Proposed EQS for Water Framework Directive Annex VIII substances: zinc *(For consultation)*

by
Water Framework Directive - United Kingdom Technical Advisory Group
(WFD-UKTAG)

Publisher: **Water Framework Directive - United Kingdom Technical Advisory Group (WFD-UKTAG)**
SNIFFER
25 Greenside Place
Edinburgh
EH1 3AA
Scotland
www.wfduk.org

Author(s):
Dawn Maycock, Adam Peters, Graham Merrington & Mark Crane

Research performed:
2010

Dissemination Status:
Publicly available

Keywords:
Zinc, Environmental Quality Standard, Water Framework Directive, Predicted No Effect concentrations, freshwater, marine water

Research Contractor:
wca environment limited, Brunel House, Volunteer Way, Faringdon, Oxfordshire SN7 7YR, Tel: 01367 244311

Environment Agency's Project Manager:
Bruce Brown/Lindsey Sturdy, Evidence Directorate

Collaborators:
Environment Agency
Delphine Haesaerts, IZA Europe
Frank Van Assche, IZA Europe

Environment Agency Science Project Number:
SC080021/8o

Use of this report

The development of UK wide classification methods and environmental standards that aim to meet the requirements of the Water Framework Directive (WFD) is being sponsored by the UK Technical Advisory Group (UKTAG) for the WFD on behalf of its member and partners.

This technical document has been developed through a collaborative project, managed by the Environment Agency, and has involved the members and partners of UKTAG. It provides background information to support the ongoing development of the standards and classification methods.

While this report is considered to represent the best available scientific information and expert opinion available at the time of its completion, it does not necessarily represent the final or policy positions of UKTAG or any of its partner agencies.

Executive Summary

The UK Technical Advisory Group (UKTAG) has commissioned a programme of work to derive Environmental Quality Standards (EQSs) for substances falling under Annex VIII of the Water Framework Directive (WFD). This report proposes Predicted No Effect Concentrations (PNECs) for zinc using the methodology described in Annex V of the Directive and the more recent guidance drafted by the European Commission. There are existing EQSs for zinc, but the methods used to derive these are not considered to comply with the requirements of Annex V and so are unsuitable for deriving Annex VIII EQSs.

The PNECs described in this report are based on a technical assessment of the available ecotoxicity data for zinc, along with any data that relate impacts under field conditions to exposure concentrations.

EU Risk Assessment Reports (EU RARs) have been compiled for zinc metal, zinc oxide, zinc distearate, zinc chloride, zinc sulphate and trizinc bis(orthophosphate). Toxicity data reviewed in the EU RAR for zinc metal were not subjected to additional quality assessment when used in this report. This is because they had already been assessed by the authors of the risk assessment and by an international advisory forum of experts from EU Member States. Additional freshwater chronic ecotoxicity data and field data have become available since the completion of the EU RAR. This report builds upon these data and recommends a revised freshwater PNEC.

Short-term standards have not been derived in the current report because maximum allowable concentrations for metals are not considered a priority.

The feasibility of implementing the PNECs in this report as EQSs has not been considered at this stage. However, this would be an essential step before a regulatory EQS can be recommended.

Properties and fate in water

Zinc is a naturally occurring element that exists mainly as sulphides, silicates and carbonates. Zinc plays an essential role in organisms, where its internal concentration can be regulated to a limited extent depending on the concentrations to which it is exposed. Effects of deficiency or toxicity may occur if the concentrations deviate from those that the organism can regulate.

In water, zinc exists in the +2 oxidation state in forms that are dependent on physicochemical parameters, such as pH, hardness and the content of dissolved organic carbon. Bioavailability may be affected by organic and inorganic complexation, with anions such as chloride (Cl^-) and carbonate (CO_3^{2-}), and by the competition of cations (e.g. Ca^{2+} and H^+) with zinc at biological receptors.

Availability of data
Abiotic factors have been taken into account for freshwater data selection. Data from natural and artificial waters are acceptable if the major physicochemical characteristics (in particular pH and hardness) are similar to the ranges encountered in UK freshwaters.

Background zinc concentrations are also taken into account. However, the literature references used for the aquatic toxicity dataset of the EU RAR do not usually contain data on the background concentration of zinc in the test water and, in some cases, data on pH or hardness are lacking. Therefore, tests conducted in artificial waters were excluded when there was no information on pH or hardness. Those tests conducted in natural waters were used unless there were indications that any of these three parameters (background concentration, pH and hardness) deviated substantially from real environmental conditions.

Chronic no observed effect concentration (NOEC) values from data on 25 species covering eight taxonomic groups (unicellular and multicellular algae, sponges, rotifers, molluscs, crustaceans, insects, fish and amphibians) were available to derive normalized long-term $PNEC_{add,\ freshwater}$.

Data for 36 species covering eight taxonomic groups (unicellular and multicellular algae, annelids, cnidarians, crustaceans, echinoderms, molluscs, fish, and nematodes) were used to derive the chronic $PNEC_{add,saltwater}$ from geometric "species mean" values.

Only four valid studies were available on benthic organisms.

Derivation of PNECs
The chronic effects data evaluated and used for the EU RAR have been reported along with data that have been reported since the completion of the RAR.

The "added risk" approach is appropriate when deriving PNECs for zinc because zinc is a naturally occurring substance which organisms will have been exposed to over an evolutionary timescale. Furthermore, zinc is ubiquitous in aquatic environments. The added risk approach takes account of ambient background concentrations and the PNEC ($PNEC_{add}$) applies only to the additional contribution over and above the ambient background level (i.e. the value at which toxic effects occur, ignoring contributions from background concentrations). A practical consequence of this is that when assessing compliance with such an EQS it will be necessary to consider ambient background zinc concentrations at a regional, river basin, or waterbody scale.

A $PNEC_{add,freshwater}$ derived in the EU RAR for soft waters (those with a hardness <24 mg l^{-1} $CaCO_3$) has been reassessed in a recent Environment Agency project (Environment Agency 2010). This report demonstrated that existing matched biology and chemistry data, and chemical speciation modelling, do not show that there is a clear requirement for a difference in approaches between waters with hardnesses of greater than or less than 24 mg l^{-1} $CaCO_3$. It appears that similar principles for the competition between Zn and major cations such as Ca and Mg,

and bioavailability reduction through Zn binding to DOC, can be applied across the complete range of water hardnesses in the UK.

A research programme conducted as part of the EU RAR developed quantitative methods for taking into account the bioavailability of zinc because of water and sediment chemistry. These methods use biotic ligand models (BLMs) for water. The freshwater value is expressed as a bioavailable concentration to take account of the influences of water quality on the availability and hence toxicity of zinc.

The proposed freshwater and sediment PNECs given below are supported by field data.

Long-term PNEC for freshwaters

Algae appear to be the most-sensitive taxonomic group, followed by crustaceans, sponges, rotifers and fish.

The key input parameters for Zn BLM are DOC and Ca concentrations and pH. North West Region is the most sensitive of the 10 Regions (six in England, one in Wales and three in Scotland) for which there is data, followed by Wales and the South West. The PNEC values were calculated from the annual averages of pH (mean), DOC (median) and Ca (mean) of at least six samples for each individual site (approximately 100 sites for each Region). Setting the Generic HC5 to a predefined level of protection for the whole of Great Britain, such as the level for 95% protection of 14.2 µg l^{-1}, has limitations in that the selected value represents a rather lower level of protection (approximately 68%) in the North West Region. Consequently the value was selected so as to provide 95% protection for the most sensitive region, which would ensure a high level of protection if applied on a UK basis.

An AF of 1 is recommended in order to derive the $PNEC_{add}$ from the generic PNEC value of 10.9 µg l^{-1}. Thus, the $PNEC_{add,freshwater\_lt}$ can be calculated as follows:

$PNEC_{add,freshwater\_lt}$ = 10.9 µg l^{-1}/AF (1) = 10.9 µg l^{-1} zinc (bioavailable)

The proposed PNEC is above the very lowest toxicity values observed under some test conditions. Field evidence does not, however, suggest that freshwater algae, such as benthic diatoms, are especially sensitive to zinc toxicity. Compliance with the above EQS is assessed in conjunction with consideration of the pH, Ca and DOC for the water as the standard is expressed as a bioavailable concentration.

A separate $PNEC_{add,freshwater\_lt}$ can also be derived using an assessment factor approach. Because data are available for three taxonomic groups, an assessment factor of 10 is recommended. When applied to the lowest reliable NOEC of 5 µg l^{-1} for algae, this results in a $PNEC_{add,freshwater\_lt}$ of 0.5 µg l^{-1} zinc (dissolved). This value is more stringent than that derived by the SSD approach.

The existing EQSs for total zinc are banded according to water hardness, with values ranging between 8 and 125 µg l^{-1} for the protection of "sensitive taxa". The $PNEC_{add,freshwater\_lt}$ derived using the SSD approach is comparable to the most stringent value from this range; the $PNEC_{add,freshwater\_lt}$ based on an assessment factor approach is lower.

Long-term PNEC for saltwaters

Based on abiotic factors, freshwater and saltwater can be regarded as different environments, each with organisms adapted to that environment. Thus, the freshwater and saltwater data in the EU RAR were not combined to derive a general $PNEC_{add,saltwater}$.

There are 36 species NOECs (using geometric means where applicable) available to construct an SSD to estimate an HC5 for saltwaters. The median 5th percentile cut-off value of 6.76 µg l^{-1} Zn is calculated with a lower 95% CL of 3.6 µg l^{-1} and an upper 95% CL of 10.9 µg l^{-1}. Based on comparison with assessment factors applied to HC5 values in European risk assessments for metals with similar data profiles, an assessment factor of 2 is considered to be appropriate for the derivation of the PNEC from the HC5.

$PNEC_{add,saltwater}$ **= 6.76 µg l^{-1}/AF (2) = 3.4 µg l^{-1} zinc (dissolved)**

Alternatively, using an assessment factor approach to derive a PNEC, the lowest reliable long-term NOEC is the 24-day survival NOEC of 5.6 µg l^{-1} for the crustacean, *Holmesimyis costata.* As long-term NOECs for at least three marine species representing three trophic levels (i.e. algae, crustaceans, and fish) plus data of the same quality for more than two further marine groups (i.e. annelids, molluscs, and echinoderms) are available, the appropriate assessment factor in accordance with the TGD is 10. This results in a $PNEC_{add,saltwater\_lt}$ of 0.56 µg l^{-1} zinc (dissolved).

Both derivations result in a PNEC that is lower than the existing EQS for dissolved Zn of 40 µg l^{-1}, which was derived by applying an assessment factor of 4 to a chronic data value of 166 µg l^{-1} obtained for the mysid shrimp *Americamysis bahia* (formerly *Mysidopsis bahia*).

PNEC for secondary poisoning

Based on data on the bioaccumulation of zinc in animals and on biomagnification, the EU RAR concludes that secondary poisoning is not relevant in the effects assessment of zinc.

PNEC for sediments

According to the EU RAR, only four reliable chronic NOEC values for benthic organisms (the insect *Chironomus tentans*, the annelid *Tubifex tubifex* and the crustacean *Hyalella azteca*) in the range of 488 – 1100 mg kg^{-1} sediment dw are available. These benthic species represent three taxonomic groups of invertebrates with different living and feeding conditions, therefore, an assessment factor of 10 should be applied to the lowest chronic NOEC.

This gives a $PNEC_{add,sediment}$ of 49 mg zinc kg^{-1} dw (equivalent to a $PNEC_{add,sediment}$ of 11 mg zinc kg^{-1} wet weight (ww)).

Summary of proposed PNECs

Receiving medium/exposure scenario	**Proposed PNEC (µg l^{-1})**	**Existing EQS (µg l^{-1})**
Freshwater/long-term	0.5 (dissolved) (AF approach) 10.9 (bioavailable) (SSD approach)	Range 8 – 125 (total zinc) depending on hardness
Saltwater/long-term	0.56 (dissolved) (AF approach), 3.4 (dissolved)(SSD approach)	40 (dissolved zinc)
Freshwater sediment/long-term	49 mg kg^{-1} dw	No standard

Implementation issues

Before PNECs for zinc can be adopted as EQSs, it will be necessary to address the following issues:

1. To implement the proposed PNECs using the added risk approach, it would be necessary to determine background concentrations of zinc at a regional, river basin or waterbody scale.

Contents

1. Introduction

The UK Technical Advisory Group (UKTAG) supporting the implementation of the Water Framework Directive (2000/60/EC)[1] is a partnership of UK environmental and conservation agencies. It also includes partners from the Republic of Ireland. UKTAG has commissioned a programme of work to derive Environmental Quality Standards (EQSs) for substances falling under Annex VIII of the Water Framework Directive (WFD). This report proposes Predicted No Effect Concentrations (PNECs) for zinc using the methodology described in Annex V of the Directive and the more recent guidance drafted by the European Commission (EC 2009). There are existing EQSs for zinc, but the methods used to derive these are not considered to comply with the requirements of Annex V and so are unsuitable for deriving Annex VIII EQSs.

The PNECs described in this report are based on a technical assessment of the available ecotoxicity data for zinc, along with any data that relate impacts under field conditions to exposure concentrations.

EU Risk Assessment Reports (EU RARs) have been compiled for zinc metal, zinc oxide, zinc distearate, zinc chloride, zinc sulphate and trizinc bis(orthophosphate). Freshwater toxicity data taken from the EU RAR for zinc metal (EU RAR 2008) were not subjected to additional quality assessment for this report. This is because they had already been assessed by the authors of the risk assessment and by an international advisory forum of experts from EU Member States. Study summaries, based on information available in the RAR, are provided in Appendix I. The freshwater dataset taken from the RAR has been updated with several relevant studies that have become available after the closure of the RAR databases. These additional studies have been quality assessed using the same reliability criteria as used in the RAR. The relevance criteria were subject to amendment to include tests conducted in waters with hardness less than 24 mg $CaCO_3$ l^{-1}. Detailed summaries are provided in Appendix II.

The marine ecotoxicity data reported in the RAR were not subject to quality assessment. A comprehensive review of the marine data cited in the RAR, together with more recent studies, was undertaken as a separate task (Environment Agency 2009e). Given the large amount of available data, only those considered of suitable quality for use in the derivation of a marine PNEC have been detailed in this report. Study summaries are provided in Appendix III.

Short-term standards have not been derived in the current report because maximum allowable concentrations for metals are not considered a priority.

The feasibility of implementing these PNECs as EQSs has not been considered at this stage. However, this would be an essential step before a regulatory EQS can be recommended. Appendix IV includes discussion of the types of bioavailability corrections for concentrations of zinc in water and sediments that are recommended when assessing compliance with a Zn EQS.

[1] *Official Journal of the European Communities*, **L327**, 1–72 (22/12/2000). Can be downloaded from http://www.eu.int/comm/environment/water/water-framework/index_en.html

1.1 Properties and fate in water

Zinc is a naturally occurring element that exists mainly as sulphides, silicates and carbonates. Zinc plays an essential role in organisms, where its internal concentration can be regulated to a limited extent depending on the concentrations to which an organism is exposed. Effects of deficiency or toxicity may occur if the concentrations deviate from those that the organism can regulate.

In water, zinc exists in the +2 oxidation state in forms that are dependent on physicochemical parameters, such as pH, hardness and the content of dissolved organic carbon. Bioavailability and toxicity may be affected by organic and inorganic complexation, with anions such as chloride (Cl^-) and carbonate (CO_3^{2-}), and by the competition of cations (e.g. Ca^{2+} and H^+) with zinc at biological receptors.

2. Results and observations

2.1 Identity of substance

Table 2.1 gives the chemical name and Chemical Abstracts Service (CAS) number for the substance of interest.

Table 2.1 Substance covered by this report

Name	CAS Number
Zinc metal (in the form of the divalent ion)	7440-66-6

2.2 PNECs proposed for derivation of quality standards

The PNECs contained in this report refer to the “added” bioavailable concentration (freshwaters) and dissolved concentration (saltwaters) of zinc to the natural background level. Differences in zinc bioavailability/toxicity because of water or sediment chemistry can be accounted for by correcting measured environmental concentrations using the biotic ligand models (BLMs) for water and the acid volatile sulphide (AVS) approach for sediment (Appendix IV). However, the practicability of the latter correction method for compliance monitoring in the UK needs to be assessed.

Table 2.2 lists proposed PNECs, obtained using the methodologies described in the Technical Guidance Document (TGD) issued by the European Chemicals Bureau (ECB) on risk assessment of chemical substances (ECB 2003), the recent guidance drafted by the European Commission (EC 2009), and existing EQSs obtained from the literature (Hunt and Hedgecott 1992; Mance and Yates 1984).

Section 2.6 summarises the effects data identified from the literature for zinc. The use of these data to derive the values given in Table 2.2 is explained in Section 3.

Table 2.2 Proposed overall PNECs for environmental quality standard setting (as total dissolved zinc)

PNEC	TGD deterministic approach (AFs)	TGD probabilistic approach (SSDs)	Existing EQS		
Freshwater long-term	0.5 µg l^{-1} (dissolved) (Section 3.1.1)	10.9 µg l^{-1} (generic PNEC based on bioavailable Zn) (Section 3.2.1)	*$CaCO_3$*	*EQS 1*	*EQS 2*
			0–50 mg l^{-1}	8 µg l^{-1}	75 µg l^{-1}
			50–100 mg l^{-1}	50 µg l^{-1}	175 µg l^{-1}
			100–150 mg l^{-1}	75 µg l^{-1}	250 µg l^{-1}
			150–200 mg l^{-1}	75 µg l^{-1}	250 µg l^{-1}
			200–250 mg l^{-1}	75 µg l^{-1}	250 µg l^{-1}
			>250 mg l^{-1}	125 µg l^{-1}	500 µg l^{-1}
			(all as total AA)		

PNEC	TGD deterministic approach (AFs)	TGD probabilistic approach (SSDs)	Existing EQS
Saltwater long-term	0.56 µg l^{-1} (dissolved) (Section 3.1.2)	3.4 µg l^{-1} (dissolved) (Section 3.2.2)	40 µg l^{-1} (AA) (dissolved)
Freshwater sediment long-term	49 mg kg^{-1} dw (see Section 3.5.1)	-	-
Freshwater secondary poisoning	No PNEC derived (trigger criteria not met)	-	-
Saltwater secondary poisoning	No PNEC derived (trigger criteria not met)	-	-

AA = annual average
AF = assessment factor
SSD = species sensitivity distribution
dw = dry weight
EQS1 = Protection of freshwater fish – salmonids
EQS 2 = Proection of freshwater fish – coarse fish

2.3 Hazard classification

Table 2.3 gives the R-phrases (Risk-phrases) and labelling for zinc powder or zinc dust (stabilised).

Table 2.3 Hazard classification

R-phrases and labelling	Reference
N; R50-53; S60-61	ECB 2005

2.4 Physical and chemical properties

Table 2.4 summarises the physical and chemical properties of the substance of interest.

Table 2.4 Physical and chemical properties of zinc (details taken from EU RAR 2008)

Property	Zn
CAS number	7440-66-6
Substance name	Zinc
Molecular formula	Zn
Molecular structure	Zn
Molecular weight	65.38
Colour/form	Solid metal
Odour	-
Melting point (°C)	420°C

Property	Zn
Boiling point (°C)	908°C
Vapour pressure	31 Pa at 450°C
Density/specific gravity	7.14 at 20°C
Henry's Law constant	N/A
Water solubility	Insoluble (as metal)
Solubilities	Soluble in acid, alkali, acetic acid

2.5 Environmental fate and partitioning

Table 2.5 summarises the information obtained from the EU RAR on the environmental fate and partitioning of zinc.

Table 2.5 Environmental fate and partitioning of zinc (details taken from EU RAR 2008)

Property	Value
Abiotic fate	Not applicable
Biodegradation	Not applicable
Partition coefficient (log Kow) $K_{susp–water}$ (suspended matter–water partition coefficient) Kp_{susp} (solids–water partition coefficient of suspended matter)	Not applicable 27501 $m^3 m^{-3}$ (calculated) 110 $m^3 kg^{-}1$

2.6 Effects data

2.6.1 Data sources and data validation

The critical freshwater data given in the recent EU Risk Assessment Report (EU RAR) on zinc metal (EU RAR 2008) were used as the basis for the derivation of the $PNEC_{add}$.[2] Additional data that have become available since the RAR have been reviewed and added.

The saltwater dataset listed in the Zn RAR was first published in a report by Cleven *et al* (1993). This report was described as a systematic review and critical evaluation of the most relevant data on zinc. The long-term tests (semi-chronic and chronic toxicity) resulting in NOEC values were evaluated on the basis of the original literature. These data were then used in the Zn RAR without further consideration. The International Zinc Association (IZA 2009) carried out a comprehensive review of the ecotoxicological data

[2] Since zinc is a naturally occurring element, the PNEC in the EU RAR was determined by applying the added risk approach. In this approach, the PNEC is determined on the basis of the amount of zinc that may be added to the natural background concentration ($C_{backgrnd}$) by anthropogenic activities without exerting adverse effects ($PNEC_{add}$). See Section A4.1 for details.

from original papers, published in peer-reviewed international journals. Literature and environmental databases, including ECOTOX (USEPA), MARITOX, ECETOC and BIOSIS and relevant review articles were searched. Very detailed summaries were provided for all the studies reviewed and criteria for endpoint selection were clearly set out. In reviewing the report produced by IZA, original papers were reviewed where considered necessary to confirm reported information, and for any study that provided the *only* endpoint for a taxonomic group. An additional literature search for saltwater chronic data was not carried out (Environment Agency 2009e).

For the evaluation of effects through the food chain (secondary poisoning), mammalian and avian toxicity data were taken from a number of recent reviews such as:

- the human health part of the EU RAR (2004) (the primary data source);
- World Health Organization (WHO) *Environmental Health Criteria 221: Zinc* (WHO 2001); and
- the International Uniform Chemical Information Database (IUCLID) dataset for zinc (IUCLID 2000).

Literature searches from 2001 (for avian data) and 2004 (mammalian data) to June 2005 (the date of production of an earlier draft of the Zn EQS report) were conducted to locate any new effects data.

All aquatic toxicity data reported in Tables 2.7 and 2.10 are expressed as “dissolved” zinc and not as the tested compound because zinc itself is considered to be the causative factor for toxicity (EU RAR 2008). For aquatic organisms, which are mainly exposed via water, the zinc ion and other dissolved zinc species are relevant for toxicity. Thus, the dissolved zinc concentration in water is a better indicator of aquatic toxicity than the total zinc concentration. However, the dissolved fraction[3] may also contain forms of zinc that are of low bioavailability.

The aquatic toxicity data used for derivation of the $PNEC_{add}$ were evaluated for the EU RAR on the basis of reliability (quality) and relevance criteria as outlined in Section 3.3.1.1 of the RAR (EU RAR 2008). Only tests that passed the quality criteria were considered further. These criteria are in agreement with internationally accepted guidelines for:

- testing of chemicals;
- monitoring of exposure concentrations;
- presence of dose–response relationships;
- investigation of toxicological endpoints that refer to effects at the population level (e.g. survival, growth, reproduction); and
- appropriate duration of chronic tests, i.e. in relation to the generation time and life-cycle of the test organism.

[3] The dissolved fraction of a substance in water is defined as the fraction that passes through a 0.45 µm filter.

The following approach was also used to select data (especially the chronic NOEC values).

- If several chronic NOEC values (from different tests) based on the same toxicological endpoint were available for one species, these values were averaged by calculating the geometric mean (GM). This resulted in a “species mean” NOEC.

- If several chronic NOEC values based on different toxicological endpoints were available for one species, the lowest value was selected. If more than one value for the same endpoint was available, the lowest value was determined as the geometric mean (see above).

- If NOEC values for different life stages of a specific organism were available and it became evident from these data that a distinct life stage was more sensitive, the result for the most sensitive life stage was selected.

- Only the results of tests in which the organisms were exposed to soluble zinc salts were used. This excluded tests with metal mixtures and “insoluble” zinc salts (ZnO, $ZnCO_3$).

- Unbounded NOEC values (i.e. no effect was found at the highest concentration tested) were not used.

The data and outcomes of the RAR have been subject to extensive peer review by others in both industry and regulatory agencies. Therefore no further assessment of studies selected as being of reliable quality within the RAR has been made here.

2.6.2 Toxicity to freshwater organisms

Abiotic factors that influence the speciation of zinc (and thus may influence bioavailability and toxicity) vary considerably in the freshwater environment. Hardness, pH and alkalinity[4] are usually considered to be the major factors influencing zinc aquatic toxicity. Detailed studies have demonstrated the protective effects of complexation by DOC and competing cations like Ca^{2+}, Mg^{2+}, Na^{+} and H^{+} on zinc toxicity to fish, crustaceans and algae (van Sprang et al. 2009).

Abiotic factors of the test waters were taken into account in the EU RAR (2008) when selecting freshwater data, as follows.

- Results from both natural and artificial test waters were accepted, provided that the major physicochemical characteristics (in particular pH and hardness) were similar to the ranges that are encountered in natural freshwaters. In addition, the background zinc concentration has been taken into account. The values for pH, hardness and background zinc concentration given in Table 2.6 were used for data selection in the EU RAR (these depart from the current OECD guidelines developed at the OECD Workshop on Aquatic Toxicity Testing of Sparingly Soluble Metals, Inorganic Metal Compounds and Minerals (OECD 1995)).

[4] In natural freshwaters the pH is proportional to alkalinity. Alkalinity, and hence pH, is proportional to hardness.

Table 2.6 Values for pH, hardness and background zinc concentration used for data selection in EU RAR (2008)

Factor	Value	
	Minimum	**Maximum**
pH	6	9
Hardness	24 mg l^{-1} (as $CaCO_3$)	250 mg l^{-1} (as $CaCO_3$)
Background zinc concentration	Around 1 µg l^{-1} (for soluble zinc)	

- The selected ranges of the three criteria do not cover all European aquatic systems. In particular, hardness may be lower in some areas of the UK. In addition, other abiotic parameters differ from the "mean" situation in European freshwaters. The EU RAR includes the derivation of a distinct $PNEC_{add}$ for soft waters. A recent report for the Environment Agency (Peters et al. 2009) concluded that analysis of the available data did not show any clear requirement for a difference in approach between waters with Ca concentrations of less than 7 mg l^{-1} and waters with Ca concentrations of greater than 7 mg l^{-1}. It would appear that similar principles, in terms of the competition between Zn and major cations such as Ca and Mg, and bioavailability reduction through Zn binding to DOC can still be applied across the complete range of conditions. As a consequence of this, data rejected in the RAR was reviewed. However, no studies were found that were rejected solely on the basis of low hardness criteria that could be usefully added to the dataset for the derivation of the PNEC. Additional reliable studies conducted in soft waters have been included.

