (1991; 1992). The test medium contained K and Mg salts, glucose and HEPES buffer. The EC50 range is resulting from a ring test with 20 laboratories and different conservation methods of the inoculate. Inoculating "wet" freeze dried suspensions, deep frozen cultures or freshly grown organisms did not have a significant influence on the test result. "Microtox" inoculate (lyophilized cells) reconstituted in 2% NaCl gave lower effect concentrations (average of 10 laboratories) probably because K^+ and Mg^{2+} (competing ions for Zn uptake) ions were not present. No incubation time reported.

[9] Standard Microtox test. Concentrations and EC50 (476 mg/l) were expressed as $ZnSO_4.7H_2O$, resulting in a EC50 of 108 mg Zn/l.

[10] Standard Microtox test. Medium not specified.

[11] The two EC50 values given are independent results. Total and viable cells were counted microscopically. Several (identical) concentration ranges of seawater with toxicant (+ controls),were incubated. Every hour the incubation of a complete concentration range (+ controls) was stopped. The control sample (and its corresponding concentration range) that had the longest incubation time before total cell numbers started to decrease was used to determine the number of viable counts (in all samples of the concentration range).

[12] Growth test with *Vibrio.fisheri*, closely followed DIN 38412 (1991, 1992). Test medium consisted of 50% mineral medium + peptone + yeast + glycerin and 50% sewage water. pH 7. Concentrations and EC50 (>700 mg/l) were expressed as $ZnSO_4.7H_2O$, resulting in a EC50 of >160 mg Zn/l.

[13] Medium was distilled water without $CaCO_3$, hardness = "0" mg/l (as $CaCO_3$). Concentrations and NOEC (3.33 mg/l) were expressed as $ZnSO_4$, resulting in a NOEC of 1.33 mg Zn/l. Mortality was scored microscopically.

[14] "Defined test medium". The rotating activity of flagella was microscopically counted. Non-activity was the toxicity parameter. Effect was scored when flagella motility of >90% of the cells was eliminated.

[15] Growth inhibition experiments carried out according to NEN 6509. Growth of an inoculum in 2x diluted nutrient broth is measured as absorbance at 650 nm. Metals were added to sterilized sewage, which was added to an equal volume of nutrient broth. No addition of glucose reported. The inhibition of colony formation was scored in a plate count test. The inoculated cells on membrane filters were put on pads (placed on nutrient agar) saturated with sterilized sewage solution to which zinc chloride was added.

[16] Zinc chloride dissolved in deionized water was tested in the Microtox test under standard conditions.

[17] Zinc chloride was added to sterilized sewage samples, which were tested in the Microtox test under standard conditions.

Table 3.3.2.d Toxicity of zinc metal powder to freshwater organisms: NOEC and EC values

Organism & life stage	Test compound & purity	Test-water	pH	Hard-ness	Exp.-time	Criterion	Result (% of filtrate conc.) (I)	Result (mg Zn/l; **dissolved**)
Algae (unicellular)								
Pseudokirchneriella subcapitata	Zn powder 98.4%	art.	7.4	24	72-h	E_rC50_g	18.78%	0.15 (Cd)
						NOE_rC_g	3.05%	0.05 (Cd)
						NOE_bC_g	3.05%	0.05 (Cd)
						Van Woensel '94a [1]		
Crustaceans								
Daphnia magna age <24 h	Zn powder 98.4%	art	7.7	262	48-h	$NOEC_i$	9.76%	0.15 (Cd)
						Vos, '94 [2]		
Fish								
Brachydanio rerio length 3.32 ± 0.18 cm	Zn powder 98.4%	art.	7.6	253	96-h	$NOEC_s$	>100 mg/l dispersion	>2.36 (Cd)
						Van Ginneken, '94c [3]		

Abbreviations and footnotes Table 3.3.2.d; see further the "list of abbreviations Table 3.3.2.a to 3.3.2.d"

All tests: static test system

g = growth (r: growth rate; b: biomass);

i = immobility;

s = survival

(I): The zinc concentration in the undiluted filtrate, prepared from a 100 m Zn/l dispersion, is expressed as 100% (see further footnotes).

Cd: Measured dissolved-zinc concentration in test water, based on analyses of zinc in 0.40-0.45 µm filtered test waters.

[1] No statistics reported. Test conducted according to OECD-guideline 201 and under GLP. Culture medium: Bold's Basal Medium. According to Coleman et al. (1971) (see footnote [6] in Table 3.3.2.c) this medium contains a very high background zinc concentration of 1880 µg/l. Test medium according to OECD-guideline No. 201 (nominal background zinc concentration: 1.5 µg/l; hardness 24 mg/l (as $CaCO_3$)), but EDTA was omitted. No data reported on the acclimation of the algae to the test medium. Test compound: zinc powder (median diameter 13.4 µm; 0.5% residue on 45 µm filter). Growth parameter: cell number (specific growth rate and biomass). The actual background concentration of zinc in the test medium was ≤ 10 µg/l. In the test, a control, a filtrate of a 100 mg Zn/l dispersion of the metallic zinc powder and a series of four dilutions of the filtrate were tested. The filtrate was prepared by filtering the 100 mg Zn/l dispersion of zinc powder, after 24 hour stirring, over a 0.45 µm membrane filter (Millipore). If the concentration of the test substance in the filtrate is expressed as 100% then the following concentrations expressed in % were tested: 0%, 0.95%, 3.05%, 9.76%, 31.25% and 100%; the actual zinc concentrations were ≤ 10, 50, 50, 90, 230 and 760 µg/l, respectively, based on 72-h measurements. The nominal 72-h EC50 for growth rate was 18.78% of the filtrate; the actual value (interpolation from dissolved-Zn measurements in the test solutions) was 150 µg/l (see also Table 3.3.2.d). The nominal 72-h NOEC for both growth rate and biomass was 3.05% of the filtrate (actual dissolved-Zn concentration: 50 µg/l); at the next higher concentration (9.76% of the filtrate; actual dissolved-Zn concentration 90 µg/l), growth rate and biomass were reduced 27% and 69%, respectively. This test is also included in Table 3.3.2.a – Part I.

Note that in the test report the algal species is named *Selenastrum capricornutum*; the currently used species name is *Pseudokirchneriella subcapitata.*

[2] No statistics reported. Test conducted according to OECD-guideline 202 and under GLP. Test medium according to EEC standard No. L.251/155 Part C2, 1.6.1.2. annex (1984), to which micro-nutrients were added, but EDTA was omitted. Test compound: zinc powder (median diameter 13.4 µm; 0.5% residu on 45 µm filter). In the test, a control, a filtrate (0.45 µm filter) of a 100 mg Zn/l dispersion of the metallic zinc powder and a series of four dilutions of the filtrate were tested, using a dilution factor of 3.2. The filtrate was prepared by filtering the 100 mg Zn/l dispersion of zinc powder, after 24 hour stirring, over a 0.45 µm membrane filter (Millipore). If the concentration of the test substance in the filtrate is expressed as 100%, then the following dilutions were tested: 31.25%, 9.76%, 3.05% and 0.95%. Toxicological endpoint: immobilisation. At the LOEC (31.25% nominal; actual dissolved-Zn concentration 500 µg/l), 14 out of 20 daphnids were immobile. Probit analysis for deriving an EC50 could not be applied, as no two partial immobility values were obtained. Therefore the EC50 was reported as the range of the NOEC and the LOEC, i.e. 9.76% nominal (0.15 mg dissolved-Zn/l) < 48-h EC50 < 31.25% nominal (0.5 mg dissolved-Zn/l).

The test medium contains a nominal background concentration of 0.0044 mg $ZnSO_4.7H_2O$/l (0.001 mg Zn/l). The actual dissolved-Zn concentration in the test medium was <0.01 mg/l (0-h measurement) and 0.04 mg/l (48-h measurement), respectively. Actual dissolved-Zn concentrations: based on zinc measurements in 0.45 µm filtered test water. The above NOEC and LOEC are based on averages of the 0-h and 48-h measurement (no or virtually no difference beween the 0-h and 48-h NOEC and LOEC measurements, respectively, in contrast to the 0-h and 48-h control measurements).

[3] No statistics reported. Limit test conducted according to OECD-guideline 203 and under GLP. Test medium according to EEC-guideline 79-831, Annex V, part C.1. (1984). Test compound: zinc powder (median diameter 13.4 µm; 0.5% residu on 45 µm filter). In the test, a control, a 100 mg Zn/l dispersion of the metallic zinc powder and a filtrate (0.45 µm filter) of a 100 mg Zn/l dispersion were tested. The filtrate was prepared by filtering the 100 mg Zn/l dispersion of zinc powder, after 24 hour stirring, over a 0.45 µm membrane filter (Millipore). No effects on survival and behaviour were observed in any group.

Actual dissolved background zinc concentration in test medium: <0.01 mg/l (0-h and 24-h measurements) and 0.04 mg/l (96-h measurement), respectively. Actual dissolved-Zn concentrations in filtrate and dispersion: 1.51 Zn/l (average value 0-h to 96-h measurements; range 1.50-1.52 mg/l) and 2.36 mg/l (average value 0-h to 96-h measurements; range 1.44-2.98 mg/l), respectively. Actual dissolved-Zn concentrations: based on zinc measurements in 0.4 µm filtered test waters. The measured total-Zn concentration in the dispersion was 12.8, 3.9 and 15.4 mg/l at the 0-h, 24-h and 96-h measurement, respectively, indicating that it was impossible to take a representative sample of the dispersion.

List of abbreviations Table 3.3.2.a to Table 3.3.2.d

A	Analysis of zinc in test water: +: Zinc analysed. -: Zinc not analysed or: no data reported on analysis.
Test type	S: static; R: renewal; F: flow-through (continuous flow).
Test water	Freshwater organisms: art.: artificial (reconstituted) test water. Saltwater organisms: asw: artificial (reconstituted) sea water nsw: natural sea water.
Hardness	Expressed as mg $CaCO_3$/l, unless stated otherwise. In a number of cases the hardness was not reported in the publication, but calculated from the calcium and magnesium concentration in the test water.
Exposure time	d: day(s); h: hour(s); m: month(s); min.: minute(s); w: week(s); yr: year(s).
Criterion	LC50: Median lethal concentration, i.e. the concentration which is calculated from a series of test concentrations to cause mortality in 50% of the organisms exposed to that concentration. EC50: Median effect concentration, i.e. the concentration which is calculated from a series of test concentrations to cause a particular response in 50% of the organisms exposed to that concentration.

EC(..%): At the concentration indicated (usually the only concentration tested), the toxicological endpoint was inhibited by ..%. Example: EC (21%).

NOEC: No observed effect concentration, i.e the highest concentration (in a series of test concentrations) without effect. If a statistical analysis of the toxicity data was reported, the NOEC is the highest concentration showing no statistically significant (at $p < 0.05$) effect compared to the control.

If no statistical analysis of the data was reported, the NOEC is the highest concentration showing less than 10% effect compared to the control.

In subscript the toxicological endpoint or endpoints are indicated at each NOEC (e.g. NOECg is NOEC for growth; NOECr,s is NOEC for reproduction and survival).

NOECe : NOEC values marked by "superscript e" are EC10 values (considered to be equivalent to NOEC values) or have been estimated from the LOEC (lowest observed effect concentration) in case the "real" NOEC could not be derived directly from the data reported.

NOECe values have been estimated mainly if more than one test was available for one test

species, resulting in both NOEC and LOEC values for the same toxicological endpoint, to allow the calculation of the geometric mean NOEC relating for this endpoint.

The following application factors have been used to derive a NOECe:

- in case the LOEC resulted in 11% to 20% effect: factor of 2;
- in case the LOEC resulted in 21% to 30% effect: factor of 3.

"Species mean" NOEC: In case several NOEC values (from different tests) are available for a certain species, the NOEC values **printed bold** have been used to calculate a geometric mean NOEC (for the most sensitive endpoint).
See next page for further explanation of the derivation of NOEC and $NOEC^e$ values.

Table 3.3.2.a – (freshwater) Part 1: The ("species mean") NOEC values that are **printed bold** and **underlined** have been used as input data in the ecotoxicological extrapolation methods used to derive the Predicted No Effect Concentration ($PNEC_{add,\ aquatic}$) for zinc in surface water, see RAR Zn Metal section 3.3.1.3.

NOEC values:
≥ Unbounded NOEC, i.e. no effect was found at the highest concentration used in the test) (thus the "real" NOEC may be higher). Unbounded NOEC values are not used for $PNEC_{add}$ derivation.
Cn Nominal zinc concentration in test water.
Cb Background zinc concentration in test water.
actual Analysed zinc concentration in test water.
Cu Unknown; reported NOEC from review, without data on analysis of zinc in test water.

See next page for data on the selection, derivation and reliability of the freshwater chronic NOEC values. For additional data on the selection of the freshwater chronic NOEC values, based on reliability and relevance criteria, see RAR Zinc Metal section 3.3.1.1 (sources and selection of ecotoxicological data) and section 3.3.2.1 (Toxicity to aquatic organisms).

Selection of chronic NOEC values (RAR Zn Metal section 3.3.1.1)

For the selection of chronic NOEC values used to derive $PNEC_{(add)}$ values, the following approach has been taken:

- Toxicological endpoints, which may affect the species at the population level, are taken into account. In general, these endpoints are survival, growth and reproduction. The toxicity results are commonly expressed as an acute LC50 or EC50 (usually derived from toxicity tests with a duration of four days or less) or as a chronic NOEC (usually derived from toxicity tests with a duration of more than four days). With respect to the NOEC values it is noted that the fact whether or not a NOEC is considered a chronic NOEC is not determined exclusively by the above exposure time limit of four days, but also by the generation time of the test species. For unicellular algae and other microorganisms (bacteria; protozoa), an exposure time of four days or considerably less already covers one or more generations, especially in water, thus for these kinds of species, chronic NOEC values may be derived from experiments during less than four days. On the other hand, for organisms that have a long generation time, for example fish, an exposure time of just over four days is much too short to derive a chronic NOEC. It will be clear that for PNEC derivation a full life-cycle test, in which all relevant toxicological endpoints are studied, is normally preferred to a test covering not a full life cycle and/or not all relevant endpoints. However, the results of a test, which is more limited than a full life-cycle test may be used, see further the points below.
- If for one species several chronic NOEC values (from different tests) based on the same toxicological endpoint are available, these values are averaged by calculating the geometric mean, resulting in the "species mean" NOEC. With respect to this it is noted that the NOEC values should be from equivalent tests, for example from tests with similar exposure times. However, NOEC values derived from tests with a relatively short exposure time may be used together with NOEC values derived from tests with a longer exposure time if the data indicate that a sensitive life stage was tested in the former tests.
- If for one species several chronic NOEC values based on different toxicological endpoints are available; the lowest value is selected. The lowest value is determined on the basis of the geometric mean if more than one value for the same endpoint is available (see above).
- In some cases, NOEC values for different life stages of a specific organism are available. If from these data it becomes evident that a distinct life stage is more sensitive, the result for the most sensitive life stage is selected. The life stage of the organisms is indicated in the tables as the life stage at start of the test (e.g. fish: yearlings) or as the life stage(s) during the test (e.g. eggs → larvae, which is a test including the egg and larval stage).

Derivation of NOEC values – freshwater (*) (RAR Zn Metal section 3.3.1.2)

The methods that have been used for the derivation of NOEC values, being "real" NOEC values or NOEC values derived from effect concentrations, are essentially the same as outlined in the EU TGD (Part II, Chapter 3, Table 15)(EC, 2003) .

If possible, "real" NOEC values were derived from the data reported, i.e. the NOEC is one of the concentrations actually used in the test. In order of preference:

3) Statistical analysis: the NOEC is the highest concentration (in a series of test concentrations) showing no statistical significant effect (inhibition) compared to the control. Significance level: p = 0.05 (optional: the p = 0.01 level if reported instead of the p = 0.05 level).
   If no statistical analysis has been applied: the NOEC is the highest concentration that results in $\leq$ 10% inhibition compared to the control.

   In both cases there must be a consistent concentration-effect relationship, i.e the LOEC is the concentration at which and above which statistical significant toxicity is found (1) or, when no statistical analysis has been applied (2), >10% inhibition is found.

If the "real" NOEC could not be derived from the data reported, the following procedure was used to derive the NOEC. In order of preference:

2) The NOEC is set at the EC10 level.
   a) Especially in more recent references on ecotocological data there is increasing preference for the benchmark dose approach. Hence, a benchmark dose (usually the EC10) was reported in a number of references instead of the NOEC. The EC10, which is calculated from the concentration-effect relationship, is used as NOEC equivalent, unless the "real" NOEC was also reported or could be derived from the data reported.
   b) Furthermore, a number of EC10 values was calculated by the rapporteur; the EC10 values were derived from a logistic, sigmoidal dose response model according to Haanstra et al. (1985):

$$Y = c / \{1+\exp [b.(X – a)]\}$$

2) The NOEC is derived from the LOEC
   If the EC10 was not reported and could not be calculated, the NOEC was derived from the LOEC using the following "extrapolation" factors:
   a) NOEC = LOEC/2, in case inhibition is >10% but $\leq$20%, e.g. LOEC = EC(15%).
   b) NOEC = LOEC/3, in case inhibition is >20% but $\leq$30% e.g. LOEC = EC(25%).
   If the percentage inhibition at the LOEC is >30% or in case the percentage inhibition at the LOEC is unknown, no NOEC is derived.

With respect to "rule 2b" it is noted that the EU TGD does not mention the derivation of a NOEC from a LOEC in case inhibition at the LOEC is >20%, while in this RAR the derivation of a NOEC from a LOEC up to 30% effect has been used in some aquatic toxicity studies. The use of the higher effect level is justified by the use of a higher extrapolation factor.

Reliability of NOEC values – freshwater (*) (RAR Zn Metal section 3.3.2.1)

All freshwater NOEC values (including EC10 values) used for freshwater $PNEC_{add,\ aquatic}$ derivation have been checked for reliability on the basis of the range of test concentrations, as follows:

- If the NOEC is <100 µg/l, the separation factor between the NOEC and LOEC should not exceed a factor of 3.2.
- If the EC10 is used as NOEC equivalent, the EC10 should not be more than 3.2-times lower than the lowest concentration used in the test.

It is noted that the results of all tests met these criteria, thus no tests had to be rejected because of the above reliability criteria.

(*) The saltwater NOEC values (from Janus, 1993) were not updated and not checked for reliability based on the criteria used in the RAR Zn Metal for the freshwater NOEC values, as no saltwater $PNEC_{add,\ aquatic}$ was derived. However, the unbounded NOEC values for saltwater were rejected, as those for freshwater.

ANNEX 3.3.2.B. FRESHWATER (MODEL) ECOSYSTEM STUDIES

Table 3.3.2.i – Part A: Field studies: NOEC and LOEC values

Taxa / organisms	Test comp.	Test system and water characteristics	Exp. time	Criterion	Result (µg Zn/l)	
Periophyton, Zooplankton, Invertebrates Several single species and multiple species studies, see below.	$ZnSO_4$	Field-located artificial streams (holding capacity 20 L) New river water, pH 8.1-8.4; hardness 66-89 mg/l Flow-through system	30-d	NOEC (MS) LOEC (MS)	25 Cn (actual: ≤20) * 50 Cn (actual: 34-87) Overall results, based on Belanger et al., 1986 Farris et al., 1989 Farris et al., 1994 Genter et al., 1987, see below.	Total Zn
Corbicula sp. (adults, juveniles) Several tests, in different seasons	$ZnSO_4$	Field-located artificial streams (holding capacity 20 L) New river water, pH 8.1-8.4; hardness 66-89 mg/l Flow-through system	30-d	$NOEC_g$ (SS) $LOEC_g$ (SS) $NOEC_s$ (SS)	25 Cn (actual: ≤20) * 50 Cn (actual: 43-87) 500 Cn (actual: >504) Belanger et al., 1986 [1]	Total Zn
Corbicula sp.	$ZnSO_4$	Field-located artificial streams (holding capacity 20 L) New River water, pH 8.3; hardness 71 mg/l Flow-through system	30-d	$LOEC_{g,e}$ (SS) $LOEC_s$ (SS)	50 Cn (actual: 34) 1000 Cn (actual: 1100) Farris et al., 1989 [2]	Total Zn
Corbicula fluminea (clam); *Mudalia dilatata* (snail) Several tests, in different seasons	$ZnSO_4$	Field-located artificial streams (holding capacity 20 L) untreated New River water, pH 8.1-8.4; hardness 67-89 mg/l	30-d	$NOEC_e$ (SS) $LOEC_e$ (SS)	25 Cn (actual: ≤20) 50 Cn (actual: 35-87) Farris et al., 94 [3]	Total Zn
Periphyton, Algae Several tests, in different seasons	$ZnSO_4$	Field-located artificial streams New River water pH 8.1 - 8.4; hardness 70 - 89 mg/l Flow-through system	30-d	LOEC (MS) **	50 Cn (actual: 35-87) ** Genter et al., 1987 [4]	Total Zn
Algae, cladocera, copepoda, rotifera	not reported	In situ tests in Lake Michigan, 18 L carboys with or without Zn treatment were suspended in the lake. Hardness Lake Michigan water: around 70 mg/l	14-d	LOEC (MS) **	15 Cn (actual: 17) ** Marshall et al., 1983 [5]	Total Zn, but 94% dissolved at this concentration.

* In the tests performed in the fall of 1984, the actual total-Zn concentration in the control was 94 µg/l, wich is an outlier compared with the ≤ 20 µg/l (i.e. at or usually below the detection limit of 20 µg/l) measured in the other seasons (spring and summer) in which further tests were performed.

** LOEC = lowest concentration used in the test; A NOEC can not be derived. .

NOEC (SS) and LOEC (SS): single-species NOEC and LOEC

NOEC (MS) and LOEC (SS) = multiple-species NOEC and LOEC

n.r. = not reported

Enhdpoints: g = growth, e = enzyme activity (cellulolytic activity, which is a general stress indicator), s = survival

Hardness (mg/l): total (Ca + Mg) hardness, as mg $CaCO_3$

For footnotes Table 3.3.2.i – Part A, see next pages.

Footnotes Table 3.3.2.i – Part A

[1] - [4]: Belanger et al. (1986), Genter et al., (1987), Farris et al. (1989), Farris et al. (1994)

The results from these single-species and multiple-species studies listed in Table 3.3.2.i – Part A are all based on tests performed in field-located artificial streams filled with natural New river water (and a 2 c m layer of coarse sediment).

Exposure

The actual zinc concentrations measured in the water are for total recoverable zinc. According to the underlying data, the actual total-Zn concentration in the control streams in the different tests was usually below 20 µg/l (detection limit), see further below.

According to IND (referring to Shiller & Boyle, 1985), the natural background dissolved-Zn concentration in New river wate (Virginia, United States) is expected to be very low: in the order of <0.2 µg/l, based on very detailed analysis of similar small rivers in the same area. However, based on measurements in some rivers in Virginia and Louisiana, Shiller & Boyle report zinc concentrations of 0.3-3 µg/l; there is no reference to New river specifically. Thus there is no evidence that the background Zn concentration in New river water is considerably below 1 µg/l.

Belanger et al. (1986): Streams with coarse sand sediment (2-9 mm diameter), except in spring 1984)
Nominal total-Zn concentrations
0-50-1000 µg/l in the tests performed in spring and summer 1984
0-50-500-1000 µg/l in the test performed in fall 1984
0-25-100 µg/l in the test performed in spring 1984
Control treatments
Spring 1984: Actual total-Zn concentration in the control was 20 ± 0 µg/l (20 µg/l = detection limit)
Summer 1984: Actual total-Zn concentration in the control was 28 ± 16 µg/l
Fall 1984: Actual total-Zn concentration in the control was 94 ± 228 µg/l
Spring 1985: Actual total-Zn concentration in the control was 20 ± 0 µg/l)20 µg/l = detection limit)
25 µg/l treatment (nominal total-Zn) (NOEC)
Spring 1985: Actual total-Zn concentration was 20 ± 0 µg/l (20 µg/l = detection limit)
50 µg/l treatments (nominal total-Zn) (LOEC)
Spring 1984: Actual total-Zn concentration was 43 ± 60 µg/l
Summer 1984: Actual total-Zn concentration was 35 ± 12 µg/l
Fall 1984: Actual total-Zn concentration was 87 ± 109 µg/l
Mean concentrations: based on day 0, 5, 10, 20 and 30 measurements.

Genter et al. (1987)

Tests performed in spring, summer and fall 1984; Test concentrations and further water characteristics identical to those reported by Belanger et al. (1986).

Farris et al. (1989)

Test performed in summer 1984. Nominal total-Zn concentrations: 0-50-1000 µg/l.

Control treatment
Summer 1984: Actual total-Zn concentration in the control was < 20 µg/l
(never above 20 µg/l = detection limit)

50 µg/l treatment (nominal total-Zn) (LOEC)
Summer 1984: Actual total-Zn concentration was 34 ± 4 µg/l
Mean concentration: based on day 0, 5, 10, 20 and 30 measurements.
Farris et al. (1994)
Tests performed in spring, summer and fall 1984, and spring 1995. Test concentrations and further water characteristics identical to those reported by Belanger et al. (1986).
Results
Based on the results of these four studies in field-located artificial streams in New river water (see also further below), an overall NOEC (MS) of 25 µg/l (nominal; the actual total-Zn concentration at this treatment was ≤20 µg/l) and an overall LOEC (MS) of 50 µg/l (nominal added-Zn concentration; the actual total-Zn concentration at this treatment was 34-87 µg/l). The overall NOEC (MS) is identical to the NOEC (MS) of 20 µg/l (actual concentration) derived by Versteeg et al. (1999) from these four studies.

[1] Belanger et al. (1986)P: single-species tests with *Corbicula sp.*

Field-collected *Corbicula sp.* were exposed to Zn concentrations of 0 to 1000 µg/l in field-located artificial streams with natural New river water (in the field study) or dechlorinated tap water (in the laboratory study). Four tests in the field-located streams were performed, in different seasons (spring 1984, summer 1994, fall 1984, and spring 1985). The first test was performed with juvenile and adult clams, respectively; the other tests were performed with adult clams. Coarse sand sediment (2.5-9.0 mm diameter) was added in the field tests, except in the test performed in the spring of 1984.

Each test included a control and 2 or 3 of the following nomimal Zn concentrations: 25-50-100-500-1000 µg/l, see also [1] - [4] above. Statistics ($p = 0.05$) on growth data only (shell length gain and and total weight gain, measured on days 0, 5, 10, 20 and 30. Mortality and Zn accumulation in the clams were also studied. In all tests at least 30 field-collected clams (collected from the New river) were used in the control and exposure groups.

Culture and test conditions: clams were collected from the New river in Virginia and the test was conducted in outdoor artificial streams containing New river water (this river water was also used in some single-species laboratory tests with *Ceriodaphnia dubia,* see Belanger & Cherry, '90; Table 3.3.2.A) and coarse sand sediment diameter 2-9 mm); the cages with clams were buried in the sand. Prior to the test there was a 2-w acclimation period in the artificial streams.

All exposures in the field-located streams to 50 µg/l, in spring, summer and fall of 1984, respectively, resulted in significantly ($p<0.05$) decreased weight gain and/or shell length ($LOEC_g$: 50 µg/l), as well as all exposures to higher Zn concentrations. In the test that was performed in the spring of 1985, with nominal test concentrations of 0-25-100 µg/l (measured: ≤20- ≤20-120 µg/l), no effect on growth was observed at 25 µg/l ($NOEC_g$). The actual total-Zn concentrations measured in the control and the 25 µg/l nominal concentration in this test were both below the limit of detection (20 µg/l, from Farris et al., 1989). At 1000 µg/l, the percentage mortality ranged from 9% to 50% ($LOEC_s$); in the test that included a concentration of 500 µg/l, the percentage mortality was only 2% ($NOEC_s$). In all tests, the measured total-Zn concentration at 50 µg/l and higher concentations were 70% to 180% of the nominal concentrations.

Belanger et al. (1986) also performed a laboratory study in artificial streams containing dechlorinated tap water; the results of this laboratory study could not be used because growth in all groups, including the control, was very poor.

Note: there appers to be an overlap in the data for growth and survival of Corbicula sp. reported by Belanger et al. (1986) and Farris et al. 1989 (see below), although the reported actual total-Zn concentration at 50 µg/l nominal are slightly different.

[2] Farris et al. (1989): single-species test with *Corbicula sp.*

Field-collected *Corbicula sp*, collected from New river, were exposed to Zn concentrations of 0 (control), 50 and 1000 µg/l in field-located artificial streams with natural New river water (in the field study of summer 1984, see also Belanger et al., 1986) or in laboratory artificial streams with dechlorinated tap water (in the laboratory study). Coarse sand sediment (2.5-9.0 mm diameter) was added in the field study. In the laboratory study, algae (previously unexposed to Zn) were added for feeding. Cellullolytic activity (i.e. cellulase activity, including endocellulase and exocellulase activity), growth (shell length and weight gain; measured in the field study only, as the clams did not growth well in the laboratory stream) and bioaccumulation of Zn were measured after 0, 5, 10, 20 and 30 days. The background Zn concentration was never above the detection limit (20 µg/l). In the field study, length gain was significantly reduced (>50%; $p<0.05$) compared to the control at the 50 µg/l treatment ($LOEC_{g;}$ actual concentration 34 µg/l), while weight gain was significantly reduced (>90%; $p<0.05$) only at the highest test concentration (1000 µg/l; actual 1100 µg/l). The LOEC for growh ($LOEC_g$: 50 µg/l) listed in Table 3.3.2.i– Part A is based on length gain, the most sensitive growth endpoint. No mortality was observed at the 50 µg/l treatment, while 50% mortality was observed at the 1000 µg/l treatment (no statistics reported for mortality). At the 50 µg/l treatment ($LOEC_e$) cellulase activity was significantly ($p<0.05$) reduced in the field study, but not in the laboratory study (further results of the laboratory study not included in the table of this Annex).

[3] Farris et al. (1994): single-species tests wih *Corbicula sp.* and *Mudalia dilatata*

Field-collected *Corbicula sp.* and *M. dilatata* were exposed to to zinc in field-located artificial streams with natural New river water and coarse sediment. The clams and snails were collected rom New river. Four tests were performed, in the spring, summer and fall of 1984 and the spring of 1985, respectively, at the same test concentrations (range 25-1000 µg/l) as used by Belanger et al. (1986), see also [1] – [4] above. Three replicates were used in all but one test which had no replicates). Three further test were performed in laboratory-located artificial streams containing dechlorinated tap water. In the laboratory study, algae (previously unexposed to Zn) were added for feeding of *Corbicula*. During acclimatization (10-14 days), precolonized rocks from the New River provided substrate and food for *Mudalia* grazing.

All exposures of *Corbicula sp.* and *Mudalia dilatata* to 50 µg/l ($LOEC_{e)}$ in spring, summer and fall of 1984, respectively, resulted in significantly ($p<0.05$) decreased cellulolytic activity (i.e. cellulase activity, including endocellulase and exocellulase activity), as well as all exposures to higher Zn concentrations. In the test that was performed in the spring of 1985, with nominal test concentrations of 0-25-100 µg/l (measured: $\leq$20- $\leq$20-70 µg/l), no adverse effect on on cellulolytic activity was observed at 25 µg/l ($NOEC_e$).

[4] Genter et al. (1987): multiple-species tests with algal-periphyton

Tests in field-located artificial streams with New River water. Three test were performed, in the spring, summer and fall of 1984, respectively, at the same test concentrations (range 25-1000 µg/l) as used by Belanger et al. (1986),see also [1] to [4] above. Three replicates were used per treatment. In each stream two side-by-side screen chambers were placed, one of which contained 80-120 snails to examine periphyton response to snail grazing. Samples were analyzed for algae and diatom composition, as wel as biovolume density per taxon.

Significant changes ($p<0.05$) in species composition (from diatoms to green or blue algae) compared to the control occurred in all three seasons at the 50 µg/l treatment (LOEC), the lowest Zn concentration tested. In order to derive a NOEC from the LOEC, data on quantitative differences in biovolume density are required; these were only given in Fig. 2 in the publication, thus no reliable NOEC or EC10 can be derived. In the fall 1984 test, the actual Zn concentration in the control was slighly higher than that in the 50 µg/l treatment (94 versus 87 µg/l).

[5] Marshall et al. (1983): multiple-species test with algae, cladocerans, copepoda and rotifera

In situ tests, performed in Lake Michigan. Zinc test compound: not reported. The pH and hardness of Lake Michigan water were not reported. Based on the reported US EPA standard for Cd in freshwater. i.e. around 0.88 µg/l at Lake Michigans total hardness (Muhlbaier & Tisue, 1981), and the equation for the US EPA Final Chronic Value for Cd in freshwater, $e^{(\{0.7852\ [\ln \text{hardness}]) - 3.49\}}$, the total hardness of Lake Michigan water is estimated to be around 70 mg/l (as $CaCO_3$). The background Zn concentration was estimated by Marshall et al. (1983) to be around 1 µg/l, based on a 'personal communication'.

Polyethylene carboys (18 L) were filled with lake water from 4-9 m depth and suspended in the lake at equal average depths of ~7 m for both the treatments and controls. In the 1st experiment, Zn concentrations of 30, 60 and 90 µg/l were tested (8 replicates) plus a control, in the 2nd experiment 15, 30, 60 and 90 µg/l plus a control (8 replicates) were tested. After 2 weeks, the carboys were collected and examined for: Zn concentration, dissolved oxygen, chlorophyll *a*, micronutrients (NO_3-N, NH_4-N, SiO_4-Si and PO_4-P), primary production, quantitative zooplankton analysis and dry weight of zooplankton. Zooplankton investigations resulted in population density countings for 5 cladoceran species, 10 copepod species and 13 rotifer species. The 15 µg/l treatment (the lowest nominal Zn concentration tested) can be identified as the LOEC. In this 15 µg/l Zn treatment (actual Zn concentration: 17 µg/l) a significant ($p<0.05$) reduction relative to the control was observed for various parameters. An effect of >50% decrease relative to the control was observed for: oxygen content, primary production and chlorphyll *a*. A 50% decrease in population density relative to the control was observed for one cladoceran species (*Holopedium gibberum*), two copepod species in nauplii stages (Calanoid nauplii and Cyclopoid nauplii) and four rotatorian species (*Gastropus stylifer*, *Polyarthra vulgaris*, *Conochilis unicornis* and *Collotheca mutabilis*). In conclusion, the LOEC is 17 µg/l (actual concentration) with >50% effect for several parameters. A reliable NOEC or EC10 can not be derived.

Note that Emans et al. (1993) derived a NOEC (MS) of 1.7 µg/l (actual concentration) from this study, estimated from the LOEC (17 µg/l; actual concentration) using an assessment factor of 10 (NOEC = LOEC/10). Based on the RAR criteria, this NOEC is considered to be unreliable (high assessment of 10 and NOEC extrapololated far below the lowest test concentration).

Additional field studies: Nosov et al. (1981) and Williams & Mount (1965)

Not included in Table 3.3.2.i - Part A, as no useful results can be derived from these studies, due to the high zinc concentrations used in the tests (both studies) and the high backgrond zinc concentration in the control (Williams & Mount, 1965).

Nosov et al. (1981)

The effect of zinc on the community structure of natural phytoplankton populations (green and blue-green algae, diatoms and other algal species) was studied in 3-week tests in tanks with natural water. At the lowest concentration tested (100 µg/l, added as zinc chloride) the growth of some species was stimulated while that of other species was delayed or inhibited.

The results show species-dependent differences in sensitivity, resulting in changes in community structure. The growth of all groups of algal species was reduced at 1000 µg/l (Nosov, 1981).

Williams and Mount (1965)

Periphytic communities were grown on glass slides in four artificial outdoor streams. The amount of algal species was decreased with increasing Zn concentration (130 (control), 1100, 2800 and 6500 µg Zn/l) compared to the control. The percentage of decomposers and producers increased with increasing Zn concentration.

Changes in community structure were also observed in a 14-week study in which periphytic communities (bacteria, fungi, algae and ciliate protozoa) were collected at 2-week intervals from 4 outdoor channels supplied with running pond water containing average zinc concentrations of 130 (control), 1100, 2800 and 6500 µg/l.

The diversity of primary producers (algal species) decreased and the percentage of decomposers and consumers increased in all three treated streams; this effect increased with increasing zinc concentration. Hardness of the pond water averaged 170 mg/l (as $CaCO_3$); the pH ranged from 7 to 9, depending on the test concentration (Williams and Mount, 1965).

Table 3.3.2.i – Part B: Laboratory studies: NOEC and LOEC values

Taxa / organisms	Test comp.	Test system and water characteristics	Exp. time	Criterion	Result (µg Zn/l)	
Periphyton	**$ZnSO_4$**	**Polyethylene tanks containing 7.5 L dechlorinated tap water (pH 7.8; hardness 74 mg/l) and peryphyton from Pandapas pond (pH 7.1; hardness 13 mg/l) Flow-through system**	**21-d**	**LOEC (MS) **** (primary production and community respiration) NOEC (MS) (species richness of protozoans) LOEC (MS) (species richness of protozoans)	**50 Cn (actual: 73) **** 50 Cn (actual: 73) *(= overall MS NOEC according to Versteeg et al., 1999)* 225 Cn (actual: 172) Niederlehner & Cairns, 1993 [6]	**Dissolved Zn** (acid-labile fraction)
Microbial communities	**$ZnSO_4$**	**Polyethylene tanks containing 7.5 L dechlorinated tap water (pH 8.0; hardness 65 mg/l) and microbial communities from Pandapas pond (pH 7.1; hardness 13 mg/l) Flow-through system**	**28-d**	**LOEC (MS)` **** (total P, total biomass and and DO) NOEC (algal biomass) NOEC (MS) (species richnes of protozoans)	**3 Cn (actual: 4.2) **** 3 Cn (actual: 4.2) 10 (actual: 11)) *(= overall MS NOEC accordingt to Versteeg et al., 1999)*	**Dissolved Zn** (acid-labile fraction)
					Pratt et al., 1987 [7]	
Periphyton	**$ZnCl_2$**	**Glass aquaria containing water and periphyton from river Göta Älv (pH 6.1-7.1; hardness around 24 mg/l i.e. soft water) Flow-through system**	**28-d**	**NOEC (MS)**	**7.8 (bacterial activity)** 9.8 (total biomass) 11 (photosynthesis) 27 (algal biomass: chlorophyll *a)* 27 (species richness) 117 (species composition)	**Total Zn** (Actual concen-tration)

					Paulsson et al., 2000a [8]	
Phytoplankton	**$ZnCl_2$**	**Samples of water and phytoplankton from Lake of Alpnach (pH 7.6-8.7; hardness 280-340 mg/l) Static system**	**1-d**	**NOEC (MS) LOEC (MS)** (photosyn-thesis)	**14 Cn 27 Cn**	**Total Zn** (Nominal concen-tration).
		Samples of water and phytoplankton from Lake of Lucerne (pH 7.6-8.7; hardness 170-220 mg/l) Static system		**NOEC (MS) LOEC (MS)** (photosyn-thesis)	**11 Cn 21 Cn** Gächter, 1976 [9]	

** LOEC = lowest concentration used in the test; A NOEC can not be derived. .

NOEC (MS) and :LOEC (SS) = multiple-species NOEC and LOEC

Hardness (mg/l): total (Ca + Mg) hardness, as mg $CaCO_3$

For footnotes Table 3.3.2.i – Part B, see next pages

[6] Niederlehner and Cairns (1993): multiple-species test with periphyton
Field-collected periphyton communities grown on polyurethane-foam (PF) artificial substrates in Pandapas pond (Virgina, United States; pH 7.1; hardness 13 mg/l; TOC content 5 mg/l; background Zn concentration 13 µg/l) were used as species sources (epicencenters) in the laboratory in tanks with dechlorinated tap water (pH 7.8; hardness 74; TOC content 0.4 mg/l; background Zn concentration 1.3 µg/l, based on previous determinations). In the tanks barren PF substrates were placed. Three tanks were set up at each of the three treatments (nominal added-Zn concentrations: 0-50-225 µg/l). The test water was refreshed continuously (volume replacement in 2.4 d). At the LOEC (50 µg/l nominal; actual dissolved-Zn concentration: 73 µg/l, the lowest Zn concentration tested), a significant ($p<0.05$) reduction in gross primary production (GPP, as mg O_2 per liter substrate contents per 12 h: around 50% inhibition) and community respiration (CR, as mg O_2 per liter of substate contents per 24 h: around 60% inhibition) was observed compared to the control. A reliable NOEC or EC10 can not be derived for these effects. Species richness of protozoan communities was not significantly different from the control at this Zn treatment, but was significant impaired at 225 µg/l (actual dissolved-Zn concentration: 172 µg/l). The dissolved-Zn concentration measured in the controls was below the detection limit of 25 µg/l (actually, the dissolved-Zn concentration will have been around 1.3 µg/l, see above).

The actual Zn concentrations were measured in the beginning of colonization, after treatment of the water samples with nitric acid to 0.25%. Based on the data on the zinc measurements reported by Pratt, Niederlehner, Bowers and Cairns (Pratt et al., 1987, see footnote [7]), it is assumed that the Zn analyses were performed after filtering of the acidified water samples through a 0.45 µm membrane filter, thus the measured concentrations are assumed to be for acid-labile dissolved-Zn[43].

Note that Versteeg et al. (1999) derived a NOEC (MS) of 73 µg/l (actual concentration) from this studuy, based on the results for species richness.

[7] Pratt et al. (1987): multiple-species test with microbial communities

Field-collected periphyton communities grown on polyurethane-foam (PF) artificial substrates in Pandapas pond (Virgina, United States; pH 7.1; hardness 13 mg/l; TOC content 5 mg/l;

[43] The U.S. EPA defines acid-soluble metal as the metal fraction that passes through a 0.45 µm membrane filter after the sample is acidified to pH 1.5 to 2.0 with nitric acid. The U.S. EPA prefers to express Water Quality Criteria for metals as acid-soluble metal, to take into account the bioavailable, see for example the Ambient Aquatic Life Water Quality Criteria Document for Zinc (U.S. EPA, 1987).

background Zn concentration 13 µg/l) were used as species sources (epicencenters) in the laboratory in tanks with dechlorinated tap water (pH 8,0; hardness hardness 65 mg./l); TOC content 0.4 mg/l; background Zn concentration 1.3 µg/l, based on previous determinations). The above data on Pandapas water and the data on TOC and background Zn concentration are from Niederlehner and Cairns (1993), see footnote [6]. In the tanks barren PF substrates were placed. Three tanks were set up at each of the six treatments (nominal added-Zn concentrations: 0-3-10-30-100-300 µg/l). The mesasured dissolved-Zn concentrations (mean values) were <2.0-4.2-11-30-89-280 µg/l. The test water was refreshed continuously (volume replacement in 2.4 d).

Concentration-related effects were found for both biomass-related endpoints and protozoan species richness.

Total phosphate, total biomass (dry weight) and dissolved oxygen (DO) content were all three significantly ($p<0.05$) decreased at the nominal Zn concentration of 3 µg/l (LOEC; actual dissolved-Zn concentration: 4.2 µg/l, the lowest Zn concentration tested). No reliable NOEC or EC10 can be derived for these effects. At the nominal Zn concentration of 10 µg/l (actual dissolved-Zn concentration: 11 µg/l), algal biomass was significantly reduced. After 21-d of exposure, species richness of protozoans was reduced about 10% at 10 µg/l (NOEC species richness) and >10 % at 30 µg/l (LOEC species richness). No statistics reported for species richness (only a graphical picture of the total number of protozoan species was given. After 7-d and 14-d of exposure, species richness was somewhat more reduced than after 21-d of exposure, thus the NOEC for species richness may be 3 µg/l rather than 10 µg/l (nomimal concentration).

The actual Zn concentrations were measured after the water samples were acidified to pH <2 and subsequently filtered through a 0.45 µm membrane filter, thus the measured concentrations are assumed to be for acid-labile dissolved-Zn.

Note that Versteeg et al. (1999) derived a NOEC (MS) of 10 µg/l (nominal concentration) from this study, based on the results for species richness.

[8] Paulsson et al. (2000a): multiple-species test with periphyton

River Göta Älv water (general characteristics: pH 7.1, total Ca concentration 0.194 mM (8 mg/l), P-PO_4 concentration 0.2 µM (19 µg/l), DOC concentration 0.33 mM, background total-Zn concentration 0.4 µM (26 µg/l, from Paulsson, 2000) and its contents of indigenousa microbiota was continuously taken from the river and pumped to 22 L glass aquaria in the laboratory. Before reaching the aquaria, the water was sieved through a nylon net with a mesh size of 1 mm to prevent larger organisms from entering. A flow distributor maintained a water flow of 221 ml/minute, giving a mean residence time of 99 minutes. A total of 12 aquaria (8 zinc treatments at nominal concentrations of 3-9-21-51-130-325-845-2080 µg/l and 4 controls) were used in the 4-w test. The actual total-Zn concentrations in the water during the test were 5.9 µg/l in the controls and 7.2-9.1-18-40-98-234-630-1625 µg/l in the Zn treatments, being the mean values of 3 weekly measurements in the zinc treatments (after 2, 3, and 4 weeks, respectively) and 12 measurements in the controls. The pH of the water during the test was 6.1-7.1 and the water temperature was 12-15°C. During the test, the algae and bacteria in the incoming water settled on glass discs. After 4-w exposure the following endpoints were measured: total biomass dry weight, algal biomass (measured as chlorphyll *a* , ^{3}H-thymidine incorporation (bacterial acitivity), photosynthesis and species composition. The no-effect levels were determined by the study authors as follows. The mean of control activity with 95% confidence intervals was calculated, giving a no-effect 'baseline'. A linear regression line with 95% confidence limits was fitted through the effect data (log actual total-Zn concentration versus effect parameter). The intercept of these two lines is considered to be

the NOEC. The intercept of the 95% confidence limits of no-effect baseline and dose-effect relationship provide a 95% confidence range for the NOEC values.The NOEC values and their respective ranges are: bacterial activity: 7.8 (0.72-29) µg/l, total biomass dry weight 9.8 (4.1-18) µg/l, photosynthesis: 11 (4.8-20) µg/l, algal biomass (chlorophyll *a*): 27 (12-45) µg/l, species richness: 27 (16-41) µg/l and species composition: 117 (72-163) µg/l.

Zn analyses

The actual total-Zn concentrations were measured after treatment of the water samples with 0.5% HNO_3 and 0.5% H_2O_2, followed by a 3-h digestion in a Metrohm UV digester of 550 W).

Note: The Thesis by Paulsson (2000) includes further data on water characteristics of river Göta Älv, including data on chx-Zn, volt-Zn and cc-Zn concentrations, in addition to the background total-Zn concentration).

Hardness

The total hardness of around 24 mg/l (as $CaCO_3$) was not reported by Paulsson et al. (2000a), but in the Thesis by Paulsson (2000), a total-Ca concentration of 0.194 mM (being 8 mg/l) is reported for river Göta Älv. Based on data on the ratio of calcium and magnesium in European waters, the Ca/Mg ratio at the reported low Ca concentration is assumed to be 7:1, resulting in a Mg concentration of 1.1 mg/l (0.045 mM) and a total hardness of around 0.24 mM, being 24 mg/l (as $CaCO_3$).

Further results

The study by Paulsson et al (2000a) shows effects on biomass-related enpoints at total-Zn concentrations of around 10 µg/l. However, the PICT response, (measured by the assimilation ratio, i.e. C incorporated per hour in bacteria and chlorophyll a in algae) was not affected untill exposure to 630 µg/l. According to Paulsson et al. (2000a) this indicates that there was no direct zinc effect on photosynthesis below this concentration, which is consistent with the increase in community tolerance (PICT) to zinc for both bacteria and algae between 630 and 1600 µg/l. The study authors hypothise that this discrepancy in effect concentrations between biomass-related eindpoints and other, structure-related eindpoints (including (PICT) is due to an interaction between zinc and phosphorus leading to nutrient depletion and a concomitant decrease in biomass.

To further study the effects of zinc in phosphorus limited (12-15 µg/l) river Göta Älv, Paulsson et al. (2000b) performed a second microcosm experiment in river Göta Älv water, following the test design of Paulsson et al. (2000a). The results of the second experiment support the hypothesis of a zinc-induced phosphorus deficiency. There also appears to be an interaction between zinc and nitrogen and carbon. Paulsson et al. (2000b) concluded that zinc might be an environmental hazard in phosphorus-limited environments at concentrations above 0.1-0.2 µM of total zink (6.5-13 µg/l). The results of this second experiment are not included in Table 3.3.2.i- Part B.

[9] Gächter (1976): mutiple-species test with phytoplankton

Field-collected water plus phytoplankton samples from two pre-alpin lakes (the eutrophe Lake of Alpanach and the mesotrophe Lake of Lucerne) were collected in monthly intervals during a 1-year period. Characteristics of the Lake of Alpnach: pH 7.6-8.7, Ca concentration 2.8-3.4 *mval*/l, alkalinity 2.3-2.8 *mval/l*, DOC content 1.6-2.3 mg C/l, background zinc concentration 1.3-3.9 µg/l. Characteristics of the Lake of Lucerne: pH 7.6-8.7, Ca concentration 1.7-2.2 *mval/l*, alkalinity 1.6-1.9 *mval/l*, DOC content 0.7-1.9 mg C/l, background zinc concentration

1.0-3.3 µg/l. The hardness values listed in Table 3.3.3.1 – Part B are calculated from the Ca concentrations, assuming that the reported unit *mval/l* is equal to mMol Ca *(+ Mg?)*.

In the laboratory, subsamples of the phytoplankton were exposed for 24 hours to nominal Zn concentrations of 0-33-65-130-325-650-1300 µg/l (actual concentrations not determined), in the presence of ^{14}C-bicarbonate, after which the photosynthese was measured. The results listed in Table 3.3.2.i – Part B are based on the combined results of all measurements (12 in Lake Alpnach and 11 in Lake Lucerne, from February to December).

According to Gächter, a 20% reduction in phytoplankton photosynthesis could be analytically established with certainty. For the Lake of Alpnach, EC(20%) values ranged from 6.5 to 77 µg/l, resulting in an arithmetic and geometric mean EC(20%) of 36 and 27 µg/l, respectively. The latter value is considered to be the LOEC for this lake and the NOEC (14 µg/l) has been estimated from the LOEC using an assessment factor of 2. For the Lake of Lucerne, EC(20%) values ranged from 6.5 to 97 µg/l, resulting in an arithmetic and geometric mean EC(20%) of 33 and 21 µg/l, respectively. The latter value is considered to be the LOEC for this lake and the NOEC (11 µg/l) has been estimated from the LOEC using assessment factor of 2. On the average, the test concentration of 130 µg/l resulted in around 50% inhibition of photosynthesis in the Lake of Alpnach and 60% inhibition of photosynthesis in the Lake of Lucerne.

Note that Emans et al. (1993) derived a NOEC (MS) of 4.3 µg/l from this study. It is not clear where this value is based on. In the publication of Gächter (1976) it is stated that phytoplankton photosynthesis was not adversely affected if the concentration increase above the background levels did not exceed 5.10^{-8} mole Zn/l. which is 3.3 µg/l. It may be that Emans and al. (1993) considered this value to be NOEC and made a typing errror or added the lowest background zinc concentration (1.0 µg/l) to the value of 3.3 µg/l.

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ANNEX 3.3.2.C. DERIVATION OF SOFT WATER $PNEC_{add, aquatic}$

RIVM, August 18, 2003 – FINAL PROPOSAL
(TEST072-077_ENV_NL11, discussed and approved at TMIII/2003)

Evaluation of aquatic toxicity studies from the 'soft water testing programme' and application of the results for deriving a $PNEC_{add, aquatic}$ for soft waters

1. Background

The present paper includes the final proposal for a $PNEC_{add, aquatic}$ for soft waters. This paper builds on previous proposals (TEST072-077_env_NL6, TEST072-077_env_NL7 and TEST072-077_env_NL10) and comments from Member States and Industry (e.g. CTEST072-077_env_DK1, CTEST72-75_env_FIN1, CTEST72-75_env_FIN2, CTEST072-077_env_IND2, COM072-077_env_IND11, STK J.No. 914/03 (Letter from Norway of July 3, 2003), CTEST072-077_env_S2, COM072-077_env_S5, and e-mail of July 4 from the UK).

2. Evaluation of the aquatic toxicity studies and derivation of 'water effect ratios' (WER[44]s)

The studies with alga *Pseudokirchneriella subcapitata,* daphnid *Daphnia longispina*, and brown trout *Salmo trutta,* reported in Muyssen et al. (2003) and Källqvist et al. (2003) have been evaluated by the Rapporteur. The toxicity of zinc to each of the three species was tested in two natural soft waters, viz. Lake Maridalsvann (mean hardness 8 mg $CaCO_3$/L) and Lake Sandungen (mean hardness 6 mg $CaCO_3$/L). Testing was also done in the same two waters adjusted to a medium hardness of 100 mg $CaCO_3$/L). Table 1 shows the water characteristics of the two natural soft waters.

44 In the USA the Environmental Protection Agency released a streamlined procedure for determining site-specific values for a Water-Effect Ratio (WER), a criteria adjustment factor accounting for the effect of site-specific water characteristics on pollutant bioavailability and toxicity to aquatic life (see the *1994 Interim Guidance on Determination and Use of Water-Effect Ratios for Metals* (EPA-823-B-94-001). In the USA the Water Effect Ratio is determined as the toxicity observed in the Site water LC (Lethal Concentration) ÷ Lab water LC. In the present study the water from the lakes are taken as the "Site waters". It was recognised that no lab water could be found that could act as a generic European surface water. Therefore, the "Site waters" were adjusted to a hardness of 100 mg/L $CaCO_3$ to mimic a generic European surface water, and are thus used as "Lab waters".

Table 1. Chemical characterisation of sampling sites (December 2002) (from Muyssen et al., 2003)

Sampling date		**02.12.2002**		**12.12.2002**	
Location		**L. Maridalsvann**	**L. St. Sandungen**	**L. Maridalsvann**	**L. St. Sandungen**
pH		6.59	6.22	6.72	6.54
Conductivity	mS/m	2.47	1.88	2.62	1.96
Alkalinity	mmol/L	0.104	0.093	0.11	0.094
Tot. N	µg/L	385	245	415	285
NO_3	µg N/L	210	115	205	125
TOC	mg/L	3.9	4	3.9	4.1
Cl	mg/L	1.7	0.93	1.77	1
SO_4	mg/L	2.86	2.38	2.91	2.39
Al-reactive	µg/L	33	39	31	37
Al	µg/L	30	36	25	35
Ca	mg/L	2.57	1.93	2.49	1.91
K	mg/L	0.33	0.28	0.37	0.31
Mg	mg/L	0.424	0.317	0.413	0.317
Na	mg/L	1.49	1.02	1.57	1.08
Zn	µg/L	11	7.5	7.9	8.6

Based on the results of the aquatic toxicity tests as described and summarised in Annex 1, a 'water effect ratio' (WER), defined as the NOEC (or LOEC) derived from the test performed in the medium hardness water divided by the NOEC (or LOEC) derived in the original soft water, has been calculated for each test. From these WERs, arithmetic and geometric mean WERs were calculated, as follows:

i) For each species: mean value of the 2 WERs for the 2 test waters (either based on NOECs or LOECs).
ii) For each test water: mean value of the 3 WERs for the 3 species (either based on NOECs or LOECs).
iii) For the combined WERs: mean values of the total of 6 WERs (either based on NOECs or LOECs).

The results of these calculations are listed in Table 2.

Table 2. 'Water Effect Ratios' (WERs)

	WER based on NOEC	**WER based on LOEC**
P. subcapitata		
Maridalsvann	1.6	1.6
Sandungen	1.1	1.0
Arithmetic mean (n =2)	*1.4*	*1.3*
Geometric mean (n = 2)	*1.3*	*1.3*
D. longispina		
Maridalsvann	2.2	2.2
Sandungen	4.4	4.4
Arithmetic mean (n = 2)	*3.3*	*3.3*
Geometric mean (n = 2)	*3.1*	*3.1*
S. trutta (*)		
Maridalsvann	1.1	1.0
Sandungen	4.5	4.7
Arithmetic mean (n = 2)	*2.8*	*2.9*
Geometric mean (n =2)	*2.2*	*2.2*
Maridalsvann		
Arithmetic mean (n = 3)	1.6	1.6
Geometric mean (n = 3)	1.6	1.5
Sandungen		
Arithmetic mean (n =3)	*3.3*	*3.4*
Geometric mean (n= 3)	*2.8*	*2.7*
All Tests		
Arithmetic mean (n = 6)	2.5	2.5
Geometric mean (n = 6)	2.1	2.0

(*) Based on hatching time, the most sensitive endpoint for S. trutta.

Application of the results for deriving a $PNEC_{add, aquatic}$ for soft waters

As indicated in TEST072-077_env_NL7 and discussed in a subgroup meeting during TM II '03 (Norway, Finland, Sweden, Belgium, Industry and Rapporteur), the soft-water PNECadd,

aquatic will be derived from the generic PNECadd, aquatic by dividing the generic PNECadd, aquatic by a 'water effect ratio' (WER), as follows[45]:

soft-water $PNEC_{add, aquatic}$ = generic $PNEC_{add, aquatic}$ / WER

3.1. Choice of WER

The Rapporteur proposes to use the arithmetic mean WER of 2.5, calculated from the 6 available tests (3 species and 2 test waters), based on the following considerations:

i) The use of a mean WER based on all available tests is in conformity with the use of all available 'species mean' NOECs for generic $PNEC_{add, aquatic}$ derivation.
ii) Based on the low number of tests and the dependency of the NOEC and LOEC values (and thus the resulting WERs) of the separation factor between the concentrations tested, the use of the arithmetic mean WER is considered to be more appropriate than the use of the geometric mean WER.
iii) The generic $PNEC_{add, aquatic}$ is based on tests in a variety of test waters, including test waters with a relatively low hardness (starting with a hardness of 24 mg $CaCO_3$/L. Thus the use of the highest WER (4.7) is considered to be too conservative.

3.2. Soft water $PNEC_{add, aquatic}$ (provisional value)

The use of the arithmetic mean WER of 2.5 and the generic $PNEC_{add, aquatic}$ of 7.8 µg/L (final proposal August 2003) results in a soft water $PNEC_{add, aquatic}$ of 3.1 µg/L. Note that the values are for dissolved zinc.

When the standard assessment factor approach would be used on the results of the soft water testing programme in natural waters (Annex 1), this would result in a soft water $PNEC_{add, aquatic}$ of 4.2 µg/L (based on the lowest NOEC of 42 µg/L, for daphnid *Daphnia longispina* and an assessment factor of 10). This indicates that the use of the arithmetic mean WER of 2.5 on the generic $PNEC_{add, aquatic}$ is not likely to underestimate the toxicity in low hardness natural waters[46].

Application of the soft water $PNEC_{add, aquatic}$ in the risk characterisation

As already indicated in TEST072-077_env_NL7, the Rapporteur proposes to use the soft water $PNEC_{add, aquatic}$ for all soft freshwaters with a hardness below 24 mg $CaCO_3$/L (the minimum value for hardness used in the RAR Zn Metal for the selection of NOEC values for $PNEC_{add, aquatic}$ derivation), including waters from other parts of Europe than the Nordic Countries.

In case no information on the hardness of a freshwater is available, expert judgement (e.g. on expected water characteristics of the geographical location) will be used to estimate whether the hardness of that water is $\geq$24 mg $CaCO_3$/L [where the EU generic $PNEC_{add, aquatic}$ will be used] or <24 mg $CaCO_3$/L [where the soft water $PNEC_{add, aquatic}$ will be used]. Regarding the above it is emphasised that in case data on hardness are lacking, this does not mean that the soft water $PNEC_{add, aquatic}$ will be used as a default in the risk characterisation; the use of the

[45] The application of the WER on the generic PNECadd, aquatic intrinsically assumes that the justification for a safety factor of 2, as applied on the generic HC5 to get the PNECadd, aquatic, also applies to the PNECadd, aquatic for soft waters. See also footnote 1, where the present WER-approach is explained.

46 The standard assessment factor approach was used earlier in a discussion paper prepared by the rapporteur, resulting in a preliminary soft water $PNEC_{add, aquatic}$ of 1.4 µg/L, derived from the lowest 'species mean' NOEC of 14 µg/L for alga *Pseudokirchneriella subcapitata*, based on the results of 5 test performed in artificial test waters with a hardness up to 24 mg $CaCO_3$/L (Sijm & Janus, 2002).

generic $PNEC_{add,\ aquatic}$ remains the starting point of the risk assessment. Thus, the soft water $PNEC_{add,\ aquatic}$ will only be used in case there are sufficient indications that the water is most likely a soft water.

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ANNEX 3.3.2.C – APPENDIX.

EVALUATION OF THE 'SOFT WATER TESTING PROGRAMME'

Evaluation of Final report "Ecotoxicity of zinc to algae and daphnids tested in natural soft surface water" April 2003 by Muyssen, Bossuyt and Janssen of Ghent University, Laboratory of Environmental Toxicology and Aquatic Ecology and of final report "Effect of zinc on the early life stages of brown trout (Salmo trutta) at different levels of water hardness" May 2003 by Källqvist, Rosseland, Hytterød and Kristiansen of the Norwegian Institute for Water Research.

Algae

Pseudokirchneriella subcapitata was gradually acclimated from standard ISO medium to ISO medium with adjusted hardness of 5 mg/l and of 100 mg/l over a period of ten weeks. After the acclimation period, toxicity tests were performed according to OECD-guideline no. 201. Algae acclimated to ISO-medium with hardness 5 mg/l (as CaCO3) were tested in two natural soft waters from Lake Maridalsvann and from Lake Sandungen, respectively, and algae acclimated to ISO-medium with hardness 100 mg/l were tested in the same two natural waters but with hardness adjusted to 100 mg/l. Six concentrations in a logarithmic series and a control were tested for each medium. Three replicates per concentration were tested and the control was replicated 6 times. Growth rates (μ) were statistically evaluated in each test by one way ANOVA followed by Dunnett's test (significance level: $p < 0.05$).

Growth rates in natural soft waters and natural waters with adjusted hardness to 100 mg/l were similar. NOEC- and LOEC-values were higher in the high hardness waters compared to the natural soft waters. NOEC- and LOEC-values are given in the table below. It should be noticed that for Lake Maridalsvann natural water p-values originating from the Dunnett's test for 50 μg/l zinc but especially 38 μg/l zinc, concentrations that are a factor 2-3 lower than the LOEC set at present, approached 0.05 very near (Table A, Muyssen et al. 2003). For Lake Sandungen natural water a p-value of 0.052 was found for 86 μg/l zinc (Table B; Muyssen et al. 2003) and for Lake Maridalsvann water with adjusted hardness a p-value of 0.066 was found for 81 μg/l zinc (Table C; Muyssen et al. 2003). One should be aware during evaluation of ratios of soft and hard water NOEC- (or LOEC-) values that only a slight change in growth rates may change differences between treatments from just not significant into significant. This may explain the relatively low ratios between the results in hard and soft waters compared to those for daphnids and fish (see further below for the results for daphnids and fish).

	A	Test-type	Test-comp.	Test-water	pH	Hardness	Exp. time	Criterion	Result (µg Zn/l)
Pseudokirchneriella subcapitata	+	S	$ZnCl_2$	Maridalsvann natural low	6.7	8.0	72-h	$NOEC_r$ $LOEC_r$	50 99
				Sandungen natural low	6.4	6.1	72-h	$NOEC_r$ $LOEC_r$	86 164
				Maridalsvann H = 100 mg/l	6.7	100	72-h	$NOEC_r$ $LOEC_r$	81 160
				Sandungen H = 100 mg/l	6.4	100	72-h	$NOEC_r$ $LOEC_r$	93 161

Reference: Muyssen et al., 2003

Daphnids

Daphnia longispina was gradually acclimated from its natural water (hardness 20-40 mg/l, as $CaCO_3$) to the two natural soft waters from Lake Maridalsvann and from Lake Sandungen, respectively and to the natural soft waters with hardness adjusted to 100 mg/l, over a period of 7 weeks. After the acclimation period, acute and chronic tests were performed following OECD guidelines no. 202 and no 211, respectively. For the chronic test, five concentrations in a logarithmic series and a control were tested for each medium. Ten replicates per concentration were tested with one daphnid per replicate; each test was performed in duplicate.

Three times a week, age-specific survival (l_x) and reproduction (m_x) were recorded. The chronic test was terminated after 21 days and intrinsic rate of natural increase r_m and net reproduction R_0 were calculated. R_0-values were log10-transformed. The endpoints r_m and R_0 were statistically evaluated in each test by one way ANOVA followed by Dunnett's test (significance level: $p < 0.05$).

The NOEC- and LOEC-values were higher in the high hardness waters compared to the natural soft waters. Calculated NOEC- and LOEC-values are shown in the table below. It is noted that in all four tests with *Daphnia longispina*

survival was the most sensitive endpoint, i.e. up to the concentration where R_0 and r_m were zero due to mortality of the test organisms, no significant differences were found between the test concentrations and the control.

	A	Test-type	Test-comp.	Test-water	pH	Hardness	Exp. time	Criterion	Result (µg Zn/l)
Daphnia longispina	+	S	$ZnCl_2$	Maridalsvann natural low	6.7	8.0	21-d	$NOEC_{R0}$	42
								$LOEC_{R0}$	93
								$NOEC_{rm}$	42
								$LOEC_{rm}$	93
				Sandungen natural low	6.4	6.1	21-d	$NOEC_{R0}$	48
								$LOEC_{R0}$	94
								$NOEC_{rm}$	48
								$LOEC_{rm}$	94
				Maridalsvann H = 100 mg/l	6.7	100	21-d	$NOEC_{R0}$	91
								$LOEC_{R0}$	203
								$NOEC_{rm}$	91
								$LOEC_{rm}$	203
				Sandungen H = 100 mg/l	6.4	100	21-d	$NOEC_{R0}$	209
								$LOEC_{R0}$	412
								$NOEC_{rm}$	209
								$LOEC_{rm}$	412

Reference: Muyssen et al., 2003

Brown trout

Effect of water hardness on early life stages of brown trout (*Salmo trutta*) was tested following OECD guideline no. 210 (1992). The early life stage test was performed with two soft waters from Lake Maridalsvann and from Lake Sandungen, respectively, and with the same two natural waters but then with hardness adjusted to 100 mg/l (as $CaCO_3$). Five concentrations of zinc and one control were tested for each of the four dilution waters. At the start of the experiment eggs were mixed and dry-fertilised with milt. For each treatment, two groups of dry fertilised eggs were placed in separate chambers of a channel (l x w x d: 52 x 8 x 8 cm) of 1.66 l with water flow of 5 ml/min. The test was terminated after 116-119 days. The length of the exposure period was adjusted in order to obtain approximately the same degree-days (defined as the product of time (in days) and mean temperature (in ^0C)) in each treatment.At that time, most of the fry showed start-feeding behaviour. At test termination, all surviving fry was measured for length and weight.

The endpoint hatching was statistically evaluated in each test by the t-test; the larval growth data (length and weight) were statistically evaluated in each test by the Dunnett's test (significance level: $p < 0.05$).

Percentage of dead fertilised eggs showed minor effect of zinc exposure in Lake Sandungen in the two highest exposure concentrations of 100 and 250 µg/l. In Lake Marisdalsvann water adjusted to hardness 100 mg $CaCO_3$ also an increased percentage of lethality of fertilised eggs was observed at 500 and 1000 µg/l. Percentage of dead larvae among hatched larvae showed a rather irregular pattern. For instance, percentage of dead larvae among hatched larvae was 0, 5.3, 3.3, 1.7, 0 and 10.1% in the control, 50, 100, 250, 500 and 1000 µg/l, respectively. There seems to be no relationship with zinc exposure. Time to start of hatching and to the end of hatching showed the clearest trend with zinc exposure; time to hatching was prolonged by zinc exposure in all dilution waters in the highest exposure concentrations. Differences in length and weight of the larvae at the end of the exposure period were generally small. In Lake Sandungen length was significantly increased at 25 µg/l and significantly decreased at 100 and 250 µg/l. In Lake Maridalsvann an increase in length was found at 25 and 50 µg/l and no significant decrease in any of the experimental units. In the highest concentration of the 2 dilution waters with adjusted hardness length was significantly decreased compared to the control. Larval weight had only significantly decreased compared to the control in the highest zinc concentration of the two natural soft waters.

For the derivation of NOEC- and LOEC-values, increases in weight or length were not considered. The NOEC- and LOEC values listed in the table below are based on the most sensitive endpoint hatching,

	A	Test-type	Test-comp.	Test-water	pH	Hardness	Exp. time	Criterion	Result (µg Zn/l)
Salmo trutta	+	F	$ZnSO_4 \cdot 7 H_2O$	Maridalsvann natural low	6.7	8.6	116-119 days (659-675 degree-days) *	$NOEC_{hatching}$ $LOEC_{hatching}$	61 108
								$NOEC_{length}$	-
								$LOEC_{length}$	-
								$NOEC_{weight}$	-
								$LOEC_{weight}$	-
				Sandungen natural low	6.5	6.7	116-118 days (661-672 degree-days) *	$NOEC_{hatching}$ $LOEC_{hatching}$	56 106
								$NOEC_{length}$	56
								$LOEC_{length}$	106
								$NOEC_{weight}$	106
								$LOEC_{weight}$	253
				Maridalsvann H = 100 mg/l	6.7	100	116-118 days (660-671 degree-days) *	$NOEC_{hatching}$ $LOEC_{hatching}$	57 108
								$NOEC_{length}$	502
								$LOEC_{length}$	1003
								$NOEC_{weight}$	-[a]
								$LOEC_{weight}$	-[a]
				Sandungen H = 100 mg/l	6.5	100	116-119 days (662-671 degree-days) *	$NOEC_{hatching}$ $LOEC_{hatching}$	250 496
								$NOEC_{length}$	496
								$LOEC_{length}$	997
								$NOEC_{weight}$	496[a]
								$LOEC_{weight}$	997[a]

[a]NOEC- or LOEC-values not in accordance with Källqvist et al. 2003 Table 15 but in accordance with data presented in other tables and text.

* Degree-days is a widely used measurement of biological and especially embryonic development in poikilothermic animal like fish. It represents the product of time in days needed to reach a certain developmental stage (in this case: the larval stage) and the temperature in ^{0}C.

Reference: Källqvist et al., 2003

ANNEX 3.3.2.D. SEDIMENT TOXICITY DATA BASE

Table 3.3.2.e. Toxicity of zinc to freshwater benthic macroinvertebrates: NOEC values from single-species laboratory studies in spiked-sediment – water systems
Part I: Studies useful for $PNEC_{add, sediment}$ derivation
Part II: Studies not useful for $PNEC_{add, sediment}$ derivation

Table 3.3.2.f- Part A. Toxicity of zinc to freshwater benthic macroinvertebrates (single-species laboratory studies in spiked-sediment – water systems): NOEC and LOEC values related to SEM/AVS and SEM-AVS, and (SEM-AVS)/f_{oc} (Short-term and long-term studies, from Table 3.3.2.e)

Table 3.3.2.f- Part B. Chronic toxicity of cadmium to estuarine benthic macroinvertebrates (single-species laboratory studies in spiked-sediment – water systems): NOEC and LOEC values related to SEM/AVS and SEM-AVS, and (SEM-AVS)/f_{oc}

Table 3.3.2.f- Part C. Colonisation of zinc-spiked sediments by benthic macroinvertebrates (long-term field studies): NOEC and LOEC values related to SEM/AVS and SEM-AVS, and (SEM-AVS)/f_{oc}.

Table 3.3.2.f- Part D. Colonisation of cadmium-spiked and metal-spiked sediments by benthic macroinvertebrates (long-term field and laboratory studies): NOEC and LOEC values related to SEM/AVS and SEM-AVS, and (SEM-AVS)/f_{oc}.

Table 3.3.2.g. Toxicity of zinc to microbe-mediated processes in anaerobic freshwater sediments: NOEC, EC and IC values

Table 3.3.2.e. Toxicity of zinc to freshwater benthic macroinvertebrates: NOEC values from single-species laboratory studies in spiked-sediment – water systems

Part I: Studies useful for $PNEC_{add, sediment}$ derivation

(*See Table 3.3.3.f – Part A for additional data on these studies)*

Organism & life stage	A	Test-type	Test-comp.	Sediment	f_{oc}	Clay %	Temp. °C	Exp.-time	Criterion	Result (mg Zn/kg d.w.)
Oligochaetes										
Tubifex tubifex adults	+	S	$ZnCl_2$	pond sediment, background Zn 34 mg/kg d.w	0.01-0.02	-	23	4-w	$NOEC_r$ $NOEC_r$ $NOEC_s$ $NOEC_s$	1,135 (actual) 1,101 (act.-Cb) 2,610 (actual) 2,576 (act.-Cb)
									Farrar & Bridges, 2003 [4]	
Crustaceans										
Hyalella azteca 1-w old	+	R	$ZnCl_2$	stream sediment, background Zn 22 mg/kg d.w	0.02	8	23	6-w	$NOEC_s$ **$NOEC_s$** 4-w $NOEC_g$ $NOEC_g$ 6-w $NOEC_r$ $NOEC_r$	510 (actual) **488 (actual-Cb)** ≥1,000 (actual) ≥978(actual-Cb) ≥1,000 (actual) ≥978 (actual-Cb)
									Nguyen et al., 2005 [9]	
Insects										
Chironomus tentans P (newly hatched larvae) → F_1 [lc]	+	R	$ZnCl_2$	lake sediment, background Zn 55 mg/kg d.w. ("SEM" zinc)	-	-	23	8-w	$NOEC_{s,g,e,r}$ $NOEC_{s,g,e,r}$	850 (actual) 795 (actual-Cb)
									Sibley et al. 1996 [1]	
Chironumus tentans <1-d old	+	R	$ZnCl_2$	pond sediment, background Zn 30 mg/kg d.w	0.01	-	23	3-w	$NOEC_g$ $NOEC_g$ $NOEC_s$ $NOEC_s$	639 (actual) 609 (act.-Cb) 2,420 (actual) 2,390 (act.-Cb)
									Farrar & Bridges 2002, 2003 [6]	

(Table 3.3.2.e: To be continued in Part II: Studies not useful for $PNEC_{add, sediment}$ derivation)

Table 3.3.2.e. Toxicity of zinc to freshwater benthic macroinvertebrates: NOEC values (*)

from single-species laboratory studies in spiked-sediment – water systems

Part II: Studies not useful for $PNEC_{add, sediment}$ derivation

*(See Table 3.3.3.f – Part A for additional data on most of these studies **)*

Organism & life stage	A	Test-type	Test-comp.	Sediment	f_{oc} %	Clay %	Temp. °C	Exp.-time	Criterion	Result (mg Zn/kg d.w.)
Crustaceans										
Hyalella azteca 1-w old	+	R	$ZnCl_2$	pond sediment, background Zn 31 mg/kg d.w	0.02	-	23	4-w	$LOEC_g$ $LOEC_g$ $NOEC_s$ $NOEC_s$ Farrar & Bridges, 2001, 2002, 2003 [5] Not useful: Q	252 (actual) 221 (actual-Cb) 936 (actual) 905 (actual-Cb)
Hyalella azteca 1-2 w old	+	R	$ZnCl_2$	lake sediment, background Zn 48 mg/kg d.w	0.01-		23	10-d	$NOEC_{s,g}$ $NOEC_{s,g}$ Farrar & Bridges, 2002 [7] Not useful: Q	229 (actual) 181(actual-Cb)
Hyalella azteca < 1 w old	+	S	$ZnCl_2$	harbour sediment, background Zn 1,500 mg/kg d.w.	0.05-		25	4-w	$NOEC_s$ $NOEC_s$ $NOEC_g$ $NOEC_g$ Borgmann & Norwood '97 [3] Not useful: R	2,700 (actual) 1,200(act.-Cb) ≥4,600 (actual) ≥3,100(act.-Cb)
Hyalella azteca 4-5 w old	+	S	$ZnCl_2$	harbour sediment, background Zn 1,500 mg/kg d.w.	0.05-		25	7-d	$NOEC_s$ $NOEC_s$ Borgmann & Norwood '97 [3] Not useful: R, Q	4,600 (actual) 3,100 (act.-Cb)
Hyalella azteca -	+	R	$ZnCl_2$	pond sediment, background Zn 25 mg/kg d.w. ("SEM" zinc)	0.116	-		10-d	$NOEC_s$ $NOEC_s$ Liber et al. 1996 [2] Not useful: Q	≥750 (actual) ≥725 (actual-Cb)
Insects										
Chironumus tentans 2nd to 3th instar	+	R	$ZnCl_2$	lake sediment, background Zn 48 mg/kg d.w	0.01-		23	10-d	$NOEC_g$ $NOEC_g$ $NOEC_s$ $NOEC_s$ Farrar & Bridges 2002, [8] Not useful: Q	435 (actual) 387 (act.-Cb) 1,805 (actual) 1,757 (act.-Cb)
Chironomus tentans -	+	R	$ZnCl_2$	pond sediment, background Zn 25 mg/kg d.w. ("SEM" zinc)	0.116	-		10-d	$NOEC_{s,g}$ $NOEC_{s,g}$ Liber et al. 1996 [2] Not useful: Q	≥750 (actual) ≥725 (actual-Cb)

Toxicological endpoints: e = emergence; g = growth; r = reproduction ; s = survival

For footnotes: see next pages. For further information: see the "list of abbreviations Table 3.3.2.e to 3.3.2.g"

* NOEC values, except the LOEC for growth derived in the 4-w *Hyalella azteca* study by Farrar & Bridges (2001, 2002, 2003).

** The study by Borgmann & Norwood (1997) is not included in Table 3.3.3.f – Part A, as this study was performed in polluted harbour sediment with a very high 'background' Zn concentration of 1,500 mg/kg dw.

Footnotes Table 3.3.2.e

[1] Sibley et al. (1996): Chironomus tentans (8-w test)

Statistics: p = 0.05 Test method referring to Benoit et al.,1993, Benoit et al. 1997 and Sibley et al. 1997. Nowadays the test method used is implemented in EPA method 100.5: Life-cycle Test for Measuring the Effects of Sediment-associated Contaminants in *Chironomus tentans* (EPA/600/R-99/064, EPA, 2000). The test was conducted in a sediment-water intermittent renewal system using zinc-spiked lake sediment and overlying water that was renewed twice daily (at 12-h intervals, over a 1-h period , according to the data reported by Benoit et al., 1993 and Sibley et al., 1997). The amounts of sediment and water per 300 ml test beaker were 100 and 150 ml, respectively (sediment/water ratio: 1: 1.5). Life-cycle test with endpoints survival (larvae, pupae and adults), growth (dry weight of larvae and adults), adult emergence and reproduction (number of eggs per female and hatching success).

For the NOEC values in the (pore) water derived from this study, see Table 3.3.2.a in Annex 3.3.2.A.

Organisms and replicates: The test was started with newly hatched larvae. In the test, 144 animals from laboratory culture were used per treatment (12 replicates of 12 animal/beaker), of which 4 replicate "growth beakers" were used for the determination of 20-d larval survival and growth, 6 replicate "reproduction beakers" were used for determination of adult emergence and reproduction (egg counts and hatching success) and 2 replicate "chemistry beakers" were used for determinations of AVS, SEM and pore-water zinc at day 20. Emergence and reproduction were monitored until 10 days past the last recorded emergence in a given treatment. The collection of eggs and the determination of hatching success occurred in samples of the overlying water in a given treatment.

Toxicological endpoints: Survival, growth (dry weight), emergence and reproduction (number of eggs).

Sediment: Unpolluted West Bearskin Lake sediment (Minnesota). Characteristics: AVS concentration 3.9 mmol S/kg dry weight and SEM concentration 1.0 mmol/kg dry weight, of which 70% zinc (0.7 mmol/kg dry weight, corresponding to 45 mg Zn/kg d.w.) No data on the other metals present in the sediment (comprising 30% of the molar SEM concentration) and on general sediment characteristics such as the organic carbon content and texture.

Overlying water: Lake Superior water; this water was used for culturing and testing. Characteristics (reported by Biesinger & Christensen, 1972): pH 7.7, hardness 45 mg/l and background zinc concentration 0.8 µg/l.

Spiking and equilibrium: The sediment was spiked with $ZnCl_2$ solutions in deionised water. Stabilisation of the spiked sediments was determined by monitoring the concentration of zinc in the pore water over a 2-w period. During this time the sediments were shaken manually twice a day. After this time the sediments were prepared and introduced in the test system on the day prior to test initiating by adding the test organisms. The nominal test concentrations, expressed as SEM/AVS molar ratios, were 0.18 (control)-0.4-0.8-4-8-16.

Metal and AVS analyses during the test: "SEM"-zinc and AVS concentrations in sediment and zinc concentrations in pore water were determined at day 0 (start of test), day 20 (coincident with larval survival and growth measurements; samples taken from the two "chemistry beakers") and day 56 (end of test; samples taken from two of the "reproduction beakers"). The samples used for the day 0 measurements of sediment and pore-water were taken from the spiking containers; the samples used for the day 20 and day 56 measurements were taken from the 0-1 cm and 1-2 cm horizons of the sediment samples in the test beakers.

Pore-water dissolved-Zn concentrations were determined in 0.45 µm Millipore-filtered supernatants of centrifuged sediment samples.

Actual "SEM"-zinc concentrations in the 0-2 cm horizon in the sediment: 0.84 (control)-2.1-3.5-13-29-41 mmol/kg dry weight (arithmetic mean value of day 0, 20 and 56 measurements, which were very similar for a given treatment and also very similar for the 0-1 cm and 1-2 cm horizon), equal to 55 (control)-140-230-850-1,900-2,700 mg "SEM"-Zn/kg d.w.

Note: Metal concentrations in sediment were reported as SEM or SEM-zinc. It is assumed that only zinc was analysed in the exposure groups, because no other metals were mentioned specifically.

Actual AVS concentrations in the 0-2 cm horizon of the sediment: 5.2 (control)-4.8-5.1-7.1-6.8-6.3 mmol/kg dry weight (arithmetic mean value of day 20 and 56 measurements, which were very similar for a given treatment and also very similar for the 0-1 and 0-2 cm horizon). The day 0 measurements have been excluded from the calculations of the mean AVS concentrations, since the day 0 measurements were always lower than the day 20 and day 56 measurements (with a difference of a factor of 2-6). In the highest two exposure groups there appeared to be a further increase in AVS concentration between day 20 and 56, but the increase was small (on the average within 40%). These temporal increases were ascribed to (i) enhanced stability of zinc sulphide relative to that of iron sulphide (concurrent with a positive correlation between "SEM"-zinc and AVS), (ii) increased anaerobic conditions in the overlying water due to microbial decomposition of food, resulting in the formation of sulphide, and (iii) the degree of larval activity: the primary increase in AVS was observed in the highest two concentrations, at which only a few or no larvae survived. The absence of bioturbation in conjuction with the build up of food would have promoted a reducing environment and a subsequent increase in AVS.

Molar SEM/AVS ratios: 0. 2 (control)-0.4-0.7-1.8-2.8-6.5.

Actual dissolved-Zn concentrations in the pore water: 29 (sediment-water control)-31-56-166-4,200-10,000 µg/l (arithmetic mean value of day 20 and 56 measurements, which were usually similar for a given measurements and usually also similar for the 0-1 cm and 1-2 cm layer; each value represents the mean value of 4 measurements per exposure concentration). The concentrations in the pore water at a given treatment were much more variable (both in time and in the two layers) than those in sediments. At the highest three test concentrations, pore water measurements on day 0 showed zinc concentrations of 38,000, 480,000 and 950,000 µg/l, which are 1- to 3-orders of magnitude higher than the concentrations at day 20 and 56. According to the study authors, these very high concentrations on day 0 are probably due to non-equilibrium between zinc in sediment and water and thus not representative for the true exposure received by the organisms; therefore the results of 20-d and 56-d measurements were used for effect assessment.

Other analyses during the test: Dissolved oxygen (DO) levels and pH values in the overlying water were determined in all treatments twice a week throughout the test, but the results were not reported in detail. According to the study authors, DO levels in the overlying water declined steadily in all treatments up to the time of emergence (day 24), resulting in levels as low as 1.1 mg/l (but generally remained above 2.0 mg/l) in the treatments with SEM-Zn concentrations up to 850 mg/kg d.w. and as low as 0.5 mg/l in some replicates of the highest two treatments (SEM-Zn concentrations 1,900 and 2,700 mg/kg d.w). Following initiation of emergence, DO levels increased to 3-4 mg/l, but remained consistently low at the highest two concentrations. The low DO levels at the highest two concentrations are assumed to be related to the lack of bioturbation and the build of of food (because little or no larvae survived at these concentrations) rather than to the test system used.

Toxicity results: No significant effects on any of the endpoints were found up to the actual "SEM"-zinc concentration of 13 mmol/kg d.w. (850 mg "SEM"-Zn/kg d.w.); at this NOEC the SEM/AVS ratio was 1.8 and the SEM-AVS value was 5.9. Larval survival in the control and the lowest three test concentrations was ≥85 after 20 days and ≥75% after 56 days (determined by back calculation of mortality in larvae, pupae and adults). The actual "SEM"-zinc concentration of 29 mmol "SEM"-zinc/kg d.w. (1,900 mg "SEM"-Zn/kg d.w) resulted in 85% larval mortality and in reduced growth and no emergence of the surviving larvae); at this LOEC the SEM/AVS ratio was 4.3 and the SEM-AVS value was 22. .

Additional data: On request of the rapporteur, Sibley submitted additional data on this study, amongst others the raw data on dissolved oxygen (DO) levels measured in the overlying water during the test, as the low DO levels measured in the highest two Zn treatments may have affected the results of the study. From the total of 374 measurements of the DO level, 54 (14%) were belowc 1.5 mg/l and only 11 (3%) were below 1.0 mg/l. Values below 1.5 mg/l and 1.0 mg/l occurred 4 and 6 weeks after the start of the study (thus in the second part of the study) and all values below 1.0 mg/l were found in the highest two Zn treatments. At the beginning of the emergence period, most DO levels were between 3.0 and 4.0 mg/l, then dropping to levels that were generally between 1.0 and 3.0 mg/l. According to Sibley and the data in EPA-guideline 100.5, *C. tentans* is very tolerant to low DO levels in water and sediment and periodic depressions of DO levels at levels as low as 1.5 mg/l are not likely to result in adverse effects. Thus it is quite unlike that the low DO levels, which occurred primarily at the end of the study in the highest two Zn treatments, resulted or contributed to the adverse effects found at these treatments. Most likely, the low DO levels at the highest two Zn treatments were due to the lack of bioturbation due to the high larval mortality. Based on the data and because all validity criteria from EPA-guideline 100.5 with respect to control survival, growth, emergence and reproduction were met, the study and study result ($NOEC_{s,g,e,r}$ of 850 mg SEM-Zn/kg d.w.; actual concentration) are considered to be valid.

The pH values in the overlying water (Lake Superior water) during the test were usually near 7.5, with a total range of 6.5-7.8 and the hardness was around 40 mg/l (as $CaCO_3$).

[2] Liber et al. (1996): *Hyalella azteca* (10-d test) and *Chironomus tentans* (10-d test)

Statistics: p = 0.05. Test method referring to Benoit et al. 1993 and Ankley et al. 1993. The tests with *Hyalella azteca* endpoint: survival) and *Chironomus tentans* (endpoints: survival and growth) were performed in the framework of a 1-yr field study on the colonisation of zinc-spiked sediments by benthic macroinvertebrates (see Table 3.3.2.f- Part C). The laboratory tests were run for each of the five sampling dates used in the field study and were performed in intact sediment cores, either directly in the core tubes used to collect the sample from the field or (in some *H. azteca* tests) in 300 ml test beakers. The overlying water was renewed three to nine times per day. In each series of tests three different controls were included: a manipulated field control (CM: sediment handled as the zinc-spiked sediments), an unmanipulated field control (CU: sediment undisturbed until sampling for testing) and a laboratory control (CS: using silica sand for *C. tentans* and Lake West Bearskin sediment for *H. azteca*; see Sibley et al. 1996 (footnote [1]) for characteristics of Lake West Bearskin sediment). No data were reported on the life stage of the organisms tested.

Organisms and replicates per test: In each test, 30 *H. azteca* (3 replicates of 10 animals per core tube or beaker) or 18 *C. tentans* (3 replicates of 6 animals per core tube) were used per treatment. No data on the life stage of the animals.

Toxicological endpoints: *Hyalella azteca*: survival; *Chironomus tentans*: survival and growth

Sediment: Unpolluted pond sediment (Duluth, Minnesota). Characteristics (0-6 cm horizon): sand 59%, silt 35%, clay 6%, TOC 11%, moisture content 72%, AVS concentration 3.5 mmol/kg dry weight and SEM concentration 0.65 mmol/ kg dry weight, of which 60% is zinc (0.38 mmol/kg d.w., corresponding to 25 mg/kg d.w.).

Overlying water: Dechlorinated tap water originating from Lake Superior for *C. tentans* and filtered Lake Superior water for *H. azteca.* Characteristics for Lake Superior water (reported by Biesinger & Christensen, 1972): pH 7.7, hardness 45 mg/l and background zinc

concentration 0.8 µg/l.

Spiking and equilibrium: The sediment was spiked by adding zinc chloride in distilled water. Stabilisation of the spiked sediments was determined by monitoring the concentration of zinc in the pore water over a 9-day period. In this perid the sediments were homogenised daily. After this period the samples were transferred to test trays and placed in the test location in the pond. The nominal test concentrations, expressed as SEM, were 0.8 (control)-1.5-3-6-12 µmol/kg d.w.

Metal and AVS analyses during the test: SEM and AVS concentrations in sediment and zinc concentrations in pore water were determined on each of the five sampling dates. The sediment samples were taken from the 0-6 cm horizon of the intact cores (sampled under water, in situ) and divided into 2-cm sections. Pore-water samples, also collected in situ, were taken at sediment depths of 1, 3, and 5 cm.

Actual SEM concentrations in the 0-2 cm horizon of the sediment: 0.7 (control: CU and CM))-0.8-1.4-2.4-5.4-11.9 mmol/kg dry weight (arithmetic mean of the five measurements, which showed minimal temporal changes at a given treatment), corresponding to 0.4 (control)-0.5-1.1-2.1-5.2-11.6 mmol "SEM"-Zn /kg d.w., equal to 25 (control)-33-72-140-340-750 mg "SEM"-Zn / kg d.w.

Actual AVS concentrations in the 0-2 cm horizon of the sediment: 3.5 (control: CU and CM)-4.4-4.8-5.2-7.5-10.9 mmol/kg dry weight (arithmetic mean of the five measurements, which also showed minimal temporal changes at a given treatment: maximum difference of a factor of 2). These and the further data on AVS concentrations show that the concentrations increase with increasing sediment depth and increasing SEM concentration. The total range of AVS concentrations in the three horizons ranged from 2.4 to 15 mmol/kg d.w. (factor 6 difference).

Note: The SEM and AVS concentrations in the unmanipulated control (CU) and manipulated control (CM) were very similar. Additional data on the SEM concentrations (averaged over the three sediment depths) and AVS concentrations (in the 2-4 and 4-6 cm sediment horizon) were not reported in detail but in graphical representations.

Molar SEM/AVS ratios in the 0-2 cm horizon of the sediment: 0.3 (control)-0.3-0.3-0.5-0.8-1.1.

At the highest concentration in sediment (750 mg Zn/kg d.w.), the pore-water concentrations on the first sampling date (3 weeks after the sediment trays were placed in the pond) were 18, 160 and 210 µg/l at sediment depths of 1, 3 and 5 cm, respectively. One month later and onwards, pore-water concentrations usually were below the detection limit of 6 µg/l (as well as the pore-water concentrations at the lower exposure levels); the maximum concentration in this period was 30 µg/l.

Other analyses during the tests: No data on dissolved oxygen (DO) levels, pH values, or other water characteristics during the tests.

Toxicity results: Detailed data were only reported on the survival data for *C.* tentans. According to the study authors, no significant zinc-related effects were found on *H. azteca* survival or on *C. tentans* survival and growth (statistical analysis: zinc-treated groups versus manipulated control). The highest SEM concentration was 11.9 mmol/kg dry weight; at this concentration the SEM/AVS ratio was 1.1 and the SEM-AVS value was 1.0. The SEM concentration of 11.9 mmol/kg d.w. corresponds to 11.6 mmol zinc (750 mg Zn/kg d.w.).

It is noted that survival in field controls, including manipulated and unmanipulated controls, was variable (44-94% for *C. tentans)* and less than the desired level of 80%. Survival on zinc-treated groups was also variable (0-94% for *C. tentans*). The low survival in both control and treated groups collected from the field could usually be explained by natural causes (predation or growth of fungus and filamentous algae covering the sediment). Mean control survival in laboratory control sediments was ≥ 89% for *C. tentans* and 40-100% for H. azteca.

The study as such is valid but both tests are rejected for chronic NOEC derivation, based on the following Quality criteria:

i) Both for *H. azteca* and *C. tentans* a 10-d test is a short-term test which cannot be used to derive a chronic NOEC value.

ii) Both tests resulted in an unbounded NOEC.

iii) In the *H. azteca* test only endpoint survival was studied.

Note that the resullts of these two tests have been used in Table 3.3.2.f-Part A, that include short- and long-tem single-species studies in Zn-spiked sediments, for the evaluation of the AVS-approach (see RAR section 3.3.2.1.1).

[3] Borgmann & Norwood (1997): *Hyalella azteca* (7-d test and 4-w test).

Statistics: only on toxicity-related bioaccumulation data (body burden in relation to survival), not on toxicity data as such. Test method referring to Borgmann & Norwood 1993. The tests were conducted in a sediment-water static system using zinc-spiked harbour sediment. The amounts of sediment and water per 250 ml test beaker were 40 and 160 mg/l, respectively (sediment/water ratio 1:4). Test beakers were covered with petri dishes and gently aerated throughout the tests; the water was not changed, but evaporated water was replaced with double-distilled water. Both 1-w tests (endpoint: survival) and 4-w tests (endpoints: survival and growth: wet weigth) were conducted twice, the second half a year later than the first.

Organisms and replicates per test : The tests were started with 0- to 1-w old amphipods in the 4-w tests and 4- to 5-w old amphipods in the 1-w tests. In each test, 40 animals from laboratory culture were used per treatment (2 replicates of 20 animals/beaker).

Toxicological endpoints: 7-d test: survival; 4-w test: survival and growth (wet weight).

Sediment: Zinc-polluted Hamilton Harbour sediment (Ontario), collected from site 1; sediments from this reference site consistently supported high survival and growth in 4-w tests over a 2-yr period (see also Borgmann & Norwood 1993), despite the high zinc level. Characteristics: Moisture content 73%, density 1.22 kg/l, organic matter content 9.1% (weight loss on ashing at 500 ^{0}C), equivalent to 5% TOC, and 'background' zinc concentration 1,500 mg/kg dry weight. For data on measured levels of other metals (Pb, Ni, Cu, Cr, Co and Cd) and organic pollutants (DDE, PCBs, and PAH) in this Hamilton Harbour sediment, see Borgmann & Norwood (1993).

Overlying water: Dechlorinated tap water originating from Lake Ontario; pH 7.9-8.6, hardness 130 mg/l and background zinc concentration 6 µg/l. This water was used for culturing and testing.

Spiking and equilibrium time: The sediment was spiked by mixing equal volumes of sediment and a 50 mM $ZnCl_2$ solution (acidified with HCl), by rotating the mixture for 24 h at 4 rpm on a mechanical mixer. The spiking solution was made in experimental water. After spiking the sediment was allowed to settle, the excess water was decanted and the sediment was neutralised with NaOH. Spiked sediments of lower concentrations were then made by mixing 10%, 18%, 32%, or 56% of the spiked sediment with control sediment. No data on equilibrium time after spiking of the sediment.

Metal and AVS analyses during the test: Actual total-Zn concentrations in the sediment: 1,500 (control)-2,400-2,700-4,600-6,400-8,400 mg/kg dry weight. Actual total-Zn concentrations in the overlying water, determined at the end of the exposure period: 117 (sediment-water control)-139-166-208-525-1,763 µg/l. The control Zn concentration in the overlying water in the sediment-water control (117 µg/l) is 20-times higher than the native background Zn concentration (6 µg/l).

The actual total-Cu concentration in control sediment and overlying water at the end of the exposure period were 74 mg/kg dry weight and 15 µg/l, the latter being about 4-times higher than the native background Cu concentration in the test water (4 µg/l).

The results of the metal analyses are mean values, averaged over the four tests (two 1-w and two 4-w tests).

No data reported on AVS (also not in in Borgmann & Norwood, 1993).

Other analyses during the test : No data on dissolved oxygen (DO) levels, pH values, or other water characteristics during the test.

Toxicity results: The results of the two 1-w tests were very similar, resulting in the same NOEC (4,600 mg/kg d.w., for survival). The results of the two 4-w tests were also very similar and resulting in the same NOEC (2,700 mg/kg d.w. for survival and ≥4.600 mg/kg d.w. for growth, respectively), although the 4-w survival at the LOEC for survival (4,600 mg/kg) was 65% in the first 4-w test and 0% in the second 4-w test (mean value: 33%). The mean 4-w survival in the control and lowest two test concentrations (including the NOEC) was 91%, 86% and 94%, respectively.

For the NOEC values in the (overlying) water derived from this study: see Table 3.3.2.in Annex 3.3.2.A.

The study as such is valid, but both tests are rejected based on Relevance criterion (Tests performed in polluted harbour sediment with a very high 'background' zinc concentration of 1,500 mg/kg dw. Using zinc-polluted sediment could cause misinterpreted results, either underestimated or overestimated toxicity.). Furthermore, for *H. azteca* the 10-d test is a short-term test which cannot be used to derive a chronic NOEC value. In addition, only endpoint survival was studied in this short-term test (Quality criterion).

[4] Farrar & Bridges (2003): *Tubifex tubifbex* (28-d test)

Statistics: p = 0.05. The test was conducted following ASTM method E 1706–Annex 4: Guidance for Conducting Sediment Toxicity Tests with *Tubifex tubifex* (ASTM/E 1706, ASTM, 2000). The test was performed in a static sediment-water system (no renewal of overlying water) using zinc-spiked sediment and overlying water (250 ml beakers containing 100 ml sediment and 100 ml overlying water).

Organisms and replicates per test: 24 animals per treatment (6 replicates of 4 animals; sexually mature adults).

Toxicological endpoints: Survival and reproduction (number of young).

Sediment: Unpolluted pond sediment (Denton, Texas), background total-Zn concentration 34 mg/kg dw. (0.52 mmol/kg dry weight), being the arithmetic mean of the day 0 and day 28 measurements during the test. TOC: 1%-2%. The sediment is from the same location as that used in the 28-d *Hyalella azteca* test (see footnote [5]) and the 20-d *Chironomus tentans* test (see footnote [6]). No data on sediment texture (sand, silt and clay content).

The total-SEM (divalent metals) background concentration in the sediment was 44 mg/kg dw (Zn: 19 mg/kg dw, Cu: 14 mg/kg dw, Pb: 11 mg/kg dw, Ag: 0.3 mg/kg dw, Cd: ND, Ni: ND), equal to 0.57 mmol/kg dw (0.291, 0.220, 0.053 and 0.005 mmol/kg dw for Zn, Cu, Pb and Ag, respectively).

Overlying water: Dechlorina ted tap water (no further data reported).

Spiking and equilibrium: The 300 μm sieved and re-homogenated sediment was spiked by adding zinc chloride in 20 ml de-ionised water while mixing. After the addition of zinc, the sediment was mixed for an additional 1-2 hours to ensure homogenous distribution of zinc within the sediment. The spiked sediments were stored for 1 month prior to the test.

Metal and AVS analyses during the test: Total-Zn, SEM-Zn and AVS concentrations in sediment and the Zn concentrations in the pore water were determined on test days 0 and 28. The data below for the actual total-Zn concentrations in the sediment are the arithmitic mean values of the day 0 and day 28 measurements for each treatment.

Note: The results of the measurements of the SEM-Zn, AVS and TOC concentrations in the sediment and the Zn concentrations in the pore water during the test have not (yet) been reported by Farrar & Bridges.

Nominal Zn concentrations: 0 (control)-500-1000-2000-4000-8000-10000 mg/kg dry weight. Actual total-Zn concentrations: 34 (control)-629-1135-2610-4405-8105-18050 mg/kg dw., equal to 0.5 (control)-9.7-17.5-40.1-67.8-125-278 mmol/kg dw. Per treatment, the measured zinc concentrations at day 0 and day 28 were very similar.

Other analyses during the tests: Water quality measurements (dissolved oxygen, hardness, alkalinity, pH, ammonia and conductivity) were measured at several intervals during the test; the results of the measurements were not reported, but it was stated that all water quality parameters fell within acceptable ranges.

Toxicity results: Data were reported for survival and reproduction (number of cocoons and number of young per surviving parent animal) at each treatment. The mean percent survival was 95% (control)-95%-95%-95%-10%-0%-0%, fulfilling the validity criterion for control survival (<10% mortality) and resulting in a $LOEC_s$ of 4405 mg/kg dw and a $NOEC_s$ of 2610 mg/kg dw (actual total-Zn concentrations).

The number of young was significantly ($p < 0.05$) reduced at the actual total-Zn concentration of 2610 mg/kg dw, based the mean number of young/surviving parent animal: 1.0 (control)-1.0-1.1-0.0-0.0-n.d-n.d (n.d: no data for the highest two test concentrations, as there were no surviving parent animals at those concentrations). This results in a $LOEC_r$ of 2610 mg/kg dw and a $NOEC_r$ of 1135 mg/kg dw (actual total-Zn concentrations), as the mean number of cocoons/surviving parent animal was not affected at concentrations up to 2610 mg/kg dw. The validity criterion for reproduction (coefficient of variation (CV) for the reproductive endpoints in control sediment must be less than 25%) was met.

[5] Farrar & Bridges (2001, 2002, 2003): *Hyalella azteca* (28-d test).

Statistics: p = 0.05. The test was conducted following EPA method 100.4: *Hyalella azteca* 42-d test for Measuring the Effects of Sediment-associated Contaminants on Survival, Growth and Reproduction (EPA/600/R-99/064, EPA, 2000), with the following deviations: i) reproduction not studied, ii) 28-d test duration instead of 42 days and iii) aeration was used. The test was performed in a sediment-water intermittent renewal system using zinc-spiked sediment and overlying water (300 ml beakers containing 75 ml sediment and 125 ml overlying water). Renewal of overlying water: two intermittent volume exchanges per day.

Organisms and replicates per test: 60 animals per treatment (6 replicates of 10 animals; 1-w old).

Toxicological endpoints: Survival and growth (dry weight).

Sediment: Unpolluted pond sediment (Denton, Texas). Characteristics: TOC 2%, AVS concentration 5.8 mmol/kg dry weight, background total-Zn concentration 31 mg/kg dry weight (0.48 mmol/kg dry weight) and SEM-Zn concentration 10.5 mg/kg dry weight (0.17 mmol/kg dry weight). No data on sediment texture (sand, silt and clay content).

The total-SEM (divalent metals) background concentration in the sediment was 44 mg/kg dw (Zn: 19 mg/kg dw, Cu: 14 mg/kg dw, Pb: 11 mg/kg dw, Ag: 0.3 mg/kg dw, Cd: ND, Ni: ND), equal to 0.57 mmol/kg dw (0.291, 0.220, 0.053 and 0.005 mmol/kg dw for Zn, Cu, Pb and Ag, respectively).

Overlying water: Dechlorinated tap water (no further data reported).

Spiking and equilibrium: The 300 µm sieved and re-homogenated sediment was spiked by adding zinc chloride in 20 ml de-ionised water while mixing. After the addition of zinc, the sediment was mixed for an additional 1-2 hours to ensure homogenous distribution of zinc within the sediment. The spiked sediments were stored for 1 month prior to the test.

Metal and AVS analyses during the test: Total-Zn, SEM-Zn and AVS concentrations in sediment and the Zn concentrations in the pore water were determined on test days 0 and 28. The data below are the arithmitic mean values of the day 0 and day 28 measurements for each treatment.

Nominal Zn concentrations: 0 (control)-250-500-1000-2000-4000 mg/kg dry weight. Actual total-Zn concentrations: 31 (control)-252-484-936-1810-3445 mg/kg dw., equal to 0.5 (control)-3.9-7.4-14.3-27.7-52.7 mmol/kg dw. Actual SEM-Zn concentrations: 10 (control)-222-484-853-2035-3625 mg/kg dw, equal to 0.2 (control)-3.4-7.4-13.1-31.3-55.8 mmol/kg dw. Per treatment, the measured zinc concentrations at day 0 and day 28 were very similar.

Actual AVS concentrations: 5.8 (control)-0.6-3.3-5.3-4.3-6.9 mmol/kg dw. In some treatments the AVS concenrations at day 0 and day 28 differed considerably, up to factor of 10. In the control and lowest two Zn treatments the AVS concentration decreased between day 0 and 28, while in the highest three Zn treatments the AVS concentration increased.

Note that this 28-d test with *Hyalla azteca* and the 20-d test with *Chironomus tentans* (see foootnote [6]) were performed in pond sediment from the same location, but that different batches were used (collected at different times), resulting in different levels of TOC and especially AVS. In the *H. azteca* test the AVS concentrations in the sediment samples were on average about 10-times lower than those in the *C. tentans* test and the TOC concentrations in the sediment samples used in the *H. azteca* test were about 2-times higher than those in the *C. tentans* test.

Actual Zn concentrations in the pore water: 11 (control)-62-144-231-4590-18600 µg/l. In the control and lowest three Zn treatments the pore water Zn concentrations on day 0 and day 28 were similar (within a factor of 2), while in the highest two Zn treatments the pore water Zn concentrations strongly decreased between day 0 and day 28 (with a factor of 40 and 30, respectively).

Other analyses during the tests: Water quality measurements (dissolved oxygen, hardness, alkalinity, pH, ammonia and conductivity) were measured at several intervals during the test; the results of the measurements were not reported, but it was stated that all water quality parameters fell within acceptable ranges.

Toxicity results: Detailed data were reported for survival and growth for each replicate per treatment. The mean percent survival was 93% (control)-83%-75%-78%-3%-0%, fulfilling the validity criterion for control survival (<20% mortality) and resulting in a $LOEC_s$ of 1810 mg/kg dw and a $NOEC_s$ of 936 mg/kg dw (actual total-Zn concentrations).

Growth was significantly ($p < 0.05$) reduced at the lowest test concentration and dose-related further reduced at the higher test concentrations, based on the mean individual dry weights: 0.32 (control)-0.24-019-0.16-0.15-n.d mg (n.d.: no data for the highest test concentration, as there were no surviving animals at that concentration). At the $LOEC_g$ (actual total-Zn concentration of 252 mg/kg dw, equivalent to an added total-Zn concentration of 221 mg/kg dw (actual-Cb: 252-31 mg/kg dw), growth was reduced by 25%. The control growth (individual dry weigt: 0.35 mg) fulfilled the validity criterion for this endpoint (individual dry weight: >0.15 mg).

In the December 2004 draft version of this RAR, an estimated $NOEC_g^e$ of 84 mg/kg dw (actual total-Zn concentration) was derived from the $LOEC_g$ of 252 mg/kg dw ($NOEC_g^e = LOEC_g/3 = 252/3 = 84$ mg/kg dw), equivalent to a $NOEC_g^e$ of 74 mg/kg dw for added Zn ($NOEC_g^e = LOEC_g/3 = 221/3 = 74$ mg/kg dw). The $NOEC_g^e$ of 74 mg/kg dw for added Zn was used as key study for $PNEC_{add,\ sediment}$ derivation, i.e $PNEC_{add,\ sediment}$ was $NOEC_g^e/2 = 74/2 = 37$ mg/kg dw. Although the derivation of an estimated NOEC from a LOEC at which 20-30% inhibition is found (as is the case in this study), the estimated $NOEC_g^e$ from this study now has been rejected, as a new valid long-term study with *Hyalella azteca* has been performed; this new study (Nguyen et al., 2005, see Table 3.3.2.e-Part I and footnote [9] does not indicate that growth is the most sensitive endpoint for *H. azteca* exposed to zinc. Also the rejected long-term *Hyalella azteca* study by Borgmann & Norwood, 1997 (see Table 3.3.2.e-Part II and footnote [3]) does not indicate that growth is the most sensitive endpoint for *H. azteca* exposed to zinc.

The study as such is valid, but rejected, based on quality criterion (Growth was affected at the lowest concentration tested, but the sensitivity for endpoint growth compared to endpoint survival is not confirmed by the results of the two other long-term tests with *H. azteca*).

Note that the results of this test have been used in Table 3.3.2.f-Part A, that include short- and long-tem single-species studies in Zn-spiked sediments, for the evaluation of the AVS-approach (see RAR section 3.3.2.1.1).

[6] Farrar & Bridges (2002, 2003): *Chironomus tentans* (20-d test)

Statistics: p = 0.05. The test was conducted following EPA method 100.5: Lyfe-cycle test for Measuring the Effects of Sediment-associated Contaminants on *Chironomus tentans* (EPA/600/R-99/064, EPA, 2000), with the following deviations: i) reproduction not studied, ii) 20-d exposure instead of ≥50 days (the 20-d exposure for the assessment of survival and growth is according to the guideline, as no reproduction was studied, and iii) aeration was used. The test was performed in a sediment-water intermittent renewal system using zinc-

spiked sediment and overlying water (300 ml beakers containing 75 ml sediment and 125 ml overlying water). Renewal of overlying water: two intermittent volume exchanges per day.

Organisms and replicates per test: 72 animals per treatment (6 replicates of 12 animals; <1-d old larvae).

Toxicological endpoints: Survival and growth (dry weight) .

Sediment: Unpolluted pond sediment (Denton, Texas). Characteristics: TOC 1%, AVS concentration 37 mmol/kg dry weight, total-Zn concentration 30 mg/kg dry weight (0.47 mmol/kg dry weight) and SEM-Zn concentration 9.0 mg/kg dry weight (0.14 mmol/kg dry weight). No data on sediment texture (sand, silt and clay content).

The total-SEM (divalent metals) background concentration in the sediment was 44 mg/kg dw (Zn: 19 mg/kg dw, Cu: 14 mg/kg dw, Pb: 11 mg/kg dw, Ag: 0.3 mg/kg dw, Cd: ND, Ni: ND), equal to 0.57 mmol/kg dw (0.291, 0.220, 0.053 and 0.005 mmol/kg dw for Zn, Cu, Pb and Ag, respectively).

Overlying water: Dechlorinated tap water (no further data reported).

Spiking and equilibrium: The 300 µm sieved and re-homogenated sediment was spiked by adding zinc chloride in 20 ml de-ionised water while mixing. After the addition of zinc, the sediment was mixed for an additional 1-2 hours to ensure homogenous distribution of zinc within the sediment. The spiked sediments were stored for 1 month prior to the test.

Metal and AVS analyses during the test: Total-Zn, SEM-Zn and AVS concentrations in sediment and the Zn concentrations in the pore water were determined on test days 0 and 28. The data below are the arithmitic mean values of the day 0 and day 20 measurements for each treatment.

Nominal Zn concentrations: 0 (control)-250-500-1000-2000-4000 mg/kg dry weight. Actual total-Zn concentrations: 30 (control)-348-639-1255-2420-4910 mg/kg dw., equal to 0.5 (control)-5.3-9.8-19.2-37.0-75.1 mmol/kg dw. Actual SEM-Zn concentrations: 9 (control)-366-767-1440-2745-5505 mg/kg dw, equal to 0.1 (control)-5.6-11.7-22.0-42.0-84.2 mmol/kg dw. Per treatment, the measured zinc concentrations at day 0 and day 20 were very similar.

Actual AVS concentrations: 37 (control)-41-40-48-53-66 mmol/kg dw. Per treatment, the measured AVS concentrations at day 0 and day 20 were similar (in all treatments within a factor of 2) to very similar.

Note that the 28-d test with *Hyalla azteca* (see footnote [5]) and this 20-d test with *Chironomus tentans* were performed in pond sediment from the same location, but that different batches were used (collected at different times), resulting in different levels of TOC and especially AVS. In the *H. azteca* test the AVS concentrations in the sediment samples were on average about 10-times lower than those in the *C. tentans* test and the TOC concentrations in the sediment samples used in the *H. azteca* test were about 2-times higher than those in the *C. tentans* test.

Actual Zn concentrations in the pore water: 60 (control)-60-110-360-270-24700 µg/l. In the control and lowest four treatments the pore water Zn concentrations on day 0 and day 20 were similar (within a factor of 2 or 3), while in the highest Zn treatment the pore water Zn concentrations strongly decreased between day 0 and day 20 (with a factor of 30).

Other analyses during the tests: Water quality measurements (dissolved oxygen, hardness, alkalinity, pH, ammonia and conductivity) were measured at several intervals during the test; the results of the measurements were not reported, but it was stated that all water quality parameters fell within acceptable ranges.

Toxicity results: Data were reported for survival and growth at each treatment. The mean percent survival was 93% (control)-85%-93%-81%-86%-0%, fulfilling the validity criterion for control survival (<20% mortality) and resulting in a $LOEC_s$ of 4910 mg/kg dw and a $NOEC_s$ of 2420 mg/kg dw (actual total-Zn concentrations).

Growth was significantly ($p < 0.05$) reduced at the actual total-Zn concentration of 1255 mg/kg dw and dose-related further reduced at the higher test concentrations, based on the mean individual dry weights: 2.71 (control)-2.58-2.17-1.63-1.25-n.d. mg (n.d.: no data for the highest test concentration, as there were no surviving animals at that concentration). This results in a $LOEC_g$ of 1255 mg/kg dw and a $NOEC_g$ of 639 mg/kg dw (actual total-Zn concentrations). The validity criterion for growth (a minimum individual dry weight of 0.6 mg/animal) was met.

[7] = Farrar & Bridges (2002): *Hyalella azteca* (10-d test)

Statistics: $p = 0.05$. The test was conducted following EPA method 100.1: *Hyalella azteca* 10-day Survival and Growtth Test for Sediments (EPA/600/R-99/064, EPA, 2000), with the following deviations: i) growth was determined using wet weigt instead of dry weight, and ii) aeration was used. The test was performed in a sediment-water intermittent renewal system using zinc-spiked sediment and overlying water (300 ml beakers containing 75 ml sediment and 125 ml overlying water). Renewal of overlying water: two intermittent volume exchanges per day.

Organisms and replicates per test: 60 animals per treatment (6 replicates of 10 animals; 1-2 weeks old).

Toxicological endpoints: Survival and growth (wet weight).

Sediment: Unpolluted Brown's Lake sediment (Vicksburg, Mississippi). Characteristics: TOC 1%, AVS concentration 1.1 mmol/kg dry weight, total-Zn concentration 48 mg/kg dry weight (0.74 mmol/kg dry weight) and SEM-Zn concentration 19 mg/kg dry weight (0.29 mmol/kg dry weight). No data on sediment texture (sand, silt and clay content).

The total-SEM (divalent metals) background concentration in the sediment was 64 mg/kg dw (Zn: 41 mg/kg dw, Cu: 11 mg/kg dw, Pb: 12 mg/kg dw, Ag: 0.4 mg/kg dw, Cd: 0.1 mg/kg dw, Ni: ND), equal to 0.86 mmol/kg dw (0.624, 0.175, 0.057, 0.004, and 0.001 for Zn, Cu, Pb, Ag and Cd, respectively).

Overlying water: Dechlorinated tap water (no further data reported).

Spiking and equilibrium: The 300 µm sieved and re-homogenated sediment was spiked by adding zinc chloride in 20 ml de-ionised water while mixing. After the addition of zinc, the sediment was mixed for an additional 1-2 hours to ensure homogenous distribution of zinc within the sediment. The spiked sediments were stored for 2 weeks prior to the test.

Metal and AVS analyses during the test: Total-Zn, SEM-Zn and AVS concentrations in sediment were determined on test days 0 and 10. The data below are the arithmitic mean values of the day 0 and day 10 measurements for each treatment. The Zn concentrations in the pore water were only determined on day 10.

Nominal Zn concentrations: 0 (control)-250-500-1000-2000-4000 mg/kg dry weight. Actual total-Zn concentrations: 48 (control)-229-398-984-1690-3260 mg/kg dw, equal to 0.7 (control)-3.5-6.8-15.0-27.6-49.9 mmol/kg dw. Actual SEM-Zn concentrations: 19 (control)-226-461-984-1835-3300 mg/kg dw, equal to 0.3 (control)-3.5-7.1-15.0-28.1-50.5 mmol/kg dw. Per treatment, the measured zinc concentrations at day 0 and day 10 were very similar.

Actual AVS concentrations: 1.1 (control)-1.9-1.8-2.4-2.3-2.4 mmol/kg dw. Per treatment, the measured AVS concentrations at day 0 and day 10 were very similar.

Note that this 10-d test with *Hyalla azteca* was performed in the same batch of the sediment as used in the 10-d test with *Chironomus tentans* (see footnote [8]), resulting in (virtually) the same TOC, Total-Zn, SEM-Zn and AVS levels at the corresponding treatments.

Actual Zn concentrations in the pore water (only determined on day 10): 280 (control)-820-3000-4020-12100-76700 µg/l.

Other analyses during the tests: Water quality measurements (dissolved oxygen, hardness, alkalinity, pH, ammonia and conductivity) were measured at several intervals during the test; the results of the measurements were not reported, but it was stated that all water quality parameters fell within acceptable ranges.

Toxicity results: Data were reported for survival and growth at each treatment. Both survival and growth (mean individual wet weight) were significantly ($p < 0.05$) reduced at the actual total-Zn concentraton of 398 mg/kg dw, resulting in a $LOEC_{s,g}$ of 398 mg/kg dw and a $NOEC_{s,g}$ of 229 mg/kg dw (actual total-Zn concentrations). The validity criteria for control survival (<20% mortality) and control growth (measurable growth in the control sediment) were met.

The study as such is valid but the test is rejected for chronic NOEC derivation, based on the following Quality criterion:

For H. azteca a 10-d test is a short-term test which cannot be used to derive a chronic NOEC value.

Note that the results of these two tests have been used in Table 3.3.2.f-Part A, that include short- and long-tem single-species studies in Zn-spiked sediments, for the evaluation of the AVS-approach (see RAR section 3.3.2.1.1).

[8] = Farrar & Bridges (2002): Chironomus tentans (10-d test)

Statistics: p = 0.05. The test was conducted following EPA method 100.2: Chironomus tentans 10-d Survival and Growtth Test for Sediments (EPA/600/R-99/064, EPA, 2000), with the following deviations: i) growth was determined using wet weigt instead of ash-free dry weight, and ii) aeration was used. The test was performed in a sediment-water intermittent renewal system using zinc-spiked sediment and overlying water (300 ml beakers containing 75 ml sediment and 125 ml overlying water). Renewal of overlying water: two intermittent volume exchanges per day.

Organisms and replicates per test: 60 animals per treatment (6 replicates of 10 animals; 2nd to 3th instar).

Toxicological endpoints: Survival and growth (wet weight).

Sediment: Unpolluted Brown's Lake sediment (Vicksburg, Mississippi). Characteristics: TOC 1%, AVS concentration 1.1 mmol/kg dry weight, total-Zn concentration 48 mg/kg dry weight (0.74 mmol/kg dry weight) and SEM-Zn concentration 22 mg/kg dry weight (0.34 mmol/kg dry weight). No data on sediment texture (sand, silt and clay content).

The total-SEM (divalent metals) background concentration in the sediment was 64 mg/kg dw (Zn: 41 mg/kg dw, Cu: 11 mg/kg dw, Pb: 12 mg/kg dw, Ag: 0.4 mg/kg dw, Cd: 0.1 mg/kg dw, Ni: ND), equal to 0.86 mmol/kg dw (0.624, 0.175, 0.057, 0.004, and 0.001 for Zn, Cu, Pb, Ag and Cd, respectively).

Overlying water: Dechlorinated tap water (no further data reported).

Spiking and equilibrium: The 300 µm sieved and re-homogenated sediment was spiked by adding zinc chloride in 20 ml de-ionised water while mixing. After the addition of zinc, the sediment was mixed for an additional 1-2 hours to ensure homogenous distribution of zinc within the sediment. The spiked sediments were stored for 2 weeks prior to the test.

Metal and AVS analyses during the test: Total-Zn, SEM-Zn and AVS concentrations in sediment were determined on test days 0 and 10. The data below are the arithmitic mean values of the day 0 and day 10 measurements for each treatment. The Zn concentrtins in the pore water were only determined on day 10.

Nominal Zn concentrations: 0 (control)-250-500-1000-2000-4000 mg/kg dry weight. Actual total-Zn concentrations: 48 (control)-234-435-968-1805-3250 mg/kg dw, equal to 0.7 (control)-3.6-6.6-14.8-27.6-49.8 mmol/kg dw. Actual SEM-Zn concentrations: 22 (control)-232-456-968-1810-3325 mg/kg dw, equal to 0.3 (control)-3.5-7.0-14.8-27.7-50.9 mmol/kg dw. Per treatment, the measured zinc concentrations at day 0 and day 10 were very similar.

Actual AVS concentrations: 1.1 (control)-1.9-1.8-2.3-1.8-2.3 mmol/kg dw. Per treatment, the measured AVS concentrations at day 0 and day 10 were very similar.

Note that the 10-d test with *Hyalla azteca* (see footnote[7]) was performed in the same batch of the sediment as used in this 10-d test with *Chironomus tentans*, resulting in (virtually) the same TOC, Total-Zn, SEM-Zn and AVS levels at the corresponding treatments.

Actual Zn concentrations in the pore water (only determined on day 10): 1200 (control)-2460-3580-8780-15500-54200 µg/l.

Other analyses during the tests: Water quality measurements (dissolved oxygen levels, hardness, alkalinity, pH, ammonia and conductivity) were measured at several intervals during the test; the results of the measurements were not reported, but it was stated that all water quality parameters fell within acceptable ranges.

Toxicity results: Data were reported for survival and growth at each treatment. Survival was significantly ($p < 0.05$) reduced at the actual total-Zn concentraton of 3250 mg/kg dw (highest test concentration), resulting in a $LOEC_s$ of 3250 mg/kg dw and a $NOEC_s$ of 1805 mg/kg dw (actual total-Zn concentrations). Growth (mean individual wet weight) was significantly ($p < 0.05$) reduced at the actual total-Zn concentraton of 968 mg/kg dw, resulting in a $LOEC_g$ of 968 mg/kg dw and a $NOEC_g$ of 435 mg/kg dw (actual total-Zn concentrations). The validity criterion for control survival (<30% mortality) was met. The validity criterion for control growth (minimum ash-free dry weight of 0.48 mg) could not be checked, as only the mean individual wet weight per treatment (11 mg/animal in the control sediment) was reported. The test is assumed to be valid.

The study as such is valid but the test is rejected for chronic NOEC derivation, based on the following Quality criterion:

For *C. tentans* a 10-d test is a short-term test which cannot be used to derive a chronic NOEC value.

Note that the results of these two tests have been used in Table 3.3.2.f-Part A, that include short- and long-tem single-species studies in Zn-spiked sediments, for the evaluation of the AVS-approach (see RAR section 3.3.2.1.1).

[9] Nguyen et al. (2005): *Hyalella azteca (6-w test)*

Statistics: $p = 0.05$. The test was conducted following EPA method 100.4: *Hyalella azteca* 42-d test for Measuring the Effects of Sediment-associated Contaminants on Survival, Growth and Reproduction (EPA/600/R-99/064, EPA, 2000), with the following deviation: only 28-d

growth was studied instead of 28-d and 42-d growth. The test was performed in a sediment-water renewal system using zinc-spiked sediment and overlying water (500 ml beakers containing 200 g sediment and 275 ml overlying water). Renewal of overlying water: two times a week. Note that according to the guideline the actual exposure to the spiked sediment lasted for 28 days, followed by a 14-d water-only exposure to clean water.

Organisms and replicates per test: 100 animals per treatment (10 replicates of 10 animals from laboratory culture; 1-w old), of which 8 replicates for toxicity assessmentd and 2 replicates for chemical analyses:

- 4 replicates for 28-d survival and growth;
- 4 replicates for 42-d survival and 42-d reproduction;
- 2 replicates for chemical analyses.

Toxicological endpoints: Survival, growth (dry weight) and reproduction (number of young).

Sediment: Unpolluted forest stream sediment (Belgium ??) Characteristics: TOC 1.3% (control) to 1.6-1.7% (Zn-treatments), AVS concentration 5.5 mmol/kg dry weight, background total-Zn concentration 55 mg/kg dry weight (0.84 mmol/kg dry weight). Background concentrations of other divalent metals: 20 mg/kg dw (0.01 mmol/kg dw) for Pb, 17.5 mg/kg dw (0.3 mmol/kg dw) for Ni, 8 mg/kg dw (0.12 mmol/kg dw) for Cu and 0.6 mg/kg dw (0.005 mmol/kg) for Cd. Total-SEM concentration: 0.49 mmol/kg dw. Sediment texture: 8% clay, 36% silt and 56% sand.

Overlying water (culture and test medium): Borgmanns medium *(ASTM, 1994; Borgmann, 1996; not checked)* containing $NaHCO_3$ (84 mg/l), $CaCl_2.2H_2O$ (147 mg/l), $MgSO_4.7H_2O$ (62 mg/l), KCl (3.7 mg/l) and NaBr (1.0 mg/l). The values for pH and total hardness measured during the test were 6.9-7.8 and 125-250 mg/l (as $CaCO_3$), respectively.

Spiking and equilibrium: The sediment was spiked by adding zinc chloride in a small volume of de-ionised water; the ratio of the zinc solution (ml) and the sediment sample (g) was smaller than 1:10. After the addition of zinc, the sediment was mixed by means of rolling in a plastic bag (to avoid air contact) for 15 minutes. Zinc measurements showed the effectiveness of the mixing procedure, as the analytical results showed that the coefficient of variation of the sediment Zn concentrations between replicate samples was 8-13%. The spiked sediments were stored for 40 days prior to the test, under the same conditions as used during the exposure period.

Metal and AVS analyses during the test: Total-Zn, SEM-Zn and AVS concentrations in sediment and the Zn concentrations in the pore water were determined on test days 0 and 28. The data below are the arithmetic mean values of the day 0 and day 28 measurements for each treatment, measured in the whole sediment.

Nominal Zn concentrations: 0 (control)-56-100-180-320-560-1000-1800 mg/kg dry weight, based on the results of a range-finding test. Actual total-Zn concentrations (reported in mg/kg dw): 22 (control)-74-144-212-358-510-1000-1423 mg/kg dw., equal to 0.3 (control)-1.1-2.2-3.2-5.5-7.8-15.3-21.8 mmol/kg dw. Actual SEM-Zn concentrations: 16 (control)-63-106-186-290-475-791-1230 mg/kg dw, equal to 0.2 (control)-1.0-1.6-2.8-4.4-7.3-12.1-18.8 mmol/kg dw (SEM-Zn concentrations reported by Nguyen et al (2005) in mmol/kg dw.). Per treatment, the measured total-Zn or SEM-Zn zinc concentrations at day 0 and day 28 were very similar. SEM-Zn measurements were also performed separately in the 0-1 cm and 1-4 cm sediment layer, with (very) similar results as the corresponding values for whole sediment.

Actual AVS concentrations: 5.7 (control)-7.7-7.6-6.7-6.9-8.5-8.4-8.1 mmol/kg dw. Per treatment, the measured AVS concentrations at day 0 and day 28 were (very) similar. AVS measurements were also performed separately in the 0-1 cm and 1-4 cm sediment layers, with at the higher Zn treatments similar results as the corresponding values for whole sediment. In the lower Zn treatments the AVS concentrations in the 0-1 cm layer were up to around 2 times lower than those in the 1-4 cm layer and whole sediment.

Actual Zn concentrations in the pore water: 32 (control)-49-35-52-40-34-1060-9560 µg/l on day 0 and 12 (control)-82-52-65-77-61-275-3066 µg/l on day 28, showing a decrease with a factor of around 3 in the control and highest two Zn treatments between day 0 and day 28 and similar concentrations (within a factor of 2) on day 0 and day 28 in the other Zn treatments.

Other analyses during the tests: Water quality measurements (dissolved oxygen, temperature, pH, hardness, ammonia, conductivity, and Zn were measured in the overlying water two times a week, before renewal; the results were reported for day 0, day 6, day 12, day 18, day 24 and day 28. The Zn concentrations were 18 (control)-16-18-9-13-28-183-2689 µg/l on day 0, and 4 (control)-4-4-4-6-34-134-938 µg/l on day 28, usually showing a decrease with a factor of 2 to 4 during the 28-d exposure period. The values for pH and total hardness measured during the test were 6.9-7.8 and 125-250 mg/l (as $CaCO_3$), respectively. The dissolved oxygen content was always $\geq$3.8 and also the results for the other parameters confirmed the validity of the test.

In pore water, the pH was 6.4-7.3 and the hardness was 215-400 mg/l (as $CaCO_3$). based on day 0 and day 28 measurements. In addition, conditivity and ammonia were determined in the pore water.

Toxicity results:

Range-finding test (10 days)

In the range-finding test (3 replicates of 10 animals), the mean percent survival was 100% (control)-100%-100%-63%-0%-0% at nominal Zn concentrations of 0 (control)-100-500-1000-2000-4000 mg/kg. No other endpoints were studied.

Final test (42 days)

Detailed data were reported for survival (day 28 and day 42), growth (day 28) and reproduction (day 28, day 35 and day 42) for each replicate per treatment. The mean percent 42-d survival was 95% (control)-98%-90%-98%-100%-98%-65%-0%, fulfilling the validity criterion for control survival (<20% mortality on day 28) and resulting in a $LOEC_s$ of 1000 mg/kg dw and a $NOEC_s$ of 510 mg/kg dw (actual total-Zn concentrations).

Growth (mean individual dry weight) and reproduction (number of young per female) were not affected up to 1000 mg/kg, i.e. up to the $LOEC_s$. At the highest Zn concencentration (1423 mg/kg dw, actual total-Zn concentration), growth and reproduction could not be measured, as there were no surviving animals after 28 days of exposure. Based on this, the $NOEC_g$ and the $NOEC_r$ both are $\geq$1000 mg/kg dw (actual total-Zn concentration). The control growth (individual dry weight: 0.18 mg) and control reproductive performance (6.2 young per female) fulfilled the validity criteria for these endpoints (individual dry weight: >0.15 mg and >2 young per female, respectively.

Table 3.3.2.f- Part A. Toxicity of zinc to freshwater benthic macroinvertebrates (single-species laboratory studies in spiked-sediment – water systems): NOEC and LOEC values related to SEM/AVS and SEM-AVS, and (SEM-AVS)/f_{oc} (Short-term and long-term studies, from Table 3.3.2.e)

Organism	Test-Comp.	Sediment	Dura-tion	Criterion	SEM* (mmol/kg d.w.)	SEM/AVS* (molar ratio)	SEM-AVS* (molar difference)	(SEM-AVS)/f_{oc} (mmol/kg_{oc})	Reference [footnote]
Zinc-spiked freshwater sediments									
Tubifex tubifex adults	$ZnCl_2$	pond sediment **f_{oc}: 0.01-0.02**	4-w	$NOEC_r$	17.5 (actual)	No data	No data	No data	Farrar & Bridges, 2003 [4]
				$NOEC_r$	17.0 (actual-Cb)				
				$LOEC_r$	40.1 (actual-Cb)				
				$LOEC_r$	39.6 (actual-Cb)				
Hyalella azteca 1-w old	$ZnCl_2$	stream sediment **f_{oc}: 0.02**	6-w	$NOEC_s$	7.3 (actual)	0.9	-1.2	-60	Nguyen et al., 2005 [9]
				$NOEC_s$	7.1 (actual-Cb)	0.8	-1.4	-70	
				$LOEC_s$	12.1 (actual)	1.4	3.7	185	
				$LOEC_s$	11.9 (actual-Cb)	1.4	3.5	175	
Hyalella azteca 1-w old	$ZnCl_2$	pond sediment **f_{oc}: 0.02**	4-w	$LOEC_g$	3.4 (actual)	12.3	2.8	140	Farrar & Bridges, 2002, 2003 [5]
				$LOEC_g$	3.2 (actual-Cb)	11.6	2.6	130	
Hyalella azteca 1-2 w old	$ZnCl_2$	lake sediment **f_{oc}: 0.01**	10-d	$NOEC_{s,g}$	3.5 (actual)	1.9	1.6	160	Farrar & Bridges, 2002, 2003 [7]
				$NOEC_{s,g}$	3.2 (actual-Cb)	1.7	1.3	130	
				$LOEC_{s,g}$	7.1 (actual)	4.0	5.3	530	
				$LOEC_{s,g}$	6.8 (actual-Cb)	3.8	5.0	500	
Hyalella azteca	$ZnCl_2$	pond sediment **f_{oc}: 0.11**	10-d	$NOEC_s$	≥11.9 (actual)	≥1.1	≥1.0	≥9.1	Liber et al., 1996 [2]
					≥11.2 (actual-Cb)	≥1.0	≥0.3	≥2.7	

To be continued

Table 3.3.2.f- Part A. (continued) Toxicity of zinc to freshwater benthic macroinvertebrates (single-species laboratory studies in spiked-sediment – water systems): NOEC and LOEC values related to SEM/AVS and SEM-AVS, and (SEM-AVS)/f_{oc} (Short-term and long-term studies, from Table 3.3.2.e)

Organism	Test-Comp.	Sediment	Duration	Criterion	SEM* (mmol/kg d.w.)	SEM/AVS* (molar ratio)	SEM-AVS* (molar difference)	(SEM-AVS)/f_{oc} (mmol/kg_{oc})	Reference [footnote]
Chironomus tentans P (newly hatched larvae → F1 [lc]	$ZnCl_2$	lake sediment **f_{oc}: 0.05 (default; no actual data)**	8-w	$NOEC_{s,g,e,r}$	13 (actual)	1.8	5.9	118	Sibley et al., 1996 [1]
					12 (actual-Cb)	1.7	4.9	98	
				$LOEC_{s,g,e,r}$	29 (actual)	4.3	22	440	
					28 (actual-Cb)	4.1	21	420	
Chironomus tentans 1-d old	$ZnCl_2$	pond sediment **f_{oc}: 0.01**	3-w	$NOEC_g$	11.7 (actual)	0.3	-28.0	-2800	Farrar & Bridges, 2002, 2003 [6]
				$NOEC_g$	11.6 (actual-Cb)	0.3	-28.0	-2800	
				$LOEC_g$	22.0 (actual)	0.5	-26.2	-2620	
				$LOEC_g$	21.9 (actual-Cb)	0.5	-26.2	-2620	
Chironomus tentans 2^{nd} to 3^{th} instar	$ZnCl_2$	lake sediment **f_{oc}: 0.01**	10-d	$NOEC_g$	7.0 (actual)	4.0	5.2	520	Farrar & Bridges, 2002, 2003 [8]
				$NOEC_g$	6.7 (actual-Cb)	3.8	4.9	490	
				$LOEC_g$	14.8 (actual)	6.4	12.5	1250	
				$LOEC_g$	14.5 (actual-Cb)	6.3	12.2	1220	
Chironomus tentans	$ZnCl_2$	pond sediment **f_{oc}: 0.11**	10-d	$NOEC_{s,g}$	≥11.9 (actual)	≥1.1	≥1.0	≥9.1	Liber et al., 1996 [2]
					≥11.2 (actual-Cb)	≥1.0	≥0.3	≥2.7	

Table 3.3.2.f- Part B. Chronic toxicity of cadmium to estuarine benthic macroinvertebrates (single-species laboratory studies in spiked-sediment – water systems): NOEC and LOEC values related to SEM/AVS and SEM-AVS, and (SEM-AVS)/f_{oc}

Organism	Test-Comp.	Sediment	Dura-tion	Criterion	SEM* (mmol/kg d.w.)	SEM/AVS* (molar ratio)	SEM-AVS* (molar difference)	(SEM-AVS)/f_{oc} (mmol/kg_{oc})	Reference [footnote]
Cadmium-spiked estuarine sediment									
Lepocheirus plumulosus	-	estuarine	28-d	$NOEC_{s, g, r}$	12.2 (actual)	1.2	1.9	63	
P (newly hatched		sediment		$LOEC_s$	17.3 (actual)	2.0	8.7	290	
→ F1 [lc]		**f_{oc}: 0.03**						DeWitt et al., 1996 [3]	

Footnotes Table 3.3.2.f – Part A and B

* Further information on AVS and SEM concentrations:

Sibley et al. (1996)

SEM is "SEM"-zinc; Cb is total SEM (i.e. all metals measured in the control sediment). The SEM/AVS and SEM-AVS values were calculated from the SEM and AVS level at the given exposure concentration.

Liber et al. (1996)

SEM and Cb are total SEM (i.e. all metals measured during the study), but at the NOEC and LOEC nearly all SEM is "SEM"-zinc, the only metal added. The SEM/AVS and SEM-AVS values were calculated from the SEM and AVS level at the given exposure concentration.

DeWitt et al. (1996)

SEM is "SEM"-cadmium. The SEM/AVS and SEM-AVS values were calculated from the SEM and AVS level at the given exposure concentration.

Farrar & Bridges (2002, 2003)

SEM and Cb are "SEM-Zn". The SEM/AVS and SEM-AVS values were calculated from the SEM and AVS level at the given exposure concentration.

Note: The SEM/AVS and SEM-AVS values listed in Table 3.3.2.f for the tests by Farrar & Bridges may deviate somewhat from the values that can be calculated from the data in the footnotes of Table 3.3.2.e. The reason for this is the following:

In Table 3.3.2.f, the molar ratio (SEM/AVS) is the arithmetic mean value of SEM/AVS at the start of the test and that at the end of the test. The molar difference (SEM-AVS) was calculated likewise.

In the footnotes of Table 3.2.2.e, the SEM and AVS levels are given separately, each as arithmetic mean value of the value (SEM or AVS) at the beginning of the test and that at the end of the test.

Nguyen et al. (2005)

SEM and Cb are "SEM-Zn". The SEM/AVS and SEM-AVS values were calculated from the SEM and AVS level at the given exposure concentration. In Table 3.3.2.f, the molar ratio (SEM/AVS) is the arithmetic mean value of SEM/AVS at the start of the test and that at the end of the test. The molar difference (SEM-AVS) was calculated likewise The SEM/AVS and SEM-AVS values listed in Table 3.3.3.f (calculated by the rapporteur) slightly deviate from the corresponding values reported by Nguyen et al. (2005), but in all cases, no effect was found at SEM/AVS values <1 and SEM-AVS values <0, while an effect was found at SEM/AVS values >1 and SEM-AVS values >0.

Toxicological endpoints: e = emergence; g = growth; r = reproduction ; s = survival

For further information: see the "list of abbreviations Table 3.3.2.a to 3.3.2.i"

Footnotes

[1] Sibley et al. (1996): *Chironomus tentans* (8-w test)

See footnote [1] of Tabl 3.3.2.e.

[2] Liber et al. (1996): *Hyalella azteca*(10-d test) and *Chironomus tentans* (10-d test)

See footnote [2] of Table 3.3.2.e.

[3] DeWitt et al. (1996): *Leptocheirus plumulosus* (28-d test)

Statistics: p = 0.05. SEM/AVS normalisation was tested using sediment spiked with cadmium under static-renewal conditions for 28 d with newborn amphipods.

Organisms and replicates: The test was started with newly hatched larvae. Per replicate 20 newborn *L. plumulosus* were added. Amphipods were fed 3 times a week with live microalgae, yeast, alfalfa and commercial fishfood, simultaneously with renewal of overlying water. Mortality, growth and reproduction was assessed at the end of the 28-days exposure period.

Sediment: Fine-grained (<250-µm grain size, 3% total organic carbon (TOC) and AVS concentration 19.3 mmol/kg d.w. No data on the other metals present in the sediment.

Overlying water: 800 mL 20 λ seawater per test unit, constant aeration. Water was renewed 3 times per week.

Spiking and equilibrium: After spiking with cadmium (no data on test compound) to reach nominal test concentrations of 0, 203, 407, 813, 1627, 3254 and 6508 mg Cd/kg d.w., i.e. molar Cd/AVS ratios of 0, 0.093, 0.187, 0.375, 0.75, 1.5 and 3.0, respectively, the sediments were stored for 6 days. Eight days prior to test initiation, 175 g (2 cm thickness) sediment was placed to exposure chambers. Overlying water was replaced daily prior to start of the test.

Metal and AVS analyses during the test: overlying water was sampled on days 0, 1,4,6,13,20 and 27 of exposure and pore water was sampled weekly to determine dissolved Cd-concentrations. AVS and SEM_{Cd} concentrations were determined of weekly taken sediment cores. Sediment cores were sectioned into 3 sections, i.e. 0 –6 mm, 6 – 12 mm and 12 to bottom (20 mm). AVS and SEM were determined following the cold-acid purge-and-trap technique. SEM_{Cd} and dissolved Cd were measured by ICP-AES or GFAAS.

Average actual dissolved-Cd concentrations in the porewater: 1.7 (control), 31.7, 23.4, 202, 178, 43200, 140000 µg/l.

Actual SEM_{Cd}/AVS molar ratio: 0, 0.34, 0.74, 1.55, 1.31, 2.23, 4.82 according to the authors, but recalculation by the Rapporteur resulted in actual SEM_{Cd}/AVS molar ratios of 0, 0.32, 0.62, 1.13, 1.19, 2.03 and 3.22. Measured SEM_{Cd}/AVS were consistenly higher than nominal ratios within each spiked sediment due to reduction in AVS concentration in all treatments.

Toxicity results:

Mortality in control was 5%. No significant differences compared to the control in mortality, growth or fertility in treatments with actual SEM_{Cd}-concentrations of ≤1370 µg/g d.w. (i.e. 1.19 SEM_{Cd}/AVS molar ratio and SEMCd-AVS 1.9 µmol/g d.w.). Complete mortality occurred at the 2 highest SEM_{Cd}-concentrations.

[4] Farrar & Bridges (2003): *Tubifex tubifex* (28-d test)

See footnote [4] of Table 3.3.2.e.

For *T. tubifex* the NOEC and LOEC values are based on total measured Zn, not on SEM-Zn as in the other tests from Farrar & Bridges (no data on SEM-Zn and AVS levels are available yet).

[5] Farrar & Bridges (2001,2002, 2003): *Hyalella azteca* (28-d test)

See footnote [5] of Table 3.3.2.e.

[6] Farrar & Bridges (2003): *Chiromus tentans* (20-d test)

See footnote [6] of Table 3.3.2.e.

[7] Farrar & Bridges (2003): *Hyalella azteca* (10-d test)

See footnote [7] of Table 3.3.2.e.

[8] Farrar & Bridges (2003): *Chironomus tentans* (10-d test)

See footnote [8] of Table 3.3.2.e.

[9] Nguyen et al. (2005): *Hyalella azteca* (42-d test)

See footnote [9] of Table 3.3.2.e.

Table 3.3.2.f- Part C. Colonisation of zinc-spiked sediments by benthic macroinvertebrates (long-term field studies): NOEC and LOEC values related to SEM/AVS and SEM-AVS, and (SEM-AVS)/f_{oc}.

Taxa	Test-Reference Comp.	Sediment*	Dura-tion	Criterion	SEM* (mmol/kg d.w.)	SEM/AVS* (molar ratio)	SEM-AVS* (molar difference)	(SEM-AVS)/f_{oc}* (mmol/kg_{oc})	[footnote]
Zinc-spiked freshwater sediment (field study at one site in the United States)									Liber et al., 1996 [1]
<u>Overall data</u>	$ZnCl_2$								
Chironomidae		pond sediment,	1-yr	$NOEC_{eco}$	11.9 (actual)	1.1	1.0	9.1	
Oligochaeta,		background SEM			11.2 (actual-Cb)	1.0	0.3	2.7	
Bivalvia,		0.7 mmol/kg d.w.							
Nematoda		(60% Zn *(molar)*)							
		f_{oc}: 0.11							
<u>Data per sampling period</u>									
July 1993		background SEM		$NOEC_{eco}$	12.4 (actual)	1.6	4.5	41.3	
		0.7 mmol/kg d.w.			11.7 (actual-Cb)	1.5	3.8	35	
August 1993		background SEM		$NOEC_{eco}$	12.8 (actual)	1.1	-0.4	-3.7	
		0.7 mmol/kg d.w.			12.1 (actual-Cb)	0.9	-1.1	-10	
October 1993		background SEM		$NOEC_{eco}$	2.3 (actual)	0.5	-2.8	-25.7	
		0.4 mmol/kg d.w.			1.9 (actual-Cb)	0.4	-3.2	-29	
				$LOEC_{eco}$	5.3 (actual)	0.6	-3.5	-32.1	
					4.9 (actual-Cb)	0.6	-3.9	-36	
May 1994		background SEM		$NOEC_{eco}$	14.0 (actual)	1.3	3.2	29.4	
		0.8 mmol/kg d.w.			13.2 (actual-Cb)	1.2	2.4	22	
July 1994		background SEM		$NOEC_{eco}$	4.7 (actual)	0.7	-2.8	-25.7	
		0.7 mmol/kg d.w.			4.0 (actual-Cb)	0.5	-3.5	-32	
				$LOEC_{eco}$	11.0 (actual)	1.1	1.1	10.1	
					10.3 (actual-Cb)	1.0	0.4	3.7	

To be continued

Table 3.3.2.f- (continued) Part C. Colonisation of zinc-spiked sediments by benthic macroinvertebrates (long-term field studies): NOEC and LOEC values related to SEM/AVS and SEM-AVS, and (SEM-AVS)/f_{oc}.

Taxa	Test-Reference Comp.	Sediment	Dura-tion	Criterion	SEM* (mmol/kg d.w.)	SEM/AVS* (molar ratio)	SEM-AVS* (molar difference)	(SEM-AVS)/f_{oc} ($mmol/kg_{oc}$)	[footnote]	
Zinc-spiked freshwater sediments (field studies at four different sites in Europe)									Burton et al. 2003 [2]	
	$ZnCl_2$									
Pallanza		river,	6-w	$LOEC_{eco}$	2.8 (actual)	43	2.7	1503		
sampling period: September 2002		background SEM					2.5 (actual-Cb)		41	2.4
1333										
Oligochaeta,		0.3 mmol/kg d.w.								
Chironominae		**f_{oc}: 0.0018**								
Tanypodinae										
Caenidae										
Dryopoidae										
Ankeveen		lake,	12-w	$NOEC_{eco}$	7.2 (actual)	0.2	-28.3	-316		
sampling period: June 2002		background SEM			5.3 (actual-Cb)	0.1	-30.2	-338		
Ceratopogonidae		2.0 mmol/kg d.w.		$LOEC_{eco}$	33.0 (actual)	0.7	0	0		
Phryganea		**f_{oc}: 0.02-0.09**			31.0 (actual-Cb)	0.9	-2.0	-576		
Oecetis										
Sphaeridae										
Bythinia										

To be continued

Table 3.3.2.f- (continued) Part C. Colonisation of zinc-spiked sediments by benthic macroinvertebrates (long-term field studies): NOEC and LOEC values related to SEM/AVS and SEM-AVS, and (SEM-AVS)/f_{oc}.

Taxa	Test-Reference Comp.	Sediment	Dura-tion	Criterion	SEM* (mmol/kg d.w.)	SEM/AVS* (molar ratio)	SEM-AVS* (molar difference)	(SEM-AVS)/f_{oc} ($mmol/kg_{oc}$)	[footnote]
Zinc-spiked freshwater sediments (field studies at four different sites in Europe) (continued)									Burton et al. 2003
	$ZnCl_2$								[2]
Schmallenberg		lake,							
Oligochaeta		**f_{oc}: 0.03-0.08**							
Chironominae									
Tanypodinae									
Ceratopogonidae									
Haliplus									
sampling period									
June 2002		background SEM	12-w	$NOEC_{eco}$	17 (actual)	1.5	6.0	61	
		0.6 mmol/kg d.w.			16.4 (actual-Cb)	1.5	5.4	55	
September 2002		background SEM	24-w	$NOEC_{eco}$	2.1 (actual)	0.5	-2.3	-43	
		1.0 mmol/kg d.w			1.1 (actual-Cb)	0.3	-3.4	-87	
				$LOEC_{eco}$	9.9 (actual)	1.8	4.5	92	
					8.9 (actual-Cb)	1.6	3.5	72	
December 2002		background SEM	37-w	$NOEC_{eco}$	5.2 (actual)	0.6	-3.8	-44	
		1.1 mmol/kg d.w			4.1 (actual-Cb)	0.5	-4.9	-58	
				$LOEC_{eco}$	10.2 (actual)	1.7	4.1	52	
					9.1 (actual-Cb)	1.5	3.0	38	

To be continued

Table 3.3.2.f- (continued) Part C. Colonisation of zinc-spiked sediments by benthic macroinvertebrates (long-term field studies): NOEC and LOEC values related to SEM/AVS and SEM-AVS, and (SEM-AVS)/f_{oc}.

Taxa	Test-Reference Comp.	Sediment	Dura-tion	Criterion	SEM* (mmol/kg d.w.)	SEM/AVS* (molar ratio)	SEM-AVS* (molar difference)	(SEM-AVS)/f_{oc} ($mmol/kg_{oc}$)	[footnote]
Zinc-spiked freshwater sediments (field studies at four different sites in Europe) (continued)									Burton et al. 2003 [2]
	$ZnCl_2$								
Biesbosch		riverine, **f_{oc}: 0.01-0.09**							
sampling period									
June 2002		background SEM	12-w	$LOEC_{eco}$	3.5 (actual)	1.6	1.3	14	
		1.5 mmol/kg d.w			2.0 (actual-Cb)	0.9	-0.2	-2.5	
September 2002		background SEM	24-w	$NOEC_{eco}$	4.0 (actual)	2.9	2.6	154	
		0.8 mmol/kg d.w			3.2 (actual-Cb)	2.4	1.9	111	
				$LOEC_{eco}$	10.0 (actual)	8.3	8.8	576	
					9.2 (actual-Cb)	7.7	8.0	526	
December 2002		background SEM	37-w	$LOEC_{eco}$	3.9 (actual)	2.3	2.2	148	
		0.7 mmol/kg d.w			3.2 (actual-Cb)	1.9	1.5	101	

Table 3.3.2.f- Part D. Colonisation of cadmium-spiked and metal-spiked sediments by benthic macroinvertebrates (long-term field and laboratory studies): NOEC and LOEC values related to SEM/AVS and SEM-AVS, and (SEM-AVS)/f_{oc}.

Taxa	Test-Reference Comp.	Sediment	Dura-tion	Criterion	SEM* (mmol/kg d.w.)	SEM/AVS* (molar ratio)	SEM-AVS* (molar difference)	(SEM-AVS)/f_{oc} (mmol/kg_{oc})	[footnote]
Cadmium-spiked freshwater sediment (field study)									
Chironomidae, Diptera, Oligochaeta Nematoda	$CdCl_2$	lake sediment, background SEM 0.7 mmol/kg d.w. (75% Zn *(molar)*) **f_{oc}: 0.05 default; no actual data**	14-m	$NOEC_{eco}$	5.7 (nominal+Cb) 5.0 (nominal)	11.4 10	5.2 4.5	104 90	Hare et al. 1994 [3]
Cadmium-spiked marine sediment (laboratory study)									
Crustacea (Harpacticoda), Nematoda, Annelida, Chordata, Amphipoda	$CdCl_2$	marine sediment, background SEM 3.2 mmol/kg d.w. (60% Zn *(molar)*) **f_{oc}: 0.01**	17-w	$NOEC_{eco}$ $LOEC_{eco}$	4.9 (nominal+Cb) 1.7 (nominal) 17.2 (nominal+Cb) 14.0 (nominal)	0.3 0.1 0.9 0.7	-10 -13 -2.9 -6.1	-1000 -1300 - 290 - 610	Hansen et al. 1996b [4]
Metal mixture (Zn,Ni, Cd, Pb, Cu)-spiked marine sediment (field study)									
Annelida (primarily Polychaeta) Mollusca (Bivalvia and Gastropoda) Crustacea (primarily amphipoda)	-	marine sediment background SEM **pm (plus main metal)** **f_{oc}: 0.01**	4-m	$NOEC_{eco}$	27 (nominal)	3	18	1800	Boothman et al. 2001 [5]

Footnotes Table 3.3.2.f – Part C and D

$NOEC_{eco}$: "Overall" $NOEC_{ecosystem}$ (does not exclude effects on lower taxa, such as the species, genus or family level; see footnotes).

Note: Bioaccumulation data from the above studies have not been evaluated.

* Further information on AVS and SEM concentrations:

Liber et al. 1996: SEM and Cb are total SEM (i.e. all metals measured during the study), but at the NOEC and LOEC nearly all SEM is "SEM"-zinc, the only metal added. The SEM/AVS and SEM-AVS values were calculated from the AVS level at the given exposure concentration. (SEM-AVS)/fOC-values are calculated with overall fOC of 0.11. No temporal trend was reported for fOC-values.

Burton et al. 2003: SEM is total SEM (i.e. all metals measured during the study). The SEM/AVS and SEM-AVS values were calculated from the AVS level at the given exposure concentration. For Cb SEM, SEM in the control is taken. Foc-data are based on Table 1, SEM/AVS-data on Table 2 and (SEM-AVS)/fOC-values are based on Table 4 and of the original report.

Hare et al. 1994: SEM is "SEM"-cadmium; Cb is total SEM (i.e. all metals measured in the control sediment). The SEM/AVS and SEM-AVS values were calculated from the control AVS level. The actual AVS level in Cd-spiked sediment may be higher (see e.g. Liber et al., 1996) and thus the SEM/AVS and SEM-AVS values may be lower.