The literature references used for the aquatic toxicity dataset of the EU RAR (2008) do not usually contain data on the background concentration of zinc in the test water. In addition, data on pH or hardness are lacking in a number of cases. Thus, a stringent application of the limits for all three parameters given in Table 2.6 (especially the zinc concentration) would have reduced the dataset considerably. This was not considered to be acceptable from a practical point of view. The following approach was therefore adopted in the EU RAR:

- When information was given on pH, hardness and background zinc concentration, the selection criteria described in Table 2.6 were used.
- When no information was given on these parameters:
  - Tests that were conducted in **artificial** waters were excluded when data on pH or hardness were lacking.
  - Tests that were conducted in **natural** waters were used unless there were clear indications that the water's pH, hardness and background zinc concentration deviated strongly from real environmental conditions.

For example, tests were excluded when carried out in waters that received special treatment to remove zinc (and other cations such as calcium and manganese). On the other hand, tests carried out in untreated natural US waters (e.g. water from Lake

Superior) that were reported to contain a background zinc concentration considerably less than 1 µg l^{-1} (depending on natural seasonal variations) were not excluded.

Data on other abiotic factors such as particulate matter or dissolved organic carbon (DOC) were rarely reported. This limited the use of these abiotic factors as selection criteria.

Freshwater toxicity data on zinc are available for various taxonomic groups including algae, invertebrates and fish as required for the application of the assessment factor approach specified in the EU Technical Guidance Document (TGD) (EC 2003). Long-term data are available for eight taxonomic groups: algae (unicellular and multicellular), amphibians, crustaceans, fish, insects, molluscs, rotifers, and sponges. Long-term data suitable for use in a species sensitivity distribution are presented in Table 2.7. The chronic NOEC values (using geometric means where applicable) of 25 species are shown in Table 2.8 before normalization and normalized to two ‘river-basin specific physico-chemistries (see Section 3.2.1).

Table 2.7 Summary of reliable long-term aquatic toxicity data for freshwater organisms.

Zinc salt	Scientific name	Common name	Taxo-nomic group	Effect	End-point	Test duration	Conc. µg l^{-1}	Exposure	Nom/ Meas	Comments	Reference
Algae (unicellular)											
Zn	*Pseudo-kirchneriella subcapitata*	Green alga	ALG	Growth rate	NOEC	3 d	50.0	s	m	OECD medium* pH 7.4 hardness 24 mg $CaCO_3$ l^{-1}	Van Woensel 1994
ZnO	*Pseudo-kirchneriella subcapitata*	Green alga	ALG	Growth rate	NOEC	3 d	24.0	s	m	OECD medium* pH 7.5 hardness 24 mg $CaCO_3$ l^{-1}	Van Ginneken 1994a
$ZnCl_2$	*Pseudokirchneriella subcapitata*	Green alga	ALG	Growth rate	EC10	3 d	5.4	s	m	OECD medium* pH 7.5 hardness 24 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003
$ZnCl_2$	*Pseudokirchneriella subcapitata*	Green alga	ALG	Growth rate	EC10	3 d	5.2	s	m	OECD medium* pH 7.5 hardness 112 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003
$ZnCl_2$	*Pseudokirchneriella subcapitata*	Green alga	ALG	Growth rate	EC10	3 d	5.5	s	m	OECD medium* pH 7.5 hardness 162 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003
$ZnCl_2$	*Pseudokirchneriella subcapitata*	Green alga	ALG	Growth rate	EC10	3 d	5.5	s	m	OECD medium* pH 7.5 hardness 212 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003
$ZnCl_2$	*Pseudokirchneriella subcapitata*	Green alga	ALG	Growth rate	EC10	3 d	5.2	s	m	OECD medium* pH 7.5 hardness 62 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003
$ZnCl_2$	*Pseudokirchneriella subcapitata*	Green alga	ALG	Growth rate	EC10	3 d	8.6	s	m	OECD medium* pH 7.5 hardness 112 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003

Zinc salt	Scientific name	Common name	Taxo-nomic group	Effect	End-point	Test duration	Conc. µg l^{-1}	Exposure	Nom/ Meas	Comments	Reference
$ZnCl_2$	*Pseudokirchneriella subcapitata*	Green alga	ALG	Growth rate	EC10	3 d	7.7	s	m	OECD medium* pH 7.5 hardness 162 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003
$ZnCl_2$	*Pseudokirchneriella subcapitata*	Green alga	ALG	Growth rate	NOEC	3 d	8.5	s	m	OECD medium* pH 7.5 hardness 212 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003
$ZnCl_2$	*Pseudokirchneriella subcapitata*	Green alga	ALG	Growth rate	EC10	3 d	6.8	s	m	OECD medium* pH 7.5 hardness 24 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003
$ZnCl_2$	*Pseudokirchneriella subcapitata*	Green alga	ALG	Growth rate	EC10	3 d	7.9	s	m	OECD medium* pH 7.5 hardness 24 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003
$ZnCl_2$	*Pseudokirchneriella subcapitata*	Green alga	ALG	Growth rate	NOEC	3 d	7.4	s	m	OECD medium* pH 7.5 hardness 24 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003
$ZnCl_2$	*Pseudo-kirchneriella subcapitata*	Green alga	ALG	Growth rate	EC10	3 d	4.9	s	m	OECD medium* pH 7.5 hardness 24 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003
$ZnCl_2$	*Pseudokirchneriella subcapitata*	Green alga	ALG	Growth rate	NOEC	3 d	124	s	m	OECD medium* pH 6.2 hardness 24 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003
$ZnCl_2$	*Pseudokirchneriella subcapitata*	Green alga	ALG	Growth rate	NOEC	3 d	74	s	m	OECD medium* pH 6.8 hardness 24 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003
$ZnCl_2$	*Pseudokirchneriella subcapitata*	Green alga	ALG	Growth rate	NOEC	3 d	41	s	m	OECD medium* pH 7.1 hardness 24 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003

Zinc salt	Scientific name	Common name	Taxo-nomic group	Effect	End-point	Test duration	Conc. µg l^{-1}	Exposure	Nom/ Meas	Comments	Reference
$ZnCl_2$	*Pseudokirchneriella subcapitata*	Green alga	ALG	Growth rate	EC10	3 d	15	s	m	OECD medium* pH 7.4 hardness 24 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003
$ZnCl_2$	*Pseudokirchneriella subcapitata*	Green alga	ALG	Growth rate	NOEC	3 d	10	s	m	OECD medium* pH 7.7; hardness 24 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003
$ZnCl_2$	*Pseudokirchneriella subcapitata*	Green alga	ALG	Growth rate	NOEC	3 d	9.4	s	m	OECD medium* pH 7.8 hardness 24 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003
$ZnCl_2$	*Pseudokirchneriella subcapitata*	Green alga	ALG	Growth rate	NOEC	3 d	58	s	m	River water DOC 2.9 mg l^{-1} pH 6.2 hardness 28 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003
$ZnCl_2$	*Pseudokirchneriella subcapitata*	Green alga	ALG	Growth rate	NOEC	3 d	91	s	m	River water DOC 2.5 mg l^{-1} pH 6.3 hardness 27 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003, 2005
$ZnCl_2$	*Pseudokirchneriella subcapitata*	Green alga	ALG	Growth rate	NOEC	3 d	73	s	m	River water DOC 3.7 mg l^{-1} pH 6.4 hardness 27 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003, 2005
$ZnCl_2$	*Pseudokirchneriella subcapitata*	Green alga	ALG	Growth rate	EC10	3 d	27	s	m	Lake water DOC 5.9 mg l^{-1} pH 8.0 hardness 239 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003, 2005
$ZnCl_2$	*Pseudokirchneriella subcapitata*	Green alga	ALG	Growth rate	NOEC	3 d	105	s	m	Ditch water DOC 22 mg l^{-1} pH 7.4 hardness 144 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003, 2005

Zinc salt	Scientific name	Common name	Taxo-nomic group	Effect	End-point	Test duration	Conc. µg l^{-1}	Exposure	Nom/ Meas	Comments	Reference
$ZnCl_2$	*Pseudo-kirchneriella subcapitata*	Green alga	ALG	Growth rate	NOEC	3 d	28	s	m	Lake Maridalsvann water pH 6.7 hardness 8mg $CaCO_3$ l^{-1}	Muyssen et al. 2003
$ZnCl_2$	*Pseudo-kirchneriella subcapitata*	Green alga	ALG	Growth rate	NOEC	3 d	67	s	m	Lake Maridalsvann water pH 6.7 hardness 100 mg $CaCO_3$ l^{-1}	Muyssen et al. 2003
$ZnCl_2$	*Pseudo-kirchneriella subcapitata*	Green alga	ALG	Growth rate	NOEC	3 d	65	s	m	Lake Sundungen water pH 6.4 hardness 6.1 mg $CaCO_3$ l^{-1}	Muyssen et al. 2003
$ZnCl_2$	*Pseudo-kirchneriella subcapitata*	Green alga	ALG	Growth rate	NOEC	3 d	86	s	m	Lake Sundungen water pH 6.4 hardness 100 mg $CaCO_3$ l^{-1}	Muyssen et al. 2003
$ZnSO_4 \cdot 7H_20$	*Chlorella* sp.	Green alga	ALG	Growth rate	EC10	48 hours	349	s	m	Artificial water pH 6 hardness 43 mg $CaCO_3$ l^{-1}	Wilde et al. 2006
$ZnSO_4 \cdot 7H_20$	*Chlorella* sp.	Green alga	ALG	Growth rate	EC10	48 hours	104	s	m	Artificial water pH 6.5 hardness 43 mg $CaCO_3$ l^{-1}	Wilde et al. 2006
$ZnSO_4 \cdot 7H_20$	*Chlorella* sp.	Green alga	ALG	Growth rate	EC10	48 hours	92	s	m	Artificial water pH 7 hardness 43 mg $CaCO_3$ l^{-1}	Wilde et al. 2006

Zinc salt	Scientific name	Common name	Taxo-nomic group	Effect	End-point	Test duration	Conc. µg l^{-1}	Exposure	Nom/ Meas	Comments	Reference
$ZnSO_4 \cdot 7H_20$	*Chlorella* sp.	Green alga	ALG	Growth rate	EC10	48 hours	15	s	m	Artificial water pH 7.5 hardness 43 mg $CaCO_3$ l^{-1}	Wilde et al. 2006
$ZnSO_4 \cdot 7H_20$	*Chlorella* sp.	Green alga	ALG	Growth rate	EC10	48 hours	4.9	s	m	Artificial water pH 8 hardness 43 mg $CaCO_3$ l^{-1}	Wilde et al. 2006
Algae (multicellular)											
$ZnCl_2$	*Cladophora glomerata*	Green alga	ALG	Growth	NOEC	3 d	60	s	n	pH 8.4 hardness >35 mg $CaCO_3$ l^{-1}	Whitton 1967
Sponges											
$ZnCl_2$	*Ephydatia fluviatilis*	Sponge	POR	Develop-ment	NOEC	7 d	43	s	n	Elendt M4 pH 8 hardness 250 mg $CaCO_3$ l^{-1}	Van de Vyver 2001
$ZnCl_2$	*Ephydatia muelleri*	Sponge	POR	Develop-ment	NOEC	7 d	43	s	n	Elendt M4 pH 8 hardness 250 mg $CaCO_3$ l^{-1}	Van de Vyver 2001
$ZnCl_2$	*Spongilla lacustris*	Sponge	POR	Develop-ment	NOEC	7 d	65	s	n	Elendt M4 pH 8 hardness 250 mg $CaCO_3$ l^{-1}	Van de Vyver 2001
$ZnCl_2$	*Eunapius gragilis*	Sponge	POR	Develop-ment	NOEC	7 d	43	s	n	Elendt M4 pH 8 hardness 250 mg $CaCO_3$ l^{-1}	Van de Vyver 2001
Rotifers											
$ZnCl_2$	*Anuraeopsis fissa*	Rotifer	ROT	Popu-lation growth	NOEC	~ 20 d	48	ss	m	EPA medium pH 7.1-7.3 hardness 80-100 mg $CaCO_3$ l^{-1}	Azuara-García et al. 2006

Zinc salt	Scientific name	Common name	Taxo-nomic group	Effect	End-point	Test duration	Conc. µg l^{-1}	Exposure	Nom/ Meas	Comments	Reference
$ZnCl_2$	*Brachionus rubens*	Rotifer	ROT	Popu-lation growth	NOEC[1]	~ 20 d	24	ss	m	EPA medium pH 7.1-7.3 hardness 80-100 mg $CaCO_3$ l^{-1}	Azuara-García et al. 2006
Molluscs											
$ZnCl_2$	*Dreissena polymorpha*	Zebra mussel	MOL	Survival	NOEC	10 weeks	379	ss	m	Lake water pH 7.9 hardness 270 mg $CaCO_3$ l^{-1}	Kraak et al. 1994
$ZnCl_2$	*Potamopyrgus jenkinsi*	Jenkins' Spire Snail	MOL	Growth	NOEC	16 weeks	60	ss	m	Lake water pH 8 hardness 160 mg $CaCO_3$ l^{-1}	Dorgelo et al. 1995
Crustaceans											
-	*Ceriodaphnia dubia*	Water flea	CRU	Repro-duction	NOEC	7 days	25	ss	n	River water pH 6 hardness 81 mg $CaCO_3$ l^{-1}	Belanger and Cherry 1990
-	*Ceriodaphnia dubia*	Water flea	CRU	Repro-duction	NOEC[1]	7 days	25	ss	n	River water pH 8 hardness 81 mg $CaCO_3$ l^{-1}	Belanger and Cherry 1990
-	*Ceriodaphnia dubia*	Water flea	CRU	Repro-duction	NOEC	7 days	25	ss	n	River water pH 9 hardness 81 mg $CaCO_3$ l^{-1}	Belanger and Cherry 1990
-	*Ceriodaphnia dubia*	Water flea	CRU	Repro-duction	EC10	7 days	40	ss	n	River water pH 6 hardness 118 mg $CaCO_3$ l^{-1}	Belanger and Cherry 1990
-	*Ceriodaphnia dubia*	Water flea	CRU	Repro-duction	NOEC	7 days	50	ss	n	River water pH 8 hardness 118 mg $CaCO_3$ l^{-1}	Belanger and Cherry 1990

Zinc salt	Scientific name	Common name	Taxo-nomic group	Effect	End-point	Test duration	Conc. µg l^{-1}	Exposure	Nom/ Meas	Comments	Reference
-	*Ceriodaphnia dubia*	Water flea	CRU	Repro-duction	EC10	7 days	45	ss	n	River water pH 9 hardness 118 mg $CaCO_3$ l^{-1}	Belanger and Cherry 1990
-	*Ceriodaphnia dubia*	Water flea	CRU	Repro-duction	EC10	7 days	29	ss	n	River water pH 6 hardness 168 mg $CaCO_3$ l^{-1}	Belanger and Cherry 1990
-	*Ceriodaphnia dubia*	Water flea	CRU	Repro-duction	NOEC	7 days	50	ss	n	River water pH 8 hardness 168 mg $CaCO_3$ l^{-1}	Belanger and Cherry 1990
-	*Ceriodaphnia dubia*	Water flea	CRU	Repro-duction	NOEC[2]	7 days	33	ss	n	River water pH 9 hardness 168 mg $CaCO_3$ l^{-1}	Belanger and Cherry 1990
$ZnCl_2$	*Ceriodaphnia dubia*	Water flea	CRU	Repro-duction	NOEC	4 days	50	ss	n	Little Miami River water pH 8 hardness 169 mg $CaCO_3$ l^{-1}	Masters et al. 1991
$ZnCl_2$	*Ceriodaphnia dubia*	Water flea	CRU	Repro-duction	NOEC	4 days	14	ss	n	Little Miami River water pH 8 hardness 169 mg $CaCO_3$ l^{-1}	Masters et al. 1991
$ZnCl_2$	*Ceriodaphnia dubia*	Water flea	CRU	Repro-duction	NOEC	7 days	50	ss	n	Little Miami River water pH 8 hardness 169 mg $CaCO_3$ l^{-1}	Masters et al. 1991
$ZnCl_2$	*Ceriodaphnia dubia*	Water flea	CRU	Repro-duction	NOEC	7 days	100	ss	n	Little Miami River water pH 8 hardness 169 mg $CaCO_3$ l^{-1}	Masters et al. 1991

Zinc salt	Scientific name	Common name	Taxo-nomic group	Effect	End-point	Test duration	Conc. µg l^{-1}	Exposure	Nom/ Meas	Comments	Reference
$ZnCl_2$	*Daphnia magna*	Water flea	CRU	Repro-duction	NOEC	21 days	97	ss	m	Well water pH 7.5 hardness 52 mg $CaCO_3$ l^{-1}	Chapman et al. 1980
$ZnCl_2$	*Daphnia magna*	Water flea	CRU	Repro-duction	NOEC	21 days	43	ss	m	Well water pH 7.7 hardness 104 mg $CaCO_3$ l^{-1}	Chapman et al. 1980
$ZnCl_2$	*Daphnia magna*	Water flea	CRU	Repro-duction	NOEC	21 days	42	ss	m	Well water pH 8.4 hardness 211 mg $CaCO_3$ l^{-1}	Chapman et al. 1980
$ZnSO_4 \cdot 7H_20$	*Daphnia magna*	Water flea	CRU	Repro-duction	EC10	7 weeks	31	ss	n	Pond water pH 8.4 hardness 52 mg $CaCO_3$ l^{-1}	Paulauskis and Winner 1988
$ZnSO_4 \cdot 7H_20$	*Daphnia magna*	Water flea	CRU	Repro-duction	EC10	7 weeks	33	ss	n	Pond water DOC 0.75 mg l^{-1} pH 8.4 hardness 52 mg $CaCO_3$ l^{-1}	Paulauskis and Winner 1988
$ZnSO_4 \cdot 7H_20$	*Daphnia magna*	Water flea	CRU	Repro-duction	NOEC	7 weeks	84	ss	n	Pond water DOC 1.5 mg l^{-1} pH 8.4 hardness 52 mg $CaCO_3$ l^{-1}	Paulauskis and Winner 1988
$ZnSO_4 \cdot 7H_20$	*Daphnia magna*	Water flea	CRU	Repro-duction	NOEC	7 weeks	83	ss	n	Pond water pH 8.3 hardness 102 mg $CaCO_3$ l^{-1}	Paulauskis and Winner 1988
$ZnSO_4 \cdot 7H_20$	*Daphnia magna*	Water flea	CRU	Repro-duction	NOEC	7 weeks	159	ss	n	Pond water pH 8.3 hardness 197 mg $CaCO_3$ l^{-1}	Paulauskis and Winner 1988
$ZnSO_4 \cdot 7H_20$	*Daphnia magna*	Water flea	CRU	Repro-duction	NOEC	7 weeks	208	ss	n	Pond water DOC 1.5 mg l^{-1} pH 8.3	Paulauskis and Winner 1988

Zinc salt	Scientific name	Common name	Taxo-nomic group	Effect	End-point	Test duration	Conc. µg l^{-1}	Exposure	Nom/ Meas	Comments	Reference
										hardness 197 mg $CaCO_3$ l^{-1}	
$ZnCl_2$	*Daphnia magna*	Water flea	CRU	Repro-duction	NOEC[1]	21 days	35	ss	n	Lake Superior water pH 7.7 hardness 45 mg $CaCO_3$ l^{-1}	Biesinger and Christensen 1972
$ZnCl_2$	*Daphnia magna*	Water flea	CRU	Repro-duction	NOEC	21 days	74	ss	m	Lake Superior water pH 7.7 hardness 45 mg $CaCO_3$ l^{-1}	Biesinger et al. 1986
$ZnCl_2$	*Daphnia magna*	Water flea	CRU	Repro-duction	NOEC	21 days	310	ss	n	Lake IJssel water pH 8.1 hardness 225 mg $CaCO_3$ l^{-1}	Enserink et al. 1991
$ZnCl_2$	*Daphnia magna*	Water flea	CRU	Repro-duction	EC10	17 days	420	f	n	Lake IJssel water pH 8.1 hardness 225 mg $CaCO_3$ l^{-1}	Enserink et al. 1991
-	*Daphnia magna*	Water flea	CRU	Repro-duction	NOEC	21 days	100	ss	n	Lake Maggiore water pH 7.7 hardness 65 mg $CaCO_3$ l^{-1}	Münzinger and Monicelli 1991
-	*Daphnia magna*	Water flea	CRU	Repro-duction	NOEC	21 days	100	ss	n	Lake Maggiore water pH 7.7 hardness 65 mg $CaCO_3$ l^{-1}	Münzinger and Monicelli 1991
-	*Daphnia magna*	Water flea	CRU	Repro-duction	EC10	21 days	25	ss	n	Lake Maggiore water pH 7.7 hardness 65 mg $CaCO_3$ l^{-1}	Münzinger and Monicelli 1991

Zinc salt	Scientific name	Common name	Taxo-nomic group	Effect	End-point	Test duration	Conc. µg l^{-1}	Exposure	Nom/ Meas	Comments	Reference
$ZnCl_2$	*Daphnia magna*	Water flea	CRU	Repro-duction	NOEC	21 days	82	ss	m	Artificial water pH 6.6 hardness 50 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003
$ZnCl_2$	*Daphnia magna*	Water flea	CRU	Repro-duction	NOEC	21 days	50	ss	m	Artificial water pH 6.6 hardness 75 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003
$ZnCl_2$	*Daphnia magna*	Water flea	CRU	Repro-duction	NOEC	21 days	54	ss	m	Artificial water pH 6.6 hardness 125 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003
$ZnCl_2$	*Daphnia magna*	Water flea	CRU	Repro-duction	NOEC	21 days	92	ss	m	Artificial water pH 6.6 hardness 225 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003
$ZnCl_2$	*Daphnia magna*	Water flea	CRU	Repro-duction	NOEC	21 days	48	ss	m	Artificial water pH 6.6 hardness 75 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003
$ZnCl_2$	*Daphnia magna*	Water flea	CRU	Repro-duction	NOEC	21 days	152	ss	m	Artificial water pH 6.6 hardness 125 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003
$ZnCl_2$	*Daphnia magna*	Water flea	CRU	Repro-duction	NOEC	21 days	155	ss	m	Artificial water pH 6.6 hardness 175 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003
$ZnCl_2$	*Daphnia magna*	Water flea	CRU	Repro-duction	NOEC	21 days	156	ss	m	Artificial water pH 6.6 hardness 225 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003
$ZnCl_2$	*Daphnia magna*	Water flea	CRU	Repro-duction	NOEC	21 days	143	ss	m	Artificial water pH 6.6 hardness 50 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003

Zinc salt	Scientific name	Common name	Taxo-nomic group	Effect	End-point	Test duration	Conc. µg l^{-1}	Exposure	Nom/ Meas	Comments	Reference
$ZnCl_2$	*Daphnia magna*	Water flea	CRU	Repro-duction	NOEC	21 days	136	ss	m	Artificial water pH 6.6 hardness 50 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003
$ZnCl_2$	*Daphnia magna*	Water flea	CRU	Repro-duction	NOEC	21 days	143	ss	m	Artificial water pH 6.6 hardness 50 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003
-	*Daphnia magna*	Water flea	CRU	Repro-duction	NOEC	21 days	155	ss	m	Artificial water pH 7.2 hardness 250 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2005
$ZnCl_2$	*Daphnia longispina*	Water flea	CRU	Repro-duction	NOEC	21 d	37	s	m	Lake Maridalsvann water pH 6.7 hardness 8 mg $CaCO_3$ l^{-1}	Muyssen et al. 2003
$ZnCl_2$	*Daphnia longispina*	Water flea	CRU	Repro-duction	NOEC	21 d	82	s	m	Lake Maridalsvann water pH 6.7 hardness 100 mg $CaCO_3$ l^{-1}	Muyssen et al. 2003
$ZnCl_2$	*Daphnia longispina*	Water flea	CRU	Repro-duction	NOEC	21 d	41	s	m	Lake Sundungen water pH 6.4 hardness 6.1 mg $CaCO_3$ l^{-1}	Muyssen et al. 2003
$ZnCl_2$	*Daphnia longispina*	Water flea	CRU	Repro-duction	NOEC	21 d	199	s	m	Lake Sundungen water pH 6.4 hardness 100 mg $CaCO_3$ l^{-1}	Muyssen et al. 2003

Zinc salt	Scientific name	Common name	Taxo-nomic group	Effect	End-point	Test duration	Conc. µg l^{-1}	Exposure	Nom/ Meas	Comments	Reference
-	*Hyalella azteca*	Amphi-pod	CRU	Survival	NOEC	10 weeks	36	ss	m	Dechlorinated tap water pH 7.9 – 8.6 hardness 130 mg $CaCO_3$ l^{-1}	Borgmann et al. 1993
Insects											
$ZnCl_2$	*Chironomus tentans*	Midge	INS	Emer-gence	NOEC	8 weeks	137	ss	m	Lake water pH 7.7 hardness 45 mg $CaCO_3$ l^{-1}	Sibley et al. 1996
Vertebrates (fish and amphibians)											
$ZnSO_4$ ·$7H_20$	*Brachydanio rerio*	Zebra fish	FIS	Hatch-ability	NOEC	14 days	2900	ss	n	Artificial water pH 7.5 hardness 100 mg $CaCO_3$ l^{-1}	Dave et al. 1987
$ZnSO_4$ ·$7H_20$	*Brachydanio rerio*	Zebra fish	FIS	Hatch-ability	NOEC	14 days	180	ss	n	Artificial water pH 7.5 hardness 100 mg $CaCO_3$ l^{-1}	Dave et al. 1987
$ZnSO_4$ ·$7H_20$	*Brachydanio rerio*	Zebra fish	FIS	Hatch-ability	NOEC	14 days	720	ss	n	Artificial water pH 7.5 hardness 100 mg $CaCO_3$ l^{-1}	Dave et al. 1987
$ZnSO_4$ ·$7H_20$	*Brachydanio rerio*	Zebra fish	FIS	Hatch-ability	NOEC	14 days	180	ss	n	Artificial water pH 7.5 hardness 100 mg $CaCO_3$ l^{-1}	Dave et al. 1987
$ZnSO_4$ ·$7H_20$	*Brachydanio rerio*	Zebra fish	FIS	Hatch-ability	NOEC	14 days	180	ss	n	Artificial water pH 7.5 hardness 100 mg $CaCO_3$ l^{-1}	Dave et al. 1987
$ZnSO_4$ ·$7H_20$	*Brachydanio rerio*	Zebra fish	FIS	Hatch-ability	NOEC	14 days	180	ss	n	Artificial water pH 7.5 hardness 100 mg $CaCO_3$ l^{-1}	Dave et al. 1987

Zinc salt	Scientific name	Common name	Taxo-nomic group	Effect	End-point	Test duration	Conc. µg l^{-1}	Exposure	Nom/ Meas	Comments	Reference
$ZnSO_4 \cdot 7H_20$	*Brachydanio rerio*	Zebra fish	FIS	Hatch-ability	NOEC	14 days	2900	ss	n	Artificial water pH 7.5 hardness 100 mg $CaCO_3$ l^{-1}	Dave et al. 1987
$ZnSO_4 \cdot 7H_20$	*Brachydanio rerio*	Zebra fish	FIS	Hatch-ability	NOEC	14 days	2900	ss	n	Artificial water pH 7.5 hardness 100 mg $CaCO_3$ l^{-1}	Dave et al. 1987
$ZnSO_4 \cdot 7H_20$	*Brachydanio rerio*	Zebra fish	FIS	Hatch-ability	NOEC	14 days	1400	ss	n	Artificial water pH 7.5 hardness 100 mg $CaCO_3$ l^{-1}	Dave et al. 1987
$ZnSO_4 \cdot 7H_20$	*Jordanella floridae*	Flag fish	FIS	Growth	NOEC	14 weeks	26	f	m	Lake Superior water pH 7.5 hardness 44 mg $CaCO_3$ l^{-1}	Spehar 1976
$ZnSO_4 \cdot 7H_20$	*Jordanella floridae*	Flag fish	FIS	Growth/ repro-duction	NOEC	14 weeks	75	f	m	Lake Superior water pH 7.5 hardness 44 mg $CaCO_3$ l^{-1}	Spehar 1976
$ZnSO_4 \cdot 7H_20$	*Oncorhynchus mykiss*	Rainbow trout	FIS	Survival	NOEC	2 years	130	f	m	Dechlorinated tap water pH 6.8 hardness 26 mg $CaCO_3$ l^{-1}	Sinley et al. 1974
$ZnSO_4 \cdot 7H_20$	*Oncorhynchus mykiss*	Rainbow trout	FIS	Survival	NOEC	25 days	25	f	m	Dechlorinated tap water pH 6.8 hardness 26 mg $CaCO_3$ l^{-1}	Sinley et al. 1974
$ZnCl_2$	*Oncorhynchus mykiss*	Rainbow trout	FIS	Survival	NOEC	72 days	440	f	m	Well water pH 7.0 hardness 27 mg $CaCO_3$ l^{-1}	Cairns et al. 1982

Zinc salt	Scientific name	Common name	Taxo-nomic group	Effect	End-point	Test duration	Conc. µg l^{-1}	Exposure	Nom/ Meas	Comments	Reference
$ZnCl_2$	*Oncorhynchus mykiss*	Rainbow trout	FIS	Survival	NOEC	30 days	39	f	m	Artificial water pH 7.5 hardness 30 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003
$ZnCl_2$	*Oncorhynchus mykiss*	Rainbow trout	FIS	Survival	NOEC	30 days	95	f	m	Artificial water pH 7.5 hardness 30 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003, De Schamphelaere and Janssen 2004
$ZnCl_2$	*Oncorhynchus mykiss*	Rainbow trout	FIS	Survival	NOEC	30 days	45	f	m	Artificial water pH 7.7 hardness 45 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003, De Schamphelaere and Janssen 2004
$ZnCl_2$	*Oncorhynchus mykiss*	Rainbow trout	FIS	Survival	NOEC	30 days	151	f	m	Artificial water pH 7.7 hardness 139 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003, De Schamphelaere and Janssen 2004
$ZnCl_2$	*Oncorhynchus mykiss*	Rainbow trout	FIS	Survival	NOEC	30 days	159	f	m	Artificial water pH 7.7 hardness 229 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003, De Schamphelaere and Janssen 2004
$ZnCl_2$	*Oncorhynchus mykiss*	Rainbow trout	FIS	Survival	NOEC	30 days	256	f	m	Artificial water pH 6.7 hardness 29 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003, De Schamphelaere and Janssen 2004
$ZnCl_2$	*Oncorhynchus mykiss*	Rainbow trout	FIS	Survival	NOEC	30 days	157	f	m	Artificial water pH 7.6 hardness 28 mg	De Schamphelaere et al. 2003, De

Zinc salt	Scientific name	Common name	Taxo-nomic group	Effect	End-point	Test duration	Conc. µg l^{-1}	Exposure	Nom/ Meas	Comments	Reference
										$CaCO_3$ l^{-1}	Schamphelaere and Janssen 2004
$ZnCl_2$	*Oncorhynchus mykiss*	Rainbow trout	FIS	Survival	NOEC	30 days	974	f	m	Artificial water pH 7.9 hardness 190 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003, De Schamphelaere and Janssen 2004
$ZnCl_2$	*Oncorhynchus mykiss*	Rainbow trout	FIS	Survival	NOEC	30 days	771	f	m	Ditch water DOC 23 mg l^{-1} pH 7.8 hardness 104 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003, 2005
$ZnCl_2$	*Oncorhynchus mykiss*	Rainbow trout	FIS	Survival	NOEC	30 days	696	f	m	Lake water DOC 6.2 mg l^{-1} pH 8.1 hardness 176 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003, 2005
$ZnCl_2$	*Oncorhynchus mykiss*	Rainbow trout	FIS	Survival	NOEC	30 days	324	f	m	River water DOC 3.9 mg l^{-1} pH 6.8 hardness 28 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003, 2005
$ZnCl_2$	*Oncorhynchus mykiss*	Rainbow trout	FIS	Survival	NOEC	30 days	370	f	m	River water DOC 4.3 mg l^{-1} pH 6.2 hardness 23 mg $CaCO_3$ l^{-1}	De Schamphelaere et al. 2003, 2005
$ZnSO_4 \cdot 7H_20$	*Phoxinus phoxinus*	Minnow	FIS	Survival/ growth	NOEC	5 months	50	f	m	Dechlorinated tap water pH 7.5 hardness 70 mg $CaCO_3$ l^{-1}	Bengtsson 1974
$ZnSO_4 \cdot 7H_20$	*Pimephales promelas*	Fathead minnow	FIS	Repro-duction	NOEC	8 months	78	f	m	Lake water pH 7-8 hardness 46 mg	Benoit and Holcombe 1978

Zinc salt	Scientific name	Common name	Taxo-nomic group	Effect	End-point	Test duration	Conc. µg l^{-1}	Exposure	Nom/ Meas	Comments	Reference
										$CaCO_3$ l^{-1}	
$ZnSO_4 \cdot 7H_20$	*Salvelinus fontinalis*	Brook trout	FIS	Hatch-ability	NOEC	3 years	530	f	m	Lake water pH 7.0-7.7 hardness 45 mg $CaCO_3$ l^{-1}	Holcombe et al. 1979
$ZnSO_4 \cdot 7H_20$	*Salmo trutta*	Brown trout	FIS	Hatching success	NOEC	~ 120 days	51	f	m	Lake Store Sandungen water pH 6.7 hardness 8 mg $CaCO_3$ l^{-1}	Källqvist et al. 2003
$ZnSO_4 \cdot 7H_20$	*Salmo trutta*	Brown trout	FIS	Hatching success	NOEC	~ 120 days	243	f	m	Lake Store Sandungen water pH 6.2–6.6 hardness 100 mg $CaCO_3$ l^{-1}	Källqvist et al. 2003
$ZnSO_4 \cdot 7H_20$	*Salmo trutta*	Brown trout	FIS	Hatching success	NOEC	~ 120 days	54	f	m	Lake Maridalsvann water pH 6.4 hardness 6.1 mg $CaCO_3$ l^{-1}	Källqvist et al. 2003
$ZnSO_4 \cdot 7H_20$	*Salmo trutta*	Brown trout	FIS	Hatching success	NOEC	~ 120 days	51	f	m	Lake Maridalsvann water pH 6.6–6.9 hardness 100 mg $CaCO_3$ l^{-1}	Källqvist et al. 2003
$ZnSO_4 \cdot 7H_20$	*Cottus bairdii*	Mottled sculpin	FIS	Survival	NOEC	30 days	169	f	m	Dechlorinated tap water/ well water pH 7.5 hardness 154 mg $CaCO_3$ l^{-1}	Brinkman and Woodling 2005

Zinc salt	Scientific name	Common name	Taxo-nomic group	Effect	End-point	Test duration	Conc. µg l^{-1}	Exposure	Nom/ Meas	Comments	Reference
$ZnCl_2$	*Rhinella arenarum*	South American toad	AMP	Survival	LC10	21 days	840	ss	n	Artificial water hardness 90 mg $CaCO_3$ l^{-1}	Brodeur et al. 2009

* OECD medium with no EDTA
LOEC = lowest observed effect concentration; NOEC = no observed effect concentration
LCx = concentration lethal to X% of the organisms tested; ECx = concentration effective against X% of the organisms tested
$NOEC^1$ = NOEC derived from LOEC/2; $NOEC^2$ = NOEC derived from LOEC/3
ALG = algae, AMP = amphibians, CRU = crustaceans, FIS = fish, INS = insects, MOL = molluscs, POR = porifera, ROT = rotifera
s = static; ss = semi-static; f = flow-through; m = measured test concentration; n = nominal value test concentration

Table 2.8 Species NOEC values before normalization and normalized for two specific 'river-basin' water chemistries

Taxonomic groups/species	Species NOEC values (µg l^{-1})		
	Before normalization	**Normalized to UK(1) soft water**	**Normalized to UK(2) hard water**
Algae (unicellular)			
*P. subcapitata**	19.7	314.9	7.7
Chlorella sp.*	47.6	841.9	13.8
Algae (multicellular)			
C. glomerata	60	8918.7	127.7
Sponges			
E. fluviatilis	43	31.3	71.2
E. muelleri	43	31.3	71.2
S. lacustris	65	48.1	106.7
E. fragilis	43	31.3	71.2
Molluscs			
D. polymorpha	400	133.9	281.8
P. jenkinsi	75	16.4	39.1
Crustaceans			
*C., dubia**	36.9	10.5	25.6
*D. magna**	90.1	93.1	199.3
*D. longispina**	70.5	101.2	215.7
H. azteca	42	19.9	46.8
Insects			
C. tentans	137	236.5	487.6
Rotifers			
A. fissa	48	77.2	166.6
B. rubens	24	38.2	85.9
Fish			
*D. rerio**	666.1	1215.8	1935.2
*J.floridae**	44.2	121.1	205.1
P. phoxinus	50	107.3	182.7
P. promelas	78	237.5	391.0
*O. mykiss**	189.3	423.2	685.4
S. fontinalis	530	1993.4	3156.9
S. trutta	76.4	155.1	259.4
C. bairdi	169	266.9	437.8
Amphibians			
R. arenarum	840	2708.2	4278.5

* Geometric mean values.

Toxicity observed in mesocosm and field studies

It is important to compare the results from single-species toxicity data from laboratory tests with the results of (model) ecosystem studies and field studies, if these are available. This section, which is taken from the EU RAR (2008), describes the available literature on model ecosystems and field studies.

- A study in outdoor artificial streams resulted in a nominal multi-species no observed effect concentration (NOEC) of 25 µg l^{-1} (actual total zinc concentration: ≤20 µg l^{-1} (detection limit), pH ~8.1–8.4, hardness 60–90 mg l^{-1}). The nominal lowest observed effect concentration (LOEC) was 50 µg l^{-1} (actual total zinc concentration: 34–87 µg l^{-1}). Effects on periphyton, zooplankton and macroinvertebrates (clams and snails) were studied in this model ecosystem (Belanger et al. 1986, Farris et al. 1989 and 1994, Genter et al. 1987).

- A study in a laboratory flow-through system with periphyton resulted in a NOEC of around 10 µg l^{-1} (actual total zinc concentration) for the most sensitive, biomass-related endpoints: bacterial activity (^{3}H-incorporation), periphyton photosynthetic activity (^{14}C-incorporation), and periphyton dry weight (dw). The pH of the water was 6.1–7.1. The NOEC for algal biomass (chlorophyll a content) and species richness (the number of different taxa or groups of taxa) was 27 µg l^{-1}; for species composition (relative abundance) the NOEC was 117 µg l^{-1} (Paulsson et al. 2000).

  According to the authors of the study, 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 of 630 µg l^{-1}.

- Two further studies in laboratory flow-through systems with periphyton resulted in effects on biomass-related endpoints at actual concentrations of 73 µg l^{-1} (nominal: 50 µg l^{-1}) and 4.2 µg l^{-1}, the lowest concentrations tested (pH 7.78, hardness 73.8 mg l^{-1}) (Niederlehner and Cairns 1993, Pratt et al. 1987, respectively). In the former study, species richness was not significantly affected at 73 µg l^{-1}. In the latter study, species richness was lower at 4.2 µg l^{-1} than at 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^{-1}, respectively, probably based on species richness.

- A field study with phytoplankton and zooplankton (Lake Michigan water, pH and hardness not reported; Marshall et al. 1983) and a laboratory study with phytoplankton (pH 7.6 – 8.7, hardness 280 – 340 mg l^{-1}; Gächter 1976) resulted in effects at 17 µg l^{-1} (several endpoints were studied, including quantitative analysis of zooplankton) and 33 µg l^{-1} (photosynthesis). From these two studies, Emans et al. (1992) derived multi-species NOEC values of 1.7 and 3.3 µg l^{-1}, respectively, using NOEC = LOEC/10. However, the values derived by Emans et al. (1992) are considered to be unreliable based on the RAR criteria of high assessment factor of 10 and NOEC extrapolated far below the lowest test concentration.

These field and mesocosm studies show effects in the low range of 10–20 µg l^{-1} zinc (and at higher concentrations, depending on the endpoint).

2.6.3 Toxicity to saltwater organisms

Chronic toxicity to saltwater organisms

Selection of ecotoxicity data for quality was done using the systematic approach recommended by Klimisch et al. (1997) which has become an accepted method for data evaluation. Particular issues when considering the reliability and relevance of saltwater zinc toxicity data are:

- *Test substance* – All the endpoints that were eventually selected for use in the SSD were from tests conducted with readily soluble salts, mostly $ZnSO_4$ or $ZnCl_2$, with the exception of one endpoint where the form of the test material is described only as zinc. It is generally accepted that under laboratory conditions the majority of zinc present in the test system is in the dissolved fraction and therefore the results have been treated as dissolved zinc concentrations.

- *Test medium – background concentrations.* Natural or artificial seawater were considered to be acceptable as test media. Where natural seawater was used consideration was given to the zinc background concentration. Toxicity studies from unpolluted test media were regarded as reliable. Tests that were performed in media containing relatively high background zinc concentrations were excluded (zinc concentrations >10 µg Zn l^{-1}). In reality, the majority of the studies did not mention background concentrations and results were based on nominal concentrations. Such results have been treated as "added" concentrations in the IZA report (2009) and in the current analysis. For studies where results are based on measured concentrations the amount of zinc in the control (i.e. the background level) has been deducted from the result to comply with the principles of the added risk approach.

- Allied to discussions on background concentrations of zinc is acclimation of test organisms (ICMM 2007). Theoretically, one can consider both physiological adaptation during an organism's lifespan and genetic adaptation of a population over several generations as factors which might justify the adoption of particular background levels as "safe" benchmarks for local setting of EQS (Bryan et al. 1987). Tests using organisms taken from wild populations that had been exposed to high zinc concentrations were rejected on the grounds of possible adaptation that could affect their sensitivity to zinc in the test media. In reviewing the studies for this report, the origin of test species has been considered on a case-by-case basis, taking test design into account.

- *Test medium – ethylenediamine tetraacetate (EDTA)*. Chelating agents are compounds that exhibit a strong affinity towards divalent and trivalent metal ions, due to formation of multiple bonds between the ligands of the complexing agent and the metal ion. The presence of chelators in the test medium has the potential to affect the toxicity of zinc. The stability constant varies for complexes formed between a specific chelating agent and different metal ions. The equilibrium constants for the complexation of zinc(II) with some complexing agents are given in Table 2.9 (Martell and Smith 1974). The higher the K value the greater the affinity of the complexing agent is for the metal ion. The presence of chelators other than EDTA (e.g. nitrilo triacetic acid (NTA), citrate) was regarded as acceptable. EDTA has been shown to

decrease the toxicity of zinc and, since the minimum EDTA concentration which will affect the toxicity of zinc in any particular test cannot be readily determined; all tests where the test media contained EDTA were regarded as unreliable.

Table 2.9 Equilibrium constants for the complexation of zinc

Complexing agent	Log K
EDTA	16.44
NTA	10.66
Citric acid	4.98
Oxalic acid	3.43

Saltwater toxicity data on zinc are available for various taxonomic groups including algae, invertebrates and fish as required for the application of the assessment factor approach specified in the EU Technical Guidance Document (TGD) (EC 2003). Long-term data are available for eight taxonomic groups: algae (unicellular and multicellular), annelids, cnidarians, crustaceans, echinoderms, fish, molluscs, and nematodes. Long-term data suitable for use in a species sensitivity distribution (SSD) are presented in Table 2.10. The "species mean" NOEC values of 36 species (Table 2.11) were used to derive the $PNEC_{add,saltwater}$.

Where sufficient data were presented in a paper (tabulated or graphical) an EC10 was recalculated using the Toxicity Relationship Analysis Program (TRAP) from the US EPA National Health and Environmental Effects Research Laboratory (NHEERL). The piecewise-tailed least squares nonlinear regression analysis model was the analysis option selected from the TRAP program to calculate EC10 values.

Two studies of particular note where recalculation of EC10 values was performed were for the algal studies by Strömgren (1979) and Fisher and Frood (1980). Strömgren (1979) examined the growth rate for five species of intertidal brown macroalgae. The experiments were carried out in the laboratory with a continuous flow of natural seawater which had a background concentration of 7 – 9 µg Zn l^{-1}. Concentrations presented in the paper were additions to this background value. The concentration series used in the exposures do not meet current guideline criteria as the intervals between adjacent concentrations are more than a factor of 3.2 at the lower end. The test series was 25, 100, 1400, 2900, 7000 and 14000 µg Zn l^{-1}. Additional test concentrations of 250, 1000, 10000 and 100000 µg Zn l^{-1} were used in the exposures with *Ascophyllum nodosum*. Although some detail is provided in the text the results are primarily presented in graphical form. Except for *Fucus vesiculosus*, stimulation of growth was observed at the lower concentrations (at either 25 or 100 µg Zn l^{-1}). In all cases the data selected met the requirements for model fit at the highest significance level (99 per cent, p = 0.01). In accepting the TRAP calculation the results were also compared against the LOEC value and associated percentage inhibition reported by the study author.

Baumann et al. (2009) investigated the effects of zinc on photosynthetic activity, measured as pulse amplitude modulation (PAM) chlorophyll fluorescence yield in seven species of green, red and brown macroalgae over a 14-day period. The data were assigned as Klimisch 3 data as the difference factor between tested concentrations was high (0, 0.1, 1 and 10 µmol l^{-1}) and there was only one replicate per concentration containing three plants. There were insufficient data presented in the paper to allow for the calculation of a notional EC10. The highest concentration (654 µg l^{-1}) reduced the yield of *Ascophyllum nodosum*, *Fucus vesiculosus*, *Cladophora rupestris*, *Ulva intestinalis*, *Chondrus crispus*, *Palmaria palmata* and *Polysiphonia lanosa* significantly by day four. No effects were seen at the other concentrations tested (≤ 65.4 µg l^{-1}). This result supports the re-calculated EC10 values for *A. nodosum* and *F. vesiculosus* of 69.4 and 71.0 µg l^{-1}, respectively (Strömgren 1979).

Fisher and Frood (1980) conducted a series of experiments to determine whether diatom clones isolated from contaminated waters were more metal-resistant than clones from cleaner waters. The diatoms, *Skeletonema costatum, Chaetoceros compressum, Nitzschia closterium* and *Asterionella japonica*, were exposed to different coastal waters collected either from Corio Bay or Bass Strait near Melbourne, Australia. For each species two clones were cultured: one from clean waters (Bass Strait) and one from metal contaminated waters. *C. compressum* and *A. japonica* were collected from Hobson's Bay (metal contaminated) and the other two species were collected from Corio Bay (metal contaminated). Cultures were established several months prior to the experiments and maintained in complete f/2 medium prepared from filtered surface Bass Strait water.

Mean (over a period of 10 years) zinc levels in unfiltered water averaged 32 µg l^{-1} in Corio Bay and 16 µg l^{-1} in Hobson's Bay. No similar data were presented for Bass Strait. The tests were carried out in filtered water and measured concentrations of zinc are reported as 1.5 µg $l^{-1}$and 5.2 µg l^{-1} for Bass Strait and Corio Bay, respectively.

The clones collected from Corio and Hobson's Bays were kept for several months in f/2 medium prepared from filtered Bass Strait water. Given the short generation time and the fact that background concentrations of zinc at Corio Bay were measured at 5.2 µg l^{-1}, suggesting the algal clones were collected when background concentrations were low, results from tests using these clones have also been included.

EC10 values were calculated based on the tabulated data presented in the paper. As only three test concentrations were used (20.0, 40.0 and 60.0 µg l^{-1}), and growth stimulation was observed in some tests, the data did not always fit the model well. For these purposes a significance level of 70% (p = 0.30) was accepted if the result was also substantiated by the LOEC and associated inhibition was observed. It is worth noting that the authors could find no trend of enhanced metal tolerance and the results appear to have been influenced by the amount of dissolved organic compounds (DOC) in the water.

The species NOEC values of 36 species presented in Table 2.11were used to derive the $PNEC_{add,saltwater}$.

Table 2.11 Species NOEC values used to derive the $PNEC_{add,saltwater}$

Species	Species NOEC values (µg l^{-1})
Algae (unicellular)	9.6*; 15*; 19.3*; 41.1*
Algae (multicellular)	11.9; 69.4; 71.0; 100.6; 190.2; 313; 409.9; 719.8
Cnidarians	300
Annelids	33.3; 100; 100; 100
Molluscs	11.9*; 13.3; 20.4; 22.9; 55.0; 57.6; 84.9*
Crustaceans	5.6; 61.5; 101; 101; 297
Echinoderms	10; 10; 16*; 50; 160
Nematodes	250
Fish	25

* Geometric mean values.

Toxicity observed in mesocosm and field studies

It is important to compare the results from single-species toxicity data from laboratory tests with the results of (model) ecosystem studies and field studies, if these are available.

Davies and Sleep (1979) studied the effect of zinc on carbon fixation rates of natural phytoplankton communities present in the English Channel. A series of three samples of different biological composition (100% diatoms - predominant species *Rhizosolenia alata*; 60% dinoflagellates/40% diatoms – predominant species *Scrippsiella* aff. *trochoidea* and *R. stolterfothii*; and 60% diatoms/40% dinoflagellates – predominant species as previous sample) were taken in July 1978, at approximately weekly intervals, at the same location. The tests with zinc (plus ^{65}Zn as a radioactive tracer) were carried out in natural sea water. The zinc background concentration in seawater was 0.4, 0.8 and 7.6 µg l^{-1} in the three samples. The higher zinc concentration in the third sample was taken two days after substantial rainfall in the area, leading to increased land drainage. A pre-incubation period of phytoplankton assemblages to zinc levels was designed in order to equilibrate the populations with the experimentally added zinc before measuring their carbon fixation rates. Due to adsorption onto the bottles, the zinc concentrations in the water decreased between 5 and 10% during the carbon fixation measurements, the biggest losses occurring at the lowest concentrations. Means of the zinc concentrations at the beginning and end of the test were used for calculating effect values. The study authors determined that differences in the carbon fixation rates due to experimental manipulation of water samples and possible differences in illumination accounted for ≤10%, therefore fixation rates less than 90% of the mean control value were attributed to inhibition caused by the presence of zinc. The lowest concentrations of zinc which caused detectable inhibition of carbon fixation (i.e. rates lower than 90% of the mean control values) were reported as being in the range of 10 to 15 µg l^{-1}. From the dose-response curves presented in the paper, estimated EC10 levels were in the range of 7 to 13 µg l^{-1}.

A similar study on phytoplankton communities in Kiel Fjord, North Sea, and a coastal area of the North Atlantic Ocean was carried out by Wolter et al. (1984). Surface samples were taken in Kiel Fjord during the spring and autumn plankton bloom. Zinc was added to

subsamples to give concentrations in the range of 4.3 to 304.3 µg l^{-1}. The North Sea samples were collected during a cruise in 1981. The added zinc concentrations were 0.29 to 1.45 µg l^{-1}. The added metal concentrations were lower than in the Kiel Fjord experiments due to lower background concentrations in the water compared to those in Baltic Sea water, although the background concentration values are not given for either water. In all cases the samples contained mixed phytoplankton populations which were dominated by diatoms. Zinc reduced plankton activity in the Kiel Fjord samples at concentrations > 100 µg l^{-1} added Zn. Carbon fixation measurements carried out 4 and 24 hours after metal addition to the North Sea samples were not reduced at any test concentration.