Hansen et al.1996: SEM is "SEM"-cadmium; Cb is total SEM (i.e all metals measured in the control sediment). The SEM/AVS and SEM-AVS values were calculated from the AVS level at the given exposure concentration. The background total-SEM concentration (3.2 mmol/kg .d.w.) is higher than the added "SEM"-Cd concentration at the NOEC level. Therefore the results were expressed as nominal concentrations, to allow an estimate of the total SEM concentration. The actual "SEM"-cadmium concentrations (NOEC: 1.5 mmol /kg d.w. and LOEC 12 mmol/kg d.w.) were very similar than the nominal values.

Boothman et al. 2001: SEM is total SEM (in this study, equimolar quantities of Zn, Ni, Cd, Pb, and Cu were added to the sediment. The nominal test concentrations, expressed as total SEM/AVS molar ratios, were 0.1-0.8-3.

Footnotes

[1] Liber et al. 1996: A 1-yr field study (July 1993 to July 1994) on the in situ colonisation of zinc-spiked freshwater sediments by benthic macroinvertebrates. Major taxa in the natural environment (in order of decreasing numbers): Chironomidae (insects), Bivalvia (bivalve molluscs), Oligochaeta (annelid worms) and Nematoda (roundworms). The abundances (number of animals: mean ± SD) for each of these taxa, as well as the abundances for some Chironomidae and Oligochaeta families and for all taxa together (total abundance) were determined at the five different sample times during the study. Statistics: $p = 0.05$ (unpaired t-test), comparing each treatment group with the manipulated control (CM). Furthermore, community diversity and similarity indices were calculated at the taxonomic levels of families and higher (also compared to CM). The bioaccumulation of zinc was studied in Chironomidae.

Field location: An unpolluted 2-ha mesotrophic pond near Duluth (Minnesota, U.S.). The lake has a maximum depth of 4-5 m and a rich and diverse invertebrate fauna. Exposure occurred in a 6.5 x 6.5 m enclosure, at a water depth of 40-90 cm.

Sediment characteristics: Sand 59%, silt 35%, clay 6%, TOC 11% (corresponding to 19% OM), moisture content 72%, AVS concentration 3.5 mmol/kg dry weight and SEM concentration 0.65 mmol/ kg dry weight, of which 60% is zinc (0.38 mmol/kg d.w., corresponding to 25 mg/kg d.w.). These data (for the 0-6 cm horizon) are based on measurements during the test. Pre-test samples collected in the pond in February and May 1993 showed AVS concentrations varying from 0.4 to 8 mmol S/kg dry weight and TOC concentrations from 1 to 11%, depending on the place and time of collection.

Pond water characteristics: no data.

Porewater characteristics: pH 6.8-7.1, hardness 160-315 mg/l (as $CaCO_3$), ammonia concentration 0.8-4.3 mg/l, and dissolved organic carbon (DOC) concentration 215 mg/l. The hardness and ammonia concentration increased with depth (analyses at sediment depth of 1, 3 and 5 cm).

Methods: Sediment was collected with a 15x15 grab sampler from the 90 predesignated tray locations within the enclosure, sieved, pooled and homogenised. Subsamples of the sediment were spiked by adding zinc chloride in distilled water. Stabilisation of the spiked sediments was determined by monitoring the concentration of zinc in the porewater over a 9-day period. In this period the sediments were homogenised daily. After this period the samples were transferred to 4-liter test trays that were placed in the test location in the pond on 1 July 1993. The trays contained 4 kg of sediment and had holes covered with 1-mm mesh screens to allow for aqueous and gaseous diffusion and for colonisation from below the sediment surface. The nominal test concentrations, expressed as SEM, were 0.8 (control)-1.5-3-6-12 µmol/kg d.w. The control included a manipulated control (CM: sediment handled as the zinc-spiked sediments) and an unmanipulated control (CU: sediment undisturbed until sampling) along with the five treatment groups (Z1 to Z5).

The trays for invertebrate determinations were sampled on 22 July 1993 (day 21), 23 August 1993 (day 53), 1 October 1993 (day 92), 13 May 1994 (day 317) and 6 July 1994 (day 371).

Replicates: A total of 105 trays were used: 15 trays per treatment (3 replicates for each of the 5 sampling dates).

Metal and AVS analyses during the test: SEM and AVS concentrations in sediment and zinc concentrations in porewater were determined on each of the five sampling dates. The sediment samples were taken from the 0-6 cm horizon of the intact cores (sampled under water, in situ) and divided into 2-cm sections. Porewater samples, also collected in situ, were taken at sediment depths of 1, 3, and 5 cm.

Actual SEM concentrations in the 0-2 cm horizon of the sediment: 0.7 (control: CU and CM))-0.8-1.4-2.4-5.4-11.9 mmol/kg dry weight (arithmetic mean of the five measurements, which showed minimal temporal changes at a given treatment), corresponding to 0.4 (control)-0.5-1.1-2.1-5.2-11.6 mmol "SEM"-Zn /kg d.w., equal to 25 (control)-33-72-140-340-750 mg "SEM"-Zn / kg d.w.

The AVS concentrations in the 0-2 cm horizon of the sediment: 3.5 (control: CU and CM)-4.4-4.8-5.2-7.5-10.9 mmol/kg dry weight (arithmetic mean of the five measurements, which also showed minimal temporal changes at a given treatment: maximum difference of a factor of 2). These and the further data on AVS concentrations show that the concentrations increase with increasing sediment depth and increasing SEM concentration. The total range of AVS concentrations in the three horizons ranged from 2.4 to 15 mmol/kg d.w. (factor 6 difference).

Note: The SEM and AVS concentrations in the unmanipulated control (CU) and manipulated control (CM) were very similar. Additional data on the SEM concentrations (averaged over the three sediment depths) and AVS concentrations (in the 2-4 and 4-6 cm sediment horizon) were not reported in detail but in graphical representations.

Molar SEM/AVS ratios in the 0-2 cm horizon of the sediment: 0.3 (control)-0.3-0.3-0.5-0.8-1.1.

At the highest concentration in sediment (750 mg Zn/kg d.w.), the porewater zinc concentrations on the first sampling date (3 weeks after the sediment trays were placed in the pond) were 18, 160 and 210 µg Zn/l at sediment depths of 1, 3 and 5 cm, respectively. One month later and onwards, porewater concentrations usually were below the detection limit of 6 µg/l (as well as the porewater concentrations at the lower exposure levels); the maximum concentration in this period was 30 µg/l.

Toxicity results: On all sampling dates, abundances of the different taxa in zinc-spiked sediments generally were similar to those in control sediments. However, the total abundance (all taxa) and the abundance of a number of individual taxa were lower, although not statistically significant, at the highest SEM concentration (11.9 mmol/kg d.w., corresponding to 750 mg "SEM"- Zn/kg d.w.) on the first two sampling dates. This initial effect is in conformity with the initial high porewater zinc concentrations measured. On the following sampling dates, the abundances at this concentration were usually similar or higher than the control values. The total abundance at the third sampling date was reduced, although again not statistically significant, due to a reduction in Chironomidae. Significant reductions of abundance were only observed for the Oligochaeta family Naididae (absent or significantly reduced at the SEM concentration of 11.9 mmol/kg d.w. on the third and fifth sampling date and at the SEM concentration of 5.4 mmol/kg d.w. (corresponding to 340 mg "SEM"-Zn/kg d.w.) on the third sampling date and for the Oligochaeta family Tubificidae (significantly reduced at the SEM concentration of 11.9 mmol/kg d.w. on the fifth sampling date. Community compositions (e.g. similarity, diversity, evenness) in zinc-spiked sediments also strongly resembled those in control sediments. Based on all data, including the normal variation throughout the study year, the highest test concentration (SEM concentration 11.9 mmol/kg d.w.) is considered as $NOEC_{ecosystem}$.

The toxicity results are in conformity with the low amounts of accumulated zinc in Chironomidae (determined on 3 of the 5 sampling dates) and the low zinc concentrations in porewater.

Liber et al. 1996 – Additional information

AVS and "SEM"-Cd measurements outside the test trays were made in undisturbed sediment cores (CU locations) approximately monthly throughout the study. The AVS concentrations (analysed in the 0-2, 2-4, and 4-6 horizon) ranged from 2 to 20 mmol/kg d.w. (factor 20 difference), with a peak in the winter months.

Measurements of dissolved oxygen (DO) in the water under the ice in January and February 1994 showed DO levels as low as 0.1-0.5 mg/l at 5-10 cm above the sediment surface and 0.7-0.8 mg/l at 30-50 cm above the sediment surface. In March, the DO levels were around 1 mg/ at both depths and in April around 9 mg/l. The data suggest that the system may temporarily have approached anoxia. On basis of SEM/AVS-ratios, no great differences between manipulated control and zinc-spiked sediments are expected, i.e. only in the highest tested concentrations SEM/AVS-ratios of >1 were found. However, even SEM/AVS-ratios in the highest tested concentrations were near 1 (0.7-1.6).

[2] Burton et al. 2003: long-term field studies (2002) on the in situ colonisation of zinc-spiked sediments by benthic macroinvertebrates.

Further information provided by the authors of the study in addition to the study report has been included in the evaluation of the study.

Four sites were selected that represented a range of freshwater systems with a range of acid volatile sulphides- (AVS-) levels. Test sites included two lakes and two rivers with varying levels of AVS, grain size distribution, and other physicochemical parameters. Selected sites were Rio Bugnano, Oggebio, Italy (river with low AVS); Biesbosch/River Meuse, the Netherlands (riverine with high AVS); Schmallenberg, Germany (lake with lower AVS) and Ankeveense Plassen, Utrecht, the Netherlands (lake with high AVS).

	flow	SEM (mmol/kg DW)	AVS (mmol/kg DW)	TOC (%)	US soil classification
Schmallenberg	none	1.4	0.5	3-8	silty loam
Biesbosch	low	2.5	11.0	1-9	sand/ loamy sand
Ankeveen	low/none	1.7	31.7	2-9	clay
Pallanza	high	0.4	<0.03	0.18	sandy loam/ loamy sand

Sediment samples were taken from the Schmallenberg site to be spiked with a range of zinc concentrations in order to perform 14-day bioassays with *Chironomus riparius*. Based on the results of the bioassays it was decided to spike the sediments of the four sites at 400 mg zinc/kg and at 1200 mg/kg to capture a no-effect and high-effect range that bracketed AVS:SEM ranges found at the test sites during preliminary AVS- and SEM-sampling.

Methods: Sampling for zinc spiking for usage in the colonisation study took place early March 2002. All sediment samples were spiked with zinc chloride. The sediments were stored under nitrogen gas. Subsamples were collected and analysed for AVS, SEM and dry weight, prior to spiking and after spiking. Sediments spiked with Zn or water were returned to their original collection site on March 22 and the following 7 days. At the site, the sediments were mixed and samples were added to colonisation and *in situ* toxicity trays. A sediment subsample was retained for AVS/SEM analyses.

Trays used to study colonisation were 16*11* 6 cm or 10.5*15.5*6 cm (surface area 175 cm^2 and 162 cm^2 resp.). Four replicate trays were used for each treatment (low and high zinc level and unspiked) and for each of three exposure periods. Colonisation trays were placed in wired baskets to secure the colonisation trays. Openings on the wire baskets were large, i.e. 1.6 by 3.5 cm openings. When possible, the baskets were dug into the sediments to place the top of the colonisation trays at the level of the original sediment surface. Colonisation was allowed for 11-12 (sampling in June), 23-24 (sampling in September) and 37 weeks (sampling in December/January). The tray design did not work for the Italian situation (fast flowing river). The sediment was eroded from the tray over time. The colonisation experiment was started again at the end of July 2002 with similar colonisation trays but now the trays were covered with finer mesh (mesh openings of 4 mm). Due to experimental misfortune, colonisation could only be determined at one period at the Italian site; after 6 weeks of colonisation (September). Due to unfortunate destruction of colonisation trays, only the first sampling period succeeded for the Ankeveen site (11-12 weeks colonisation period, sampling in June).

During each sampling period, 4 colonisation trays from each treatment were sacrificed. The content of each tray was transferred into a bottle containing 70% ethanol. In addition, grab sampling took place from sediments located within 1 meter of the colonisation trays. Sediments were subsampled for chemical analysis. As written in the report, samples for chemical analysis were taken after ethanol was added to the sediment. Sediments were analysed for AVS, SEM, NH_3, and organic carbon. Benthic organisms were identified to the lowest practical taxon. Major taxa abundances of control and treatments were compared with ANOVA followed by Tukey's test (sign. differences at $p<0.05$). Additional statistical analyses were performed in the framework of reviewing this study using the Bray-Curtis similarity index, statistically tested using non-parametric permutation based statistics (ANOSIM).

Metal and AVS analyses during the test: solubilised metals during HCl acidification and bulk metals were determined using ICP AES. For analyses of bulk metals, the metals were extracted from the sediments by digestion with several acids in a microwave oven. AVS were removed from solution with a nitrogen purging stream and collected in a gas-washing bottle. Amount of sulphide was determined spectrophotometrically by reaction with N,N-dimethyl-p-phenylenediamine to form methylene blue.

Results:

Pallanza-site (Italy) (only data for one sampling period available): AVS was <0.02 mmol/kg DW in the reference sediments and total zinc 56 mg/kg DW. AVS in the zinc-spiked sediments were similarly low (0.06 and 0.12 mmol/kg DW for low- and high-zinc treatments, respectively). Zinc-levels in the spiked sediments were 175 and 270 mg/kg DW for the low- and the high-zinc sediments, respectively. No data of physical and chemical conditions of background samples were provided.

Colonisation trays were covered with a finer mesh to reduce loss of spiked sediment, but the changed design enhanced build-up of small particles within the trays. The benthic community in the colonisation trays showed higher macroinvertebrate diversity compared to the diversity in the grab samples of adjacent sediments. In the reference samples were present Oligochaeta, Chironominae, Tanypodinae, Ceratopogonidae, Caenidae, Dryopoidae, Trichoptera, Gammarus, Lymnaea, Erpobdella, Nematomorpha and Tricladida present. Species diversity was dramatically decreased in the zinc-treated colonisation trays compared to the reference trays. Oligochaeta, Chironominae and Dryopoida were significantly lower in low-zinc and high-zinc treatments compared to the reference samples. For the Pallanza site, the $NOEC_{ecosystem}$ could not be established and the $LOEC_{ecosystem}$ 175 mg Zn total/kg DW.

Ankeveen-site (Netherlands) (only data of one sampling period available): AVS is much higher in the reference samples compared to the background samples, resulting in higher SEM/AVS ratios (>1) in the background samples. Reference AVS was similar to AVS in the zinc-spiked sediments (all within the range of 33-35.5 mmol/kg DW). Total zinc levels were 131 mg/kg DW in the reference sediment, 296 mg/kg DW in the low-zinc treatment and 913 mg/kg in the high-zinc treatment. SEM/AVS-ratios were low in reference and zinc-spiked sediments, i.e. 0.06-0.7.

Benthic community in the reference trays contained Ceratopogonidae, Phryganea, Oecetis, Sphaeridae and Bythinia. Number of Chironominae and total number of individual organisms was significantly elevated in the high-zinc treatment in comparison to the reference treatments. Significant shifts in feeding mode for both zinc exposures were observed towards a higher proportion of collector/gatherers, caused by an increase in Oligochaeta and Chironominae. Extra statistics using the Bray-Curtis similarity index, statistically analysed using non-parametric permutation based statistics (ANOSIM) showed that both low- and high

zinc-treated colonisation trays differed significantly from the control colonisation trays, although differences between controls and low-zinc treatments were only boarderline. Therefore, $NOEC_{ecosystem}$ and $LOEC_{ecosystem}$ are expected to be both near the lowest zinc-concentration and $NOEC_{ecosystem}$ is considered to be the low-zinc concentration with total zinc of 296 mg/kg DW, SEM of 7.23 mmol/kg DW and SEM/AVS of 0.2 en de $LOEC_{ecosystem}$ the high-zinc concentration with total zinc of 913 mg/kg DW, SEM of 33.0 mmol/kg DW and SEM/AVS of 0.7.

Schmallenberg-site (Germany): Comparison of physical and chemical properties of background samples and reference samples is only possible for the sampling period of June 2002 because background data of the other sampling periods are lacking. Mean particle size of the background samples is smaller compared to the reference samples. Metal concentrations are lowered in the reference samples as well as AVS-levels compared to the background samples. AVS in the reference units ranged between 0.4 and 4.6 mmol/kg DW and SEM between 0.6 and 1.2 mmol/kg DW during the whole colonisation period, resulting in SEM/AVS-ratios of 0.2-2.5. Total zinc-levels in the reference sediments were relatively high: 119-153 mg/kg DW. Zinc-levels in the low-zinc treatment were 345-358 mg/kg DW and in the high-zinc treatment 620-823 mg/kg DW. AVS-levels in the low-zinc and the high zinc treatment were a factor 1.5 to 14 higher compared to the reference. SEM/AVS-ratios in the low-zinc and high-zinc treatments were comparable to SEM/AVS-ratios in reference sediments, ranging from 0.5 to 2.5.

In June, Oligochaeta, Chironominae, Tanypodinae, Ceratopogonidae and Haliplus were present in the reference samples. Zinc-treatment did not significantly affect the benthic community. Therefore, no $LOEC_{ecosystem}$ could be established for the June sampling period and the $NOEC_{ecosystem}$ is considered to be the highest test concentration, i.e. total zinc of 823 mg/kg DW, SEM of 17 mmol/kg DW and SEM/AVS of 1.5.

In September, number of Chironominae and Tanypodinae were significantly higher in the high-zinc treatment colonisation trays. Extra statistics using the Bray-Curtis similarity index, statistically analysed using non-parametric permutation based statistics (ANOSIM) confirmed that the high-zinc treatment differed from the control. Therefore, $NOEC_{ecosystem}$ is considered to be the low-zinc treatment (total zinc of 358 mg/kg DW, SEM of 2.1 mmol/kg DW and SEM/AVS of 0.5) and the $LOEC_{ecosystem}$ (total zinc of 700 mg/kg DW, SEM of 9.9 mmol/kg DW and SEM/AVS of 1.8) as the high-zinc treatment.

In December, number of Tanypodinae was significantly elevated in the low- and high-zinc treatments and numbers of Ceratopogonidae and Haliplus were lowered in the high-zinc treatment compared to the reference sediments. ANOSIM showed only significant differences between the highest zinc-treatment and the control. The $NOEC_{ecosystem}$ is considered to be the low-zinc treatment (total zinc of 345 mg/kg DW, SEM of 5.2 mmol/kg DW and SEM/AVS of 0.6) and the $LOEC_{ecosystem}$ (total zinc of 620 mg/kg DW, SEM of 10.2 mmol/kg DW and SEM/AVS of 1.7) as the high-zinc treatment.

Biesbosch-site (Netherlands): AVS in the reference units ranged between 1.0 and 5.1 mmol/kg DW and SEM between 0.7 and 1.5 mmol/kg DW during the colonisation period, resulting in SEM/AVS-ratios of 0.3-0.7. Zinc levels in the reference sediment were 53-67 mg/kg DW. AVS-levels in the low-zinc and the high-zinc treatments were comparable to AVS-levels in the reference sediment. SEM was elevated compared to the reference sediment resulting in SEM/AVS-ratios between 1.6 and 2.9 for the low-zinc treatment and between 1.6 and 9.7 for the high-zinc levels. SEM/AVS-ratios seemed to increase from June to December. Zinc levels were 617-794 mg/kg DW throughout the colonisation period in the high-zinc treatment (1200 mg/kg DW nominal concentration) and 232-322 mg/kg DW in the low-zinc

treatment (400 mg/kg DW nominal concentration). Background data for physical and chemical variables were only available for the June 2002 sampling period. Water content of the background sediment was clearly higher compared to the reference samples. AVS-level in the background sediment was lower than in the reference samples resulting in higher SEM/AVS-ratios in the background sediment.

In June, Oligochaeta, Chironominae, Tanypodinae, Ceratopogonidae and a high number of other taxa were present in the reference samples. Number of Chironominae was significantly higher in the reference sediment compared to the high-zinc treatment. Number of taxa, number of Caenis and Helobdella were lower in the reference trays compared to the low-zinc treatment. Both low- and high-zinc treatments affected the benthic community, confirmed by additional statistical analysis with ANOSIM. Therefore, $NOEC_{ecosystem}$ for the June sampling period could not be established within this field experiment and the $LOEC_{ecosystem}$ is at the low-zinc level (total zinc of 322 mg/kg, SEM of 3.5 mmol/kg DW and SEM/AVS of 1.6).

In September, Oligochaeta, Caenis, Trichoptera, Polychaeta among many more taxa were present in the reference samples. Number of taxa, number of individuals and number of Ceratopogonidae, chironominae and Unionidae (t1) were significantly elevated in the reference sediments compared to high-zinc treatment. Sphaeriidae were significantly lowered in both low- and high-zinc treatments compared to the reference treatments. Both low- and high-zinc treatment significantly affected the benthic community, although the influence of the low-zinc treatment was only minor. Additional calculations with ANOSIM showed only significant differences between high-zinc treatment and control colonisation trays. Therefore, the $NOEC_{ecosystem}$ is considered to be the low-zinc treatment (total zinc of 69 mg/kg DW, SEM of 4.0 mmol/kg DW and SEM/AVS of 2.9) and the $LOEC_{ecosystem}$ (total zinc of 617 mg/kg DW, SEM of 10.0 mmol/kg DW and SEM/AVS of 8.3) as the high-zinc treatment.

In December, Tanypodinae, Unionidae (t1), Sphaeriidae, Planorbidae, Potamopyrgus, and a number of other taxa were present in the reference colonisation trays. Number of taxa, number of individuals, Chironominae, Ceratopogonidae, Valvata and Potamopyrgus were significantly higher in the reference sediments compared to both low- and high-zinc treatments. Dreissena and Bythinia were present in significantly higher numbers in the reference sediments compared to the high-zinc treatment. Statistics with ANOSIM confirmed that both low- and high-zinc treatment differed significantly from the control. Therefore, $NOEC_{ecosystem}$ for the December sampling period was not established within this field experiment and the $LOEC_{ecosystem}$ was at the low-zinc level (total zinc of 232 mg/kg, SEM of 3.9 mmol/kg DW and SEM/AVS of 2.3).

[3] Hare et al. 1994**:** A 1-yr field study (August 1990 to October 1991) on the in situ colonisation of cadmium-spiked freshwater sediments by macroinvertebrates. Major taxa in the natural environment (in order of decreasing numbers: Chironomidae (insects), Diptera (insects), Oligochaeta (annelid worms) and Nematoda (roundworms). The abundances (number of animals: mean ± SE) for each of these taxa, as well as the abundances for some Chironimidae and Diptera genera or species, some other (minor) taxa and for all taxa together (total abundance) were determined at the end of the study. The bioaccumulation of cadmium was studied in several genera and individual species, after a depuration period in the laboratory. The significance of trends in abundances or bioaccumulation among the five treatments was tested by ANOVA ($p = 0.05$). Variation about mean values is represented by standard deviations (SD) in the case of bioaccumulation data or by standard errors (SE) in the case of the more variable insect abundance data. Statistical data for individual species were reported only for the most abundant species, i.e. insect species.

Field location: A 1 km^2, multibasin, Precambrium shield lake (Lake Tantaré) near Québec City (Canada), located in an ecological reserve.. The lake is ice-covered from around November to May. Exposure occurred at a water depth of 15 m, where the water temperature is 4-6 ^{0}C (year-round).

Sediment characteristics: The test sediment, sampled below the top 0-10 cm of the native sediment, had an AVS concentration of 0.5 mmol/kg d.w. and a SEM concentration of 0.66 mmol/kg d.w., calculated from 0.5 mmol Zn/kg d.w. (33 mg/kg d.w.), 0.08 mmol Pb/kg d.w. (17 mg/kg d.w.), 0.0.5 mmol Cu/kg d.w. (3.2 mg/kg d.w.) and 0.025 mmol Cd (28 mg/kg d.w.). The molar Cd/AVS ratio was 0.05. No further data on sediment characteristics.

In the top 0-10 cm layer of the sediment (not used in the study) the total-zinc concentration was 300 mg/kg dry weigt and the total-lead concentration 200 mg/kg d.w. These relatively high metal levels, which are typical for thousands of shield lakes in southern Ontario and Québec, probably originate from atmospheric fall-out. No data reported on the cadmium concentration in the top layer. The AVS concentration in the top layer was 5 mmol/kg d.w.

Lake water characteristics: Soft (hardness 3 mg/l, as $CaCO_3$) and acidic (pH 5.5-5.6).

Hardness calculated from Ca (20 µmol/l) and Mg (10 µmol/l), resulting in the very low value of 3 mg/l. Incorrect data on Ca and/or Mg?

Porewater characteristics: pH 5.3-5.6 at the sediment-water interface to 6.3 at 5 cm below the interface.

Methods: Sediment was collected with a 30x30x30 grab sampler from the 15 m deep test location. After removal of the top 0-10 cm layer (to eliminate the metal-rich layer and most of the resident animals) the sediment was pooled and homogenised. Subsamples of the sediment were spiked by adding $Cd(NO_2).4H_2O$. The samples were transferred to 8-liter test trays that were placed in the test location in the lake on 31 August 1990. The trays were placed into the bottom sediment, leaving 1 cm visible above the sediment surface, after which the lids were removed. The nominal test concentrations, expressed as molar Cd/AVS ratio, were 0.05-(control)-0.1-0.5-2-10. Based on the control AVS level of 0.5 mmol/kg d.w, this corresponds to nominal cadmium concentrations of 0.025 (control)-0.05-0.25-1-5 mmol/kg d.w., equal to 2.8 (control)-5.6-28-112-560 mg Cd/kg d.w. The actual molar Cd/AVS ratios, determined at the start (before transfer to the lake), were within 10% of the nominal values, except at 0.1 which was actually 0.125 (thus 25% higher than the nominal value). The trays for invertebrate determinations were sampled on 7-11 October 1991.

Replicates: A total of 60 trays were used: 12 trays per treatment, of which 9 replicates per treatment for biological sampling (at termination) and 3 trays per treatment for SEM and SVS analyses (1 on each sampling date).

Metal and AVS analyses during the test: "SEM"-cadmium and AVS concentrations in sediment and zinc concentrations in 0.2 µm filtered porewater were determined at start and on three sampling dates (May, August and October 1991, thus about 9, 12 and 14 months after the start of the study, the last sampling date coincident with the biological sampling date). On each sampling date, one of the trays per treatment was used for sampling. Porewater and overlying-water samples, collected in the intact cores (under water, in situ) were taken at 1-cm intervals, the porewater to a sediment depth of 10 cm and the overlying water to a height of 5 cm above the sediment-water interface. After the transfer of the trays to the lake surface, sediment samples were taken from the 0-9 cm horizon of the intact cores and divided into 3-cm sections.

The results of the "SEM"-cadmium and AVS measurements were mostly reported in graphical representations from with the actual concentrations can not be derived with high accuracy. Therefore, not all information can be given exactly as actual concentrations.

The actual "SEM"-cadmium concentrations in the sediments were close to nominal concentrations and showed minimal temporal changes. The AVS concentrations also showed minimal temporal changes, but increased with increasing depth. The AVS concentrations ranged from 0.1 to 0.4 mmol/kg d.w. (factor 4 difference). Thus, molar Cd/AVS ratios decreased with increasing depth, also resulting in a difference of a factor of 4 at a given "SEM"-cadmium concentration..

At the highest concentration in sediment, the dissolved-Cd concentrations in the porewater were usually 100- 400 nM (11-55 µg/l), either similar at different sediments depths (May and October) or with a peak at around 2 cm sediment depth (August); the concentrations appeared to decrease over time. At the next lower two sediment concentrations the porewater concentrations were usually <20 nM (<2.2 µg/l) and <10 nM (< 1.1 µg/), respectively, and at the lowest two sediment concentrations <5 nM (<0.6 µg/l). The dissolved-Cd concentrations in the overlying water were only "somewhat" elevated at the highest exposure concentration and at the lower exposure levels generally below the detection limit of 0.28 nM (0.03 µg/l).

Toxicity results: The mean total abundance in the test trays, viz. 1,980 (control)-2,080-1,780-2,440-1,770, was not significantly related to cadmium exposure. Taken individually (at the taxon or species level), the abundances of most taxa or species also did not appear to be related to exposure. Only the number of C*hironomus (salinarius gp) sp.,* which is one of most abundant Chironomidae species, were strongly reduced at the highest exposure level (mean number of 9 versus 47 in the control group and 40-63 in the other groups). Larvae of this species burrow deep in the sediment and have there guts filled with sediment, indicating a high exposure via sediment intake. Considering all data, the highest test concentration (SEM concentration 5.7 mmol/kg d.w., i.e the nominal Cd concentration of 5 mmol/kg d.w. plus the background SEM concentration of 0.7 mmol/kg d.w.) is considered as $NOEC_{ecosystem}$.

The toxicity results are in conformity with the low cadmium concentrations in porewater and overlying water.

Hare et al. 1994 – additional data

AVS and "SEM"-Cd measurements outside the test trays were made in undisturbed sediment cores collected on June 1990, both in profundal sediment (from a water depth of 15 m, collected near the test location) and in shallow littoral sediment (from a water depth of 5 m). These sediment cores comprised the 0-15 cm horizon and were divided into 1-cm sections. In these measurements the AVS concentrations also increased with increasing depth, up to around 5 cm depth at which the maximum concentration was found. In the profundal sediment the AVS concentrations ranged from 0.2 – 4 mmol/kg d.w. (factor 20 difference) and in the littoral sediment from 0.8 to 2.4 mmol/kg d.w. (factor 3 difference).

[4] Hansen et al. 1996b: A 17-w laboratory study (4 July – 21 October 1991) on the colonisation of cadmium-spiked marine sediments by benthic macroinvertebrates. The abundances of macroinvertebrates of major taxa that colonised the sediments, viz. Crustaceans (Harpacticoda), Nematoda, Annelida, Chordata and Amphipoda) were usually determined down to the species level. In addition, the abundances of periphyton (diatoms) were determined. Analysis of variance was used to detect differences in the abundances of periphyton and individual macroinvertebrate species and phyla (at $p = 0.05$). Maximum species richness was estimated using the jackknife procedure. Cluster analyses were

performed to compare the kinds of macroinvertebrate species present or absent for each replicate or treatment using the simple matching coefficient.

Sediment characteristics: Silt 70%, clay 24%, sand 6%, TOC 1%, AVS concentration 17.2 mmol/kg dry weight and SEM concentration 3.2 mmol/ kg dry weight, calculated from 2.0 mmol Zn/kg d.w (130 mg/kg d.w.), 0.63 mmol Cu/kg d.w. (40 mg/kg d.w.), 0.29 mmol Pb/ kg d.w. (60 mg/kg d.w.), 0.25 mmol Ni/kg d.w. (15 mg/kg d.w.) and 0.004 mmol Cd/kg d.w (0.4 mg/kg d.w), resulting in a SEM/AVS ratio of 0.2. The sediment was sampled from Long Island Sound (Milford, U.S.) and has proven biologically acceptable as control sediment in previous U.S. EPA tests.

Overlying water characteristics: Unfiltered seawater (salinity 29-32 ^{0}/oo, temperature 16-24 ^{0}C) from central Long Island Sounds.

Methods: The sediment was defaunted by freezing. Subsamples of the sediment were spiked by adding cadmium chloride in seawater, homogenised and stored for 26 days prior to test initiation. After this period the samples were transferred to aquaria. The aquaria received 4 liter of sediment, resulting in a sediment layer with a depth of 8 cm. The nominal test concentrations, expressed as molar "SEM"-Cd/AVS ratios, were 0-(control)-0.1-0.8-3. Based on the control AVS level of 17.2 mmol/kg d.w., this corresponds to nominal cadmium concentrations of 0-(control)-1.7-14-52 mmol/kg d.w., equal to 0-(control)-190-1,600-5,800 mg /kg d.w.

A flow-through system delivered 200 ml unfiltered seawater per minute to each aquarium (300 volume additions per day) A drain hole maintained water depth over the sediment at 2 cm. Colonisation occurred by the unfiltered seawater that contained planktonic larvae and other life stages of benthic organisms. The aquaria for biological determinations were sampled on day 80 (periphyton: diatoms) and day 118 (invertebrates). Those for chemical analyses on days 14, 28, 56, and 117.

Replicates: A total of 48 aquaria were used: 12 aquaria per treatment, of which 9 replicates for biological sampling (at termination) and 4 aquaria per treatment for "SEM"-cadmium and AVS analyses (1 for each sampling date).

Metal and AVS analyses during the test: :"SEM"-cadmium and AVS concentrations in sediment and cadmium concentrations in porewater were determined on day 0 and on each of the four sampling dates during the study (days 14, 28, 56, and 117). Porewater samples, collected in intact sediment cores in the aquaria (under water) were taken just below the sediment-water interface and at a sediment depth of 6 cm. After removal of the cores, the sediment samples were taken and divided into 0.6 cm sections (0-3 cm horizon) and further into 2 cm sections (3-8 cm horizon), with the exception of the cores sampled on day 14; these cores were divided into a variety of horizons.

Actual "SEM"-cadmium concentrations in the 0-8 cm horizon of the sediment: 0-(control)-1.5-12-44 mmol/kg dry weight (arithmetic mean of the five measurements, which showed minimal temporal changes at a given treatment), equal to 0-(control)-170-1,300-4,900 mg "SEM"-Cd /kg d.w.

Actual AVS concentrations in the 0-8 cm horizon of the sediment: 16.7 (control)-14.9-20.1-16.8 mmol/kg dry weight (arithmetic mean of the five measurements, which also showed minimal temporal changes at a given treatment), resulting in a mean value of 17.2 mmol/kg d.w. The AVS concentrations increased with increasing sediment depth, except at the highest exposure level. The total range of AVS concentrations ranged from <1 to 23 mmol/kg d.w. (factor >20 difference). Also the "SEM"-Cd concentrations increased with increasing sediment depth, but less than the AVS concentrations. As the overall result, the "SEM"-

Cd/AVS ratios in the surface layer of the sediments were considerably higher than in deeper sediment layers (details given in graphical representations).

"SEM"-Cd/AVS ratios in the 0-8 cm of the sediment: 0 (control)-0.1-0.6-2.6, thus within 15% of the nominal values, except the median exposure level (30% lower than the nominal value of 0.8).

The actual cadmium concentrations in the porewater were <4 µg/l (maximum: 7 µg/l) and <6 µg/l (maximum 10 µg/l) in the control and lowest exposure concentrations, respectively (with 70% and 50% of the measurements below the detection limit of 3 µg/l) and increased to 53 µg/l (range 20-157 µg/l) and 108,000 µg/l (range 28,000-174,000 µg/l) in the higher exposure concentrations.

Other analyses during the tests: No data on dissolved oxygen (DO) levels, pH values, or other water characteristics during the tests.

Toxicity results: At the highest exposure concentration (actual "SEM"-Cd 44 mmol/kg d.w, equal to 4.900 mg "SEM"-Cd/kg. d.w.), the total number of different species that colonised the sediment was significantly reduced (19 species versus 37 in the control and 33 or 39 in the other exposure groups), as well as the number of Annelida species. Furthermore, the abundances of Annelida, Crustacea (Harpacticoda) and Nematoda, as well as the abundances of some specific species of Annelida (Polychaete species) and Crustacea (Harpacticoid bb) were significantly reduced (Nematoda were not determined down to the species level). The total abundance (thus the total number of individual organisms) at this concentration was about half that in the control, but the difference was not statistically significant. At the median exposure concentration (actual 12 mmol "SEM"-Cd/kg d.w., equal to 1,300 mg "SEM"-Cd/kg d.w.) the abundances of Nematoda and Annelida (as well as the abundances of some specific polychaete species) were significantly reduced. No significant effects on macroinvertebrate community or abundances were found at the lowest exposure concentration (actual 1.5 mmol "SEM"-Cd/kg d.w., equal to 170 mg "SEM"-Cd/kg d.w.)

The total number of diatoms decreased with increasing concentrations, but was only significantly reduced at the highest exposure level.

Based on all data, the concentration of 1.5 mmol "SEM"-Cd/kg d.w. (170 mg "SEM"-Cd/kg d.w.) is considered as $NOEC_{ecosystem}$. It is noted, however, that the next higher exposure concentrations was eight times higher; thus the NOEC cannot be derived with high accuracy.

[5] Boothman et al. 2001: A four month field study on the *in situ* colonisation of cadmium, copper, lead, nickel and zinc spiked marine sediments by benthic invertebrates. Nominal test concentrations of the spiked sediments, expressed as SEM/AVS molar ratios, were 0.1-0.8-3.

Sediment: Narragansett Bay sediment. Characteristics: 77% silt/clay, 23% sand, 1.2% organic carbon and AVS concentration 9 mmol/kg d.w.

Methods: Sediment from Narragansett Bay was sieved to remove particles larger than 2 mm in diameter and frozen to defaunate. Equimolar quantities of Zn, Ni, Cd, Pb and Cu were added to sediment. Trays of 14 x 35 x 10 cm were filled with control or metal-spiked sediment and placed at approximately 10 m depth late May 1992. Five chemistry and eight biological replicates were prepared for each of the four treatments (control and 3 metal treatments). At the end of the experiment, all biological replicates were retrieved and brougth to the laboratory. Five additional core samples were collected from sediments adjacent to the experimental trays for comparison of ambient biota with those recolonizing the experimental sediments.

Number of individuals and species from each treatment were compared by ANOVA, using a general linearized models procedure on both untransformed and transformed as log(x+1). Where statistically significant differences were found, means of individual classes were compared using Duncan's multiple range test (p=0.05).

Metals and AVS analyses during the test: After 0, 15, 27, 56 and 119 d of exposure, one chemistry replicate was retrieved for chemical analysis of AVS and SEM. Cores were taken from the chemistry replicates, which were divided into horizons of 0-3 cm (surface sediment) and 6-10 cm (subsurface sediment). AVS was measured by a purge-and-trap method with sulfide specific electrode detection, according to Boothman and Helmstetter (1992). Samples of interstitial waters were collected for determination of dissolved metals.

SEM- and AVS-concentrations during the experiment: The addition of metals decreased the concentrations of AVS in the sediment. The authors of the study suggest that this is because the sulfides of nickel and copper do not react with 1 M HCl, thus lowering the amount of sulfide recoverable from the sediment as AVS. Concentrations of AVS decreased with time in surface sediments. Concentrations of SEM did not change with time or depth in the control and SEM/AVS = 0.1 treatment, but decreased with time in surface sediments of the SEM/AVS = 0.8 and 3.0 treatments. The AVS concentration in control sediment was 9 mmol/kg. In the control and SEM/AVS = 0.1 treatments, the SEM-AVS concentration was <0 mmol/kg d.w. through the whole experiment in both horizons. In the SEM/AVS = 0.8 treatment, the SEM-AVS concentration decreased with time from 3.4 to 1.5 mmol/kg d.w. in the surface sediment and from 2.3 to –0.8 mmol/kg d.w. in the subsurface sediment. In the SEM/AVS = 3.0 treatment, the SEM-AVS concentration decreased with time from 16.6 to 8.7 mmol/kg d.w. in the surface sediment and ranged from 18 to 24 mmol/kg d.w in the subsurface sediment without clear decrease.

Toxicity results: The total abundance and the abundances of the different taxa and individual species which colonised the initially defaunted sediments were very similar in all treatments. Major phyla were Annelida and Mollusca. The only statistically significant difference (p = 0.05) among the treatments was the decrease (by almost 70%) of the gastropod *Nassurius trivitatus* in the highest treatment relative to the others.

Based on all data, the highest test concentration (SEM concentration of 27 mmol/kg d.w., based on an AVS concentration of 9 mmol/kg d.w. and a SEM/AVS ratio of 3) is considered as $NOEC_{ecosystem}$.

Table 3.3.2.g. Toxicity of zinc to microbe-mediated processes in anaerobic freshwater sediment: NOEC, EC and IC values *

Toxicol. endpoint	A type	Test-comp	Sediment	OM %	Clay %	Temp. °C	Exp.-time	Criterion	Result (mg Zn/kg/d.w)	
Methane production [2]										
	-	S	$ZnCl_2$	harbour sediment	5	28	20	7 d	EC10	48 (Cn)
				Cb: 800 mg/kg					EC50	110 (Cn)
	-	S	$ZnCl_2$	harbour sediment	18	23	20	7 d	EC10	1,780 (Cn)
				Cb: 800 mg/kg					EC50	4,490 (Cn)
									Van Vlaardingen & Van Beelen '92 [1]	
Mineralization of specific substrates [3]										
Acetate	-	S	$ZnCl_2$	harbour sediment	8	26	20	4 h	NOEC	≥ 3,500 (Cn)
				Cb: 800 mg/kg					Van Vlaardingen en Van Beelen '94 [1]	
4-Clorophenol	-	S	$ZnCl_2$	harbour sediment	4	27	20	4 h	EC10	11 (Cn)
				Cb: 800 mg/kg					EC50	30 (Cn)
									IC10	8 (Cn)
									IC50	21 (Cn)
Benzoate	-	S	$ZnCl_2$	harbour sediment	7	25	20	2 h	EC10	59 (Cn)
				Cb: 800 mg/kg					EC50	180 (Cn)
									IC10	42 (Cn)
									IC50	130 (Cn)
									Van Beelen & Van Vlaardingen '94 [1]	
Chloroform	-	S	$ZnCl_2$	harbour sediment	± 7	± 25	20	7 d	EC10	840 (Cn)
				Cb: 800 mg/kg					EC50	2,600 (Cn)
									IC10	700 (Cn)
									IC50	2,100 (Cn)
Chloroform	-	S	$ZnCl_2$	harbour sediment	± 7	± 25	20	16 d	EC10	25 (Cn)
				Cb: 800 mg/kg					EC50	320 (Cn)
									IC10	11 (Cn)
									IC50	150 (Cn)
									Van Beelen et al. '94a [1] [4]	

* All studies have been rejected for $PNEC_{add, sediment}$ derivation, see below.

Toxicity of zinc to microbe-mediated processes in freshwater sediment

Data on microbial toxicity tests conducted in anaerobic sediment-water systems are summarised in Table 3.3.2.g. The data include methane production (which is the last stage of the anaerobic degradation of organic matter) and the mineralization of specific organic substrates of either natural or anthropogenic origin. The microbial toxicity tests do not include single-species (growth) tests. All tests were conducted in anaerobic 1:1 (w/w) wet sediment-water slurries and used zinc chloride as test compound. The sediment samples, originating from the river Rhine estuary (location Gorinchem, The Netherlands), were collected about 10 years ago at several sampling dates during a period of several years, which explains differences in characteristics such as organic matter content. The 'background' zinc concentration (Cb: 800 mg/kg d.w.) in the sediment is based on the analysis of some (and not all of the samples) and thus may vary as well. This high 'background' Zn concentration is mainly caused by historical pollution, as the Zn concentration in the river Rhine clearly decreased during the last decades.

The results in Table 3.3.2.g include one unbounded NOEC value (≥ 3,500 mg/kg d.w.) and several EC10, EC50, IC10, and IC50 values, reported by Van Beelen and co-workers; all values were expressed as mg Zn/kg dry sediment. The EC10 and EC50 values (the concentrations that cause 10% and 50% inhibition of mineralisation at a certain incubation

time) varied between 11 to 1,780 mg/kg d.w. and 30 to 4,490 mg/kg d.w., respectively. The IC10 and IC50 values (the concentrations that cause 10% and 50% inhibition of the first-order mineralization rate) are 8 to 700 mg/kg d.w and 21 to 2,100 mg/kg d.w., respectively. In contrast to the EC10 and EC50 values, the IC10 and IC50 do not depend on the incubation time and the substrate half-life and, therefore, are considered by Van Beelen and co-workers to be more suitable toxicity endpoints than the EC10 and EC50, see also footnote 1 of Table 3.3.2.g. However, IC10 and IC50 values are not standard criteria in microbial toxicity tests.

It is noted that the two tests for methane production, as well as the two tests for chloroform mineralization, showed highly variable results, although the tests were conducted in similar sediment samples, collected from the same location. In both the methane production tests and the chloroform mineralization tests, however, the sediment samples were collected at different sampling dates and thus (will) show differences in characteristics such as organic matter content (e.g. 5% versus 18% in the methane production tests). According to Van Vlaardingen en Van Beelen (1992) and Van Beelen et al. (1994a), the variation for the same toxicological endpoint can be ascribed to the variation in sorption of zinc.

All tests in Table 3.3.2.g have been rejected for $PNEC_{add,\ sediment}$ derivation, as the test were performed in polluted sediment samples (relevance criterion, see RAR Zn Metal section 3.3.1.1). Furthermore, data on effects of Zn (or other pollutants) on microbe-mediated processes in sediments are limited to the work of Van Beelen and co-workers, thus these tests need further development and discussion. Based on this, no further evaluation of some additional data on IC10 and IC50 values for zinc in sediments, also based on anaerobic mineralization tests performed by Van Beelen and co-workers, has been made. These additional data have been summarised in Janus (1993).

Footnotes Table 3.3.2.g

[1] EC10, EC50, IC10, and IC50 values: reported by the study authors. The EC10 and EC50 values (the concentrations that cause 10% and 50% inhibition of mineralization at a certain incubation time) were calculated using a logistic dose-response curve. According to several publications by Van Beelen and co-workers, the EC10 and EC50 values may increase with exposure time, because resistent species of the microbial community continue to grow and to mineralize the substrate. This occurs when the incubation time is much longer than the control half-live of substrate. When the EC10, the EC50, the incubation time of the toxicity test and the half-life of the substrate mineralization are known, the IC10 and IC50 (the concentrations that cause 10% and 50% inhibition of the first-order mineralization rate) can be calculated. The mathematical derivation is described in detail by Van Beelen et al. (1991). In contrast to the EC10 and EC50 values, the IC10 and IC50 are not dependent on the incubation time and the substrate half life and, therefore, are considered by Van Beelen and co-workers to be more suitable toxicity endpoints than the EC10 and EC50.

All tests were conducted in 1:1 (w/w) wet sediment-water slurries. The harbour sediment (from the river Rhine estuary; location Gorinchem), sampled at 0.1 to 1 m below the sediment-water interface, was primarily composed of methanogenic mud. It is emphasised that sediment samples, including those used in the first two tests (methane production) and the last two tests (chloroform mineralization) were collected on different sampling dates (during a total period of several years regarding all the tests listed in Table 3.3.2.g), which explains differences in sediment characteristics such as organic matter content (calculated from the reported organic carbon content) and differences in test results for the same toxicological endpoint. The pH KCl of the sediment samples ranged from 7.4 to 8.1. The ‘background’ zinc concentration (Cb: 800 mg/kg d.w.) is based on analysis of some (and not all of the samples) and thus may vary as well.

Test compound ($ZnCl_2$; purity ≥ 98%) added as portions of a 100 g/l stock solution in 0.1 N HCl, 2 h before the addition of the substrate.

In the tests, low concentrations of the substrates were used (around 1 to 3 µg/l slurry to simulate environmentally relevant conditions. According to several publications by Van Beelen and co-workers, it has been shown that the use of high substrate concentrations in microbial tests can lead to an underestimation of the sensitivity of the natural process, because high substrate concentrations may result in a unnatural rapid growth of resistent microorganisms, which obscures the effects of the toxicant.

[2] Methane production, mediated by a specific group of strictly anaerobic bacteria, is the last stage of the anaerobic decomposition of organic matter. Without the activity of these bacteria, the degradation of organic matter cannot be carried out completely and acetic acid (and, to a lesser extent, other organic acids) would accumulate. It is noted, however, that inhibition of any of the processes carried out by the microbial community degrading organic matter, can lead to inhibition of the methane production.

[3] Mineralization of specific substrates: measured by CO_2 production and/or substrate disappearance.

[4] The organic matter content (± 7%) and clay content (± 25%) are based on the ranges reported in Van Beelen et al. (1994a): 5-9% (*reported as 3-5% organic carbon*) and 23-28%, respectively. Specific data for the two different samples were not reported.

Data on the chloroform mineralization tests were originally published in RIVM-report 714206002. According to the data in that report (cited in Janus, 1993), the EC10 and EC50 of 25 and 320 mg/kg, for added zinc (Cn), correspond to an EC10 and EC50 of 452 and 955 mg/kg, respectively, for total zinc, i.e. for the background zinc concentration (Cb) plus added zinc (Cn). The concentration-response curves indicate that the background concentration of zinc in the sediment also contributes to the effect, although less than the added concentration of zinc.

List of abbreviations Table 3.3.2.e to Table 3.3.2.g

A — Analysis of zinc in test sediment:
+: Zinc analysed.
-: Zinc not analysed or: no data reported on analysis.

Test type — S: static; R: renewal; F: flow-through (continuous flow).

Exposure time — d: day(s); h: hour(s); m: month(s); min.: minute(s); w: week(s); yr: year(s).

Criterion — LC50: Median lethal concentration, i.e. the concentration which is calculated from a series of test concentrations to cause mortality in 50% of the organisms exposed to that concentration.
EC50: Median effect concentration, i.e. the concentration which is calculated from a series of test concentrations to cause a particular response in 50% of the organisms exposed to that concentration.
EC(..%): At the concentration indicated (usually the only concentration tested), the toxicological endpoint was inhibited by ..%. Example: EC (21%).
NOEC: No observed effect concentration, i.e the highest concentration (in a series of test concentrations) without effect. If a statistical analysis of the toxicity data was reported, the NOEC is the highest concentration showing no statistically significant (at $p < 0.05$) effect compared to the control.
If no statistical analysis of the data was reported, the NOEC is the highest concentration showing less than 10% effect compared to the control.
In subscript the toxicological endpoint or endpoints are indicated at each NOEC (e.g. $NOEC_g$ is NOEC for growth; $NOEC_{r,s}$ is NOEC for reproduction and survival).
$NOEC^e$: NOEC values marked by "superscript e" are EC10 values (considered to be equivalent to NOEC values) or have been estimated from the LOEC (lowest observed effect concentration) in case the "real" NOEC could not be derived directly from the data reported.
$NOEC^e$ values have been estimated mainly if more than one test was available for one test
species, resulting in both NOEC and LOEC values for the same toxicological endpoint, to allow the calculation of the geometric mean NOEC relating for this endpoint.
The following application factors have been used to derive a $NOEC^e$:
- in case the LOEC resulted in 11% to 20% effect: factor of 2;
- in case the LOEC resulted in 21% to 30% effect: factor of 3.
See next page for further explanation of the derivation of NOEC and $NOEC^e$ values.

Table 3.3.2.e – Part 1: The NOEC value that has been **printed bold** and **underlined** has been used to derive the Predicted No Effect Concentration for zinc in sediment ($PNEC_{add, sediment}$) see RAR Zn Metal section 3.3.2.2.3).

NOEC values: ≥ Unbounded NOEC, i.e. no effect was found at the highest concentration used in the test) (thus the "real" NOEC may be higher). Unbounded NOEC values are not used for $PNEC_{add}$ derivation.

Cn Nominal zinc concentration in test sediment.
Cb Background zinc concentration in test sediment.
actual Analysed zinc concentration in test sediment.

See next page for data on the selection, derivation and reliability of chronic NOEC values. For additional data on the selection of the chronic NOEC values, based on reliability and relevance criteria, see RAR Zinc Metal section 3.3.1.1 (sources and selection of ecotoxicological data) and section 3.3.2.1 (Toxicity to aquatic organisms).

Selection of chronic NOEC values (RAR Zn Metal section 3.3.1.1)
For the selection of chronic NOEC values used to derive $PNEC_{(add)}$ values, the following approach has been taken:

- Toxicological endpoints, which may affect the species at the population level, are taken into account. In general, these endpoints are survival, growth and reproduction. The toxicity results are commonly expressed as an acute LC50 or EC50 (usually derived from toxicity tests with a duration of four days or less) or as a chronic NOEC (usually derived from toxicity tests with a duration of more than four days). With respect to the NOEC values it is noted that the fact whether or not a NOEC is considered a chronic NOEC is not determined exclusively by the above exposure time limit of four days, but also by the generation time of the test species. For unicellular algae and other microorganisms (bacteria; protozoa), an exposure time of four days or considerably less already covers one or more generations, especially in water, thus for these kinds of species, chronic NOEC values may be derived from experiments during less than four days. On the other hand, for organisms that have a long generation time, for example fish, an exposure time of just over four days is much too short to derive a chronic NOEC. It will be clear that for PNEC derivation a full life-cycle test, in which all relevant toxicological endpoints are studied, is normally preferred to a test covering not a full life cycle and/or not all relevant endpoints. However, the results of a test, which is more limited than a full life-cycle test may be used, see further the points below.
- If for one species several chronic NOEC values based on different toxicological endpoints are available; the lowest value is selected.

Derivation of NOEC values (RAR Zn Metal section 3.3.1.2)
The methods that have been used for the derivation of NOEC values, being "real" NOEC values or NOEC values derived from effect concentrations, are essentially the same as outlined in the EU TGD (Part II, Chapter 3, Table 15)(EC, 2003) .

If possible, "real" NOEC values were derived from the data reported, i.e. the NOEC is one of the concentrations actually used in the test. In order of preference:

1) Statistical analysis: the NOEC is the highest concentration (in a series of test concentrations) showing no statistical significant effect (inhibition) compared to the control. Significance level: $p = 0.05$ (optional: the $p = 0.01$ level if reported instead of the $p = 0.05$ level).
2) If no statistical analysis has been applied: the NOEC is the highest concentration that results in $\leq$ 10% inhibition compared to the control.

In both cases there must be a consistent concentration-effect relationship, i.e the LOEC is the concentration at which and above which statistical significant toxicity is found (1) or, when no statistical analysis has been applied (2), >10% inhibition is found.

If the "real" NOEC could not be derived from the data reported, the following procedure was used to derive the NOEC. In order of preference:

1) The NOEC is set at the EC10 level.
   a) Especially in more recent references on ecotocological data there is increasing preference for the benchmark dose approach. Hence, a benchmark dose (usually the EC10) was reported in a number of references instead of the NOEC. The EC10, which is calculated from the concentration-effect relationship, is used as NOEC equivalent, unless the "real" NOEC was also reported or could be derived from the data reported.
   b) Furthermore, a number of EC10 values was calculated by the rapporteur; the EC10 values were derived from a logistic, sigmoidal dose response model according to Haanstra et al. (1985):

$$Y = c / \{1+\exp [b.(X - a)]\}$$

2) The NOEC is derived from the LOEC
   If the EC10 was not reported and could not be calculated, the NOEC was derived from the LOEC using the following "extrapolation" factors:
   a) NOEC = LOEC/2, in case inhibition is >10% but ≤20%, e.g. LOEC = EC(15%).
   b) NOEC = LOEC/3, in case inhibition is >20% but ≤30% e.g. LOEC = EC(25%).
   If the percentage inhibition at the LOEC is >30% or in case the percentage inhibition at the LOEC is unknown, no NOEC is derived.

With respect to "rule 2b" it is noted that the EU TGD does not mention the derivation of a NOEC from a LOEC in case inhibition at the LOEC is >20%, while in this RAR the derivation of a NOEC from a LOEC up to 30% effect has been used in some aquatic toxicity studies. The use of the higher effect level is justified by the use of a higher extrapolation factor.

Reliability of NOEC values (RAR Zn Metal section 3.3.2.1)
The NOEC values (including EC10 values) that are useful for $PNEC_{add, sediment}$ derivation have been checked for reliability on the basis of the range of test concentrations, as follows:

- If the NOEC is <100 mg/kg dry weight, the separation factor between the NOEC and LOEC should not exceed a factor of 3.2.
- If the EC10 is used as NOEC equivalent, the EC10 should not be more than 3.2-times lower than the lowest concentration used in the test.

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ANNEX 3.3.3.A . TERRESTRIAL TOXICITY DATA BASE

Table 3.3.3.a. Toxicity of zinc to soil microbe-mediated processes: NOEC and EC values
Part I: Studies useful for $PNEC_{add.\ terrestrial}$ derivation
Part II: Studies not useful for $PNEC_{add.\ terrestrial}$ derivation

Table 3.3.3.b. Chronic toxicity of zinc to soil invertebrates: NOEC values
Part I: Studies useful for $PNEC_{add,\ terrestrial}$ derivation
Part II: Studies not useful for $PNEC_{add,\ terrestrial}$ derivation

Table 3.3.3.c. Toxicity of zinc to soil invertebrates: LC50 and EC50 values

Table 3.3.3.d. Chronic toxicity of zinc to soil plants: NOEC and EC values
Part I: Studies useful for $PNEC_{add,\ terrestrial}$ derivation
Part II: Studies not useful for $PNEC_{add,\ terrestrial}$ derivation

Table 3.3.3.a Toxicity of zinc to soil microbe-mediated processes: NOEC and EC values

Part I: Studies useful for $PNEC_{add.\ terrestrial}$ derivation

Toxicologal endpoint	Test-comp	Soil type	pH	OM %	Clay %	Temp. ^0C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)		(Estimated) **NOEC (Cn)** used for $PNEC_{add}$ derivation
C-mineralization (respiration)											
Respiration	$ZnSO_4$	silt loam (+ 1% sludge + 1% alfalfa)	6.9	2	44[e]	25	3-m	EC10	12 19	(Cn) (Cn + Cb)	**17** Chang & Broadbent '81 [4]
Respiration	$ZnSO_4$	silty loam (Crider)	6.7	3	27	20	45-d	NOEC	33	(Cn)	**110** [36a]
	$ZnSO_4$	- (Rifle)	6.2	64	-	20	45-d	NOEC	327	(Cn)	**327**
	$ZnSO_4$	clay (Toledo)	7.0	6	51	20	45-d	NOEC	33	(Cn)	**165** [36b]
	$ZnSO_4$	silty loam (Walla Walla)	7.2	2	21	20	45-d	NOEC	33	(Cn)	**110** [36c]
	$ZnSO_4$	sandy loam (Sharpsburg)	8.2	5	11	20	45-d	NOEC	3	(Cn)	**17**]36d] Lighthart et al '83 [36]
Respiration *(EU soil)*	$ZnCl_2$	sandy loam (+ 1% straw)	5.2	2	8	22	4-w	NOEC	50 >54	(Cn) (Cn + Cb)	**50**
								EC50	2,073 >2,077	(Cn) (Cn + Cb)	
									Saviozzi et al. '95 [32]		
C-mineralization (respiration) of specific substrates											
Acetate *(EU soil)*	$ZnCl_2$	sand (Flevopolder; surface soil)	7.4	1	1	10	18-h	EC10	303 346	(Cn) (Cn + Cb)	**303**
							18-h	EC50	1,510 1,553	(Cn) (Cn + Cb)	
							18-h	IC10	0.7 44	(Cn) (Cn + Cb)	-
							18-h	IC50	12 55	(Cn) (Cn + Cb)	
									Van Beelen et al. 94b [34a]		
Glutamic acid (EU soil)	$ZnCl_2$	humic sand (Wageningen)	5.5	4	-	22?	2-d	NOEC	100	(Cn)	**100** Notenboom & Posthuma '94 [48] Postuma et al., '98
Glutamic acid (EU soil)	$ZnCl_2$	sand (Budel reference soil #11)	3.4	4	12 (1?)	22?	2-d	NOEC	100 111	(Cn) (Cn + Cb)	**100** Notenboom & Posthuma '95 [48] Posthuma et al. '98
Glutamic acid (EU soil)	$ZnCl_2$	sand (PANH)	4.9	2	3	22?	2-d	NOEC	30 53	(Cn) (Cn + Cb)	**30** Notenboom & Posthuma '95 [48, 48a]

(to be continued)

Table 3.3.3.a Toxicity of zinc to soil microbe-mediated processes: NOEC and EC values (continued) Part I: Studies useful for $PNEC_{add.\ terrestrial}$ derivation

Toxicologal endpoint	Test-comp	Soil type	pH	OM %	Clay %	Temp. ^0C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)	(Estimated) **NOEC (Cn)** used for $PNEC_{add}$ derivation
C-mineralization (respiration) of specific substrates (continued)										
Glutamic acid (EU soil)	$ZnCl_2$	sand (PANH)	6.0	2	3	22?	2-d	NOEC	55 (Cn) 71 (actual) Notenboom & Posthuma '95 [48, 48b] Posthuma et al. '98	**55**
Glucose	$ZnCl_2$	sandy clay	6.7	2	4[e]	28	96-h	NOEC	300 (Cn) >314 (Cn + Cb) Ohya et al. '85 [20]	**300**
Glucose *(EU soil)*	$ZnSO_4$	sandy loam	5.7	1	14	20	9-w	NOEC	80 (Cn) 121 (Cn + Cb) Stadelmann & Santschi-Fuhrimann '87 [43]	**80**
Glucose *(EU soil)*	$ZnCl_2$ **1 ***	loamy sand (Gudow; Cb 7 mg/kg)	3.0	9	7	20	3-d	NOEC EC10 EC50	240 (Cn) 247 (Cn + Cb) 256 (Cn) 263 (Cn + Cb) 731 (Cn) 738 (Cn + Cb) Smolders et al. '03 [44,46]	**240**
Glucose *(EU soil)*	$ZnCl_2$ **3 ***	loamy sand (Houthalen, Cb 8 mg/kg)	3.4	3	5	20	3-d	NOEC EC10 EC50	30 (Cn) 38 (Cn + Cb) 33 (Cn) 41 (Cn + Cb) 139 (Cn) 147 (Cn + Cb) Smolders et al. '03 [44,46]	**30**
Glucose *(EU soil)*	$ZnCl_2$ **6 ***	?? (Rhydtalog, Cb 83 mg/kg)	4.8	13	-	20	3-d	NOEC EC10 EC50	800 (Cn) 883 (Cn + Cb) 780 (Cn) 863 (Cn + Cb) 3,569 (Cn) 3,652 (Cn + Cb) Smolders et al. '03 [44,46]	**800**
Glucose *(EU soil)*	$ZnCl_2$ **8 ***	sandy clay (Souli I, Cb 37 mg/kg)	4.8	1	38	20	3-d	NOEC EC10 EC50	100 (Cn) 137 (Cn + Cb) 70 (Cn) 107 (Cn + Cb) 1,018 (Cn) 1.055 (Cn + Cb) Smolders et al. '03 [44,46]	**100**

(to be continued)

Table 3.3.3.a Toxicity of zinc to soil microbe-mediated processes: NOEC and EC values (continued) Part I: Studies useful for $PNEC_{add.\ terrestrial}$ derivation

Toxicologal endpoint	Test-comp	Soil type	pH	OM %	Clay %	Temp. ^0C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)		(Estimated) **NOEC (Cn)** used for $PNEC_{add}$ derivation
C-mineralization (respiration) of specific substrates (continued)											
Glucose *(EU soil)*	$ZnCl_2$ **9 ***	sandy loam (Kövlinge II, Cb 26 mg/kg)	5.1	4	9	20	3-d	NOEC	400 426	(Cn) (Cn + Cb)	**400**
								EC10	124 150	(Cn) (Cn + Cb)	
								EC50	633 659	(Cn) (Cn + Cb)	
									Smolders et al. '03 [44,46]		
Glucose *(EU soil)*	$ZnCl_2$ **11 ***	?? (De Meern, Cb 155 mg/kg)	5.2	17	-	20	3-d	NOEC	1,300 1,455	(Cn) (Cn + Cb)	**1,300**
								EC10	1,238 1.393	(Cn) (Cn + Cb)	
								EC50	3,035 3,190	(Cn) (Cn + Cb)	
									Smolders et al. '03 [44,46]		
Glucose *(EU soil)*	$ZnCl_2$ **12 ***	clay (Aluminusa; Cb 53 mg/kg)	5.4	1	51	20	3-d	NOEC	600 653	(Cn) (Cn + Cb)	**600**
								EC10	549 602	(Cn) (Cn + Cb)	
								EC50	2,068 2,121	(Cn) (Cn + Cb)	
									Smolders et al. '03 [44,46]		
Glucose *(EU soil)*	$ZnCl_2$ **13 ***	?? (Zeveren, Cb 76 mg/kg)	5.7	6	-	20	3-d	NOEC	1,400 1,476	(Cn) (Cn + Cb)	**1,400**
								EC10	227 303	(Cn) (Cn + Cb)	
								EC50	6,936 7,012	(Cn) (Cn + Cb)	
									Smolders et al. '03 [44,46]		
Glucose *(EU soil)*	$ZnCl_2$ **14 ***	sandy clay loam (Woburn, Cb 99 mg/kg)	6.4	7	21	20	3-d	NOEC	300 399	(Cn) (Cn + Cb)	**300**
								EC10	653 752	(Cn) (Cn + Cb)	
								EC50	1,643 1,742	(Cn) (Cn + Cb)	
									Smolders et al. '03 [44,46]		
Glucose *(EU soil)*	$ZnCl_2$ **15 ***	silt loam (Ter Munck, Cb 54 mg/kg)	6.8	2	15	20	3-d	NOEC	50 104	(Cn) (Cn + Cb)	**50**
								EC10	111 165	(Cn) (Cn + Cb)	
								EC50	392 446	(Cn) (Cn + Cb)	
									Smolders et al. '03 [44,46]		
Glucose *(EU soil)*	$ZnCl_2$ **17 ***	silty clay loam (Rots, Cb 51 mg/kg)	7.4	2	27	20	3-d	NOEC	100 151	(Cn) (Cn + Cb)	**100**
								EC10	211 262	(Cn) (Cn + Cb)	
								EC50	568 619	(Cn) (Cn + Cb)	
									Smolders et al. '03 [44,46]		

(to be continued)

Table 3.3.3.a Toxicity of zinc to soil microbe-mediated processes: NOEC and EC values (continued) Part I: Studies useful for $PNEC_{add.\ terrestrial}$ derivation

Toxicologal endpoint	Test-comp	Soil type	pH	OM %	Clay %	Temp. ^0C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)		(Estimated) **NOEC (Cn)** used for $PNEC_{add}$ derivation
C-mineralization (respiration) of specific substrates (continued)											
Glucose *(EU soil)*	$ZnCl_2$ **18 ***	clay (Souli II, Cb 51 mg/kg)	7.4	4	46	20	3-d	NOEC	100	(Cn)	**<u>100</u>**
									151	(Cn + Cb)	
								EC10	189	(Cn)	
									240	(Cn + Cb)	
								EC50	946	(Cn)	
									997	(Cn + Cb)	
									Smolders et al. '03 [44,46]		
Glucose *(EU soil)*	$ZnCl_2$ **19 ***	silt loam (Marknesse, Cb 80 mg/kg)	7.5	2	26	20	3-d	NOEC	100	(Cn)	**<u>100</u>**
									180	(Cn + Cb)	
								EC10	179	(Cn)	
									259	(Cn + Cb)	
`								EC50	517	(Cn)	
									597	(Cn + Cb)	
									Smolders et al. '03 [44,46]		
Glucose *(EU soil)*	$ZnCl_2$ **22 ***	loam (Guadalajara Cb 27 mg/kg)	7.5	1	25	20	3-d	NOEC	100	(Cn)	**<u>100</u>**
									127	(Cn + Cb)	
								EC10	95	(Cn)	
									122	(Cn + Cb)	
								EC50	355	(Cn)	
									382	(Cn + Cb)	
									Smolders et al. '03 [44,47]		
maize residue *(EU soil)*	$ZnCl_2$ **1 ***	loamy sand (Gudow; Cb 7 mg/kg)	3.0	9	7	20	28-d	NOEC	120	(Cn)	**<u>120</u>**
									127	(Cn + Cb)	
								EC10	78	(Cn)	
									85	(Cn + Cb)	
								EC50	5,115	(Cn)	
									5,122	(Cn + Cb)	
									Smolders et al. '03 [44,46]		
maize residue *(EU soil)*	$ZnCl_2$ **5 ***	sandy clay loam (Zegveld, Cb 191mg/kg)	4.7	40	24	20	28-d	NOEC	200	(Cn)	**<u>200</u>**
									391	(Cn + Cb)	
								EC10	38	(Cn)	
									229	(Cn + Cb)	
								EC50	16,804	(Cn)	
									16,995	(Cn + Cb)	
									Smolders et al. '03 [44,46]		
maize residue *(EU soil)*	$ZnCl_2$ **6 ***	?? (Rhydtalog, Cb 83 mg/kg)	4.8	13	-	20	28-d	NOEC	469	(Cn)	**<u>469</u>**
									552	(Cn + Cb)	
								EC10	160	(Cn)	
									243	(Cn + Cb)	
								EC50	8,187	(Cn)	
									8,270	(Cn + Cb)	
									Smolders et al. '03 [44,46]		
maize residue *(EU soil)*	$ZnCl_2$ **9 ***	sandy loam (Kövlinge II, Cb 26 mg/kg)	5.1	4	9	20	28-d	NOEC	50	(Cn)	**<u>50</u>**
									76	(Cn + Cb)	
								EC10	30	(Cn)	
									56	(Cn + Cb)	
								EC50	1,552	(Cn)	
									1,578	(Cn + Cb)	
									Smolders et al. '03 [44,46]		

(to be continued)

Table 3.3.3.a Toxicity of zinc to soil microbe-mediated processes: NOEC and EC values (continued) Part I: Studies useful for $PNEC_{add.\ terrestrial}$ derivation

Toxicologal endpoint	Test-comp	Soil type	pH	OM %	Clay %	Temp. ^0C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)		(Estimated) **NOEC (Cn)** used for $PNEC_{add}$ derivation
C-mineralization (respiration) of specific substrates (continued)											
maize residue *(EU soil)*	$ZnCl_2$ **11 ***	?? (De Meern, Cb 155 mg/kg)	5.2	17	-	20	28-d	NOEC	1,300 1,455	(Cn) (Cn + Cb)	**1,300**
								EC10	817 972	(Cn) (Cn + Cb)	
								EC50	3,767 3,922	(Cn) (Cn + Cb)	
									Smolders et al. '03 [44,46]		
maize residue *(EU soil)*	$ZnCl_2$ **13**	?? (Zeveren, Cb 76 mg/kg)	5.7	6	-	20	28-d	NOEC	1,400 1,476	(Cn) (Cn + Cb)	**1,400**
								EC10	1,068 1,144	(Cn) (Cn + Cb)	
								EC50	6,077 6,153	(Cn) (Cn + Cb)	
									Smolders et al. '03 [44,46]		
maize residue *(EU soil)*	$ZnCl_2$ **15 ***	silt loam (Ter Munck, Cb 54 mg/kg)	6.8	2	15	20	28-d	NOEC	38 92	(Cn) (Cn + Cb)	**38**
								EC10	18 72	(Cn) (Cn + Cb)	
								EC50	17,144 17,198	(Cn) (Cn + Cb)	
									Smolders et al. '03 [44,46]		
maize residue *(EU soil)*	$ZnCl_2$ **17 ***	silty clay loam (Rots, Cb 51 mg/kg)	7.4	2	27	20	28-d	NOEC	150 201	(Cn) (Cn + Cb)	**150**
								EC10	76 127	(Cn) (Cn + Cb)	
								EC50	3,065 3,116	(Cn) (Cn + Cb)	
									Smolders et al. '03 [44,46]		
maize residue *(EU soil)*	$ZnCl_2$ **18 ***	clay (Souli II, Cb 51 mg/kg)	7.4	4	46	20	28-d	NOEC	600 651	(Cn) (Cn + Cb)	**600**
								EC10	636 687	(Cn) (Cn + Cb)	
								EC50	2,757 2,808	(Cn) (Cn + Cb)	
									Smolders et al. '03 [44,46]		
maize residue *(EU soil)*	$ZnCl_2$ **19 ***	silt loam (Marknesse, Cb 80 mg/kg)	7.5	2	26	20	28-d	NOEC	150 230	(Cn) (Cn + Cb)	**150**
								EC10	122 202	(Cn) (Cn + Cb)	
								EC50	1,766 1,846	(Cn) (Cn + Cb)	
									Smolders et al. '03 [44,46]		
maize residue *(EU soil)*	$ZnCl_2$ **22 ***	loam (Guadalajara Cb 27 mg/kg)	7.5	1	25	20	28-d	NOEC	300 327	(Cn) (Cn + Cb)	**300**
								EC10	183 210	(Cn) (Cn + Cb)	
								EC50	2,080 2,107	(Cn) (Cn + Cb)	
									Smolders et al. '03 [44,46]		

(to be continued)

Table 3.3.3.a Toxicity of zinc to soil microbe-mediated processes: NOEC and EC values (continued) Part I: Studies useful for $PNEC_{add.\ terrestrial}$ derivation

Toxicologal endpoint	Test-comp	Soil type	pH	OM %	Clay %	Temp. ^0C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)		(Estimated) **NOEC (Cn)** used for $PNEC_{add}$ derivation
N-mineralization											
N-mineralization	$ZnSO_4$	loam (Webster)	5.8	4	23	30	3-w	EC (14%)	327	(Cn)	**164**
	$ZnSO_4$	silty clay (Judson)	6.6	5	45	30	3-w	EC (12%)	327	(Cn)	**164**
	$ZnSO_4$	clay loam (Harps)	7.8	6	30	30	3-w	EC (15%)	327	(Cn)	**164**
	$ZnSO_4$	silty clay (Okoboji)	7.4	9	34	30	3-w	EC (14%)	327	(Cn)	**164**
									Liang & Tabatabai '77 [7, 7a]		
N-mineralization	$ZnSO_4$	silt loam (+ 1% sludge + 1% alfalfa)	6.9	2	44[e]	25	3-m	NOEC	100	(Cn)	**100**
									107	(Cn + Cb)	
									Chang & Broadbent '82 [8]		
N-mineralization *(EU soil)*	$ZnSO_4$	- (forest)	3.4	8	10[e]	20	7-w	EC(30%)	700	(Cn)	**233**
									Necker and Kunze '86 [38]		
Ammonification *(EU soil)*	$ZnSO_4$	sandy loam	7.1	3	17	30	3-w	NOEC	1,000	(Cn)	**1,000**
									1,057	(Cn + Cb)	
									Premi & Cornfield '69 [9]		
Nitrification	$ZnSO_4$	clay loam (Harps)	7.8	6	30	30	10-d	EC (24%)	327	(Cn)	**109**
									Liang & Tabatabai '78 [7,10]		
Nitrification *(EU soil)*	$ZnSO_4$	sandy loam	7.1	3	17	30	3-w	NOEC	100	(Cn)	**100**
									157	(Cn + Cb)	
									Premi & Cornfield '69 [9]		
Nitrification	$ZnSO_4$	clay loam (Decatur)	5.5	2	28	30	7-w	NOEC	100	(Cn)	**100**
									236	(Cn + Cb)	
	$ZnSO_4$	sandy loam (Cecil)	6.2	2	8	30	7-w	NOEC	100	(Cn)	**100**
									124	(Cn + Cb)	
	$ZnSO_4$	loamy sand (Leefield)	5.1	1	2	30	7-w	NOEC	10	(Cn)	**50**
									17	(Cn + Cb)	
									Wilson '77 [11]		

(to be continued)

Table 3.3.3.a Toxicity of zinc to soil microbe-mediated processes: NOEC and EC values (continued) Part I: Studies useful for $PNEC_{add.\ terrestrial}$ derivation

Toxicologal endpoint	Test-comp	Soil type	pH	OM %	Clay %	Temp. ^0C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)		(Estimated) **NOEC (Cn)** used for $PNEC_{add}$ derivation
N-mineralization (continued)											
Nitrification *(EU soil)*	$ZnCl_2$ **5 ***	sandy clay loam (Zegveld, Cb 191mg/kg)	4.7	40	24	20	7-d	NOEC	400	(Cn)	**400**
									591	(Cn + Cb)	
								EC10	506	(Cn)	
									697	(Cn + Cb)	
								EC50	944	(Cn)	
									1,135	(Cn + Cb)	
									Smolders et al. '03 [44.45]		
Nitrification *(EU soil)*	$ZnCl_2$ **6 ***	?? (Rhydtalog, Cb 83 mg/kg)	4.8	13	-	20	7-d	NOEC	257	(Cn)	**257**
									340	(Cn + Cb)	
								EC10	517	(Cn)	
									600	(Cn + Cb)	
								EC50	852	(Cn)	
									935	(Cn + Cb)	
									Smolders et al. '03 [44.45]		
Nitrification *(EU soil)*	$ZnCl_2$ **8 ***	sandy clay (Souli I, Cb 37 mg/kg)	4.8	1	38	20	28-d	NOEC	50	(Cn	**50**
									87	(Cn + Cb)	
								EC10	77	(Cn)	
									114	(Cn + Cb)	
								EC50	189	(Cn)	
									226	(Cn + Cb)	
									Smolders et al. '03 [44.45]		
Nitrification *(EU soil)*	$ZnCl_2$ **9 ***	sandy loam (Kövlinge II, Cb 26 mg/kg)	5.1	4	9	20	14-d	NOEC	50	(Cn)	**50**
									76	(Cn + Cb)	
								EC10	51	(Cn)	
									77	(Cn + Cb)	
								EC50	224	(Cn)	
									250	(Cn + Cb)	
									Smolders et al. '03 [44.45]		
Nitrification *(EU soil)*	$ZnCl_2$ **11 ***	?? (De Meern, Cb 155 mg/kg)	5.2	17	-	20	4-d	NOEC	424	(Cn)	**424**
									579	(Cn + Cb)	
								EC10	436	(Cn)	
									591	(Cn + Cb)	
								EC50	1,046	(Cn)	
									1,201	(Cn + Cb)	
									Smolders et al. '03 [44.45]		
Nitrification *(EU soil)*	$ZnCl_2$ **12 ***	clay (Aluminusa; Cb 53 mg/kg)	5.4	1	51	20	14-d	NOEC	38	(Cn)	**38**
									91	(Cn + Cb)	
								EC10	43	(Cn)	
									96	(Cn + Cb)	
								EC50	199	(Cn)	
									252	(Cn + Cb)	
									Smolders et al. '03 [44.45]		
Nitrification *(EU soil)*	$ZnCl_2$ **13 ***	?? (Zeveren, Cb 76 mg/kg)	5.7	6	-	20	7-d	NOEC	-		
								EC10	206	(Cn)	**206**
									282	(Cn + Cb)	
								EC50	409	(Cn)	
									485	(Cn + Cb)	
									Smolders et al. '03 [44.45]		