Table 2.10 Summary of reliable long-term aquatic toxicity data for saltwater organisms used to derive the $PNEC_{add,saltwater}$

Zinc salt	Scientific name	Common name	Taxonomic group	Effect	End-point	Test duration	Conc. (µg l^{-1})	Exposure	Nom/ meas	Comments	Reference
ALGAE											
$ZnCl_2$	*Ascophyllum nodosum*	Brown alga	ALG (macro)	Growth	EC10	10 d	69.4	f	n	T=6.4 - 6.8°C SW - 33.4 - 33.5‰ BG= 7 - 9 µg l^{-1}	Strömgren 1979
$ZnSO_4$	*Asterionella japonica*	Diatom	ALG	Growth	EC10	4 d	20.6	s	n	T=17°C FSW - 35‰ BG= 1.5 µg l^{-1}	Fisher et al. 1981
$ZnSO_4$	*Asterionella japonica*	Diatom	ALG	Growth	EC10	3 d	15.05	s	n	T=17 ± 1°C UV irradiated FSW - 35‰ BG= 1.5 µg l^{-1}	Fisher and Frood 1980
$ZnSO_4$	*Asterionella japonica*	Diatom	ALG	Growth	EC10	3 d	16.58	s	n	T=17 ± 1°C UV irradiated FSW - 35‰ BG= 5.2 µg l^{-1}	Fisher and Frood 1980
$ZnSO_4$	*Asterionella japonica*	Diatom	ALG	Growth	EC10	3 d	2.15	s	n	T=17 ± 1°C FSW - 35‰ DOC 1.46 mg l^{-1} BG= 1.5 µg l^{-1}	Fisher and Frood 1980
$ZnSO_4$	*Asterionella japonica*	Diatom	ALG	Growth	EC10	3 d	29.14	s	n	T=17 ± 1°C FSW - 35‰ DOC 1.6 mg l^{-1} BG= 5.2 µg l^{-1}	Fisher and Frood 1980
$ZnSO_4$	*Asterionella japonica*	Diatom	ALG	Growth	EC10	3 d	11.21	s	n	T=17 ± 1°C FSW - 35‰ DOC 2.1 mg l^{-1} BG= 1.5 µg l^{-1}	Fisher and Frood 1980
$ZnSO_4$	*Asterionella japonica*	Diatom	ALG	Growth	EC10	3 d	46.95	s	n	T=17 ± 1°C FSW - 35‰ DOC 1.9 mg l^{-1} BG= 5.2 µg l^{-1}	Fisher and Frood 1980
	Asterionella japonica						**14.98**			**GEOMEAN**	
Zn	*Ceramium tenuicorne*	Red alga	ALG (macro)	Growth	EC10	7 d	11.9	s	n	T=26°C NSW= 20‰	Eklund 2005

Zinc salt	Scientific name	Common name	Taxonomic group	Effect	End-point	Test duration	Conc. (µg l^{-1})	Exposure	Nom/ meas	Comments	Reference
$ZnSO_4$	*Chaetoceros compressum*	Diatom	ALG	Growth	EC10	3 d	7.13	s	n	T=17°C FSW - 35‰ DOC=1.56 mg l^{-1} BG= 1.5 µg l^{-1}	Fisher and Frood 1980
$ZnSO_4$	*Chaetoceros compressum*	Diatom	ALG	Growth	EC10	3 d	56.51	s	n	T=17°C FSW - 35‰ DOC=2.61 mg l^{-1} BG= 5.2 µg l^{-1}	Fisher and Frood 1980
$ZnSO_4$	*Chaetoceros compressum*	Diatom	ALG	Growth	EC10	3 d	17.77	s	n	T=17°C FSW - 35‰ DOC=1.03 mg l^{-1} BG= 1.5 µg l^{-1}	Fisher and Frood 1980
	Chaetoceros compressum						**19.27**			**GEOMEAN**	
$ZnCl_2$	*Fucus serratus*	Brown alga	ALG (macro)	Growth	EC10	10 d	409.9	f	n	T=6.4 - 6.8°C SW - 33.4 - 33.5‰ BG= 7 - 9 µg l^{-1}	Strömgren 1979
$ZnCl_2$	*Fucus spiralis*	Brown alga	ALG (macro)	Growth	EC10	10 d	100.6	f	n	T=6.4 - 6.8°C SW - 33.4 - 33.5‰ BG= 7 - 9 µg l^{-1}	Strömgren 1979
$ZnCl_2$	*Fucus vesiculosus*	Brown alga	ALG (macro)	Growth	EC10	10 d	71.0	f	n	T=6.4 - 6.8°C SW - 33.4 - 33.5‰ BG= 7 - 9 µg l^{-1}	Strömgren 1979
$ZnSO_4$	*Macrocystis pyrifera*	Giant kelp	ALG (macro)	Growth	NOEC	2 d	190.2	s	m	T=11.5 - 17°C UV treated FSW – 34 - 36‰	Anderson and Hunt 1988
$ZnSO_4$	*Nitzschia closterium*	Diatom	ALG	Growth	EC10	3 d	51.71	s	n	T=17°C FSW - 35‰ DOC=3 mg l^{-1} BG= 1.5 µg l^{-1}	Fisher and Frood 1980
$ZnSO_4$	*Nitzschia closterium*	Diatom	ALG	Growth	EC10	3 d	53.48	s	n	T=17°C FSW - 35‰ DOC=1.91 mg l^{-1} BG= 5.2 µg l^{-1}	Fisher and Frood 1980

Zinc salt	Scientific name	Common name	Taxonomic group	Effect	End-point	Test duration	Conc. ($\mu g\ l^{-1}$)	Exposure	Nom/ meas	Comments	Reference
$ZnSO_4$	*Nitzschia closterium*	Diatom	ALG	Growth	EC10	3 d	12.33	s	n	T=17°C FSW - 35‰ DOC=2.62 mg l^{-1} BG= 1.5 µg l^{-1}	Fisher and Frood 1980
$ZnCl_2$	*Nitzschia closterium*	Diatom	ALG	Growth	EC10	3 d	84	s	m	T=27°C FSW - 34‰ BG= < 10 µg l^{-1}	Johnson et al. 2007
	Nitzschia closterium						**41.14**			**GEOMEAN**	
$ZnCl_2$	*Pelvetia canaliculata*	Brown alga	ALG (macro)	Growth	EC10	10 d	719.8	f	n	T=6.4 - 6.8°C SW - 33.4 - 33.5‰ BG= 7 - 9 µg l^{-1}	Strömgren 1979
$ZnSO_4$	*Skeletonema costatum*	Diatom	ALG	Growth	EC10	3 d	1.43	s	n	T=17°C UV irradiated FSW - 35‰ BG= 1.5 µg l^{-1}	Fisher and Frood 1980
$ZnSO_4$	*Skeletonema costatum*	Diatom	ALG	Growth	EC10	3 d	7.2	s	n	T=17°C UV irradiated FSW - 35‰ BG= 5.2 µg l^{-1}	Fisher and Frood 1980
$ZnSO_4$	*Skeletonema costatum*	Diatom	ALG	Growth	EC10	3 d	11.63	s	n	T=17°C FSW - 35‰ DOC=1.46 mg l^{-1} BG= 1.5 µg l^{-1}	Fisher and Frood 1980
$ZnSO_4$	*Skeletonema costatum*	Diatom	ALG	Growth	EC10	3 d	70.24	s	n	T=17°C FSW - 35‰ DOC=2.19 mg l^{-1} BG= 5.2 µg l^{-1}	Fisher and Frood 1980
	Skeletonema costatum						**9.58**			**GEOMEAN**	
$ZnNO_3$	*Ulva pertusa*	Green alga	ALG (macro)	Sporulation	NOEC	5 d	313.0	s	n	T=15°C ASW - 35‰	Han and Choi 2005
INVERTEBRATES											
$ZnCl_2$	*Capitella capitata*	Polychaete worm	ANN	Repro-duction	NOEC	2 mo	100.0	ss	n	T=15 & 20°C FSW - 32‰ BG= 8 µg l^{-1}	Reish et al. 1977
$ZnSO_4$	*Ctenodrilus serratus*	Polychaete worm	ANN	Repro-duction	NOEC	21 d	100.0	s	n	SW	Reish and Carr 1978

Zinc salt	Scientific name	Common name	Taxonomic group	Effect	End-point	Test duration	Conc. ($\mu g\ l^{-1}$)	Exposure	Nom/ meas	Comments	Reference
$ZnCl_2$	*Neanthes arenaceao-dentata*	Polychaete worm	ANN	Repro-duction	NOEC	7 mo	33.3	ss	n	T=15 & 20°C FSW - 32‰ BG= 8 µg l^{-1}	Reish et al. 1977
$ZnSO_4$	*Ophryotrocha diadema*	Polychaete worm	ANN	Repro-duction	NOEC	28 d	100	s	n	SW	Reish and Carr 1978
$ZnSO_4$	*Allorchestes compressa*	Amphipod	CRU	Survival	LC10	28 d	61.5	f	m	T=19°C SW - 31‰	Ahsanullah and Williams 1991
$ZnSO_4$	*Holmesimysis costata*	Mysid shrimp	CRU	Survival	NOEC	24 d	5.6	ss	n	T=12 ± 2°C SW – 33 ± 2 ‰	Hunt et al. 1997
$ZnSO_4$	*Americamysis bahia*	Gulf coast shrimp	CRU	Survival	NOEC	7 d	101.0	ss	n	T=26 - 27°C FSW - 30‰	Harmon and Langdon 1996
$ZnSO_4$	*Mysidopsis intii*	Pacific shrimp	CRU	Growth	NOEC	7 d	101.0	ss	n	T=20°C FSW - 34‰	Harmon and Langdon 1996
$ZnSO_4$	*Tigriopus brevicornis*	Copepod	CRU	Repro-duction	NOEC	10 d	297	s	n	T=20°C SW – 34-36‰	Le Dean and Devineau 1987
$ZnSO_4$	*Arbacia lixula*	Sea urchin	ECH	Develop-ment	NOEC	38 h	10.0	s	n	T=20°C FSW – 38 ‰	Cesar et al. 2002
$ZnCl_2$	*Asterias amurensis*	Northern pacific seastar	ECH	Fertility	NOEC	20 + 60 min	50.0	s	n	T=15°C FSW	Lee et al. 2004
$Zn(NO_3)_2$	*Paracentrotus lividus*	Mediterrane an sea urchin	ECH	Develop-ment	EC10	3 d	23.0	s	n	T=18°C ASW - 35‰	Novelli et al. 2003
$Zn(NO_3)_2$	*Paracentrotus lividus*	Mediterrane an sea urchin	ECH	Develop-ment	EC10	2 d	17.7	s	n	T=22°C FSW - 34‰	Radenac et al. 2001
$ZnSO_4$	*Paracentrotus lividus*	Mediterrane an sea urchin	ECH	Develop-ment	NOEC	28 h	10.0	s	n	T=20°C FSW – 38 ‰	Cesar et al. 2002
	Paracentrotus lividus						**15.97**			**GEOMEAN**	
$ZnSO_4$	*Sphaerechinus granularis*	Sea urchin	ECH	Develop-ment	NOEC	28 h	10.0	s	n	T=20°C FSW – 38 ‰	Cesar et al. 2002
$ZnSO_4$	*Sterechinus neumayeri*	Antartic sea urchin	ECH	Develop-ment	NOEC	20-23 d	160.0	s	n	T=0 ± 0.5°C FSW – 34 ± 1‰	King and Riddle 2001
$ZnCl_2$	*Crassostrea cacullata*	Oyster	MOL	Growth	EC10	4 d	22.9	ss	n	T=22 - 23°C FSW - 34‰	Watling 1982
$ZnCl_2$	*Crassostrea gigas*	Oyster	MOL	Growth	EC10	4 d	57.6	ss	n	T=22 - 23°C FSW - 34‰	Watling 1982
$ZnCl_2$	*Crassostrea margaritacea*	Oyster	MOL	Growth	EC10	4 d	13.3	ss	n	T=22 - 23°C FSW - 34‰	Watling 1982

Zinc salt	Scientific name	Common name	Taxonomic group	Effect	End-point	Test duration	Conc. ($\mu g\ l^{-1}$)	Exposure	Nom/ meas	Comments	Reference
$ZnCl_2$	*Haliotis ruba*	Blacklip abalone	MOL	Develop-ment	EC10	2 d	20.4	s	n	T=20°C FSW	Gorski and Nugegoda 2006
$ZnSO_4$	*Haliotis rufescens*	Red abalone	MOL	Metamor-phosis	NOEC	9 d	19.0	f	n	T=14 – 17.5°C UV treated NSW – 33 - 36‰	Anderson et al. 1988
$ZnSO_4$	*Haliotis rufescens*	Red abalone	MOL	Metamor-phosis	NOEC	10 d	7.48	f	n	T=15°C NSW	Conroy et al. 1996
	Haliotis rufescens						**11.92**			**GEOMEAN**	
$ZnCl_2$	*Mytilus gallo-provincialis*	Blue mussel	MOL	Develop-ment	NOEC	2 d	80.0	s	n	T=20°C FSW	Beiras and Albentosa 2004
$ZnSO_4$	*Mytilus gallo-provincialis*	Blue mussel	MOL	Develop-ment	NOEC	2 d	90.0	s	n	T=20°C SW - 38‰	Pavicic et al. 1994
	Mytilus gallo-provincialis						**84.85**			**GEOMEAN**	
$ZnCl_2$	*Ruditapes decussatus*	Grooved carpet shell clam	MOL	Develop-ment	EC10	2 d	55.0	s	n	T=20°C ASW - 34‰ DOC=0 mg l^{-1}	Beiras and Albentosa 2004
$ZnSO_4$	*Eirene viridula*	Medusa	CNI	Develop-ment	NOEC	3 mo	300	ss	n	T=20°C FSW - 30‰	Karbe 1972
$ZnCl_2$	*Monhystera disjuncta*	Nematode	NEM	Repro-duction	NOEC	4 d	250	s	n	T=17°C ASW - 30‰	Vranken et al. 1991
VERTEBRATES											
$ZnSO_4$	*Clupea harengus*	Atlantic herring	FIS	Develop-ment	NOEC	27 d	25	ss	n	T=8°C ASW - 21‰	Somasundaram *et al*. 1984

NOEC = no observed effect concentration
ECx = concentration effective against X% of the organisms tested
ALG = algae, ANN = annelid, CNI = cnidarian, CRU = crustaceans, ECH = echinoderm, FIS = fish, MOL = molluscs, NEM = nematodes
S = static; ss = semistatic; f = flow-through; m = measured test concentration; n = nominal value test concentration
FSW = filtered seawater
ASW = artificial seawater
NSW = natural seawater
SW = seawater (no other details given)
T = temperature
BG = background Zn concentration
DOC = dissolved organic carbon
UV = ultra violet

2.6.4 Toxicity to sediment-dwelling organisms

The content of this section is an abridged and modified adaptation of the text in Section 3.3.2.2.2 (toxicity of zinc in freshwater sediments) of the EU RAR (2008).

For benthic organisms, only four valid chronic NOEC values could be identified in the EU RAR:

- one study with the oligochaete *Tubifex tubifex* (4-week $NOEC_{reproduction}$ 1100 mg kg^{-1} dw) (Farrar and Bridges 2003);
- two with the insect *Chironomus tentans* (3-week $NOEC_{growth}$ of 609 mg kg^{-1} dw (Farrar and Bridges 2002, 2003) and 8-week $NOEC_{survival\_growth\_emergence\_reproduction}$ of 795 mg kg^{-1} dw (Sibley et al. 1996));
- one with the crustacean *Hyalella azteca* (6-week $NOEC_{survival}$ 488 mg kg^{-1} dw) (Nguyen et al. 2005).

These NOEC values are expressed as the added concentration.

The four tests were performed in unpolluted sediments with a background zinc concentration of 22 to 55 mg kg^{-1} dw. In addition to survival at least one other endpoint (growth or reproduction) was studied in each test.

Thus, the lowest chronic **NOEC for benthic organisms is 488 mg kg^{-1} dw**.

2.6.5 Endocrine-disrupting effects

No data were found on the effects of zinc compounds on the endocrine system.

2.6.6 Mode of toxic action of relevant zinc species

Zinc plays an essential role in organisms. This implies that organisms have a (specific) minimum requirement for zinc to supply their needs. Regulating mechanisms exist in organisms which are capable of supplying the required zinc by maintaining a constant internal level independent of the external concentration. However, the regulating capacity of this mechanism is limited and, if an organism is exposed to levels below or above the concentration at which it can regulate its internal concentration, effects of deficiency or toxicity may occur (EU RAR 2008).

Zinc occurs within organisms in two different protein combinations:

- as a metalloenzyme in which zinc is an integral part of an enzyme system;
- as a metal–protein complex in which zinc is loosely bound to a protein.

The latter acts as its carrier and transport mechanism in the body (metallothionine) (WHO 2001).

Excessive exposure of the body to zinc leads to disturbances of the functional state of individual organs and systems. Intoxication is manifested by (WHO 2001):

- a reduction in the content of free SH groups in serum;
- reduced activity of hepatic arginase;
- reduced prothrombin index.

Zinc metal and dust are carcinogenic. However, zinc appears to be potentially carcinogenic only when a high concentration of insoluble material has caused necrosis and is followed by a regenerative response. There is no indication that occupational exposure to zinc increases the incidence of any type of cancer. Zinc may sustain tumour growth, but it appears not to be teratogenic except perhaps at very high doses. However, zinc can modify the teratogenic potential of other metals. Studies on the mutagenicity of zinc strongly suggest that zinc does not represent a mutagenic risk (WHO 2001).

3. Calculation of PNECs as a basis for the derivation of quality standards

Physicochemical water parameters such as pH, hardness and organic carbon content influence zinc speciation. The bioavailability and toxicity may be affected by:

- organic and inorganic complexation (anions such as Cl^- and CO_3^{2-}) of zinc; and
- competition of cations (e.g. Ca^{2+} and H^+) with zinc at receptors of aquatic organisms (i.e. so-called biotic ligands).

To compare environmental concentrations measured in a particular water body with an EQS that may be derived on the basis of the proposed PNECs in this report, it is necessary to correct the measured environmental concentration for zinc bioavailability. This can be performed by using the approaches described in Appendix IV.

Based on abiotic factors (physicochemical 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, freshwater and saltwater data are not combined to derive a general $PNEC_{add}$ for surface water. Instead, two separate PNECs for freshwater and saltwater are derived on the basis of validated freshwater and saltwater datasets, respectively.

3.1 Derivation of PNECs by the TGD assessment factor (AF) method

3.1.1 PNECs for freshwaters

PNEC accounting for the annual average concentration

Algae appear to be the most sensitive taxonomic group in the freshwater database, followed by crustaceans, sponges/rotifers and fish (Figures 3.2 and 3.3). Insects and molluscs may be slightly less sensitive. However, the difference in the sensitivities of the most susceptible representatives of these taxonomic groups is just over an order of magnitude (5 versus 137 µg l^{-1} for a total of 25 different species in the freshwater database; Table 2.7).

Using the assessment factor method to derive a $PNEC_{freshwater}$ requires that an assessment factor of 10 is applied to the lowest reliable NOEC or EC10 (4.9 µg l^{-1} for *Pseudokirchneriella subcapitata* and *Chlorella* sp.). Hence, the $PNEC_{add,freshwater\_lt}$ is as follows:

$PNEC_{add,freshwater\_lt}$ = 4.9 µg l^{-1}/AF (10) = 0.5 µg l^{-1} zinc (dissolved)

However, there are sufficient freshwater ecotoxicity data to allow a PNEC to be derived from the HC5 of an SSD, and as a result of this the PNEC derived by the assessment factor (AF) method is not recommended for adoption as an EQS.

3.1.2 PNECs for saltwaters

Freshwater and saltwater are considered to be different environments in the EU RAR (EC 2003) because of differences in the various abiotic physicochemical factors, including natural background concentrations of essential and other elements. The RAR therefore recommends that the freshwater and saltwater effects databases should not be combined. This recommendation is followed in this report, although no obvious differences in the sensitivity of freshwater or saltwater species of the same taxonomic group are noticeable (Sections 2.6.2 and 2.6.3).

PNEC accounting for the annual average concentration

The lowest reliable species long-term NOEC is the 24 day survival NOEC of 5.6 µg l^{-1} for the crustacean, *Holmesimysis costata* (Hunt *et al*. 1997). Lower, individual EC10 values of 1.43 and 2.15 µg $l^{-1}$for the diatoms *Skeletonema costatum* and *Asterionella japonica* are included in the overall dataset (Fisher and Frood 1980). The wide range of values reported for these two species and the fact that these EC10 values were extrapolated far below the lowest test concentration make them less suitable.

As long-term NOECs for at least three marine species representing three trophic levels (i.e. algae, crustaceans, and fish) plus data of the same quality for more than two further marine groups (i.e. annelids, molluscs, and echinoderms) are available, the appropriate assessment factor in accordance with the TGD is 10. Hence, the $PNEC_{add,saltwater\_lt}$ can be calculated as follows:

$PNEC_{add,saltwaterlt}$ = 5.6 µg l^{-1}/AF (10) = 0.56 µg l^{-1} zinc (dissolved)

However, there are sufficient marine ecotoxicity data to allow a PNEC to be derived from the HC5 of an SSD, and as a result of this the PNEC derived by the AF method is not recommended for adoption as an EQS.

3.2 Derivation of PNECs by the TGD probabilistic approach (SSD method)

3.2.1 Annual average PNEC for freshwaters

Identification of the Generic or Reference PNEC for the UK

The current draft EQS guidance (EC 2009) is imprecise about how the reference EQS should be set, and there are indications that it should be set for the 10^{th} to 90^{th} percentile ranges of abiotic conditions, and also that it should be set to be protective of the most sensitive waterbodies which will be monitored for EQS compliance. It is this latter statement that the Environment Agency has attempted to meet in the derivation of a Zn EQS.

The key input parameters for Zn BLM are DOC and Ca concentrations and pH. The Ca concentration can be considered as a useful indicator of hardness, with the relationship between Ca and total water hardness generally being considered to be relatively constant. North West Region is the most sensitive of the 10 Regions (six in England, one in Wales and three in Scotland) for which we have data, followed by Wales and the South West. The greatest degree of potential under-protection was observed for the North West Region (32%). The PNEC values were calculated from the annual averages of pH (mean), DOC (median) and Ca (mean) of at least six samples for each individual site (approximately 100 sites for each Region). The Environment Agency monitoring data was collected in 2007 to 2008 for Scotland and 2000 to 2009 for England and Wales. The use of the mean is consistent with requirements under the WFD, whereas a median value for DOC was chosen as it is less likely to be sensitive to outliers and skewed data. Various percentiles of the calculated Zn HC5 for individual sites across the whole of Great Britain (n = 916) and the North West Region (n = 103) are given in Table 3.1.

Table 3.1 Frequency distribution of Zn PNEC values (µg l^{-1}) for Great Britain and North West Region

Percentile	Great Britain	North West
5^{th}	14.15	10.92
10^{th}	16.56	11.59
15^{th}	18.29	12.01
25^{th}	21.85	12.85
50^{th}	31.01	23.46
75^{th}	41.21	41.35
90^{th}	54.61	57.78
95^{th}	64.47	63.50

Setting the Generic HC5 to a predefined level of protection for the whole of Great Britain, such as the level for 95% protection of 14.2 µg l^{-1}, has limitations in that the selected value represents a rather lower level of protection (approximately 68%) in the North West Region. Consequently the value was selected so as to provide 95% protection for the most sensitive region, which would ensure a high level of protection if applied on a UK basis. The Generic HC5 for the UK is therefore set at 10.9 µg l^{-1} bioavailable Zn, and is considered to be protective of sensitive water quality conditions.

All the chronic toxicity data from Table 2.7 were normalized to ‘river-basin’ specific physico-chemistry before being used as input data for the calculation of the ‘river-basin’ specific HC5-50 values. A schematic overview of the procedure for normalization of ecotoxicity data with Zn biotic ligand models is shown in Figure 3.1.

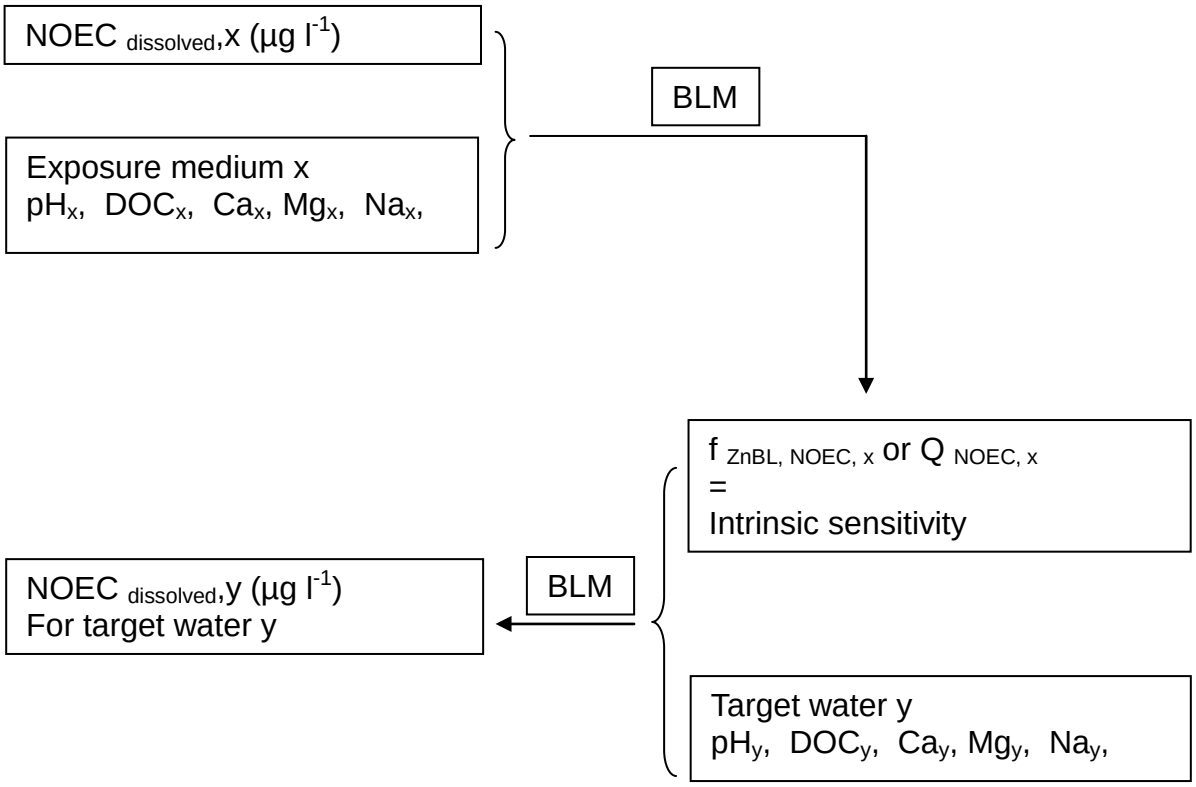

Figure 3.1 Schematic overview of the procedure for normalization of ecotoxicity data with biotic ligand models, for normalization of NOEC values obtained in exposure medium *x* to the physico-chemistry of target water *y* (taken from van Sprang et al. 2009).

Two UK waters with different combinations of water chemistry have been chosen by way of examples (Table 3.2). The HC5s are the closest ones to the selected value for the generic PNEC that we have (HC5 = 9 and 13 µg l^{-1}, Figures 3.2 and 3.3, respectively) and bracket the generic PNEC of 10.9 µg l^{-1}. These two examples demonstrate that they can occur for different combinations of water chemistry. The two examples are a soft water (slightly lower HC5) and a hard water. The first site is on the Carnon River near Redruth in the Fal hydrometric area, and would currently have an EQS of 50 µg l^{-1} total zinc. The second site is on the River Bourne near Andover in the Test hydrometric area, and would currently have an EQS of 125 µg l^{-1} total zinc.

The normalised data meets all the goodness of fit tests for log-normal distribution (Anderson Darling AD, Kolgomorov Smornov KS, and Cramer von Misses CvM) at all significance levels (Table 3.3).

Table 3.2 Physico-chemical data for two selected UK waters

Test	UK NGR	pH	DOC	Ca	Mg	Na	K	SO_4	Cl	Alk
			mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}
UK (1)	SW7620041800	5.45	0.403	15.27	3.72	12.14	2.42	15.47	19.90	33.12
UK (2)	SU4357547363	8.00	0.433	105.94	7.85	31.33	5.93	65.27	47.55	189.50

Table 3.3 Hazard Concentration (HC5) values and SSD goodness-of-fit for freshwater Zn toxicity dataset

Site	*n*	***Hazard Concentration HC_5 (µg l^{-1})***			***SSD goodness-of-fit (acceptance at 5% significance)***		
		Median	Lower limit	Upper limit	A-D	K-S	CvM
UK soft	25	9.0	3.1	19.3	✓	✓	✓
UK hard	25	13.3	5.1	26.4	✓	✓	✓

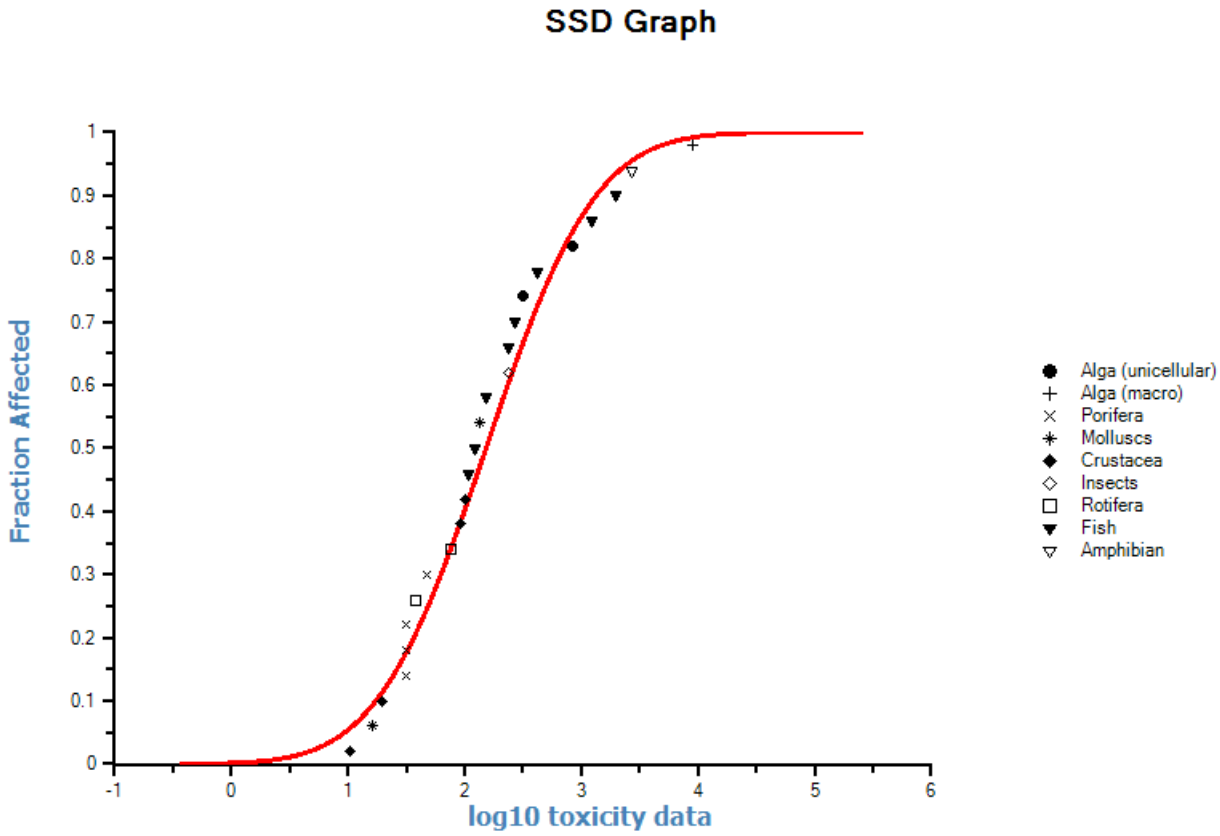

Figure 3.2 Log-normal Species sensitivity distribution of freshwater organisms in UK soft water

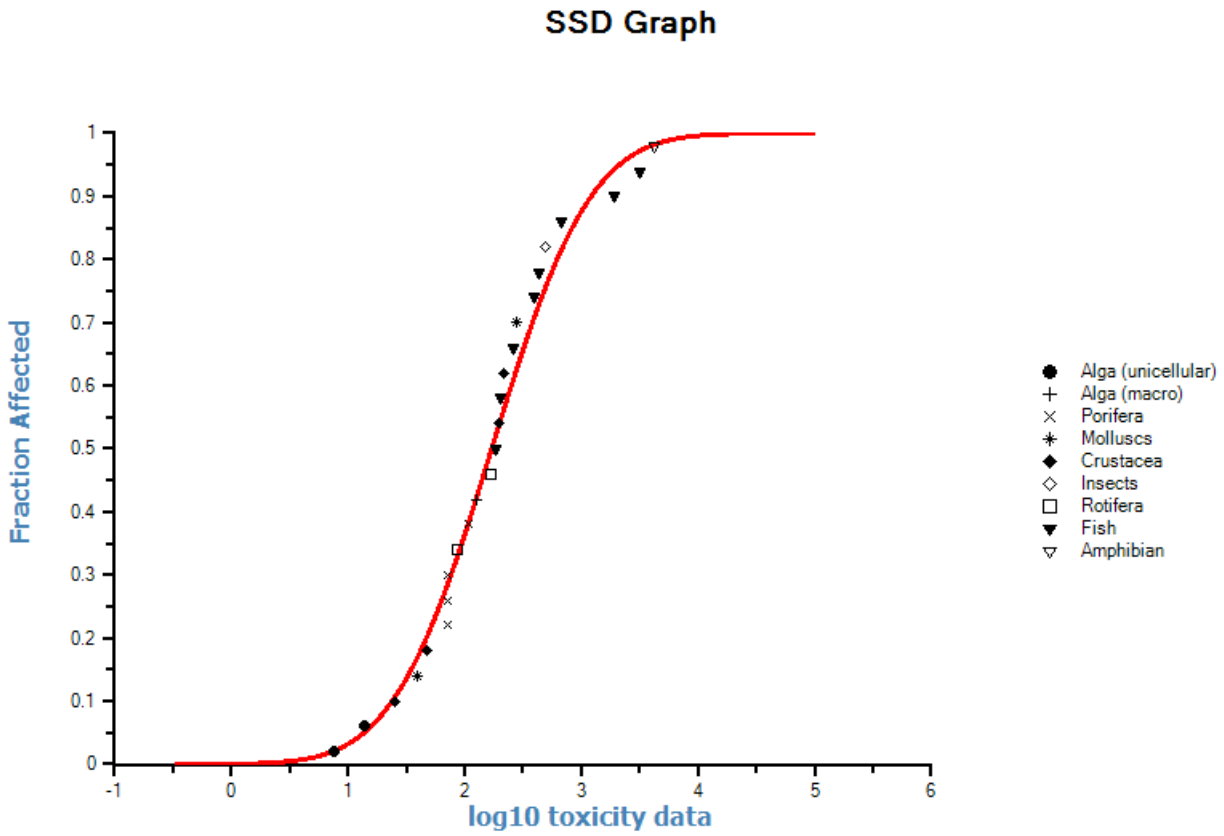

Figure 3.3 Log-normal Species sensitivity distribution of freshwater organisms in UK hard water

The difference between the two SSDs is that the bioavailability of zinc to different organisms responds slightly differently to changes in water quality. Invertebrates are expected to be the most sensitive under low pH and low hardness conditions, whereas algae are expected to be more sensitive under high pH conditions. DOC affects all types of organisms similarly, with the most sensitive conditions occurring at low DOC concentrations.

The use of statistical extrapolation is preferred for $PNEC_{add}$ derivation rather than the use of an assessment factor on the lowest NOEC. With regard to the size of the assessment factor, there are a number of reasons to use an assessment factor smaller than 5.

According to the TGD (ECB 2003), an assessment factor of 1–5 should be applied in order to derive the PNEC from the 5th percentile of the SSD. The size of this assessment factor needs to be justified by taking into account aspects such as:

- data comprehensiveness and quality;
- fit to the assumed distribution;
- the occurrence of NOEC values below the 5th percentile of the SSD;

- the results of field tests (if available);
- the results of the conventional assessment factor method.

The critical assessment of these parameters leading to a justified proposal for the size of the assessment factor to be used for the calculation of the PNEC from the 5th percentile of the SSD is summarised below:

- The number of chronic NOEC values (*n* = 25 species NOEC values, using geometric means where applicable) meets the general requirement for the number of input data (minimum requirement: 10 NOEC values, preferably more than 15 NOEC values, for different species covering at least 8 taxonomic groups).

- Chronic NOEC values are available for two unicellular algal species, one multicellular algal species (macro alga), four porifera species, two mollusc species, four crustacean species, one insect species, two rotifer species, eight fish species and one amphibian species. The database includes all eight taxonomic groups (families) mentioned in the US EPA list taken as a starting point. The EU RAR further recommends that primary producers (algae and higher plants) are included since these are not included in the US EPA list.

  Algae are included in the current database, but higher plants are not. However, the database includes one NOEC for the macro alga *Cladophora glomerata*, which may be taken instead of higher plants. Moreover, a long-term freshwater study with four different species of higher plants (*Elodea nuttallii*, *Callitrische platycarpa*, *Spirodela polyrhiza* and *Lemna gibba*) is available; however, this study is not included in the data because it resulted in unbounded $NOEC_g$ values (all four ≥650 µg l^{-1}). Thus, aquatic higher plants do not appear to be very sensitive to zinc toxicity in comparison to algae or animals, and the lack of useful NOEC values for higher plants is considered acceptable.

- The normalised data meets all the goodness of fit tests for log-normal distribution (Anderson Darling AD, Kolgomorov Smornov KS, and Cramer von Misses CvM) at all significance levels.

- The PNEC derivation process under the ESR programme identified algae as being potentially very sensitive to the effects of zinc, and this was a key factor in the decision to apply an assessment factor of 2 to the HC5 value in order to derive the PNEC. A review of UK ecological monitoring data suggests that zinc does not cause effects on primary producers that are observable in WFD ecological assessment methods. EA ecological monitoring data is available for benthic macroinvertebrates, fish, benthic diatoms and aquatic macrophytes. This was matched to EA chemical monitoring data which was available for total and dissolved metals and bioavailability modifying factors. These data are shown below along with the proposed generic PNEC (vertical line) and the thresholds for high (dotted horizontal line) and good ecological status (solid horizontal line) in Figures 3.4 to 3.8 for diatoms, macrophytes, fish, invertebrates (N-Taxa), and invertebrates (ASPT), respectively.

Whilst some of the ecological metrics shown above do suggest a possible downward trend with increasing bioavailable zinc exposure (e.g. macrophytes and N-Taxa), none of the metrics indicate that there is any limitation to the achievement of high ecological status where the proposed generic PNEC for bioavailable zinc is met. Limited numbers of sites with matched biological and chemical monitoring data and higher zinc exposures prevent the derivation of a threshold directly from the field data. Whilst this may not necessarily ensure complete ecotoxicological protection, because the community metrics may not necessarily respond to the loss of a single zinc sensitive taxa, the proposed PNEC is not expected to restrict the potential for achievement of WFD ecological goals.

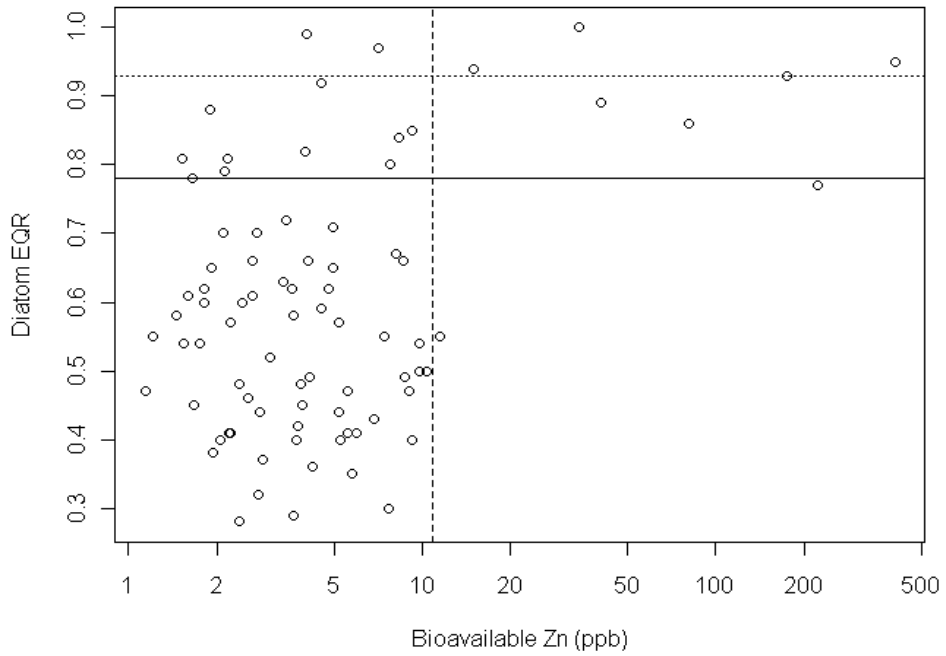

Figure 3.4 Diatom community quality as a function of bioavailable zinc exposure.

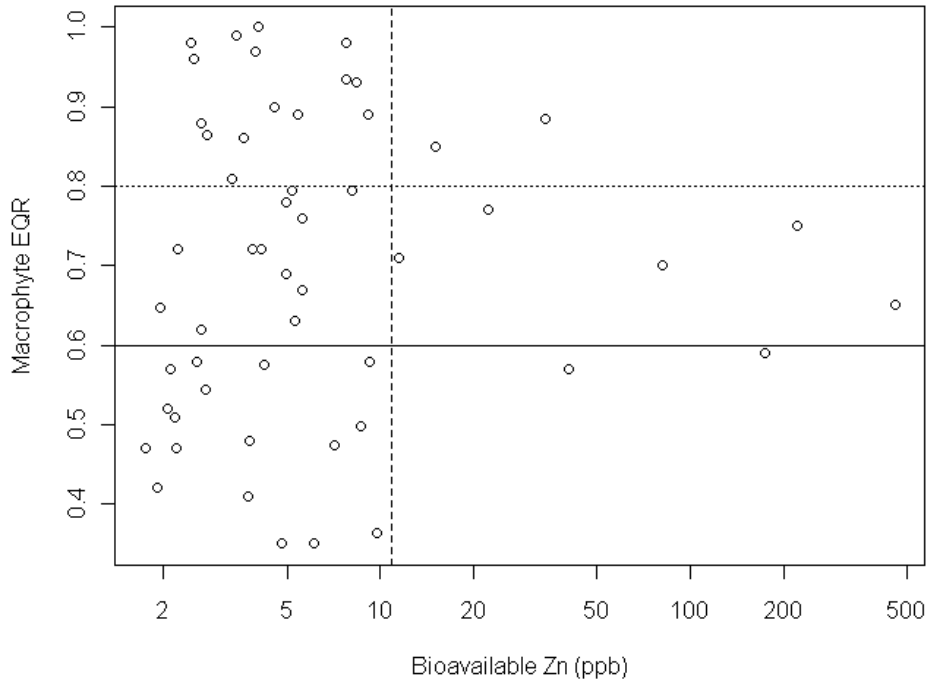

Figure 3.5 Macrophyte community quality as a function of bioavailable zinc exposure.

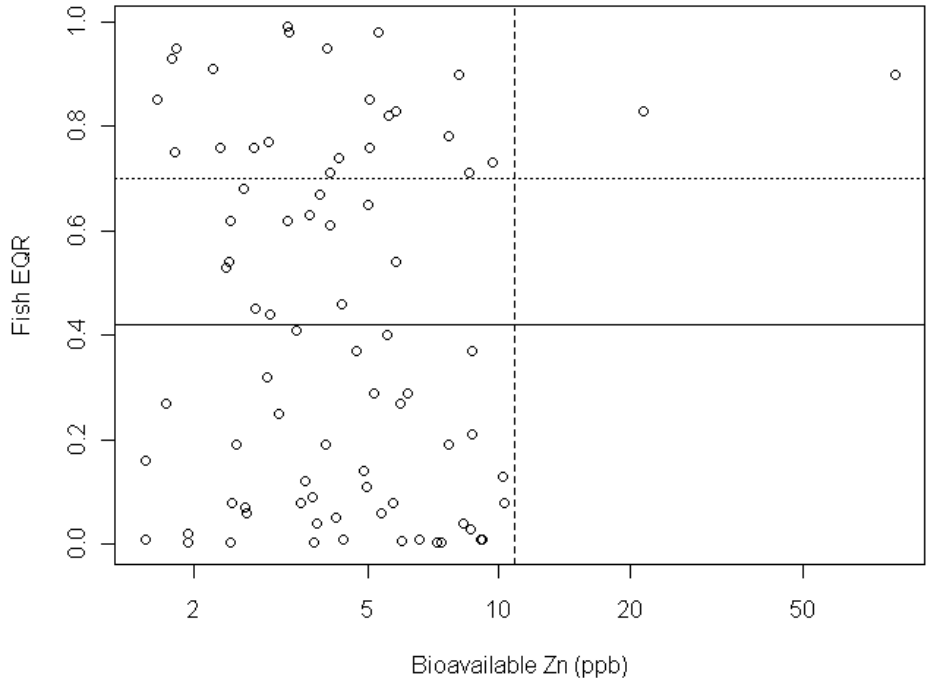

Figure 3.6 Fish community quality as a function of bioavailable zinc exposure.

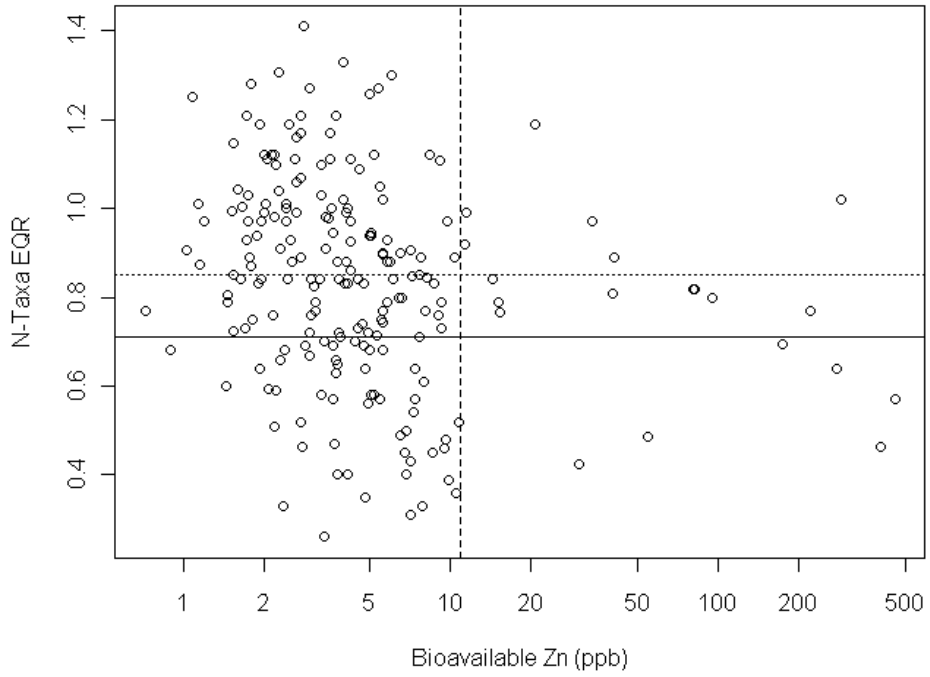

Figure 3.7 Benthic macroinvertebrate community quality (expressed as N-Taxa) as a function of bioavailable zinc exposure.

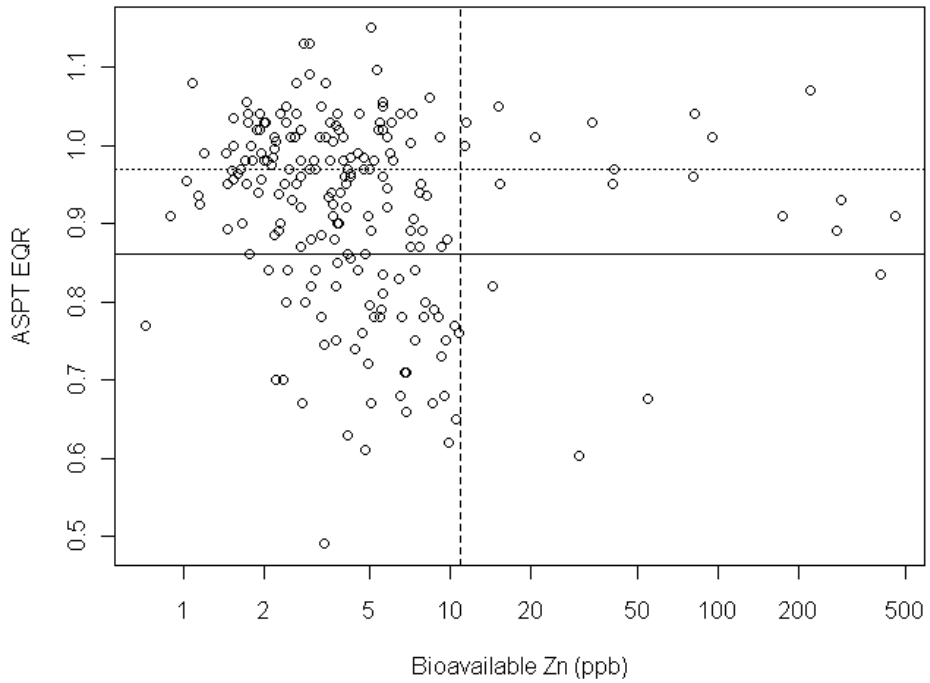

Figure 3.8 Benthic macroinvertebrate community quality (expressed as ASPT) as a function of bioavailable zinc exposure.

- There is a relatively large ecotoxicity database, resulting in a relatively high reliability and the generic PNEC value is established from a reliable database of water quality conditions in Great Britain (Environment Agency 2009f). This is also shown by the small difference between the 50% CL and the 95% CL (approximately a factor of 2). This would support an AF smaller than 5. It is estimated that less than one percent of locations in Great Britain have water chemistries that are more sensitive to zinc toxicity than the conditions that the generic PNEC are aimed to protect.

- 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). 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 were not conducted in natural waters, but in artificial (reconstituted) freshwaters. Tests in artificial waters with deviating characteristics were excluded, as well as tests in artificial waters without information on the characteristics. Therefore, the data properly reflect UK aquatic compartments. This would also support an AF smaller than 5.

- The median 5th percentile value of 10.9 µg l^{-1} may not be sufficiently protective. In 14 of the 27 accepted tests with the alga *Pseudokirchneriella subcapitata* and in one of the 5 accepted tests with the alga *Chlorella* sp., a NOEC below this value was found. This would support an AF higher than 1, although the “species mean” NOEC values (17 µg l^{-1} for *P. subcapitata* and 47.6 µg l^{-1} for *Chlorella* sp.) are both higher than the generic PNEC value. Furthermore, all 14 tests with *P. subcapitata* that resulted in a NOEC below the generic PNEC value (all from the study by De Schamphelaere et al. 2003) were performed in artificial test water with a very low DOC concentration. DOC was found to be an important mitigating factor for the toxicity of this algal species. This would support an AF smaller than 5.

- The results of laboratory and field (model) ecosystem studies with zinc show that major effects on ecosystems are unlikely at the generic PNEC level. However, in some ecosystem or field studies effects on biomass-related endpoints were found in the range of 10–20 µg l^{-1}. Thus, effects were found at close to or above the generic PNEC value for the proposed generic PNEC. Effects on species richness (i.e. community structure) were less sensitive and were mostly found above the generic PNEC level (see Section 2.6.2 for an overview of the ecosystem studies). This would support an AF smaller than 5.

- An analysis of ecological monitoring data from England and Wales suggests that ecological communities, including primary producers (diatoms and macrophytes), and consumers (benthic macroinvertebrates and fish) did not show any indication of under protection of the ecology where the PNEC is met. This would support an AF smaller than 5.

In conclusion, an AF of 1 is recommended in order to derive the $PNEC_{add}$ from the generic PNEC value of 10.9 µg l^{-1}. Thus, the $PNEC_{add,freshwater\_lt}$ can be calculated as follows:

$PNEC_{add,freshwater\_lt}$ = 10.9 µg l^{-1}/AF (1) = 10.9 µg l^{-1} zinc (bioavailable)

The proposed PNEC is above the very lowest toxicity values observed under some test conditions. Field evidence does not, however, suggest that freshwater algae, such as benthic diatoms, are especially sensitive to zinc toxicity (see Figure 3.3).