(to be continued)

Table 3.3.3.a Toxicity of zinc to soil microbe-mediated processes: NOEC and EC values (continued) Part I: Studies useful for $PNEC_{add.\ terrestrial}$ derivation

Toxicologal endpoint	Test-comp	Soil type	pH	OM %	Clay %	Temp. ^0C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)		(Estimated) **NOEC (Cn)** used for $PNEC_{add}$ derivation
N-mineralization (continued)											
Nitrification *(EU soil)*	$ZnCl_2$ **14 ***	sandy clay loam (Woburn, Cb 99 mg/kg)	6.4	7	21	20	4-d	NOEC	75	(Cn)	**<u>75</u>**
									174	(Cn + Cb)	
								EC10	241	(Cn)	
									340	(Cn + Cb)	
								EC50	464	(Cn)	
									563	(Cn + Cb)	
									Smolders et al. '03 [44.45]		
Nitrification *(EU soil)*	$ZnCl_2$ **15 ***	silt loam (Ter Munck, Cb 54 mg/kg)	6.8	2	15	20	4-d	NOEC	150	(Cn)	**<u>150</u>**
									204	(Cn + Cb)	
								EC10	113	(Cn)	
									167	(Cn + Cb)	
								EC50	267	(Cn)	
									321	(Cn + Cb)	
									Smolders et al. '03 [44.45]		
Nitrification *(EU soil)*	$ZnCl_2$ **17 ***	silty clay loam (Rots, Cb 51 mg/kg)	7.4	2	27	20	4-d	NOEC	300	(Cn)	**<u>300</u>**
									351	(Cn + Cb)	
								EC10	336	(Cn)	
									387	(Cn + Cb)	
								EC50	710	(Cn)	
									761	(Cn + Cb)	
									Smolders et al. '03 [44.45]		
Nitrification *(EU soil)*	$ZnCl_2$ **18 ***	clay (Souli II, Cb 51 mg/kg)	7.4	4	46	20	23-d	NOEC	150	(Cn)	**<u>150</u>**
									201	(Cn + Cb)	
								EC10	542	(Cn)	
									593	(Cn + Cb)	
								EC50	748	(Cn)	
									799	(Cn + Cb)	
									Smolders et al. '03 [44.45]		
Nitrification *(EU soil)*	$ZnCl_2$ **19 ***	silt loam (Marknesse, Cb 80 mg/kg)	7.5	2	26	20	4-d	NOEC	300	(Cn)	**<u>300</u>**
									380	(Cn + Cb)	
								EC10	262	(Cn)	
									342	(Cn + Cb)	
								EC50	513	(Cn)	
									593	(Cn + Cb)	
									Smolders et al. '03 [44.45]		
Nitrification *(EU soil)*	$ZnCl_2$ **22 ***	loam (Guadalajara Cb 27 mg/kg)	7.5	1	25	20	10-d	NOEC	75	(Cn)	**<u>75</u>**
									102	(Cn + Cb)	
								EC10	87	(Cn)	
									114	(Cn + Cb)	
								EC50	275	(Cn)	
									302	(Cn + Cb)	
									Smolders et al. '03 [44.45]		
Denitrification	$Zn(NO_3)_2$	silt loam	6.8	3	28	28	3-w	NOEC	100	(Cn)	**<u>100</u>**
									Bollag & Barabasz '79 [21]		

(to be continued)

Table 3.3.3.a Toxicity of zinc to soil microbe-mediated processes: NOEC and EC values (continued) Part I: Studies useful for $PNEC_{add.\ terrestrial}$ derivation

Toxicologal endpoint	Test-comp	Soil type	pH	OM %	Clay %	Temp. ^0C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)		(Estimated) **NOEC (Cn)** used for $PNEC_{add}$ derivation
Enzyme activities											
Amidase	$ZnSO_4$	clay	7.5	-	18	28	12-w	NOEC	200	(Cn)	**200**
	$ZnSO_4$	sand	7.4	-	2	28	12-w	NOEC	200	(Cn)	**200**
									Hemida et al. '97 [31]		
Arylsulphatase	$ZnSO_4$	clay loam (Nicollet)	6.2	5	29	-	30-min	EC(20%)	1,640	(Cn)	**820**
	$ZnSO_4$	clay loam (Harps)	7.8	6	30	-	30-min	EC10	140	(Cn)	**140**
	$ZnSO_4$	clay (Webster)	5.8	4	23	-	30-min	NOEC	164	(Cn)	**164**
	$ZnSO_4$	silty clay (Okoboji)	7.4	9	34	-	30-min	EC (17%)	1,640	(Cn)	**820**
									Al-Khafaji and Tabatabai '79 [7, 39]		
Arylsulphatase *(EU soils)*	$ZnCl_2$	sand	7.7	2	2	20	6-w	EC10	105	(Cn)	**105**
									119	(Cn + Cb)	
							1.5-yr	EC10	311	(Cn)	
									325	(Cn + Cb)	
							6-w	EC50	904	(Cn)	
									918	(Cn + Cb)	
							1.5-yr	EC50	372	(Cn)	
									386	(Cn + Cb)	
	$ZnCl_2$	sandy loam	5.1	6	9	20	6-w	EC10	728	(Cn)	**728**
									745	(Cn + Cb)	
							1.5-yr	EC10	800	(Cn)	
									817	(Cn + Cb)	
							6-w	EC50	2,171	(Cn)	
									2,188	(Cn + Cb)	
							1.5-yr	EC50	943	(Cn)	
									960	(Cn + Cb)	
	$ZnCl_2$	silty loam	7.4	2	19	20	6-w	EC10	151	(Cn)	**151**
									254	(Cn + Cb)	
							1.5-yr	EC10	2,704	(Cn)	
									2,807	(Cn + Cb)	
							6-w	EC50	1,287	(Cn)	
									1,390	(Cn + Cb)	
							1.5-yr	EC50	4,323	(Cn)	
									4,426	(Cn + Cb)	
	$ZnCl_2$	clay	6.8	3	60	20	6-w	EC10	2,353	(Cn)	**2,353**
									2,579	(Cn + Cb)	
							1.5-yr	EC10	1,014	(Cn)	
									1,240	(Cn + Cb)	
							6-w	EC50	5,525	(Cn)	
									5,751	(Cn + Cb)	
							1.5-yr	EC50	2,821	(Cn)	
									3,047	(Cn + Cb)	
	ZnCl	sandy peat	4.3	13	5	20	6-w	EC10	-		
							1.5-yr	EC10	7,930	(Cn)	
									7,968	(Cn + Cb)	
							6-w	EC50	-		
							1.5-yr	EC50	9,620	(Cn)	
									9,658	(Cn + Cb)	
									Haanstra & Doelman '91 [6, 18]		

(to be continued)

Table 3.3.3.a Toxicity of zinc to soil microbe-mediated processes: NOEC and EC values (continued) Part I: Studies useful for $PNEC_{add.\ terrestrial}$ derivation

Toxicologal endpoint	Test-comp	Soil type	pH	OM %	Clay %	Temp. ^0C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)		(Estimated) **NOEC (Cn)** used for $PNEC_{add}$ derivation
Enzyme activities (continued)											
Dehydrogenase *(EU soils)*	$ZnSO_4$	sand	6.9	3	-	20	3-m	EC10	76 >91	(Cn) (Cn + Cb)	**76**
	$ZnSO_4$	alluvial soil	7.1	2	-	20	3-m	NOEC	500 >508	(Cn) (Cn + Cb)	**500**
									Maliszewska et al '85 [19]		
Dehydrogenase	$ZnSO_4$	- (unenriched)	-	2	-	27	24-h	NOEC EC10	30 145	(Cn) (Cn)	**145**
	$ZnSO_4$	- (+ 1% alfalfa)	-	2	-	27	24-h	NOEC EC10	30 48	(Cn) (Cn)	**48**
									Rogers & Li '85 [40]		
Nitrate reductase	$ZnSO_4$	sand	7.4	-	2	-	12-w	EC10	34	(Cn)	**67**
									Hemida et al. '97 [31]		
Phosphatase *(EU soil)*	$ZnSO_4$	-	4.7	-	-	22	1-h	EC10	508	(Cn)	**508**
									Svenson '86 [41]		
Phosphatase *(EU soils)*	$ZnCl_2$	sandy loam	5.1	6	9	20	6-w	EC10	1,341 1,358	(Cn) (Cn + Cb)	**1,341**
							1.5-yr	EC10	570 587	(Cn) (Cn + Cb)	
							6-w	EC50	3,342 3,359	(Cn) (Cn + Cb)	
							1.5-yr	EC50	2,969 2,986	(Cn) (Cn + Cb)	
	$ZnCl_2$	silty loam	7.4	2	19	20	6-w	EC10	2,623 2,726	(Cn) (Cn + Cb)	**2,623**
							1.5-yr	EC10	300 403	(Cn) (Cn + Cb)	
							6-w	EC50	2,963 3,066	(Cn) (Cn + Cb)	
							1.5-yr	EC50	4,872 4,975	(Cn) (Cn + Cb)	
	$ZnCl_2$	clay	6.8	3	60	20	6-w	EC10	160 386	(Cn) (Cn + Cb)	**160**
							1.5-yr	EC10	36 262	(Cn) (Cn + Cb)	
							6-w	EC50	3,623 3,849	(Cn) (Cn + Cb)	
							1.5-yr	EC50	2,845 3,071	(Cn) (Cn + Cb)	
									Doelman & Haanstra '89 [6]		
Phosphatase (acid-)	$ZnSO_4$	loam (Webster)	5.8	4	23	-	30-min	NOEC	164	(Cn)	**164**
Phosphatase (alkaline-)	$ZnSO_4$	silty clay (Okoboji)	7.4	9	34	-	30-min	NOEC	164	(Cn)	**164**
									Juma & Tabatabai '77 [7, 13]		

(to be continued)

Table 3.3.3.a Toxicity of zinc to soil microbe-mediated processes: NOEC and EC values (continued) Part I: Studies useful for $PNEC_{add.\ terrestrial}$ derivation

Toxicologal endpoint	Test-comp	Soil type	pH	OM %	Clay %	Temp. ^0C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)		(Estimated) **NOEC (Cn)** used for $PNEC_{add}$ derivation
Enzyme activities (continued)											
Phytase *(EU soil)*	$ZnSO_4$	-	4.7	-	-	22	1-h	NOEC	590 Svenson '86 [41]	(Cn)	**590**
Pyrophosphatase	$ZnSO_4$	loam (Clarion)	4.6	3	24	-	30-min	NOEC	1,640	(Cn)	**1,640**
		clay loam (Nicollet)	6.2	5	29	-	30-min	NOEC	1,640	(Cn)	**1,640**
		clay loam (Okoboji)	7.4	9	34	-	30-min	NOEC	1,640 Stott et al. '85 [7, 42]	(Cn)	**1,640**
Urease	$ZnSO_4$	loam (Webster)	5.8	4	23	37	30-min	EC (23%)	327	(Cn)	**109**
	$ZnSO_4$	clay loam (Harps)	7.8	6	30	37	30-min	NOEC	33	(Cn)	
								EC10	52	(Cn)	**52**
	$ZnSO_4$	silty clay (Okoboji)	7.4	9	34	37	30-min	NOEC	33	(Cn)	
								EC10	64 Tabatabai '77 [7, 14]	(Cn)	**64**
Urease *(EU soils)*	$ZnCl_2$	sand	7.7	2	2	20	6-w	EC10	70	(Cn)	**70**
									84	(Cn + Cb)	
							1.5-yr	EC10	160	(Cn)	
									174	(Cn + Cb)	
							6-w	EC50	420	(Cn)	
									434	(Cn + Cb)	
							1.5-yr	EC50	290	(Cn)	
									304	(Cn + Cb)	
	$ZnCl_2$	sandy loam	5.1	6	9	20	6-w	EC10	30	(Cn)	**30**
									47	(Cn + Cb)	
							1.5-yr	EC10	1	(Cn)	
									18	(Cn + Cb)	
							6-w	EC50	480	(Cn)	
									497	(Cn + Cb)	
							1.5-yr	EC50	110	(Cn)	
									127	(Cn + Cb)	
	$ZnCl_2$	silty loam	7.4	2	19	20	6-w	EC10	30	(Cn)	**30**
									133	(Cn + Cb)	
							1.5-yr	EC10	-		
							6-w	EC50	1,030	(Cn)	
									1,133	(Cn + Cb)	
							1.5-yr	EC50	-		
	$ZnCl_2$	clay	6.8	3	60	20	6-w	EC10	460	(Cn)	**460**
									686	(Cn + Cb)	
							1.5-yr	EC10	8	(Cn)	
									234	(Cn + Cb)	
							6-w	EC50	1,780	(Cn)	
									2,006	(Cn + Cb)	
							1.5-yr	EC50	90	(Cn)	
									316	(Cn + Cb)	
	$ZnCl_2$	sandy peat	4.3	13	5	20	6-w	EC10	-		
							1.5-yr	EC10	5	(Cn)	
									43	(Cn + Cb)	
							6-w	EC50	-		
							1.5-yr	EC50	70	(Cn)	
									108	(Cn + Cb)	
									Doelman & Haanstra '86 [6]		

(Table 3.3.3.a: to be continued in Part II: studies <u>not</u> useful for $PNEC_{add,\ terrestrial}$ derivation)

Table 3.3.3.a Toxicity of zinc to soil microbe-mediated processes: NOEC and EC values

Part II: Studies not useful for $PNEC_{add.\ terrestrial}$ derivation

Toxicologal endpoint	Test-comp	Soil type or substrate	pH	OM %	Clay	Temp. °C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)
C-mineralization (respiration)									
Respiration	$ZnCl_2$	loamy sand (95%) / oak litter (5%) intact microcosm	4.8	2	3[e]	22	3-w	NOEC	48 (Cn) 110 (Cn + Cb) Chaney et al. '78 [1]
Respiration	$ZnCl_2$	litter [fir needles]	-	77[e]	0[e]	22	4-w	NOEC	100 (Cn) 111 (Cn + Cb) Spalding '79 [2]
Respiration *(EU soil)*	$ZnSO_4$	loamy sand	4.9	4	5	30	8-w	EC10	2 (Cn) 31 (Cn + Cb) Cornfield '77 [3]
Respiration *(EU soil)*	ZnO	sand	6.0	4	6	30	5-m	EC(16%)	1,000 (Cn) 1,074 (Cn + Cb)
	ZnO	sand (+ 1% straw)	6.0	4	6	30	5-m	NOEC	1,000 (Cn) 1,074 (Cn + Cb) Bhuiya & Cornfield '72 [5]
Respiration *(EU soils)*	$ZnCl_2$	sand	7.7	2	2	20	8-w	EC50	500 (Cn) 514 (Cn + Cb)
							1.5-yr	NOEC	150 (Cn) 164 (Cn + Cb)
	$ZnCl_2$	sandy loam	5.1	6	9	20	1-yr	EC50	1,250 (Cn) 1,267 (Cn + Cb)
							1-yr	NOEC	150 Cn) 167 (Cn + Cb)
	$ZnCl_2$	silty loam	7.4	2	19	20	1.5- yr	NOEC	3,000 (Cn) 3,103 (Cn + Cb)
	$ZnCl_2$	clay	6.8	3	60	20	1.5-yr	NOEC	400 (Cn) 626 (Cn + Cb)
	$ZnCl_2$	sandy peat	4.3	13	5	20	8-w	EC50	8,000 (Cn) 8,038 (Cn + Cb)
							1.5-yr	NOEC	400 (Cn) 1,038 (Cn + Cb) Doelman & Haanstra '83; '84 [6] [6a]
Respiration	-	-	-	-		-	-	NOEC	51 Bååth ’89 [17, 50]
Respiration	-	peat	5.6	71	-	21	125-d	EC(25%)	108 (actual) Mathur & Rayment ’77 [16]

(to be continued)

Table 3.3.3.a Toxicity of zinc to soil microbe-mediated processes: NOEC and EC values (continued) Part II: Studies not useful for $PNEC_{add.\ terrestrial}$ derivation

Toxicologal endpoint	Test-comp	Soil type or substrate	pH	OM %	Clay	Temp. °C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)
C-mineralization (respiration) of specific substrates									
Glucose *(EU soil)*	$ZnSO_4$	-	5.0	6	9	25	2-w	NOEC	≥5,000 (Cn) Denneman & van Gestel '90 [17,35]
Glucose *(EU soil)*	$ZnCl_2$ **5**	sandy clay loam (Zegveld, Cb 191mg/kg)	4.7	40	24	20	28-d	NOEC EC10 EC50	≥1,200 (Cn) ≥1,391 (Cn + Cb) - - Smolders et al. '03 [44, 47]
Glutamic acid *(EU soils)*	$ZnCl_2$	sand	7.7	2	2	20	1.5-yr	EC50	400 (Cn) 414 (Cn + Cb)
	$ZnCl_2$	sandy loam	5.1	6	9	20	1.5-yr	NOEC	≥1,000 (Cn) ≥1,017 (Cn + Cb)
	$ZnCl_2$	silty loam	7.4	2	19	20	1.5-yr	NOEC	400 (Cn) 503 (Cn + Cb)
	$ZnCl_2$	clay	6.8	3	60	20	1.5-yr	NOEC EC50	400 (Cn) 626 (Cn + Cb) 1,500 (Cn) 1,726 (Cn + Cb)
	ZnCl	sandy peat	4.3	13	5	20	1.5-yr	NOEC	≥1,000 (Cn) ≥1,038 (Cn + Cb) Doelman & Haanstra '83 [6, 33a]
Glutamic acid *(EU soils)*	$ZnCl_2$	sand	7.7	2	2	20	1.5-yr	EC10	92 (Cn) 106 (Cn + Cb)
	$ZnCl_2$	silty loam	7.4	2	19	20	1.5-yr	NOEC	≥1,000 (Cn) ≥1,103 (Cn + Cb)
	ZnCl	sandy peat	4.3	13	5	20	1.5-yr	NOEC	≥1,000 (Cn) ≥1,038 (Cn + Cb)
	$ZnCl_2$	clay	6.8	3	60	20	1.5-yr	NOEC	400 (Cn) 626 (Cn + Cb) Haanstra & Doelman '84 [6, 33b]
Glutamic acid (EU soil)	$ZnCl_2$	sand (PANH-aged)	7.1	2	3	22?	2-d	NOEC	115 (actual) 91 (actual-Cb) Notenboom & Posthuma '95 [48, 48c] Posthuma et al. '98
Acetate	$ZnCl_2$	sand (PANH-aged)	7.1	2	3	-	-	EC10	236 (actual) 212 (actual-Cb) Van Beelen & Notenboom '96 [49] Posthuma et al. '98
Acetate *(EU soil)*	$ZnCl_2$	sand (Flevopolder; subsoil)	8.2	2	1	10	4-d 4-d 4-d 4-d	EC10 EC50 IC10 IC50	59 (Cn) 71 (Cn + Cb) 87 (Cn) 99 (Cn + Cb) 39 (Cn) 51 (Cn + Cb) 62 (Cn) 74 (Cn + Cb)

(to be continued)

Table 3.3.3.a Toxicity of zinc to soil microbe-mediated processes: NOEC and EC values (continued) Part II: Studies not useful for $PNEC_{add.\ terrestrial}$ derivation

Toxicologal endpoint	Test-comp	Soil type or substrate	pH	OM %	Clay °C	Temp.	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)
C-Mineralization (respiration) of specific substrates (continued)									
Acetate *(EU soil)*	$ZnCl_2$	sand (De Peel; subsoil)	4.0	0.3	1	10	-	EC10 IC10	≥1,000 (Cn) ≥1,004 (Cn + Cb) ≥1,000 (Cn) ≥1,004 (Cn + Cb) Van Beelen et al. '94b [34a]
Acetate *(EU soil)*	$ZnCl_2$	humic sand (De Peel; surface soil)	2.8	4	1	10	-	EC10 IC10	≥1,000 (Cn) ≥1,006 (Cn + Cb) ≥1,000 (Cn) ≥1,006 (Cn + Cb) Van Beelen et al. '94b [34a]
Acetate *(EU soil)*	$ZnCl_2$	sand	3.8	1	0.5	10	2-d	NOEC	≥1,110 (Cn) Van Beelen & Fleuren-Kemilä '93 [34b]
maize residue *(EU soil)*	$ZnCl_2$ **3 ***	loamy sand (Houthalen, Cb 8 mg/kg)	3.4	3	5	20	28-d	NOEC EC10 EC50	≥720 (Cn) ≥728 (Cn + Cb) 31 (Cn) 39 (Cn + Cb) 28,080 (Cn) 28,088 (Cn + Cb) Smolders et al. '03 [44,46][
maize residue *(EU soil)*	$ZnCl_2$ **8 ***	sandy clay (Souli I, Cb 37 mg/kg)	4.8	1	38	20	28-d	NOEC EC10 EC50	≥1,200 (Cn) ≥1,237 (Cn + Cb) - - Smolders et al. '03 [44,46][
maize residue *(EU soil)*	$ZnCl_2$ **12 ***	clay (Aluminusa; Cb 53 mg/kg)	5.4	1	51	20	28-d	NOEC EC10 EC50	≥1,800 (Cn) ≥1853 (Cn + Cb) 1,158 (Cn) 1,211 (Cn + Cb) 12,100 (Cn) 12,153 (Cn + Cb) Smolders et al. '03 [44,46][
maize residue *(EU soil)*	$ZnCl_2$ **14 ***	sandy clay loam (Woburn, Cb 99 mg/kg)	6.4	7	21	20	28-d	NOEC EC10 EC50	≥1,800 (Cn) ≥1,899 (Cn + Cb) 3,511 (Cn) 3,610 (Cn + Cb) 13,077 (Cn) 13,176 (Cn + Cb) Smolders et al. '03 [44,46]

(to be continued)

Table 3.3.3.a Toxicity of zinc to soil microbe-mediated processes: NOEC and EC values (continued) Part II: Studies not useful for $PNEC_{add.\ terrestrial}$ derivation

Toxicologal endpoint	Test-comp	Soil type or substrate	pH	OM %	Clay	Temp. °C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)
N-mineralization									
N-mineralization *(EU soil)*	ZnO	sand	7.7	4	6	30	6-w	EC(32%)	1,000 (Cn) 1074 (Cn + Cb) Bhuiya and Cornfield '74 [37]
Ammonification *(EU soil)*	ZnO	sand	6.0	2	-	20	2-w	NOEC	1,000 (Cn?)
	ZnO	sand	7.0	2	-	20	2-w	EC (mod.)	1,000 (Cn?)
	ZnO	sand	7.7	2	-	20	2-w	EC (great)	1,000 (Cn?) Doelman & Haanstra '83 [17, 51]
Ammonification *(EU soil)*	$ZnSO_4$	sand	-	-	-	30	4-w	EC (20%)	2,500 (Cn?) Doelman & Haanstra '83 [17, 52]
Ammonification *(EU soi)l*	$ZnCO_3$	sandy loam *(end of test:*	7.1 *7.4)*	3	17	30	3-w	NOEC	1,000 (Cn) 1,057 (Cn + Cb)
		(end of test:	7.1 *8.5)*	3	17	30	3-w	NOEC	≥10,000 (Cn) ≥10,057 (Cn + Cb) Premi & Cornfield '69 [9, 9a]
Nitrification *(EU soil)*	$ZnCO_3$	sandy loam *(end of test:*	7.1 *7.4)*	3	17	30	3-w	NOEC	1,000 (Cn) 1,057 (Cn + Cb)
		(end of test:	7.1 *8.5)*	3	17	30	3-w	NOEC	≥10,000 (Cn) 10,057 (Cn + Cb) Premi & Cornfield '69 [9, 9a]
Nitrification *(EU soil)*	ZnO	sand	7.7	4	6	30	6-w	EC(33%)	1,000 (Cn) 1,074 (Cn + Cb) Bhuiya & Cornfield '74 [37]
Nitrification	--	-	-	-	-	-	-	NOEC	500 (Cn?) Bååth '89 [17, 50]
Nitrification	$ZnSO_4$	loam (Webster)	5.8	4	23	30	10-d	EC (58%)	327 (Cn)
	$ZnSO_4$	silty clay (Okoboji)	7.4	9	34	30	10-d	EC (39%)	327 (Cn) Liang & Tabatabai '78 [7,10]

(to be continued)

Table 3.3.3.a Toxicity of zinc to soil microbe-mediated processes: NOEC and EC values (continued) Part II: Studies not useful for $PNEC_{add.\ terrestrial}$ derivation

Toxicologal endpoint	Test-comp	Soil type or substrate	pH	OM %	Clay	Temp. °C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)	
Enzyme activities										
Amylase	$ZnCl_2$	litter [fir needles]	-	77[e]	0[e]	-	4-w	NOEC	≥1,000 ≥1,111 Spalding '79 [2]	(Cn) (Cn + Cb)
Arylsulphatase *(EU soils)*	$ZnCl_2$	sand	7.7	2	2	20	< 1-w ? [12]	EC50	900 914	(Cn) (Cn + Cb)
	$ZnCl_2$	sandy loam	5.1	6	9	20	< 1-w ? [12]	EC50	2180 2197	(Cn) (Cn + Cb)
	$ZnCl_2$	silty loam	7.4	2	19	20	1-1,5-yr	EC50	4390 4493	(Cn) (Cn + Cb)
	$ZnCl_2$	clay	6.8	3	60	20	1-1.5-yr	EC50	2860 3086 Doelman & Haanstra '83 [6]	(Cn) (Cn + Cb)
Cellulase	$ZnCl_2$	litter [fir needles]	-	77[e]	0[e]	-	4-w	NOEC	≥1,000 ≥1,111 Spalding '79 [2]	(Cn) (Cn + Cb)
Nitrate reductase	$ZnSO_4$	clay	7.5	-	18	-	12-w	EC(43%)	200 Hemida et al. '97 [31]	(Cn)
Phosphatase *(EU soils)*	$ZnCl_2$	sand	7.7	2	2	20	6-w 1.5- yr 6-w 1.5-yr	EC10 EC10 EC50 EC50	4 5 220 170 Doelman & Haanstra '89 [6]	(Cn) (Cn) (Cn) (Cn)
Phosphatase *(EU soils)*	$ZnCl_2$	sand	7.7	2	2	20	1-1.5-yr	EC50	150 164	(Cn) (Cn + Cb)
	$ZnCl_2$	sandy loam	5.1	6	9	20	1-1.5-yr	EC50	2,970 2,987	(Cn) (Cn + Cb)
	$ZnCl_2$	silty loam	7.4	3	19	20	< 1-w ? [12]	EC50	2,760 2,863	(Cn) (Cn + Cb)
	$ZnCl_2$	clay	6.8	3	60	20	1-1.5-yr	EC50	2,710 2,936 Doelman & Haanstra '83 [6, 6b]	(Cn) (Cn + Cb)
Phosphatase	-	peat	5.6	71	-	21	125-d	EC (37%)	108 Mathur & Rayment '77 [16]	(actual)
Phosphatase	-	-	-	-	-	-		EC (28%)	71 Bååth '89 [17, 50]	(Cn)
Phosphatase (acid -)	$ZnSO_4$	clay loam (Harps)	7.8	6	30	-	30-min	EC (32%)	1,640	(Cn)
	$ZnSO_4$	silty clay (Okoboji)	7.4	9	34	-	30-min	EC (33%)	1,640	(Cn)
Phosphatase (alkaline-)	$ZnSO_4$	clay loam (Harps)	7.8	6	30	-	30-min	EC (59%)	1,640 Juma & Tabatabai '77 [7, 13]	(Cn)

(to be continued)

Table 3.3.3.a Toxicity of zinc to soil microbe-mediated processes: NOEC and EC values (continued) Part II: Studies not useful for $PNEC_{add.\ terrestrial}$ derivation

Toxicologal endpoint	Test-comp	Soil type or substrate	pH	OM %	Clay °C	Temp.	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)	
Enzyme activities (continued)										
Protease	$ZnCl_2$	sandy loam	7.4	3	19	20	1-1.5-yr	EC50	3,250	(Cn)
									3,303	(Cn + Cb)
									Doelman & Haanstra '83 [6]	
Urease	$ZnSO_4$	-	6.5	4	31	-	5-h	NOEC	≥50	(Cn)
(check soil)	$ZnSO_4$	-	7.3	5	31	-	5-h	NOEC	≥50	(Cn)
									Bremner & Douglas '71[15]	
Urease	$ZnCl_2$	sand	7.7	2	2	20	1-1.5-yr	EC50	290	(Cn)
(EU soils)									304	(Cn + Cb)
	$ZnCl_2$	sandy loam	5.1	6	9	20	1-1.5-yr	EC50	45	(Cn)
									62	(Cn + Cb)
	$ZnCl_2$	silty loam	7.4	3	19	20	1-1.5-yr	EC50	3,200	(Cn)
									3,353	(Cn + Cb)
	$ZnCl_2$	clay	6.8	3	60	20	1-1.5-yr	EC50	85	(Cn)
									311	(Cn + Cb)
	$ZnCl_2$	sandy peat	4.3	13	5	20	1-1.5-yr	EC50	60	(Cn)
									98	(Cn + Cb)
									Doelman & Haanstra '83 [6, 6b]	
Urease	$ZnSO_4$	silty loam (Weller)	5.1	3	17	37	30-min	EC (61%)	327	(Cn)
	$ZnSO_4$	clay loam (Nicollet)	6.2	6	30	37	30-min	EC (33%)	327	(Cn)
	$ZnSO_4$	silty clay loam (Luton)	6.8	7	42	37	30-min	EC (51%)	327	(Cn)
									Tabatabai '77 [7, 14]	
Urease	$ZnSO_4$	clay	7.5		18	-	12-w	EC(65%)	200	(Cn)
	$ZnSO_4$	sand	7.4		2	-	12-w	EC(57%)	200	(Cn)
									Hemida et al. '97 [31]	
Xylanase	$ZnCl_2$	litter [fir needles]	-	77[e]	0[e]	-	4-w	NOEC	≥1,000	(Cn)
									≥1,111	(Cn + Cb)
									Spalding '79 [2]	

For footnotes: see next pages; for further information see the "list of abbreviations Table 3.3.2.a to 3.3.2.d"

Footnotes Table 3.3.3.a
General remarks

pH values listed in Table 3.3.3.a - Part I: initial (native) pH values of the soil, thus before treatment!

See footnotes for pH changes due to treatment.

(Table 3.3.3.a - Part II: not checked for pH changes, unless tests from the studies are also included in Part I of Table 3.3.3.a).

The data on reported soil characterisics mentioned in the footnotes are focussed on data that can be used to indicate the soil type (textural class), organic matter content and clay content, if these data are not reported as such, and on the total background zinc concentration (Cb) in the soil. Thus, the data on sand, silt, and clay content are used to indicate the soil type, the organic carbon content is used to calculated the organic matter content and the CEC is used to estimate the clay content (provided the organic matter content is known). In some references additional soil characteristics than those mentioned in the table and footnotes are reported, for example the concentrations of micro- and macro-elements.

Solid test compound: usually reported as finely ground powder. Soil samples: top soil samples, unless stated otherwise.

EU-soil = European soil

[1] Chaney et al. '78: Respiration

Statistics: p = 0.05..Substrate: Intact field-collected microcosms of soil and litter from an unpolluted black oak forest area (background zinc level in the litter and top 2.5 cm of soil: 62 mg/kg). Mineral soil accounted for approximately 95% of the dry weight of the microcosms and litter (ranging from intact leaves to highly decomposed fragments) for 5%. Test compound added in aqueous solution and evenly distributed over the surface of the microcosms with a burette. The exposure concentrations (0-100-1000 mg/kg d.w.; nominal) and the above background concentration are related to the microcosms (soil + litter).

Reported soil characteristics: CEC 6.3 meq/100 g, organic carbon content 1.9% (equivalent to 3.2% organic matter) and pH 4.8.

Rejected, based on Relevance criterion (measurement of litter microbial respiration rather than soil microbial respiration).

[2] Spalding et al. '79: Respiration and enzyme activities

Statistics: p = 0.05. Test compound added in aqueous solution. The needle litter contained 23% ash (residue on ignition at 550 ^{o}C for 2 hours).

Rejected, based on Relevance criterion (measurement of litter microbial activity rather than soil microbial activity). Furhermore, the tests for enzyme activities (amylase activity, cellulase activity and xylanase activity) resulted in unbounded NOEC values (Quality criterion).

[3] Cornfield '77: Respiration

Statistics: p = 0.05. Test compound added as a solid. Test concentrations: 0-10-100 mg/kg. The test was performed in previously air-dried soil samples that were remoistened before start. Respiration (cumulative CO_2 production) was measured for 8 weeks and results reported for 2- and 8-weeks exposure. The 8-w EC10 (2 mg/kg) was calculated by the rapporteur from the reported study results after 8 weeks: 21% inhibition at 10 mg/kg and 45% inhibition at

100 mg/kg. For the 2-w exposure period, the results were 20% inhibition at 10 mg/kg and 24% inhibition at 100 mg/kg; no EC10 can be calculated from these data. Both after 2- and 8-w exposure the effects at both concentrations were statistically significant compared to the control. According to Doelman & Haanstra (1984) drying of the soil eliminates a certain part of the microbial community and thus after remoistening of the soil more nutrient is available for the remaining microorganisms. This may explain the lower percentage of inhibition found in the 2-w exposure to 100 mg/kg compared to the 8-w exposure. In addition, the test compound was added as a solid, which may have resulted in a lower bioavailability in the 2-w exposure. Thus, preference is given to the results of the 8-w exposure, because the 2-w exposure may underestimate the toxicity. It is noted that the 8-w EC10 (2 mg/kg) is 5-times lower than the lowest test concentration and thus not valid. Option: 8-w NOEC = LOEC/3 (21% inhibition at 10 mg/kg) = 3 mg/kg.

Soil: EU-soil; top soil (0-20 cm) samples collected in an unpolluted area in southwest England. The soil was obtained from arable land where vegtables were grown for 15 years and which had been limed every 3 or 4 years to maintain pH at about 5. Reported soil characteristics: 82% sand, 10% silt, 5% clay, 2.1% organic carbon (equivalent to 3.6 organic matter), pH water 4.9. After treatment the soil pH was within 0.2 pH units of the initial value. Background zinc concentration: Originally reported by Cornfield (1977) to be 2.6 mg/kg d.w. However, the soil from the same site was resampled in 200l; the new measurement show a background zinc concentration (average of four samples) of 29 mg/kg d.w. in the topsoil (sandy loam) and 17 mg/kg d.w. in the subsoil (sand). Data from McGrath et al. (2001), Paper with comments on the microbial data base in the draft RAR Zn Metal of 29 June 2001; the paper ("Appendix 3") was part of the industry comments.

Rejected, based on Quality criteria: The NOEC (3 mg/kg) estimated from this study is considerably lower than the next lowest NOEC in the microbial data base used for $PNEC_{add, terrestrial}$ derivation (17 mg/kg d.w., derived from respiration studies in two other soils: see Chang & Broadbent, 1981 and Lighthart et al., 1983), thus the result of the Cornfield (1977) study may be an outlier. Moreover, the validity of the study has been questioned because the original and further measurement of the background Zn concentration in the soil show a considerable difference.

[4] Chang & Broadbent '81: Respiration

No statistics reported. Test compound added in aqueous solution. Test concentrations: 0-50-100-200-400 mg/kg. Parameter: cumulative CO_2 production (7 determinations of CO_2 were made during the experimental period of 3 months). The CO_2 production decreased with increasing dose and exposure time. At the lowest nominal test concentration (50 mg/kg), the cumulative CO_2 production was reduced around 30% (derived from graphical representation: dose-response curve). The EC10 (12 mg/kg; nominal), was reported by the study authors (Chang & Broadbent, 1981). The reported EC10 is 4-times lower than lowest test concentration (test range: 0-50-100-200-400 mg/kg), thus unreliable.

Alternative NOEC used for PNECderivation: NOEC = LOEC/3 (30% inhibition at 50 mg/kg) = 50/3 = 17 mg/kg,.

Soil: non-EU soil (Yolo silt loam; Typic Xerorthent); top 15 cm samples, assumed to be collected in California, U.S. (based on the location of the research institute). Reported soil characteristics: organic carbon content 1.3% (equivalent to 2.2 % organic matter) and CEC 27.5 meq/100 g, resulting in an estimated clay content of 44%. Soil background zinc level: 7 mg/kg. The soil was amended with 1% sewage sludge (zinc level 450 mg/kg d.w.) and 1% ground alfalfa, on a dry weight basis resulting in a higher microbial activity (respiration rate)

compared to unamended soil (in spite of relatively high levels of zinc and other metals in sludge compared to the levels in soil). Due to the amendment of the soil with 1% sludge and 1% alfalfa, the "background" zinc level in the control substrate increased to more than 13 mg/kg, see further below (soil analysis).The amendment with sludge and alfalfa will have increased the organic matter content in the substrate to a maximum of 4%. The soil pH value is from the N-mineralization study reported by Chang & Broadbent (1982).

Soil analysis: At the end of the experimental period, 10-gsoil samples were extracted sequentially with 25 mlwater for 1 h, 25 ml of1 M KNO_3 for 2 h, 25 ml of DTPA solution *(containing 0.005 M DTPA, 0.01 M $CaCl_2$, 0.1 M triethanolamine, pH adjusted to 7.3)* and 25 ml1 M HNO_3 for 2 h. The percentage of cumulative recovery of zinc by the four sequential extractions was 24%, 41%, 67% and 61% at the added zinc concentration of 50, 100, 200 and 400 mg/kg, respectively. In the control soil amended with 1% sludge and 1% alfalfa, the sequentially extracted zinc concentration was 13 mg/kg, thus the total zinc concentration will have been higher.

[5] Bhuyia & Cornfield '72: Respiration

Statistics: p = 0.05. Respiration (CO_2 release) measured during the last 3 months of the 5-month incubation period following soil treatment. Test compound (zinc oxide) added as a solid. Test concentrations: 0 and 1000 mg/kg. Reported soil (Bagshot sand) characteristics: organic carbon content 2.2% (equivalent to 3.7% organic matter) and clay content 5.5; soil pH: 6.0 (in water). Treatment did not alter soil pH by more than 0.3 units. Test performed in unamended soil and in soil amended with 0.5% of finely-ground oat straw (on a wet weight basis), resulting in a higher microbial activity (respiration rate) compared to unamended soil. The amendment with straw, containing 40% organic carbon, will have increased the organic carbon content of the substrate to a maximum of 4.4%. .

Rejected, based on Relevance criterion (test compound: "insoluble" zinc salt: ZnO).

[6] = All Doelman & Haanstra studies studies (respiration and enzyme activities), i.e. Doelman & Haanstra (1983), Doelman & Haanstra (1984), Haanstra & Doelman (1984), Doelman & Haanstra (1986), Doelman & Haanstra (1989) and Haanstra & Doelman (1999).

Statistics p = 0.05. Test compound added as a solid. Test concentrations: 0-55-150-400-1,000-3,000-8,000 mg/kg (all studies, except the soil respiration study: in that study the lowest test concentration reported for zinc was 150 mg/kg). The study included both "short-term" measurements carried out during the first 6-8 weeks after the addition of zinc to the soil samples and long-term measurements conducted 1-1.5 years later. The results were reported as NOEC, EC10 and/or EC50 values (calculated by the study authors). Tests were started with field-moist soil samples .

Soils: EU-soils; top soil (0-10 cm) samples of the five different soil types used in the study were collected from several parts of the Netherlands. The sandy soil was obtained from fallow land. The sandy loam, silty loam and clay soil were obtained from arable land after the harvest of a potato crop. The sandy peat soil was obtained from a marshy pasture. The pH values of the soils listed in the table are the initial pH KCl values measured before treatment of the soils; the initial pH H_2O values are 7.0 (sand), 6.0 (sandy loam), 7.7 (silty loam), 7.5 (clay) and 4.4 (sandy peat). According to Doelman & Haanstra (1984), the pH KCL values at the end of the tests were within 1 unit of the initial values, except for 3,000 and 8,000 mg/kg in the sandy soil (decreased from 7.7 to 5.8 and 5.5).

Background zinc concentrations: 14 mg/kg in sand, 17 mg/kg in sandy loam, 103 mg/kg in silty loam, 226 mg/kg in clay, and 38 mg/kg in sandy peat.

Only the 6-w results (EC10 values) were used for PNEC derivation: all 1.5-yr results (NOEC or EC10 values, see Part I and Part II of Table 3.3.3.a) were rejected, whether the 6-w EC10 was lower than the 1.5-yr EC10 derived from the same test or not. The 1.5-yr NOEC and EC10 values were rejected based on Relevance criterion (tests in aged soil).

The 6-w EC10 for phosphatase activity in the sand soil was rejected based on Quality criterion (the 6-w EC10 of 4 mg/kg is far below the lowest test concentration of 55 mg/kg).

Further note: In the Doelman & Haanstra publications on the arylsulphatase, phosphatase and urease activity, graphical representations of the dose-response curves (calculated with the logistic response model used) were given in addition to the EC10 and EC 50 values, but the data onderlying the dose response curves are not given. In some of the phosphatase and urease activity tests, the reported EC10 value is far below the lowest test concentration of 55 mg/kg, especially regarding the 1.5-yr test results.

[6a] Doelman & Haanstra, ’83; ’84: Respiration

Statistics: p = 0.05. Test concentrations: 0-150-400-1,000-3,000-8,000 mg/kg.

Starting 2-7 days after the addition of Zn, respiration rate (CO_2 production per 24 h) was measured after 2, 4 and 8 weeks (all soils) and further after about 1 year (sandy loam soil) or 1.5 year (the other 4 soils).

- In the silty loam soil, respiration rate after 1.5 yr was increased 19% at 400 mg/kg and 26% at 1,000 mg/kg (statistically significant at both concentrations; no data on the respiration rate at 150 mg/kg.

- In the sandy peat soil, respiration rate after 1.5 yr was inhibited 9%, 4%, 11%, 26% and 51% at 150, 400, 1,000, 3,000 and 8,000 mg/kg, respectively, all statistically different compared to the control except for the 4% inhibition at 400 mg/kg. . The NOEC was set at 400mg/kg (and not at 150 mg/kg) because there was only a clear concentration-effect response from 400 mg/kg and onward

- All soils: There was no statistical analysis of the respiration rate data after "short-term" exposure (2, 4, and 8 weeks) and for most soils, data for one or more of these short-tem exposure times were not available; therefore, the short-term data were not used to derive NOEC values. In the sandy loam soil and the sandy peat soil, the short-term effects were similar to the long-term effects. In the other soils the results were variable, e.g. in the silty loam soil, respiration was increased at short-term exposure to the highest test concentration (while decreased after long-term exposure to this and the next lower concentration) and in the sandy soil and the clay soil, the effect after short-term exposure was usually more severe than that at long exposure. The 8-w EC50 values were reported in Doelman & Haanstra '83.

Regression analysis of the data showed that the Fe content in soil was the main abiotic factor related to the effect of zinc on respiration, followed by the clay content (the other abiotic factors studied were pH, CEC, organic matter, lime, and Mn).

[7] = All Tabatabai and co-workers studies (N-mineralization and emzyme activities)

One or more of the soils mentioned belowe were used in the studies by Tabatabai and co-workers, i.e. Tabatabai (1977), Liang & Tabatabai (1977), Liang & Tabatabai (1978), Juma & Tabatabai (1977), Al-khafaji & Tabatabai (1979) and Stott, Dick and Tabatabai (1985).

In the tests conducted by these authors, only one or two test concentrations were used next to the control. In the tests in which two concentrations were tested, there was always a concentration-related effect (either no effect at the lowest concentration and effect at the highest, or effect at both concentrations, with the highest inhibition at the highest concentration). The results were reported as percentage inhibition. i.e. EC(..%) In some of these studies the difference required for significance (LSD) compared to the control was reported (at p = 0.05 and p = 0.01). In some tests, ≤10% inhibition was found to be statistically different from the control. However, as statistical data were not reported in all publications of Tabatabai & co-workers, the rapporteur considered all concentrations that resulted in ≤10% inhibition to be NOEC values, regardless the statistical results. In some cases an EC10 could be calculated by the rapporteur.

Soils: non-EU-soils; top (0-15 cm) soil samples of different soils assumed to be collected in the U.S (based on the location of the research institute (Ames, Iowa, U.S) One or more of the following soils were used in each study: Webster, Judson, Harps, Okoboji, Weller, Nicollet, Luton and Clarion. The soil types listed in the table are based on the data reported on the sand, silt and clay content (combined data several publications; not all aforementioned data were reported in each reference). The pH values listed in the table are the initial pH H_2O values measured before treatment of the soils. The organic matter content was calculated from the organic carbon content in the sample. Some data on the clay content, organic carbon content and pH value of the soils reported in the different references by Tabatabai and co-workers show some slight differences, probably based on analyses of different samples of each soil. For consistency reasons one fixed set of characteristics has been listed in the table for each soil. No data on background zinc concentrations in the soils.

Test compound added in aqueous solution. The tests were performed in previously air-dried soil samples that were remoistened before start.

[7a] = Liang & Tabatabai '77: N-mineralization

Statistics: the difference required for significance (LSD) was indicated at p = 0.05 and p = 0.01) for both ammonium-N and nitrate-N; in all four soils at least one parameter was significantly different compared to the control. No statistical data were given for the combined data on N-mineralization on which the percentages inhibition (reported by the study authors) is based. Test compound added in aqueous solution, together with ammonium-N added as $(NH_4)_2SO_4$. Test concentrations: 0 and 327 mg/kg (reported as 5 µMol/g).

[8] Chang & Broadbent '82: N-mineralization

No statistics reported. Test compound added in aqueous solution. Test concentrations: 0-50-100-200-400 mg/kg. N-mineralization parameters: organic-N, inorganic-N, and nitrate-N, measured after 2, 4, 8 and 12 weeks of exposure.

Soil: non-EU soil (Yolo silt loam; Typic Xerorthent). Reported soil charcteristics: pH 6.9; zinc backgrounds concentrtation 7 mg/kg. For urther data on soil characteristics. soil amendments (with 1% sludge and 1% alfalfa) and soil zinc analyses method: see footnote 4. After 2 weeks of incubation the percentage of added zinc extractable in water and KNO_3 was 0.8% to 2.4% and the percentage of cumulative recovery of zinc by the four sequential extractions was 40%, 68% and 61% at the added zinc concentration of 100, 200 and 400 mg/kg, respectively. The cumulative recovery of zinc after 2 weeks of incubation (reported in this study: Chang & Broadbent, 1982) and after 12 weeks of incubation (reported in Chang & Broadbent, 1981, see footnote 4) are very similar. According to Chang & Broadbent 1982) the fraction of water-soluble, "exchangeble" (KNO_3 extractable) and "available" (DTPA extractable) of zinc and other metals used in the study decreased in time, indicating gradual

conversion to less soluble forms (HNO_3 extractable). With exception of the above data on water and KNO_3 extractable zinc after 2 weeks of incubation, no further data on this subject were reported.

[9] Premi and Cornfield '69: Ammonification and nitrification

Statistics: p = 0.05. Test compounds (zinc sulphate and zinc carbonate, tespectively) added as a solid. Test concentrations: 0-100-1,000-10,000 mg/kg. The tests were performed in previously air-dried soil samples that were remoistened before start. N-mineralization parameters studied: ammonium-N, nitrite-N and nitrate-N. The results listed in the table are from aerobic tests conducted in aerated soil samples at 50% WHC. The effects of zinc on ammonifcation were also studied in an anaerobic test in un-aerated soil samples at 100% WHC, resulting in the same NOEC as under aerobic concditions.

Soil: Eu-soil; top soil samples from an agricultural soil were collected in England (based on the location of the research institute:London).

Reported soil characteristics: 2.0% organic carbon (equivalent to 3.4% organic matter, 17% clay; pH 7.1. At the end of the exposure period the pH was 7.0, 6.2 and 5.6 in the zinc sulphate treated soil (decreasing with increasing zinc sulphate concentration) and 7.2, 7.4 and 8.5 in the zinc carbonate treated soil (increasing with increasing zinc carbonate concentration), under aerobic conditions. Under anarobic conditions the pH values in the zinc sulphate treated soils were 7.3, 6.8 and 5.7. Background zinc concentration: 57 mg/kg d.w.

[9a] **Rejected, based on Relevance criterion (test compound: "insoluble" zinc salt: $ZnCO_3$).**

[10] Liang & Tabatabai '78: Nitrification

Statistics: the difference required for significance (LSD) was indicated at p = 0.05 and p = 0.01) for the two nitrification parameters studied (nitrite-N and nitrate-N), but no statistics on the combined data on which the percentage inhibition (reported by the study authors) is based. Test compound added in solution. Test concentrations: 0 and 327 mg/kg (reported as 5 µMol/g). After treatment of the soils the pH was within 0.1 units of the initial value.

The tests in Webster soil and Okoboji soil were rejected based on Quality criterion (>30% inhibition at the LOEC; no NOEC or EC10 can be derived).

[11] Wilson '77: Nitrification

Statistics: p = 0.05. Test compound added in solution. Test concentrations 0-10-100-1,000 mg/kg. The tests were performed in previously air-dried soil samples that were remoistened before start. Inorganic-N (as NH_4Cl) was added to all soil samples. The N-mineralization parameters studied were (i) ammonium-N and (ii) nitrite-N + nitrate-N; measurements were made every week during the 7-w exposure time. For each of the soils, the nitrite-N + nitrate-N concentration have been corrected by subtracting the average initial value in that particular soil. The ammonium-N concentrations were not corrected since the initial value in the soils was nearly zero.

In all three soils (Decatur, Cecil and Leefield), the 1,000 mg/kg level complete eliminated nitrification.

In the sandy loam soil (Cecil), the level of 100 mg/kg resulted in a statistical significant inhibition of nitrification during the second and third weeks of exposure, but not in the remaining period. (NOEC set at 100 mg/kg).

In the loamy sand soil (Leefield), the level of 100 mg/kg resulted in a statistical significant inhibition of nitrification during the third, fourth, fifth and seventh weeks of exposure, thus during most of the total exposure time of 7 weeks (NOEC set at 10 mg/kg). Measured by the inhibition of nitrite-N + nitrate-N formation, the percentage inhibition of nitrification was around 25%-35% at 3-5 weeks, less than 10% after 6 weeks and 20% after 7 weeks (derived from graphical representation: dose-response curve). Based on the 7-w results, the NOEC for Leefield soil would be 10 mg/kg. Because of the low reliability of this NOEC, an alternative NOEC of 50 mg/kg (NOEC = LOEC/2 (20% inhibition at 100 mg/kg) = 100/2 = 50 mg/kg) has been derived for this soil and used for PNEC derivation. See RAR section 3.3.3.1 for general requirements and methods for alternative NOEC derivation in case the "real" NOEC is unreliable (Quality criterion). An EC10 can be calculated for Leefield soil, but not with a high accuracy: the percentages inhibition must be derived from the graphical representation (dose-response curve) in the reference. Thus, no EC10 was calculated.

Soils: non-EU soils, assumed to be collected in Georgia, U.S. (based on the location of the research institute). Before the start of the tests, the pH values of the clay loam soil (Decatur; initial pH 5.5) and the loamy sand soil (Leefield; initial pH 5.1) were increased to 6.8 and 7.4, respectively. After the 7-w exposure time, the pH values of these two soils decreased around one unit, to 5.5-6.1 in the clay loam soil (Decatur) and to 6.0-6.2 in the loamy sand soil (Leefield). In the sandy loam soil (Cecil; initial pH 6.2) the pH remained within one unit: 5.3-5.8 after the 7-w exposure time. Background zinc concentrations in the soils: 136 mg/kg in the clay loam soil (Decatur), 24 mg/kg in the sandy loam soil (Cecil) and 7 mg/kg in the loamy sand soil (Leefield).

[12] Doelman & Haanstra '83: Arylsulphatase and phosphatase activity

In the test with an exposure time of <1 w, the enzyme activity was measured "directly" after addition of zinc to the soil.

[13] Juma & Tabatabai '77: Phosphatase activity.

No statistics reported. Test compound added in solution. Test concentrations: 0-164-1,640 mg/kg (reported as 2.5 and 25 µMol/g. In some tests only the highest concentration was tested along with the control. Phosphatase activity was measured 30 minutes after the addition of zinc to the soil (after this 30 minutes exposure time the soil was treated with toluene (bacteriostat), buffer (pH 6.5 and 11 for acid and alkaline phosphatase measurements, respectively) and sodium p-nitrophenyl phosphatese, after which the phosphatase activity was measured for 1 hour at 37 ^{0}C, referring to the method of *Tabatabai & Bremmer (1969)* and *Eivazi & Tabatabai 1977 (not checked).*

The tests for acid phosphatase activity in Harps soil and Okoboji soil and the test for alkaline phosphatase activity in Harps soil were rejected based on Quality criterion (>30% inhibition at the LOEC; no NOEC or EC10 can be derived).

[14] Tabatabai '77: Urease activity

No statistics reported. Test compound added in aqueous solution. Test concentrations: 0-33-327 mg/kg (reported as 0.5 and 5 µMol/g). In some tests only the highest concentration was tested along with the control. Urease activity was measured 30 minutes after the addition of zinc to the soil (after this 30 minutes exposure time the soil was treated with toluene (bacteriostat), buffer (pH 9) and urea, after which the urease activity was measured for 2 hours at 37 ^{0}C, refering to the method of *Tabatabai & Bremner (1972) (not checked).* The EC10 values were calculated by the rapporteur from the reported study results: in Harps 7% and 34% inhibition at 33 and 327 mg/kg, respectively; in Okoboji soil 6% and 30% inhibition at 33 and 327 mg/kg, respectively. In addition, the rapporteur calculated EC50 values: 721 mg/kg for Harps soil and 907 mg/kg for Okoboji soil.

Because of the low reliability of the NOEC values for Harps soils and Okoboji soil, alternative NOEC values of 52 mg/kg (NOEC = EC10) and 64 mg/kg (NOEC = EC10) were derived for Harps soil and Okoboji soil, respectively, and used for PNEC derivation. See RAR section 3.3.3.1 for general requirements and methods for alternative NOEC derivation in case the "real" NOEC is unreliable (Quality criterion).

The tests in Weller soil, Nicolett soil and Luton soil were rejected based on Quality criterion

(>30% inhibition at the LOEC; no NOEC or EC10 can be derived).

[15] Bremner & Douglas '71: Urease activity

No statistics reported. Test compound added in aqueous solution.

Rejected, based on Quality criterion (unbounded NOEC values).

[16] Mathur & Rayment '77: Respiration and phoshatase activity

Statistics ($p = 0.01$). Mixed-metal exposure: experimental plots in the peat soil which was limed to pH 5.5 in the field and further treated for 7 or 8 years with a yearly amendment of 560 kg NPF fertilizer per hectare, with or without 2% fritted trace element mixture (FTE) containing 7% Zn, 3% Cu, 3% B, 18% Fe, 7.5% Mn and 0.2% Mo. Respiration (cumulative CO_2 production, measured in the laboratory during 60 to 125 days at different temparatures) was statistically significant reduced in soil amended with NPF + FTE compared to control soil amended with NPF alone; the percentage inhibition ranged from around 10% to 30%. Compared to virgin soil untreated with either NPF or FTE, amendments with NPF alone or with NPF + FTE resulted in a stimulation of respiration. Phosphatase activity (measured in the laboratory in soil samples stored under different conditions) was also reduced in soil amended with NPF + FTE compared to control soil amended with NPF alone; the percentage inhibition ranged from around 10% to 40%. Based on the relative increase in Cu concentration compared to that of Zn and based on the results of additional tests on the effects of Cu on respiration and phosphatase activity, it appears that Cu is the causative factor rather than zinc (Mathur and Rayment (1977). Refering to Tyler ((1976), Mathur & Rayment stated that the results of a field study in a conifer forest in the vicinity of a brass mill in Sweden iindicate, that Cu was more responsible for reduced respiration rate (litter decomposition) phosphatase activity and rate of P mineralization than zinc at nearly equal concentrations.

Soil: non-EU soil; top soil samples (0-15 cm) were collected in southeast Newfoundland (Canada). Reported soil characteristics: 42% organic carbon (equivalent to 71% organic matter). The CEC of this soil is 113 meq/100g). At the high %OM of this soil (71%), no clay content can be calculated from

{CEC (meq/100 g) = 2,5 x %OM + 0,5 %xClay}, as this equation results in a negative value for the clay content.

The pH value of 5.6 is the pH of the limed soil amended with NPF + FTE; the pH of the limed soil amended with NPF alone was 5.4. The pH of the unlimed and unamended virgin soil was 3.2.

Background zinc concentration in the unamended virgin soil: 78 mg/kg d.w. The zinc concentration measured in control soil amended with NPF alone was 70 mg/kg d.w.; the zinc concentration measured in soil amended with NPF + FTE was 109 mg/kg d.w.

Rejected, based on Relevance criterion (mixed-metal exposure).

[17] Bååth '89: Respiration, nitrification, and phosphatase activity; Denneman & Van Gestel '90: Glucose mineralization; Doelman & Haanstra '83: Ammonification

Secondary literature sources; original publications not available, thus the quality of the study and the reported results could not be checked.

Rejected, based on Quality criterion (data from secondary literature source).

[18] Haanstra & Doelman '91: Arylsulphatase activity

Test compound added as a solid. Test concentrations: 0-55-150-400-1,000-3,000-8,000 mg/kg. At the end of the tests, the pH values were within one unit of the initial values, except in sandy soil at 3,000 and 8,000 mg/kg (decreased from 7.7 to 5.8 and 5.5, respectively). Arylsulphatase activity was measured 6 weeks and 1.5 year after the addition of zinc to the soil (measurement of arylsulphatase activity: for 2 hours at 30 ^{o}C, refering to the method of *Tabatabai & Bremmer, 1970 (not checked)*.

The results of this study show variable results with respect to short-term (6-w) and long-term (1.5-yr) effects on arylsulphatase activity. In the sandy soil, the sandy loam soil and the clay soil, the long-term EC50 was statistically significant lower than the short-term EC50, while in the silty loam soil the long-term EC50 was statistically significant higher than the short-term EC50. The EDR values (the dose range of EC10 and EC90) usually showed the same trend for long-term versus short-term effects in a particular soil, but the results were variable. For example, in the sandy soil the long-term EC10 was higher than the short-term EC10, while the long-term EC90 was more than one order of magnitude lower than the short-term EC90. The EDR values did not show a statistically significant change over time, but due to a poor estimate of one of the model parameters, no precise and meaningful comparison between long-term and short-term EDR values could be made.

In the sandy peat, a soil with a high organic matter content, 6-w criteria could not be calculated (the iteration proces did not converge, all measurements were at the same level).

[19] Maliszewska et al. '85: Dehydrogenase activity

No statistics reported. No data on the physical forrm in which the test compound was added. Test concentrations: 0-200-500-1,000-5,000-10,000 mg/kg. The EC10 values were calculated by the rapporteur.

Dehydrogenase acivity:

In sandy soil the activity was dose-related reduced, with 33% inhibition at 200 mg/kg, the lowest test concentration. The NOEC was set equal to the EC10: 76 mg/kg. This EC10 was calculated with the top of the curve fitted (thus not fixed at control value of 0% effect, because control performance in the duplicates may vary) and the bottom of the curve fixed at 100% effect. With both the top and the control value of the curve fitted (thus not fixed at 0% and 100% effect, respectively), the EC 10 is 59 mg/kg. The r^2 values of the two dose-reponse curves are very similar: 0.9800 and 0.9833, respectively.

In alluvial soil the activity was increased at the lowest two concentratations (+72% at 200 mg/kg and +53% at 500 mg/kg) and dose-related decreased at the higher concentrations (30%, 66% and 77% inhibition at 1,000, 5,000 and 10,000 mg/kg, respectively). The NOEC is set at 500 mg/kg. Based on these data the following EC10 values were calculated: i) 248 mg/kg (top of curve fixed at increased activity at 200 mg/kg; $r^2 = 0.63$), ii) 1,242 mg/kg (top of curve fixed at control activity at 0 mg/kg; $r^2 = 0.52$) and iii) 453 mg/kg (top of curve fitted; $r^2 = 0.73$). In the calculations i) and iii), stimulation is taken into account, in contrast to calculation ii). In all calculations the bottom of the curve was fixed at 100% effect.

Additional data: also data on the effects on microbial numbers have been reported by Maliszewska et al. '85.

Not used for PNEC derivation, as only tests measuring the effect on microbe-mediated processes in soil with the native microbial population were used in the RAR Zn and not tests measuring the effect on microbial numbers or microbial diversity.

Soils: EU-soils; samples assumed to be collected in Poland (based on the location of the research institute).

Reported soil characteristics for sandy soil: total C content 1.8% (assumed to be organic-C, equivalent to 3% organic matter) and pH H_2O 6.9. Alluvial soil: 1.1% total C content (equivalent to 1.9% organic matter) and pH H_2O 7.1. DTPA-extractable background zinc concentrations (at pH 7.2): 15 mg/kg in the sandy soil and 8 mg/kg in the alluvial soil. No data on the molarity of the DTPA solution.

[20] Ohya et al. '85: Glucose mineralization

No statistics reported. Test compound added in aqueous solution, together with U-(^{14}C)-glucose (5 g/kg soil, as C) and $(NH_4)_2$ SO_4 (0,5 g/kg soil, as N). Test concentrations: 0-100-300-1,000 mg/kg. The test was performed in previously air-dried soil samples that were remoistened before start. Parameter: cumulative CO_2 production of both labelled and unlabellled CO_2, representing amended glucose mineralization and soil organic carbon mineralization, respectively. The CO_2 measurements were made daily during the 96-h exposure time.

Mineralization: Within the 96-h exposure period, the total CO_2 production (from glucose mineralization + soil organic carbon respiration) was most clearly inhihibited within the initial 24 h of the incubation (13%, 33% and 44% inhibition at 100, 300 and 1,000 mg/kg, respectively, especially due to the inhibition of the glucose mineralization, intially the major carbon source. The cumulative total CO_2 production during 96 hour was inhibited 5%, 7% and 12% at 100, 300 and 1,000 mg/kg, respectively. Based on these data and additional data on the effect of 1,000 mg/kg on glucose mineralization and soil organic carbon carbon mineralization separately, the NOEC is set at 300 mg/kg.

Additional data: also data on the effects on microbial numbers have been reported by Ohya et al., '85; see also Ohya et al. '86. Not used for PNEC derivation, as only tests measuring the effect on microbe-mediated processes in soil with the native microbial population were used in the RAR Zn and not tests measuring the effect on microbial numbers or microbial diversity.

Soil: non-EU soil; top (0-10 cm) soil samples collected in Japan (Osaka). The soil, a diluvial, non-vulcanic sandy clay loam, was obtained from a paddy. repored soil characteristics: organic carbon content 1.2% (equivalent to 2% organic matter) and CEC 6.9 meq/100 g, resulting in an estimated clay content of 4%. HCl- (0.1 N)-extractable background zinc concentration: 14 mg/kg.

[21] Bollag & Barabasz '79: Denitirification

No statistics reported. Test compound added in solution (together with nitrate-N) to air-dried soil samples. Test concentratons: 0-10-50-100-250-500 mg/kg. The test was performed under anaerobic (helium) conditions. Denitrification parameters (nitrate, nitrite, nitrous oxide and nitrogen gas) were measured daily during the 3-w exposure time; the 1-w, 2-w and 3-w results were reported in graphical representations (block digrams) At 250 and 500 mg/kg there was a clear inhibition of denitrifictaion, as indicated by the accumulation of nitrite and N_2O, and a decrease in N_2 formation.

Additional denitrification tests were conducted in autoclaved soil to which an inoculum of bacterial species Pseudomonas aeruginosa, Pseudomonas denitrificans or unidentified Pseudomonas sp. was added. These single-species denitrification tests resulted in a 4-d LOEC of 50 and a 4-d NOEC of 10 mg/kg, which is lower than the NOEC of 100 mg/kg found in the abovementioned denitrification test in native (unautoclaved) soil, which is a multi-species test. Not used for PNEC derivation, as only tests measuring the effect on microbe-mediated processes in soil with the native microbial population were used in the RAR Zn and not tests measuring the effect on specific microbial species

Soil: non-EC soil, assumed to to be collected in the U.S. (Pensylvania, based on the location of the research institute).Reported soil characteristics: organic carbon content 1.8% (equivalent to 3.1% OM), clay content 28% and pH 6.8.. No data on background zinc concentration.

Footnotes [22]-[30]: Cancelled; these footnotes mentioned application factors used to derive NOECs from EC(..%) values. See RAR section 3.3.1.2 for Derivation of NOEC values (methods).

[31] Hemida et al. 97: Amidase activity, nitrate reductase activity and urease activity

Statistics**:** p = 0.05. Test compound added in solution. Test concentrations:0-200-2,000 mg/kg. Measurements after 1, 4 and 12 weeks of exposure. Cb test soils: not reported.

Amidase activity: In both soils significantly decreased at 2,000 mg/kg after 1, 4, and 12 weeks of exposure (at 200 mg/kg: amydase activity significantly increased after 1 and 4 weeks).

Nitrate reductase activity:

In clay soil significantly decreased at 200 mg/kg after 4 and 12 weeks of exposure (38% and 43% inhibition, respectively) and significantly decreased at 2,000 mg/kg after 1, 4, and 12 weeks of exposure (45%, 60% and 52%, respectively). Because the 12-w results at 200 and 2,000 mg/kg did not differ ≥15% (and were not significantly different from each other), no EC10 was calculated.

In sandy soil significantly decreased at 200 mg/kg after 1 and 12 weeks of exposure (27% and 23% inhibition, respectively) and significantly decreased at 2,000 mg/kg after 1, 4, and 12 weeks of exposure (51%, 41% and 51% respectively). The 12-w EC10 (34 mg/kg) was calculated by the rappporteur. Based on the low reliability of this EC10 (34 mg/kg) ,which is 6-times lower than the lowest test concentration (200 mg/kg), an alternative NOEC of 67 mg/kg (NOEC = LOEC/3 (23% inhibition at 200 mg/kg) = 200/3 = 67 mg/kg was derived for this soil and used for PNEC derivation. See RAR section 3.3.3.1 for general requirements and methods for alternative NOEC derivation in case the EC10 is unreliable (Quality criterion).

Urease activity: In clay soil significantly decreased at 200 mg/kg after 1, 4 and 12 weeks of exposure (61%, 64% and 65% inhibition, respectively) and 100% inhibition at 2.000 mg/kg. In sandy soil significantly decreased at 200 mg/kg after 1, 4 and 12 weeks of exposure (59%, 51% and 57% inhibition, respectively) and 100% inhibition at 2,000 mg/kg. Because of the complete inhibition at the highest concentration, no EC10 values can be calculated.

Microbial populations: Both test concentrations (200 and 2,000 mg/kg) resulted in significant effects on the total counts of one or more microbial groups studied (glycophilic fungi, thermophilic and thermotolerant fungi, cellulose-decomposing fungi, bacteria and actinomycetes). The effects (both decreased and increased numbers of organisms) depended on both the exposure time and soil type. Not used for PNEC derivation, as only tests measuring the effect on microbe-mediated processes in soil with the native microbial population were used in the RAR Zn and not tests measuring the effect on microbial numbers or microbial diversity.

Soils: non-EU soils; samples were collected in agricultural soils (cultivated with wheat) in Egypt. The pH value is the pH H_2O.

The test for nitrate reductase activity in clay soil and the tests for urease activity in sand and clay soil were rejected based on Quality criterion (>30 inhibition at the LOEC; no NOEC or EC10 can be derived).

[32] Saviozzi et al. ’95: Respiration

No statistics reported. Test compound added in solution. Test concentrations: 0-50-100-250-500-1,000 mg/kg. Respiration (cumulative CO_2 production was periodically measured during the 28-d exposure time.

The EC20 and EC50 based on the nominal zinc concentration were 275 and 2,073 mg/kg, respectively. Based on the extractable zinc concentration of zinc in the soil the EC20 and EC50 were 203 and 1,530 mg/kg, respectively. The extractable concentrations (extracted with 0.5 M ammonium acetate + EDTA solution at pH 4.65) were determined at the end of the test.

Soil: EU soil; top soil (5-15 cm) samples were collected in Italy in the province of Pisa. Reported soil characteristics: organic carbon content 1.4% (equivalent to 2.4% OM), clay content 8%, CEC 13 meq/100 g., pH 5.2 . Soil type derived from the reported clay (8%), sand (72%) and silt (20%) content. After treatment the pH value in zinc treated soil was within 0.4 units. The extractable background concentration of zinc in the soil was 3.5 mg/kg d.w. (extracted with 0.5 M ammonium acetate + EDTA solution at pH 4.65).

[33a] Doelman & Haanstra ’83: Glutamic acid mineralization

Rejected, based on relevance criterion (tests in aged soils; see also footnote [6])

Further note that data reported by Doelman & Haanstra '83 refer to the maximum glutamic acid mineralization rate. In a reevaluation of this study by Haanstra & Doelman '84 (see below), the results refer to the mean glutamate mineralization rate, which is considered to be a

more relevant criterion (see Van Beelen & Doelman '97 and ISO / TC 190 ISC4 n149 for arguments underlying the statement that the mean mineralization rate is more relevant than the maximum rate).

[33b] Haanstra & Doelman, '84b: Glutamic acid mineralization

Statistics: p = 0.10 and 0.05. Test concentrations 0-55-400-1,000 mg/kg (no data on the physical form of the added $ZnCl_2$, but assumed to be added as a solid, see the other publications of Doelman & Haanstra: footnote [6]). The data reported by Haanstra & Doelman '84 refer to the mean glutamic acid mineralization rate (decomposition time, in hours). The decomposition time, determined 1.5 yr after the addition of zinc to the soil, was defined as the time from mixing the soil with glutamic acid untill the maximum glutamic mineralization rate was reached. The shape of the peak was described according to the maximum mineralization rate (*peak height*, in ml CO_2/kg/h) and the *peak width* at half maximum. Glutamic acid was added to the soil at a concentration of 10 mMol/kg, i.e. about 1,500 mg/kg).

In the sandy soil, the mean mineralization rate was statistically significantly inhibited (p = 0.05) at all three test concentrations (12%, 50% and 89% inhibition, respectively). From these data the EC10 was calculated by the rapporteur (and in addition the EC50: 361 mg/kg)

In the silty loam soil, the mean mineralization rate was increased at all three concentrations (statistically significant at 55 mg/kg at p = 0.05 and 1,000 mg/kg at p = 0.1, but not at 400 mg/kg) , resulting in an unbounded NOEC of 1,000 mg/kg.

In the clay soil the mean mineralization rate was statistically significantly inhibited at 400 mg/kg (p = 0.10) and 1,000 mg/kg (p = 0.05). The NOEC was set at 400 mg/kg..

In the sandy peat soil the mean mineralization rate was not statistically significantly affected at any concentration, resulting in an unbounded NOEC of 1,000 mg/kg.

Rejected, based on relevance criterion (tests in aged soils; see also footnote [6|)

[34a] Van Beelen et al., '94b: Acetate mineralization

The EC10 and IC10 values were reported by the study authors. The IC10 (the concentration that causes 10% inhibition of first-order mineralization rate) is not dependent on the incubation time and the uninhibited substrate half life, in contrast to the EC10. Because the IC10 (or, for example, the IC50) is not a standard criterion in soil toxicity tests, the IC10 values have not been used for PNEC derivation. For more details on IC versus EC values, see footnote 1 of Table 3.3.2.f.

The tests were conducted in (10 g soil + 10 ml water) slurries. According to Van Beelen (personal communication) the use of soil/water slurries facilitates the mixing of the test compound with the soil, but there appears to be no significant difference between the results of soil and slurrie tests. Fresh soil samples were used to prepare the slurry. Test compound added in an aqueous solution. Highest test concentration: 1,000 mg/kg; no further data on test concentrations. After 2 h of preincubation with $ZnCl_2$, [^{14}C] acetate was added at a concentration of 1 µg/l soil slurry, to simulate the low acetate concentration under real environmental conditions, thus preventing an unnatural rapid growth of resistent microorganisms, which could obscure the effect of zinc. Parameter: $^{14}CO_2$ production. For the acid De Peel topsoil and subsoil, a value of >1,000 mg/kg was reported for the EC10 and IC10. The microbial populations in the alkaline Flevopolder soil was more sensitive. The difference in sensitivity in the soils could not be explained by differences in sorption of zinc (which was highest in Flevoland soil; partition coëfficiënt of both soils reported), but may be

attributed to differences in chemical speciation (formation of hydroxide complexes, see also Van Beelen and Fleuren-Kemilä, 1993). *The relatively low toxicity of zinc for the microflora in acid soils has been observed earlier by Bewley & Stosky (1983) and Wilke (1990); not checked; cited in Van Beelen and Fleuren-Kemilä, 1993).*

Soils: EU soils; topsoil samples (surface soil) and subsoil samples (collected from a depth of around 150 cm) were collected from 2 different sites in The Netherland (Flevopolder forest soil: calcareous soil reclaimed from the sea; De Peel forest soil: humic sand). Soil characterisics: reported for both the topsoil and subsoil samples. The organic matter content was calculated from the reported organic carbon content in the sample. The pH values are pH KCl values; the pH H_2O values are 8.2 in Flevoland topsoil, 8.3 in Flevoland subsoil, 3.8 in De peel topsoil, and 4.5 in De Peel subsoil. Background zinc concentrations: 43 mg/kg in Flevoland topsoil, 12 mg/kg in Flevoland subsoil, 6 mg/kg in De Peel topsoil, and 4 mg/kg in De Peel subsoil.

The surface soils of both sites showed a higher number of bacteria and a higher acetate mineralization rate compared to the subsoils, which may result in a higher sensitive of microbial populations in subsoils**. Because tests in subsoils are not common practice in soil microbial tests, the results of the subsoil tests have been rejected for PNEC derivation (Relevance criterion). Furthermore, the tests in de De Peel subsoil and De Peel surface soil resulted in unbounded NOEC values (Quality criterion).**

[34b] Van Beelen & Fleuren- Kemilä '93: Acetate mineralization

Test design (material and methods): see footnote [34a]. No detailed information on the test with zinc, but reported that there was no effect at the highest test concentration: 1,110 mg/kg.

Soil: EU soil; top soil (0-10 cm) samples collected from a fir forest soil in The Netherlands (near Bilthoven). Reported soil characteristics: organic carbon content 0.5% (equivalent to 0.9% organic matter), clay content 0.5%, CEC 1.1-1.6 meq/100 g, pH KCl 3.8 (the pH H_2O is 4.4). No data on the background zinc concentration.

Rejected, based on Quality criterion (unbounded NOEC).

[35] Denneman & Van Gestel '90: Glucose mineralization

Denneman & Van Gestel (1990) reported 3% and 7% inhibition at 1,000 and 5,000 mg/kg, respectively.

Study originally reported by Babich & Stotsky (1983) (not checked).

Rejected, based on Quality criteria (secondary literature source and unbounded NOEC).

[36] Lighthart et al. '83: Respiration

No statistics reported. Test compound added in aqueous solution. Test concentrations 0-3.3-33-327-3,270 mg/kg (reported as 0-0.05-0.5-5.0-50 mMol/kg) The tests were performed in previously air-dried soil samples that were remoistened before start. Distilled water plus a 1 mL microbial inoculum of a filterate from 100 g of fresh active soil mixed in 1 L of distilled water was added. After 9-d of preincubation, the zinc solutions were added. No data on the origin of the microbial inoculum: indigenous or the same for all 5 soils tested??. Respiration was periodically measured by CO_2 production. The onset of respiration inhibition was defined by the study authors as occurring between one and two standard deviations of the control, i.e. 4-8% inhibition, but detailed data were not reported and the NOEC values, based on the rapporteur's criteria could only be derived from poor graphical representations (block diagrams) giving the percentage of inhibition or stimulation per test concentration). The

diagram showed, however, clear concentration-effect relationships with respect to the effect of zinc (at least with respect to inhibition at the concentrations that did not result in stimulation, see below).

It is noted that Toledo soil, Walla Walla Soil and Crider soil may be zinc deficient, as indicated by a clear stimulation of respiration at 0.05 and/or 0.5 mMol/kg (3 and 33 mg/kg) the lowest two concentrations tested. In Sharpsburg soil and Rifle soil there appears to be a (very slight) stimulation of respiration at the lowest concentration tested.

Because of the low reliability of the NOEC values for Crider soil, Toledo soil, Walla Walla soil and Sharpburg soil, alternative NOEC values were derived for these soils (see below) and used for PNEC derivation. See RAR section 3.3.3.1 for general requirements and methods for alternative NOEC derivation in case the "real" NOEC is unreliable (Quality criterion).

[36a] Alternative NOEC for Crider soil: NOEC = LOEC/3 (>20% and <30% inhibition at 330 mg/kg)

= 330/3 = 110 mgkg.

[36b] Alternative NOEC for Toledo soil: NOEC = LOEC/2 (>10% and <20% inhibition at 330 mg/kg)

= 330/2 = 165 mg/kg.

[36c] Alternative NOEC for Walla Walla soil: NOEC = LOEC/3 (>20% and <30% inhibition at 330 mg/kg)

= 330/3 = 110 mg/kg.

[36d] Alternative NOEC for Sharpsburg soil: NOEC = LOEC/2 (>10% and <20% inhibition at 33 mg/kg)

= 33/2 = 17 mg/kg.

Further note Lighthart et al. study: EC10 values can be calculated (4 test concentrations), but not with a high accuracy: the percentages inhibition must be derived from poor graphical representations (block diagrams). Thus, no EC10 values were calculated.

Soils: Non-EU soils, collected in the U.S, in different states. Five different soils were used (Crider, Rifle, Toledo, Walla Walla and Sharpsburg); the soil types listed in the table are based on the data reported on the sand, silt and clay content in each soil. Rifle soil: no clay content reported. The CEC of this soil is 125 mmol/kg (12.5 meq/100g). At the high %OM of this soil (64%), no clay content can be calculated from

{CEC (meq/100 g) = 2,5 x %OM + 0,5 %xClay}, as this equation results in a negative value for the clay content.

Soil zinc analyses: The background zinc concentrations in the soils were not analysed. Soil solutions were analysed from a 1:1 saturation extract after filtration through a Whatman No. 42 filter paper.. The results were then recalculated to a concentraion at 70% of the WHC, resulting in the following concentrations: 148 µg/l in Crider soil, 10,330 µg/l in Rifle soil, 150 µ/l in Toledo soil, 177 µg/l in Walla walla soil, and 84 µg/l in Sharpsburg soil (reported by the study authors as 2.26, 15.8, 2.30, 2.71 and 1.29 µM).

[37] Bhuyia & Cornfield '74: N-mineralization and nitrification

pH = pH H_2O, No statistics reported. Test compound (zinc oxide) added as a solid, mixed through soil. Test concentrations: 0 and 1000 mg/kg. Background concentration is 74 mg/kg

total Zn. The soil was brought to pH 7.7 with addition of $CaCO_3$. Before start of the incubation, the soils were preincubated for 2 w at 18-22ºC.

Soil: EU or non-EU soil? Soil type: "Pseudogley-Braunerde".

Rejected, based on Relevance criterion (test compound: "insoluble" zinc salt: ZnO).

The publication includes more data: tests at three different pH values; Zn analysis in EDTA extract.

[38] Necker & Kunze '86: N-mineralization

No statistics reported Test concentrations 0 and 700 mg/kg. N-mineralization parameters studied: ammonium-N and nitrate-N (seperately and summed).

The study included a long-term test in which the effect of zinc on N-mineralization was measured in the laboratory 1 year after zinc (300-700-3,000 mg/kg) had been added to the soil in the field at (measurement period was 7 weeks) and a "short-term" test in which the effect of zinc (one concentration: 700 mg/kg) on N-mineralization was measured during a 7-w period that started immediately after the treatment of the soil samples with zinc in the laboratory. The result listed in the table is the result of the short-term test, resulting in around 30% inhibition of N-mineralization, based on nitrate-N and summed inorganic-N (percentage inhibition derived from graphical representation: block diagram). The long-term test resulted in a stimulation of N-mineralization, especially of nitrification, with the highest stimulation at the highest concentration tested: at 3,000 mg/kg the amount of nitrate-N was almost three times higher than that in the control. Both test were conducted without a supplement of organic-N. In the presence of a supplement of organic-N (pepton), a stimulation of around 50% was found for ammonification at all three concentration in the long-term test. In the long-term test, the test compound solution was sprayed on the soil. No data on the physical form (solid or solution) of zinc added in the short-term test.

Soil: EU-soil; both the zinc-treated samples used in the long-term study and the unpolluted samples used in the short-term study were collected in a forest in Germany. Reported soil characteristics: 4.7% organic carbon (equivalent to 8 % OM) and CEC 25 meq/100 g), resulting in an estimated clay content of 10%. The pH value listed in the table is the pH KCl; the pH H_2O value is 4.4. No data on background zinc concentration.

[39] Al-Khafaji & Tabatabai, '79: Arylsulphatase activity

Statistics: the difference required for significance (LSD) was indicated at p = 0.05 and p = 0.01. Test compound added in aqueous solution. Test concentrations: 0-164-1,640 mg/kg (reported as 0-2.5-25 µMol/g). In some tests only the highest concentration was tested along with the control. Arylsulphatase activity was measured 30 minutes after the addition of zinc to the soil (after this 30 minutes exposure time the soil was treated with toluene (bacteriostat), buffer (pH 5.8) and potassium p-nitrophenyl sulphate, after which the arylsulphatase activity was measured for 1 hour at 37 ^{0}C, referring to the method of *Tabatabai & Bremner (1970) (not checked)*.

In all four soils the addition of zinc resulted in a stastistically significant inhibition of the arylsulphatase activity at the concentration (1640 mg/kg) or concentrations (164 and 1,640 mg/kg) tested. The EC10 value for Harps soil was calculated by the rapporteur from the reported study results: 11% and 36% inhibition at 164 and 1,640 mg/kg, respectively. In Webster soil the NOEC was set at 164 mg/kg, although the 10% inhibition found at this concentration was statistically significant (no lower concentrations were tested, however).

[40] Rogers & Li, '85: Dehydrogenase activity

No statistics reported. Test compound added in solution, together with glucose and 2,3,4-triphenyl tetrazolium chloride (TTC); the solution was added to air-dried soil samples. Test concentrations: 0-30-150-300-500-1,000-3,000-5,000 mg/kg. Duplicate tests were performed in soil enriched with 1% alfalfa and in unenriched soil. In all four tests the NOEC was 30 mg/kg. The results of the duplicate tests were very similar, in enriched soil resulting in average inhibition percentages of 7%, 35%, 44%, 66% , 84%, 96% and 97% at the consecutive test concentrations and in unenriched soil resulting in average inhibition percentags of 0%, 13%, 30%, 52%, 81%, 100% and 100%. From these data the EC10 values were calculated by the rapporteur. Combining the data for enriched and unenriched soil results in an EC10 of 88 mg/kg. In addition, EC50 values were calculated by the rapporteur: 291 mg/kg for enriched soil, 466 for unenriched soil, and 381 mg/kg based on the combined data.

The study authors reported EC50 values of 177 and 346 mg/kg in alfalfa enriched and unenriched soil, respectively.

Because of the low reliability of the NOEC values, alternative NOEC values of 135 mg/kg (NOEC = EC10) and 48 mg/kg (NOEC = EC10) were derived for unenriched soil and enriched soil (+ 1% alfalfa), respectively.

See RAR section 3.3.3.1 for general requirements and methods for alternative NOEC derivation in case the "real" NOEC is unreliable (Quality criterion).

Soil: non-EU soil sampled from Section 24 of the Rocky Mountain Arsenal *(U.S. or Canada?).* Except for the organic carbon content (1.3%) no data on soil type or soil characteristics were reported.

[41] Svenson '86: Phosphatase and phytase activity

No statistics reported. Test compound added in solution (together with sodium acetate and sodium phytate in the phytase test and together with nitrophenyl phosphate in the phosphatase test); the solution was added to air-dried soil samples. Test concentrations: 0-590-3,530-10,460-35,320 mg/kg (reported as 0-9-54-160-540 μMol/g**).** In the phosphatase activity test these exposure levels resulted in 15%, 19%, 30% and 48% inhibition, respectively. From these data the EC10 was calculated by the rapporteur (and in addition the EC50: 50,000 mg/kg).

Soil: EU soil; top soil (0-5 cm) samples collected in a spruce forest in Odensala parish, 40 km NW of Stockholm, Sweden. No data reported on soil type or soil characteristics. The pH listed in the table is the (phytase control) value after the 1 hour incubation .

[42] Stott et al. '85: Pyrophosphatase activity

Statistics: the difference required for significance (LSD) was indicated at p = 0.05 and p = 0.01. Test compound added in aqueous solution. Test concentrations: 0-164-1,640 mg/kg (reported as 0-2.5-25 μMol/g) in the loam soil Clarion. In the other two soils, Nicollet and Okoboji, only the highest concentration was tested along with the control. Pyrophosphatase activity was measured 30 minutes after the addition of zinc to the soil (after this 30 minutes exposure time the soil was treated with buffer (pH 8) and PPi, after which the pyrophosphtase activity was measured for 5 hours at 37 ^{0}C, referring to the method of *Dick & Tabatabai (1978) (not checked).*

In Clarion, Nicollet and Okoboji soil, the addition of 1,640 mg/kg resulted in 7%, 10% and 9% inhibition of the pyrophosphatase activity, respetively, which was statistically significant in all three soils. However, since the effect was ≤ 10% and no other concentrations were

tested except for the 10-times lower concentration of 164 mg/kg in Clarion soil (resulting in 1% effect which was not significant), the NOEC for all three soils was set at 1,640 mg/kg.

[43] Stadelmann & Santschi-Fuhrimann, '87: Glucose mineralization

No statistics reported. Test compound added in aqueous solution. Test concentrations: 0-1-10-33-67-80-100-200-333-500-666-800-1,000-3,333-10,000 mg/kg.. Glucose added to soil: 5 g/kg d.w. Glucose mineralization (CO_2 production in 24 h) was measured after 1, 31, and 63 days of exposure; these exposure periods resulted in the same NOEC (80 mg/kg).

Soil: EU soil; top soil 0-10 cm) samples collected in a pasture ("naturwiese") in Switserland. Reported soil characteristics: organic carbon content 0.7% (equivalent to 1.2% oprganic matter),. clay content 14%, CEC 11.3 meq/100 g; pH H_2O 5.7. At the end of the test, the pH values ranged from 4.3 at the highest concentration to 5.0 at the lowest concentrations. Background zinc concentration: 41 mg/kg.

[44] Smolders et al. 03: Glucose mineralization, maize residue mineralization and nitrification

The study was performed i) to study the effects of abiotic factors on the toxicity of zinc in freshly-spiked soils to soil microbial processes (see Table 3.3.3.a) and plants (see Table 3.3.3.d), and ii) to study the difference in zinc toxicity in freshly-spiked soils and field-polluted soils.

Statistics used for NOEC derivation: ANOVA (Duncan test), $p < 0.05$. Each test included 7 treatments (control and 6 zinc concentrations, chosen on the basis of the expected sensitivity of the soil). The lowest Zn treatments were used in soils 1 and 3, viz. 0-15-30-60-120-240-720 mg/kg dw (nominal added-Zn concentrations, Cn).

The actual total-Zn concentrations in soil were determined in three soils (no. 6: Rhydtalog soil, no. 11: De Meern soil, no. 13: Zeveren soil), to confirm the nominal concentrations. For Rhydtalog soil and Zeveren soil the actual total-Zn concentrations were in good agreement wth the calculated total-Zn concentrations (nominal + Cb), but for the De Meern soil the actual concentrations were less than the nonimal concentration. Since Zn application was performed consecutively in each soil using the same stock solution, Smolders et al. (2003) considered it appropiate to report the toxicity values (NOEC, EC10 and EC50 values) as nominal concentrations. This is considered acceptable. The EC10 and EC50 values were calculated with the logistic response model from Doelman & Haanstra (1989). This is the same model as published earlier by Haanstra et al. (1985) and this model is also used by the rapporteur for the calculation of EC10 values.

The zinc concentrations in the soil solutions (pore water) were not measured in this microbial test, but calculated from the measurements in the growth tests performed by Smolders et al., (2003) with wheat, *Triticum aestium*, see Table 3.3.3.d-Part II.

Soils: 15 uncontamined EU soils, top soil (plough layer in cultivated soils and 0-20 cm layer in undisturbed soils) collected from arable land or non-arable land (forest, woodland, heath land, grassland, olive orchard) all over Europe. The uncontamined soils were selected to cover the relevant ranges of abiotic factors influencing Zn bioavailability in soils, including pH and cation exchange capacity. Background Zn concentrations: 7 to 191 mg/kg d.w. pH = pH $CaCl_2$. The Rhydtalog, De Meern and Zeveren soil are the uncontaminated reference soils of the field-polluted transect soils with Zn contamination due to corrosion of galvanized pylons.

See further footnotes [45], [46] and [47].

* Soil No. in study (see Table 3.3.3.a)

[45] Smolders et al. (2003): Nitrification **

The test is a modification of ISO 14238 (1995): Soil quality – Determination of Nitrogen Mineralisation and Nitrification in Soils and the Influence of Chemicals on this Proces. This guideline is also mentioned in OECD 216: Soil Microorganisms: Nitrogen Transformation Test. The test measures the Potential Nitrification Rate (PNR), which is the nitrification at unlimited substrate (NH_4^+) availability. The test is most sensitive to Zn in the initial period after NH_4 addition, i.e. as long as NH_4^+ is still abundanly present. The PNR generally increases with with increasing soil pH; the test duration was therefore varied (from 4 days up to 28 days, depending on the ntrification rate in the soil. No data reported on the number of replicates used at each treatment, but the raw data of the study indicate that at least two replicates per treatment were used.

The air-dried soil samples were pre-incubated for 14 days to the test conditions with respect to temperature and moisture content. Following spiking of the soils with aqueous $ZnCl_2$ solutions, a period of 3 days was allowed for equilibrium, after which the soils were amended with 100 mg NH_4-N/kg wet soil.

Results: In Gudow soil (pH 3.0 and Houthalen soil (pH 3.4), the PNR was undetectable, most likely due to the low pH values of these soils. Hence, no toxicity values could be derived for these 2 soils. For the other 13 soils, reliable NOEC, EC10 and EC50 values could be derived. For 12 soils, the EC10 values were very similar than the corresponding NOEC values or up to a factor of 2 higher. In Woburn soil the EC10 was 3 times higher than the NOEC.

In Smolders et al. (2003), the toxicity values (NOEC, EC10 and E50 values) were also reported in terms of soil solution Zn concentration.

See further RAR section 3.3.3.1.1 for the results of this study with respect to the influence of abiotic soil factors on the toxicity of zinc.

* Soil No. in study (see Table 3.3.3.a)

** Further information provided by the authors of the study in addition to the study report (Smolders et al., 2003) has been included in the evaluation of the study. The further information included the raw data for each test, i.e. the results (mean and SD) for nitrification at each test concentration, expressed as added-Zn (Cn) and total-Zn (Cn + Cb)..

[46] Smolders et al. (2003): Maize residue mineralization **

This test was performed according to OECD guideline 217: Soil Microorganisms: Carbon Transformation Test, with the following deviations: i) maize root residue instead of glucose was used as mineralization substrate and ii) the respiration rate was measured as the cumulative CO_2 release in the 28-d test period (In OECD 217 the respiration rate is measured during a 12-h period, starting 28 days after the addition of the test substance to the soil). ^{14}C-labelled maize residue was used as mineralization substrate; the use of ^{14}C-labelled plant material allows discrimination between CO_2 release due to the decomposition of the plant residue and that from carbonate dissolution.

The air-dried soil samples were pre-incubated for 5 days to the test conditions with respect to temperature and moisture content. Following spiking of the soils with aqueous $ZnCl_2$ solutions, a period of 2 days was allowed for equilibrium, after which the soils were amended with ground maize resudue (30 mg substrate per 40 g soil sample). Two replicates were used at each treatment.

Results: In many soils, the percentage inhibition of the respiration at the highest test concentration was <30%. The EC50 values were therefore estimated by extrapolation outside

the concentration range tested and the confidence intervals for the EC50 values are wide. The lack of strong inhibition and the concommittant less successful fitting of the response model also inflated the variance on EC10 values. For 9 soils, the EC10 values were very similar than the corresponding NOEC values or up to a factor of 2 lower. For the further soils, the EC10 values were up to factor of 5 lower than the corresponding NOEC values (excluding unbounded NOEC values). According to Smolders et al. (2003), the insensitivy of the tests can be attributed to the relative long test duration. Nevertheless, the tests and NOEC values derived from these tests are considered valid (amongst others because the NOEC values are in the same range as those from other microbial soil tests), **except the tests in Houthalen soil, Souli I soil, Aluminosa soil and Woburn soil: these 4 tests resulted in unbounded NOEC values, thus rejected based on Quality criterion.**

In Smolders et al. (2003), the toxicity values (NOEC, EC10 and E50 values) were also reported in terms of soil solution Zn concentration.

See further RAR section 3.3.3.1.1 for the results of this study with respect to the influence of abiotic soil factors on the toxicity of zinc.

* Soil No. in study (see Table 3.3.3.a)

** Further information provided by the authors of the study in addition to the study report (Smolders et al., 2003) has been included in the evaluation of the study. The further information included the raw data for each test, i.e. the results (mean and SD) for respiration at each test concentration, expressed as added-Zn (Cn) and total-Zn (Cn + Cb).

[47] Smolders et al. (2003): Glucose mineralization **

This test was performed according to OECD guideline 217: Soil Microorganisms: Carbon Transformation Test, with the following deviation: the respiration rate (CO_2 release) was measured during a 24-h period, starting 48 hours after the addition of zinc to the soil (In OECD 217 the respiration rate is measured during a 12-h period, starting 28 days after the addition of the test substance to the soil). The relatively short test duration was chosen in this study to increase the sensitivity of the test in comparison with the Smolders et al. (2003)

28-d respiration test with maize residue, see footnote [46]. ^{14}C-labelled glucose was used as mineralization substrate; the use of ^{14}C-labelled glucose allows discrimination between CO_2 release due to the decomposition of the substrate and that from carbonate dissolution.

The air-dried soil samples were pre-incubated for 6 days to the test conditions with respect to temperature and moisture content. Following spiking of the soils with aqueous $ZnCl_2$ solutions, the soils were incubated for 48 hours. The soils were then amended with glucose (1 g/kg wet soil) and incubated for an additional 24 hours in which the respiration rate was measured. No data reported on the number of replicates at each treatment.

Results: For 12 soils, the EC10 values were very similar than the corresponding NOEC values or up to a factor of 2 higher. In Kövlinge soil the EC10 was 3-times lower than the NOEC and in Zeveren soil the EC10 was 6-times lower than the NOEC. The tests and NOEC values derived from these tests are considered valid, **except the test in Zegveld soil: this test resulted in an unbounded NOEC, thus rejected based on Quality criterion.**

In Smolders et al. (2003), the toxicity values (NOEC, EC10 and E50 values) were also reported in terms of soil solution Zn concentration.

See further RAR section 3.3.3.1.1 for the results of this study with respect to the influence of abiotic soil factors on the toxicity of zinc.

* Soil No. in study (see Table 3.3.3.a)

** Further information provided by the authors of the study in addition to the study report (Smolders et al., 2003) has been included in the evaluation of the study. The further information included the raw data for each test, i.e. the results (mean and SD) for respiration at each test concentration, expressed as added-Zn (Cn) and total-Zn

(Cn + Cb).

[48] Notenboom & Posthuma ’94; ‘95; Posthuma et al. ’98: Glutamic acid mineralization

Tests performed in the framework of the Dutch research project “Validation of toxicity data and risk limits for soils” (see also RAR sections 3.3.3.1.1 and 3.3.3.1.4). The final summarising report of the whole project is published by Posthuma et al. (1998). This report (and underlying references) include EC50 values; the EC50 values are not included in Table 3.3.3.a.

Soils: EU soils, collected in the Netherlands (Budel, Wageningen and Panheel). See also footnote [8] in Table 3.3.3.b.

[48a] PAHN: Test performed in: “freshly” laboratory-spiked Panheel soil. Cb = 23 mg/kg.

[48b] PAHN: Test performed in “freshly” field-spiked Panheel soil collected from the field plot in July 1994 (some weeks after spiking). Cb = 16 mg/kg. Nominal NOEC (55 mg/kg; added Zn) is the mean result of 3 plots sampled, resulting in NOEC values of 100, 32 and 32 mg/kg, respectively. Actual NOEC (71 mg/kg): also based on the mean result of the 3 plots; the height of the actual NOEC (71 mg/kg) is in conformity with Cn (55 mg/kg) plus Cb (23 mg/kg): this results in a calculated total-Zn concentration of 78 mg/kg (NOEC).

[48c] PAHN-aged: Test performed in aged field-spiked Panheel soil collected from the field plot in March 1995 (around one year after spiking). Cb = 24 mg/kg. the NOEC is based on actual concentrations (mean result of the 3 plots); result reported only as actual, not as Cn. **Rejected, based on Relevance criterion (test in aged soil).**

[49] Van Beelen & Notenboom ’96; Posthuma et al. ’98: Acetate mineralization

Test performed in the framework of the Dutch research project “Validation of toxicity data and risk limits for soils” (see also footnote [48]).

PAHN-aged: Test performed in aged field-spiked Panheel soil collected from the field plot in February 1995 (around one year after spiking). Cb = 24 mg/kg.

Rejected, based on Relevance criterion (test in aged soil).

Additional acetate mineralization tests performed in this soil: (Not used for PNEC derivation):

1. Slurry test (10 g soil + 10 ml water, see footnote 34a): EC10 =261 mg/kg (very similar result)
2. Suspension test (30 mg soil + 50 ml Tris buffer): EC10 = 31 mg/kg (According to the study authors, sorption of added Zn to the soil is minimal).
3. The acetate mineralization was also measured in a single species test in this soil to which, after sterilisation, a pure culture of bacterium *Pseudomonas putida* was added; this test resulted in an EC10 of 363 mg/kg (actual). There are additional *P. putida* single-species acetate mineralisation tests (in OECD soil, resulting in additional EC10-values.

[50] Bååth ’89: Respiration, nitrificatiopn and phosphate activity

Rejected, based on Quality criterion (data from secondary literature source) and Relevance criteria (no data on zinc compound tested and no data on soil type and soil characteristics).

[51] Doelman & Haanstra ’83: Ammonification

Rejected, based on Quality criterion (data from secondary literature source) and Relevance criterion

(test compound: “insoluble” zinc salt: ZnO).

[52] Doelman & Haanstra ’83: Ammonification

Rejected, based on Quality criterion (data from secondary literature source) and Relevance criterion

(no data on soil characteristics;; only the soil type (sand) was reported).

Table 3.3.3.b Chronic toxicity of zinc to soil invertebrates: NOEC values

Part I: Studies useful for $PNEC_{add, terrestrial}$ derivation

Organism	Test-comp	Soil type	pH	OM %	Clay %	Temp. °C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)	**NOEC (Cn) used for $PNEC_{add}$ derivation**
Oligochaetes (Annelids)										
Aporrectodea caliginosa adults	$ZnSO_4$	- (Egypt)	7.1	22	-	25	8-w	$NOEC_{r(c)}$ $EC10_{r(c)}$ $NOEC_s$ Khalil et al., 1996 [21]	600 (Cn) 568 (Cn) ≥1,600 (Cn)	**600**
Eisenia andrei adults	$ZnCl_2$	art.soil (OECD)	6.0	10	20	20	21-d	$NOEC_{r(c,j)}$ $NOEC_{g(f)}$ Van Gestel et al., 1993 [18]	320 (Cn) ≥1,000 (Cn)	**320**
Eisenia fetida adults	$Zn(NO_3)_2$	art. soil (OECD)	6	8	8	20	2-w	$EC10_g$ Neuhauser et al., 1985 [1]	300 (Cn)	
Eisenia fetida adults	$Zn(NO_3)_2$	art. soil (OECD)	6.0	10	20	15 15 20 20	21-d 14-d 21-d 14-d	$NOEC_{r(c)}$ $NOEC_s$ $NOEC_{r(c)}$ $NOEC_s$ Spurgeon et al., 1997 [10]	350 (Cn) 1,200 (Cn) 350 (Cn) 1,200 (Cn)	**350** **350**
Eisenia fetida adults	$ZnCl_2$	art. soil (OECD)	6.1	10	20	20	14-d 21-d 21-d	$NOEC_s$ $NOEC_{r(c)}$ $NOEC_g$ Spurgeon & Hopkin, 1995 [17, 23]	442 (Cn) 237 (Cn) ≥400 (Cn)	 **237**
Eisenia fetida adults	$Zn(NO_3)_2$	art. soil (OECD)	6.3	10	20	20	56-d 56-d	$NOEC_s$ $NOEC_{r(c)}$ Spurgeon et al., 1994 [20, 23]	289 (Cn) 199 (Cn)	 **199**
Eisenia fetida adults	$Zn(NO_3)_2$	art.soil (OECD)	6.0	5	20	20	21-d	$NOEC_s$ $NOEC_{r(c)}$ $NOEC_s$ $NOEC_{r(c)}$ $NOEC_s$ $NOEC_{r(c)}$ 5.0 5 $NOEC_s$ $NOEC_{r(c)}$ $NOEC_s$ $NOEC_{r(c)}$ $NOEC_s$ $NOEC_{r(c)}$ 4.0 5 $NOEC_s$ $NOEC_{r(c)}$ $NOEC_s$ $NOEC_{r(c)}$ $NOEC_s$ $NOEC_{r(c)}$ Spurgeon & Hopkin, 1996b [22, 23]	274 (Cn) 97 (Cn) 702 (Cn) 553 (Cn) 1,048 (Cn) 484 (Cn) 366 (Cn) 85 (Cn) 256 (Cn) 183 (Cn) 368 (Cn) 414 (Cn) 197 (Cn) 115 (Cn) 168 (Cn) 161 (Cn) 184 (Cn) 223 (Cn)	 **97** **553** **484** **85** **183** **414** **115** **161** **223**

(to be continued)

Table 3.3.3.b Chronic toxicity of zinc to soil invertebrates: NOEC values (continued) Part I: Studies useful for $PNEC_{add, terrestrial}$ derivation

Organism	Test-comp	Soil type	pH	OM %	Clay %	Temp. °C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)		**NOEC (Cn) used for $PNEC_{add}$ derivation**
Oligochaetes (Annelids) (continued)											
Eisenia fetida adults	$ZnCl_2$ **1 ***	loamy sand (Gudow; Cb 7 mg/kg) *(EU soil)*	3.0	9	7	20	28-d	$NOEC_{r(c)}$	180	(Cn)	**<u>180</u>**
									187	(Cn+Cb)	
								$EC10_{r(c)}$	130	(Cn)	
									137	(Cn+Cb)	
								$EC50_{r(c)}$	250	(Cn)	
									257	(Cn+Cb)	
								Lock et al., 2003 [24, 25]			
Eisenia fetida adults	$ZnCl_2$ **3 ***	loamy sand (Houthalen, Cb 8 mg/kg) *(EU soil)*	3.4	3	5	20	28-d	$NOEC_{r(c)}$	100	(Cn)	**<u>100</u>**
									108	(Cn+Cb)	
								$EC10_{r(c)}$	96	(Cn)	
									104	(Cn+Cb)	
								$EC50_{r(c)}$	120	(Cn)	
									128	(Cn+Cb)	
								Lock et al., 2003 [24, 25]			
Eisenia fetida adults	$ZnCl_2$ **5 ***	sandy clay loam (Zegveld, Cb 191 mg/kg) *(EU soil)*	4.7	40	24	20	28-d	$NOEC_{r(c)}$	1,000	(Cn)	**<u>1,000</u>**
									1,191	Cn+Cb)	
								$EC10_{r(c)}$	1,150	(Cn)	
									1,341	Cn+Cb)	
								$EC50_{r(c)}$	1,820	(Cn)	
									2,011	Cn+Cb)	
								Lock et al., 2003 [24, 25]			
Eisenia fetida adults	$ZnCl_2$ **6 ***	?? (Rhydtalog, Cb 83 mg/kg) *(EU soil)*	4.8	13	-	20	28-d	$NOEC_{r(c)}$	320	(Cn)	**<u>320</u>**
									403	(Cn+Cb)	
								$EC10_{r(c)}$	486	(Cn)	
									569	(Cn+Cb)	
								$EC50_{r(c)}$	915	(Cn)	
									998	(Cn+Cb)	
								Lock et al., 2003 [24, 25]			
Eisenia fetida adults	$ZnCl_2$ **8 ***	sandy clay (Souli I, Cb 37 mg/kg) *(EU soil)*	4.8	1	38	20	28-d	$NOEC_{r(c)}$	560	(Cn)	**<u>560</u>**
									597	(Cn+Cb)	
								$EC10_{r(c)}$	503	(Cn)	
									540	(Cn+Cb)	
								$EC50_{r(c)}$	649	(Cn)	
									686	(Cn+Cb)	
								Lock et al., 2003 [24, 25]			
Eisenia fetida adults	$ZnCl_2$ **9 ***	sandy loam (Kövlinge II, Cb 26 mg/kg) *(EU soil)*	5.1	4	9	20	28-d	$NOEC_{r(c)}$	320	(Cn)	**<u>320</u>**
									346	(Cn+Cb)	
								$EC10_{r(c)}$	243	(Cn)	
									269	(Cn+Cb)	
								$EC50_{r(c)}$	381	(Cn)	
									407	(Cn+Cb)	
								Lock et al., 2003 [24, 25]			
Eisenia fetida adults	$ZnCl_2$ **11 ***	?? (De Meern, Cb 155 mg/kg) *(EU soil)*	5.2	17	-	20	28-d	$NOEC_{r(c)}$	560	(Cn)	**<u>560</u>**
									715	(Cn+Cb)	
								$EC10_{r(c)}$	747	(Cn)	
									902	(Cn+Cb)	
								$EC50_{r(c)}$	1,520	(Cn)	
									1,675	(Cn+Cb)	
								Lock et al., 2003 [24, 25]			

(to be continued)

Table 3.3.3.b Chronic toxicity of zinc to soil invertebrates: NOEC values (continued) Part I: Studies useful for $PNEC_{add, terrestrial}$ derivation

Organism	Test-comp	Soil type	pH	OM %	Clay %	Temp. °C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)		**NOEC (Cn) used for $PNEC_{add}$ derivation**
Oligochaetes (Annelids) (continued)											
Eisenia fetida adults	$ZnCl_2$ **13 ***	?? (Zeveren, Cb 76 mg/kg) *(EU soil)*	5.7	6	-	20	28-d	$NOEC_{r\,(c)}$	1,000	(Cn)	**1,000**
									1,076	(Cn+Cb)	
								$EC10_{r\,(c)}$	1,040	(Cn)	
									1,116	(Cn+Cb)	
								$EC50_{r\,(c)}$	1,310	(Cn)	
									1,386	(Cn+Cb)	
								Lock et al., 2003 [24, 25]			
Eisenia fetida adults	$ZnCl_2$ **14 ***	sandy clay loam (Woburn, Cb 99 mg/kg) *(EU soil)*	6.4	7	21	20	28-d	$NOEC_{r\,(c)}$	560	(Cn)	**560**
									659	(Cn+Cb)	
								$EC10_{r\,(c)}$	629	(Cn)	
									728	(Cn+Cb)	
								$EC50_{r\,(c)}$	1,060	(Cn)	
									1,159	(Cn+Cb)	
								Lock et al., 2003 [24, 25]			
Eisenia fetida adults	$ZnCl_2$ **15 ***	silt loam (Ter Munck, Cb 54 mg/kg) *(EU soil)*	6.8	2	15	20	28-d	$NOEC_{r\,(c)}$	180	(Cn)	**180**
									234	(Cn+Cb)	
								$EC10_{r\,(c)}$	79	(Cn)	
									133	(Cn+Cb)	
								$EC50_{r\,(c)}$	275	(Cn)	
									329	(Cn+Cb)	
								Lock et al., 2003 [24, 25]			
Eisenia fetida adults	$ZnCl_2$ **19 ***	silt loam (Marknesse, Cb 80 mg/kg) *(EU soil)*	7.5	2	26	20	28-d	$NOEC_{r\,(c)}$	180	(Cn)	**180**
									260	(Cn+Cb)	
								$EC10_{r\,(c)}$	122	(Cn)	
									202	(Cn+Cb)	
								$EC50_{r\,(c)}$	577	(Cn)	
									657	(Cn+Cb)	
								Lock et al., 2003 [24, 25]			
Eisenia fetida adults	$ZnCl_2$ **22 ***	loam (Guadalajara, Cb 27 mg/kg) *(EU soil)*	7.5	1	25	20	28-d	$NOEC_{r\,(c)}$	560	(Cn)	
									587	(Cn+Cb)	
								$EC10_{r\,(c)}$	346	(Cn)	**350**
									373	(Cn+Cb)	
								$EC50_{r\,(c)}$	531	(Cn)	
									558	(Cn+Cb)	
								Lock et al., 2003 [24, 25]			
Eisenia foetida			*(n = 25)*			*geometric mean*		$NOEC_{r\,(c)}$	*280*	*(Cn)*	

(to be continued)

Table 3.3.3.b Chronic toxicity of zinc to soil invertebrates: NOEC values (continued) Part I: Studies useful for $PNEC_{add, terrestrial}$ derivation

Organism	Test-comp	Soil type	pH	OM %	Clay %	Temp. °C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)		**NOEC (Cn) used for $PNEC_{add}$ derivation**
Insects											
Folsomia candida 10-d juveniles	$ZnCl_2$	art. soil (OECD)	6.0	10	20	18	4-w	$NOEC_{g\,(d,f)}$	567	(actual)	
									565	(actual-Cb)	
								$NOEC_{r\,(j)}$	368	(actual)	
									366	(Cn)	**366**
								$EC10_{g\,(f)}$	738	(actual)	
									736	(Cn)	
								$EC10_{r\,(j)}$	269	(actual)	
									267	(Cn)	
	$ZnCl_2$	sand (PANH) *(EU soil)*	6.0	2	2	19	4-w	$NOEC_{g\,(d,f)}$	298	(actual)	
									275	(Cn)	
								$NOEC_{r\,(j)}$	298	(actual)	
									275	(Cn)	**275**
								$EC10_{g\,(f)}$	159	(actual)	
									136	(Cn)	
								$EC10_{r\,(j)}$	136	(actual)	
									113	(Cn)	
	$ZnCl_2$	sand (perc.) (PANH-perc) *(EU soil)*	6.0	2	2	18	4-w	$NOEC_{g\,(d,f)}$	457	(actual)	
									436	(Cn)	
								$NOEC_{r\,(j)}$	335	(actual)	
									314	(Cn)	**314**
								$EC10_{g\,(f)}$	305	(actual)	
									284	(Cn)	
								$EC10_{r\,(j)}$	355	(actual)	
									334	(Cn)	
								Smit & Van Gestel, 1998 [8]			
Folsomia candida adults	$Zn(NO_3)_2$	art. soil (OECD)	6.0	10	20	20	4-w	$NOEC_s$	3,000	(Cn	
								$NOEC_{r\,(j)}$	620	(Cn)	**620**
			5.0					$NOEC_s$	6,500	(Cn)	
								$NOEC_{r\,(j)}$	300	(Cn)	**300**
			4.5					$NOEC_s$	300	(Cn)	
								$NOEC_{r\,(j)}$	300	(Cn)	**300**
								Sandifer & Hopkin, 1996 [12]			
Folsomia candida adults	$Zn(NO_3)_2$	art. soil (OECD)	6.0	10	20	15	6-w	$NOEC_s$	300	(Cn)	
								$NOEC_{r\,(j)}$	300	(Cn)	**300**
								Sandifer & Hopkin, 1997 [13]			
Folsomia candida 10-d juveniles	$ZnCl_2$	art. soil (OECD)	6.0	10	20	20	6-w	$EC10_{g\,(f)}$	840	(Cn)	
							4-w	$EC10_{r\,(j)}$	399	(Cn)	**399**
							6-w	$EC10_{r\,(j)}$	423	(Cn)	
								Van Gestel & Hensbergen, 1997 [15]			

(to be continued)

Table 3.3.3.b Chronic toxicity of zinc to soil invertebrates: NOEC values (continued) Part I: Studies useful for $PNEC_{add, terrestrial}$ derivation

Organism	Test-comp	Soil type	pH	OM %	Clay %	Temp. °C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)		**NOEC (Cn)** used for $PNEC_{add}$ derivation
Insects (continued)											
Folsomia candida 10-d juveniles	$ZnCl_2$ **3 ***	loamy sand (Houthalen, Cb 8 mg/kg) *(EU soil)*	3.4	3	5	20	28-d	$NOEC_{r(j)}$	32	(Cn)	**32**
									40	(Cn+Cb)	
								$EC10_{r(j)}$	30	(Cn)	
									38	(Cn+Cb)	
								$EC50_{r(j)}$	64	(Cn)	
									72	(Cn+Cb)	
								Lock et al., 2003 [24, 26]			
Folsomia candida 10-d juveniles	$ZnCl_2$ **5 ***	sandy clay (Zegveld, Cb 191mg/kg) *(EU soil)*	4.7	40	24	20	28-d	$NOEC_{r(j)}$	1,000	Cn)	**1,000**
									1,191	(Cn+Cb)	
								$EC10_{r(j)}$	520	(Cn)	
									711	(Cn+Cb)	
								$EC50_{r(j)}$	1,390	(Cn)	
									1,581	(Cn+Cb)	
								Lock et al., 2003 [24, 26]			
Folsomia candida 10-d juveniles	$ZnCl_2$ **6 ***	?? (Rhydtalog, Cb 83 mg/kg) *(EU soil)*	4.8	13	-	20	28-d	$NOEC_{r(j)}$	320	(Cn)	**320**
									403	(Cn+Cb)	
								$EC10_{r(j)}$	88	(Cn)	
									171	(Cn+Cb)	
								$EC50_{r(j)}$	395	(Cn)	
									478	(Cn+Cb)	
								Lock et al., 2003 [24, 26]			
Folsomia candida 10-d juveniles	$ZnCl_2$ **8 ***	sandy clay (Souli I, Cb 37 mg/kg) *(EU soil)*	4.8	1	38	20	28-d	$NOEC_{r(j)}$	100	(Cn)	**100**
									137	(Cn+Cb)	
								$EC10_{r(j)}$	63	(Cn)	
									100	(Cn+Cb)	
								$EC50_{r(j)}$	248	(Cn)	
									285	(Cn+Cb)	
								Lock et al., 2003 [24, 26]			
Folsomia candida 10-d juveniles	$ZnCl_2$ **11 ***	?? (De Meern, Cb 155 mg/kg) *(EU soil)*	5.2	17	-	20	28-d	$NOEC_{r(j)}$	300	(Cn)	**300**
									455	(Cn+Cb)	
								$EC10_{r(j)}$	303	(Cn)	
									458	(Cn+Cb)	
								$EC50_{r(j)}$	1,440	(Cn)	
									1,600	(Cn+Cb)	
								Lock et al., 2003 [24, 26]			
Folsomia candida 10-d juveniles	$ZnCl_2$ **12 ***	clay (Aluminusa, Cb 53 mg/kg) *(EU soil)*	5.4	1	51	20	28-d	$NOEC_{r(j)}$	320	(Cn)	**320**
									373	(Cn+Cb)	
								$EC10_{r(j)}$	209	(Cn)	
									262	(Cn+Cb)	
								$EC50_{r(j)}$	682	(Cn)	
									735	(Cn+Cb)	
								Lock et al., 2003 [24, 26]			
Folsomia candida 10-d juveniles	$ZnCl_2$ **13 ***	?? (Zeveren, Cb 76 mg/kg) *(EU soil)*	5.7	6	-	20	28-d	$NOEC_{r(j)}$	320	(Cn)	**320**
									396	(Cn+Cb)	
								$EC10_{r(j)}$	89	(Cn)	
									165	(Cn+Cb)	
								$EC50_{r(j)}$	586	(Cn)	
									662	(Cn+Cb)	
								Lock et al., 2003 [24, 26]			

(to be continued)

Table 3.3.3.b Chronic toxicity of zinc to soil invertebrates: NOEC values (continued) Part I: Studies useful for $PNEC_{add, terrestrial}$ derivation

Organism	Test-comp	Soil type	pH	OM %	Clay %	Temp. °C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)		**NOEC (Cn)** used for $PNEC_{add}$ derivation
Insects (continued)											
Folsomia candida 10-d juveniles	$ZnCl_2$ **17 ***	silty clay loam (Rots, Cb 51 mg/kg) *(EU soil)*	7.4	2	27	20	28-d	$NOEC_{r(j)}$	560	(Cn)	**560**
									611	(Cn+Cb)	
								$EC10_{r(j)}$	588	(Cn)	
									639	(Cn+Cb)	
								$EC50_{r(j)}$	903	(Cn)	
									954	(Cn+Cb)	
								Lock et al., 2003 [24, 26]			
Folsomia candida 10-d juveniles	$ZnCl_2$ **18 ***	clay (Souli II, Cb 51 mg/kg) *(EU soil)*	7.4	4	46	20	28-d	$NOEC_{r(j)}$	1,000	(Cn)	**1,000**
									1,051	(Cn+Cb)	
								$EC10_{r(j)}$	1,210	(Cn)	
									1,261	(Cn+Cb)	
								$EC50_{r(j)}$	1,500	(Cn)	
									1,551	(Cn+Cb)	
								Lock et al., 2003 [24, 26]			
Folsomia candida 10-d juveniles	$ZnCl_2$ **20 ***	loam (Guadalajara, Cb 27 mg/kg) *(EU soil)*	7.5	1	25	20	28-d	$NOEC_{r(j)}$	320	(Cn)	**320**
									347	(Cn+Cb)	
								$EC10_{r(j)}$	139	(Cn)	
									166	(Cn+Cb)	
								$EC50_{r(j)}$	593	(Cn)	
									620	(Cn+Cb)	
								Lock et al., 2003 [24, 26]			
Folsomia candida				*(n = 18)*		*geometric mean*		*$NOEC_{r(j)}$*	*320*	*(Cn)*	

(Table 3.3.3.b: To be continued in Part II: Studies not useful for PNEC derivation)

Table 3.3.3.b Chronic toxicity of zinc to soil invertebrates: NOEC values

Part II: Studies not useful for $PNEC_{add, terrestrial}$ derivation

Organism	Test-comp	Soil type or substrate	pH	OM	Clay	Temp. °C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)
Oligochaetes (Annelids)									
Eisenia fetida < 2-w old	sol. Zn salts	soil+manure	-	50[e]	0[e]	25	6-w	$NOEC_{g,r}$	1,000 (Cn) Neuhauser et al. '84 [2]
Eisenia fetida hatchlings	$ZnSO_4$	soil+ sludge	6.5	-	-	24	8-w	$NOEC_{s,g}$	1,500 (Cn) Hartenstein et al.'81 [3]
Eisenia fetida		soil+manure	-	50[e]	0[e]	22			
	ZnO						8-w	$NOEC_{s,g,r}$	2,000[est.] (Cn)
	$Zn(Ac)_2$						8-w	$NOEC_{s,g,r}$	1,000 (Cn)
	$ZnCl_2$ or $Zn(NO_3)_2$						8-w	$NOEC_{s,g,r}$	1,000[est.] (Cn)
	$ZnSO_4$ or $ZnCO_3$						8-w	$NOEC_{s,g,r}$	250[est.] (Cn) Malecki et al.'82 [4]
Eisenia fetida	$Zn(Ac)_2$	soil+manure	-	50[e]	0[e]	22	20-w	$NOEC_{s,g,r}$	2,500 (Cn) Malecki et al.'82 [5]
Eisenia fetida hatchlings	metal mixt.	soil (contam.) *(EU soil)*	6.7	27	-	20	5-w	$NOEC_s$	2,790 (actual)
							12-w	$NOEC_g$	2,790 (actual)
			6.3	22	-		16-w	$NOEC_g$	7,950 (actual)
			6.7	27	-		12-w	$NOEC_m$	2,790 (actual)
			7.4	19	-		20-w	$NOEC_{tr}$	1,850 (actual) Spurgeon & Hopkin, 1996a [14]
Eisenia fetida	metal mixt.	soil (contam.) *(EU soil)*	6.6	17	-	20	14-d	$NOEC_s$	≥32,871 (actual)
							21-d	$NOEC_{r(c,j)}$	1,848 (actual)
							21-d	$NOEC_g$	2,793 (actual)
	metal mixt.	art. soil (OECD)	6.1	10	20	20	14-d	$NOEC_s$	1,047 (Cn)
							21-d	$NOEC_{r(c)}$	833 (Cn)
							21-d	$NOEC_g$	777 (Cn) Spurgeon & Hopkin, 1995 [17, 17a]
Eisenia fetida adults	$ZnCl_2$ **12 ***	clay (Aluminusa, Cb 53 mg/kg) *(EU soil)*	5.4	1	51	20	28-d	$NOEC_{r(c)}$	180 (Cn) 233 (Cn+Cb)
								$EC10_{r(c)}$	21 (Cn) 74 (Cn+Cb)
								$EC50_{r(c)}$	173 (Cn) 226 (Cn+Cb) Lock et al., 2003 [23,24]
Eisenia fetida adults	$ZnCl_2$ **17 ***	silty clay loam (Rots, Cb 51 mg/kg) *(EU soil)*	7.4	2	27	20	28-d	$NOEC_{r(c)}$	560 (Cn) 611 (Cn+Cb)
								$EC10_{r(c)}$	326 (Cn) 377 (Cn+Cb)
								$EC50_{r(c)}$	557 (Cn) 608 (Cn+Cb) Lock et al., 2003 [23,24]
Eisenia fetida adults	$ZnCl_2$ **18 ***	clay (Souli II, Cb 51 mg/kg) *(EU soil)*	7.4	4	46	20	28-d	$NOEC_{r(c)}$	560 (Cn) 611 (Cn+Cb)
								$EC10_{r(c)}$	572 (Cn) 623 (Cn+Cb)
								$EC50_{r(c)}$	760 (Cn) 811 (Cn+Cb) Lock et al., 2003 [23,24]

(to be continued)

Table 3.3.3.b Chronic toxicity of zinc to soil invertebrates: NOEC values (continued) Part II: Studies not useful for $PNEC_{add, terrestrial}$ derivation

Organism	Test-comp	Soil type	pH	OM %	Clay %	Temp. °C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)	**NOEC (Cn)** used for $PNEC_{add}$ derivation
Gastropods (Molluscs)										
Arion ater 5-7 g	$ZnCl_2$	feed	-	95[e]	0[e]	20	4-w	$NOEC_{f,s,g}$ Marigomez et al.'86 [6]	300	(Cn)
Crustaceans (Arthropods)										
Porcellio scaber	$Zn(NO_3)_2$	feed	-	95[e]	0[e]	-	10-w	$NOEC_{f,.g,r}$ Denneman & Van Gestel, '90 [7]	400	(Cn)
Insects										
Folsomia candida *(EU soil)*	$ZnCl_2$	sand (aged) (PANH-aged)	6.0	2	3	18	4-w	$NOEC_{g\,(f)}$ $NOEC_{r\,(j)}$ $EC10_{g\,(f)}$ $EC10_{r\,(j)}$ Smit & Van Gestel, 1998 [8, 8a]	709 685 709 (actual) 685 800 776 1,059 1,035	(actual) (actual-Cb) (actual) (actual-Cb) (actual) (actual-Cb)
Folsomia candida 10-d juveniles	$ZnCl_2$ **1 ***	loamy sand (Gudow; Cb 7 mg/kg) *(EU soil)*	3.0	9	7	20	28-d	$NOEC_{r\,(j)}$ $EC10_{r\,(j)}$ $EC50_{r\,(j)}$ Lock et al., 2003 [23,25]	56 63 11 18 76 83	(Cn) (Cn+Cb) (Cn) (Cn+Cb) (Cn) (Cn+Cb)
Folsomia candida 10-d juveniles	$ZnCl_2$ **9 ***	sandy loam (Kövlinge II, Cb 26 mg/kg) *(EU soil)*	5.1	4	9	20	28-d	$NOEC_{r\,(j)}$ $EC10_{r\,(j)}$ $EC50_{r\,(j)}$ Lock et al., 2003 [23,25]	320 346 100 126 325 351	(Cn) (Cn+Cb) (Cn) (Cn+Cb) (Cn) (Cn+Cb)
Folsomia candida 10-d juveniles	$ZnCl_2$ **14 ***	sandy clay loam (Woburn, Cb 99 mg/kg) *(EU soil)*	6.4	7	21	20	28-d	$NOEC_{r\,(j)}$ $EC10_{r\,(j)}$ $EC50_{r\,(j)}$ Lock et al., 2003 [23,25]	1,000 1,099 806 905 1,270 1,369	(Cn) (Cn+Cb) (Cn) (Cn+Cb) (Cn) (Cn+Cb)
Folsomia candida 10-d juveniles	$ZnCl_2$ **15 ***	Silt loam (Ter Munck, Cb 54 mg/kg) *(EU soil)*	6.8	2	15	20	28-d	$NOEC_{r\,(j)}$ $EC10_{r\,(j)}$ $EC50_{r\,(j)}$ Lock et al., 2003 [23,25]	320 374 43 97 247 301	(Cn) (Cn+Cb) (Cn) (Cn+Cb) (Cn) (Cn+Cb)
Folsomia candida 10-d juveniles	$ZnCl_2$ **19 ***	silt loam (Marknesse, Cb 80 mg/kg) *(EU soil)*	7.5	2	26	20	28-d	$NOEC_{r\,(j)}$ $EC10_{r\,(j)}$ $EC50_{r\,(j)}$ Lock et al., 2003 [23,25]	1,000 1,080 491 571 1,140 1,220	(Cn) (Cn+Cb) (Cn) (Cn+Cb) (Cn) (Cn+Cb)

Table 3.3.3.c Toxicity of zinc to soil invertebrates: LC50 and EC50 values

(Effect concentrations, not used for $PNEC_{add, terrestrial}$ derivation)

Organism	Test-comp	Soil type	pH	OM %	Clay %	Temp. °C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)	
Oligochaetes (Annelids)										
Aporrectodea caliginosa adults	$ZnSO_4$	- (Egypt)	7.1	22	-	25	8-w	$EC50_r$	826	(Cn)
								LC50	3,610	(Cn)
								Khalil et al., 1996 [21]		
Eisenia andrei	-	Soil (contam.) (Budel) *(EU soil)*				20	?	EC50 ?	2,553	
	-	art. soil (OECD)	5.5	10	20	20	?	EC50 ?	429	
								Posthuma & Notenboom, 1996		
Eisenia andrei adults	$ZnCl_2$	art.soil (OECD)	6.0	10	20	20	21-d	$EC50_{r\,(c)}$	659	(Cn)
								$EC50_{r\,(j)}$	512	(Cn)
								Van Gestel et al., 1993 [18]		
Eisenia fetida adults	$Zn(NO_3)_2$	art.soil (OECD)	6.0	8	8	20	14-d	LC50	662	(Cn)
								Neuhauser et al., 1985 [1]		
Eisenia fetida adults	$Zn(NO_3)_2$	art. soil (OECD)	6.0	10	20	15	14-d	LC50	1,598	
						20	14-d	LC50	1,235	
						25	14-d	LC50	1,131	
						15	21-d	$EC50_r$	382	
						20	21-d	$EC50_r$	308	
						25	21-d	EC50	234	
								Spurgeon et al., 1997 [10]		
Eisenia fetida adults	$Zn(NO_3)_2$	art. soil (OECD)	6.3	10	20	20	14-d	LC50	1,010	
							56-d	LC50	745	
							56-d	$EC50_r$	276	
								Spurgeon et al., 1994 [20]		
Eisenia fetida adults	$Zn(NO_3)_2$	art. soil (OECD)	6.0	5	20	20	21-d	LC50	620	
				10				LC50	791	
				15				LC50	1,613	
			5.0	5				LC50	591	
				10				LC50	601	
				15				LC50	992	
			4.0	5				LC50	451	
				10				LC50	617	
				15				LC50	474	
			6.0	5	20	20	21-d	$EC50_r$	136	
				10				$EC50_r$	462	
				15				$EC50_r$	592	
			5.0	5				$EC50_r$	199	
				10				$EC50_r$	343	
				15				$EC50_r$	548	
			4.0	5				$EC50_r$	142	
				10				$EC50_r$	189	
				15				$EC50_r$	230	
								Spurgeon & Hopkin, 1996b [22]		
Eisenia fetida hatchlings	metal mixt.	soil (contam.) *(EU soil)*	-	-	-	20	8-w	LC50	1,860	(actual)
							5-w	$EC50_g$	3,120	(actual)
							8-w	$EC50_{r\,(c)}$	637	(actual)
								Spurgeon & Hopkin, 1996a [14]		

(to be continued)

Table 3.3.3.c (continued) Toxicity of zinc to soil invertebrates: LC50 and EC50 values (Effect concentrations, not used for $PNEC_{add, terrestrial}$ derivation)

Organism	Test-comp	Soil type	pH	OM %	Clay %	Temp. °C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)	
Oligochaetes (Annelids) (continued)										
Eisenia fetida	$ZnCl_2$	art. soil (OECD)	6.1	10	20	20	14-d	LC50	1,078	(Cn)
							21-d	$EC50_r$	357	(Cn)
							21-d	$EC50_g$	>400	(Cn)
	metal mixt.	soil (contam.) *(EU soil)*	6.6	17	-	20	14-d	LC50	>32,871	
							21-d	$EC50_r$	3,605	
							21-d	$EC50_g$	22,371	
	metal mixt.	art. soil (OECD)	6.1	10	20	20	14-d	LC50	1,730	
							21-d	$EC50_r$	1,001	
							21-d	$EC50_g$	740	
								Spurgeon & Hopkin, 1995 [17]		
Eisenia fetida	$Zn(NO_3)_2$	soil	-	-	-	-	14-d	LC50	1.08 E-02	
								Callahan et al., 1994 [19]		
Enchytraeus crypticus adults	$ZnCl_2$	art. soil (OECD)	6.4	10	20	17	4-w	$EC50_r$	188	
								$EC50_r$	336	
								Posthuma et al., 1997 [11]		
Enchytraeus crypticus	-	art. soil (OECD)	5.5	10	20	20	?	EC50 ?	273	
								EC50 ?	251	
								EC50 ?	186	
							?	EC50 ?	254	
							?	EC50 ?	212	
							?	EC50 ?	361	
	-	soil (contam.) (Budel) *(EU soil)*	-	-	-	20	?	EC50 ?	205	
								Posthuma & Notenboom, 1996		
Nematods (Annelids)										
Caenorhabditis elegans adults *(U.S. soils)*	$ZnCl_2$	sandy loam	6.2	1.7	16	20	24-h	LC50	360	
		sandy loam	5.1	3.0	16			LC50	255	
		loam	6.1	3.4	20			LC50	392	
		clay loam	6.2	2.2	39			LC50	549	
								Donkin & Dusenbery, 1994		

(to be continued)

Table 3.3.3.c Toxicity of zinc to soil invertebrates: LC50 and EC50 values
(continued) (Effect concentrations, not used for $PNEC_{add, terrestrial}$ derivation)

Organism	Test-comp	Soil type	pH	OM %	Clay %	Temp. °C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)	
Insects										
Folsomia candida 10-d juveniles	$ZnCl_2$	art. soil (OECD)	6.0	10	20	18	4-w	$EC50_{g\,(f)}$	1,228	(actual)
								$EC50_r$	473	(actual)
		sand (PANH) *(EU soil)*	6.0	2	2	19	4-w	$EC50_{g\,(f)}$	526	(actual)
								$EC50_r$	261	(actual)
		sand (perc.) (PANH-perc) *(EU soil)*	6.0	2	2	18	4-w	$EC50_{g\,(f)}$	584	(actual)
								$EC50_r$	534	(actual)
		sand (aged) (PANH-aged) *(EU soil)*	6.0	2	3	18	4-w	$EC50_{(}$	2,178	(actual)
								Smit & Van Gestel, 1998 [8]		
Folsomia candida 10-d juveniles		sand *(EU soil)* PAHN soil ?	4.6-5.3	2	2	18	4-w	LC50	625	[9a]
							10-w	LC50	476	[9b]
							4-w	LC50	670	[9c]
							10-w	LC50	1,085	[9d]
							4-w	$EC50_{g\,(f)}$	500	[9a]
							4-w	$EC50_{g\,(f)}$	618	[9c]
							4-w	$EC50_{g\,(d)}$	476	[9a]
							4-w	$EC50_{g\,(d)}$	577	[9c]
							4-w	$EC50_r$	184	[9a]
							10-w	$EC50_r$	389	]9b]
							4-w	$EC50_r$	258	[9c]
							10-w	$EC50_r$	291	[9d]
								Smit et al., 1998 [9]		
Folsomia candida adults	$Zn(NO_3)_2$	art. soil (OECD)	6.0 5.0 4.5	10	20	20	4-w	$EC50_r$ $EC50_r$ $EC50_r$	900 600 590	
								Sandifer & Hopkin, 1996 [12]		
Folsomia candida adults	$Zn(NO_3)_2$	art. soil (OECD)	6.0	10	20	15	6-w	$EC50_r$	590	
								Sandifer & Hopkin, 1997 [13]		
Folsomia candida 10-d juveniles	$ZnCl_2$	art. soil (OECD)	6.0	10	20	20	2-w	$EC50_{g(f)}$	1,509	
							4-w	$EC50_{g(f)}$	1,220	
							6-w	$EC50_{g(f)}$	1,661	
							2-w	$EC50_{g(d)}$	1,160	
							4-w	$EC50_{g(d)}$	1,202	
							6-w	$EC50_{g(d)}$	1,444	
							4-w	$EC50_r$	626	
							6-w	$EC50_r$	683	
								Van Gestel & Hensbergen, 1997 [16]		

For footnotes: see next pages; for further information see the "list of abbreviations Table 3.3.3.a to Table 3.3.3.d"

Further notes regarding the LC50 and EC50 values for soil invertebrates

- **The LC50 and EC50 values listed in Table 3.3.3.c are usually based on the nominal zinc concentrations (Cn). A large number of the LC50 and EC50 values are from studies from which also chronic NOEC and/or EC10 values could be derived, see Table 3.3.3.b.**
- **All EC50 values for *Eisenia fetida* and *Folsomia candida* from Lock et al. (2003) are listed in Table 3.3.3.b, not in Table 3.3.3.c.**

Abbreviations and footnotes Table 3.3.3.b and Table 3.3.3.c

Test compounds

$Zn(Ac)_2$ = Zinc acetate (soluble)

$ZnCl_2$ = Zinc chloride (soluble)

$Zn(NO_3)_2$ = Zinc nitrate (soluble)

$ZnSO_4$ = Zinc sulphate (soluble)

$ZnCO_3$ = Zinc carbonate ("insoluble")

ZnO = Zinc oxide ("insoluble")

Toxicological endpoints

f = feeding activity (consumption)

g = growth

g (d) growth based on dry weight

g (f) = growth based on fresh weight

m= sexual maturity;

r = reproduction;

r (c): reproduction based on the number of cocoons (e.g. cocoons/worm/week)

r (j)= reproduction based on the number of juveniles (e.g. juveniles/week, or juveniles/parent animal/week)

t= time to reach sexual maturity

[1] Neuhauser et al. (1985): *Eisenia fetida* (Table 3.3.3.b and 3.3.3.c)

No statistics reported. No data on test concentrations, but it was reported that at least five concentrations were tested. The NOEC = EC10 was estimated from the regression equation reported by the study authors: % weight loss = 0.05 C_{metal} – 5. Growth parameter: weight. Test compound added to the soil in aqueous solution.

Soil: Artificial OECD soil. No data on background Zn concentration.

[2] Neuhauser et al. (1984): *Eisenia fetida* (Table 3.3.3.b)

Statistics: p = 0.05. Worms were exposed to 30 g soil covered with 20 g contaminated manure. The NOEC indicated is the average result of 4 tests using the acetate, chloride, nitrate and sulphate salt; the average value was reported by Neuhauser (1984). The experimental result is expressed as mg Zn/kg dry manure. No data on soil characteristics.

Rejected, based on Relevance criterion (exposure through manure).

[3] Hartenstein et al. (1981): *Eisenia fetida* (Table 3.3.3.b)

Statistics applied ("p" not reported). The NOEC refers to growth; survival was less affected. Worms were exposed to 50 g soil (dry weight basis) covered with 50 g contaminated wet sludge (Zn salt added as a solid). After 4 weeks of exposure the remaining sludge was removed and a fresh supply of Zn-treated sludge was added. Soil and sludge pH ranged from 6.5 to 7, independent of treatment. The experimental result is expressed as mg Zn/kg dry sludge.

Soil: silt loam; no further soil characteristics reported.

Rejected, based on Relevance criterion (exposure through sludge).

[4] Malecki et al. (1982): *Eisenia fetida* (Table 3.3.3.b)

Statistics: p = 0.05. Worms were exposed to 30 g soil covered with 20 g contaminated manure. The zinc-manure mixture was removed and replenished at 4 and 6 weeks. The experimental results are expressed as mg Zn/kg dry manure. The NOEC values marked by "est." were estimated from the lowest effect concentration, using a factor of 2 (NOEC = LOEC/2).

- Test with ZnO: the NOEC refers to both growth and reproduction; survival was less affected.

- Test with Zn acetate: the NOEC refers to reproduction; survival and growth were less affected.

- Test with $ZnCl_2$ or $Zn(NO_3)_2$: the NOEC refers to growth and reproduction; survival was less affected.

- Test with $ZnSO_4$ or $ZnCO_3$: the NOEC refers to reproduction; survival and growth were less affected.

Rejected, based on Relevance criterion (exposure through manure).

[5] Malecki et al. (1982): *Eisenia fetida* (Table 3.3.3.b)

Statistics: p = 0.05. The NOEC refers to reproduction; survival and growth were less affected. Worms were exposed to 90 g soil covered with 100 g contaminated manure. The experimental result is expressed as mg Zn/kg dry manure. No data on soil characteristics.

Rejected, based on Relevance criterion (exposure through manure).

[6] Marigomex et al. (1986): *Arion ater* (Table 3.3.3.b)

Statistics: regression analysis. Feed (natural diet): equiproportional, grounded mixture of lettuce, apple, carrot and pumpkin with a 1.5% agar aqueous solution mixed with ZnCl . Feeding activity tended to decrease at 300 mg/kg during the last week of exposure, but body weight and survival were not affected.

Rejected, based on Relevance criterion (exposure through feed).

[7] Denneman & van Gestel (1990): *Porcellia scaber* (Table 3.3.3.b)

The NOEC refers to growth; feeding activity and reproduction were less affected. Feed: mixture of carrots and potatoes.

Rejected, based on Relevance criterion (exposure through feed).

[8] Smit & Van Gestel (1998): *Folsomia candida* (Table 3.3.3.b and Table 3.3.3.c)

Tests performed in the framework of the Dutch research project “Validation of toxicity data and risk limits for soils” (see also RAR sections 3.3.3.1.1 and 3.3.3.1.4). The final summarising report of the whole project is published by Posthuma et al. (1998).

Statistics (p = 0.05) applied for NOEC derivation for growth and reproduction. The study authors also derived EC10 values for growth and reproduction, using the Van Brummelen et al. (1996) modification of the logistic model of Haanstra et al. (1985). The results were reported as actual concentrations (thus Cn = actual-Cb). Test compound added in solution to the soils (in test 1 and test 2: immediately before use in test).

- Test 1: in artifical OECD soil (Cb: 2 mg/kg).

Nominal test concentrations 0-160-256-410-655-1,049-1,678 mg/kg.

- Test 2: in field-collected Panheel soil : PANH (Cb = 23 mg/kg d.w.)

Nominal test concentrations 0-160-256-410-655-1,049-1,678 mg/kg.

- Test 3: in field-collected Panheel soil that after the addition of Zn was percolated with deionized water (4 times the pore volume): PAHN-perc (Cb = 21 mg/kg).

Nominal test concentrations 0-160-256-410-655-1,049-1,678 mg/kg.

- Test 4: in field-collected Panheel soil that after the addition of Zn was placed in uncovered outdoor plots for approximalely 20 months: PAHN-aged (Cb = 24 mg/kg d.w.).(aged soil)

Nominal test concentrations 0-32-100-180-320-560-1,000-1,800-3,200 mg/kg.

Panheel soil: EU-soil, collected in The Netherlands (Panheel, province of Limburg).

Soil analysis: total Zn and exchangeble Zn (0.01 $CaCl_2$ and water-soluble Zn).

Results for growth: With respect to the NOEC, fresh weigt and dry weight were equally sensitive in OECD, PANH and PANH-perc. In PANH-aged the NOEC for fresh weigth (709 mg/kg; listed in Table 3.3.3.b) was somewhat lower than that for dry weight (1,198 mg/kg). The EC10 values were only reported for fresh weights.

Results for survival: Control survival averaged 88% in PANH-aged, 71% PANH-perc, 63% in OECD and 56% in PANH. At the lowest Zn concentration of 160 mg/kg in OECD, PANH and PANH-perc, survival was >80%. At the highest Zn concentration of 1,678 mg/kg in OECD and PANH-perc, survival was 68% and 62%, respectively. At the highest Zn concentration in PANH-aged, survival was 90%. In OECD, PANH-perc and PAHN-aged there was no relationship between survvial and Zn exposure. In PANH , survival was inversely related with Zn exposure and 100% mortality was found at the highest Z concentration (LC50: 699 mg/kg)

Tests in this study by Smit & Van Gestel (1998): Survival, growth (fresh and dry weight) and reproduction were determined according to Smit & Van Gestel (1996): Appl. Soil Ecol. 3, 127-136.

[8a] Rejected based on Relevance criterion (test performed in aged soils).

[9] Smit et al. (1998): *Folsomia candida* (Table 3.3.3.c)

Statistics: logistic, Tukey's test ($p < 0.05$)

a: 5 mg dried baker's yeast was applied on top of the soil

b: 5 mg dried baker's yeast was homogeneously mixed with the soil

c: pollen grains on top of the soil

d: no food during the experiment

[10] Spurgeon et al. (1997): *Eisenia fetida* (Table 3.3.3.b and 3.3.3.c)

Statistics: probit analysis and linear interpolation technique ($p< 0.05$, $p< 0.01$ or $p< 0.001$). Test compound added in solution to air-dried soil. Test concentrations 0-190-350-620-1,200-2,000 mg/kg. Toxicological endpoints: survival of parent worms and reproduction (cocoon production: niumber of cocoons/worm/week.

In this study a third test (not listed in the table) was performed at 25 ^{0}C. In this test, all test concentrations resulted in a statistically significant inhibition of reproduction, with 41%

inhibition at the lowest concentration tested (190 mg/kg). The test at 25 ^{0}C is considered to be not valid since OECD Guideline 207 (acute toxicity test) recommend a test temperature of 20 ^{0}C for the recommended species *E. fetida.*

Soil: artificial OECD soil. No data on background Zn concentration.

[11] Posthuma et al. (1997): *Eisenia fetida* (Table 3.3.3.c)

Actual concentration; background concentration 12 mg Zn/kg dry wt. The EC50 of 188 mg Zn/kg dry wt. is the result from a range finding study.

[12] Sandifer & Hopkin (1996): *Folsomia candida* ((Table 3.3.3.b and Table 3.3.3.c)

Statistics: $p \leq 0.05$. Test compound added in solution to air-dried soil. The study included three test with zinc, conducted in artificial OECD soil with different pH values. Test concentrations 0-100-190-350-620-1,200-2,000-3,000-6,500-10,000 mg/kg in the test at pH 6.0, and 0-100-300-1,000-2000-3,000-6,500-10,000 mg/kg in the tests at pH 5 and pH 4.5. The actual concentrations were reported to be within 10% of the nominal concentrations; no further data on actual concentrations reported. Toxicological endpoints: adult survival and reproduction (number of juveniles).

Soil: Artificial OECD soil; no data on background Zn concentration.

[13] Sandifer & Hopkin (1997): *Folsomia candida* (Table 3.3.3.b and Table 3.3.3.c)

Statistics: $p \leq 0.05$. Test compound added in solution to air-dried soil. The study included three tests with zinc, conducted at three different temperatures in artifical OECD soil at pH 6. The 4-w test at 20 ^{0}C were already included in Sandifer & Hopkin (1996), see footnote [12]. Test concentrations: 0-100-300-1,000-3,000-10,000 mg/kg in the tests at 15 ^{0}C and 25 ^{0}C. The actual concentrations were reported to be within 10% of the nominal concentrations; no further data on actual concentrations reported. The exposure time was 4 weeks at 20 ^{0} and 6 weeks at 15 ^{0}C. Toxicological endpoints: adult survival and reproduction (number of juveniles).

In the test at 25 ^{0}C the juvenile production was increased at the concentrations up to 1,000 mg/kg (but statistically significant only at 300 mg/kg) and no reproduction occurred at 3,000 mg/kg (while survival was not effected at this concentration) This test is considered to be not valid, since the control juvenile production is too low at this temperature (number of juveniles/10 parent animals: 30 at 25 ^{0}C versus 800 and 500 at 20 ^{0}C and 15 ^{0}C, respectively).

Soil: Artificial OECD soil; no data on background Zn concentration.

The test at 25 ^{0}C was rejected, based on Quality criterion (too low control performance).

[14] Spurgeon & Hopkin (1996a): *Eisenia fetida* (Table 3.3.3.b and Table 3.3.3.c)

Statistics: US EPA linear interpolation technique ($p < 0.01$ and $p< 0.05$). Soil samples were collected from 7 contaminated sites in the vicinity of a smelting works and from an uncontaminated site that served as control. Zinc levels in the contaminated soils ranged from 925 to 32,900 mg/kg; these soils were also contaminated with other metals (Cd, Cu, and Pb). The zinc level in the uncontaminated soil was 38 mg/kg. In this study: no added zinc, but high soil concentrations of zinc and other metals from metal deposition by the smelting works. The results were reported as NOEC values (Table 3.3.3.b – Part II) and L(E)C50 values (Table 3.3.3.c). The LC50 and EC50 values reported in Table 3.3.3.c are the lowest values calculated during the 20-w study.

Soil: EU soil sampled in the UK(northeast of the Avonmouth smelter).

Rejected , based on Relevance criterion (metal mixture exposure, in soil contaminated by emissions from a smelting works)

[15] Van Gestel and Hensbergen (1997): *Folsomia candida* (Table 3.3.3.b)

The EC10 values for growth (fresh and dry weight) of the parent animals and reproduction (number of juveniles) were derived by the study authors, using the Van Brummelen et al. (1996) modification of the logistic model of Haanstra et al. (1985). No EC10 or EC50 could be derived for survival (as the effect on survival was not dose-related), but survival was less sensitive than growth and reproduction. Test compound added in solution; after that the soils were equilibrated for 1-2 days before use in the test. Test concentrations: 0-100-200-400-800-1,600-3,200 mg/kg. Toxicological endpoints: survival and growth (fresh and dry weight) of the parent animals and reproduction (number of juveniles). The NOEC for dry weigth (840 mg/kg; listed in Table 3.3.3.b) was slighly lower than that for fresh weight (882 mg/kg).

Soil: artificial OECD soil (Cb 14 mg/kg).

Soil analysis: total Zn and water-soluble Zn. The actual total-Zn concentrations were not reported.

See also Table 3.3.3.c and footnote [16]

[16] Van Gestel & Hensbergen (1997): *Folsomia candida* (Table 3.3.3.c)

The EC50 values are based on thre total-Zn concentration.

See also Table 3.3.3.b and footnote [15].

[17] Spurgeon & Hopkin (1995): *Eisenia fetida* (Table 3.3.3.b and Table 3.3.3.c)

Statistics: p = 0.05. The reported NOEC values are 'estimated' NOEC values, derived by the study authors, see footnote [23] for further explanation. No 'real' NOEC values can be derived from the data reported for these tests.

Test in artifial OECD soil (exposure to Zn): Test compound added in solution. Test concentrations 0-100-400-2,000-10,000 mg/kg. After a 1-w pre-incubation period in untreated artificial soil, adult worms were exposed for 3 weeks. At the end of this period, after which growth (% increase in body weight) and reproduction (cocoon production) were assessed. The survival of the parent worms was assessed after 2 weeks. The cocoons were incubated in untreated artificial for 5 weeks to assess hatchability (% fertile cocoons), the number of juveniles/fertile cocoon and, hence, juvenile production rate (number of juveniles/worm/week). The NOEC values for reproduction listed in the table are for cocoon production, the most sensitive reproductive parameter, see also below.

Soil: artificial OECD soil. No data on background Zn concentration.

In this study, two series of similar tests were performed with exposure to metal mixtures of Zn, Cd, Cu and Pb in a transect of a field-contaminated soil collected in the vicinity of a smelting works, see also footnote [14] and exposure to metal mixtures of Zn, Cd, Cu and Pb at the same concentrations as those found in the field transect soil.

The reported NOEC values from the Zn test in artificial soil and those from the test series with mixed-metal exposure in artificial soil are 'estimated' NOEC values, derived by the study authors, see footnote [23] for explanation. No 'real' NOEC values can be derived from the data reported for these tests. The results (EC50 values and 'estimated' NOEC values) for reproduction are for cocoon production, reported to be the most sensitive reproductive parameter (see also below); no detailed results for the further studied reproductive endpoints were reported.

The NOEC values derived from the test series in a transect of field-contamined soils are 'real' NOEC values; the unbounded NOEC for survival is the highest zinc concentration measured in the field-contaminated soil. The detailed results for all studied reproductive parameters indicate that the number of cocoons/worm and the number of juveniles/worm were more sensitive than hatchability or the number of juveniles/cocoon.
Tests in field-contaminated soils:

Reproduction (c, j): 'Real' NOEC is 1,848 mg/kg; 'estimated NOEC' is 1,879 mg/kg.

Growth: 'Real' NOEC is 2,793 mg/kg; 'estimated NOEC' is 5,444 mg/kg.

[17a] **Rejected, based on Relevance criterion (metal mixture exposure, either in soil contaminated by emissions from a smelting works or in soil experimentally contaminated with a mixture of metal salts.**

[18] Van Gestel et al. (1993): *Eisenia andrei.* (Table 3.3.3.b and 3.3.3.c)

Statistics: p = 0.05. Test concentrations 0-100-180-320-560-1000 mg/kg. After a 1-w pre-incubation period in untreated artificial soil, adult worms were exposed for 3 weeks. At the end of this period, cocoon production (no.of cocoons/week) was asssessed and the worms were transferred to untreated artifial soil for a 3-w recovery period, after which growth (% increase in fresh body weight during the whole test period) of the worms was assessed. Cocoons were incubated in untreated artificial for 5 weeks to assess hatchability (% fertile cocoons), the number of juveniles/fertile cocoons and, hence, juvenile production rate (number of juveniles/worm/week). For further data on the test design (material & methods), see Van Gestel et al., 1992a: Ecotox Environ. Saf. 23: 206-220.

The NOEC for reproduction listed in Table 3.3.3.b is based on cocoon production (number of cocoons/worm/week) and the number of juveniles/worm/week. The other reproductive parameters, i.e. hatchability (% fertile cocoons) and the number of juveniles/fertile cocoon) were less sensitive (NOEC 560 mg/kg; not listed in Table 3.3.3.b).

With regard to the effect on growth it is noted that there was a dose-related increase, the highest concentration showing a statistically significant stimulation of growth compared to the controls.

Soil: artificial OECD soil. No data on background Zn concentration.

[19] Callahan et al. (1994): *Eisenia fetida* (Table 3.3.3.c)

Secondary literature source (review of lethal toxicity of 62 chemicals to 4 species of earthworms). A Weibull function was used for describing the mean concentration-response curve. Note that the 14-LC50 (0.108 E-02 mg/kg) reported by Callahan et al. (1994) for *E. fetida* is more than 100,000-times lower than the other zinc LC50 and EC50 values for this soil species or other soil invertbrates and thus the LC50 reported by Callahan et al. (1994) is considered to be very unreliable.

[20] Spurgeon et al. (1994): *Eisenia fetida.* (Table 3.3.3.b and 3.3.3.c)

Statistics: p = 0.05. The reported NOEC values are 'estimated' NOEC values, derived by the study authors, see footnote [23] for further explanation. No 'real' NOEC values can be derived from the data reported for these tests.

Test compound added in solution. Test concentrations: 0-100-400-2,000-10,000 mg/kg. The mortality and growth of the parent worms and reproduction (number of cocoons) were assessed on a weekly basis. The cocoons were incubated on moist filter paper untill all juveniles had emerged, to assess hatchability. The NOEC for reproduction listed in Table

3.3.3.b is based on cocoon production; hatchability was clearly less sensitive (no effect found).

Soil: artificial OECD soil. No data on background Zn concentration.