3.2.2 Annual average $PNEC_{add}$ for marine water bodies (saltwater)

Based on the 36 species NOECs (using geometric means where applicable) presented in Table 2.11 and use of the program ETX 2.0 (Van Vlaardingen et al., 2004) for deriving an SSD (Figure 3.9), the median (i.e. 50 per cent confidence) 5th percentile cut-off value of 6.76 µg l^{-1} Zn is calculated with a lower 95% CL of 3.6 µg l^{-1} and an upper 95% CL of 10.9 µg l^{-1}.

The assumption that the input data are normally distributed is accepted at the highest level (p = 0.01) using the Anderson–Darling Goodness-of-Fit, the Kolmogorov–Smirnov test and the Cramer van Mises tests for normality.

An HC5 of 6.84 µg l^{-1} Zn was also derived from the same dataset fitted with a Burr type III model using BurrliOZ software (Figure 3.10).

According to the ECHA Guidance (2008a), an assessment factor of 1–5 should be applied in order to derive the PNEC from the 5th percentile of the SSD. The size of this assessment factor needs to be justified by taking into account aspects such as:

- data comprehensiveness and quality;
- fit to the assumed distribution;
- the occurrence of NOEC values below the 5th percentile of the SSD;
- the results of field tests (if available);
- the results of the conventional assessment factor method.

There are several reasons in the case of a saltwater zinc PNEC to use an assessment factor smaller than 5 and higher than 1.

All goodness of fit tests for a normal distribution of the log transformed data (Anderson–Darling, Kolmogorov–Smirnov, and Cramer van Mises) are accepted at the highest significance level (99%, p = 0.01). There is a relatively large database, resulting in high reliability of the HC5 value. This is also shown by the small difference between the 50% CL and the 95% CL (less than a factor of 2). This would support an AF smaller than 5.

The number of chronic NOEC values (*n* = 36 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, for different species covering at least 8 taxonomic groups). Chronic NOEC values are available for four unicellular algal species, eight multicellular algal species, one cnidarian species, four annelid species, seven mollusc species, five crustacean species, five echinoderm species, one nematode species and one fish species. The input database does not include all groups recommended in ECHA (2008a) for setting up an SSD, but this guidance is specifically for the freshwater compartment. Higher plants and insects are not represented in the dataset of saltwater species, but these taxonomic groups are considered to be of marginal relevance for the assessment of saltwater. Lepper (2005) suggests that the same assessment factor on the result of the SSD (the 5% cut-off value) that is considered appropriate for inland waters can be applied for transitional, coastal and territorial waters if the data set used to establish the SSD comprises long-term NOECs for at least two additional marine taxonomic groups other than fish, crustaceans and algae (e.g. echinoderms, molluscs, or cnidarians), showing that these additional marine groups are not more sensitive than other taxa. Representatives of all three of these taxonomic groups are available.

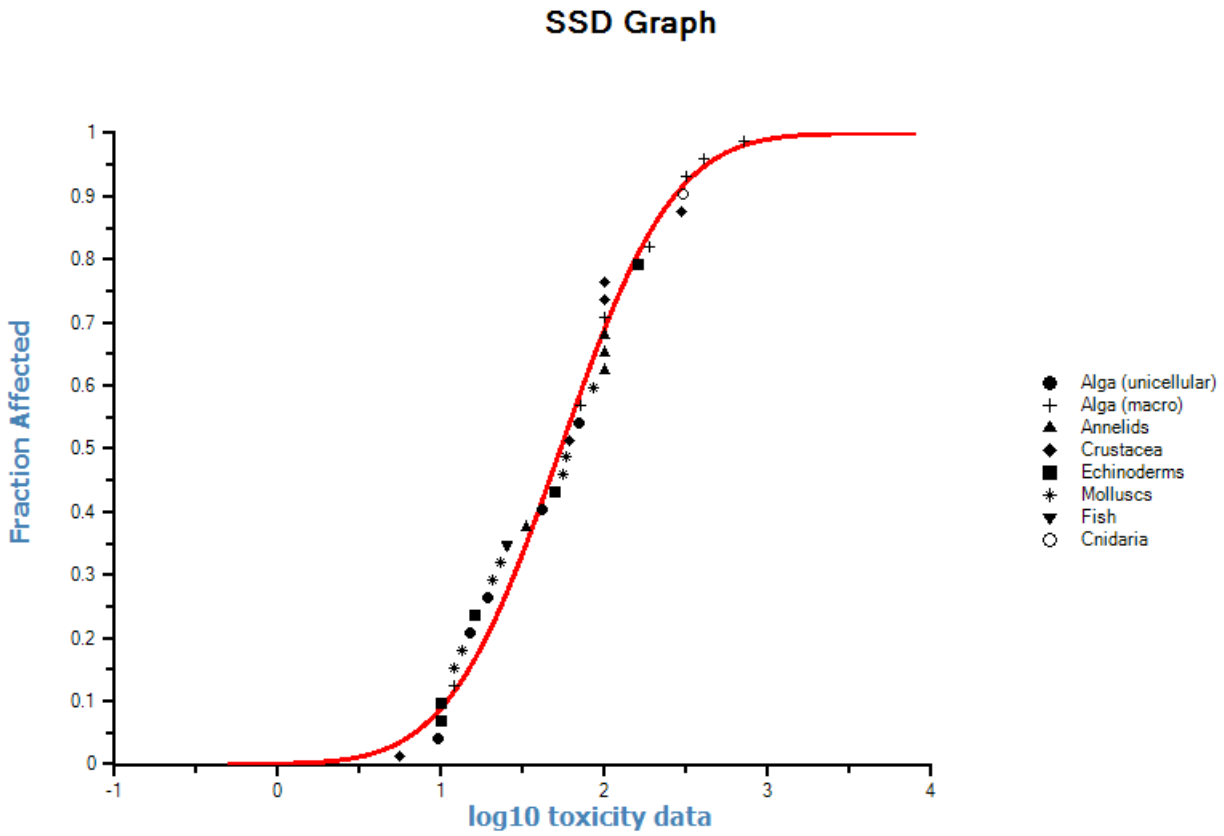

Figure 3.9 Species sensitivity distribution of selected chronic marine Zn endpoints.

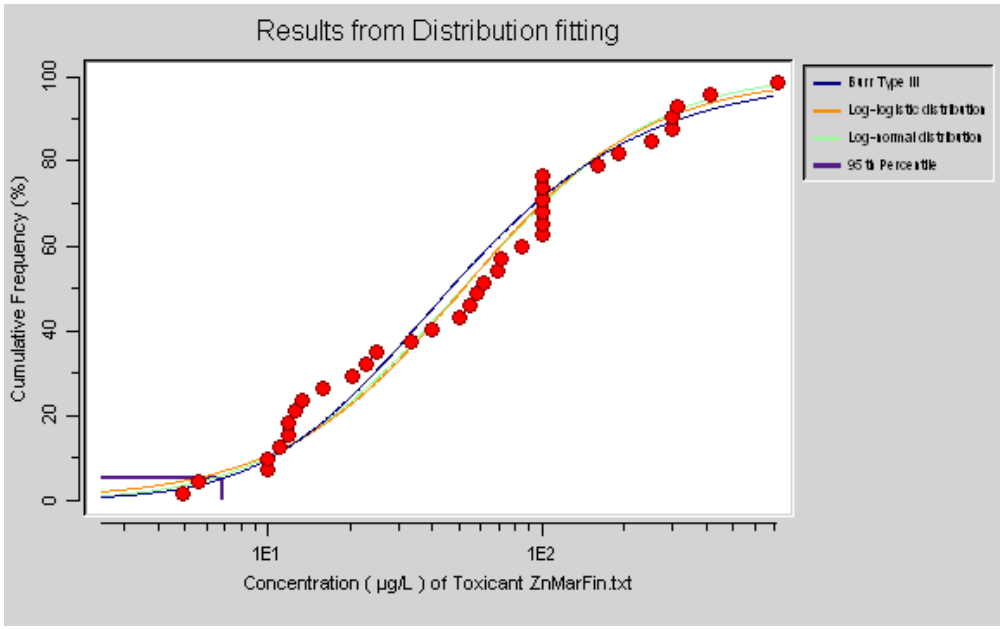

Figure 3.10 Burr type III Species sensitivity distribution of saltwater organisms (input data as presented in Table 2.11)

Zinc plays an essential role in organisms. This implies that organisms have a (specific) minimum requirement for zinc to supply their needs. Regulating mechanisms exist in organisms which are capable of supplying the required zinc by maintaining a constant internal level independent of the external concentration. However, the regulating capacity of this mechanism is limited and, if an organism is exposed to levels below or above the concentration at which it can regulate its internal concentration, effects of deficiency or toxicity may occur. The HC5 calculated from this SSD is very similar to the HC5 values of 6.1 and 6.09 µg l^{-1} calculated using data presented in the RAR and by IZA, respectively, suggesting that no single species or taxonomic group is influencing the SSD. The sensitivity displayed by species within a taxonomic group varies so there are representatives across the distribution.

NOECs below the HC5 do not generally occur when the sample size is below 10-20, but the availability of more data points increases the probability of NOECs below the value of the HC5. Only one NOEC value used in the derivation of the SSD falls below the HC5. This is the 24 day survival NOEC of 5.6 µg l^{-1} for the crustacean *Holmesimysis costata* (Hunt et al. 1997). The authors carried out chronic 24 day static renewal tests starting with juveniles ~ 72 h old, the results of which were compared to a 7 day growth and survival test initiated at the same time. Six zinc concentrations were used (5.6, 10.0, 18.0, 32.0, 56.0 and 100 µg Zn l^{-1}) plus a control, each replicated eight times. The solutions were renewed after days 2 and 6 in both exposures, and again every 96 h in the 24 day test. Although survival in controls was not different for the two exposure periods, survival values in all toxicant concentrations above the long-term NOEC of 5.6 µg l^{-1} were significantly lower in the 24 day test when compared to the 7 day test (ANOVA, $p < 0.0001$). The LC50 for the 24 day exposure was 7.8 µg l^{-1}. Growth was not significantly inhibited at zinc concentrations equal to the NOEC in either test. From the graphical representation of the data (Figure 3.11) it would appear that there was considerable variation within replicates with the confidence intervals appearing to overlap

for the control and 5.6 µg l^{-1} test concentration, indicating that the HC5 should be sufficiently protective of this species.

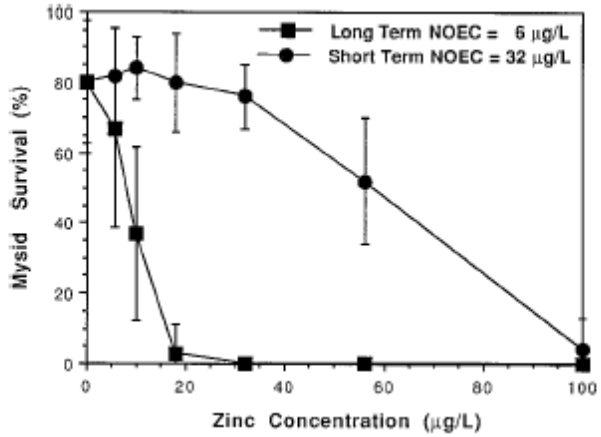

Figure 3.11 Dose response curves for mysid survival for 7 and 24 day Zn exposures (from Hunt et al. 1997).

In addition, individual EC10 values of 1.43 and 2.15 µg l^{-1} for the diatoms *Skeletonema costatum* and *Asterionella japonica*, respectively, are included in the overall dataset. If valid data exist for the same endpoints and the same species it is generally accepted practice to calculate the geometric mean and to use this value together with that for other species. The use of a mean value reduces over-precaution which may arise if the most sensitive data points are always used, especially when additional valid data are available. The fact that not all the NOECs for *S. costatum* and *A. japonica* fell below the HC5 reduces uncertainties associated with how protective a PNEC set at the HC5 would be for these particular species.

From the two field ecosystem studies the lowest concentrations of zinc which caused detectable inhibition of carbon fixation (i.e. rates lower than 90% of the mean control values) were reported as being in the range of 7 to 13 µg l^{-1}. Diatoms are among the more sensitive species in the chronic NOEC database (e.g. *Asterionella japonica, Chaetoceros compressum*, and *Skeletonema costatum*). Also, in the freshwater database, unicellular algae and algal communities were among the more sensitive organisms. The results of the above experiments using natural phytoplankton communities, dominated by diatoms suggest that the HC5 value is sufficiently protective of such assemblages and supports the selection of an assessment factor of less than 5.

Under the old TGD (EC 2003) the PNEC derived using the HC5 should be compared with the results from the assessment factor method as prescribed in the TGD. The lowest species long-term NOEC is the 24 day survival NOEC of 5.6 µg l^{-1} for the crustacean, *Holmesimysis costata* (Hunt et al. 1997), which results in a $PNEC_{add,saltwater\_lt}$ = 0.56 µg Zn l^{-1} (dissolved) (Section 3.1.2).

There are 36 species NOECs (using geometric means where applicable) available to construct an SSD to estimate an HC5 of 6.76 µg l^{-1} Zn for saltwaters. Based on the discussion above it is considered that there is a reliable dataset with a good range of taxonomic groups, including those that are exclusively marine. Comparison with

assessment factors applied to HC5 values in European risk assessments for metals with similar data profiles, an assessment factor of 2 is considered to be appropriate for the derivation of the PNEC from the HC5:

$PNEC_{add,saltwater}$ = 6.76 µg l^{-1}/AF (2) = 3.4 µg l^{-1} zinc (dissolved)

3.3 Derivation of existing EQSs

The standards proposed in the 1984 Zn EQS report (Mance and Yates 1984) and subsequently adopted (Department of the Environment and Welsh Office 1989) for the long-term protection of freshwater life were for total zinc and were banded according to water hardness.

The European Inland Fisheries Advisory Commission (EIFAC) originally proposed the values for the protection of salmonid and cyprinid fish in waters other than those designated as European Community fisheries. Because the toxicity of zinc to fish has a logarithmic linear relationship with water hardness, it was recommended that EQS values for intermediate hardness should be calculated by simple linear interpolation between the relevant hardness-related values. The standards are expressed as annual averages and are shown in Table 3.4.

The few data available at that time for the chronic effects of zinc on marine species were limited to invertebrates, though acute data were also reported for fish. The lowest acute value was a 96-hour LC50 of 166 µg l^{-1} for the larvae of the mollusc *Mercenaria mercenaria*, which was identical to the chronic value obtained for the mysid shrimp, *Americamysis bahia* (formerly *Mysidopsis bahia*). An assessment factor of 4 was applied to this chronic value because of the possibility of other invertebrates with greater sensitivity to the long-term effects of exposure to zinc and the likelihood of increased toxicity of zinc to invertebrates at low salinities. This resulted in a long-term saltwater EQS of 40 µg l^{-1} dissolved zinc expressed as an annual average.

Revisions to these EQSs were presented in a report in 1992 (Hunt and Hedgecott 1992), but these latter values were never adopted.

Table 3.4 Existing EQS values for zinc (Department of the Environment and Welsh Office 1989)

Receiving medium	Annual average concentration (µg l^{-1})
Freshwater	
Protection of sensitive freshwater life:	
0–50 mg l^{-1} $CaCO_3$	8 (dissolved)
50–100 mg l^{-1} $CaCO_3$	50 (dissolved)
100–250 mg l^{-1} $CaCO_3$	75 (dissolved)
>250 mg l^{-1} $CaCO_3$	125 (dissolved)
Protection of other freshwater life:	
0–50 mg l^{-1} $CaCO_3$	75 (dissolved)
50–100 mg l^{-1} $CaCO_3$	175 (dissolved)
100–250 mg l^{-1} $CaCO_3$	250 (dissolved)
>250 mg l^{-1} $CaCO_3$	500 (dissolved)
Saltwater	40 (total)

3.5 Derivation of PNECs for sediment

3.5.1 PNEC derivation by the TGD assessment factor approach

According to the EU RAR (2008), only four reliable chronic NOEC values are available for benthic organisms (the insect *Chironomus tentans*, the annelid *Tubifex tubifex* and the crustacean *Hyalella azteca*). These are all in the range of 488 – 1100 mg kg^{-1} sediment dw expressed as the added concentration (Section 2.6.4).

These benthic species represent three taxonomic groups of invertebrates with different living and feeding conditions. According to the TGD (EC 2003) an assessment factor of 10 should therefore be used, which results in:

$PNEC_{add,sediment}$ = 488 mg zinc kg^{-1} dw/AF (10) = 49 mg zinc kg^{-1} dw

This value is equivalent to a **wet weight based $PNEC_{add,sediment}$ of 11 mg kg^{-1}.**[5]

3.5.2 PNEC derivation by the TGD species sensitivity distribution approach

Because only four reliable chronic NOEC values for benthic organisms are available, statistical extrapolation cannot be applied to derive a reliable $PNEC_{add,sediment}$.

[5] The TGD defines wet suspended particulate matter (SPM) as 90% volume/volume (v/v) water (density 1 kg l^{-1}) and 10 per cent v/v solids (density 2.5 kg l^{-1}), thus giving a wet density of (0.9 × 1) + (0.1 × 2.5) = 1.15 kg l^{-1}. The dry weight of solids is therefore 0.25 kg (per litre wet SPM) and the wet/dry ratio is 1.15:0.25 = 4.6.

3.6 Secondary poisoning of predators

3.6.1 Mammalian and avian toxicity data

Available information on mammalian and avian toxicity is summarised in Table 3.5.

Table 3.5 Most sensitive mammal and bird oral toxicity data relevant for the assessment of secondary poisoning

Study and result	Details
Sub-chronic toxicity to mammals	
Van Reen 1953 Cited in WHO 2001 **Sub-chronic LOAEL = 0.5% zinc oxide in diet**	Rats received zinc oxide in their diet for 15 days at a dose of 0, 0.5 or 1%. Death in the top dose group and reduced body weight (bw), fat content of the liver and impaired bone development at both doses, resulted in a LOAEL of 0.5%.
Chronic toxicity to mammals	
Maita et al. 1981 Cited in EU RAR 2004 **Chronic NOAEL = 3000 mg zinc sulphate kg^{-1} diet (104 mg zinc kg^{-1} bw d^{-1})**	Male and female ICR mice received zinc sulphate in their diet at concentrations ranging from 300–30000 mg kg^{-1} diet for 13 weeks. At 30000 mg kg^{-1} diet, haematological and biochemical effects were observed and gross pathology and histopathology showed changes in kidney, thyroid, gastrointestinal tract and pancreas. The NOAEL was set at 3000 mg zinc sulphate kg^{-1} diet, which corresponds to 104 mg zinc kg^{-1} bw d^{-1}. A LOAEL was also set at 1107 mg zinc kg^{-1} bw d^{-1}.
Maita et al. 1981 Cited in EU RAR 2004 **Chronic NOAEL = 3000 mg zinc sulphate kg^{-1} diet (53.5 mg zinc kg^{-1} bw d^{-1})**	Male and female Wistar rats received zinc sulphate in their diet at concentrations ranging from 300–30000 mg kg^{-1} diet for 13 weeks. At 30000 mg kg^{-1} diet, haematological effects and pancreatic damage were observed. The NOAEL was set at 3000 mg zinc sulphate kg^{-1} diet, which corresponds to 53.5 mg zinc kg^{-1} bw d^{-1}. A LOAEL was also set at 564 mg zinc kg^{-1} bw d^{-1}.
Edwards and Buckley 1995 Cited in EU RAR 2004 **Chronic NOAEL = 31.52 mg zinc monoglycerolate kg^{-1} bw d^{-1} (13.26 mg zinc kg^{-1} bw d^{-1})**	Female Sprague–Dawley rats received zinc monoglycerolate in their diets at levels of up to 1% (31.52–758.73 mg kg^{-1} bw d^{-1}) for 13 weeks. At 0.2% (127.52 mg kg^{-1} bw d^{-1}), effects on the pancreas, spleen and clinical chemical parameters were observed. The NOAEL was set at 31.52 mg zinc monoglycerolate kg^{-1} bw d^{-1}, which corresponds to 13.26 mg zinc kg^{-1} bw d^{-1}. A LOAEL was also set at 53.65 mg zinc kg^{-1} bw d^{-1}.
Aughey et al. 1977 Cited in EU RAR 2004 and WHO 2001 **Chronic LOAEL = 0.5 g zinc sulphate l^{-1} water (22.6 mg zinc kg^{-1} bw d^{-1})**	C3H mice received zinc sulphate in their drinking water at 0.5 g l^{-1} (~100 mg zinc sulphate/kg bw/day, or 22.6 mg zinc kg^{-1} bw d^{-1}) for 14 months. Plasma zinc, glucose and insulin, tissue zinc and histological, histochemical and electron microscopy examinations were made. No effects other than pancreatic hypertrophy and pituitary gland hypertrophy were observed.

Aulerich et al. 1991 Cited in EU RAR 2004 and EHC 2001 **Chronic NOEL = 1500 mg zinc sulphate kg^{-1} diet**	Adult and juvenile mink (three per sex per group) received zinc sulphate in their diets at 0, 500, 1000 or 1500 mg kg^{-1} for 144 days. No effects were observed on food consumption, body weights, haematological parameters, fur quality or survival. Similarly, no histological lesions were seen in liver, pancreas or kidney. Thus, the NOEL was set at the highest dose tested.
Zaporowska and Wasilewski 1992 Cited in EU RAR 2004 and WHO 2001 **Chronic LOAEL = 0.12 mg ml^{-1} zinc drinking water (12 mg zinc kg^{-1} bw d^{-1})**	Rats received zinc chloride in their drinking water for 4 weeks at a dose of either 0 or 0.12 mg ml^{-1} zinc in water (~12 mg zinc kg^{-1} bw d^{-1}). Decreased body weight, anaemia and increased lymphocyte count were observed at the 0.12 mg ml^{-1} zinc.
Straube et al. 1980 Cited in EU RAR 2004 and WHO 2001 **Chronic LOAEL = 500 mg zinc oxide kg^{-1} diet**	Ferrets (3–5 per group) received zinc oxide in their diets as 0, 500, 1500 or 3000 mg kg^{-1} for up to 3 months. Bodyweight loss, reduced food intake and death were observed on days 9–13 at 3000 mg kg^{-1} and on days 7–21 at 1500 mg kg^{-1}. Diffuse nephrosis and active haemopoieses in bone marrow and spleen were also observed in the 3000 and 1500 mg kg^{-1} diet groups. Pancreatitis was seen in one animal in each group at 3000 and 1500 mg kg^{-1}. No toxicity was observed at 500 mg kg^{-1}, apart from some evidence of effects on red blood cells.
The EU RAR 2004 and WHO 2001 state that "no adequate long-term carcinogenicity studies are available' and 'no adequate evidence has been found to indicate that zinc salts administered orally…are tumorigenic", respectively.	
Effects on reproduction of mammals	
Samanta and Pal 1986 Cited in EU RAR 2004 **Reproductive LOAEL = 4000 mg zinc kg^{-1} diet (200 mg zinc kg^{-1} bw d^{-1})**	Male Charles Foster rats received zinc sulphate in their diet at a concentration of 4000 mg zinc kg^{-1} diet (~200 mg zinc kg^{-1} bw d^{-1}) for 30–32 days before mating. A significantly decreased number of females conceived and a decreased number of live births, increased testes and sperm zinc levels, and reduced sperm motility were observed. However, sperm viability was unaffected.
Pal and Pal 1987 Cited in EU RAR 2004 and WHO 2001 **Reproductive LOAEL = 4000 mg zinc kg^{-1} diet (200 mg zinc kg^{-1} bw d^{-1})**	Female Charles Foster rats received zinc sulphate in their diet at 4000 mg zinc kg^{-1} diet (~200 mg zinc kg^{-1} bw d^{-1}) for various periods. Exposure for 18 days post coitus decreased the incidence of conception. However, exposure for 21–26 days before mating and throughout gestation for 18 days had no effect. This difference is thought to be due to adaptation to zinc feeding in the latter scenario. No increases in stillbirths or malformations were observed in any group.
Evenson et al. 1993 Cited in WHO 2001 **Reproductive NOAEL = 500 mg zinc chloride kg^{-1} diet**	Weanling male rats received 4 (low), 12 (normal) or 500 (high) mg zinc chloride kg^{-1} diet for 8 weeks. Testicular cell development was the only effect examined and excess zinc was observed to have no effects on it. Thus, the NOAEL was set at the highest dose tested.
Embryotoxicity and teratogenicity	

FDRL 1973 Cited in EU RAR 2004 **Developmental NOAEL = 12 mg anhydrous zinc sulphate kg^{-1} bw d^{-1} or 6.8 mg zinc sulphate heptahydrate kg^{-1} bw d^{-1})**	Female mice received daily doses of 0, 0.3, 1.4, 6.5 or 30 mg zinc sulphate kg^{-1} bw during days 6–15 of gestation. No effects were seen on implantation, maternal or foetal survival, or foetal abnormalities. Thus, the NOAEL was set at the highest dose tested. Because the form of the zinc sulphate used was not stated, two NOAELs have been provided, one on the assumption that the anhydrous form was used and one on the assumption that the heptahydrate was used.
FDRL 1973 Cited in EU RAR 2004 **Developmental NOAEL = 17 mg anhydrous zinc sulphate kg^{-1} bw d^{-1} or 9.6 mg zinc sulphate heptahydrate kg^{-1} bw d^{-1}**	Female rats received daily doses of 0, 0.4, 2.0, 9.1 or 42.5 mg zinc sulphate kg^{-1} bw during days 6–15 of gestation. No effects were seen on implantation, maternal or foetal survival, or foetal abnormalities. Thus, the NOAEL was set at the highest dose tested. Due to the fact that the form of the zinc sulphate used was not stated, two NOAELs have been provided, one on the assumption that the anhydrous form was used and one on the assumption that the heptahydrate was used.
FDRL 1973 Cited in EU RAR 2004 **Developmental NOAEL = 35.2 mg anhydrous zinc sulphate kg^{-1} bw d^{-1} or 19.9 mg zinc sulphate heptahydrate kg^{-1} bw d^{-1}**	Female hamsters received daily doses of 0, 0.9, 4.1, 19 or 88 mg zinc sulphate kg^{-1} bw during days 6–15 of gestation. No effects were seen on implantation, maternal or foetal survival, or foetal abnormalities. Thus, the NOAEL was set at the highest dose tested. Due to the fact that the form of the zinc sulphate used was not stated, two NOAELs have been provided, one on the assumption that the anhydrous form was used and one on the assumption that the heptahydrate was used.
FDRL 1974 Cited in EU RAR 2004 **Developmental NOAEL = 24 mg anhydrous zinc sulphate kg^{-1} bw d^{-1} or 13.6 mg zinc sulphate heptahydrate kg^{-1} bw d^{-1}**	Female rabbits received daily doses of 0, 0.6, 2.8, 13 or 60 mg zinc sulphate kg^{-1} bw during days 6–18 of gestation. No effects were seen on implantation, maternal or foetal survival, or foetal abnormalities. Thus, the NOAEL was set at the highest dose tested. Due to the fact that the form of the zinc sulphate used was not stated, two NOAELs have been provided, one on the assumption that the anhydrous form was used and one on the assumption that the heptahydrate was used.
Ketcheson et al. 1969 Cited in EU RAR 2004 and WHO 2001 **Developmental LOAEL = 0.2% zinc oxide in diet**	Pregnant albino rats received zinc oxide in their diet at either 0.2 or 0.5% for the entire gestation period and the first 14 days of lactation. Maternal body weight, gestation period and number of viable pups per litter were unaffected at either dose level at birth or on day 14. No malformations were observed in any pup, but there was a dose-related reduction in pup bodyweight. Additionally there were some alterations in iron and copper distribution in newborn pups at both treatment levels.
Sub-chronic toxicity to birds	

Hussein et al. 1988 Cited in WHO 2001 **Sub-chronic LOAEL = 15,000 mg zinc oxide kg^{-1} diet**	Japanese quail (*Coturnix coturnix japonica*) received zinc oxide in their diet at a concentration of 15000 mg kg^{-1} for 7 days. Effects observed included reduced body weight, decreased egg production, reduced eggshell breaking strength and induced moulting.
Stahl *et al.* 1989 Cited in WHO 2001 **Sub-chronic NOAEL = 100 mg zinc kg^{-1} diet**	Hens received zinc in their diets at concentrations of 37 (control), 100, or 2000 mg kg^{-1} for 21 days. There were no treatment-related deaths, but the highest dose caused decreased growth rate, anaemia, decreased tissue copper and iron levels, and increased tissue zinc levels.
Stahl et al. 1990 Cited in WHO 2001 **Sub-chronic NOAEL = 2,028 mg zinc kg^{-1} diet**	Hens received zinc in their diet at 48, 228 or 2028 mg kg^{-1} for 12–44 weeks. Effects at 12 or 44 weeks were unreported. However at 3 weeks, no effects were observed on egg production, feed conversion, feed consumption, hatchability or progeny growth.
Long-term toxicity to birds	
Dewar et al. 1983 Cited in WHO 2001 **Chronic LOAEL = 2,000 mg zinc kg^{-1} diet**	Two-week-old chicks received zinc in their diet at concentrations of 74 (control), 2000, 4000 or 6000 mg kg^{-1} for 4 weeks. Gizzard and pancreatic lesions were increased in all groups, and high mortality was observed in the top dose group.
Donmex et al. 2002 **Chronic NOAEL = 500 mg l^{-1} zinc drinking water**	New born male broiler chicks received zinc sulphate in their drinking water at concentrations of 0, 125, 500 or 1000 mg l^{-1} zinc for 60 days. In the two top dose groups, serum triiodothyronine and thyroxine levels and the diameters of follicles of the thyroid gland were reduced. The authors stated that birds receiving the 1000 mg l^{-1} level of zinc showed signs of toxicity; thus the NOAEL was set at 500 mg l^{-1} zinc.