[21] Khalil et al. (1996): *Aporrectodea caliginosa* (Table 3.3.3.b and 3.3.3.c)

No statistics reported for NOEC derivation. Test compound added in solution. Test concentrations 0-2,500-3,000-3,500-4,000-4,500-5,000 mg/kg in the lethal toxicity test and 0-300-600-1,000-1,600 mg/kg in the reproduction toxicity test. The toxicological endpoints in the reproduction experiment were survival of the parent worms and reproduction (cocoon production). The EC10 and EC50 for reproduction were derived by the study authors, using the log-logistic model of Haanstra et al. (1985) and are based on the cumulative cocoon production in weeks 3-8 (6-w period). The NOEC for reproduction was derived by the rapporteur from graphs showing very similar results for the 3-8 and total 8 weeks period. The worms used in the tests were collected in the same area as the soil samples.

Soil: non-EU soil sampled in Egypt (Al-Qanater Al-Khahiriya city). No data on the background zinc concentration in the soil.

[22] Spurgeon & Hopkin (1996b): *Eisenia fetida* (Table 3.3.3.b and 3.3.3.c**).**

Statistics: p = 0.05. The reported NOEC values are 'estimated' NOEC values, derived by the study authors, see footnote [23] for further explanation. No 'real' NOEC values can be derived from the data reported for these tests.

Test compound added in solution. Test concentrations: 0-(100)-190-350-620-(1,000)-1,200-2,000-(3,600) mg/kg. Before the addition of zinc, the laboratory-cultured worms were acclimated to the relevant artificial soil (differing in organic matter content and pH) for one week. Toxicological endpoint: survival of the parent worms and reproduction (number of cocoons). Hatchability (cocoon viability) and the number of juveniles emerging per fertile cocoon were not recorded since no effects due to zinc were found for these parameters during earlier toxicity tests (Spurgeon & Hopkin, 1995; Spurgeon et al., 1994).

Soil: artificial OECD soil. The organic matter content (5%, 10% and 15%) was adjusted by varying the S*pagnum* peat content; the pH value (4, 5, and 6) was adjusted by varying the quantity of calcium carbonate. Background Zn concentration varying from 0.2 to 2 mg/kg.

Soil analysis: Data on total and water-extractable Zn.

[23] Spurgeon et al. (1994), Spurgeon & Hopkin (1995) and Spurgeon & Hopkin (1996b):

Eisenia fetida

(Table 3.3.3.b)

The NOEC values listed in the studies by Spurgeon et al. (1994), Spurgeon & Hopkin (1995) and Spurgeon & Hopkin (1996b) are 'estimated' NOEC values, Stricly speaking a NOEC value should correspond to the highest test concentration causing no significant effect compared to the controls. In the above-mentioned studies, 'estimated' NOEC values have been calculated by the study authors by adjusting the mean within the William's (1971, 1972) test to determine the mean value giving a p = 0.05 value. This mean value was then used to estimate the NOEC value by assuming a straight line relationship between the highest concentration causing no significant effect and the lowest concentration giving a significant effect. (above procedure described in Spurgeon et al., 1994).

[24] Lock et al. (2003**):** *Eisenia fetida* and *Folsomia candida* (Table 3.3.3.b)

Statistics used for NOEC derivation (endpoint reproduction): ANOVA followed by the Dunnett-test ($p < 0.05$). Nominal test concentrations: a control and 5 consecutive Zn concentrations in the range of 56-100-180-320-560-1000-1800 mg/kg d.w. for added Zn. The results for total Zn as reported by Lock et al. (2003) are expressed as added Zn (Cn) plus background Zn (Cb). The actual Zn concentrations in soil and pore water were measured at the end of the tests**.** The actual total-Zn concentrations in the soils were in good agreement with the calculated total-Zn concentrations (Cn + Cb).

The study was performed i) to study the effects of abiotic factors on the chronic toxicity of zinc in freshly-spiked soils to *E. fetida* and *F. candida (* one soft-bodied and one hard-bodied terrestrial invertebrate), and ii) to study the difference in zinc toxicity in freshly-spiked soils and field-polluted soils.

Soils: 15 uncontamined EU soils, top soil (plough layer in cultivated soils and 0-20 cm layer in undisturbed soils) collected from arable land or non-arable land (forest, woodland, heath land, grassland, olive orchard) all over Europe. The uncontamined soils were selected to cover the relevant ranges of abiotic factors influencing Zn bioavailability in soils, including pH and cation exchange capacity. Background Zn concentrations: 7 to 191 mg/kg d.w. pH = pH $CaCl_2$. The Rhydtalog, De Meern and Zeveren soil are the uncontaminated reference soils of the field-polluted transect soils with Zn contamination due to corrosion of galvanized pylons.

It is noted that some of the NOEC values derived by Lock et al. (2003) were revised by the rapporteur, see the separate Appendix on the Lock et al. (2003) study for further explanation. All EC10 and EC50 values in Table 3.3.3.b (Part 1 and Part II) are the values originally reported by Lock et al. (2003).

See also footnotes [25] and [26] and the Appendix on the Lock et al. (2003) study.

[25] Lock et al. (2003): *Eisenia fetida* (Table 3.3.3.b) **

The test was performed according to ISO 11268-2 (1996): Soil quality – Effects of pollutants on earthworms (*Eisenia fetida*) – Part 2: Determination of effects on reproduction. Number of test animals per treatment: 40

(4 replicates of 10 adult worms with fully developed clitellium). Reproductive endpoint: number of cocoons

instead of the number of live offspring `(juveniles) that is mentioned as reproductive endpoint in the ISO guideline.

The test duration of 28 days is according to the ISO guideline, although in the ISO test the 28-d reproduction period is preceded by a 28-d period in which mortality and growth (biomass) of the parent worms is determined (survival and growth were not included as endpoints in this study). Further note that in all other *E. fetida* tests of which the results were used for $PNEC_{add, terrestrial}$ derivation, the test duration was usually lower than 28 days, i.e. 14 to 21 days.

* Soil No. in study (see Table 3.3.3.b)

** Further information provided by the authors of the study in addition to the study report (Lock et al., 2003) has been included in the evaluation of the study. The further information included the purity of the test compound (ZnCl2, purity 98%), data on spiking and equilibrium (following spiking of the soils with aqueous solutions of ZnCl2, a period of at least 24 hours was allowed for equilibrium before animals were added to the test soils) and the raw data for each test, i.e. the results for cocoon production for each replicate at each test concentration (expressed total Zn). In the controls the mean number of cocoons per 10 parent animals was >30, except in Rots soil (mean number of cocoons: 15) and Souli II soil (mean

number of cocoons: 14). The number of live offspring (juveniles) was not determined, but assuming a mean number of 2 juveniles/cocoon (estimated from the other studies with E. fetida), the number of juveniles per 10 parent animals will have been >30 in all tests, thus fulfilling this validity criterion for reproduction. Furthermore, the results of the other E. fetida tests and the E. andrei test in Table 3.3.3.b clearly show that cocoon production is the most sensitive reproductive endpoint for Eisenia species and also more sensitive than mortality and growth. According to a general statement by Lock, the validity criterion for control survival was also met in the tests, which is consistent with the results for reproduction. Thus, the tests are valid, **except the test in Aluminosa soil (rejected because no reliable NOEC or EC10 could be derived from this test) and the tests in Rots soil and Souli II soil (rejected because the coefficient of variance for reproductive performance in the control was 80%, thus strongly exceeding the second validity criterion for reproduction: the coefficient of variation should not exceed 30%). Thus these 3 tests were rejected based on Quality criteria.**

See also the separate Appendix on the Lock et al. (2003) study for further explanation (see next pages).

[26] Lock et al. (2003): *Folsomia candida* (Table 3.3.3.b) **

The test was performed according to ISO 11267 (1999): Soil quality – Inhibition of reproduction of Collembola (*Folsomia candida*). Number of test animals per treatment: 40 (4 replicates of 10 synchronised animals of 10-12 days old). Reproductive endpoint: number of live offspring (juveniles). Test duration: 28 days.

* Soil No. in study (see Table 3.3.3.b)

** Further information provided by the authors of the study in addition to the study report (Lock et al., 2003) has been included in the evaluation of the study. The further information included the purity of the test compound ($ZnCl_2$, purity 98%), data on spiking and equilibrium (following spiking of the soils with aqueous solutions of $ZnCl_2$, a period of at least 24 hours was allowed for equilibrium before animals were added to the test soils) and the raw data for each test, i.e. the results for juvenile production for each replicate at each test concentration (expressed total Zn). In the controls the mean number of juveniles per 10 parent animals was >100, thus fulfilling this validity criterion for reproduction. According to a general statement by Lock, the validity criterion for control survival was also met in the tests, which is consistent with the results for reproduction. Thus, the tests are valid, **except the tests in Gudow soil, Ter Munck soil and Markness soil (rejected because no reliable NOEC or EC10 could be derived from these tests) and the tests in Kovlinge II soil and Woburn soil (rejected because the coefficient of variance for reproductive performance in the control was >50%, thus strongly exceeding the second validity criterion for reproduction: the coefficient of variation should not exceed 30%). Thus these 5 tests were rejected based on Quality criteria.**

See also the separate Appendix on the Lock et al. (2003) study for further explanation (see next pages).

Appendix on Lock et al. (2003): Laboratory zinc ecotoxicity testing for soil invertebrates (Table 3.3.3.b)

As discussed at TM III '03, the Lock et al. (2003) study as such was considered to be valid by the rapporteur. However, there were serious doubts on the validity of a number of NOEC values derived by Lock et al. (2003). Therefore, the study results were evaluated by the rapporteur on the basis of the 'raw' data (including for each test the results for all four replicates at each test concentration), to be able to check the validity of the tests and the NOEC values.

Lock et al. (2003) as well as the rapporteur used ANOVA and the Dunnett test to derive NOEC values. Lock et al. (2003) as well as the rapporteur used the log-logistic response model from Haanstra et al. (1985) to derive EC10 and EC50 values. Both with respect to NOEC values and EC10 values, these evaluations resulted in a number of (considerable) differences.

Based on these latter findings, the following rules were used by the rapporteur for the selection and derivation of NOEC values from the Lock et al. (2003) study.

1. Validity criterion: Tests in which the coefficient of variance (CV47) for the reproductive performance in the control was >40% are rejected, regardless the results of the statistical evaluations. According to the test guidelines the CV should not be >30%, but a somewhat less stringent limit (40%) was used because of the following:
   i) This validity criterion could not be checked for the other invertebrate studies (no raw data or specific data on CV available).
   ii) The Lock et al. (2003) study shows no very clear relationship between the reliability of the NOEC values and the CV.
   iii) Using a limit value of 30% would strongly reduce the number of useful NOEC values from this study (rejection of 11 of the 30 tests), while the limit of 40% results in the rejection of 4 tests.

2. If the ANOVA and Dunnett analyses by Lock et al. (2003) and the rapporteur of a particular test resulted in the same NOEC, the NOEC is accepted, unless the NOEC is equal to or higher than the EC50. In that case the NOEC is set equal to the EC10, provided that a reliable EC10 could be calculated, i.e. the EC10 values calculated by Lock et al. (2003) and the rapporteur must be very similar and the 90% confidence interval of the EC10 must be within reasonable limits. Furthermore, the EC10 may not be more than a factor of 3.2 lower than the lowest test concentration (general rule applied in the RAR Zn Metal).

3. If the ANOVA and Dunnett analyses by Lock et al. (2003) and the rapporteur resulted in NOEC values that differ no more than one concentration step (and the highest NOEC is equal to or higher than the EC50), the lowest NOEC is selected.

4. If the ANOVA and Dunnett analyses by Lock et al. (2003) and the rapporteur resulted in NOEC values that differ more than one concentration step, both NOEC values are considered to be unreliable and rejected. In that case the NOEC is set equal to the EC10, provided that a reliable EC10 could be calculated (see earlier rule 2). If neither a reliable NOEC nor a reliable EC10 could be calculated, the test is rejected.

[47] Coefficient of variance = (standard deviation / average) x 100%

Results for NOEC values

Based on the above rules, 8 of the 30 tests were rejected and 4 of the NOEC values of the remaining 22 tests were revised. For a summary of the result for each test, see Tables 1 and 2.

Results for EC50 and EC10 values

Table 3 shows for 9 of the 30 tests the EC50 and EC10 values that were calculated by Lock et al. (2003) and the rapporteur, i.e for the tests with a 'doubtful' NOEC (these teste were not rejected directly on the basis of the validity criteria for reproductive performance). These data show that with respect to EC50 values the results of the two calculations are similar to very similar, except for the *F. candida* test in Marknesse soil. With respect to the EC10 values the results of the two calculations are mostly similar to very similar, but in 3 of the 9 calculations there was a large to very large difference between the two EC10 values, especially in the *F. candida* test in Marknesse soil.

Table 1. *Eisenia fetida* tests: NOEC values derived by Lock et al. (2003) and the rapporteur, and the selected NOEC values included in the NOEC data base for $PNEC_{add, terrestrial}$ derivation in the RAR Zn Metal

			NOEC-Lock et al. (2003) and NOEC-Rapporteur based on ANOVA and Dunnett test (p < 0.05 is significant)			**Selected for $PNEC_{add, terrestrial}$**
			All NOEC values: added Zn concentration (Cn), in mg/kg dry soil.	**Lock et al. (2003)**	**Rapporteur**	
Soil	**CV of control**	**Cb**	**Remark**	**NOEC-Lock**	**NOEC-Rapporteur**	**NOEC-RAR**
Gudow	24	7	NOEC-Rapporteur: >EC50	**180**	≥320	**180**
Houthalen	35	8		**100**	**100**	**100**
Zegveld	2	191		**1000**	**1000**	**1000**
Rhydtalog control	11	83		**320**	560	**320**
Souli I	13	37		**560**	**560**	**560**
Kovlinge II	6	26		**320**	**320**	**320**
De Meern control	12	155		**560**	1000	**560**
Aluminusa	19	53	Test rejected: No reliable NOEC or EC10 (Lock: 21; Rapporteur: 8). NOEC-Lock: >EC50.	180	<56	
Zeveren control	25	76		**1000**	**1000**	**1000**
Woburn	10	99		**560**	**560**	**560**
Ter Munck	11	54		**180**	**180**	**180**
Rots	80	51	Test rejected: Coefficient of variance in control is >40%. i.e. 80%	560	≥1000	
Souli II	80	51	Test rejected: Coefficient of variance in control is >40%, i.e. 80%	560	560	
Marknesse	23	80		**180**	**180**	**180**
Guadalajara	39	27	NOEC >EC50. Revised: selected NOEC = EC10 (equal value calculated by both Lock and the rapporteur)	560	560	**350**

Table 2. *Folsomia candida* tests: NOEC values derived by Lock et al. (2003) and the rapporteur, and the selected NOEC values included in the NOEC data base for $PNEC_{add, terrestrial}$ derivation in the RAR Zn Metal

			NOEC-Lock et al. (2003) and NOEC-Rapporteur based on ANOVA and Dunnett test ($p < 0.05$ is significant)			**Selected for $PNEC_{add, terrestrial}$**
			All NOEC values: added Zn concentration (Cn), in mg/kg dry soil.	**Lock et al. (2003)**	**Rapporteur**	
Soil	**CV of control**	**Cb**	**Remark**	**NOEC-Lock**	**NOEC-Rapporteur**	**NOEC-RAR**
Gudow	18	7	Test rejected: No reliable NOEC or EC10 (Lock: 11; Rapporteur: 16).	56	≥320	
Houthalen	34	8		56	**32**	**32**
Zegveld	31	191		**1000**	**1000**	**1000**
Rhydtalog control	24	83		**320**	**320**	**320**
Souli I	29	37		**100**	180	**100**
Kovlinge II	52	26	Test rejected: Coefficient of variance in control is >40%, i.e. 52%	320	≥560	
De Meern control	19	155	NOEC >EC50. Selected NOEC = EC10 (equal value calculated by both Lock and the rapporteur)	1800	1800	**300**
Aluminusa	31	53	NOEC-Rapporteur: >EC50	**320**	≥1000	**320**
Zeveren control	26	76		560	320	**320**
Woburn	71	99	Test rejected: Coefficient of variance in control is >40%, i.e 71%	1000	≥1800	
Ter Munck	32	54	Test rejected: No reliable NOEC or EC10 (Lock: 43; Rapporteur: 12). NOEC-Lock: >EC50.	320	<100	
Rots	26	51		**560**	**560**	**560**
Souli II	36	51		**1000**	**1000**	**1000**
Marknesse	22	80	Test rejected: No reliable NOEC or EC10 (Lock: 43; Rapporteur: 12). NOEC-Lock: >EC50.	1000	<180	
Guadalajara	14	27		**320**	**320**	**320**

Table 3. EC10 and EC50 calculations for a number of tests
(Input: added-Zn concentrations, Cn)

E. fetida in Gudow soil
Lock E10 = 130 (90% CI: 45-373); Rapporteur EC10 = 135 (90% CI: 19-923)
Lock EC50 = 250 (90% CI: 157-401); Rapporteur EC50 = 254 (90% CI: 107-601)
E. fetida in Aluminosa soil
Lock EC10 = 21 (90%CI: 8.6-49); Rapporteur EC10 = 8 (90% CI: 1-60)
Rapporteur EC10 (8) is 7-times lower than the lowest test concentration (56).
Lock EC50 = 173 (90% CI: 129-232); Rapporteur EC50 = 124 (90% CI: 66-232)
E. fetida in Guadalajara soil
Lock EC10 = 346 (90%CI: 133-899); Rapporteur EC10 = 346 (90% CI: 189-634)
Lock EC50 = 531 (90% CI: 387-728); Rapporteur EC50 = 522 (90% CI: 427-641)
F. candida in Gudow soil
Lock EC10 = 11 (90% CI: 2.4-50; Rapporteur EC10 =16 (90% CI: 2.7-88)
Lock EC50 = 76 (90% CI: 42-140); Rapporteur EC50 = 74 (90% CI: 33-165)
F. candida in Houthalen soil
Lock EC10 = 30 (90% CI: 22-40); Rapporteur EC10 = 30 (90% CI: 28-31)
Lock EC50 = 64 (CI: 56-73); Rapporteur EC50: 64 (90% CI: 63-66)
F. candida in De Meern soil
Lock EC10 = 303 (90% CI: 99-929); Rapporteur EC10 = 296 (35-2506)
Lock EC50 = 1440 (90% CI: 916-2230); Rapporteur EC50 = 1445 (90% CI: 610-3420)
F. candida in Aluminosa soil
Lock EC10 = 209 (90% CI 59-741); Rapporteur EC10 = 248 (90% CI: 32-1888)
Lock EC50 = 682 (90% CI: 399-1170); Rapporteur EC50 = 678 (90% CI: 277-1660)
F. candida in Ter Munck soil
Lock EC10 = 43 (90% CI: 11-169); Rapporteur EC10 = 12 ((90% CI: 0.6-249)
Rapporteur EC10 (12) is 8-times lower than the lowest test concentration (100).
Lock EC50 = 247 (190% CI: 165-369); Rapporteur EC50 = 176 (90% CI: 78-397)
F. candia in Marknesse soil
Lock EC10 = 491 (90% CI: 200-1210); Rapporteur EC10 = 1.4 (90% CI: 0.0001 - 19000)
Lock EC50 = 1140 (90% CI: 868-1490); Rapporteur EC50 = 372 (90% CI: 51 - 2700)

Table 3.3.3.d Chronic toxicity of zinc to soil plants: NOEC and EC values

Part I: Studies useful for $PNEC_{add, terrestrial}$ derivation

Organism	Test-comp.	Substrate (soil (type)	pH	OM %	Clay %	Temp. °C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)		(estimated) **NOEC (Cn)** used for $PNEC_{add}$ derivation	
Medicago sativa (alfalfa)	$Zn(NO_3)_2.6H_2O$	silt loam	7.5	-	-	26-30	67-d	$NOEC_{y(p)}$ Boawn & Rasmussen, 1971 [8]	300	(Cn)	**300**	
Zea mays (corn)	$ZnSO_4.7\ H_2O$	sandy loam H) [without P]	4.9	3	16	-	6-w	$NOEC_{y(s)}$ MacLean , 1974 [1,2]	50 103	(Cn) (Cn + Cb)	**83**	[1a]
Zea mays (field corn)	$Zn(NO_3)_2.6H_2O$	silt loam	7.5	-	-	26-30	28-d	$NOEC_{y(p}$	300	(Cn)	**300**	
Zea mays **?** (sweet corn)	$Zn(NO_3)_2.6H_2O$	silt loam	7.5	-	-	26-30	28-d	$NOEC_{y(p)}$ Boawn & Rasmussen, 1971 [8]	200	(Cn	**200**	
Zea mays					*(n = 3)*	*geometric mean*		*$NOEC_y$*		*(Cn)*	***170***	
Lactuca sativa (lettuce)	$Zn(NO_3)_2.6H_2O$	silt loam	7.5	-	-	26-30	40-d	$NOEC_{y(p)}$ Boawn & Rasmussen, 1971 [8]	400	(Cn)	**400**	
Avena sativa (oat)	$Zn(Ac)_2$	loamy soil (c1)	5.6	2	12	-	5-m	$NOEC_{y(gr)}$	100 147	(Cn) (Cn + Cb)	**100**	
	$Zn(Ac)_2$	loamy soil (c2)	5.4	2	40	-	5-m	$NOEC_{y(gr)}$	200 257	(Cn) (Cn + Cb)	**200**	
	$Zn(Ac)_2$	sandy loam (s1)	5.0	3	4	-	5-m	$NOEC_{y(gr)}$	200 215	(Cn) (Cn + Cb)	**200**	
	$Zn(Ac)_2$	sandy loam (s2)	5.4	7	5	-	5-m	$NOEC_{y(gr)}$	400 428	(Cn) (Cn + Cb)	**400**	
(EU soils)								De Haan et al. 1985 [3]				
Avena sativa					*(n = 4)*	*geometric mean*		*$NOEC_y$*		*(Cn)*	***200***	
Hordeum vulgare (barley) seeds	$ZnCl_2$	sandy loam *(EU soil)*	5.6	8	13	14-17	48-d	$NOEC_{y(s)}$ $NOEC_{y(r)}$ Luo & Rimmer, 1995 [4]	10 ≥100	(Cn)	**33**	[4a]
Hordeum vulgare (barley) seeds)	$Zn\ SO_4.7\ H_2O$	sandy loam	7.8	1	-	-	45-d	$NOEC_{y(r)}$ $EC10_{y(r)}$ $NOEC_{y(s)}$ $EC10_{y(s)}$ Aery & Jagetiya, 1997 [5]	50 215 250 1,450	(Cn) (Cn) (Cn) (Cn)	**215**	[5a]
Hordeum vulgare (barley)	$Zn(NO_3)_2.6H_2O$	silt loam	7.5	-	-	26-30	33-d	$NOEC_{y(p)}$ Boawn & Rasmussen, 1971 [8]	100	(Cn)	**100**	
Hordeum vulgare					*(n = 3)*	*geometric mean*		*$NOEC_y$*		*(Cn)*	***89***	

(to be continued)

Table 3.3.3.d Chronic toxicity of zinc to soil plants: NOEC and EC values (continued) Part I: Studies useful for $PNEC_{add, terrestrial}$ derivation

Organism	Test-comp.	Substrate (soil (type)	pH	OM %	Clay %	Temp. °C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)		(estimated) **NOEC (Cn)** used for $PNEC_{add}$ derivation
Allium cepa (onion) seedlings → maturity	$ZnSO_4.7H_2O$	clay loam	8.3	0.5	24	-	-	$NOEC_{y\,(p)}$ Dang et al., 1990 [6]	200	(Cn)	**200**
Trigonella poenum-graceum (fenugreek) seeds	$ZnSO_4.7H_2O$	clay loam	8.3	0.5	24	-	8-w	$NOEC_{y\,(p)}$ Dang et al., 1990 [6]	200	(Cn)	**200**
Vigna mungo (blackgram) seeds	$ZnSO_4.7H_2O$	-	6.2	-	-	-	45-d	$NOEC_{y\,(r,\,st)}$	100	(Cn)	**100**
								$EC10_{y\,(r)}$	155	(Cn)	
								$EC10_{y\,(st)}$	162	(Cn)	
								$NOEC_{y\,(l)}$	150	(Cn)	
								$EC10_{y\,(l)}$ Kalyanaraman & Sivagurunathan, 1993 [7]	168	(Cn)	
Sorghum bicolor (sorghum-var. XK-125)	$Zn(NO_3)_2.6H_2O$	silt loam	7.5	-	-	26-30	35-d	$NOEC_{y(p)}$	100	(Cn)	**100**
Sorghum bicolor (sorghum-var. RS-626)	$Zn(NO_3)_2.6H_2O$	silt loam	7.5	-	-	26-30	35-d	$NOEC_{y(p)}$ Boawn & Rasmussen, 1971 [8]	200	(Cn)	**200**
Sorghum bicolor					*(n = 2)*	*geometric mean*		*$NOEC_{y(p)}$*		*(Cn)*	***140***
Triticum vulgare (wheat)	$Zn(NO_3)_2.6H_2O$	silt loam	7.5	-	-	26-30	33-d	$NOEC_{y(p)}$ Boawn & Rasmussen, 1971 [8]	200	(Cn)	**200**
Pisum sativum (Alaska pea)	$Zn(NO_3)_2.6H_2O$	silt loam	7.5	-	-	26-30	-	$NOEC_{y(p)}$ Boawn & Rasmussen, 1971 [8]	400	(Cn)	**400**
Spinacea oleracea (spinach)	$Zn(NO_3)_2.6H_2O$	silt loam	7.5	-	-	26-30	-	$NOEC_{y(p)}$ Boawn & Rasmussen, 1971 [8]	200	(Cn)	**200**
Beta vulgaris (sugarbeet)	$Zn(NO_3)_2.6H_2O$	silt loam	7.5	-	-	26-30	42-d	$NOEC_{y(p)}$ Boawn & Rasmussen, 1971 [8]	300	(Cn)	**300**
Lycopersicon esculentum (tomato)	$Zn(NO_3)_2.6H_2O$	silt loam	7.5	-	-	26-30	-	$NOEC_{y(p)}$ Boawn & Rasmussen, 1971 [8]	400	(Cn)	**400**

(to be continued)

Table 3.3.3.d Chronic toxicity of zinc to soil plants: NOEC and EC values (continued) Part I: Studies useful for $PNEC_{add, terrestrial}$ derivation

Organism	Test-comp.	Substrate (soil (type)	pH	OM %	Clay %	Temp. °C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)		(estimated) **NOEC (Cn)** used for $PNEC_{add}$ derivation
Trifolium pratense (red clover) seeds	$ZnCl_2$	art. soil (OECD)	6.2	10	20	18-24	24-d	$NOEC_{y(r,s)}$ $EC10_{y(r)}$ $EC10_{y(s)}$	100 113 133	(Cn) (Cn) (Cn)	**100**
Trifolium pratense (red clover) seeds	$ZnCl_2$	art. soil (OECD)	6.0	10	20`	18-24	24-d	$NOEC_{y(r)}$ $EC10_{y(r)}$ $NOEC_{y(,s)}$ $NOEC_{y(s)}$ Van der Hoeven & Henzen, 1994a, 1994c [9, 10]	84 84 150 130	(Cn) (Cn) (Cn) (Cn)	**84**
Trifolium pratense (red clover) seeds	$ZnCl_2$	sand (Budel reference soil # 10?) (% OM: 1%?)	5.0	5?	13?	19-27	25-d	$NOEC_{y(r,s)}$ $EC50_{y(s)}$ $EC50_{y(r)}$ Van der Hoeven & Henzen, 1994b, 1994c [9, 11]	32 40 131 68	(Cn) (Cn + Cb) (Cn) (Cn)	**32**
Trifolium pratense (red clover) seeds Test 1994	$ZnCl_2$	sand (PANH)	5.3	2	2	19-24	25-d	$NOEC_{y(r,s)}$ $NOEC_{germ.}$ $EC10_{y(s)}$ $EC10_{y(r)}$ $EC50_{y(s)}$ $EC50_{y(r)}$ Van der Hoeven & Henzen, 1994c; Hooftman & Henzen, 1996 [9, 12, 12a]	32 48 180 196 30 24 76 53	(Cn) (Cn + Cb) (Cn) (Cn + Cb) (Cn) (Cn) (Cn) (Cn)	**32**
Trifolium pratense (red clover) seeds Test 1995-1.a	$ZnCl_2$	sand (PANH)	5.3	2	2	20	25-d	$NOEC_{y(r,s)}$ $NOEC_{germ.}$ $EC50_{y(s)}$ $EC50_{y(r)}$ Hooftman & Henzen, 1996 [9, 12, 12a]	32 48 320 336 73 61	(Cn) (Cn + Cb) (Cn) (Cn + Cb) (Cn) (Cn)	**32**
Trifolium pratense (red clover) seeds Test 1995-1.b	$ZnCl_2$	sand (PANH)	5.3	2	2	20	25-d	$NOEC_{y(r,s)}$ $NOEC_{germ.}$ $EC50_{y(s)}$ $EC50_{y(r)}$ Hooftman & Henzen, 1996 [9, 12, 12a]	32 48 320 336 116 95	(Cn) (Cn + Cb) (Cn) (Cn + Cb) (Cn) (Cn)	**32**
Trifolium pratense					*(n = 6) geometric mean*			*$NOEC_{y(r)}$*		*(Cn)* (was: 55)	***45***
Vicia sativa (vetch) seeds	$ZnCl_2$	sand (Budel reference soil # 10?) (% OM = 1%?)	5.0	5?	13?	19-24	24-d	$NOEC_{y(r)}$ $NOEC_{y(s)}$ $EC50_{y(s)}$ $EC50_{y(r)}$ Van der Hoeven & Henzen. 1994b [9, 11]	32 40 100 108 176 109	(Cn) (Cn + Cb) (Cn) (Cn + Cb) (Cn) (Cn)	**32**

(Table 3.3.3.d: To be continued in Part II: Studies not useful for $PNEC_{add, terrestrial}$ derivation)

Table 3.3.3.d Chronic toxicity of zinc to soil plants: NOEC and EC values

Part II: Studies not useful for $PNEC_{add, terrestrial}$ derivation

Organism	Test-comp	Substrate (soil type)	pH	OM %	Clay %	Temp. °C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)		**NOEC (Cn)** used for $PNEC_{add}$ derivation
Medicago sativa (alfalfa)	$ZnSO_4$ .7 H_2O	sandy loam (A) [2]	7.5	4	16	-	8-w	$NOEC_{y\,(a)}$	≥250 ≥329	(Cn) (Cn + Cb)	[*]
	$ZnSO_4$ .7 H_2O	sandy loam (B) [2]	7.4	4	16	-	8-w	$NOEC_{y(s)}$	≥250 ≥330	(Cn) (Cn + Cb)	[*]
	$ZnSO_4$ .7 H_2O	sandy loam (C) [2]	7.4	8	16	-	8-w	$NOEC_{y(s)}$	≥250 ≥327	(Cn) (Cn + Cb)	[*]
	$ZnSO_4$ .7 H_2O	sandy loam (D) [2]	7.3	7	16	-	8-w	$NOEC_{y(s)}$	≥250 ≥327	(Cn) (Cn + Cb)	[*]
	$ZnSO_4$ .7 H_2O	mixed loam (E) [2]	7.4	3	23	-	8-w	$NOEC_{y(s)}$	≥250 ≥356	(Cn) (Cn + Cb)	[*]
	$ZnSO_4$ .7 H_2O	sandy loam (F) [2]	7.5	3	14	-	8-w	$NOEC_{y(s)}$	≥250 ≥322	(Cn) (Cn + Cb)	[*]
	$ZnSO_4$ .7 H_2O	sandy loam (G) [2]	7.2	10	13	-	8-w	$NOEC_{y(s)}$	≥250 ≥328	(Cn) (Cn + Cb)	[*]
	$ZnSO_4$ .7 H_2O	sandy loam (H) [2]	4.9	3	16	-	8-w	$NOEC_{y(s)}$	50 103	(Cn) (Cn + Cb)	[1b]
	$ZnSO_4$ .7 H_2O	sandy loam (I) [2]	6.8	3	16	-	8-w	$NOEC_{y(s)}$	≥250 ≥302	(Cn) (Cn + Cb)	[*]
	$ZnSO_4$ .7 H_2O	sandy loam (J) [2]	7.5	3	16	-	8-w	$NOEC_{y(s)}$	≥250 ≥301	(Cn) (Cn + Cb)	[*]
								MacLean, 1974 [1,2]			
Zea mays (corn)	$ZnSO_4$ .7 H_2O	sandy loam (A) [2]	7.5	4	16	-	6-w	$NOEC_{y(s)}$	≥250 ≥329	(Cn) (Cn + Cb)	[*]
	$ZnSO_4$ .7 H_2O	sandy loam (B) [2]	7.4	4	16	-	6-w	$NOEC_{y(s)}$	≥250 ≥ 330	(Cn) (Cn + Cb)	[*]
	$ZnSO_4$ .7 H_2O	sandy loam (C) [2]	7.4	8	16	-	6-w	$NOEC_{y(s)}$	≥250 ≥327	(Cn) (Cn + Cb)	[*]
	$ZnSO_4$ .7 H_2O	sandy loam (D) [2]	7.3	7	16	-	6-w	$NOEC_{y(s)}$	≥250 ≥327	(Cn) (Cn + Cb)	[*]
	$ZnSO_4$ .7 H_2O	mixed loam (E) [2]	7.4	3	23	-	6-w	$NOEC_{y(s)}$	≥250 ≥356	(Cn) (Cn + Cb)	[*]
	$ZnSO_4$ .7 H_2O	sandy loam (F) [2]	7.5	3	14	-	6-w	$NOEC_{y(s)}$	≥250 ≥322	(Cn) (Cn + Cb)	[*]
	$ZnSO_4$ .7 H_2O	sandy loam G) [2]	7.2	10	13	-	6-w	$NOEC_{y(s)}$	≥250 ≥328	(Cn) (Cn + Cb)	[*]
	$ZnSO_4$ .7 H_2O	sandy loam (H) [with P]	5.0	3	16	-	6-w	$NOEC_{y(s)}$	≥250 ≥303	(Cn) (Cn + Cb)	[*]
	$ZnSO_4$ .7 H_2O	sandy loam (I) [2]	6.8	3	16	-	6-w	$NOEC_{y(s)}$	≥250 ≥302	(Cn) (Cn + Cb)	[*]
	$ZnSO_4$ .7 H_2O	sandy loam (J) [without P]	7.5	3	16	-	6-w	$NOEC_{y(s)}$	≥250 ≥301	(Cn) (Cn + Cb)	[*]
	$ZnSO_4$ .7 H_2O	sandy loam (J) [with P]	6.7	3	16	-	6-w	$NOEC_{y(s)}$	10 61	(Cn) (Cn + Cb)	[1c]
								MacLean, 1974 [1,2]			

(to be continued)

Table 3.3.3.d Chronic toxicity of zinc to soil plants: NOEC and EC values

(continued) Part II: Studies not useful for PNECadd, terrestrial derivation

Organism	Test-comp	Substrate (soil type)	pH	OM %	Clay %	Temp. °C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)		
Lactuca sativa (lettuce)	$ZnSO_4$ .7 H_2O	sandy loam (A) [2]	7.5	4	16	-	5-w	$NOEC_{y(s)}$	≥250	(Cn)	[*]
									≥329	(Cn + Cb)	
	$ZnSO_4$ .7 H_2O	sandy loam (B) [2]	7.4	4	16	-	5-w	$NOEC_{y(s)}$	≥250	(Cn)	[*]
									≥330	(Cn + Cb)	
	$ZnSO_4$ .7 H_2O	sandy loam (C) [2]	7.4	8	16	-	5-w	$NOEC_{y(s)}$	≥250	(Cn)	[*]
									≥327	(Cn + Cb)	
	$ZnSO_4$ .7 H_2O	sandy loam (D) [2]	7.3	7	16	-	5-w	$NOEC_{y(s)}$	≥250	(Cn)	[*]
									≥327	(Cn + Cb)	
	$ZnSO_4$ .7 H_2O	mixed loam (E) [2]	7.4	3	23	-	5-w	$NOEC_{y(s}$	≥250	(Cn)	[*]
									≥356	(Cn + Cb)	
	$ZnSO_4$ .7 H_2O	sandy loam (F) [2]	7.5	3	14	-	5-w	$NOEC_{y(s)}$	≥250	(Cn)	[*]
									≥322	(Cn + Cb)	
	$ZnSO_4$ .7 H_2O	sandy loam (G) [2]	7.2	10	13	-	5-w	$NOEC_{y(s)}$	≥250	(Cn)	[*]
									≥328	(Cn + Cb)	
	$ZnSO_4$ .7 H_2O	sandy loam (H) [2]	4.9	3	16	-	5-w	$NOEC_{y(s)}$	10	(Cn)	[1d]
									63	(Cn + Cb)	
	$ZnSO_4$ .7 H_2O	sandy loam (I) [2]	6.8	3	16	-	5-w	$NOEC_{y()}$	≥250	(Cn)	[*]
									≥302	(Cn + Cb)	
	$ZnSO_4$ .7 H_2O	sandy loam (J) [2]	7.5	3	16	-	5-w	$NOEC_{y(s)}$	≥250	(Cn)	[*]
									≥301	(Cn + Cb)	
								MacLean, 1974 [1,2]			
Avena sativa (oat)	$Zn(Ac)_2$	loamy soil (c3)	5.2	3	58	-	5-m	$NOEC_{y(gr)}$	≥800	(Cn)	[*]
									≥936	(Cn + Cb)	
	$Zn(Ac)_2$	sandy loam (s3)	4.6	19	4	-	5-m	$NOEC_{y(gr)}$	≥800	(Cn)	[*]
									≥824	(Cn + Cb)	
(EU soils)								De Haan et al., 1985 [3]			
Phaseolus vulgaris (field bean)	$Zn(NO_3)_2$. .6H_2O	silt loam	7.0	-	-	26-30	-	$NOEC_{y(p)}$	≥500	(Cn)	[*]
Phaseolus vulgaris (snap bean)	$Zn(NO_3)_2$. .6H_2O	silt loam	7.0	-	-	26-30	-	$NOEC_{y(p)}$	≥500	(Cn	[*]
								Boawn & Rasmussen, 1971 [8]			
Pisum sativum (Perfection pea)	$Zn(NO_3)_2$. .6H_2O	silt loam	7.0	-	-	26-30	-	$NOEC_{y(p)}$	≥500	(Cn)	[*]
								Boawn & Rasmussen, 1971 [8]			
Solanum tuberosum (russet potato)	$Zn(NO_3)_2$. .6H_2O	silt loam	7.0	-	-	26-30	-	$NOEC_{y(p)}$	≥500	(Cn)	[*]
Solanum tuberosum **?** (white rose potato)	$Zn(NO_3)_2$. .6H_2O	silt loam	7.0	-	-	26-30	-	$NOEC_{y(p)}$	≥500	(Cn)	[*]
								Boawn & Rasmussen, 1971 [8]			

(to be continued)

Table 3.3.3.d Chronic toxicity of zinc to soil plants: NOEC and EC values (continued) Part II: Studies not useful for PNECadd, terrestrial derivation

Organism	Test-comp	Substrate (soil type)	pH	OM %	Clay %	Temp. °C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)	
Trifolium pratense (clover)	$Zn(NO_3)_2.6H_2O$	silt loam	7.0	-	-	26-30	84-d	$NOEC_{y(p)}$	≥500	(Cn) [*]
								Boawn & Rasmussen, 1971 [8]		
Trifolium pratense (red clover) seeds Test 1995-II	$ZnCl_2$	sand (PANH)	5.3	2	2	20	25-d	$NOEC_{germ}$	320	Cn)
								$EC50_{y(s)}$	145	(Cn)
								$EC50_{y(r)}$	146	(Cn)
								Hooftman & Henzen, 1996 [9, 12, 12b]		
Trifolium pratense (red clover) seeds Test 1995-III	$ZnCl_2$	sand (PANH)	5.3	2	2	20	25-d	$NOEC_{germ}$	320	Cn)
								$EC50_{y(s)}$	167	(Cn)
								$EC50_{y(r)}$	165	(Cn)
								Hooftman & Henzen, 1996 [9, 12, 12b]		
Trifolium pratense (red clover) seeds Test 1995	$ZnCl_2$	sand (PANH-aged)	5.3	2	2	20	25-d	$NOEC_{y(r)}$	180	(Cn)
									190	(actual)
								$NOEC_{y(s)}$	320 (Cn)	
									322	(actual)
								$NOEC_{germ.}$	≥1,000	(Cn)
									815	(actual)
								$EC50_{y(s)}$	711	(Cn)
									610	(actual)
								$EC50_{y(r)}$	516	(Cn)
								$EC50_{y(r)}$	467	(actual)
								Hooftman & Henzen, 1966b [9, 12, 12c] Posthuma et al., 1998		
Trifolium pratense (red clover) seeds Test 1996	$ZnCl_2$	sand (PANH-aged)	5.3	2	2	20	25-d	$NOEC_{y(s)}$	320	(Cn)
									320	(actual)
								$NOEC_{germ.}$	≥1,000	(Cn)
									858	(actual)
								$EC50_{y(s)}$	1,050	(Cn)
									890	(actual)
								Hooftman & Henzen, '96 [9, 12, 12c] Posthuma et al., 1998		
Triticum aestivum (wheat) seeds	$ZnCl_2$ **1**	loamy sand (Gudow; Cb 7 mg/kg)	3.0	9	7	20	21-d	$NOEC_{y(s)}$	60	(Cn)
									67	(Cn+Cb)
								$EC10_{y(s)}$	9	(Cn)
									16	(Cn + Cb)
								$EC50_{y(s)}$	61	(Cn)
									68	(Cn + Cb)
								Smolders et al., 2003 [13]		
Triticum aestivum (wheat) seeds	$ZnCl_2$ **3 ***	loamy sand (Houthalen, Cb 8 mg/kg)	3.4	3	5	20	21-d	$NOEC_{y(s)}$	120 (Cn)	
									128	(Cn+Cb)
								$EC10_{y(s)}$	34	(Cn)
									42	(Cn + Cb)
								$EC50_{y(s)}$	48	(Cn)
									56	(Cn + Cb)
								Smolders et al., 2003 [13]		

(to be continued)

Table 3.3.3.d Chronic toxicity of zinc to soil plants: NOEC and EC values (continued) Part II: Studies not useful for PNECadd, terrestrial derivation

Organism	Test-comp	Substrate (soil type)	pH	OM %	Clay %	Temp. °C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)	
Triticum aestivum (wheat seeds	$ZnCl_2$ **5 ***	sandy clay loam (Zegveld, Cb 191mg/kg)	4.7	40	24	20	21-d	$NOEC_{y(s)}$	400	(Cn)
									591	(Cn+Cb)
								$EC10_{y(s)}$	101	(Cn)
									292	(Cn + Cb)
								$EC50_{y(s)}$	1,098	(Cn)
									1,289	(Cn + Cb)
								Smolders et al., 2003 [13]		
Triticum aestivum (wheat) seeds	$ZnCl_2$ **6 ***	?? (Rhydtalog, Cb 83 mg/kg)	4.8	13	-	20	20-d	$NOEC_{y(s)}$	257	(Cn)
									340	(Cn+Cb)
								$EC10_{y(s)}$	102	(Cn)
									185	(Cn + Cb)
								$EC50_{y(s)}$	631	(Cn)
									714	(Cn + Cb)
								Smolders et al., 2003 [13]		
Triticum aestivum (wheat) seeds	$ZnCl_2$ **8 ***	sandy clay (Souli I, Cb 37 mg/kg)	4.8	1	38	20	21-d	$NOEC_{y(s)}$	600	(Cn)
									637	(Cn+Cb)
								$EC10_{y(s)}$	586	(Cn)
									623	(Cn + Cb)
								$EC50_{y(s)}$	803	(Cn)
									840	(Cn + Cb)
								Smolders et al., 2003 [13]		
Triticum aestivum (wheat) seeds	$ZnCl_2$ **9 ***	sandy loam (Kövlinge II, Cb 26 mg/kg)	5.1	4	9	20	21-d	$NOEC_{y(s)}$	200	(Cn)
									226	(Cn+Cb)
								$EC10_{y(s)}$	226	(Cn)
									252	(Cn + Cb)
								$EC50_{y(s)}$	373	(Cn)
									399	(Cn + Cb)
								Smolders et al., 2003 [13]		
Triticum aestivum (wheat) seeds	$ZnCl_2$ **11 ***	?? (De Meern, Cb 155 mg/kg)	5.2	17	-	20	17-d	$NOEC_{y(s)}$	425	(Cn)
									580	(Cn+Cb)
								$EC10_{y(s)}$	256	(Cn)
									411	(Cn + Cb)
								$EC50_{y(s)}$	1,069	(Cn)
									1,224	(Cn + Cb)
								Smolders et al., 2003 [13]		
Triticum aestivum (wheat) seeds	$ZnCl_2$ **12 ***	clay (Aluminusa, Cb 53 mg/kg)	5.4	1	51	20	21-d	$NOEC_{y(s(}$	700	(Cn)
									753	(Cn+Cb)
								$EC10_{y(s)}$	385	(Cn)
									438	(Cn + Cb)
								$EC50_{y(s)}$	1,026	(Cn)
									1,079	(Cn + Cb)
								Smolders et al., 2003 [13]		
Triticum aestivum (wheat) seeds	$ZnCl_2$ **13 ***	?? (Zeveren, Cb 76 mg/kg)	5.7	6	-	20	21-d	$NOEC_{y(s)}$	-	
								$EC10_{y(s)}$	199	(Cn)
									275	(Cn+ Cb)
								$EC50_{y(s)}$	738	(Cn)
									814	(Cn + Cb)
								Smolders et al., 2003 [13]		
*										
Triticum aestivum (wheat) seeds	$ZnCl_2$ **14 ***	sandy clay loam (Woburn, Cb 99 mg/kg)	6.4	7	21	20	21-d	$NOEC_{y(s)}$	600	(Cn)
									699	(Cn+Cb)
								$EC10_{y(s)}$	-	
								$EC50_{y(s)}$	-	
								Smolders et al., 2003 [13]		

(to be continued)

Table 3.3.3.d Chronic toxicity of zinc to soil plants: NOEC and EC values (continued) Part II: Studies not useful for PNECadd, terrestrial derivation

Organism	Test-comp	Substrate (soil type)	pH	OM %	Clay %	Temp. °C	Exp.-time	Criterion	Result in test soil (mg Zn/kg d.w.)
Triticum aestivum (wheat) seeds	$ZnCl_2$ **15 ***	silt loam (Ter Munck, Cb 54 mg/kg)	6.8	2	15	20	21-d	$NOEC_{y(s)}$	600 (Cn) 654 (Cn+Cb)
								$EC10_{y(s)}$	450 (Cn) 504 (Cn + Cb)
								$EC50_{y(s)}$	659 (Cn) 713 (Cn + Cb)
								Smolders et al., 2003 [13]	
Triticum aestivum (wheat) seeds	$ZnCl_2$ **17 ***	silty clay loam (Rots, Cb 51mg/kg)	7.4	2	27	20	21-d	$NOEC_{y(s)}$	1,200 (Cn) 1,251 (Cn+Cb)
								$EC10_{y(s)}$	1,231 (Cn) 1,282 (Cn + Cb)
								$EC50_{y(s)}$	1,777 (Cn) 1,828 (Cn + Cb)
								Smolders et al., 2003 [13]	
Triticum aestivum (wheat) seeds	$ZnCl_2$ **18 ***	clay (Souli II, Cb 51 mg/kg)	7.4	4	46	20	21-d	$NOEC_{y(s)}$	1,200 (Cn) 1,251 (Cn+Cb)
								$EC10_{y(s)}$	711 (Cn) 762 (Cn + Cb)
								$EC50_{y(s)}$	1,813 (Cn) 1,864 (Cn + Cb)
								Smolders et al., 2003 [13]	
Triticum aestivum (wheats) seeds	$ZnCl_2$ **19 ***	silt loam (Marknesse, Cb 80 mg/kg)	7.5	2	26	20	21-d	$NOEC_{y(s)}$	1,000 (Cn) 1,080 (Cn+Cb)
								$EC10_{y(s)}$	945 (Cn) 1,025 (Cn + Cb)
								$EC50_{y(s)}$	1,230 (Cn) 1,310 (Cn + Cb)
								Smolders et al., 2003 [13]	
Triticum aestivum (wheat) seeds	$ZnCl_2$ **22 ***	loam (Guadalajara, Cb 27mg/kg)	7.5	1	25	20	21-d	$NOEC_{y(s)}$	150 (Cn) 177 (Cn+Cb)
								$EC10_{y(s)}$	150 (Cn) 177 (Cn + Cb)
								$EC50_{y(s)}$	538 (Cn) 565 (Cn + Cb)
								Smolders et al., 2003 [13]	

[*] Rejected, based on Quality criterion (unbounded NOEC).

For footnotes see next page; for further information see the "list of abbreviations Table 3.3.3.a to Table 3.3.3.d".

Abbreviations and footnotes Table 3.3.3.d

Ac = acetate

germ. = germination

y = yield

y(g) = yield based on weight of grains

y(l) = yield based on weight of leaves

y(p) = yield baed on weight of whole plants

y(r) = yield based on weight of roots

y(s) = yield based on weight of shoots

y(st) = yield based on weight of stems

Note that in most tests the yield is based on dry weight.

[1] Maclean (1974): Medicago sativa, Zea mays and Lactuca sativa

Statistics: p = 0.01 (only indicated in the text, not separately for the (no) effect data in the toxicity table in the publication). Test range 0-2-10-50-250 mg Zn/kg**.** Zinc was added to the soil samples 4 weeks after remoistening of air-dried soil samples. No data on the physical form of the added test compound, but the addition of zinc was followed by a 8-w pre-incuabation period in which 500 ppm P (phosphate, as $CaH(PO_4)_2.H_2O$; in one of the test series; see also footnote [2]) and fertiliser were added. After the pre-incubation period, corn was grown during a 6-w period, followed by lettuce for 5 weeks and two successive crops of alfalfa in a 16-w period.Yield measured by dry weight of the above-ground portion of the plants. for each test, the yield results (g dry matter/pot) were given for the controls and the two highest concentrations (50 and 250 mg/kg), not for the lowest two test concentrations (2 and 10 mg/kg). From the data in he text and in the table the NOEC values could be indicated.

Soils: non-EU soils; top soil (0-15 cm) samples from three locations in Canada (Grenville, Grandy and Bainsville). The pH values are the values after treatment with organic materials, clay, lime and phosphate, see below. Background zinc concentrations (Cb): 51 to 106 mg/kg.

There are also data reported on extractable Zn in soil. Liming of the acid (pH 4.9) Bainsville soil to about the neutral point reduced the amount of extractable zinc markedly.

Greenhouse tests were performed in the following soil samples:

(A): Grenville sandy loam, unmanured and unfertilized since 1921

(B): "A" plus 2.2.% (w/w) dry alfalfa;

(C): "A" plus 2.2% (w/w) dry muck;

(D): "A" plus 2.2% (w/w) dry peat;

(E): "A" mixed with an equal quantity of a clay loam;

(F): "A" treated with a total amount of 257 metric tonnes of farmyard manure and chemical fertilizer

containing a total of 584 kg N, 498 kg P, and 489 kg K/ha since 1909;

(G): Granby sandy loam unmanured and unfertilized since 1909;

(H): Bainsville fine sandy loam;

(I): "H" plus 2,000 ppm $CaCO_3$ (limed Bainsville soil);

(J): "H" plus 6,000 ppm $CaCO_3$ (limed Bainsville soil).

Tests were conducted in the above soil samples both with and without pretreatment of the soil with 500 ppm P (added as $CaH_4(PO_4)_2.H_2O$).

[1a] Test with *Zea mays* in soil H (without P)

Low NOEC (50 mg/kg) together with high separation factor (test range 0-2-10-50-250 mg/kg).

Because of the low reliability of this NOEC, an alternative NOEC of 83 mg/kg (NOEC = LOEC/3 = 250/3 = 83 mg/kg) was derived from this test. No EC10 can be calculated from the reported data: 5% stimulation at 50 mg/kg (NOEC) and 26% inhibition at 250 mg/kg (LOEC).

See RAR section 3.3.3.1 for general requirements and methods for alternative NOEC derivation in case the "real" NOEC is unreliable (Quality criterion).

[1b] Tests with *Medicago sativa* in soil H (without P and with P supplement, respectively)

Low NOEC value (50 mg/kg in both tests) together with high separation factor (test range 0-2-10-50-250 mg/kg).

No alternative NOEC can be derived from these two tests in unlimed Bainsville soil:

* Test in soil H without P: 6% inhibition at 50 mg/kg (NOEC) and 87% inhibition at 250 mg/kg (LOEC). No EC10 can be calculated from this test.

* Test in soil H with P: 13% inhibition at 50 mg/kg (LOEC) and 53% inhibition at 250 mg/kg (LOEC). The calculated EC10 is 40 mg/kg, which is below the real NOEC.

It is futher noted that the control yield of *Z. mays* in this acid soil (unlimed Bainsville soil) is considerably lower than that in the same Bainsville soil limed with 2,000 and 6,000 ppm of $CaCO_3$ (tests I and J) and also considerably lower than that in the other soils. Moreover, in a large number of plant tests performed in the Netherlands in the framework of the "validation" study (Posthuma et al., 1998) it was shown that some plant species, including species recommended in the OECD guidline 208 (Terrestrial Plants, Growth Test) show poor growth at pH values below 5. In this OECD guideline a pH value between 5 and 7.5 is recommended.

Tests rejected, based on Quality criterion (No reliable NOEC or EC10 can be derived).

[1c] Tests with *Zea mays* in soil J (with P supplement)

Low NOEC value (10 mg/kg) together with high separation factor (test range 0-2-10-50-250 mg/kg).

No alternative NOEC can be derived from this test (51% inhibition at 50 mg/kg (LOEC) and 36% inhibition at 250 mg/kg, thus no clear dose-effect relationship) and according to the study authors there is no explanation for this "anomaly" in the results; this test is the only test of the study in which effects of zinc were found in the limed Bainsville soil receiving either 2,000 or 6,000 ppm $CaCO_3$.

Test rejected, based on Quality criterion (No reliable NOEC or EC10 can be derived).

[1d] Tests with *Lactuca sativa* in soil H (without P and with P supplement, respectively)

Low NOEC value (10 mg/kg in both tests) together with high separation factor (test range 0-2-10-50-250 mg/kg). No alternative NOEC can be derived from these two tests in unlimed Bainsville soil:

* Test in soil without P: 48% inhibition at 50 mg/kg (LOEC) and 100% inhibition at 250 mg/kg. No EC10 can be calculated from this test.

* Test in soil H without P: 34% inhibition at 50 mg/kg (LOEC) and 100% inhibition at 250 mg/kg. No EC10 can be calculated from this test.

Tests rejected, based on Quality criterion (No reliable NOEC or EC10 can be derived).

[2] Maclean (1974): *Medicago sativa, Zea mays and Lactuca sativa*

Tests were conducted in the soil samples both with and without pretreatment of the soil with 500 ppm P (see also footnote [1]; both conditions resulted in the same NOEC.

[3] De Haan et al. (1985): *Avena sativa*

Statistics: Student-Newman-Keuls multiple range test; "p" not reported. pH = pH-KCl.

Test range 0-50-100-200-400-800 mg Zn/kg d.w. Yield parameters: grain and straw dry weight. Grain yield was more affected than straw yield. Yield depression at the highest test concentration was significantly correlated with soil CEC (r = 0.9).

Soils: EU soils, collected in the Netherlands.

[4] Luo & Rimmer (1995): *Hordeum vulgare*

Statistical data were reported but not in detail for each exposure level compared to the control. Test range: 0-10-100 mg/kg d.w. Test compound added to oven-dried soil as a powdered solid and the soil was amended wit a basal dose of fertiliser (1 g NH_4NO_3/kg and 1.8 g KH_2PO_4/kg). Deionized water was used to bring the soil to field capacity. pH = pH water. Pot experiments (in greenhouse). Zinc was also tested in combinations with Cu (0 and 50 mg/kg), Pb (0 and 100 mg/kg) and/or Cd (0 and 5 mg/kg); results of these metal mixture tests are not listed in the above Table. Yield (dry weight) of shoots and roots was slightly increased at exposure to 10 mg Zn/kg (added singly or in combinations with Cd and/or Cu + Pb), but decreased at exposure to 10 mg Zn/kg in combination with Cu. The addition of 100 mg Zn/kg alone resulted in a siginificant reduction of shoot yield (23% inhibition; inhibition of root yield was <10%), while 100 mg Zn/kg in combination with one or more of the other metals did not or hardly result in shoot yield reduction ..

Soil: EU soil; top soil (0-15 cm), obtained from a farm in England (Nothcumberland).

Soil analysis: the (0.05 M $CaCl_2$) extractable background zinc concentration, measured after cropping, was 0.9 mg/kg.

[4a] Low NOEC (10 mg/kg) together with high separation factor (test range 0-10-100 mg/kg). Because of the low reliability of this NOEC, an alternative NOEC of 33 mg/kg (NOEC = LOEC/3 ((23% inhibition at 100 mg/kg) = 100/3 = 33 mg/kg) was derived from this test (no EC10 can be calculated from the reported data).

See RAR section 3.3.3.1 for general requirements and methods for alternative NOEC derivation in case the “real” NOEC is unreliable (Quality criterion).

[5] Aery & Jagetiya (1997): *Hordeum vulgare*

Statistics: p = 0.05 and 0.01. Test range: 0-10-50-250-1,250-6,250 mg/kg d.w. Test compound added to air-dried soil (test compound added as a solid or in solution: not reported; probably: solid) and the soil was amended with a basal dose of fertiliser (N, P and K at 60, 50 and 60 mg/kg, respectively). Pot experiments. Yield parameters: shoot and root dry weigt (dry matter/plant). The EC10 values (loading rate to produce 10% yield reduction) were reported by the study authors.

Soil: non-EU soil collected in India.

Soil analysis: DTPA (diethylenetriaminepentacetic) extractable Zn was measured after cropping.

[5] Low NOEC (50 mg/kg) together with high separation factor (test range 0-10-50-250-1250-6250 mg/kg). Because of the low reliability of this NOEC, an alternative NOEC of 215 mg/kg (NOEC = EC10) was derived from this test. See RAR section 3.3.3.1 for general requirements and methods for alternative NOEC derivation in case the "real" NOEC is unreliable (Quality criterion).

[6] Dang et al. (1990): *Allium cepa and Trigonella poenumgraceum*

Statistics: p = 0.05 and 0.01. Test range: 0-50-100-200-400 mg/kg. Test compound added to air-dried soil (test compound added as a solid or in solution: not reported; probably: solid) and the soil was amended with a basal dose of fertilizer (N, P and K at 60, 50 and 60 mg/kg, respectively, followed by top dressing of N at 25 mg/kg after 20 days. Pot experiment (in greenhouse). Yield parameters: fresh and dry weights of whole plants (yield/pot). The NOEC of 200 mg/kg for both *A. cepa* (onion) and *T. poenumgraceum* (fenugreek) listed in the table are based on the dry weight yields (at p = 0.05). The NOEC based on wet weight yield is 50 mg/kg for both onion and fenugreek. The NOEC value based on dry weight was selected in conformity with almost all other plant studies listed in Table 3.3.3.d, in which only dry weight yields were reported. Moreover, the fresh weight yields of the plant may have been influenced by watering and washing of the plants. The study authors reported an EC10 value (loading rate to produce 10% yield reduction) of 180 mg/kg for onion and 100 mg/kg fenugreeek; it is not clear if the EC10 values are based on fresh or dry weight yields (most probably based on the former) or based on the combined data.

Soil: non-EU soil collected in IndiaSoil analysis: (DTPA (diethylenetriaminepentacetic) extractable Zn was measured after cropping.

[7] Kalyanaraman & Sivagurunathan (1993): *Vigna mungo*

Statistics: p = 0.05 and 0.01. Test range: 0-50-100-150-250 mg/kg. Test compound added to air-dried soil (test compound added as a solid or in solution: not reported; probably in solution, since the test compound was added to air-dried soil). Soil type and characteristics (except pH) not reported. Pot experiment (in greenhouse). Yield parameters dry weight of roots, stem and leaves. The NOEC of 100 mg/kg is based on the yields of roots and stem; the NOEC for the yield of leaves was 150 mg/kg. The EC10 values (loading rate to produce 10% yield reduction) were reported by the study authors.

Soil: non-EC soil collected in India.

Soil analysis: DTPA (diethylenetriaminepentacetic) extractable Zn was measured after cropping.

[8] Boawn & Rasmussen (1971): *Eighteen species (15 field crop and 3 vegetable crop species)*

Statistics: p = 0.05. Test range: 10-100-200-300-400-500 mg/kg. The lowest test concentration (10 mg added-Zn/kg served as control) Test compound (no data on the physical form) was added to dried soil and the soil was amended with fertilizer (P and K at 200 and 100 mg/kg, added as $Ca(H_2PO_4)_2.2H_2O$ and K_2SO_4, respectively. Appropriate amounts of $Ca(NO_3)_2.4H_2O$ were applied to compensate for N added by the addition of the test compound. Pot experiments (in growth chamber), in which each of the 18 plant species (15 field crop and 3 vegetable crop species) was grown in freshly treated soil. Dark period temperature 21 ^oC (all species); photoperiod temperature 26 to 30 ^oC (depending on the species grown). Exposure period: peas, beans, potatoes, tomatoes, and spinach were grown to bud stage. Yield: dry weight of whole plants. No data reported on the life stage (seed/bean or seedling) at start of the test. Test results: reported very briefly, but for each test the percent yield decrease (compared to the 10 mg/kg control) is given for each test concentration, as well as a statistical analysis (yield decrease for significance at the p = 0.05 level).

Soil: non-EU soill; top soil (15-30 cm) samples assumed to be collected in Washington State in the U.S. (based on the location of the research institute). Reported soil characteristics: pH value (also measured after cropping): 7.5 at 10 mg/kg, decreasing to 7.0 at the highest three test concentrations. With respect to the background zinc level it was reported that the sampled 15-30 cm soil layer is "extremely low in extractable zinc".

Soil analysis: Both 0.1 N HCL extractable Zn and DTPA extractable Zn were measured after cropping. The 0.1 N HCL extractable zinc concentrations were 11, 87, 188, 280, 360 and 450 mg/kg, at nominal Zn concentrations of 10, 100, 200 300, 400 and 500 mg/kg. DTPA-extractable Zn concentrations were 5, 46, 88, 146, 195 and 246 mg/kg.

[9] Van den Hoeven & Henzen (1994a, 1994b, 1994c), Hooftman & Henzen (1996) and Posthuma et al. (1998):

Trifolium pratense and *Vicia sativa*

Tests performed in the framework of the Dutch research project "Validation of toxicity data and risk limits for soils" (see also RAR sections 3.3.3.1.1 and 3.3.3.1.4). The final summarising report of the whole project is published by Posthuma et al. (1998).

All yield toxicity values from these studies are based on wet weight yield (toxicity data for dry weight yield not reported). All tests were performed according to OECD guideline 208 (Terrestrial Plants, Growth test) and started with seeds.

[10] Van der Hoeven & Henzen (1994a, 1994c): *Trifollium pratense*

Two tests in freshly spiked OECD soil, with diffent ranges of test concentrations.

[11] Van der Hoeven & Henzen (1994b]: *Trifollium pratense* and *Vicia sativa*

Tests in freshly spiked Budel soil (spiked in the laboratory). The pH of this acid soil (native pH 3.3) was adjusted to pH 5.0).

Soil: EU-soil, collected in The Netherlands.

[12] Van den Hoeven & Henzen (1994b, 1994c), Hoofdman & Henzen (1996): *Trifolium pratense*

Tests in freshly spiked Panheel soil (PAHN: spiked in the laboratory), freshly spiked field-collected Panheel soil (PANH, spiked in the field plot) and aged field-collected Panheel soil (PAHN-aged).

Soil: EU-soil, collected in the Netherlands.

[12a] Tests performed in freshly-spiked Panheel soil (Tests 1994: soil spiked in the laboratory) and freshly spiked field-collected Panheel soil (Test 1995-I.a and Test 1995-I.b: soil spiked in the field plot).

[12b] Tests performed in field-collected Panheel soil, starting 8 weeks (Test 1995-II) and 19 weeks (Test 1995-III) after spiking of the soil (spiked in the field plot).

Rejected, as only the NOEC for germination was reported, while the other tests with *T. pratense* showed that yield of roots and shoots is a more sensitive toxicological endpoint for *T. pratense* (Relevance criterion).

[12c] Tests performed in aged field-collected Panheel soil (PAHN-aged), starting around 1 year (Test 1995) and

2 years (Test 1996) after spiking of the soil (spiked in the field plot). In the first test, visible Zn effects on shoots (chlorosis and brown leaves with black spots) were observed at 180 mg/kg and higher concentrations.

Rejected, based on Relevance criterion (tests in aged soils).

[13] Smolders et al. (2003): *Triticum aestivum* **

The study was performed i) to study the effects of abiotic factors on the toxicity of zinc in freshly-spiked soils to soil microbial processes (see Table 3.3.3.a) and plants (see Table 3.3.3.d), and ii) to study the difference in zinc toxicity in freshly-spiked soils and field-polluted soils.

Statistics used for NOEC derivation: ANOVA (Duncan test), $p < 0.05$. Each test included 7 treatments (control and 6 zinc concentrations, chosen on the basis of the expected sensitivity of the soil). The lowest Zn treatments were used in soills 1 and 3, viz. 0-15-30-60-120-240-720 mg/kg dw. The air-dried soils were mixed with deionised water and aqueous solutions of $ZnCl_2$. The seeds were pre-germinated on moist paper towels for one day and then tranferred to the soil (per treatment: two replicates of 2 seeds). After one week, the plants were thinned to one per pot, leaving one 2 plants per treatment. Test duration: 21 days. Growth based on dry weight of shoots.

The actual total-Zn concentrations in soil were determined in all soils, using boiling *aqua regia* digestion (to measure the background Zn concentration in all control soils and the total-Zn concentrations at one dose level per soil) and cold HNO_3 extraction (to measure the total-Zn concentrations at all treatments, except the control treatments). Soil total-Zn concentrations determined by cold HNO_3 extraction were in good agreement with those determined by boiling *aqua regia* digestion except in calcareous soils, in with the HNO_3 extraction method resulted in 2- to 12-fold lower total-Zn concentrations than the *aqua regia* digestion method. The nominal Zn concentrations were confirmed by the actual concentrations determined with the HNO_3 method in the non-calcareous soil (all dose levels) and with the *aqua regia* method in the 4 calcareous soils (one dose level per soil). Since Zn application was performed consecutively in each soil using the same stock solution, Smolders et al. (2003) considered it appropiate to report the toxicity values (NOEC, EC10 and EC50 values) as nominal concentrations. This is considered acceptable. The EC10 values were calculated with the logistic response model from Doelman & Haanstra (1989). This is the same model as published earlier by Haanstra et al. (1985) and this model is also used by the rapporteur for the calculation of EC10 values.

The zinc concentrations in the soil solutions (pore water) were also measured.

Soils: 15 uncontaminated EU soils, top soil (plough layer in cultivated soils and 0-20 cm layer in undisturbed soils) collected from arable land or non-arable land (forest, woodland, heath land, grassland, olive orchard) all over Europe. The uncontamined soils were selected to cover the relevant ranges of abiotic factors influencing Zn bioavailability in soils, including pH and cation exchange capacity. Background Zn concentrations: 7 to 191 mg/kg d.w. pH = pH $CaCl_2$. The Rhydtalog, De Meern and Zeveren soil are the uncontaminated reference soils of the field-polluted transect soils with Zn contamination due to corrosion of galvanized pylons.

Results: In the control soils, the plant growth was highly variable (shoot dry weights: 0.011 to 0.77 g/plant, thus differing up to a factor of 70), with very poor control growth in Gudow soil (pH 3.0) and Houthalen soil (pH 3.4), the soils with the lowest pH values. In some other soils there was also a poor control growth, but in these soils the growth appears to be less dependent on pH. The poor dose-response relationship in some tests, due to the poor control growth, resulted in some unreliable toxicity values, especially regarding the NOEC and EC10 values. **Most importantly, however, the number of plants per concentration was only 2 (two replicates with one plant each). According to OECD 208 (terrestial plants, growth test), a minimum number of 20 seeds per concentration (four replicates with five seeds each) per concentration should be used. Although not all seeds may germinate, the number of plants per concentration used in the study by Smolders et al. (2003) is considered to be much too**

small, which may explain the poor dose-relationships in some tests. Based on this, all tests from this study are rejected for PNEC derivation (Quality criterion).

It is noted that the test protocol of the study was approved by the rapporteur and the Steering group for the 'Conclusion (i)' program. However, in the test protocol a number of 3 replicates/soil was mentioned, but it was not reported (and thus not clear to the rapporteur and Steering group) that each replicate would only contain one plant.

In Smolders et al. (2003), the toxicity values (NOEC, EC10 and E50 values) were also reported in terms of soil solution Zn concentration.

See further RAR section 3.3.3.1.1 for the results of this study with respect to the influence of abiotic soil factors on the toxicity of zinc.

* Soil No. in study (see Table 3.3.3.a)

** Further information provided by the authors of the study in addition to the study report (Smolders et al., 2003) has been included in the evaluation of the study. The further information included the raw data for each test, i.e. the results (mean and SD) for growth at each test concentration, expressed as added-Zn (Cn) and total-Zn (Cn + Cb).

List of abbreviations Table 3.3.3.a to Table 3.3.3.d

Exposure time:	d: day(s); h: hour(s); w: week(s); m: month(s); yr: year(s).
Soil type	OECD artificial soil: 10% sphagnum peat, 20% kaolin clay and 70% fine sand, on a dry weight
or substrate:	basis;calcium carbonate is added to adjust pH to 6.0 ± 0.5 (OECD Guideline 207: Earthworm, Acute Toxicity Test)
	The background zinc concentration (Cb) in OECD artifial soil depends on the zinc level of the constituents used to prepare the soil. For example, background Zn concentratoins of 2 mg/kg and 14 mgkg OECD soils were reported in the studies by Smit & Van Gestel (1998) and Van Gestel & Hensbergen (1997).
	OM: organic matter. In a number of cases the %OM was calculated from the organic carbon content reported, as follows: %OM = % organic carbon x 1.7, according to Denneman and Van Gestel (1990).
OM and clay content:	"e": Actual value not reported, but calculated or estimated from the data reported, using the following assumptions and equations (unless stated otherwise):
	* CEC (cation-exchange-capacity, in meq/100 g) = (2.5 x %OM) + (0.5 x %clay), according to
	Doelman and Haanstra (1983)
	* Food/feed such as vegetables and fruits = 95% organic matter; manure = 50% organic matter
	Unless stated otherwise, the soil characteristics pH (preferably: pH KCl), %OM and/or %Clay indicated in the tables are the control values in the soil, i.e. the intitial values measured before treatment of the soils (see the footnotes for additional information, e.g. on pH changes due to treatment). These characteristics, including the pH , usually refer to the native soil. In some plant toxicity tests, however, the soil samples were limed and tested at different pH values. In those cases the pH value listed in the table is the value during the test.
	The temperature values indicated in the tables is the value during the test.
Criterion	LC50: Median lethal concentration, i.e. the concentration which is calculated from a series of test concentrations to cause mortality in 50% of the organisms exposed to that concentration.
	EC50: Median effect concentration, i.e. the concentration which is calculated from a series of test concentrations to cause a particular response in 50% of the organisms exposed to that concentration.
	EC(..%): At the concentration indicated (usually the only concentration tested), the toxicological endpoint was inhibited by ..%. Example: EC (21%).

NOEC: No observed effect concentration, i.e the highest concentration (in a series of test concentrations) without effect.

If a statistical analysis of the toxicity data was reported, the NOEC is the highest concentration showing no statistically significant (at $p < 0.05$) effect compared to the control.

If no statistical analysis of the data was reported, the NOEC is the highest concentration showing less than 10% effect compared to the control.

In subscript the toxicological endpoint or endpoints are indicated at each NOEC (e.g. $NOEC_g$ is NOEC for growth; $NOEC_{r,s}$ is NOEC for reproduction and survival).

In a number of cases, EC10 values have been used as NOEC values or the NOEC has been estimated fom the LOEC (lowest observed effect concentration) in case the "real" NOEC could not be derived directly from the data reported.

The following application factors have been used to derive a NOEC from a LOEC::

- in case the LOEC resulted in 11% to 20% effect: factor of 2;

- in case the LOEC resulted in 21% to 30% effect: factor of 3.

"Species mean" NOEC: In case several NOEC values (from different tests) are available for a certain species, the NOEC values **printed bold** and underlined have been used to calculate a geometric mean NOEC (for the most sensitive endpoint).

See next page for further explanation of the derivation of NOEC values.

The individual NOEC values that are **printed bold** and underlined have been used as input data in the ecotoxicological extrapolation methods used to derive the $PNEC_{add, terrestrial}$.

NOEC and EC values:

≥ Unbounded NOEC. i.e. no effect was found at the highest concentration used in the test (thus the real NOEC may be higher).

Cn Nominal zinc concentration in test soil.

Cb Background zinc concentration in test soil.

Cn + Cb Nominal zinc concentration (Cn) plus background zinc concentration (Cb; derived or calculated from the data reported).

See next page for data on the selection, derivation and reliability of (chronic) NOEC values.. For additional data on the selection of the chronic NOEC values, based on reliability and relevance criteria, see RAR Zinc Metal section 3.3.1.1 (sources and selection of ecotoxicological data) and section 3.3.3.1 (Toxicity to terrrestrial organisms).

Selection of chronic NOEC values (RAR Zn Metal section 3.3.1.1)
For the selection of chronic NOEC values used to derive $PNEC_{(add)}$ values, the following approach has been taken:

- Toxicological endpoints, which may affect the species at the population level, are taken into account. In general, these endpoints are survival, growth and reproduction. The toxicity results are commonly expressed as an acute LC50 or EC50 (usually derived from toxicity tests with a duration of four days or less) or as a chronic NOEC (usually derived from toxicity tests with a duration of more than four days). With respect to the NOEC values it is noted that the fact whether or not a NOEC is considered a chronic NOEC is not determined exclusively by the above exposure time limit of four days, but also by the generation time of the test species. It will be clear that for PNEC derivation a full life-cycle test, in which all relevant toxicological endpoints are studied, is normally preferred to a test covering not a full life cycle and/or not all relevant endpoints. However, the results of a test, which is more limited than a full life-cycle test may be used, see further the points below.
- If for one species several chronic NOEC values (from different tests) based on the same toxicological endpoint are available, these values are averaged by calculating the geometric mean, resulting in the "species mean" NOEC. With respect to this it is noted that the NOEC values should be from equivalent tests, for example from tests with similar exposure times. However, NOEC values derived from tests with a relatively short exposure time may be used together with NOEC values derived from tests with a longer exposure time if the data indicate that a sensitive life stage was tested in the former tests.
- If for one species several chronic NOEC values based on different toxicological endpoints are available; the lowest value is selected. The lowest value is determined on the basis of the geometric mean if more than one value for the same endpoint is available (see above).
- In some cases, NOEC values for different life stages of a specific organism are available. If from these data it becomes evident that a distinct life stage is more sensitive, the result for the most sensitive life stage is selected. The life stage of the organisms is indicated in the tables as the life stage at start of the test (e.g. earthworms juvenis or plants seeds).

Note that all NOEC values derived from microbial tests (endpoints: microbe-mediated soil processes such as respiration) have been used for $PNEC_{add,\ terrestrial}$ derivation, regardless of the exposure time.

Derivation of NOEC values (RAR Zn Metal section 3.3.1.2)
The methods that have been used for the derivation of NOEC values, being "real" NOEC values or NOEC values derived from effect concentrations, are essentially the same as outlined in the EU TGD (Part II, Chapter 3, Table 15)(EC, 2003) .

If possible, "real" NOEC values were derived from the data reported, i.e. the NOEC is one of the concentrations actually used in the test. In order of preference:

1) Statistical analysis: the NOEC is the highest concentration (in a series of test concentrations) showing no statistical significant effect (inhibition) compared to the control. Significance level: $p = 0.05$ (optional: the $p = 0.01$ level if reported instead of the $p = 0.05$ level).
2) If no statistical analysis has been applied: the NOEC is the highest concentration that results in $\leq$ 10% inhibition compared to the control.

In both cases there must be a consistent concentration-effect relationship, i.e the LOEC is the concentration at which and above which statistical significant toxicity is found (1) or, when no statistical analysis has been applied (2), >10% inhibition is found.
If the "real" NOEC could not be derived from the data reported, the following procedure was used to derive the NOEC. In order of preference:

1) The NOEC is set at the EC10 level.
   a) Especially in more recent references on ecotocological data there is increasing preference for the benchmark dose approach. Hence, a benchmark dose (usually the EC10) was reported in a number of references instead of the NOEC. The EC10, which is calculated from the concentration-effect relationship, is used as NOEC equivalent, unless the "real" NOEC was also reported or could be derived from the data reported.
   b) Furthermore, a number of EC10 values was calculated by the rapporteur; the EC10 values were derived from a logistic, sigmoidal dose response model according to Haanstra et al. (1985):

$$Y = c / \{1+\exp [b.(X – a)]\}$$

2) The NOEC is derived from the LOEC
   If the EC10 was not reported and could not be calculated, the NOEC was derived from the LOEC using the following "extrapolation" factors:
   a) NOEC = LOEC/2, in case inhibition is >10% but ≤20%, e.g. LOEC = EC(15%).
   b) NOEC = LOEC/3, in case inhibition is >20% but ≤30% e.g. LOEC = EC(25%).
   If the percentage inhibition at the LOEC is >30% or in case the percentage inhibition at the LOEC is unknown, no NOEC is derived.

With respect to "rule 2b" it is noted that the EU TGD does not mention the derivation of a NOEC from a LOEC in case inhibition at the LOEC is >20%, while in this RAR the derivation of a NOEC from a LOEC up to 30% effect has been used in some aquatic toxicity studies. The use of the higher effect level is justified by the use of a higher extrapolation factor.

Reliability of NOEC values (RAR Zn Metal section 3.3.3.2)
All NOEC values (including EC10 values) used for $PNEC_{add,\ terrestrail}$ derivation have been checked for reliability on the basis of the range of test concentrations, as follows:

- If the NOEC is <100 mg/kg, the separation factor between the NOEC and LOEC should not exceed a factor of 3.2.
- If the EC10 is used as NOEC equivalent, the EC10 should not be more than 3.2-times lower than the lowest concentration used in the test.

REFERENCES ANNEX 3.3

This list includes all references of the ecotoxicological studies summarized in Annex 3.3.2.A (Aquatic toxicity data base), Annex 3.3.2.B (Freshwater (model) ecosystem studies), Annex 3.3.2.C (Derivation of soft water $PNEC_{add, aquatic}$), Annex 3.3.2.D (Sediment toxicity data base) and Annex 3.3.3.A (Terrestrial toxicity data base). The list includes references of accepted and rejected studies and the references mentioned in the footnotes of some studies, for example on test methods.
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INDUSTRY ANNEX

ANNEX 3.4.3 Update of local exposure data for zinc metal producers and users

Disclaimer: The Industry annex 3.4.3. was found by the Rapporteur to be useful to risk management because it sheds further light on the recent local exposure data. Annex 3.4.3. has not been formally approved by either the Rapporteur or TC NES.

Table 1 Summary of the local production tonnages, emission rates and calculated C_{add} values.

Company name	Production	Emission	Emission	C_{add}	Concentr.	C_{add}	C_{add}
	tonnage	air	water	air	effluent STP (total)	water (dissolved)	Sediment
	(t/y)	(kg Zn/d)	(kg Zn/d)	(µg/m³)	(µg/l)	(µg/l)	(mg/kg$_{wwt}$)
1 Asturiana (2003)	461770	73.15	5.89	20.336	1057	39.87	953.46
20 Boliden Odda (2003)	146627	31.52	9.21	8.763	1100 (Measured)	25.94	620.41
21Boliden Kokkola (2003)	265853	31.52	0.70	8.763	56	1.33	31.84
22 Rezinal (2003)	28560	1.59	0.05	0.364	765	0.41	9.73
27 Umicore Overpelt (2004)	NOT PROVIDED						
27 total	47056	16.67	4.35	3.809	1942	18.98	453.94
28 Umicore Auby (2004)	274085	12.68	6.39	3.526	1936	5.54	132.56

Table 2. The local (PE)C_{add} values, $PNEC_{add}$ values and (PE)C_{add} / $PNEC_{add}$ ratios used in the local risk characterisation of zinc metal. The (PE)C_{add} values are not corrected for bioavailability.

Company	**PEC effluent STP (dissolved)**	**C_{add} water (dissolved)**	**C_{add} sediment**	**PEC_{add} agricultural soil**	**PEC/ PNEC STP**	**C_{add}/ $PNEC_{add}$ water**	**Cadd/ $PNEC_{add}$ sediment**	**PEC_{add}/ $PNEC_{add}$ agr. soil**
	(µg/l)	**(µg/l)**	**(mg/kgwwt)**	**(mg/kgdwt)**				
Production companies:								
Company 1 (2003)	246	39.87	953.46	9.37	0.47	5.11	119.18	0.32
Company 1: measured concentrations		170 µg/l;[48]				7.5		
Company 20 (2003)	256	25.94	620.41	4.36	0.49	3.33	77.55	0.15
Company 20: measured concentrations		6.1-11.3 µg/l, year 2003; different positions	near discharge point: 239-361 mg/kg dw; further away: 1620-1680 mg/kg dw; year 1996 data ! Further downstream in fjord: 653-944 mg/kg dw			0	2.7-6.0 14-22 40-42	
Company 21 (2003)	13	1.33	31.84	2.47	0.03	0.17	3.98	0.08
Company 21: measured concentrations		1.8 µg/l (10 km upstream from plant), year 2003	reference sampling site: 125-150 mg/kg dw (years 1987-			0.12 (=4.3-1.8 µg/l)	0.3 4.6-9.8	

[48] Production 1: Contribution of waste water from the plant only is considered, no other inputs considered (16.4% of total conc. In water)

Company	PEC effluent STP (dissolved)	C_{add} water (dissolved)	C_{add} sediment	PEC_{add} agricultural soil	PEC/ PNEC STP	C_{add}/ $PNEC_{add}$ water	Cadd/ $PNEC_{add}$ sediment	PEC_{add}/ $PNEC_{add}$ agr. soil
	(µg/l)	(µg/l)	(mg/kgwwt)	(mg/kgdwt)				
		- 4.3 µg/l (average of 18 sampling points downstream, year 2003)	1995) year 1999: downstream: different sampling points; 67; 310; 380; 500 mg/kg dw; at effluent discharge point: year 1999: 440 mg/kg dw					
COMPANY 22 (2003)	178	0.41	9.73	0.73	0.34	0.05	**1.22**	0.02
COMPANY 22: MEASURED CONCENTRATIONS (MEASURED DATA NOT FURTHER USED BECAUSE OF THE FOLLOWING REASONS; DOWNSTREAM VALUE IS MEASURED TOO FAR AWAY FROM DISCHARGE POINT (1.2 KM DOWNSTREAM). INFLUENCE OF OTHER POINT SOURCE IS VERY PROBABLE !! DOWNSTREAM RIVER SYSTEM NOT THE SAME AS UPSTREAM !		66 µg/l, year 2003, 200 m before effluent discharge point - 128 µg/l, year 2003, 1.2 km after effluent discharge point	290 mg/kg dw; year 2001, 200 m before discharge point 90.1 mg/kg dw (year 2003, at discharge point); 202 mg/kg dw (year 2003, further downstream)			-	**1.7-4.0**	
COMPANY 27								
COMPANY 27 TOTAL (2004)	452	18.98	453.94	2.22	0.87	**2.43**	**56.74**	0.07
COMPANY 27: MEASURED CONCENTRATIONS		334 µg/l; year 2004, entrance plant (sample 1), 666 µg/l, year 2004, before effluent	1030 mg/kg dw, upstream discharge point; 620 mg/kg dw;			**3.0** (=741-666)	**24-13**	

Company	PEC effluent STP (dissolved)	C_{add} water (dissolved)	C_{add} sediment	PEC_{add} agricultural soil	PEC/ PNEC STP	C_{add}/ $PNEC_{add}$ water	Cadd/ $PNEC_{add}$ sediment	PEC_{add}/ $PNEC_{add}$ agr. soil
	(µg/l)	(µg/l)	(mg/kgwwt)	(mg/kgdwt)				
		discharge point (sample 2) - 741 µg/l; year 2004; at effluent discharge point (sample 3)	downstream, year 2005					
COMPANY 28 (2004)	450	5.54	132.56	2.09	0.87	0.71	**16.57**	0.07
Company 28: measured concentrations		EURAS; year 2002: 43-70 µg/l, upstream from discharge point; EURAS: 149-164 µg/l, year 2002, downstream discharge[49]	EURAS, year 2002; 2240 mg/kg dw EURAS, year 2002; 8740 mg/kg dw			-	**57-232**	
GALVANISING:								
CHDG Company A	10	1.67	40	0.57	0.02	0.21	**5**	0.02
CHDG Company A: measured concentrations			180-155 mg/kg dw (1km - 50 m upstream; 0-3 cm depth; year ????) -				**0.4-1.1**	

49 Production 28: The significant reduction of zinc quantities released to the water system in 2003 and 2004 as compared to 2002; is due to the installation and operation of 1) a new surface water collecting system and treatment station, 2) A closed internal 'cooling waters' circuit. Therefore the monitoring data of the year 2002 are not considered to be representative for the current emission situation; and are not further used in the risk characterisation.

Company	PEC effluent STP (dissolved)	C_{add} water (dissolved)	C_{add} sediment	PEC_{add} agricultural soil	PEC/ PNEC STP	C_{add}/ $PNEC_{add}$ water	Cadd/ $PNEC_{add}$ sediment	PEC_{add}/ $PNEC_{add}$ agr. soil
	(µg/l)	(µg/l)	(mg/kgwwt)	(mg/kgdwt)				
			120; 155; 180 mg/kg dw (at, 200 m after; 500 m after discharge point; 0-3 cm depth)					
CHDG Company G1 and G2	314	0.51	12.18	0.57	0.60	0.07	**1.52**	0.02
EG Company G3	42	0.07	1.62	0.57	0.08	0.01	0.2	0.02
EG Company G3: measured concentrations		8 µg/l, 500 m upstream 6 µg/l; 8 km downstream not relevant for site				NA [50]		
ALLOY AND DIE CASTING:								
Alloy production: company 4	0.014	0.00003	0.000715	0.7	0	0.000004	0.000089	0.02
Alloy production: company 4: measured concentrations		34 µg/l after STP, also influence from other sources !! <1% contribution from site = 0.34 µg/l	110 mg/kg dw U 130 mg/kg dw D			1.0 [51]	0	

[50] EG G3: Value reported for downstream measurements 8 km downstream from discharge point, not relevant for this site !

51 Alloy 4: Site discharges to a municipal STP; contribution from the water emissions from the site to the total zinc load of the STP is <1%. Measured data refer to the surface water after the STP, hence in principle 1% of PEC should be used. Since measured data do not reflect reality, the risk characterisation should be performed on the basis of modelled data.

Company	PEC effluent STP (dissolved)	C_{add} water (dissolved)	C_{add} sediment	PEC_{add} agricultural soil	PEC/ PNEC STP	C_{add}/ $PNEC_{add}$ water	Cadd/ $PNEC_{add}$ sediment	PEC_{add}/ $PNEC_{add}$ agr. soil
	(µg/l)	(µg/l)	(mg/kgwwt)	(mg/kgdwt)				
ROLLED/WROUGHT ZINC:								
Rolled/wrought zinc: company 1	5	0.77	18.36	3.26	0.01	0.10	**2.30**	0.11
Rolled/wrought zinc: company 1: measured concentrations		<1 µg/l, year 2002, 100 m upstream of discharge point of STP 16 µg/l, year 2002, 100 m downstream of discharge point of STP	68 mg/kg dw; 100 m upstream of discharge point of STP - 54 mg/kg dw; 100 m downstream of discharge point of STP.			0- 0.2	0	
Rolled/wrought zinc: company 4	0.0016	0.000003	0.000083	0.57	0	0	0.00001	0.02

Please note that for most of the sites the sediment monitoring data reported reflect the zinc concentrations in sediment in years earlier than the emissions currently used. Eg. emissions data year 2003; monitoring data years 1996, 1999.

Table 3 Characteristics (DOC, hardness and pH) of local waters for which the $C_{local,\ add}$-$PEC_{add}/PNEC_{add}$ surface water exceeds one (without correction for bioavailability (see Table 2) (production and use of zinc metal). Corresponding bioavaialabilty factors ($BioF_{water}$) are calculated with Biotic Ligand Model for algae and fish. $BioF_{water}$s in bold represent the values that will be used in the risk characterisation for, respectively, average (50P DOC and 50P inorganics) and realistic worst case (algae 10P DOC and 90P inorganics and fish: 10P DOC and 10P inorganics) conditions. Both the uncorrected $C_{local,\ add}$-$PEC_{add}/PNEC_{add}$ and the corrected $C_{local,\ add}$-$PEC_{add}/PNEC_{add}$ (rwc and average) are presented. No bioavailability correction is performed for discharges to sea.

	Remark	DOC (mg/l)		pH			Hardness (CaCO3 mg/l)			BioF algae	BioF algae	BioF fish	BioF fish	PEC water 3)	Cadd/ PNECadd	Cadd/ PNECadd	Cadd/ PNECadd
		10P	50P	10P	50P	90P	10P	50P	90P	10-90	50-50	10-10	50-50	(µg/l)	uncorrected	r.w.c.	avg.
Production 1 (2003)	Sea														5.11	no correction	
	M Sea													170[52]	7.5	no correction	
Production 20 (2003)	Sea M Sea													 6.1-11.3	3.33 0.1	no correction no correction	
Production 21 (2003)	Sea														0.17	no correction	
	M Sea													1.8 U – 4.3 D	0.12	no correction	
Production 22 (2003)	Calc	4.4 1)	7.69	6.1 1)	6.84	7.1 1)	106.9	257	1400	0.5	0.6	**1**	**0.5**		0.05		
Production 27 t	Calc.	9.7 1)	15.3-18.2	6.5 1)	7.21-7.32	7.5 1)	46.7 1)	154-181	343 1)	0.3	0.1	**0.6**	**0.2**		2.43	1.46	0.49
Production 27 (2004)	M	9.7 1)	15.3-18.3	6.5 1)	7.21-7.33	7.5 1)	46.7 1)	154-182	343 1)	0.3	0.1	**0.6**	**0.2**	666 U-741 D	3.0	1.80	0.60
Production 27	M	9.3-9.7 U	8.2 D	7.1-7.2			106-115	U; 430 D									

[52] Production 1: Contribution of waste water from the plant only is considered, no other inputs considered (16.4% of total conc. In water)

	Remark	DOC (mg/l)		pH			Hardness (CaCO3 mg/l)			BioF algae	BioF algae	BioF fish	BioF fish	PEC water 3)	Cadd/ PNECadd	Cadd/ PNECadd	Cadd/ PNECadd
		10P	50P	10P	50P	90P	10P	50P	90P	10-90	50-50	10-10	50-50	(µg/l)	uncorrected	r.w.c.	avg.
Site-specific (2004)																	
Production 28 (2004)	Calc.	3.3 1)	5.7	7.1 1)	7.9	8.2 1)	42.8 1)	154	314 1)	0.7	**0.4**	**1**	0.4		0.71 (No risk)		
	M	3.3 1)	5.7	7.1 1)	7.9	8.2 1)	42.8 1)	154	314 1)	0.7	**0.4**	**1**	0.4	43-70 U53 149-164 D (year 2002 data , not representative)	-	-	-
CHDG A (Fin.)		5.8 1)	>10 5)	6.4	7.1	7.4	12.8	46	94	0.3	0.2	**0.9**	**0.6**		0.21		
CHDG G1 & G2		2.02	2.81	7.9	8	8.1	197	223	250	1	**0.7**	**1**	0.4		0.07		
EG G3	Calc.	2.02	2.81	7.9	8	8.1	197	223	250	1	**0.7**	**1**	0.4		0.01		
	M	2.02	2.81	7.9	8	8.1	197	223	250	1	**0.7**	**1**	0.4	NA54			
Alloy 4	Calc.														4x10-6		
Alloy 4	M	4.8 1)	7.75-8.99	7.2 1)	8.02-8.05	8.3 1)	61.5 1)	215-227	451 1)	0.5	**0.3**	**1**	0.3	34	1.0455	1.04	0.3

53 Production 28: The significant reduction of zinc quantities released to the water system in 2003 and 2004 as compared to 2002; is due to the installation and operation of 1) a new surface water collecting system and treatment station, 2) A closed internal 'cooling waters' circuit. Therefore the monitoring data of the year 2002 are not considered to be representative for the current emission situation; and are not further used in the risk characterisation.

54 EG G3: Value reported for downstream measurements 8 km downstream from discharge point, not relevant for this site !

55 Alloy 4: Site discharges to a municipal STP; contribution from the water emissions from the site to the total zinc load of the STP is <1%. Measured data refer to the surface water after the STP, hence in principle 1% of PEC should be used. Since measured data do not reflect reality, the risk characterisation should be performed on the basis of modelled data.

Table 4. Site-specific information on sediment SEM/AVS for a number of production and processing sites.

		AVS_{total}	**SEM Zn**	**SEM Zn, bioav.**	**AVS_{total}-Cb**	**RCR**
		µmol/gDW	**µmol/gDW**	**µmol/g.DW**		
Production 27		3.5-0.28	15.53-3.90	13.37-3.90	2.5—0.72	**23.4-6.8**
Production 28		179	81.2	-98	178	**-173 (=0)**
Alloy 4[56]		0.28-0.3	1.47-2.65	1.19-2.35	-0.72 - -0.74	**0.79-2.9**
Rolled zinc		0.13-0.76	1.01-1.08	0.32-0.88	-0.26—0.89	**-0.026-0.098 (= 0 – 0.098)**

56 Alloy 4: Site discharges to a municipal STP; contribution from the water emissions from the site to the total zinc load of the STP is <1%. Measured data refer to the sediment after the STP, hence in principle 1% of PEC should be used. Since measured data do not reflect reality, the risk characterisation should be performed on the basis of modelled data.

Table 5 Summary of the uncorrected and corrected local (PE)C_{add} / $PNEC_{add}$ ratios used in the local risk characterisation of zinc metal.

	Uncorrected				Corrected			
Company	**PEC/ PNEC STP**	**C_{add}/ $PNEC_{add}$ water**	**Cadd/ $PNEC_{add}$ sediment**	**PEC_{add}/ $PNEC_{add}$ agr. soil**	**C_{add}/ $PNEC_{add}$ water r.w.c.**	**C_{add}/ $PNEC_{add}$ water avg.**	**Cadd/ $PNEC_{add}$ sediment**	**PEC_{add}/ $PNEC_{add}$ agr. soil**
PRODUCTION COMPANIES:								
Company 1 (2003)	0.47	5.11	119.18	0.32	5.11		59.6	
Company 1: measured concentrations		7.5[57]			7.5			
Company 20 (2003)	not appl.	3.33	77.55	0.15	3.33		38.78	
Company 20: measured concentrations		0.1	2.7-6.0 14-22 40-42				1.3-3.0 7-11 20-21	
Company 21 (2003)	0.03	0.17	3.98	0.08			1.99	
Company 21: measured concentrations		0.12 (=4.3-1.8 µg/l)	0.3 4.6-9.8				2.3-4.9	
Company 22 (2003)	0.34	0.05	1.22	0.02			0.61	
Company 22: measured concentrations		-	1.7-4.0				0.85-2.0	
Company 27 [1)]								
Company 27 total (2004) [2)]	0.87	2.43	56.74	0.07	1.46	0.49	28.4	
Company 27: measured concentrations		3.0	24-13		1.60	0.60	23.4-6.8	
Company 28 (2004)	0.87	0.71	16.57	0.07			-173	
Company 28: measured concentrations		-[58]	57-232				-173	

57 Production 1: Contribution of waste water from the plant only is considered, no other inputs considered (16.4% of total conc. In water)

58 Production 28: The significant reduction of zinc quantities released to the water system in 2003 and 2004 as compared to 2002; is due to the installation and operation of 1) a new surface water collecting system and treatment station, 2) A closed internal 'cooling waters' circuit. Therefore the monitoring data of the year 2002 are not considered to be representative for the current emission situation; and are not further used in the risk characterisation.

	Uncorrected				Corrected			
Company	**PEC/ PNEC STP**	**C_{add}/ $PNEC_{add}$ water**	**Cadd/ $PNEC_{add}$ sediment**	**PEC_{add}/ $PNEC_{add}$ agr. soil**	**C_{add}/ $PNEC_{add}$ water r.w.c.**	**C_{add}/ $PNEC_{add}$ water avg.**	**Cadd/ $PNEC_{add}$ sediment**	**PEC_{add}/ $PNEC_{add}$ agr. soil**
GALVANISING:								
CHDG Company A	0.02	0.21	5	0.02			2.5	
CHDG Company A: measured concentrations			0.4-1.1				0.65	
CHDG Company G1 and G2	0.60	0.07	1.52	0.02			0.76	
EG Company G3	0.08	0.01	0.2	0.02				
EG Company G3: measured concentrations		NA [59]						
ALLOY AND DIE CASTING:								
Alloy production: company 4	0	4x10-6	8.9x10-5	0.02				
Alloy production: company 4: measured concentrations		1.04 [60]	0		1.04	0.3		
ROLLED/WROUGHT ZINC:								
Rolled/wrought zinc: company 1	0.01	0.10	**2.30**	0.11			**-0.026-0.098**	
Rolled/wrought zinc: company 1: measured concentrations		0-0.2	0				-0.026-0.098	
Rolled/wrought zinc: company 4	0	0	1x10-5	0.02				

59 EG G3: Value reported for downstream measurements 8 km downstream from discharge point, not relevant for this site !

60 Alloy 4: Site discharges to a municipal STP; contribution from the water emissions from the site to the total zinc load of the STP is <1%. Measured data refer to the surface water after the STP, hence in principle 1% of PEC should be used. Since measured data do not reflect reality, the risk characterisation should be performed on the basis of modelled data.

Annex 3.2.5 Refinement of the Exposure assessment and risk characterisation – regional aquatic compartment

Disclaimer: Industry Annex 3.2.5 is neither agreed to at TC-NES level nor accepted by the Rapporteur, but can be useful for the risk reduction phase. The annex is based on the contributions from and reflects the opinion of Industry to shed some further light on the possible sources of zinc and zinc metals that contribute to regional concentrations from monitoring studies.

INTRODUCTION

In the main text of the RAR an exposure assessment of the regional aquatic compartment is made using a variety of monitored data from several EU countries. These data were used by the Rapporteur without further critical analysis of their quality and relevance for the regional scenario. Also, the underlying reasons for observed elevated concentrations of zinc were not considered in detail.

The issue was last discussed at TC NES 1 '04. Since then, Industry has refined the regional exposure (section 2 of this annex), reflecting the guidance of the TGD that a further refinement of the PEC/PNEC is required until despite uncertainty, clear conclusions can be drawn.

The refinement of the regional exposure analysis includes:

- the application of criteria for the quality of the monitored data
- a consistent handling of the monitored data on a region basis
- an analysis of the relevance of the data for the regional scenario
- an analysis of possible underlying sources for the elevated zinc levels observed.

It is important to understand that, for this refinement, the same monitored data have been used as those already used in the draft RAR, version of December 2004. It is emphasised that no new data have been introduced into the Industry analysis.

The value of this further refinement of the exposure assessment by industry was recognised by the Rapporteur in the bilateral meeting of March 29, 2006. The Rapporteur, however, considered that the analysis could not be integrated in the exposure analysis of the draft RAR because the latter had been agreed at TC NES 1 '04. Instead, the Rapporteur invited Industry to prepare an analysis which will become an Annex to the RAR, because the Rapporteur recognises that it contains valuable information for risk management.

Refinement of the exposure assessment means that it is possible to refine the risk characterisation for the regional aquatic scenarios (section 3 of this annex). This refined risk characterisation results in a more precise conclusion about regional risks of zinc for the aquatic compartment and the underlying sources leading to those risks. This Annex contains the refinement of the risk characterization's initial conclusions found in the risk assessment report on zincs as called for by the TGD[61].

Industry understands the procedural reasons which prevent the refined exposure analysis and the more precise conclusions following from it becoming an integral part of the RAR but nevertheless believes that, as a refinement following the guidance of the TGD, it is of at least equal importance.

In the risk characterisation the Industry annex uses the PNECs for water and sediment as set in the main text of the RAR.

61 TGD section 5.2 and with special reference to Annexes VIII and XIII

1 EXPOSURE ASSESSMENT

1.1 PEC REGIONAL WATER

1.2 PECMODELLED, REGIONAL

The modelled PEC is based on an extensive inventory of emissions in the model region (The Netherlands). This inventory is very detailed and all emissions are updated. As such, the modelled PEC reflects the current exposure in the model region with a high degree of certainty, and should thus be used for risk characterisation. The modelled PEC is for the NL Region: 12.2 µg Zn/l total (see main text of the RAR).

The model region The Netherlands can in terms of density of population, industrial and agricultural activity and zinc use, be considered as a realistic worst case for the EU. The additional information contained in the RAR on the emissions observed in other EU regions confirms the NL analysis and its relevancy as a realistic worst case for the EU.

1.3 DERIVATION OF A PEC FROM MONITORING DATA

In this section measured zinc concentrations in EU waters will be presented and evaluated for their usefulness for risk characterisation. The zinc risk assessment compiles a wealth of monitoring data on zinc in EU waters. Given the high number of available monitored data, only good quality will be used for the risk characterisation following specific guidelines for quality and data handling and manipulation.

1.3.1 Criteria for good quality

Firstly, to ensure good quality data, a strict selection is made using the following criteria:

- *General quality*: as a rule, all reported data were assumed to be of sufficient analytical quality, identified obvious errors were rejected
- *Detection limit (DL)*: a rather broad range of detection limits is observed through the zinc monitoring databases (1-70 µg/l). In several cases, a high detection limit for zinc in water may erroneously suggest that zinc levels are higher than they are in reality. It is proposed to consider in this respect the PNEC, i.e. databases with DL > PNEC should not be used for risk characterisation. Depending on the suspended matter concentration, a total zinc concentration of 25 µg/l can correspond to the PNEC. For this reason, a DL of 25 µg Zn/l is considered as the maximum DL to be used for risk characterisation. Datasets with a DL > 25 µg Zn/l (e.g. 50 µg/l) are not used because they increase too much the uncertainty of the assessment.
- *Time of sampling*: as a rule, the most recent data reported for a given region are used for the risk characterization (>1995). Data before 1995 may be referred to for information on trends in zinc concentrations, but are not generally used for risk characterization.
- *Reported zinc measurements*: If available, the dissolved monitored concentrations were preferred over total concentrations in order to reduce the uncertainty related to the total concentration and the use of (often default) suspended matter values.

1.3.2 Data handling

Secondly, in order to ensure a proper and consistent evaluation of the various monitoring databases, the manipulation of the data was done following a set of guidelines. These guidelines deal with the following issues:

- the handling of data below DL,
- the calculation of the 90P, and
- the definition of 'regions' for assessment.

a) Treatment of data reported at <DL: If the database respects the quality criteria of reporting DL <25 µg Zn/l, the database is used, and the treatment of data set at "<DL" is treated in the following manner:

- all data in the database are used, including the data reported at <DL. This ensures a proper representation of the region under evaluation and calculation of a proper P90.
- the reported data as <DL is treated as ½ DL for 90P calculations.
- Evaluations of data excluding measurements <DL (e.g. Denzer et al., 1999) are not considered relevant for the risk characterization. The 90P calculations based on these evaluations are indeed skewed since the lower part of the distribution of zinc levels in a given region is excluded[62].

b) Regional 90P calculations: a regional 90P is calculated based on the recommendations of the TGD, by taking the average of the 90Ps across sampling stations for a region. Furthermore, if a sampling station contains only 1 sampling measurement, then the 1 sampling measurement is used as the sampling station 90P. If 2 measurements are available for a sampling station, the maximum value is used as the station 90P. If ≥3 measurements are available, a 90P is calculated for the station.

c) *Defining regions for assessment*: A region for which an average 90P is calculated should ideally make reference to an ecologically relevant area, for example a river basin (e.g. German, French and Walloon databases are treated this way). This is also in line with the Water Framework Directive and recommendations at an ECB Workshop63 where it was concluded that the river basin approach was most appropriate for regional databases such as those in the RAR for zinc. The main benefit of using this approach is that it provides a clear view of the zinc levels for a specific ecologically defined region. However, a region may also be defined by a political area for which water authorities are responsible for (e.g. Flanders).

Table 1 summarizes the monitoring data available for zinc as presented in the RAR (2004). Where possible, these data were already re-grouped by region according to the TGD; in some cases however, the data handling was conducted by other institutions or experts and therefore does not necessarily follow the data handling description as described above. These deviations are discussed in the text describing each region. The data in table 1 were subsequently checked for adequacy for use in the regional assessment by applying the quality and relevancy criteria and description for data handling as referred to in sections 2.1.2.

Table 1 Measured zinc concentrations in water. Full list of available regional datasets for zinc in water **prior to any analysis on quality or relevancy of the data.**

	Most recent regional datasets for zinc	Source

62 This approach was agreed also at the TC NES Sub Group Meeting with representatives of the former CSTEE on the environmental risk assessments of cadmium and zinc, 25 August 2004.

63 TC NES Sub Group Meeting with representatives of the former CSTEE on the environmental risk assessments of cadmium and zinc, 25 August 2004.

Location		
Netherlands	Average 90P Total zinc State waters – 40 µg/L Regional Dutch waters 1997 – 41 µg/L 1999 – 53 µg/L 2000 – 54 µg/L (Full analysis conducted by RIZA)	CIW, RIZA (1985 – 1998) RIZA 2001 – 2003
Swedish watercourses, 1989-1995	12 (90 P; total) total Swedish watercourses 3.6 (90P; total) Lakes Northern Sweden 6.4 (90P) Lakes Southern Sweden (Full analysis conducted by Swedish authorities)	(Landner and Lindeström, 1998)
France, various regions	Average 90P for 2000 , 2001 , 2002 32.5 , 28.6 , 15.2 : Rhin Meuse (NE Fr) 5.4 , 3.0 , 5.8 : Seine Normandie 2.6 , 14.2 , 5.3 : Rhone Méditerrannée Corse 63.4 , 125.1 , 57.5 : Artoie Picardie 122.7 , 164.6 , 113.2 : Adour-Garonne (full analysis conducted by INERIS)	« Réseau National des Données sur l'Eau », Office International de l'Eau, F - 87065 LIMOGES Cedex, www.rnde.tm.fr) (INERIS)
Germany, various regions	2001 Data presented by river basin Rivers — 90P Total zinc µg/L **Rhine River Basin** Altbach — 19.7 Lippe — 30 Main- Hallstadt — 10 Main- Erlabrunn — 29.3 Main- Kahl — 40 Mosel — 41 Nahe — 35 Neckar- Mannheim — 34.2 Neckar- Kochendorf — 18.7 Neckar- Poppenweiler — 28.1 Neckar- Deizisau — 14.4 Neckar- Kirchentellinsfurt — 19.8 Necker- Starzach-Börst. — 20.7 Prims — 20.7 Radolfz-Aach — <10 Regnitz — 29.3 Rhine –Bad Honnef — 16.8	LAWA, 2001

	Most recent regional datasets for zinc		Source
Location			
	Rhine- Kleve Bimmen	47.6	
	Rhine- Klobenz	19.2	
	Rhine- Mainz	55	
	Rhine- Mannheim	16.5	
	Rhine- Karlsruhe	<10	
	Rhine – Vogelgrün	14.3	
	Rhine- Dogern	<10	
	Rhine- öhningen	<10	
	Rotach	<10	
	Saar- Saarbr. Güdingen	41.5	
	Saar- Fremersdorf	30	
	Saar- Kanzem	212	
	Schwalm	56	
	Steinach	19.1	
	Stever	43	
	Swist	26	
	Weschitz	<30	
	Erft	116.8	
	Ruhr	49.4	
	Sieg- Bergheim	364	
	Sieg- Au	40	
	Wupper	65.6	
	Sieg- Netphen	<50	
	Main	<50	
	Av 90P for Rhine River Basin	42.7	
	Elbe River basin		
	Alland	<10	
	Bille	12.5	
	Bongsiel Kanal	12.3	
	Elbe- Schmilka	46	
	Elbe- Wittenberg	69.1	
	Elbe- Magdeburg	70	
	Elbe- Schnackenburg	36	
	Elbe- Grauerort	94	
	Elde	3.6	
	Grosse Roder	26	
	Havel- Potsdam	25.7	
	Havel- Krughorn	<10	
	Havel- Hennigsdorf	8.1	
	Ilm	38.5	
	Ilmenau	<10	
	Peene	3.9	

	Most recent regional datasets for zinc		Source
Location			
	Pleisse	30.5	
	Saale- Bad Dürrenberg	33	
	Saale- Trotha	35	
	Saale- Stöben Saale	119.5	
	Sachs Saale	60	
	Schwarze Elster	33	
	Schwentine	12.7	
	Spree- Spandau	22.2	
	Spree- Neuzittau	3.5	
	Spree- Cottbus	3.6	
	Stor	17.7	
	Sude	6.9	
	Teltowkanal	30.8	
	Tollense	6.9	
	Trave	14.1	
	Treene	8.6	
	Uecker	29.5	
	Unstrut- Freyburg	17	
	Warnow	3.5	
	Weisse Elster Bad Elster	44	
	Weisse Elster G-Langen	34.7	
	Wipper	47.6	
	Mulde Freiberg	110	
	Mulde Dessau	76	
	Mulde- Vereinig	97	
	Mulde- Zwickau	57	
	Saale- Gross Rosenburg	111	
	Weisse Elster Ammendorf	57	
	Av 90P for Elbe River Basin	*36*	
	Danube River Basin		
	Argen	<10	
	Donau- Hundersingen	17.4	
	Donau- Ulm-Wiblingen	23.1	
	Donau- Ulm	24.8	
	Donau- Dillingen	5.8	
	Donau- Kelheim	19.3	
	Donau- Jochenstein	20	
	Grosse Ohe	10	
	Iller	5	
	Inn	19.3	
	Lech	<5	
	Naab	20	

	Most recent regional datasets for zinc		Source
Location			
	Salzach	20	
	Schussen	16.7	
	Av 90P for Danube River Basin	*14.9*	
	Oder River Basin		
	Lausitzer Neisse	35	
	Ratzdorf Neisse	<50	
	Frankfurt Oder	<50	
	Hohenutzen Oder	<50	
	Av 90P for Oder River Basin	*35*	
	Weser River Basin		
	Hunte	54	
	Weser	45.5	
	Werra	27.2	
	Aller	70	
	Leine	30	
	Weser	181	
	Aller at Grafhorst	79	
	Oker	182	
	Aller Langlingen	131	
	Av 90P for Weser River Basin	*88.9*	
	Ems River Basin		
	Ems	20	
	Ems	40	
	Hase	28	
	Vechte	33	
	Av 90P for Ems River Basin	*30.2*	
	Maas River Basin		
	Niers	48	
	Rur- End Steinkirchen	101	
	Rur –Einruhr	86.1	
	Av 90P for Maas River Basin	*78.4*	
	*If 2001 data was not available, the preceding most recent year was used.		
Belgium, Walloon Region (2001)	Code station	Av P90 sub-basin (Zn dissolved)	DGRNE, 2001
	Scheldt River Basin		
	Dendre	14.7	

	Most recent regional datasets for zinc	Source
Location		
	Dyle-Gette 7.1 Scheldt-Lys 14.9 Haine 16.8 Senne 11.5 Meuse and Seine River Basin Upper Meuse & Oise 8.2 Ourthe 12.3 Sambre 13.3 Lesse 12.8 Lower Meuse 30.7 Meuse river Basin Amblève 12.8 Moselle 10.0 Semoi-Chiers 9.8 Vesdre 46.3	
Belgium, Flanders (1999, 2000, 2002-2003)	Average 90P for Flanders Total zinc 1999 = 146 µg/L 2000 = 110 µg/L 2002-2003 = 68.5 µg/L (Full analysis conducted by EURAS (2004))	VMM, 2003
NORDIC countries (lakes) (1995)	Finland: 4.4 µg/l (90P of total zinc) Norway: 5.9 µg/l (90P of total zinc) Sweden: 5.3 µg/l (90P of total zinc) Denmark: 12.6 µg/l (75P of total zinc) (Full analysis conducted by NIVA)	NIVA, 1999 (Report no. 4039-99)

1.3.3 Criteria for Relevancy

The zinc monitoring databases (and respective 90Ps) as presented in Table 1 are influenced by a number of factors (including local natural background, industrial point sources and/or historical contamination, and diffuse emissions) **some of which are not related to the current use pattern of zinc in the EU and are as such not relevant for the regional water assessment in a risk assessment under 793/93**. Furthermore, determining the need and type of management strategies will highly depend on the type of source of zinc emissions. Therefore, a detailed

analysis of the monitoring data is needed to determine the underlying sources of zinc emissions for proper regional assessment (based on a 90P calculation) and guidance for risk management.

To this end, according to the TGD[64], the monitoring sampling points that are influenced by documented point sources are not considered relevant for the regional scenario. Consequently, data in the monitoring database affected by identified point sources need to be separated from the data to be used for the regional assessment.

Also sampling points situated in historically contaminated areas (e.g. old mining areas) are considered not to reflect the current use pattern of zinc in the EU, and therefore must also be separated from the regional assessment.

Therefore, to summarize, the assessment is carried out by assigning data as much as possible to 4 specific categories to allow for a) clear characterisation of the observed 90P zinc concentrations and the underlying sources, and b) proper guidance to risk management. These categories are:

- local scenarios influenced by point sources,
- historically contaminated areas,
- areas influenced by naturally elevated zinc background concentrations, and
- data used for regional assessment reflecting current use patterns of zinc.

1) *Local scenarios influenced by identified point sources:* Interpretation of the zinc monitoring data is complicated by the influence of point sources. Data that are influenced by identified point sources are separated from the regional data since they refer to local situations. To identify point source emissions of zinc, the European Pollutant Emission Register (EPER, 2004) was used as a main reference
2) *Historically contaminated areas:* data from areas with historical pollution have also been treated separately since they are not related to the present day production and use of zinc and therefore outside the scope of the RA and strategy for limiting the risks[65].
3) *Data influenced by elevated natural background*: Interpretation of the zinc monitoring data may be complicated by the influence of elevated natural background areas. These documented areas are to be considered separately, in order to correctly apply the principles of the added risk approach.
4) *Data used for regional assessment*: data that have been measured under conditions where there is no identified direct influence of point sources and/or of historical contamination and/or elevated natural background are useful for regional assessment (90P calculation).

Monitoring data related to particular zinc sources (i.e. corrosion of specific structures and/or traffic) are discussed in a separate part of the RAR (2004). Strictly speaking, these data refer also to local situations. They are not further discussed in this risk assessment.

The results of the selection of the exposure data are presented in Tables 8 and 9. Data used for the regional assessment are presented in Table 8, whereas data that fall outside of the scope of the regional assessment are categorised and listed in Table 9 under "summary of data identified

64 The Technical Guidance Document (2003) mentions that "samples that are used in a regional scenario (i.e. the derivation of a regional ambient PEC) should not be directly influenced by a (point source) emission. Such sites can be used to describe the local scenario, but are not representative for regional concentrations (chapter 2.2.1, page 19)". The TGD (2003) add that 'if there is no spatial proximity between the sampling site and point sources of emission, the data represent a regional concentration' (chapter 2.2.2, page 21).
65 "Legally the risk assessment and strategy of limiting the risks includes only the production and use of Zincs. Emissions from sources like mining activities, waste disposal sites and emissions due to historical processes or the production and use of other zinc compounds fall outside the scope of the risk assessment and strategy of limiting the risks". (letter of NL Ministry of Environment VROM of 3-12-04)

as related to point sources and /or historical contamination or areas with naturally elevated zinc levels".

In the following, a detailed analysis of the monitoring data compiled in Table 1 is made. It is noted that the data in Table 1 refer to those figuring in the main text of the RAR, with exception of the data obtained before 1995.

1.4 REGION SPECIFIC ANALYSIS AND DESCRIPTION

1.4.1 The Netherlands

For the Netherlands the total zinc levels in surface waters in the period 1985-1998 are presented in Figure 1. Levels in Rhine, Meuse, Scheldt, state waters and regional waters have been collected in extensive, regular monitoring programmes of CIW/RIZA. Data refer to average 90 percentile values, i.e. the average of the 90 percentile values (based on monthly data) for the various, individual sampling stations in a particular water. 'State waters' are defined as the group of major Dutch rivers (incl. Rhine, Meuse and Scheldt) and other large inland surface waters. 'Regional waters' represent approximately 250 different sampling stations spread over the Netherlands. These regional sampling stations are selected on the basis that they are not influenced by local point sources (industry, STP effluent etc.). Trends show a gradual decrease in zinc concentrations in this period. The concentration of 41 µg/l is used in the RAR as the regional concentration, because it is considered to be representative for an ambient regional concentration. The main reason is that sampling points in this regional Dutch dataset are not directly linked with obvious point sources. The regional zinc water concentrations for the Netherlands are 53 and 54 µg/l for, respectively, 1999 and 2000 (CBS/RIVM Milieucompendium, 2004). These values are higher than the 1997 value of 41 µg/l as used in the RAR. The regional database for the Netherlands includes several smaller rivers that are influenced by historical contamination. When these data are removed from the most recent datasets (RIZA, 2001-2003 data), the regional 90P value for the Netherlands is 39 µg Zn/l. This corresponds to the 90P used for the regional background in the RAR.

For the Meuse river at the Dutch border (Eijsden) data for 1999, 2000 and 2001 indicate a further decrease in zinc concentrations compared to the data used in Figure 1. Dissolved data, if available, are used to avoid error by applying a Kp. For the Meuse river, dissolved data are available for the sampling point at Eijsden. The dissolved zinc concentration in 2001 (90P) is 8.7µg/l (RIZA public water database). For the year 2002, a 90P value of 101 µg/l total zinc is reported, corresponding to a 90P value of 14 µg/l for dissolved zinc. These dissolved values are used for the risk characterisation, since they are most recent and most reliable.

Conclusion: regional NL data are useful for risk characterisation. Most NL State water data are also useful, since no major point sources are identified by EPER (2004) Exception to this is the river Meuse sampling point at the B-NL border, where influence from Belgian industry upstream cannot be excluded (see further: "Wallonia"). There are a number of historical and/or naturally high background areas identified that were included in the "regional NL" data. They have been separated for the 90P calculations of the most recent regional dataset.

Figure 1 presents zinc levels in Dutch surface waters from 1985-1998 according to the CIW/RIZA monitoring programme.

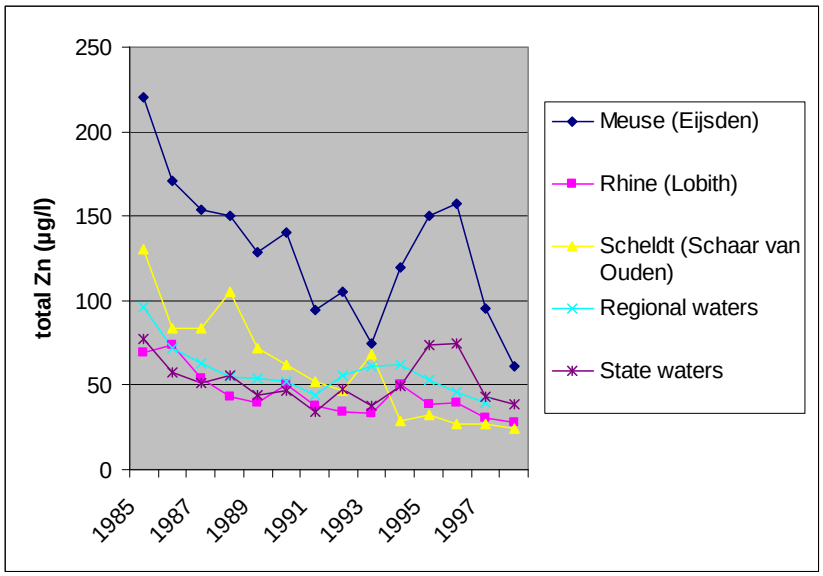

Figure 13 Total zinc concentrations (average 90th percentile values) in Dutch surface waters during the period 1985-1998 (RIVM/CBS 2000). Original data from RIZA/CIW; figure taken from RAR.

1.4.1.1 Sweden

Measurements of zinc concentrations in Swedish lakes and watercourses have been compiled (Landner and Lindeström, 1998). None of the sampling stations is situated in the immediate vicinity of a major source of metal emissions. A summary of the data is given in Table 1.

Conclusion: Since these data compile sampling stations away from point source emissions and historical contamination, and no natural high background levels have been identified, and since DLs are not influencing the 90P calculations, they are considered relevant for the regional scenario.

Much higher zinc concentrations were measured in areas near major point sources in Sweden. In the vicinity of 'traditional' mining districts leaching and erosion of mining waste leads to levels of e.g. 710 µg/l (average value) in a lake near Gruvsjön, Garpenberg during the period 1990-1996. Much lower zinc concentrations (no data given) are found outside point sources in Sweden where activities first began during 'modern' times.

1.4.2 Germany

Table 1 contains a large number of zinc concentrations (90 P values) in German surface waters (LAWA, 1998). In general, the LAWA monitoring net is designed to measure the ambient overall pollution of surface waters. Data from the LAWA monitoring net are used repeatedly in Germany for assessing and reporting the general water quality within the frame of the European environmental laws, e.g. under the Directive 76/464/EEC. Zinc levels in Germany in 1998 range from 3-291 µg/l. Much higher zinc values were reported from the period 1977-1983 in surface waters of old mining districts in Germany, e.g. in the Harz Mountains (max. 1300 µg/l Zn), in the Rheinische Schiefergebirge (max. 11,700 µg/l), near Maubach and Mechernich at the North edge of the Eifel, and near Bodenmais in Bavarian Forest (max. 10,000 µg/l) (Fauth et al., 1985).

Summary of analysis on the German data:

The 90P zinc concentrations for sampling stations along 78 German rivers are available. The data are compiled per **river basin** using the 7 main river basins (the Weser, Oder, Elbe, Rhein, Donau, Ems, and Maas river basins) identified by UBA (UmweltBundesamt) and according to

the hydrological atlas of Germany (http://www.umweltbundesamt.org/dzu/4/15/1/0002_0.html) which has further categorized in which river basin each German river falls (http://had.bafg.de).

A number of areas have been identified where influence from historical pollution is obvious and where intensive mining activities have taken place (see below). Data obtained in these areas are separated from the regional scenario category and summarised in Table 9. Elevated zinc levels are generally found in rivers that are within the catchments of former mining activities (LAWA document "Zielvorgaben zum Schutz oberirdischer Binnengewasser, Band II, LAWA 1998, 'Reasons for exceeded limit values (zinc only)'.

A comparative 90P calculation on the German river basins has been made, including and excluding the monitoring stations within catchments of former mining activities. This comparison reveals that the average 90P including the monitoring stations within catchments of former mining activities is up to a factor of 1.7 greater than the 90P calculated excluding these monitoring stations (see Table 2).

90P calculation of the German data excluding/including data influenced by histroical contamination

	# sampling stations	90P Total Conc µg/L
Germany calculations excluding historically contaminated sites		
2001 Data		
Total average 90P	*36*	23.3
Rhein River Basin°	*16**	29.1
Elbe River Basin°	*24*	19.8
Danube River Basin	*9*	13.9
Oder River Basin	*1**	35
Weser River Basin°	*4**	39.9
Ems River Basin	*2*	29
Maas River Basin°	*1*	48
Germany calculations including historically contaminated sites		
2001 Data		
Total average 90P	*45*	36.8
Rhein River Basin°	*21**	41.4
Elbe River Basin°	*32*	35.5
Danube River Basin	*9*	13.9
Oder River Basin	*1**	35
Weser River Basin°	*6**	53.5
Ems River Basin	*2*	29
Maas River Basin°	*2*	70.8
Influence of historical mining sites		
Total average 90P		1.58
Rhein River Basin°		1.42
Elbe River Basin°		1.79
Danube River Basin		1.00
Oder River Basin		1.00
Weser River Basin°		1.34
Ems River Basin		1.00
Maas River Basin°		1.48

* *excluding 1,2 and 3 data DL >50 for the Rhein, Oder and Weser River basins respectively.*

° *River basins affected by historical contamination, and number of sites removed from the 90P calculation :*
Rhein : 5 sites : Erft (NW07), Lippe, Ruhr, Sieg, Wupper
Elbe : 8 sites: Freib Mulde (SN 06), Mulde, Saale, Schwarze Elster, Vereinig Mulde, Weisse Elster, Wipper, Zwick Mulde
Danube : No historically contaminated sites were identified in this region
Oder : No historically contaminated sites were identified in this region
Weser : 2 sites: Aller, Leine
Ems : No historically contaminated sites were identified in this region
Maas : 1 site: Rur

Table 14

Analysis of the German dataset 2001 per river basin – an example of a database in which sampling stations are influenced by historical contamination and influence from point sources

1) Rhine river basin

The Rhine river basin monitoring dataset includes measuring stations from 21 rivers and includes 40 sample measurements. This is a large extensive river basin with some areas in highly industrial zones, and within old mining districts and therefore some sampling stations have not been used in the calculation of the regional 90P because of their clear influence from point sources, and historical contamination.

Historical contamination: The Erft river receives water from the Veybach river which comes from the historical mining area at Mechernich (LUA NRW Gewässergütebericht 2001). This geogenic zinc content can be measured down the whole Erft river and into the Rhine river. Also within the catchment area of the river Rhein is the river Sieg in which Pb-, Cd- and Zn-contents within suspended matter are heavily elevated because of geogenic effects and historical mining activities (LUA NRW Gewässergütebericht 2001).

Point source influences: Elevated zinc level was found in the Ruhr river near Duisburg. EPER reveals a large number of zinc emitters in the area, including the largest zinc emitter in Germany, Sachtleben in Duisburg, as well as Thyssen Krupp Stahl, DK Recycling, M.I.M, etc. for a total of nearly 60T of zinc emitted by industries in the Duisburg area. A rather elevated zinc level was also found in the Wupper river, which is influenced by a large nearby zinc emitter identified by EPER (Bayer at Leverkusen), and to some extent from other nearby industries (Bayer at Dormagen, Henkel at Dusseldorf, Bayer at Krefeld).

A regional average 90P for evaluating the impact of current use patterns of zinc was calculated without the stations mentioned above, and was based on sampling stations from 17 (out of 21) rivers (34 (out of 40) sampling measurements), resulting in a concentration of 31,4 µg/L total zinc.

2) Elbe river basin

The Elbe river basin monitoring dataset includes measuring stations from 26 rivers and includes 44 sample measurements. This is a large river basin with some areas in highly industrial zones and within old mining districts and therefore some sampling stations have not been used in the calculation of the regional 90P because of their clear influence from point sources and historical contamination.

The catchment area of the Mulde is the main source of heavy metals for the Elbe due to ores (Erzgebirge = Ore Mountains) and historical mining activities (http://www.mineral.tu-freiberg.de/geochemie/artspek/artspek.html, "Grubenwässer des Erzgebirges - Quellen von Schwermetallen für die Elbe"). The Elbe in Sachsen receives waters from the Ore-mountains as well, and further along the river, in Sachsen-Anhalt, the Mulde flows into the Elbe influencing the zinc levels in the area.

The Weisse Elster is heavily contaminated by historical pollution; remobilisation of sediments (U-mining by Sowjet company Wismut). Furthermore, the sampling station at Weisse Elster is likely influenced by the large zinc emitter identified in Elsterberg (ENKA GmbH).

Zinc levels are high in the Saale river at Rosenburg because of previous and existing copper mining in north Halle. From there, waste water containing elevated zinc levels flows continuously into the Saale and explains the high zinc levels at Rosenburg.

A regional average 90P was calculated excluding sampling measurements taken along the Mulde river as well sampling measurements in the Weisse Elster at Ammendorf and in the Saale river at Rosenburg. The average 90P was based on sampling stations from 24 (out of 26) rivers (38 (out of 44) sampling measurements), resulting in a concentration of 28.7 µg/L total zinc.

3) Danube river basin

The Danube River basin monitoring dataset includes measuring stations from 9 rivers (14 sampling measurements), including 5 measuring stations along the Danube. The monitoring stations within this river basin are thought to be representative of the current use patterns of zinc. No historical mining districts or major point sources have been identified near the sampling stations. The average 90P calculated for the Danube river basin is 15.5 µg/L total zinc.

4) Oder river basin

The Oder River basin monitoring dataset includes measuring stations from 2 rivers, the Oder and the Neisse. Measured concentrations were found <50 DL in 3 of the 4 sampling measurements. Given that the 90P for the Oder river basin is based on only 1 sampling station (90P of 35 µg/L total zinc based on the station at Lausitzer on the Neisse river), it is considered of limited value for this assessment.

5) Weser river basin

The Weser river basin monitoring dataset includes measuring stations from 6 rivers, and includes 9 sampling measurements. Extensive historical mining activities in the Hartz mountains region (according to LAWA "Zielvorgaben zum Schutz oberirdischer Binnengewässer Band II"), as well as continued metal refining activities (EPER identified point sources Harz Metal in Goslar and Salzitter AG in Salzgitter) are found within this river basin. More specifically these past and present activities affect the quality of the Oker river and a sampling station in the Aller river downstream (near the tributary of the Oker into the Aller river). Furthermore, some sampling points are located in the vicinity of identified point sources. The sampling point on the Aller river at Grafhorst is in the vicinity of Volkswagen in Wolfsburg (EPER, 2004), and the sampling point on the Weser in Nordenham is downstream of 2 zinc emitters identified in EPER (Kronos Titan GmbH & Co and Metal Europ, both in Nordenham). The average 90 was calculated based on 5 sampling measurements, excluding the sampling measurements influenced by historical contamination and point sources mentioned above, resulting in an average 90P of 45.3 µg/L total zinc.

6) Ems river basin

The Ems river basin monitoring dataset includes measuring stations from 3 rivers and includes a total of 4 sampling measurements. No historical mining districts or major point sources have been identified near the sampling stations. The average 90P calculated for this river basin is 30.3 µg/L total zinc.

7) Maas river basin

The Maas river basin monitoring dataset includes measuring stations from 2 rivers and includes a total of 3 sampling measurements. The Maas river basin is found at the German- Belgian- and German- Dutch borders area. The zinc levels in the Rur river are elevated. The sampling stations on the Rur at Einruhr and upstream (86 µg/L and 101 µg/L total zinc) are within a natural area with no identified industry and little habitation, however, it is clearly in a mineralized (Zn, Pb) region, in the old mining activities district of Stolberg (Altenberg mining industry). An average 90P for the Maas river basin was calculated based on the upstream sampling measurement on the Rur and the sampling measurement on the Niers resulting in a value of 74.5 µg/L total zinc. Given the limited number of data points in this river basin, and the clear influence from historical mining in the area, its 90P value is not used for the regional assessment.

In the river basin 90P values, presented in Table 8, data from areas influenced by historical pollution are excluded. Table 9 presents the data for these regions including the historically contaminated areas.

The EPER (2004) identifies several important industrial zinc emitters on the German rivers. Monitored data directly influenced by these emissions have been separated from the regional dataset and have been listed in Table 9. It has however not yet been possible to do a complete check of the spatial relationship between the sampling points of the LAWA dataset and the point source emitters identified by EPER (2004). Further analysis is needed in this respect.

Conclusion: Since the river basin data for Germany, with exclusion of the areas influenced by historical pollution, are still clearly influenced by point source emissions, there is uncertainty on the underlying reasons for the observed zinc levels, notably in rivers, for which only few data are

available, Therefore the latter data has not been used for regional assessment. The rivers documented by more sampling points are categorised for the purpose of this assessment as useful for the regional risk characterisation, but with reservations. Further analysis is indeed needed to assess the relationship between the zinc levels observed in these rivers and the different underlying zinc sources.

1.4.3 France

Zinc surface water concentrations have been reported for France for the years 2000-2002 (see Table 1). The average 90 P values for various regions in France ranged from 2.6 to 164.6 µg/l. A further description of the monitoring data per river basin is given below.

1) Rhin-Meuse river basin:

The average 90P values represent an average across 1, 4 and 6 stations for the years 2000, 2001 and 2002 respectively. The DL is set at 1 µg/L for this dataset. Some higher levels in this dataset point to stations located downstream from municipal waste effluent discharges (e.g. Moselle river at Sierck). The data for 2000 is not useful because all 26 measurements are taken from 1 station, and therefore it is not representative of the river basin. The datasets for 2001 and 2002 are larger and each comprise of 13 measurements per station. The data for 2002 contains the largest set of data for the Rhin-Meuse river basin (6 stations), therefore the average 90P for that year of 15.2 µg/L is used for the risk characterization.

2) Rhône-Mediterrannée River Basin:

The Rhône-Méditerrannée Corse 2000-2001 database contains 14 stations for which measurements have been taken 4 times a year in 2000 and 2001, and 3 times a year in 2002. The average 90P across stations is 2.6 , 14.2 and 5.3 µg/L total zinc. The DL for this dataset is set at 1 µg/L. It is noted that more than half of the data is reported at below the DL. No very high levels (effects of point sources,..) were observed in this river basin. An average of the average 90P across the 3 years (7.4 µg/L total zinc) is carried forward for use in the risk characterization.

3) Seine-Normandie River Basin:

The Seine-Normandie 2000-2002 dataset contains data for 3 stations for which measurements have been taken 12 to 24 times a year. Total zinc levels in this river basin are generally low (1-30 µg/L) with an average 90P across stations of 5.4 , 3.0 , 5.8 µg/L total zinc. The DL for this dataset is set at 1 µg/L. No major point source or influence of historical contamination has been identified. An average of the average 90P across the 3 years (4.7 µg/L total zinc) is carried forward for use in the risk characterization.

4) Artoie Picardie River Basin:

The Artoie Picardie 2000-2002 dataset contains data for 4 to 6 stations for which measurements have been taken 1 to 13 times a year. The average 90P across stations is 63.4, 125.1 and 57.5 for 2000, 2001 and 2002 respectively. The DL for this dataset is set at 50 µg/L. About 50% of the measurements (91 of 185 measurements) are reported at below the DL. It should be noted that the high DL skews the average 90P (may cause a bias towards a higher average 90P). Furthermore, EPER (2004) identifies several industrial point sources in this highly industrialised area e.g. the industrial zone of Northern France, surrounding Valenciennes includes the industrial emittors MetalEurope at Noyelles Godault, Umicore at Auby, Norzinco at Anzin, LME at Trith-St-Léger, Fonderie et Acérie at Denain, and other industrial sectors within the canal de la Deûle and its tributaries. Lastly, there is a possibly doubtful figure of 420 µg/l at one sampling station

(Canalized section of the Scheldt at Rouvignies) for the 2001 dataset (INERIS, 2004)[66]. Calculating the average 90P for the 2001 dataset without the doubtful figure results in an average 90P of 66.1 µg/L total zinc. This shows the importance of one data in the distribution (particularly the fact that it is the only measurement at that station), when the dataset is not very large (only 6 stations). Basically, the stations are either reported at below a very high DL, or they are in the vicinity of point sources (stations in the Scheldt river at the French/Belgian border downstream of the Deûle). For these reasons, this dataset is not considered in the risk characterization.

5) Adour-Garonne River Basin:

The Adour-Garonne 2000-2002 dataset contains data for 32 to 37 stations for which measurements have been taken 1 to 12 times a year. The average 90P across stations is 122.7, 164.6, and 113.2 for 2000, 2001 and 2002 respectively. The DL for this dataset varies between 10 and 70 µg/L. The % of measures reported at below the DL are more than 50% (and about 40% at below a DL of 50 or more µg/L). It should be noted that the high DL skews the average 90P (may cause a bias towards a higher average 90P). Furthermore, this region contains 2 stations with extremely elevated zinc levels (see table 3), influenced from point sources of zinc and historical contamination. The first is the station in the Rioux Mort, downstream of Viviez, a well known old mine with reported levels up to 3490 µg/L total zinc (and in the 2001 dataset, elevated levels were also found in 2 tributaries of the Rioux Mort). The second is a station at "La Saudrune" in Palayre, with reported levels up to 2100 µg/L. Calculating the average 90P without these 2 stations (and Rioux Mort tributaries in the 2001 dataset) the average 90Ps are 44.4, 31 and 38 µg/L total zinc for the years 2000, 2001 and 2002 respectively. The data for the region of Adour-Garonne, with exclusion of the stations influenced by point sources and historical contamination are still not considered useful for the risk characterisation because it does not pass the criteria for data quality with reference to the level of the detection limits reported (affecting ~40% of the data).

Table 3 High measured values in the Adour-Garonne dataset (all data above 400µg/L)
(Table provided by INERIS, 2004)

Station code	Description of the site	Analysis date	Results (µg/l)
s5092200	Le Lot à St-Pierre Toirac	10/07/01	530
s5092200		07/08/01	410
s5093000	Le Lot à Capdenac	10/07/01	560
s5093000		07/08/01	410
s5093550	Le Riou Mort en aval de Viviez[67]	06/08/02	2630
s5093550		09/07/02	552
s5093550		10/09/02	1960
s5093550		14/05/02	1420
s5093550		15/10/02	1990
s5093550		26/03/02	803
s5093550		03/10/00	2680
s5093550		13/06/00	820

66 INERIS comments on the French dataset of 2001 as per e-mail of H. Magaud to C. Bodard (RIVM) on 25/06/04.
67 This corresponds to a site downstream an old mine (not in use since 1986, but where amelioration concerning the releases could be made

s5093550		08/08/00	1390
s5093550		18/07/00	1620
s5093550		12/09/00	3200
s5093550		28/03/00	430
s5093550		15/05/01	547
s5093550		10/07/01	2090
s5093550		07/08/01	3490
s5093550		11/09/01	2120
s5093550		20/03/01	925
s5093550		20/11/01	1770
s5163450	La Saudrune à Palayre	03/04/02	2100
s5163450		09/02/01	560
s5163450		04/06/01	1500

Conclusion on the French dataset: The 2000-2002 data for the Rhin-Meuse, Seine Normandie and Rhone-Méditerrannée-Corse are useful for the regional scenario in the risk characterisation.

1.4.4 Walloon region in Belgium

In the Walloon Region the network contains 179 sampling locations spread over various Walloon surface waters. At 56% of the locations the zinc level is below the detection limit of 25 µg/l. The Walloon region is characterised by 3 river basins (see below). The RAR further states that *"the measured zinc concentrations in Walloon Region do not refer to total zinc levels, but to 'zinc extractible'. This 'in house' analytical technique is based on AAS and flame analysis after acidification (HNO3, pH<2), settling and decanting of the water samples (based on EPA method 7000, Sept. 1986; EPA method 7950, Sept. 1968 and Standard Methods 20th ed). A limited internal comparison of the results of analysis based on 'zinc extractible' and total zinc showed that total zinc levels tend to be (slightly) higher, but the difference is not more than 30%"*.

For the Walloon region, it is appropriate to distinguish between different subsets of the data, related to areas with specific characteristics, and repartitioned over representative sub-basins, as characterized by the 'Direction Générale des Resources Naturelles et de l'Environnement – Direction des eaux de surface', the institute responsible for this monitoring data . This repartition into river basins and sub-basins allows for a better understanding of the monitoring results and, in particular, of the sources of elevated zinc levels observed in some Walloon waters. The detail of this analysis is presented below.

The Walloon region is characterized by 3 river basins, the Scheldt, the Meuse-Seine and the Meuse River Basins. From these arise 14 sub-basins:

- The Scheldt Basin is characterized as a flat-plain, agricultural area neighbouring an industrial zone of Northern France. It contains 5 sub-basins in Wallonia; Dendre, Dyle-Gette, Scheldt-Lys, Haine, Senne.
- The Meuse-Seine River basin contains a mixture of forested valleys in the south and highly industrial centers including Charleroi, Namur and Liège along the Sambre and the Meuse in the North. It contains 5 river Basins; upstream Meuse (~prior to Namur) and Oise, Ourthe, Sambre, Lesse, downstream Meuse (~after Namur).
- The Meuse basin neighbours Germany, and is dominated by forest land and plains with little industrial activity. It contains 4 river sub-bassins: Amblève, Moselle, Semois-Chiers, Vesdre.

Handling of the Walloon dataset – an example of a region with naturally high zinc background levels, and of problems arising with databases containing high detection limits.

Firstly, all zinc 90P concentrations have been converted to dissolved zinc using the documented individual suspended matter values of each sampling station and a Kp of 110,000 l/kg (RAR)[68]. An average of the 90P has then been calculated for each sub-basin (see Table 4 below). From table 4 follows also the strong variability in suspended matter concentrations that can be observed in an area.

Table 4 Average 90P calculations for zinc per Walloon sub-region

Code station	Units	Average P90 (Total Zn)	Average P90 (Dissolved Zn)
Scheldt River Basin			
Dendre sub-basin	µg/l	118	14.7
Dyle-Gette sub-basin	µg/l	86	7.1
Scheldt-Lys sub-basin	µg/l	282	14.9
Haine sub-basin	µg/l	62	16.8
Senne sub-basin	µg/l	37	11.5
Meuse and Seine River Basin			
Upper Meuse and Oise sub-basin	µg/l	29	8.2
Ourthe sub-basin	µg/l	<25	12.3
Sambre sub-basin	µg/l	54	13.3
Lesse sub-basin	µg/l	26	12.8
Lower Meuse sub-basin	µg/l	111	30.7
Meuse river Basin			
Amblève sub-basin	µg/l	26	12.8
Moselle sub-basin	µg/l	<25	10.0
Semoi-Chiers sub-basin	µg/l	29	9.8
Vesdre sub-basin	µg/l	91	46.3

The problem of the detection limit

The Walloon database has a rather high detection limit of 25 µg/L. The % of data below this detection limit is 57%. For the calculation of the 90P of the sub-basins, the zinc levels of 57% of points are set equal to detection limit/2 (= 12.5 µg/L).

1) Scheldt River basin:

-*Dendre* is represented by 4 sampling points with an average 90P of 14.7. Two of the 4 measuring points in this sub-basin give elevated values (23-24µg/l) and are influenced by a point source industrial emissions (of which Floridienne on the Dendre is contained in the risk assessment report for ZnO as a local scenario). These sampling points are categorized as influenced by point sources, and are placed in Table 9. The other 2 data points are below the detection limit of 25 µg/l. It can be concluded that elevated zinc levels are observed due to the influence of point sources; when such influences are not present, zinc levels are < detection limit.

-*Dyle-Gette* is represented by 6 sampling points with an average 90P of 7.1. This is an agricultural area with few industries. It can be concluded that this region is mainly influenced by agricultural activity.

-*Scheldt-Lys* is represented by 7 sampling points with an average 90P of 14.9. This area is mainly agricultural. However there are a few high zinc levels in the Espierres river (1354µg/l total zinc). This river is highly contaminated from point sources and historical contamination from the industrial area in Northern France. Main industrial activities include metallurgical,

68 Zntotal = 1 + 110,000 * (Suspended Matter) * Zndissolved

inorganic, and textile. Moreover, this river has an extremely high suspended matter content (= 427 mg/kg; compare with default TGD value of 15 mg/kg), which influences significantly the Kp. When the specific Kp value is taken into the calculation, the total Zn of 1354 µg/l translates to 28 µg/l dissolved Zn. This sampling point is categorized as influenced by historical contamination, and is placed in Table 9. This sub-basin is a clear example of high levels caused by industrial point sources and historical contamination.

-*Haine* is represented by 5 sampling points with an average 90Pof 16.8. High values 11-40µg/l at Hensier) are found in the Haine River downstream of the highly industrial neighboring French city of Valenciennes, with historical metallurgical and inorganic fertilizer industries. These sampling points are categorized as influenced by historical contamination, and are placed in Table 9. The other 3 data points, not influenced by industry, are below the detection limit. It can be concluded that historical and point source influence explains the high zinc levels, other sampling stations are below the DL.

-*Senne* is represented by 7 sampling points with an average 90P of 11.5. Most levels are relatively low (between 25 and 32 total zinc). This is an area dominated by agricultural lands and contains few industries.

Conclusion for the Scheldt river basin: An average 90P of 8.1 µg µg Zn_{diss}/L is calculated for the Scheldt river basin, excluding the data influenced by point sources and historical contamination mentioned above, and using half the DL.

2) Meuse-Seine River basin:

-*Upstream Meuse and Oise* is represented by 33 sampling points with an average 90P

of 8.2. Most measurements are below the detection limit. This area is characterized by forested valleys and contains few industries.

-*Ourthe* is represented by 18 sampling points with an average 90P of 12.3. All measuring points are below the detection limit. This area is characterized by forested valleys and contains few industries. It is worth noting that despite the fact that all levels are below the DL, the average 90P is similar to the PNEC (even after correction for background). It can be concluded that the 90P value has no relationship with zinc sources but is influenced by the high detection limit.

-*Sambre* is represented by 29 sampling points with an average 90P of 13.3. Most measurements are below or near the detection limit. There are however a few high monitoring levels in the Sambre and Orneau rivers downstream from the highly industrial zones of Charleroi. This area is known for its historical coal and steel industry. Although most industrial facilities have closed down, there are still a few operating steel industries. These 3 sampling points are categorized as influenced by historical contamination, and are placed in Table 9. It can be concluded that the high zinc levels arise from the influence of historical contaminated industrial areas and current point sources.

-*Lesse* is represented by 22 sampling points with an average 90P of 12.8. Most measurements are below the detection limit. This area contains few industries.

It can be concluded that the 90P for this sub-basin is highly influenced by the high detection limit

-*Downstream Meuse* is represented by 13 sampling points with an average 90P of 30.7. There are several high monitoring levels in the Meuse, Argenteau and the Gueule rivers downstream from the highly industrial zones of Liège and the metallurgical region of the Gueule (Plombières). This area is known for its intensive historical mining activity, based on zinc ores which are sometimes present at the surface, and zinc refining. These 3 sampling points are categorized as influenced by point sources and historical contamination (Plombières), and are

placed in Table 9. Furthermore, a more extensive analysis has been conducted on the Meuse including the region around Liège (see below). It can be concluded that this sub-basin is influenced by historical contaminated industrial areas and current point sources, as well as the erosion of the surfacing zinc ore bodies in the area.

Conclusion for the Meuse-Seine River Basin: an average 90P of 7.7 µg Zn_{diss}/L is calculated for the Meuse-Seine River Basin river basin, excluding the sampling stations influenced by point sources and historical contamination as mentioned above, and using half the DL.

3) Meuse River basin

-*Amblève* is represented by 10 sampling points with an average 90P of 12.8. Nine of the 10 sites are below the DL and the 10^{th} sample is also near the detection limit. This area is characterized by forested valleys and contains few industries. It can be concluded that the 90P for this sub-basin is close to the PNEC as a result of the high detection limit; there is no detected relationship with any zinc sources at all.

-*Moselle* is represented by 4 sampling points with an average 90P of 10. All measurements are below the DL. This is an area bordering Germany and characterized by forested valleys and contains few industries. It is concluded that the 90P is near the PNEC value as a result of the high detection limit; there is no relationship with any zinc sources

-*Semois-Chiers* is represented by 15 sampling points with an average 90P of 9.8. Most of the sampling sites are below the DL (11 sites) with slightly higher levels downstream from Arlon the major city in the region. It is concluded that the 90P is near the PNEC value as a result of the high detection limit; there is no relationship with any zinc sources.

-*Vesdre* is represented by 8 sampling points with an average 90P of 46.3. The river waters in this region are classified as 'natural waters' (=uncontaminated") by the Ministère de la region Wallonne. There is no industrial activity and population density is low. But this region is characterized by naturally acidic rivers (pH around 5) which most probably explain the higher zinc levels. According to the Ministére de la region Wallone, this is a region with naturally elevated levels of zinc[69].

It can be concluded that the elevated zinc levels in this natural area can only be explained by the documented high natural zinc background, due to specific local geological conditions. Therefore this set of 8 sampling points is removed from the generic regional analysis (Table 9).

Conclusion for the Meuse River Basin: an average 90P of 6.5 µg Zn_{diss}/L is calculated for the Meuse River Basin river basin, excluding the sampling station influenced by naturally high background levels as mentioned above, and using half the DL.

This detailed analysis on the Walloon waters provides a good understanding of zinc levels and their relationship to sources at the regional scale. This analysis also demonstrates that care should be taken in assessing risks based on monitoring datasets. To assess risks of zinc to surface waters, there must be

- a proper understanding of the limitations of the dataset, where high detection limits may suggest that the zinc concentrations are elevated to levels exceeding the PNEC, while this is not the case in reality
- a proper consideration of the local abiotic conditions (i.e. suspended solids, pH and other bioavailability parameters) at each sampling point.
- A proper understanding of the possible zinc sources to the waters in that region.

69 Personal communication between L. Regoli and Mr. Wylock of the Direction Générale des Resources Naturelles et de l'Environnement en Wallonnie.

Using 90P values of total zinc levels over a whole region with a wide variety of sources and concentrations leads to over-generalisations that are not useful for risk characterisation and risk management.

The analysis of the Walloon data identified a number of sampling points that are clearly directly influenced by point source emissions. These points have been separated from the dataset, which can subsequently be used for regional assessment. This is clearly demonstrated in the zinc levels observed in the Walloon part of the river Meuse (figure 2).

The Walloon database has a rather high detection limit of 25 µg/L. The % of data below this detection limit is 57%. For the calculation of the 90P of the sub-basins, the zinc levels of 57% of points are set equal to detection limit/2 (= 12.5µg/L).

Conclusion: average 90P values for the 3 Wallonian river basins, excluding the data influenced by point sources and historical contamination:

- Scheldt river basin:8.1 µg Zn_{diss}/L
- Meuse-Seine River Basin:7.7 µg Zn_{diss}/L
- Meuse River Basin: 6.5 µg Zn_{diss}/L

The direct influence of point source emissions is further demonstrated in the zinc levels observed in the Walloon part of the river Meuse (figure 2).

1.4.5 Meuse River in Belgium – *an example of sampling points influenced by point source emissions*

Figure 2 presents an overview of zinc concentrations in the Meuse during its course from the French-Belgium border (Heer-Agimont) through Belgium (Wallonia) (until Eijsden, the Netherlands) and then further alongside the Dutch/Belgian border (until Kinrooi). The RAR states that *"data points refer to sampling during the period 1995-2001 (90 P values). Zinc levels in this Meuse transect are found to range from 29 µg/l (Dave) to 129 µg/l (Engis). High levels are also measured in Liege and Kinrooi (both 106 µg/l). It is important to note that these data refer to average zinc concentrations of several years (1996-2000). Data for the individual years per sampling station therefore show both lower and higher values. For example: zinc levels of 188 and 163 µg/l are measured in 1997 in Engis and Liege, respectively. At the Belgian sampling point Kinrooi, levels around 190 µg/l were recorded in both 1996 and 1998.*"(RAR).

A more detailed analysis of this transect[70], essentially evaluating a) characteristics of the area where sampling points are located: rural or urban/industrial, and b) specific influences for each sampling point, making use of EPER (2004) reveals several issues that are highly relevant (table 5):

70 This transect is also covering the reference sampling point at the B-NL border at Eijsden, which is also discussed separately in the zinc RAR (e.g. figure 3.4. page 130 and 3.4.68, page 320).

Table 5 Analysis of the Belgian Meuse transect with emphasis on zinc inputs

Sampling point	km	Characteristics of the area	Zn concen-tration (µg total Zn/l)	Specific influences identified	discussion
Heer-Agimont	0	Rural	84	None	Zn levels at this point are rather high. There is EPER identified influences (over French border) local population density is low
Hastière	8	Rural	33	None	Zn level low
Lustin	26	Mainly rural	30	two small towns: Dinant and Yvoir	Zn level low
Dave	29	Rural	29	None	Zn level low
Andenne	50	rural, with Walloon capital city (Namur)	44	Namur: 106213 inhabitants, limited industry, mainly services and administration	Rise in Zn : + 15 µg/l total
Engis	75	Engis area is highly industrialised, heavy industry	129	EPER sources: -Prayon SA (fertiliser production): 28,4 T/y -Several historically contaminated industrial sites along river	-Prayon SA is largest source for Zn emission to water in Belgium (EPER) -Rise in Zn level: + 87 µg/l
Liège	90	Highly industrialised and densely populated area	106	Town: Seraing, 60579 inhabitants. EPER sources: -Cockerill-Sambre (steel): 6,7 T/y, a.o. -the Vesdre river, characterised by high natural zinc content, gives in the Ourthe and directly in the Meuse at Liège (see 3.3.5.)	-Steelworks at Seraing are EPER source Nr 4 for Belgium -Several smaller (0,2-1 T/y) point sources identified by EPER -Zn level: high
Visé	106	Highly industrialised densely populated area	75	Towns: -Liège: 185488 inh, -Herstal: 36549 inh., -EPER sources: Cockerill-Sambre steel): 6,8 T/y,	-Steelworks at Herstal are EPER source Nr 3 for B. -Zn level medium-high
Eijsden	111	Rural, limited industry	78	None identified	-Reference point B-NL border (see RAR) -Influence from industrial area Liège apparent (<10 km upstream) -Zn level medium-high
Lanaken	123	Rural Limited industry Low density	87	EPER source: -Sappi Lanaken Mill (pulp & paper): 536 kg/y At Lanaken, the river Geul	Zinc level higher compared to Eijsden : + 9 µg/l -Influence from the Geul

		population		enters the Meuse. This river has a high natural Zn content due to the local geology and carries historical contamination from old mining activity.	river apparent.
Leut Veurzen Elen Heerenlaak	136	Rural No industry Low density population	55	None identified	Zn level low, (is actually mean over 4 sampling points, which are already averaged in RAR: -Leut: 50 µg/l -Veurzen: 65 µg/l -Elen: 52 µg/l -Heerenlaak: 53 µg/l
Heerenlaak		Rural, City of Maaseik (23 504 inh.)	48	None identified	Zn level low
Kinrooi		Rural, Low density population	106	None identified	-Zn level high -Rise in Zn level compared to Heerenlaak: + 58 µg/l, yet no sources and very low density population. -This point is characterised by exceptionally high variability in Zn level (52 µg/l – 190 µg/l)

This analysis of the Meuse data demonstrates:

- the clear and significant influence of point source emissions on the monitored zinc levels. There is a clear pattern of high zinc levels associated with point source emissions, e.g. in the Engis, Liège (see figure 2 below). Belgium's most important industrial point sources of zinc are located on the Meuse between Enghis and Liège, and further downstream Liège (EPER 2004) e.g.: Prayon SA at Engis is identified as the Nr 1 zinc emitter (28,4T/y); several heavy steelworks with major zinc emissions are located upstream and downstream Liège (Cockeril-Sambre at Seraing, Cockerill-Sambre at Herstal). Several smaller zinc emitors are also identified by EPER (2004) in the Liège area and further downstream (e.g. Sappi pulp & paper mill at Lanaken, Sanifrance factory in Revin, France is an identified source upstream sampling point Agimont). The data also suggest that industrial point sources influence zinc levels downstream over distances of >10 km (e.g.: transect Enghis-Liège-Visé-Eijsden.
- that the influence of tributary rivers with an elevated natural zinc level giving in the Meuse on this transect is also another possible cause of (naturally) elevated levels of zinc: e.g.: the Vesdre (see above) gives in the Ourthe and directly after that in the Meuse at Liège; the Geul, a well-documented zinc-rich river that flows through a natural mineralised area gives in the Meuse at Lanaken.
- that the influence of a large city, without industrial emissions (Namur) on zinc levels is rather limited, compared to the influence from point sources (e.g. sampling point Andenne)

Following TGD guidance, the data points on the Meuse river that are influenced by identified point sources (Enghis, Liège and Visé) are not used for calculating the average 90P zinc concentration over the Walloon transect of the river Meuse. Using the specific Kp value for the

Meuse (based on a suspended matter content of 30 mg/l) a dissolved 90P zinc concentration of 9.9 µg/l is calculated for the Meuse in Wallonia.