LOAEL = lowest observed adverse effect level
NOAEL = no observed adverse effect level

3.6.2 PNECs for secondary poisoning of predators

Based on data in the Integrated Criteria Document for zinc (Cleven et al. 1993) on bioaccumulation of zinc in animals and on biomagnification (i.e. accumulation and transfer through the food chain), the EU RAR 2008 concluded that secondary poisoning is not relevant in the effect assessment of zinc.

The accumulation of zinc, an essential element, is regulated in animals from several taxonomic groups (e.g. molluscs, crustaceans, fish and mammals). In mammals, 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 agree 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 of bioaccumulation and biomagnification are not discussed further in the RAR (EU RAR 2008) or here.

4. Analysis and monitoring

A range of methods can be used for the analysis of zinc in environmental samples. These have been published by US bodies such as US EPA, the American Public Health Association (APHA), Association of Official Analytical Chemists (AOAC), and the National Institute for Occupational Safety and Health (NIOSH).

The most common method for analysis is inductively coupled plasma-atomic emission spectroscopy (ICP-AES), which is used to determine concentrations of zinc in:

- water (EPA methods 3120 B, 6010 C, 200.7; APHA methods 3120B, 3125B, 3130B);
- solid wastes (AOAC method 990.08); and
- soil (EPA methods 6010, 3050; AOAC 1998; APHA 1998; US EPA 1986, 1994; NIOSH 1994).

As well as the US EPA compendium CD-ROM (US EPA 1996), the National Environmental Methods Index (NEMI) provides an extensive online collection of methods (http://www.nemi.gov) in a searchable database.

Detection limits in water and solid samples are as low as 0.006 µg l^{-1} and 0.01 mg kg^{-1}, respectively (WHO 2001).

Preparation for water samples typically involves acid digestion with concentrated acids.

The concentration of zinc in soil can be determined by ICP-AES coupled with an ammonium bicarbonate–diethylenetriaminepentaacetic acid (NH_4HCO_3-DTPA) extraction procedure. Inductively coupled plasma-mass spectrometry (ICP-MS) has also been used to determine the concentration of zinc in water (e.g. EPA methods 200.8, 1638; APHA method 3125 B). Detection limits have been reported to be as low as 0.017 µg l^{-1} using the ^{66}Zn isotope and recoveries range from 99–117% (APHA 1998).

Flame atomic absorption spectroscopy (FAAS) has been used to determine zinc concentrations in natural waters (Fishman 1966). Atomic absorption spectroscopy is a rapid method of measuring zinc, with a detection limit of 5 µg l^{-1}. Brooks et al. (1967) demonstrated a simple extraction system consisting of two reagents, ammonium pyrrolidine dithiocarbamate (APDC) and methyl isobutyl ketone (MIBK), with subsequent analysis by FAAS to measure particulate and ‘soluble’ zinc in seawater. Sensitivity was in the sub-mg l^{-1} range and precision was good [3% coefficient of variation (CV)].

A range of techniques is available to determine the speciation of zinc. These have been used to determine the forms of a metal that are considered to be of particular environmental relevance, e.g. with respect to bioavailability or toxic effects. It has been established that such effects are more closely related to a portion of the total or total dissolved metal. This portion varies according to different characteristics of the water concerned and has been linked with ‘free metal ion’ or ‘dissolved inorganic forms of the metal’ (Milne 2000).

Speciation techniques have been developed to determine these environmentally relevant fractions. These rely on the separation and determination of the most chemically reactive or mobile species. However, such techniques are essentially empirical; the exact form of metal defined is not identified unequivocally. Nevertheless, speciation methods have been shown to provide a clearer indication of environmental effects than the determination of dissolved or total metal. However, speciation-based analytical approaches are unable to take account of competition at the “biotic ligand”, which can also affect bioavailability, and such approaches are therefore unlikely to be used widely, except for conditions which are beyond the application range of the ZnBLM.

Cathodic stripping voltammetry (CSV), also known as adsorption voltammetry, has been used to detect various metal ions in a 10^{-10}–10^{-11} M range in seawater (van den Berg 1986). Ammonium pyrrolidine dithiocarbamate was used as a chelating agent for zinc; because of its great sensitivity and specificity for zinc, it can be detected directly in the unaltered sample. Similarly, differential pulse cathodic stripping voltammetry (DPCSV) and differential pulse anodic stripping voltammetry (DPASV) have been used after complexation with APDC to determine zinc speciation at nanomolar concentrations in ocean waters (Donat and Bruland 1990; van den Berg 1986).

Anodic stripping voltammetry (ASV) has been used to detect zinc and other metal ions simultaneously at trace levels in atmospheric aerosols. This method is primarily used for small samples with very low concentrations of zinc and has a limit of detection of 13.7 ng l^{-1} (Casassas et al. 1991).

The methods CSV, DPCSV and ASV are attractive because of their great sensitivity and ability to discriminate between free metal (considered to be the sum of free ion and inorganic complexes – and referred to as the electrochemically labile fraction) and complexed/bound metal. These methods measure labile metal by monitoring the current produced while reducing (or oxidising) the uncomplexed metal. This labile fraction can include free (uncomplexed) metal as well as metal arising from weak complexes or complexes that can dissociate rapidly. An example of use of this technique is described by Gardner (1999).

In addition to voltammetric methods, an ion chromatographic method has been proposed for simultaneous determination of several elements including zinc in soil (Basta and Tabatabai 1990). In this method, after preliminary sample treatment the metals are separated by ion chromatography and the separated elements are quantified by ultraviolet–visible detection of zinc-PAR (4-[2-pyridylazo]resorcinol) coloured complexes. The limit of detection for zinc by this method was shown to be 5 µg kg^{-1} in soil extract (Basta and Tabatabai 1990). Precision was 2.5% CV. Other analytical methods include energy dispersive X-ray fluorescence (EDXRF).

Zinc is typically analysed in freshwater samples by ICP-MS for routine analysis, with limits of detection of 3 µg l^{-1} and below being possible. This enables zinc concentrations to be determined simultaneously along with a suite of other elements, and is likely to be the most practical approach for regulatory applications.

The lowest proposed PNEC derived for freshwaters and saltwaters and based on an HC5 is 3.4 µg l^{-1}. To provide adequate precision and accuracy the data quality requirements are that, at a third of the EQS, total error of measurement should not exceed 50%. It is likely that this would be achievable for regulatory laboratories which are already capable of achieving adequate performance for the current statutory Zn EQS of 8 µg l^{-1} (total) zinc in soft (<50 mg l^{-1} $CaCO_3$) salmonid waters.

5. Conclusions

The EU Risk Assessment Report (EU RAR) has been compiled for zinc metal, zinc oxide, zinc distearate, zinc chloride, zinc sulphate and trizinc bis(orthophosphate). The current report has used that report as a basis for PNEC derivation, but has also considered additional ecotoxicity and field data that have become available since the completion of the RAR.

5.1 Availability of data

Freshwater data selection has taken account of abiotic factors. Data from natural and artificial waters are acceptable if the major physicochemical characteristics (in particular pH and hardness) are similar to the ranges encountered in freshwaters.

Background zinc concentrations are also taken into account. However, the literature references used for the aquatic toxicity dataset of the EU RAR for zinc metal do not usually contain data on the background concentration of zinc in the test water and, in some cases, data on pH or hardness are also lacking. Therefore, tests conducted in artificial waters were excluded when there was no information on pH or hardness. Those tests conducted in natural waters were used unless there were indications that any of these three parameters (background concentration, pH, and hardness) deviated strongly from real environmental conditions.

Chronic NOEC values (using geometric means where applicable) from data on 25 species covering eight taxonomic groups (unicellular and multicellular algae, sponges, rotifers, molluscs, crustaceans, insects, fish and amphibians) to derive the long-term $PNEC_{add,freshwater}$. Data from existing mesocosm and field studies have been reported.

The EU RAR used data for 28 species covering six taxonomic groups algae (unicellular and multicellular), cnidarians, molluscs, crustaceans, annelids and echinoderms) to derive the chronic $PNEC_{add,saltwater}$ from geometric ‘species mean’ values. We have extended that in this report to 36 species from eight taxonomic groups (with nematodes and fish included as additional groups).

Only four valid studies were available on benthic organisms.

5.2 Derivation of PNECs

The chronic effects data evaluated and used for the EU RAR of zinc have been published and summary data are given in Annexes.

The added risk approach is considered appropriate when deriving PNECs for zinc as zinc is a naturally occurring substance with ubiquitous distribution in the aquatic environment. This approach takes account of background concentrations and the PNEC ($PNEC_{add}$) applies only to the “added” contribution over and above the ambient background level (i.e. the value at which toxic effects occur, ignoring contributions from

background concentrations). Adopting the added risk approach has considerable practical consequences when assessing compliance as ambient zinc background concentrations need to be estimated at the waterbody scale.

A research programme conducted as part of the EU RAR developed quantitative methods for taking into account the bioavailability/toxicity of zinc due to water and sediment chemistry, as discussed in Appendix IV of the current report. These methods use BLMs and the AVS approach; the former has been adopted by the Environment Agency of England and Wales.

For aquatic organisms, which are mainly exposed via water, the zinc ion and other dissolved species are relevant for toxicity.

The proposed PNECS are described below and summarised in Table 5.1.

5.2.1 Long-term PNEC for freshwaters

Algae appear to be the most sensitive taxonomic group, followed by crustaceans, sponges, rotifers and fish.

Using the assessment factor method to derive a $PNEC_{freshwater}$ requires that an assessment factor of 10 is applied to the lowest reliable NOEC or EC10 (4.9 µg l^{-1} for *Pseudokirchneriella subcapitata* and *Chlorella* sp.). This results in:

$PNEC_{add,freshwater\_lt}$ = 4.9 µg l^{-1}/AF (10) = 0.5 µg l^{-1} zinc (dissolved)

However, there are sufficient freshwater ecotoxicity data to allow a PNEC to be derived from the HC5 of an SSD, and as a result of this the PNEC derived by the assessment factor (AF) method is not recommended for adoption as an EQS.

The current draft EQS guidance is unclear about how the reference EQS should be set, and there are indications that it should be set for the 10^{th} to 90^{th} percentile ranges of abiotic conditions, and also that it should be set to be protective of the most sensitive waterbodies which will be monitored for EQS compliance. The key input parameters for Zn BLM are DOC and Ca concentrations and pH. North West Region is the most sensitive of the 10 Regions (six in England, one in Wales and three in Scotland) for which there is data, followed by Wales and the South West. The PNEC values were calculated from the annual averages of pH (mean), DOC (median) and Ca (mean) of at least six samples for each individual site (approximately 100 sites for each Region). Setting the Generic HC5 to a predefined level of protection for the whole of Great Britain, such as the level for 95% protection of 14.2 µg l^{-1}, has limitations in that the selected value represents a rather lower level of protection (approximately 68%) in the North West Region. Consequently the value was selected so as to provide 95% protection for the most sensitive region, which would ensure a high level of protection if applied on a UK basis.

An AF of 1 is recommended in order to derive the $PNEC_{add}$ from the generic PNEC value of 10.9 µg l^{-1}. Thus, the $PNEC_{add,freshwater\_lt}$ can be calculated as follows:

$PNEC_{add,freshwater\_lt}$ = 10.9 µg l^{-1}/AF (1) = 10.9 µg l^{-1} zinc (bioavailable)

The proposed PNEC is above the very lowest toxicity values observed under some test conditions. Field evidence does not, however, suggest that freshwater algae, such as benthic diatoms, are especially sensitive to zinc toxicity.

The existing EQSs for total zinc are banded according to water hardness, with values ranging between 8 and 125 µg l^{-1} for the protection of "sensitive taxa". The $PNEC_{add,freshwater\_lt}$ derived using the SSD approach is comparable to the most stringent value from this range; the $PNEC_{add,freshwater\_lt}$ based on a deterministic approach is lower.

5.2.2 Long-term PNEC for saltwaters

Based on abiotic factors, freshwater and saltwater can be regarded as different environments, each with organisms adapted to that environment. Thus, the freshwater and saltwater data were not combined to derive a general $PNEC_{add}$.

Using AF method the lowest reliable long-term NOEC is the 24-day survival NOEC of 5.6 µg l^{-1} for the crustacean, *Holmesimyis costata.* As long-term NOECs for at least three marine species representing three trophic levels (i.e. algae, crustaceans, and fish) plus data of the same quality for more than two further marine groups (i.e. annelids, molluscs, and echinoderms) are available, the appropriate assessment factor in accordance with the TGD is 10. This results in:

$PNEC_{add,saltwaterlt}$ = 5.6 µg l^{-1}/AF (10) = 0.56 µg l^{-1} zinc (dissolved)

However, there are sufficient marine ecotoxicity data to allow a PNEC to be derived from the HC5 of an SSD, and as a result of this the PNEC derived by the AF method is not recommended for adoption as an EQS.

There are 36 species NOECs (using geometric means where applicable) available to construct an SSD to estimate an HC5 of 6.76 µg l^{-1} Zn for saltwaters. Based on comparison with assessment factors applied to HC5 values in European risk assessments for metals with similar data profiles, an assessment factor of between 2 is considered to be appropriate for the derivation of the PNEC from the HC5:

$PNEC_{add,saltwater}$ **= 6.76 µg l^{-1}/AF (2) = 3.4 µg l^{-1} zinc (dissolved)**

All derivations result in a PNEC that is lower than the existing EQS of 40 µg l^{-1}, which was derived by applying an assessment factor of 4 to a chronic data value of 166 µg l^{-1} obtained for the shrimp *Americamysis bahia* (formerly *Mysidopsis bahia*).

5.2.3 PNEC for secondary poisoning

Based on data on the bioaccumulation of zinc in animals and on biomagnifications through the food chain, the EU RAR concludes that secondary poisoning is not relevant in the effect assessment of zinc.

5.2.4 PNEC for sediments

According to the EU RAR, only four reliable chronic NOEC values for benthic organisms (the insect *Chironomus tentans*, the annelid *Tubifex tubifex* and the crustacean *Hyalella azteca*) in the range of 488 – 1100 mg kg^{-1} sediment dw are available. These benthic species represent three taxonomic groups of invertebrates with different living and feeding conditions. Therefore, an assessment factor of 10 should be applied to the lowest chronic NOEC. This gives a $PNEC_{add,sediment}$ of 49 mg zinc kg^{-1} dw (equivalent to a $PNEC_{add,sediment}$ of 11 mg zinc kg^{-1} wet weight (ww)).

Table 5.1 Summary of proposed PNECs

Receiving medium/exposure scenario	Proposed PNEC (µg l^{-1})	Existing EQS (µg l^{-1})
Freshwater/long-term	0.5 (dissolved) (AF approach) 10.9 (bioavailable) (SSD approach)	Range 8 – 125 (total zinc) depending on hardness
Saltwater/long-term	0.56 (dissolved) (AF approach), 3.4 (dissolved)(SSD approach)	40 (dissolved zinc)
Freshwater sediment/long-term	49 mg kg^{-1} dw	No standard

5.3 Analysis

The lowest proposed PNEC derived for freshwaters and saltwaters and based on an HC5 is 3.4 µg l^{-1}. The data quality requirements are that, at a third of the EQS, total error of measurement should not exceed 50%. Current analytical methodologies provide detection limits as low as 13.7 ng l^{-1}, which suggests that they offer adequate performance to analyse zinc for compliance with the proposed PNECs.

5.4 Implementation issues

Before PNECs for zinc can be adopted as EQSs, it will be necessary to address the following issues:

1. To implement the proposed PNECs using the added risk approach, it would be necessary to determine background concentrations of zinc at a regional, river basin, or possibly the waterbody scale (Environment Agency 2008).

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List of abbreviations

AA	annual average
AF	assessment factor
APDC	ammonium pyrrolidine dithiocarbamate
ASV	anodic stripping voltammetry
AVS	Acid Volatile Sulfide
BCF	bioconcentration factor
BLM	Biotic Ligand Model
bw	body weight
CAS	Chemical Abstracts Service
CI	confidence interval
CSV	cathodic stripping voltammetry
CV	coefficient of variation
DOC	dissolved organic carbon
DPCSV	differential pulse cathodic stripping voltammetry
dw	dry weight
EC50	concentration effective against 50% of the organisms tested
EHC	Environmental Health Criteria
EQS	Environmental Quality Standard
FAAS	flame atomic absorption spectrometry
GLP	Good Laboratory Practice (OECD)
ICP-AES	inductively coupled atomic emission spectrometry
IUPAC	International Union of Pure and Applied Chemistry
LC50	concentration lethal to 50% of the organisms tested
LOAEL	lowest observed adverse effect level
LOEC	lowest observed effect concentration
lt	long term
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
OECD	Organisation for Economic Co-operation and Development
PEC	predicted effect concentration
PNEC	predicted no-effect concentration
RAR	Risk Assessment Report

SEM	Simultaneously Extracted Metals
SSD	species sensitivity distribution
st	short term
TGD	Technical Guidance Document
UKTAG	UK Technical Advisory Group
US EPA	US Environmental Protection Agency
WER	water effect ratio
WFD	Water Framework Directive

Appendix I: Freshwater toxicity data for Zn taken from the EU RAR

Authors	BELANGER, S.E. AND CHERRY, D.S.
Year	1990
Title	Interacting effects of pH acclimation, pH, and heavy metals on acute and chronic toxicity to *Ceriodaphnia dubia* (Cladocera).
Bibliographic Source	Journal of Crustacean Biology, 10, 225-235
Test material	Zinc - form not stated
Test species	*Ceriodaphnia dubia* < 24 h old
Taxonomic group	Crustacean
Exposure duration	7 days
Endpoint	NOEC or EC10
Effect parameter	Reproduction
Effect concentration (µg l^{-1})	25 – 50 µg l^{-1}
Nominal/Measured	Measured zinc concentrations not reported separately for each test, but according to the authors measured zinc concentrations were ± 15% of nominal concentrations.
Test media type	3 different rivers: New river (Virginia), Amy Bayou river (Louisiana) and Clinch river (Virginia), water 11-µm filtered before use.
Klimisch code	
Free text phrase	
Principles of method if other than guideline	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 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 $CaCO_3$ l^{-1}, respectively; the tests were performed with pH 8 acclimated daphnids, cultured in New river or Clinch river water. Background zinc concentration in all three waters was less than 20 µg l^{-1} (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^{-1}, 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^{-1} ; there is no reference to New river, Amy Bayou or Clinch river specifically.
Details on results (CI,	Toxicity tested at pH 6, 8 and 9 and different hardness

statistics, etc.):	values (81, 118 and 168 mg $CaCO_3$ l^{-1}). The two test variables were tested independently. No consistent effect of hardness and pH was found. The NOEC values listed in Table 2.7 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^{-1} Zn treatment in each test water was considered to be the control value. The NOEC values in Table 2.7 are based on comparisons (per test water) with the 0 µg l^{-1} Zn control at corresponding pH. Data on survival reported incompletely, but it would appear that survival was not affected at the test concentrations used (nominal: 0, 25 and 50 µg l^{-1} in New river water; 0, 50 and 100 µg l^{-1} in Amy Bayou and Clinch river water). NOEC = LOEC/2 (19% inhibition at 50 µg l^{-1}). An EC10 could not be calculated, as 28% was found at the lower concentration tested (25 µg l^{-1}). Further concentrations were not tested. NOEC = EC10, calculated from the two effect concentrations (16% and 49% inhibition at 50 and 100 µg l^{-1}, respectively). EC10 calculated by the rapporteur, using the logistic dose-response model according to Haanstra et al. (1985). NOEC = EC10, calculated from the two effect concentrations (13% and 53% inhibition at 50 and 100 µg l^{-1}, respectively). EC10 calculated by the rapporteur, as before. NOEC = EC10, calculated from the two effect concentrations (21% and 44% inhibition at 50 and 100 µg l^{-1}, respectively). EC10 calculated by the rapporteur, as before. NOEC = LOEC/3 (26% inhibition at 100 µg l^{-1}). An EC10 could not be derived, as 30% inhibition was found at the lower concentration tested (50 µg l^{-1}). Further concentrations were not tested.

Authors	BENGTSSON, B.-E.
Year	1974
Title	Effect of zinc on growth of the minnow *Phoxinus phoxinus*.
Bibliographic Source	OIKOS, 25, 370-373.
Test material	Zinc nitrate tetrahydrate ($ZnNO_3 \cdot 4H_2O$)
Test species	*Phoxinus phoxinus* (yearlings)
Taxonomic group	Fish
Exposure duration	5 months
Endpoint	NOEC
Effect parameter	Survival and growth
Effect concentration (µg l^{-1})	50
Nominal/Measured	Measured. Background zinc concentration 1 - 12 µg l^{-1} (total range). No data on nominal concentrations.
Test media type	Dechlorinated tap water
Klimisch code	
Free text phrase	
Principles of method if other than guideline	No details reported in RAR.
Details on results (CI, statistics, etc.):	Fish Statistics (p = 0.05) reported on growth data only. Abstract : Reduced growth occurred in yearlings and adults of the minnow *Phoxinus phoxinus* L. following exposure to zinc nitrate in fresh water over a 150-day period. Yearlings were more sensitive than adults and showed reduced growth at 0.13 ppm zinc (corresponding to 1/25 of the estimated 96-hr LC50). Suppressed growth was associated with reduced *Tubifex* consumption.

Authors	BENOIT, D.A. AND HOLCOMBE, G.W.
Year	1978
Title	Toxic effects of zinc on fathead minnows *Pimephales promelas* in soft water.
Bibliographic Source	Journal of Fish. Biology, 13, 701-708.
Test material	Zinc sulphate heptahydrate ($ZnSO_4 \cdot 7H_2O$)
Test species	*Pimephales promelas*
Taxonomic group	Fish
Exposure duration	Full life cycle
Endpoint	NOEC
Effect parameter	Reproduction
Effect concentration (µg l^{-1})	78
Nominal/Measured	Measured. Background zinc concentration 2 µg l^{-1} (mean of duplicate tanks). No data on nominal concentrations reported.
Test media type	Lake Superior water, passed through an ultraviolet sterilizer
Klimisch code	
Free text phrase	
Principles of method if other than guideline	No details reported in RAR.
Details on results (CI, statistics, etc.):	Statistics: p = 0.05. 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^{-1}. 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^{-1}. Fish exposed to 295 µg l^{-1} 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^{-1} 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) were affected at concentrations up to 295 µg l^{-1}. 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^{-1} and 145 µg l^{-1}, 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^{-1}. Eight-week larval growth of both first- and second-generation fish was not affected up to the highest test concentration (575 µg l^{-1}). Hatchability of first-generation eggs was not affected at 575 µg l^{-1}, while hatchability of offspring was affected at 295 µg l^{-1}.