Conclusion: the dissolved average 90P zinc concentration of the Meuse transect in Wallonia, excluding the points influenced by identified point source emissions, is useful for the regional risk characterisation.

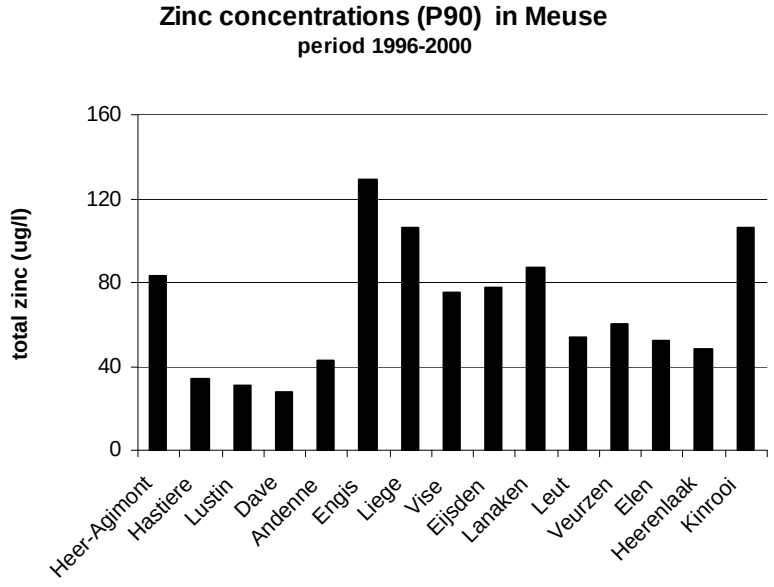

Figure 15 Zinc concentrations in surface water at various sampling points downstream the Meuse river (Belgium and the Netherlands). Data refer to the average 90P value during the period 1996-2000. From RAR.

1.4.6 Flemish region in Belgium

The RAR states that *"the monitoring network in Flanders contains a large number of sampling locations distributed over various types of surface waters in Flanders (670 sampling points in 1999 and 805 sampling points in 2000). Total zinc levels have been analysed and the data show that average 90P values amount to 146 and 110 µg/l for 1999 and 2000, respectively. Zinc concentrations above 100 µg/l are found in 41% (1999) and 27% (2000) of the locations. In 11% (1999) and 8% (2000) of the sampling locations the zinc concentrations are found to be above 200 µg/l. An important conclusion is that the measured regional zinc levels in surface water for Flanders are substantially higher than those for the Netherlands (90 P value of 41 µg/l")*. This difference can be explained by the fact that the sampling set in Flanders includes locations directly influenced by point sources and/or historical contamination" (EURAS 2004), whereas the influence of these sources on the Dutch regional data is much less.

However, more recent data is available for Flanders, and is especially important for consideration in this assessment because of the considerable investments made in recent years to increase the STP connection rates, which are known to have been very low in Flanders (38.1% in 2000; up to 57% in 2002) and which are thought to influence significantly the zinc levels in surface waters.

The VMM data for 2002-2003 includes 12,776 zinc measurements from 1,012 sampling sites. Monitoring stations are generally located for the purpose of verifying the water quality for fishing, for the production of drinking water, and for monitoring the sites located up and downstream of discharges of municipal waste water treatment plants and important industrial activities.

Because of the nature of this dataset, a thorough statistical analysis of the dataset was conducted (EURAS, 2004), including an outlier analysis according to TGD recommendations[71], and an evaluation of sampling sites influenced by point sources.

The evaluation of the point source influence in the dataset was conducted by attributing a statistical selection of sites that are not representative for the regional environment. Sampling sites were allocated as influenced by a point source(s) using the following criteria:

- sites with more than 1 outlier in both the year 2002 and 2003
- sites with more than 20% of the measurements above the outlier cut-off
- sites with a 90P> the average + (3*standard deviation), i.e. 90P > 314 µg/L (95.7 + (3*73.0).

Table 6 summarizes the results of the analysis. The comprehensive review of the analysis is in the EURAS report (EURAS 2004)

Table 6 Outlier and point sources analysis on the Flemish dataset 2002-2003.

Outlier limit value	312 µg/L
No. of individual outlier-values	289 (2.3%)
Range	313 – 11,810 µg/L
Total no. of sampling sites	1012
No. of sites with outliers	78
No. of excluded sites	48 (4.7%)[a]
Site-specific 90Ps excl. outliers:	
- range	2.5 – 234.5 µg/L
- average 90P	**68.5 µg/L**
No. of sites with a 90 P > 100 µg/L	16.9%
No. of sites with a 90 P > 200 µg/L	1.5%

[a] removed using the point source influence criteria

It is noted that this statistical evaluation of the data for outliers does not guarantee the identification of all sampling points which are influenced by point source emissions and/or historical contamination. To assess this, further detailed analysis of the database is needed.

The average suspended matter concentration in Flanders is 39 mg/L (based on VMM database). Using this value, the average 90P value of 68.5 µg total zinc/L corresponds to a value of 12.9 µg/L expressed as dissolved zinc.

- Zntot = Zndiss * (1+Kp.Cs.10^{-6})
- or 68.5 µg/l = Zndiss* (1+110000 l/kg * 0.000039 kg/l)
- or 68.5 µg/l/[5,29] = Zn dissolved = 12.9 µg/l

Taking into account the limitations of the analysis of the Flanders dataset, this 90P value is considered useful for the regional risk characterisation, with some reservation.

71 TGD recommendation for an outlier analysis uses the following statistical approach:
$\log(Xi) > \log(p_{75}) + K(\log(p_{75}) - \log(p_{25}))$ where X_i is the concentration, above which a measured value may be considered an outlier, p_i is the value of the i^{th} percentile of the statistics and K is the scaling factor. A factor of 1.5 for K is used in most statistical packages.

1.4.6.1 Outlier analysis on the Adour-Garonne Data

In order to compare the methodologies for data relevancy as used for most of the databases versus the one used as described above by EURAS for the Flemish database, the 'EURAS' outlier analysis was conducted on the Adour-Garonne database. This database was chosen because it is known to contain several high zinc levels from point source emissions and historical contamination.

Table 7 summarizes the results of comprehensive review (performed by IZA-Europe).

Table 7 Outlier and point sources analysis on the Adour-Garonne database 2000-2002.

Outlier limit value	725 µg/L
No. of individual outlier-values **Range**	17 of 879 (1.9%) 803 – 3490 µg/L
Total no. of sampling sites	102
No. of sites with outliers	5
No. of excluded sites	5 (4,9%)[a]
Site-specific 90Ps excl. outliers: **- range** **- average**	 10 – 425 µg/L **46.3 µg/L (3 years pooled together)** **2000 – 44.5 µg/L** **2001 – 57.1 µg/L** **2002 – 38 µg/L**
No. of sites with a 90 P > 100 µg/L	10 (9.8%)
No. of sites with a 90 P > 200 µg/L	6 (5.9%)
3rd criteria : the average + (3*SD)	813 µg/L

The resulting average 90Ps excluding outliers from the 2 methodologies are comparable; the EURAS methodology provides the same average 90Ps for the 2000 and 2002 datasets, however, it is different for the 2001 dataset (57.1 for the EURAS method versus 31 µg/L).

It is noted that the "EURAS" method does not identify all sampling points which are influenced by point source emissions and/or historical contamination. Table 3 describes the stations identified as having an influence from point sources and historical contamination (section 2.1.3.4. description for the Adour-Garonne region).

The "EURAS" outlier analysis does not identify the stations at the tributaries of the Rioux Mort, Lot à St-Pierre Toirac and Lot à Capdenac, as outlier stations. However, these stations are clearly influenced by the high inputs from this river. The explanation is the very high outlier threshold value that results from the Adour-Garonne database, and this in turn may be a result of the very high detection limits that cause the database to be skewed towards high values.

In conclusion, this exercise demonstrates that the EURAS outlier analysis is an alternative method for determining data points influenced by point sources and historical contamination, especially for large databases with mixed influences (point sources, historical contamination, diffuse emissions, etc) that are otherwise very labour intensive to analyse. The experience shows that the EURAS approach, as developed for the Flanders case, does not ensure the exclusion of data points influenced by e.g. point sources. This approach has its limitations in that it is highly

dependent on the data distribution and the resulting outlier threshold value. This approach and the further interpretation of the results should therefore be used with caution.

1.4.7 Other databases not used

1.4.8 European database of Denzer et al., 1999

The RAR (2004) states that "In the report *'Revised Proposal for a List of Priority Substances in the Context of the Water Framework Directive (COMMPS Procedure)' from Denzer et al. (1999) monitoring data (water and sediment) were collected for a large number of chemicals (including zinc) in the EU. Data are from 1994-1998. For surface waters, zinc dissolved measurements were received from sampling stations in Austria, Germany, Spain, UK, Italy and the Netherlands. Zinc total measurements are available in the database for sampling stations in Austria, Belgium, Germany, Finland, UK, Ireland, Portugal and Sweden. Sediment data are from Austria, Belgium, Germany, France and UK (2854 measurements from 495 sampling stations). From the total number of 11,948 measurements (340 sampling stations) for total zinc ultimately 10,809 (306 sampling stations) were used for the aggregated 90P calculation. Data were discarded if the zinc concentration was found to be below the detection limit in combination with a relatively high detection limit for that particular sampling station (for details see original report). This implies that 10% of the measurements and sampling stations were removed from the database before estimating the 90P value for total zinc. For dissolved zinc 2528 (170 sampling stations) from the original 3144 (300 sampling stations) had been discarded based on similar criteria concerning the detection limit. About 20% of the measurements and 40% of the sampling stations were thus left out for dissolved zinc. This means that, especially for dissolved zinc, a bias has occurred towards a higher overall 90P value due to omitting a significant number of sampling stations with (relatively) low zinc levels. It has to be noted, however, that data below the detection limit in combination with a (relatively) low detection limit remained in the data set… A number of German data from 1998 are presented individually in Table 8. The Denzer et al. (1999) database contains different German data than LAWA 1998 data. Only very few measurements, if any, from the LAWA network may be included in the Denzer et al. (1999) database" (RAR 2004).*

Conclusion: The Denzer et al (1999) dataset is highly heterogeneous with different detection limits, sampling procedures and site selections applied in different countries. Furthermore, a significant part of the data (<DL) has been omitted for calculating P90 as in the RAR (2004). For these reasons, the Denzer dataset should not be used as such for the risk characterisation.

Table 8 Summary of data useful for the regional scenario for risk characterization of diffuse emission patterns

Table 8. Summary of data useful for the regional scenario for risk characterization of diffuse emission patterns

Database	Source	# Sampling stations	Sampling Years	Total Zinc 90P µg/L*	Comment
Calculated					
NL Region		-	-	12.2	To be used as a **REFERENCE** dataset. Based on an extensive inventory of updated emissions in NL. As such, the modelled PEC derived from it reflects the current exposure with a high degree of accuracy.
Monitored 90P					
Rhine	CIW/RIZA	1	1998	26	Regular monitoring programme, however only 1 sampling point on the Rhein at Lobith
Meuse at B-NL border (Eijsden)	CIW/RIZA	1	2001	8,7dissolved	Levels in the Meuse at Eijsden. Levels in the Meuse have decreased significantly in the last years. It is therefore important to use the most recent data in order to assess current zinc levels.
Scheldt	CIW/RIZA	1	1998	24	Is represented by regular monitoring at Schaar van Ouden
Regional waters	CIW/RIZA	*250*	1998	41	Sampling stations are selected to avoid influence from point sources.
Regional waters	RIZA (2004)	*250*	2001-2003	39	Most recent data on regional waters in the Netherlands, excluding stations influenced by historical contamination.
State waters	CIW/RIZA	*?*	1998	40	Average 90P for a grouping of main Dutch waters (including the Rhine, Meuse, Scheldt and other large inland surfacewaters).
Swedish watercourses					
Swedish watercourses	Landner and Lindeström, 1998	76	1998 - 1996	12	It is explicitly mentioned that no influence from point sources. Error in Feb'04 Draft RAR: data are total zinc levels, not dissolved levels.
Northern Swedish Lakes	Landner and Lindeström, 1998	384	1989 - 1996	3.6	It is explicitly mentioned that no influence from point sources. Error in Feb'04 Draft RAR: data are total zinc levels, not dissolved levels.
Southern Swedish Lakes	Landner and Lindeström, 1998	781	1989 - 1996	6.4	It is explicitly mentioned that no influence from point sources. Error in Feb'04 Draft RAR: data are *total* zinc levels, not dissolved levels.
France					
Seine-Normandie	Réseau National des Données sur l'Eau RNDE, 1998	35	2000-2002	4.7	Data is considered representative of the Seine-Normandie river bassin and no influence from major point sources have been identified.
Rhin-Meuse	Réseau National des Données sur l'Eau RNDE, 1998	6	2000-2002	15.2	Data is not extensive and is a poor representation of the Rhin-Meuse river bassin. Furthermore, influence from point sources has been identified. However, it is used as a value representing the Rhin-Meuse river basin area in France.
Rhône Méditerrannée	Réseau National des Données sur l'Eau RNDE, 1998	15	2000-2002	7.4	Data is considered representative of the Rhône-Mediterranée river bassin. Older data (from 1996) had very high DL (50 µg/L), however, this is no longer an issue with the more recent dataset of 2000-2002.
Belgium					
Scheldt River basin	Direction Générale des Resources Naturelle et de l'Environnement, Direction des eaux de surface, 2001	23	2001	8,1 dissolved	Data is extensive and considered representative of the Scheldt river basin in the Walloon region. However, measuring points influenced by major point sources, historical mining areas and naturally high containing zinc levels have been identified and removed. DL is high (25 µg/L) and the 1/2 DL is used for the 90P calculation.
Meuse-Seine River Basin (West of the Meuse)	Direction Générale des Resources Naturelle et de l'Environnement, Direction des eaux de surface, 2001	111	2001	7.7 dissolved	Data is extensive and considered representative of the Scheldt river basin in the Walloon region. However, measuring points influenced by major point sources, historical mining areas and naturally high containing zinc levels have been identified and removed. DL is high (25 µg/L) and the 1/2 DL is used for the 90P calculation.
Meuse River Basin (East of the Meuse)	Direction Générale des Resources Naturelle et de l'Environnement, Direction des eaux de surface, 2001	29	2001	6.5 dissolved	Data is extensive and considered representative of the Scheldt river basin in the Walloon region. However, measuring points influenced by major point sources, historical mining areas and naturally high containing zinc levels have been identified and removed. DL is high (25 µg/L) and the 1/2 DL is used for the 90P calculation.

Database	Source	# Sampling stations	Sampling Years	Total Zinc 90P µg/L*	Comment
Meuse River in Belgium	CIW/RIZA	15	2001	9.9 dissolved	Levels in the Meuse have decreased significantly in the last years. It is therefore important to use the most recent data in order to assess current zinc levels. River bank values at Engis influenced by old abandoned metallurgical industry was omitted.
Flanders region	VMM, 2002 - 2003		2002-2003	12.9 dissolved	Most recent data available is carried through to the risk characterization. An outlier analysis and identification of point sources have been conducted and removed for further calculation of 90P. See EURAS 2004 (annex 1) for details.
Germany					
Rhein River Basin	LAWA	34	2001	31.4	Data is extensive and considered representative. Some measured data influenced by documented historically contaminated areas were removed. Errors and data with DL>50 were also removed.
Elbe River Basin	LAWA	38	2001	28.7	Data is extensive and considered representative. Some measured data influenced by documented historically contaminated areas were removed. Errors and data with DL>50 were also removed.
Danube River Basin	LAWA	14	2001	15.5	Data is extensive and considered representative. Some measured data influenced by documented historically contaminated areas were removed. Errors and data with DL>50 were also removed.
Weser River Basin	LAWA	5	2001	45.3	Number of data is rather limited but considered representative. Some measured data influenced by documented historically contaminated areas were removed. Errors and data with DL>50 as well as influenced by 1 point source were removed.
Ems River Basin	LAWA	4	2001	30.3	Number of data is rather limited but considered a representation of the Ems river basin.
Nordic Countries					
Nordic Lakes	NIVA, 2001	~4000	1995	Finland: 4.4µg/l Norway: 5.9µg/l Sweden: 5.3µg/l	NIVA report SNO 4391-2001 is the most appropriate database of zinc across Nordsic Lakes. Data is extensive however, further analysis is needed in order to correctly consider bioavailability (IZA, 2003).

*Unless otherwise indicated, the 90P represents an average 90P across sampling stations.

Table 9 Summary of data that was separated for the regional 90P calculation

Table 9. Summary of data that was separated for the regional 90P calculation

Categories and description of sampling points removed	90P total Zn µg/L	Comments
Historically contaminated areas (e.g. old mining areas)		
Dutch RIZA 2001-2003 dataset		
Historical contamination in south-eastern Holland		
Geul, Dommel and Jeker rivers	326	Old extensive mining zone at the border of Belgium and the Netherlands
German LAWA data 2001		
Historical metal industry in the Weser catchment (Harz region):		
Aller at Langlingen	131	Via Oker and Innerste rivers, the Aller receives zinc from the historic mining region of Harz
Oker	182	Within the catchment of the old mining area Harz (mining activities during the last century)
Historical metal industry in the Rhein catchment:		
Erft	116.8	Erft receives water from the Veybach which comes from the historical mining area at Mechernich. This geological zinc content can be measured down the whole river to river Rhein
Ruhr	49.4	Catchment area of the river Rhein where zinc content is related to historical mining activities. A congregation of electrogalvanizing (plating) industry is also along sections of the Ruhr
Sieg	364, 40	Catchment area of the river Rhein where zinc content within suspended matter is elevated because of geological effects and historical mining activities
Wupper	65.6	High zinc content in the lower Wupper resulting from historical pollution found in sediments dating back from early industrialisation with galvanising and metals industry
Historical mining industry in the Elbe catchment (Sachsen region):		
Freib Mulde	110	High zinc content caused by ores (natural) and by historical mining activities
Vereinig Mulde	97	High zinc content caused by ores (natural) and by historical mining activities
Zwick Mulde	57	High zinc content caused by ores (natural) and by historical mining activities
Dessau Mulde	76	Near Bitterfeld-Wolfen remobilisation of metals from sediments at former industrial sites
Historical mining in the Elbe catchment (Thüringen region):		
Weisse Elster at Ammendorf	57	High zinc content caused by historical contamination (U-mining by Soviet company Wismut). Also receives brown coal mining waste waters
Saale - Gross Rosenburg	111	High zinc levels found downstream of Halle (historical metal mining) at Rosenburg. Also influence from a viscose production plant (expected to lower emissions)
Historical mining in the Maas catchment		
Rur - Einruhr	86.1	Former mining in the area around Aachenand Stolberg, geogenic zinc content, metals industry and historical pollution
Walloon Database		
Historical mining in Northern France:		
Espierre et Grande Espierres river at Estampuis and Spiere Helkjin	219, 1354	Downstream a region highly contaminated from historical industrial activities in Northern France (metallurgical, inorganic and textile industries)
Haine river at St-Vast and Hensies	63, 170	Downstream the industrial region of the French city Valenciennes (historical metallurgical and inorganic fertilizer industries)

Categories and description of sampling points removed	90P total Zn µg/L	Comments
Historical coal and steel in Charleroi		
Sambre river and small tributaries (Tintia, Piéton) at Mornimont, Viesville and Gouy-les-Piéton	102, 197, 173	Downstream highly industrial zones of Charleroi. This area is known for its historical coal and steel industry. Most facilities are closed down, but still a few functional steel industries (EPER)
Historical mining in Eastern Belgium		
Gueule River in Plombières	593	Metallurgical region with surface zinc ore and known for its intensive historical mining and refining.
Flanders database		
Historical contamination in Noorderkempen region		A number of sampling points influenced by historical contamination have been identified. (EURAS 2004 in Annex 1)
French database		
Historical mining in France		
Adour-Garonne: sampling site at Viviez no.5093550	2800	Downstream of the old metalurgical industrial site of Viviez, with documented high historical pollution (metal industry activity since end of the 19th century).
Point sources		
French RNDE data		
Adour-Garonne: Industrial zone of Toulouse no.5163450, 5015300	326, 52,8	Downstream of industrial zone of Toulouse (various direct zinc emissions EPER)
Adour-Garonne: Industrial zone of Bordeaux no.5073000	250	Downstream of industrial zone of Bordeaux (various direct zinc emissions EPER)
*German LAWA data *(some sampling points were excluded already from historical contamination)*		
Weser at Nordenham	181	Downstream 2 identified zinc emitters; zinc smelter already considered in RAR local scenario and Kronos Titan GmbH & Co at Nordenham
Ruhr*	49.4	Sampling station influenced by nearby zinc emitter Sachtleben in Duisburg (largest zinc emitter in Germany), as well as Thyssen Krupp Stahl, DK Recycling, M.I.M, ect.. for a total of nearly 60T of zinc emitted by industries in the Duisburg area
Aller at Grafhorst	79	Downstream an identified zinc emitter; Volkswagen at Wolfsburg
Oker*	182	Contamination coming from both historical pollution and current identified emitters downstream (Harz Metall and Salzitter AG Werk in Goslar and Salzitter)
Wupper*	65.6	Sampling station influenced by zinc emitter identified (Bayer at Leverkuzen), and to some extent by other nearby industries (Bayer at Dormagen, Henkel at Dusseldorf, Bayer at Krefeld).
Weisse Elster at Ammendorf*	57	Sampling station influenced by the large zinc emitter identified in Elsterberg (ENKA GmbH).
Walloon Database		
Dendre River in the Scheldt River Bassin (Ath and Deux-Acren)	256, 166	Downstream of the ZnO producer Floridienne, already considered in the local scenario of the ZnO risk assessment
Meuse river at Engis	118	In the Liège region, downstream the largest Belgian zinc surface water emitter Prayon SA (EPER)
Argenteau River at Visé	156	Downstream Industrial region of Liège (various metallurgical and steel industry emittors; EPER 2004)
Flanders database		
Flanders datasets for 2002-2003		A number of sampling points influenced by point sources have been identified. (EURAS 2004 in Annex 1)
Elevated Natural Background		
Walloon Database		
Vesdre region of the Meuse River Bassin	91	River waters are classified as 'Natural waters' by the Ministère de la Région Wallonne. This region is characterized by naturally acidic rivers (pH ~5), which explains the naturally high zinc levels.

Categories and description of sampling points removed	90P total Zn µg/L	Comments
Other reasons for excluding data for the regional 90P calculation		
High detection Limit in the database		
German LAWA 2001 Data		
Sieg-Netphen	<50	Not used because no indication of true value and distorts 90P calculations.
Main	<50	Not used because no indication of true value and distorts 90P calculations.
Lenne	<50	Not used because no indication of true value and distorts 90P calculations.
Nidda	<50	Not used because no indication of true value and distorts 90P calculations.
Schwarzenbach	<50	Not used because no indication of true value and distorts 90P calculations.
Ratzdorf Neisse	<50	Not used because no indication of true value and distorts 90P calculations.
Frankfurt Oder	<50	Not used because no indication of true value and distorts 90P calculations.
Hohenutzen Oder	<50	Not used because no indication of true value and distorts 90P calculations.
Fulda	<50	Not used because no indication of true value and distorts 90P calculations.
France RNDE		
French 1996 data		The 1996 data for this French river bassin was not used and replaced by more recent measurments obtained with better analytical techniques and lower detection limits.
Adour-Garonne 2000-2002	174	Not used because the calculated 90P is greatly skewed from discarding 39% of the data reported at high DL >50 µg/L.
Artoie-Picardie 2000-2002	80	Not used because the calculated 90P is greatly skewed from discarding 50% of the data reported at high DL >50 µg/L.
European watercourses, Denzer et al., 1999	59.2	Extensive data from 1994-1998 (10,809 measurements) however levels below the DL are not considered (includes 30% of the dataset), and therefore this database does not represent current zinc levels in EU waters
Other datasets not used		
Norwegian rivers (1998)	0.6 - 50.2	The 90P is not reported. Reference is only made min-med-max values.
Nordic countries lakes (1995) - Denmark data	12.6	Refers to a 75P and the 90P is not reporterd.
Modelled data of the EU Region	16.8	Default EU region not justified since well documented, realistic data on regional scenarios are available
Major German and Swiss Rivers, Weijden, Middelburg, 1989	10 - 152	Data is from 1975 to 1990) and is not representative of present zinc concentrations. More recent data is available
German LAWA 2001 Data		
Oder river basin	35	Only 1 data : not considered representative for a region
Maas river bassin	74.5	Only 2 data ; not considered representative for a region

1.4.8.1 Assessing the influence of emission sources on water quality downstream

In section 2.1.3., a number of waters influenced by identified industrial point sources of zinc were separated from the regional dataset since they are defining a local, not a regional scenario. During the discussions of the RAR, the degree and spatial scale of the impact of a point source downstream a river has been an issue of debate.

Recently, the GREAT-er model, originally developed for quantifying the impact of emissions of organic substances on water quality in river systems has been applied to zinc in the catchment of the German river Ruhr (Klasmeier et al, 2006).

This analysis allows for the detailed quantification of the different sources of zinc to this basin, at a local scale and further downstream throughout the entire river catchment.

As an example, the influence of a number of different zinc sources on the zinc concentrations in the river basin is presented in Figure 3:

- a) local geology (elevated natural background): due to zinc mineralisations in the Eastern part of the catchment, Zn concentrations in the waters are elevated (> 20 µg/l). This natural input of zinc dominates the zinc profiles over the entire basin and presents > 50 % of zinc inputs to the basin (Figure 3a).
- b) local point source emissions[72] influence zinc levels clearly at the site of emission and further downstream, over distances of > 50 km (Figure 3b).
- c) diffuse emission from households (Figure 3c) have a rather limited effect on zinc concentrations (< 2,5 µg/l).

For further results and details of the study see Klasmeier et al, 2006.

72 Point source emissions are 2.8 T/y (old mining sites) and 0.86 T/y (industrial sources)

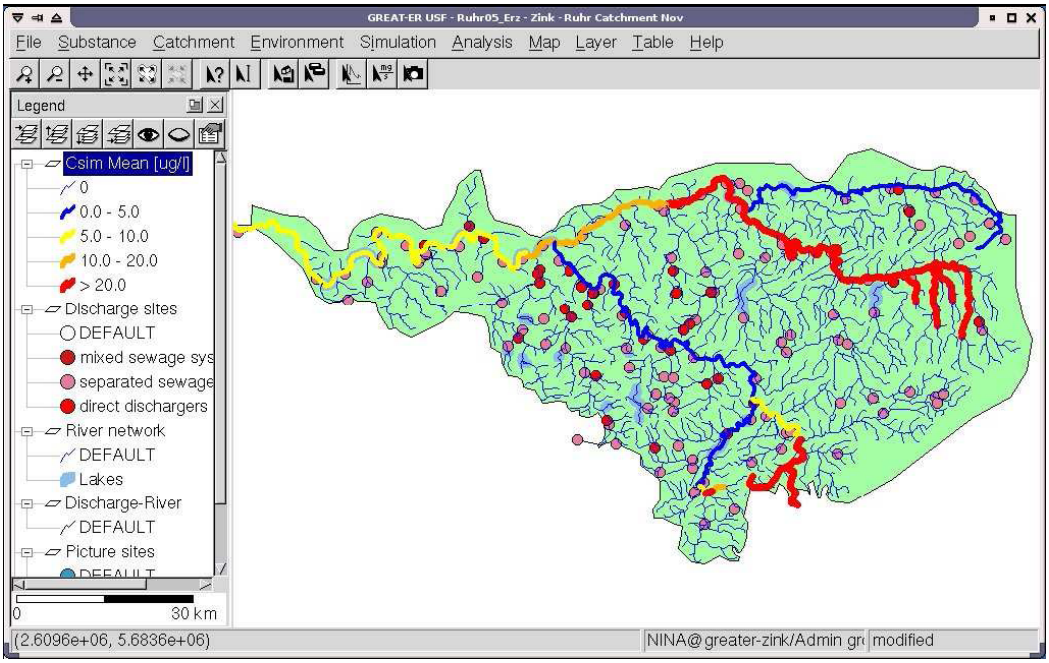

Figure 16 Influence of different zinc sources on the zinc concentrations in the Ruhr river basin : a) local geology (elevated natural background)

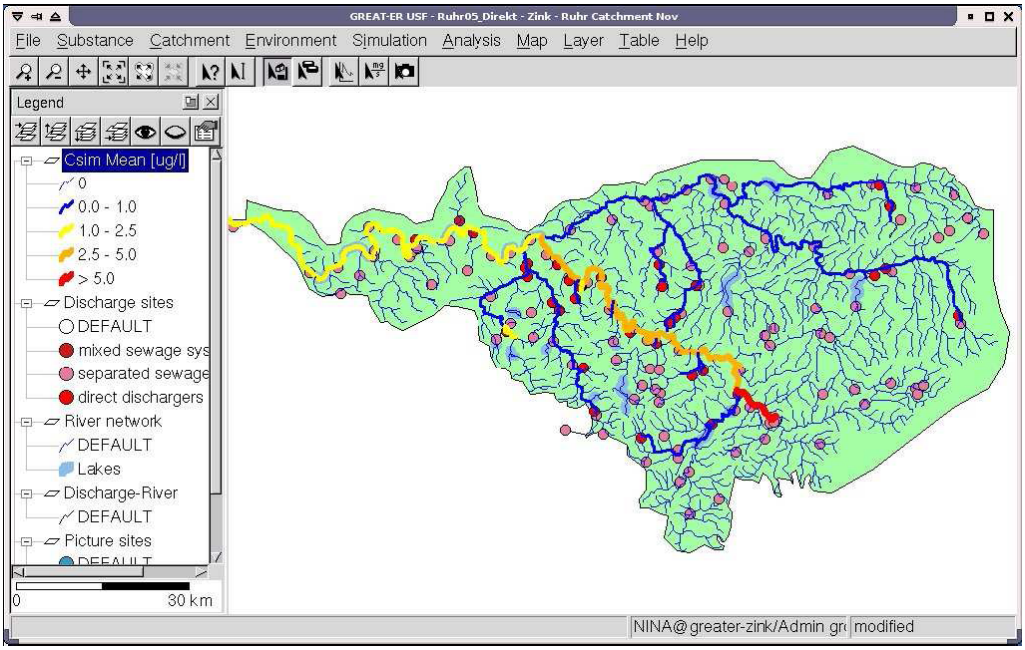

Figure 17 Influence of different zinc sources on the zinc concentrations in the Ruhr river basin : b) local point source emissions

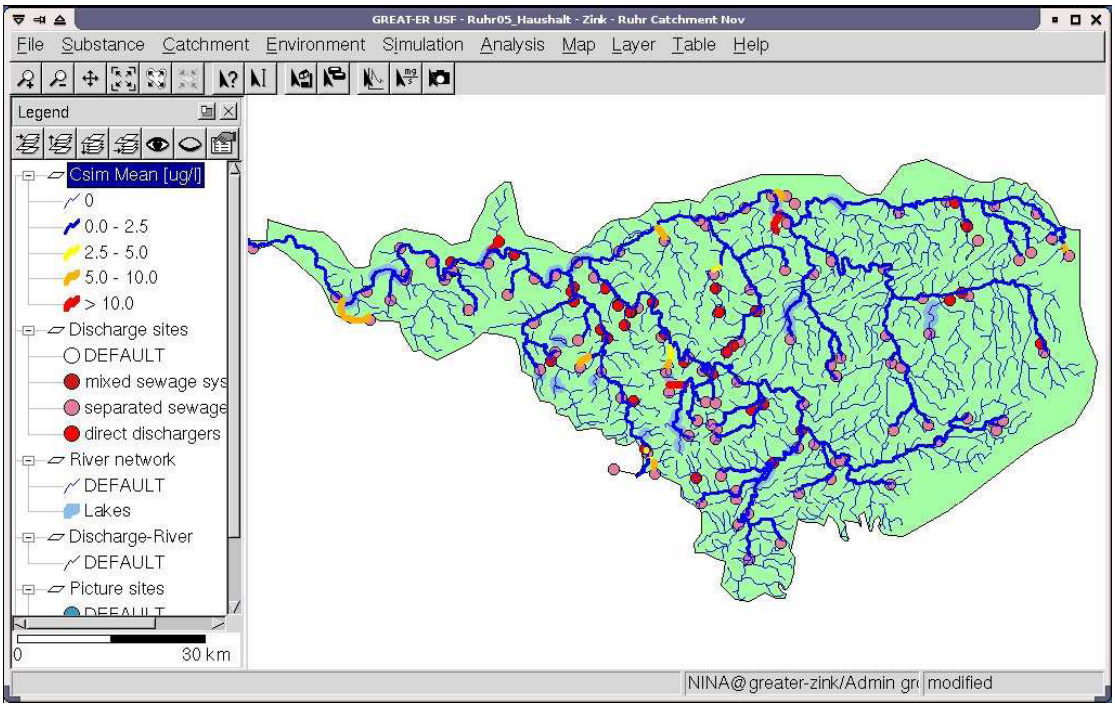

Figure 18 Influence of different zinc sources on the zinc concentrations in the Ruhr river basin : c) diffuse emission from households

Analyses as presented above of the GREAT-er type have great potential for determining the degree and scale of influence of a given source throughout a river basin. The analysis on the Ruhr river demonstrates a number of points that are very relevant for the zinc RAR, e.g.:

- the potential importance of elevated background due to local geological conditions (this is the main source explaining the zinc levels throughout this basin),
- the spatial scale of the influence of point source emissions (influences are stretching over a distance of > 50 km in this example
- diffuse emissions from the use of zinc products have a limited effect on the zinc concentrations observed in this basin. Elevated levels of zinc are related to:
    - local geology resulting in elevated background
    - influence of industrial point sources

These observations are confirming the conclusions drawn under section 3.1

1.4.9 Calculating a PEC zinc added, bio-available concentration.

Both the modelled and relevant monitoring data should be used for the risk characterisation. Considering a) that the modelled exposure assessment is based on updated and extensively detailed information on the model region (The Netherlands) and b) the difficulties in interpretation and uncertainties related to the monitoring data (in terms of e.g. background correction, influence by point sources,…), the modelled PEC is used as a reference for the validity of the monitoring data and the exposure assessment. It is noted that this is in accordance to the TGD (2002) where both data sets are considered to be complementary to each other in the complex interpretation and integration of the exposure data (TGD 2002 page 13).

The exposure information, considered relevant for the risk characterisation (Table 8) is further handled to give the $PEC_{add, bioavailable}$, by:

- Subtracting the 3-12 µg Zn/l background range from the total zinc concentrations[73], corresponding to 1.1-4.4 µg Zn (dissolved)/l (using Kp of 2.7 except when specified otherwise)
- Calculating the dissolved zinc concentration from the total zinc concentration by dividing with the Kp factor (default 2.7 except for NL waters and other specified cases)
- Correcting for the bioavailable fraction, according to the procedure, outlined in the RAR zinc, and referring to either worst-case abiotic conditions in the water, or average conditions.

This results in the 4 following combinations:

-subtraction of worst case background * worst case bioavailability

-subtraction of average background * worst case bioavailability

-subtraction of worst case background * average bioavailability

73 Not for the modelled PEC, since this already an "added" concentration. For the dissolved concentrations of the Meuse river, a dissolved background of 4.3 µg/l is subtracted, derived from the total background of 12 µg/l, and considering the Meuse-specific Kp of 16 mg/L.

-subtraction of average background * average bioavailability
For reasons of clarity, only the outer boundaries of the PECs ("worst case * worst case" and "average * average" are presented in Table 10.

Table 20 Summary of data useful for the regional scenario for risk characterization of diffuse emission patterns. 90Ps presented as Zn added, bioavailablefraction using realistic worst case and average scenarios.

Table 10. Summary of data useful for the regional scenario for risk characterization of diffuse emission patterns. 90Ps presented as Zn added, bioavailable fraction using realistic worst case and average scenarios

Database	Source	# Sampling stations	Sampling Years	Total Zinc 90P µg/L*	Total added zinc low bckgd (µg/L)	Total added zinc ave bckgd (µg/L)	Dissolved added zinc low bckgd (µg/L)	Dissolved added zinc average bckgd (µg/L)	BioF RWC	BioF Ave.	Dissolved added bioavail Zn rwc low bckgd (µg/L)	Dissolved added bioavail Zn average average bckgd (µg/L)
Calculated												
NL Region		-	-	12.2	9.2	0.2	3.4	0.1	na	na	na	na
Monitored 90P												
Rhine	CIW/RIZA	1	1998	26	23	14	5.5	3.3	1	0.8	5.5	2.7
Meuse at B-NL border (Eijden)	CIW/RIZA	1	2001	8,7dissolved			7.3	5.9	1	0.7	7.3	4.1
Meuse at B-NL border (Eijden)	RIZA (2004)	1	2002	14 (dissolved)			12.6	11.2	1	0.7	12.6	7.8
Scheldt	CIW/RIZA	1	1998	24	21	12	5.0	2.9	na	na	na	na
Regional waters	CIW/RIZA	*250*	1998	41	38	29	9.0	6.9	0.8	0.3	7.2	2.1
Regional waters	RIZA (2004)	*250*	2001-2003	39	36	27	8.6	6.4	0.8	0.3	6.9	1.9
State waters	CIW/RIZA	*?*	1998	40	37	28	8.8	6.7	0.8	0.3	7.0	2.0
Swedish watercourses												
Swedish watercourses	Landner and Lindeström, 1998	76	1998 - 1996	12	9	-	3.3	-	na	na	na	na
Northern Swedish Lakes	Landner and Lindeström, 1998	384	1989 - 1996	3.6	0.6	-	0.6**	-	na	na	na	na
Southern Swedish Lakes	Landner and Lindeström, 1998	781	1989 - 1996	6.4	3.4	-	3.4**	-	na	na	na	na
France												
Seine-Normandie	Réseau National des Données sur l'Eau RNDE, 1998	35	2000-2002	4.7	1.7	-	0.6	-	na	na	na	na
Rhin-Meuse	Réseau National des Données sur l'Eau RNDE, 1998	6	2000-2002	15.2	12.2	3.2	4.5	1.2	1	0.6	4.5	0.7
Rhône Méditerrannée	Réseau National des Données sur l'Eau RNDE, 1998	15	2000-2002	7.4	4.4	-	1.6	-	na	na	na	na

Database	Source	# Sampling stations	Sampling Years	Total Zinc 90P µg/L*	Total added zinc low bckgd (µg/L)	Total added zinc ave bckgd (µg/L)	Dissolved added zinc low bckgd (µg/L)	Dissolved added zinc average bckgd (µg/L)	BioF RWC	BioF Ave.	Dissolved added bioavail Zn rwc low bckgd (µg/L)	Dissolved added bioavail Zn average average bckgd (µg/L)
Belgium												
Scheldt River basin	Direction Générale des Resources Naturelle et de l'Environnement, Direction des eaux de surface, 2001	23	2001	8,1 dissolved			7.4	5.3	1	0.7	7.4	3.7
Meuse-Seine River Basin (West of the Meuse)	Direction Générale des Resources Naturelle et de l'Environnement, Direction des eaux de surface, 2001	111	2001	7.7 dissolved			7	4.9	na	na	na	na
Meuse River Basin (East of the Meuse)	Direction Générale des Resources Naturelle et de l'Environnement, Direction des eaux de surface, 2001	29	2001	6.5 dissolved			5.1	3.7	na	na	na	na
Meuse River in Belgium	CIW/RIZA	15	2001	9.9 dissolved			8.5	7.1	1	0.7	8.5	5.0
Flanders region	VMM, 2002 - 2003		2002-2003	12.9 dissolved			12.2	10.1	1 / 0.6	0.6 / 0.3	12.2 / 7.3	6.1 / 3.0
Germany												
Rhein River Basin	LAWA	34	2001	31.4	28.4	19.4	10.5	7.2	1	0.8	10.5	5.7
Elbe River Basin	LAWA	38	2001	28.7	25.7	16.7	9.5	6.2	0.6	0.5	5.7	3.1
Danube River Basin	LAWA	14	2001	15.5	12.5	3.5	4.6	1.3	na	na	na	na
Weser River Basin	LAWA	5	2001	45.3	42.3	33.3	15.7	12.3	0.8	0.6	12.5	7.4
Ems River Basin	LAWA	4	2001	30.3	27.3	18.3	10.1	6.8	0.7	0.4	7.1	2.7
Nordic Countries												
Nordic Lakes	NIVA, 2001	~4000	1995	Finland: 4.4µg/l Norway: 5.9µg/l Sweden: 5.3µg/l	1.4 - 2.3	-	1.4 - 2.3 **	-	na	na	na	na

*Unless otherwise indicated, the 90P represents an average 90P across sampling stations.

"-" indicates that the dissolved added zinc is close to '0', so levels are assumed at near background.

** Total = dissolved for Scandinavian Lakes (conservative assumption that suspended matter is very low in Nordic lakes (same may not be true in rivers).

1.5 REGIONAL SEDIMENTS

1.5.1 $PEC_{modelled, regional}$

The modelled PEC is based on an extensive inventory of emissions in the selected model region (The Netherlands). This inventory is very detailed and all emissions are updated. As such, the modelled PEC reflects the current exposure in the selected region with a high degree of accuracy.

Moreover, for the sediment compartment, modelling of emissions for estimating current exposure has the major benefit that it is based on the emissions, related to the present-day production and use of the substance. The modelling approach by definition excludes influences from the past. Particularly for the sediment, which is a "sink" compartment reflecting not only the emissions from the present but also those from the past, this is a major benefit as compared to exposure estimates based on measured zinc concentrations, since the latter are inevitably "contaminated" by the emissions from the past.

Given the well-documented, significant reduction in zinc emissions to the surface water observed over the last decades, the loading of sediments with zinc has also been much higher in the past. Taking into account also the continuous re-distribution of sediment layers over the river profile, this historical contamination leads to a continuous re-deposition in fresh sediment layers of zinc related to past emissions. In terms of risk, this phenomenon results in an over-estimation of the current loading of sediments with zinc, and an over-estimation of the exposure, related to the present-day production and use of the metal, which is the objective of the risk assessment under 793/93/EEC.

Considering the fact that it is based on a complete and updated dataset of present-day emissions resulting from the production and use of the substance, the modelled regional PEC is used as a reference for the other exposure information and as the main estimate of exposure for risk characterisation.

The model region The Netherlands can in terms of density of population, industrial and agricultural activity and zinc use, be considered as a realistic worst case for the EU. The additional information contained in the RAR on the emissions observed in other EU regions confirms the NL analysis and its relevancy as a realistic worst case for the EU.

In conclusion, the modelled PEC of 504mg/kg DW, derived based on a well-documented emissions inventory for the model region, is used as the main reference in the risk characterisation.

1.5.2 PECmonitored, regional

It is most important for the sediment, which is a "sink" compartment, that the monitoring data should be critically evaluated before being used for an assessment of current exposure, applying the following criteria:

- Accuracy and quality: as a rule, all reported data were assumed to be of sufficient analytical quality[74]. Zinc detection limits are not identified as an issue in the sediment.

74 In principle, attention must be given to issues that influence the comparability of the monitoring data and decrease their relevancy for the regional assessment, e.g. differences in sampling techniques, sampling depths, etc. These aspects are however not reported, so cannot further be considered.

- Time of sampling: In contrast to the modelled PEC, the available monitoring database of the RAR contains a lot of older data. Historical data show that zinc concentrations in EU sediments decreased significantly during the 1980s and 1990s. Therefore only data from 1995 on are to be considered for the assessment of the possible impact of current use pattern of zinc.
- Relevancy: the following data were considered not relevant for the regional assessment:
  - data influenced by documented point sources
  - data obtained in historically contaminated areas (e.g. old mining areas)
  - data obtained in areas with documented elevated natural background

The effect of the re-distribution of historical contamination in sediments is also an important consideration, notably in dynamic systems, e.g. rivers. This phenomenon is difficult to quantify, but is well documented, e.g. by river profiles in the Lot-Garonne area, showing the constant downstream re-distribution of Cd-contamination of sediment. This phenomenon is anticipated to play an important role in the loading of sediments of, notably the sedimentation areas of the big river systems that are mentioned in the RAR. It is therefore highly relevant to consider when interpreting monitoring data for the assessment of the risks, resulting from the current use and production of a substance .

The monitoring data presented in the RAR, provide only a scattered pattern of discrete sampling points. In the conclusion (i) programme on zinc bioavailability, a regional dataset, covering all relevant waters from the Flanders region was developed. This dataset is much more extensive than e.g. the one for the NL regional waters (RAR), and is considered therefore, from the point of view of coverage for a region, of superior relevancy for risk characterisation.

The Flanders data set however represents a mix of reference points, hot spots and moderately contaminated sediments. It is as such typical for an industrialized regional scenario including all these influences and provides a better estimation of the zinc concentrations in regional sediments subject to all these influences than a database only consisting of data from some selected rivers which are not characterized in a detailed way.

For the risk characterisation, the monitoring data figuring in the RAR were critically evaluated and a selection of relevant information was made on the criteria mentioned above.

The exposure data are presented in Table 11. In this table, the data that are considered useful for the risk characterisation, and those that are not, are indicated and presented in Table 12. Below a short description is given of the selection for each region/river.

1.5.2.1 -Modelled PEC for model region - The Netherlands

For reasons mentioned above, this PEC modelled is considered of high relevancy for the regional scenario. PECadd: 504 mg/kgDW.

1.5.2.2 -Rhine sediment at Lobith

In the RAR, the value from 1993-1997 is given. There is however, more recent information on the Rhine sediment, showing the further steady decrease of zinc concentrations in sediment (publicly available data from the NL Ministry of "Verkeer and Waterstaat"). This source mentions a Rhine sediment Zn concentration (PECtotal) of 470 mg/kgDW (Verkeer en

Waterstaat 2003). This is in better agreement with the value mentioned in the German database for the Rhine (353 mg/kgDW)

1.5.2.3 -The Denzer et al (1999) database

The Denzer et al 1999 database contains a compilation of data on zinc in sediments obtained from monitoring datasets of several countries. Included are data for France (INERIS dataset), Germany (LAWA dataset) and Belgium (VMM dataset). Unlike for the water, there is no sediment monitoring data available for other regions of Europe in Denzer et al., 1999. The monitoring data refers to sampling measurements from 1994 to 1996. The Denzer et al 1999 database is not used since for the specific regions (France, Belgium, Germany) more recent data obtained directly from INERIS, LAWA and VMM are available and discussed in detail below.

1.5.2.4 -German river sediments

According to the evaluation of the water data, some of the rivers mentioned in the RAR (2005) are influenced by historical mining sites and past industrial sites and are therefore not considered useful for the regional scenario. These are outlined in Table 5 and described in more detail below.

1.5.2.5 The region of Niedersachsen

The area in which these rivers float (Oker/Aller/Weser and Innerste/ Leine/ Aller/ Weser) is an old mining area during the last centuries (Harz); no longer operated underground mines and tailings dumps are releasing constantly metals such as zinc. The Aller river, via the Oker and Innerste rivers, receives zinc from the historical mining region of the Harz.

1.5.2.6 The region of Sachsen

At the Vereinigte Mulde (unification from Zwickauer Mulde and Freiberger Mulde, later enter the Elbe): remobilisation of sediment at higher water levels results periodically in higher zinc levels in the total zinc analysis.

1.5.2.7 The region of Sachsen-Anhalt

The rivers Mulde/ Schwarze Elster/ Schlüsselstollen and Wipper are all rivers where old mining activities existed and where metals were emitted into these rivers which flow into the Saale later.

Saale: near Merseburg-Halle remobilisation of heavy metals from sediments at former industrial sites.

In addition, for several rivers, the average 90P value is used, instead of 1 single value (e.g. for the Elbe, Neckar, Rhein, Saar and Weser).

The PECtotal values for the German rivers that were selected are given in Table 11.

1.5.2.8 -Swedish data

The Swedish data are considered useful since it is indicated that point source influence is avoided. PECs for total zinc are given in Table 11.

1.5.2.9 -The French dataset: issues related to sediment monitoring datasets.

-In Ardoie-Picardie and Rhin-Meuse, the influence of point source emissions is apparent. Sampling points in areas influenced by point source emissions and historical contamination have been identified and were not used for the 90P calculations as they are not relevant for present day emissions related to zinc use. The 90P was taken across each river basin. An average of the 90P across sites was not made because most sampling points only contained 1 or 2 values and so a 90P for the site could not be made. Instead, a 90P across all stations within each river basin was calculated.

-Ardoie Picardie is a highly industrialized region. Main industries are textiles and chemical and fertilizers industry, and some of the largest ferrous and non-ferrous metallurgy companies of France are also located in this region. Zinc levels from sampling points taken along the Deûle Canal and its' tributaries in the area (La Scarper, Canal d'Aire,…) between Lille and Valenciennes are greatly influenced by historical contamination of old metallurgical companies and fertilizers industries as well as presently operating companies emitting zinc. (EPER (European Pollution Emissions Register) refers to MetalEurope Nord at Noyelles Godault, Umicore at Auby (both figuring with a local scenario in the zinc RAR), Norzinco at Anzin, LME at Trith-St-Léger, Fonderie et Aciérie at Denain, and other industrial sectors: McCain Alimentaire at Haines, PC Loos at Loos and Roquette at Lestrem). The sampling points in the French database that are influenced by the historical contamination and point sources of this area are detailed in table 12.

Another industrial area is discharging into the English Channel at Dunkerque-Mardyck. This area includes ferrous and non-ferrous metals industries including several of Arcelor's installations and Bus Valera Comilog, all influencing the zinc levels measured in this area.

The 90P calculated for the Ardoie-Picardie river basin, without the sampling points influenced by the above mentioned historical and point sources is (PECtotal) 626 mg/kg of zinc.

- the Rhin-Meuse region contains the highly industrial zone North of Metz for which the primary industrial sector is ferrous and non-ferrous metallurgy. The South of Saarbrücken (on the French-German border) is also an industrial zone. Zinc levels from sampling points taken along the Moselle river and its tributaries North of Metz and between Metz and Nancy are influenced by the local metallurgical industry (EPER refers e.g. to direct emissions of zinc to water from Arcelor in Florange, Ispat unimétal in Amneville, Ascométal and Technilor in Hagondange, Usine Fonderie at pont-à-Mousson, and SAM at Neuves-Maison).

The 90P calculated for the Rhin-Meuse river basin, without the sampling points influenced by the above mentioned historical and point sources is 599 mg/kg of zinc (PECtotal).

Background zinc in Rhine-Meuse region

Interestingly, sampling points with zinc levels in the range of 215 – 352 mg/kgDW are found in the Vosges region. This region is characterized by forested hills and mountains. There are no industries identified, no agriculture and this region is scarcely populated. The zinc levels in this region can be considered as natural ambient zinc levels with little input from historical, point sources or diffuse emissions.

This is evidence, once again, that a general EU default background value for zinc in sediment (140 mg/kgDW) is not relevant (levels found here are more than double the proposed default value) and in this case, leads to erroneous conclusions of risk.

- the Loire-Bretagne region has rather few industry and is more agricultural in nature (especially in the Bretagne region). However, there are several metallurgical industry along the Loire river in the area between St-Etienne and Montluçon, and again near Nantes. In the area around St-Etienne and Montluçon EPER identified Trelleborg at Clermont-Ferrand (organic chemicals industry), Ugine at Geugnon, St-Rémy industry at St-Rémy, Valéo Sécurité Habitacle at Nevers. Some high zinc levels are found on the Vilaine river just downstream the discharges of the Citroën company at St-Jacques-de-la-Lande (Citroën is an identified source of zinc emission to water by EPER).

The agricultural area of Bretagne is characterized by rather low zinc levels (59 to 177 mg/kg zinc) except for the industrial zone of Nantes. Near Nantes, Polstau at Châteaudun (organic chemicals industry) and Sai Vern at Vern d'Anjou were identified by EPER.

The 90P calculated for the Loire-Bretagne river basin, without the sampling points influenced by the above mentioned point sources is 286 mg/kg of zinc (PECtotal).

These examples of the detailed analysis of the monitoring data for France demonstrate the influence on the regional data by point source emissions.

1.5.2.10 -Belgium, Flanders.

In the RAR, reference is made to VMM (2003). Within the framework of the concl (i) programme, another sub-dataset on Flanders was developed (concl (i) dataset), giving slightly lower zinc levels. The difference between the 2 is partially explained by the presence of black points near the sampling sites in the VMM dataset, as well as by the use of more aggressive extraction techniques in the past (HF vs Aqua Regia). Therefore, the VMM (2003) database is less representative for a region than the Flanders database compiled in the framework of the conclusion (i) program.

The concl (i) dataset is also most recent (sampling in 2002), so most relevant for the current emissions of zinc to this region. The relevancy of the Flanders dataset as a realistic worst case for the EU has been extensively discussed and demonstrated (EURAS 2003). This dataset is considered of superior relevancy for risk characterisation. The 90^{th} percentile of the total zinc concentration for the conclusion (i) Flanders data set (n = 200) is 535 mgZn /kg DW which is similar to the modelled regional (NL-region) concentration (PEC_{add}) of zinc (504 mg/kg DW). The advantage of this dataset is that for every sampling point, specific AVS-SEM data exist. As such, a correction for bioavailability can be made for every sampling point. The 90 P of the excess SEM values (= bioavailable zinc) is 70 mg Zn/kgDW (IZA – Europe 2003).

1.5.2.11 -The Netherlands, additional data.

In the RAR, values for 2 other sedimentation areas from the big rivers are reported. However, only average and maximum values are given, so this information is difficult to use for the risk characterisation (90P values needed). These data show also remarkably big differences within the same river systems, e.g. the Hollandsch Diep West and East are significantly different (average of 1001 versus 293 mg/kg DW). No explanation is given for this phenomenon. Since no 90P values are reported, these values are not used for the risk characterisation. Still, the reported average values are higher than the 90P levels, recently measured in the NL rivers. The range observed in these sediments is very broad (e.g. 22 – 4003 mg/kg DW in Hollands Diep West). No further information is provided. It is noted that these sampling points are located in the large sedimentation area of the "Biesbosch", receiving the waters from Rhine and Meuse.

These sediments are referred to as "freshly deposited layers", but most recent data are from 1995/1997. It is also stated that in these layers the "contribution of historical pollution is less relevant". This statement is neglecting the continuous re-distribution of older deposited zinc into new sediment layers. This "memory effect" of sediments downstream former sources is a problem with all monitored sediments, including the most recently deposited.

Data presented in the zinc RAR referring to the period before 1995 are not used for the risk characterisation.

Table 3 Regional PECadd, bioavailable for the sediment, using modelled and monitored data.

Country River and location	PEC mg/kg dw	PEC_{add} (PEC-140 mg/kg dwt)	Comment on relevancy of PEC	PEC_{add} corrected for average bioavailability	PEC_{add} corrected for RWC bioavailability
Modelled data					
Netherlands					
PEC modelled for NL	504	504	Considered highly relevant (see text)	101	252
Monitoring data					
Netherlands					
Rhine (Lobith, NL) 90P value	470	330	Most recent information used (see text)	66	165
Germany					
Elbe	1095	955		191	478
Ems	480	340		68	170
Lausitzer Neisse	680	540		270[3]	270
Main	403	263		53	132
Mosel	1029	889		178	445
Nahe	398	258		52	129
Neckar	452	312		62	156
Rhein	546	406		81	203
Saar	589	449		90	225
Spree	1010	870		174	435
Warnow	465	325		65	163
Weser	584	449		90	225
Sweden					
Northern Sweden (median)	150	10		5[3]	5
Southern Sweden (median)	240	100		50[3]	50
France					
Artoie Picardie	626	486		97	243
Rhin Meuse	599	459		92	230
Seine Normandie	463	323		65	162
Loire Bretagne	286	146		30	73
Adour Garonne	340	200		100[3]	100
Rhone Mediterranee Corse:	372	232		116[3]	116
Belgium					
Belgium, Flanders (90P value)*	604	464		93	232
Belgium Flanders (90P value from conclusion I programme)	535	268		70[2]	134

[1] See text for explanation
[2] Specific AVS correction instead of generic bioavailability factor of 0.2 for EU lowlands
[3] Default bioavailability correction of 0.5 for areas outside of EU lowlands

Table 13 . Summary of Data influenced by point sources and historical contamination in sediments

Description of sampling points	Total zinc mg/kg dw	Comments
France		
Ardoie-Picardie		
Canal de la Deûle		
no. 1079000	2200	At Don, downstream of Umicore Auby, Metal Europe
no. 1077000, 1078000	4400, 6940	At Courrière, just downstream of McCain Alimentaire discharge
no. 1076000	1700	At Flers-en-escrebieux, near Umicore Auby site
no. 1080000	2380	At Haubourdin, near PC Loos industry
no. 1081000, 1082000	3120, 1200	At Wambrechies, downstream of PC Loos industry and the other industries mentioned above
Tributaries of the Canal de la Deûle: Canal de Roubaix		
no. 1050000, 1087000		At Leers and Marquette, downstream of PC Loos and the other industries mentioned above
Tributaries of the Canal de la Deûle: La Marque		
no. 1086000		At Wasquehal, downstream of PC Loos and the other industries mentioned above
Tributaries of the Canal de la Deûle: La Scarpe		
no. 1039000	5000	At Râches, surrounding industries are Umicore, MetalEurope Nord, Fonderie et Acier, LME, Norzinco in the region between Douai and Valenciennes
no. 1040000	2590	At Marchiennes, surrounding industries are Umicore, MetalEurope Nord, Fonderie et Acier, LME, Norzinco in the region between Douai and Valenciennes
no. 1041000	1430	At Nivelles, surrounding industries are Umicore, MetalEurope Nord, Fonderie et Acier, LME, Norzinco in the region between Douai and Valenciennes
no. 1019000	3160	At junction of the Sarpe canal and the Escaut at Blégharies, surrounding industries are Umicore, MetalEurope Nord, Fonderie et Acier, LME, Norzinco in the region between Douai and Valenciennes
Tributaries of the Canal de la Deûle: Le canal d'Aire		
no. 1063900, 1063000, 1062000	3320, 2860, 1560	At Beurry Béthune, Aire sur-la-Lys and Cuinchy surrounding industries are McCain Alimentaire, PC Loos and Roquette
Tributaries of the Canal de la Deûle: Canal La Somme		
no. 1119000, 1125000	6304, 2090	Canalised at Offoy and in river, influenced by the Northern industrial zones of Douai and Valenciennes
no. 1118000	2340	At Ham, influenced by the Northern industrial zones of Douai and Valenciennes
no. 1119300	1846	At Villiers Carbonnel, influenced by the Northern industrial zones of Douai and Valenciennes
no. 1117000	4516	At Sérancourt-le-Grand, influenced by the Northern industrial zones of Douai and Valenciennes
Canal de Mardyck		
no. 1111900	2160	In the industrial zone of Dunkerque, in the vicinity of the Arcelor Sollac Atlantique and Bus Valera Comilog
Canal de Moëres		
no. 1111000	1796	In the industrial zone of Dunkerque, at Courdekerque-Branche in the vicinity of the Arcelor Sollac Atlantique and Bus Valera Comilog
Canal de Bourbourg		
no. 1109500	1069	In the industrial zone of Dunkerque, at Bourbourg in the vicinity of the Arcelor Sollac Atlantique and Bus Valera Comilog
Rhin-Meuse		
Moselle and tributaries		
no. 2092000	3249	The Fensch at Florange at emission point of Arcelor
no. 2094900	1360	The Moselle at Sierck, downstream of conglomeration of ferrous and non-ferrous industries Arcelor, Ispat unimétal, Ascométal, technilor
no. 2090000	474	The Moselle at Uckange, downstream of conglomeration of ferrous and non-ferrous industries Ispat unimétal, Ascométal, technilor
no. 2089900	4357	The Orne at Richmont, downstream of Ispat unimétal discharge

Description of sampling points	Total zinc µg/L	Comments
Southern industrial section of Saarbücken		
no. 2103800	1071	The Roselle river at Petite-Roselle, within the historical industrial zone of Saarbrücken
no. 2103850	852	The Bist at Creutzwald in the historical industrial zone of Saarbrücken
The Vosges region		
no. 2065300, 2066000	352, 254	Within forested hills of Vosges. An area without industry, agriculture or cities. Zinc levels are thought to be natural levels.
Loire-Bretagne		
Metals industry along the Loire and tributaries		
no. 4007100	2330	Loire river before Roanne at Etrat, downstream a conglomeration of metal industries; Valéo sécurité Habitacle, St-Rémy industry and Ugine
no. 4008000	941	Loire river before Roanne at Andrézieux-Bouthéon, downstream of conglomeration of metal industries Valéo sécurité Habitacle, St-Rémy industry and Ugine
no. 4009000	454	Loire river after Roanne at Veauchette, further downstream of conglomeration of metal industries Valéo sécurité Habitacle, St-Rémy industry and Ugine
no. 4004900	901	Ondaire river downstream of a slaughterhouse
no. 4178650	1314	Aulne river near Nantes, downstream Polstau and Sai Vern industries
Allier river near Clermont-Ferrand		
no. 4034400, 4036500	340, 453	Allier river downstream Trelleborg industry at Clermont Ferrand
Vilaine River after Rennes		
no. 4208000	2680	Downstream the Citroën discharges at St-Jacques-de-la-lande
no. 4207000	949	Further downstream the Citroën discharges at St-Jacques-de-la-Lande
no. 4215780	1020	Downstream a conglomeration of industries including EPER identified zinc emitter, ACI combustion at Mans
Germany		
Niedersachsen; historical metal industry in the Weser catchment (Harz region) :		
Aller	1500	Via Oker and Innerste rivers, the Aller receives zinc from the historic mining region of Harz
Sachsen; historical mining industry in the Elbe catchment :		
Vereinigte Mulde	1600	High zinc content caused by ores (natural) and by historical mining activities
Sachsen Anhalt; historical mining in the Elbe catchment (Thüringen region):		
Mulde	3230	High zinc content caused by ores (natural) and by historical mining activities
Saale	2519	High zinc levels found downstream of Halle (historical metal mining) at Rosenburg. May also be influenced by a viscose production plant
Swarzbach	1557	High zinc content caused by ores (natural) and by historical mining activities
Schwarze Elster	1033	High zinc content caused by ores (natural) and by historical mining activities
Belgium		
Province of Liège, Meuse river Basin		
Argenteau River at Visé	907	Downstream Industrial region of Liège (various metallurgical and steel industry emittors; EPER 2004)
Netherlands		
Hollandisch Diep East	2089	see text
Hollandisch Diep West	4003	see text
Dordtsche Biesbosch clay	1131 (average), 2802 (max)	Historical contamination input from receiving waters of the Rhine and Meuse rivers
Dordtsche Biesbosch sand	1904	Historical contamination input from receiving waters of the Rhine and Meuse rivers
EU waters (1994-1998) 90 P value		
Denzer et al., 1999 database (90P calculation)	1367	More recent data is available for the regions represented within this dataset

2 RISK CHARACTERISATION

2.1 REGIONAL WATERS

In this chapter, the regional PEC data that are considered adequate for the regional exposure assessment (table 8, see chapter 2.1.) are compared with the PNEC, after correction for bioavailability (table 10).

For the calculation of the PEC/PNEC ratios, the PNEC from the RAR (i.e. 7.8 µg Zn/l) is used. The resulting PEC/PNEC values are summarised in table 11.

Table 14 Summary of risk characterization for regional scenario of diffuse emission patterns

Database	PEC/PNEC of 7.8 µg/L	
	RWC scenario	Average scenario
Calculated		
NL Region	0.44	0.01
Monitored 90P		
Rhine	0.70	0.34
Meuse at B-NL border (Eijden)	0.94	0.53
Meuse at B-NL border (Eijden)	**1.6**	**1.0**
Scheldt	0.64	0.37
Regional waters	0.93	0.27
Regional waters	0.88	0.25
State waters	0.90	0.26
Swedish watercourses		
Swedish watercourses	0.43	-
Northern Swedish Lakes	0.08	-
Southern Swedish Lakes	0.44	-
France		
Seine-Normandie	0.08	-
Rhin-Meuse	0.58	0.09
Rhône Méditerrannée	0.21	-
Belgium		
Scheldt River basin	0.95	0.48
Meuse-Seine River Basin (West of the Meuse)	0.90	0.63
Meuse River Basin (East of the Meuse)	0.65	0.47
Meuse River in Belgium	**1.09**	0.64
Flanders region	**1.6 / 0.94**	0.78 / 0.38
Germany		
Rhein River Basin	**1.35**	0.74
Elbe River Basin	0.73	0.40
Danube River Basin	0.59	0.17
Weser River Basin	**1.61**	0.95
Ems River Basin	0.91	0.35
Nordic Countries		
Nordic Lakes	0.2 / 0.3	-

*Unless otherwise indicated, the 90P represents an average 90P across sampling stations.
** Total = dissolved for Scandinavian Lakes (conservative assumption that suspended matter is very low in Nordic lakes (same may not be true in rivers).
"-" indicates that the dissolved added zinc is close to '0', so levels are assumed at near background.

2.2 DISCUSSION OF THE REGIONAL PEC/PNECS

From table 13, it can be observed:

- The PEC/PNEC using the modelled emissions from the model region (The Netherlands) is < 1.
- Using the regional PECs from monitoring data, and applying the PNEC from the RAR, the vast majority of PEC/PNECs are <1.
    - A few cases of slight exceedance (risk ratio of 1-2) of the PNEC are observed only when worst case correction for background and bioavailability are combined (Meuse, Flanders). It is noted that for these waters the influence from industrial point sources and historical contamination could not be fully excluded (section 2.1.).
    - When typical (average) corrections are made for background and bioavailability, no exceedance of the PNEC of 7.8 µg/l is observed.

There are waters in the EU with elevated levels of zinc. Considering the PECs summarised in table 9, PEC/PNECs of > 1 are calculated. The main causes for this are a) emissions from point sources, and b) emissions from historical contamination. Waters influenced by these factors show consistently zinc levels above those, observed in the absence of these factors.

The regional analysis is supposed to asses the risks in areas not influenced by point sources (the latter relate to local scenarios). Influences from historical contamination also fall outside the scope of regulation 793/93.

Zinc being a natural element, special attention needs be paid to areas with higher natural zinc background, which results in higher water levels, eventually exceeding the PNEC (e.g. Vesdre area, B). The high detection limit for zinc in some water datasets may also erroneously suggest that zinc levels are higher than reality.

Finally, the observations above demonstrate that there is no evidence for a direct relationship between diffuse emissions (resulting from the use of zinc products) and elevated zinc concentrations (exceeding the PNEC) in European surface waters.

Risk conclusion – regional waters

Considering the above, a general conclusion (ii), i.e., no risk - is drawn for regional EU waters. This conclusion is drawn from a very conservative analysis. This general conclusion (ii) can be formulated more explicitly as follows:

- **Conclusion (ii) is drawn for regional surface waters in the EU where there is no direct influence of multiple industrial point sources and/or historical contamination, and/or elevated natural background. There is no evidence that measured regional concentrations reflecting the general use and application of zinc exceed the PNECadd.**
- **Conclusion (iii) is solely drawn for regional surface waters that are directly influenced by industrial point sources, and/or historical contamination and/or natural elevated background as monitored zinc concentrations for certain of these waters exceed the PNECadd.**

2.3 REGIONAL SEDIMENTS

As argued before, the modelled exposure should be used as the reference for the risk characterisation related to the current production and use of the substance. The relevant monitoring data are also used since they reflect the real sediment concentrations, noting however that these real concentrations can be the result of many different influences, including notably, influence from historical emissions, which are not the subject of risk assessments under 793/93/EEC.

In the RAR, modelled data are considered less useful, since there are many monitoring data. However, considering a) that the modelled exposure assessment is based on updated and extensively detailed information on the model region (The Netherlands) and b) the difficulties in interpretation and uncertainties related to the monitoring data (in terms of e.g. historical pollution and influence by point sources), it is proposed to use the modelled PEC as a reference for the validity of the monitoring data and the exposure assessment. It is noted that this is in accordance to the new TGD where both data sets are considered to be complementary to each other in the complex interpretation and integration of the exposure data (TGD page 13).

The exposure information, considered relevant for the risk characterisation (Table 1) is further handled to give the $PEC_{add, bioavailable}$, by:

a) Subtracting the 140 mg/kgDW background from the total zinc concentrations (not for the modelled data since these are added emissions). For this correction, the generic default value of 140 mg Zn/kg DW is used. It is noted that the subtraction of one background for all sediment values is scientifically questionable from the viewpoint of the geologically different backgrounds reported in literature (see also remark on the data from the Vosges area (France under section 2.2.2.6). By lack of more precise data, the generic value of 140 mg Zn/kgDW is however used.

b) Correcting for the bioavailable fraction, according to the procedure, outlined in the RAR zinc. To obtain the bioavailable fraction of the PECadd, it is multiplied with a generic bioavailability factor (Bio-F). For this Bio-F, 2 approaches are used in this annex, in a way similar to the bioavailability corrections done for regional water:

  - A generic average bioavailability factor of 0.2, which is derived from the extensive analysis of SEM-AVS data in Flanders (EURAS 2004). This value is applied to all waters of the EU lowland river system, and based on the coupled SEM-AVS data from Flanders.
  - A generic realistic worst case bioavailability factor of 0.5, following from the 90P values of the analysis of AVS-SEM data from Flanders and the Netherlands.

- The $PEC_{add, bioavailable}$ is now compared with a PNEC to characterise the risk according to: RCR = (PECtotal – Cb) x generic Bio-F (Eq-1)[75]

For the calculation of the PEC/PNEC ratios, the PNEC from the RAR (i.e. 49 mg Zn/kgDW) is used. The resulting PEC/PNEC values are summarised in table 14.

Results for the different European modelled and monitoring data are presented in Table 14.

75 A more refined approach is the use of the organic carbon normalised approach (Eq-2). In this approach the excess SEM_{Zn} is normalised for organic carbon and compared with a threshold value (i.e. 100 $\mu mol/g_{oc}$). Only above this threshold effects are expected to occur. This further correction is not being considered in the Zn RAR which adds significantly to the conservatism of the proposed approach. However if site specific data are available it is proposed to apply the fOC normalisation to the local scenarios.

Specifically for the interpretation of monitoring data for sediment, it is repeated that the sediment is a "sink" compartment, and therefore current monitoring data are influenced by the effect of the re-distribution of historical contamination. This phenomenon should be taken into account when assessing the risks resulting from current emissions

Risk conclusions for regional sediments

In the majority of cases, exceedance of the PNEC of 49 mg Zn/kgDW is observed. This is the case for the modelled PEC, as well as for the PECs based on monitoring data. Consequently, a concl (iii) is drawn from these data.

The application of a realistic worst case bioavailability factor or average bioavailability factor does not change these conclusions.

Table 15 Regional PeCadd, bioavailable for the sediment, using modelled and monitored data.

	PEC/ PNEC 49	
Country		
River and location	$PEC_{add,\ bioavailable}$ average conditions	$PEC_{add\ bioavailable}$ RWC conditions
Modelled data		
Netherlands		
PEC modelled for NL	2.1	5.1
Monitoring data		
Netherlands		
Rhine (Lobith, NL) 90P value	1.3	3.4
Germany		
Elbe	3.9	9.7
Ems	1.4	3.5
Lausitzer Neisse	5.5	5.5
Main	1.1	2.7
Mosel	3.6	9.1
Nahe	1.1	2.6
Neckar	1.3	3.2
Rhein	1.7	4.1
Saar	1.8	4.6
Spree	3.6	8.9
Warnow	1.3	3.3
Weser	1.8	4.6
Sweden		
Northern Sweden (median)	0.1	0.1
Southern Sweden (median)	1.0	1.0
France		
Artoie Picardie	2.0	5.0
Rhin Meuse	1.9	4.7
Seine Normandie	1.3	3.3
Loire Bretagne	0.6	1.5
Adour Garonne	2.0	2.0
Rhone Mediterranee Corse:	2.4	2.4
Belgium		
Belgium, Flanders (90P value)*	1.9	4.7
Belgium Flanders (90P value from conclusion I programme)	1.4	2.7

3 REFERENCES

Most of the references figuring in this Annex are listed already in the RAR. Additional references are:

EURAS 2004. Comments on the data analysis of the Flanders surface water database performed in the framework of the Environmental Risk Assessment for Zinc and zinc compounds (draft RAR 09-02-2004).

Klasmeier J, Huffmeyer N, Matties M. 2006. Investigation of the importance of different zinc emission pathways on for surface water concentrations in the Ruhr river basin using the geo-referenced model GREAT-er. Report to IZA-Europe, February 2006.

Réseau National des Donnés sur l'Eau. www.rnde.tm.fr

VMM 2003. Vlaamse Milieumaatschappij. VMM meetdatabank at www.vmm.be.

VMM (2001). General Water Quality Plan 2-Nete. D/2001/6871/041, Aalst, Belgium.

The report provides the comprehensive risk assessment of the substance zinc metal.

It has been prepared by the Netherlands in the frame of Council Regulation (EEC) No. 793/93 on the evaluation and control of the risks of existing substances, following the principles for assessment of the risks to humans and the environment, laid down in Commission Regulation (EC) No. 1488/94.

Part I – Environment

This part of the evaluation considers the emissions and the resulting exposure to the environment in all life cycle steps. Following the exposure assessment, the environmental risk characterisation for each protection goal in the aquatic, terrestrial and atmospheric compartment has been determined.

The environmental risk assessment concludes that there is a need for specific measures to limit the risks for the local scenarios in which no concern would be claimed but where (possibly) due to high regional background concentrations a local risk cannot be excluded. This applies to concerns for the local terrestrial environment, for micro-organisms in the sewage treatment plant and for effects on the local aquatic (including sediment) environment as a consequence of exposure arising from the production of zinc metal and from the use in continuous hot dip galvanising, electro galvanising, in brass, as die casting alloy, as rolled/wrought zinc and as zinc powder/dust. There is also a need for limiting the risks for the regional aquatic (including sediment) environment due to elevated regional zinc levels in some, but not all, regional surface waters and sediments.

Moreover there is a need for further information because of concerns for effects on the aquatic (including sediment) environment alongside motorways in the European Union. No concerns for any other local and regional scenarios, concerning secondary poisoning, micro-organisms in the sewage treatment plant and the local aquatic (including sediment) environment have been concluded. In addition there is at present no concern for the atmospheric compartment.

Part II – Human Health

This part of the evaluation is published in a separate document.

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Abstract

For zinc metal (CAS No. 7440-66-6), zinc distearate (CAS No. 557-05-1 / 91051-01-3), zinc oxide (CAS No.1314-13-2), zinc chloride (CAS No.7646-85-7), zinc sulphate (CAS No.7733-02-0) and trizinc bis(orthophosphate) (CAS No.7779-90-0) risk assessments were carried out within the framework of EU Existing Chemicals Regulation 793/93. For each compound a separate report has been prepared. It should be noted, however, that this risk assessment on zinc metal contains specific sections (as well in the exposure part as in the effect part) that are relevant for the other zinc compounds as well. For these aspects, the reader is referred to this risk assessment report on zinc.

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