Authors	BIESINGER, K.E. AND CHRISTENSEN, G.M. 1972.
Year	1972
Title	Effects of various metals on survival, growth, reproduction, and metabolism of.
Bibliographic Source	Journal of the Fisheries Research Board of Canada 29, 1691-1700.
Test material	Zinc chloride ($ZnCl_2$)
Test species	*Daphnia magna* (< 24 h old)
Taxonomic group	Crustacean
Exposure duration	21 days
Endpoint	NOEC
Effect parameter	Reproduction
Effect concentration (µg l^{-1})	35
Nominal/Measured	Zn concentrations analyzed but effects data based on nominal concentrations.
Test media type	Culture and test medium: Lake Superior water, strained through # 20 bolting cloth.
Klimisch code	
Free text phrase	
Principles of method if other than guideline	No details reported in RAR.
Details on results (CI, statistics, etc.):	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^{-1} (LOEC). The NOEC (35 µg l^{-1}) 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^{-1} for the parent animals (no further data on survival reported) and the 3-w EC50 of 102 µg l^{-1} for reproduction, survival was less sensitive to Zn than reproduction. Growth (body weight) of the parent animals after a 3-w exposure was less sensitive than reproduction, with 28% weight reduction at 175 µg l^{-1} (growth was studied at 12 Zn concentrations but only the result at 175 µg l^{-1} was reported. Background zinc concentration in Lake Superior water: 0.8 µg l^{-1} (mean), with a range of 1 to 2.7 µg l^{-1} *(lowest level reported should be 0.1 µg l^{-1} ?)*; pH: 7.7 (mean), with a range of 7.4 to 8.2; total hardness 45 mg l^{-1} (mean), with a range of 44 to 53 mg l^{-1}; alkalinity 42 mg l^{-1} (mean), with a range of 41 to 50 mg l^{-1}. These water characteristics are not study specific but based on general data on Lake Superior water characteristics mentioned in Biesinger and Christensen ‘72. 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.

Authors	BIESINGER, K.E., CHRISTENSEN, G.M. AND FIANDT, J.T.
Year	1986
Title	Effects of metal salt mixtures on *Daphnia magna* reproduction.
Bibliographic Source	Ecotoxicology and Environmental Safety, 11, 9-14.
Test material	Zinc chloride ($ZnCl_2$)
Test species	*Daphnia magna* (< 24 h old)
Taxonomic group	Crustacean
Exposure duration	21 days
Endpoint	NOEC
Effect parameter	Reproduction
Effect concentration (µg l^{-1})	74
Nominal/Measured	Measured.
Test media type	Lake Superior water, strained through # 20 bolting cloth.
Klimisch code	
Free text phrase	
Principles of method if other than guideline	No details reported in RAR.
Details on results (CI, statistics, etc.):	Statistics: p = 0.05. Reproductive parameter: total number of young. In one out of two test performed, reproduction was considerably reduced (40-50%) at 74 µg l^{-1} (actual concentration), but this effect was not statistically significant. Tests were conducted at sublethal concentrations (see also Biesinger & Christensen, 1972).

Authors	BORGMANN, U., NORWOOD, W.P. AND CLARKE, C.
Year	1993
Title	Accumulation, regulation and toxicity of copper, zinc, lead and mercury in *Hyalella azteca*.
Bibliographic Source	Hydrobiologia, 259, 79-89.
Test material	Zinc – form not stated
Test species	*Hyalella azteca* (< 1 week old)
Taxonomic group	Crustacean
Exposure duration	10 weeks
Endpoint	NOEC
Effect parameter	Survival and reproduction
Effect concentration (µg l^{-1})	42
Nominal/Measured	Measured zinc concentrations (6, 13, 21, 42, 108, 185 and 316 µg l^{-1}) were only 40-60% of nominal zinc concentrations (0, 32, 56, 100, 180, 320 and 560 µg l^{-1}) 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).
Test media type	Dechlorinated tap water, originating from Lake Ontario.
Klimisch code	
Free text phrase	
Principles of method if other than guideline	No details reported in RAR.
Details on results (CI, statistics, etc.):	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.

Authors	CAIRNS, M.A., GARTON, R.R. AND TUBB, R.A.
Year	1982
Title	Use of fish ventilation frequency of estimate chronically safe toxicant concentrations.
Bibliographic Source	Transactions of the American Fisheries Society, 111, 70-77.
Test material	Zinc chloride ($ZnCl_2$)
Test species	*Oncorhynchus mykiss* (eggs)
Taxonomic group	Fish
Exposure duration	72 days
Endpoint	NOEC
Effect parameter	Survival
Effect concentration (µg l^{-1})	440
Nominal/Measured	Measured
Test media type	UV-sterilized well water, diluted with water treated by reverse osmosis to reduce hardness. Background zinc concentration in test medium <5 µg/l.
Klimisch code	
Free text phrase	
Principles of method if other than guideline	No details reported in RAR.
Details on results (CI, statistics, etc.):	Statistics applied. Before incubation eggs were disinfected by dipping in Wescodyne disinfectant. No data on nominal concentrations reported.

Authors	CHAPMAN, G.A., OTA, S. AND RECHT, F. 1980.
Year	1980
Title	Effects of water hardness on the toxicity of metals to *Daphnia magna*
Bibliographic Source	(Status Report 1980). U.S. EPA, Corvallius, Oregon 97330.
Test material	Zinc chloride ($ZnCl_2$)
Test species	*Daphnia magna* (< 24 h old)
Taxonomic group	Crustacean
Exposure duration	21 days
Endpoint	NOEC
Effect parameter	Survival and reproduction
Effect concentration (µg l^{-1})	42 - 97
Nominal/Measured	Measured
Test media type	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.
Klimisch code	
Free text phrase	
Principles of method if other than guideline	No details reported in RAR.
Details on results (CI, statistics, etc.):	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. Separate cultures were maintained at each water hardness, so it appears that the animals were acclimated to the hardness of the water before testing.

Authors	DAVE, G., DAMGAARD, B., GRANDE, M., MARTELIN, J.E., ROSANDER, B. AND VIKTOR, T.
Year	1987
Title	Ring test of an embryo-larval toxicity test with zebrafish (*Brachydanio rerio*) using chromium and zinc as toxicants.
Bibliographic Source	Environmental Toxicology and Chemistry, 6, 61-71.
Test material	Zinc sulphate heptahydrate ($ZnSO_4 \cdot 7H_2O$)
Test species	*Brachydanio rerio* (eggs)
Taxonomic group	Fish
Exposure duration	14 days
Endpoint	NOEC
Effect parameter	Hatchability
Effect concentration (µg l^{-1})	180 - 2900
Nominal/Measured	Nominal
Test media type	Reconstituted water
Klimisch code	
Free text phrase	
Principles of method if other than guideline	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.
Details on results (CI, statistics, etc.):	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 acclimated to the test medium and other test conditions for two weeks.

Authors	DE SCHAMPHELAERE, K.A.C., HEIJERICK, D.G. AND JANSSEN, C.R.
Year	2003
Title	Development and Validation of Biotic Ligand Models for Predicting Chronic Zinc Toxicity to Fish, Daphnids and Algae.
Bibliographic Source	Final report of ILZRO project ZEH-WA-1). Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University, Belgium (Sponsor: International Lead and Zinc Research Organization, ILZRO, United States)
Test material	Zinc chloride ($ZnCl_2$)
Test species	(1) *Pseudokirchneriella subcapita* (2) *Daphnia magna* (< 24 h old) (3) *Oncorhynchus mykiss* (early juveniles 5 – 6 weeks old)
Taxonomic group	(1) Algae (2) Crustacean (3) Fish
Exposure duration	(1) 72 hours (2) 21 days (3) 30 days
Endpoint	NOEC or EC10
Effect parameter	(1) growth (2) reproduction/survival (3) survival
Effect concentration (µg l^{-1})	(1) 5.2 – 124 (2) 48 - 155 (3) 39 - 974
Nominal/Measured	Measured. The background Zn concentrations in the artificial test media were 1 – 3 µg l^{-1}. 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.
Test media type	(1) Culture water: drinking water enriched with nutrients: pH 7.5, hardness 90 mg l^{-1} (as $CaCO_3$), background Zn concentration 15-20 µg l^{-1}. Before testing the algae were pre-acclimated for 5 days under the conditions of standard OECD medium (pH 7.5, hardness 25 mg l^{-1} (as $CaCO_3$), background Zn concentration 1.4 µg l^{-1} (nominal; measured Zn concentrations <3 µg l^{-1}). 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^{-1}). 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^{-1} (fulfilling the criterion for the minimum 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^{-1}), while the standard OECD medium contains 0.6 mM Na (13.7 mg Na l^{-1}, from 50 mg $NaHCO_3$ l^{-1}). Additional calcium, magnesium or sodium was added as chloride salt. (2) 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^{-1} (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^{-1} (fulfilling the criterion for the minimum Zn concentration in artificial media). As the standard test medium was prepared from carbon-filtered and deionised water, the DOC concentration was assumed to be 0.3 mg l^{-1}, as in the fish *O. mykiss* study. Additional calcium, magnesium or sodium was added as chloride salt. In all tests of the pH series, a DOC concentration of 5 mg l^{-1} (natural DOC, from Lake Ankeveen water, see fish *O. mykiss* study) was added to the test water to control the pH value. (3) Culture water: pH 7.5, hardness 50-70 mg l^{-1} (as $CaCO_3$), background Zn concentration 5 µg/l. 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^{-1} (detection limit); DOC concentration 0.3 mg l^{-1}. 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^{-1} (fulfilling the criterion for the minimum Zn concentration in artificial media). Additional calcium, magnesium or sodium was added as chloride salt.
Klimisch code	
Free text phrase	
Principles of method if other than guideline	Statistics: p = 0.05. 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 (univariate) 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. Further information provided by the authors of the study in addition to the study report was 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. (1) Test conducted according to OECD 201. Each test included a control and 4 or 5 test concentrations, selected on the basis of the physico-chemical properties of the test water. 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 concentration in these 'reconstituted' natural waters was < 5 µg l^{-1} (detection limit). The values for pH, hardness and DOC are those measured in these 'reconstituted' 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^{-1}, respectively. 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 slightly 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 throughout 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. (2) Test conducted according to OECD 211. Culture medium: Elendt M4 ((hardness 250 mg l^{-1}, as $CaCO_3$, pH 7.5-8.5, background Zn concentration 6 µg l^{-1}, see OECD

	211). Each test included a control and 5 test concentrations, selected on the basis of the physico-chemical properties of the test water. 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). The validity criterion for control survival of the parent animals (<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 slightly 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. (3) Test conducted according to OECD 215. Before testing the fish were acclimated for 1 week to the standard test medium without zinc. Each test included a control and 4 or 5 test concentrations, selected on the basis of the physico-chemical properties of the test water. In addition to the tests listed in Table 2.7, 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^{-1}, which is clearly above the water solubility limit of zinc of around 1,000 µg l^{-1} at pH 8.5, as shown by the cloudiness of the test solution and the low (<860 µg l^{-1}) 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 concentration in these 'reconstituted' natural waters was <5 µg l^{-1} (detection limit). The values for pH, hardness and DOC are those measured in the 'reconstituted' waters during the toxicity tests and may somewhat deviate from those measured in the original waters. The validity criteria for control survival (<10% mortality) and control growth (>50% weight increase) were met in all tests.
Details on results (CI, statistics, etc.):	(1) 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 E_rC10 values 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). (2) The results of the tests are based on the dissolved-Zn concentrations measured before and after each renewal (renewal: every other day). Toxicological 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. (3) 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 2.7 are for survival, but also protective for growth.

Authors	DORGELO, J., MEESTER, H. AND VELZEN, C.
Year	1995
Title	Effects of diet and heavy metals on growth rate and fertility in the deposit-feeding snail *Potamopyrgus jenkinsi* (Smith) (Gastropoda: Hydrobiidae).
Bibliographic Source	Hydrobiologia, 316, 199-210.
Test material	Zinc chloride ($ZnCl_2$)
Test species	*Potamopyrgus jenkinsi* (Smith) (juveniles, 1.7 ± 0.1 cm length)
Taxonomic group	Mollusc
Exposure duration	16 weeks
Endpoint	NOEC
Effect parameter	Growth
Effect concentration (µg l^{-1})	75
Nominal/Measured	Nominal. Measured zinc concentrations (12, 72, 115, 189, 387 µg l^{-1}) within 15% of nominal zinc concentrations (0, 75, 100, 200, 400 µg l^{-1}) in exposure groups.
Test media type	Culture and test medium: 0.45 µm filtered Lake Maarsseveen water.
Klimisch code	
Free text phrase	
Principles of method if other than guideline	No details reported in RAR.
Details on results (CI, statistics, etc.):	Statistics: p = 0.01. Results from preliminary tests (not reported in detail) showed an almost complete suppression of growth at 200 and 400 µg l^{-1}. Hardness based on reported Ca level (64 mg l^{-1}).

Authors	ENSERINK, E.L., MAAS-DIEPEVEEN, J.L. AND VAN LEEUWEN, C.J.
Year	1991
Title	Combined effects of metals: an ecotoxicological evaluation.
Bibliographic Source	Water Research, 25, 679-687.
Test material	Zinc chloride ($ZnCl_2$)
Test species	*Daphnia magna* (< 24 h old)
Taxonomic group	Crustacean
Exposure duration	17 and 21 days
Endpoint	EC10 and NOEC
Effect parameter	Survival/reproduction
Effect concentration (µg l^{-1})	420 and 310
Nominal/Measured	Nominal
Test media type	Lake IJssel water filtered through a 25 µm mesh and UV-treated. Lake IJssel is part of the River Rhine system**.**
Klimisch code	
Free text phrase	
Principles of method if other than guideline	No details reported in RAR.
Details on results (CI, statistics, etc.):	Statistics: p = 0.01. 21 day test - because only the lowest effect concentrations with respect to growth (120 µg l^{-1}) and survival and reproduction (1,000 µg l^{-1}) 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 (F0 generation). 17 day 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).

Authors	HOLCOMBE, G.W., BENOIT, D.A. AND LEONARD, E.N. 1979.
Year	1979
Title	Long-term effects of zinc exposures on brook trout (*Salvelinus fontinalis*).
Bibliographic Source	Transactions of the American Fisheries Society, 108, 76-87.
Test material	Zinc sulphate heptahydrate ($ZnSO_4 \cdot 7H_2O$)
Test species	*Salvelinus fontinalis*
Taxonomic group	Fish
Exposure duration	3 years
Endpoint	NOEC
Effect parameter	Hatchability
Effect concentration (µg l^{-1})	530
Nominal/Measured	Measured. Exposure concentrations: 2.6, 39, 69, 144, 266, 534 and 1,360 µg l^{-1} (actual concentrations; no data on nominal concentrations reported); the highest concentration was not used for the third-generation exposure.
Test media type	Lake Superior water, passed through an ultraviolet sterilizer.
Klimisch code	
Free text phrase	
Principles of method if other than guideline	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.
Details on results (CI, statistics, etc.):	Statistics: p = 0.05. Egg fragility (force required to rupture egg chorions) was significantly reduced at 266 µg l^{-1} and higher concentrations, but according to Holcombe et al., '79, only 1,360 µg l^{-1} 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^{-1} (NOEC) and 1,360 µg l^{-1} (LOEC), based on hatching. During a separate exposure of embryos and larvae, 1,368 µg l^{-1} significantly reduced (P = 0.05) both embryo and 12-week larval survival.

Authors	KRAAK, M.H.S., WINK, Y.A., STUIJFZAND, S.C., BUCKERT-DE JONG, M.C., DE GROOT, C.J. AND ADMIRAL, W.
Year	1994
Title	Chronic ecotoxicity of Zn and Pb to the zebra mussel *Dreissena polymorpha*.
Bibliographic Source	Aquatic Toxicology, 30, 77-89.
Test material	Zinc chloride ($ZnCl_2$)
Test species	*Dreissena polymorpha* (length 1.6 – 2.2 cm)
Taxonomic group	Mollusc
Exposure duration	10 weeks
Endpoint	NOEC
Effect parameter	Survival
Effect concentration (µg l^{-1})	400
Nominal/Measured	Nominal. Measured zinc concentrations (3, 38, 101, 382, 1,266 and 2,739 µg l^{-1}) within 10% of nominal zinc concentrations (0, 40, 100, 400, 1,400 and 3,000 µg l^{-1}) in exposure groups.
Test media type	Culture and test medium: Sieved (25 µm) and filtered (through sand) Lake Markermeer water. The hardness of this lake water (270 mg l^{-1}, as $CaCO_3$, based on the reported hardness of 150 mg l^{-1}, as CaO) is somewhat higher than the upper limit of 250 mg l^{-1} (as $CaCO_3$) used as selection criterion; the test has been selected however, because Lake Markermeer is part of the river Rhine system.
Klimisch code	
Free text phrase	
Principles of method if other than guideline	No details reported in RAR
Details on results (CI, statistics, etc.):	Statistics: p = 0.05. Growth (dry weight of soft tissues) was not affected at concentrations up to 1,400 µg l^{-1} (in the two 3000 µg l^{-1} groups this could not be studied, since only one mussel survived at this concentration).

Authors	MASTERS, J.A., LEWIS, M.A., DAVIDSON, D.I. AND BRUCE, R.D.
Year	1991
Title	Validation of a four-day *Ceriodaphnia* toxicity test and statistical considerations in data analysis.
Bibliographic Source	Environmental Toxicology and Chemistry, 10, 47-55.
Test material	Zinc chloride ($ZnCl_2$)
Test species	*Ceriodaphnia dubia* (< 24 h old, 7-day test; 3 days old, 4-day test)
Taxonomic group	Crustacean
Exposure duration	4- and 7-days
Endpoint	NOEC
Effect parameter	Reproduction
Effect concentration (µg l^{-1})	14 - 100
Nominal/Measured	Nominal
Test media type	Culture and test medium: 60 µm filtered Little Miami River water
Klimisch code	
Free text phrase	
Principles of method if other than guideline	Tests with reference to the US EPA and ASTM guidelines for testing chronic survival and reproduction of Ceriodaphnia. The 7-d exposure is standard; the 4-d exposure was tested to validate a shorter alternative. Three generations of *C. dubia* were acclimated in the river water before testing.
Details on results (CI, statistics, etc.):	Statistics: p = 0.05. 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 √ 2, according to the TGD.

Authors	MÜNZINGER, A. AND MONICELLI, F.
Year	1991
Title	A comparison of the sensitivity of three *Daphnia magna* species populations under chronic heavy metal stress.
Bibliographic Source	Ecotoxicology and Environmental Safety, 22, 24-31.
Test material	Zinc – form not stated
Test species	*Daphnia magna* (< 48 h old)
Taxonomic group	Crustacean
Exposure duration	21 days
Endpoint	NOEC and EC10
Effect parameter	Reproduction
Effect concentration (µg l^{-1})	25 and 100
Nominal/Measured	
Test media type	Culture and test medium: Lago Maggiore (Italy) water filtered though a 40 µm mesh. Background zinc concentration <6 µg l^{-1}.
Klimisch code	
Free text phrase	
Principles of method if other than guideline	Three different *Daphnia* populations were tested separately. No other details reported in RAR.
Details on results (CI, statistics, etc.):	EC10, calculated by the rapporteur. No statistics reported. Reproductive parameter: number of young. In additional 21-day 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^{-1}, the only test concentration used in these additional tests.

Authors	PAULAUSKIS, J.D. AND WINNER, R.W.
Year	1988
Title	Effects of water hardness and humic acid on zinc toxicity to *Daphnia magna* Straus.
Bibliographic Source	Aquatic Toxicology, 12, 273-290.
Test material	Zinc sulphate heptahydrate ($ZnSO_4 \cdot 7H_2O$)
Test species	*Daphnia magna* Straus
Taxonomic group	Crustacean
Exposure duration	7 weeks
Endpoint	NOEC and EC10
Effect parameter	Reproduction
Effect concentration (µg l^{-1})	31 - 208
Nominal/Measured	Nominal. Nominal Zn concentrations in soft water: 0, 25, 50, 75, 100 and 125 µg l^{-1}. Nominal Zn concentrations in soft water plus DOC (0.75 mg l^{-1}): 0, (50), 75, 100, 125 and 150 µg l^{-1}. Nominal Zn concentrations in soft water plus DOC (1.5 mg l^{-1}): 0, (25), (50), (75), 100, 125, 150 and 175 µg l^{-1} (nominal). Nominal Zn concentrations in medium hardness water: 0, 75, 100, 125, 150, (175) µg l^{-1}. Nominal Zn concentrations in hard water: 0, 125, 150, 175, 200, (225), (250) µg l^{-1}. Nominal Zn concentrations in hard water plus DOC (1.5 mg l^{-1}): 0, (150), 175, 200, 225, 250, (275) µg l^{-1}.
Test media type	Soft test water (hardness 52 mg l^{-1}) 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^{-1}) and hard test waters (hardness 197 mg l^{-1}) 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^{-1}. In the tests of the DOC series, DOC was added as artificial humic acid (sodium salt).
Klimisch code	
Free text phrase	
Principles of method if other than guideline	No details reported in the RAR.
Details on results (CI, statistics, etc.):	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). Survival usually was less sensitive than reproduction (only in hard water survival was equally sensitive than reproduction or slightly more sensitive).

Authors	SIBLEY, P.K., ANKLEY, G.T., COTTER, A.M. AND LEONARD, E.N.
Year	1996
Title	Predicting chronic toxicity of sediments spiked with zinc: an evaluation of the Acid-Volatile Sulfide model using a life-cycle test with the midge *Chironomus tentans*.
Bibliographic Source	Environmental Toxicology and Chemistry, 15, 2102-2112.
Test material	Zinc chloride ($ZnCl_2$)
Test species	*Chironomus tentans*
Taxonomic group	Insect
Exposure duration	8 weeks
Endpoint	NOEC
Effect parameter	Survival/growth/emergence/reproduction
Effect concentration (µg l^{-1})	137 (actual [166] – background)
Nominal/Measured	Measured
Test media type	Sediment: Unpolluted West Bearskin Lake sediment (Minnesota). Characteristics: AVS concentration 3.9 mmol S kg^{-1} dry weight and SEM concentration 1.0 mmol kg^{-1} dry weight, of which 70% zinc (0.7 mmol kg^{-1} dry weight, corresponding to 45 mg Zn kg^{-1} 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^{-1} and background zinc concentration 0.8 µg l^{-1}. 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.
Klimisch code	
Free text phrase	
Principles of method if other than guideline	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). 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). 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 and 41 mmol kg^{-1} 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 and 2,700 mg ”SEM”-Zn kg^{-1} 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 and 6.3 mmol kg^{-1} 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 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 conjunction 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 <u>dissolved</u>-Zn concentrations in the <u>pore</u> water: 29 (sediment-water control), 31, 56, 166, 4,200 and 10,000 µg l^{-1} (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^{-1}, 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. <u>Other analyses during the test</u>: 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^{-1} (but generally

	remained above 2.0 mg l^{-1}) in the treatments with SEM-Zn concentrations up to 850 mg kg^{-1} d.w. and as low as 0.5 mg l^{-1} in some replicates of the highest two treatments (SEM-Zn concentrations 1,900 and 2,700 mg kg^{-1} d.w.). Following initiation of emergence, DO levels increased to 3-4 mg l^{-1}, 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 food (because little or no larvae survived at these concentrations) rather than to the test system used. 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 ~ 40 mg l^{-1} (as $CaCO_3$).
Details on results (CI, statistics, etc.):	Statistics: p = 0.05. Toxicity results: No significant effects on any of the endpoints were found up to the actual “SEM”-zinc concentration of 13 mmol kg^{-1} d.w. (850 mg “SEM”-Zn kg^{-1} 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”-Zn kg^{-1} d.w. (1,900 mg “SEM”-Zn kg^{-1} 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 below 1.5 mg l^{-1} and only 11 (3%) were below 1.0 mg l^{-1}. Values below 1.5 mg l^{-1} and 1.0 mg l^{-1} 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^{-1} 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^{-1}, then dropping to levels that were generally between 1.0 and 3.0 mg l^{-1}. 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^{-1} 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 because of 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^{-1} d.w.; actual concentration) are considered to be valid.

Authors	SINLEY, J.R., GOETTL, J.P. AND DAVIES, P.H.
Year	1974
Title	The effect of zinc on rainbow trout (*Salmo gairdneri*) in hard and soft water.
Bibliographic Source	Bulletin of Environmental Contamination and Toxicology, 12, 193-201.
Test material	Zinc sulphate ($ZnSO_4$)
Test species	*Oncorhynchus mykiss* (eyed eggs and fish (unexposed as eggs))
Taxonomic group	Fish
Exposure duration	Life cycle (± 2-yr ?) and 25-days
Endpoint	NOEC
Effect parameter	Survival
Effect concentration (µg l^{-1})	130 and 25
Nominal/Measured	Measured. (actual – background)
Test media type	Dechlorinated tap water; background zinc concentration 11 µg l^{-1}. No data on nominal concentrations reported.
Klimisch code	
Free text phrase	
Principles of method if other than guideline	No details reported in RAR.
Details on results (CI, statistics, etc.):	Life- cycle test: 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^{-1} versus 1.4% - 2.6% (latter value: control value) at actual concentrations up to 140 µg l^{-1}. Eggs and yolk-sac fry were less sensitive. The $NOEC_{survival}$ (140 µg l^{-1}) is in accordance with the view of Sinley et al., 74. 25-day test: mortality 8% at 71 µg l^{-1} versus 1% at 36 µg l^{-1} and 0% in the control group, respectively. Although survival was reduced less than 10% at 71 µg l^{-1}, this concentration is considered to be the LOEC and 36 µg l^{-1} the NOEC, in accordance with the view of Sinley et al. ’74.

Authors	SPEHAR, R.L. 1976.
Year	1976
Title	Cadmium and zinc toxicity to flagfish, *Jordanella floridae*.
Bibliographic Source	Journal of the Fisheries Research Board of Canada, 33, 1939-1945.
Test material	Zinc sulphate heptahydrate ($ZnSO_4 \cdot 7H_2O$)
Test species	*Jordanella floridae*
Taxonomic group	Fish
Exposure duration	14 weeks
Endpoint	NOEC
Effect parameter	Growth
Effect concentration (µg l^{-1})	Test 1: 26 and test 2: 75
Nominal/Measured	Measured. Test 1: <1 (control), 26, 51, 85, 139 and 267 µg l^{-1} (nominal concentrations not reported). Test 2: 10 (control), 28, 47, 75, 139 and 267 µg l^{-1} (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 and that the larvae were from eggs exposed to the elevated concentration of 10 µg l^{-1}; the reported data are not clear in this respect.
Test media type	Untreated Lake Superior water; background zinc concentration < 1 µg l^{-1}.
Klimisch code	
Free text phrase	To control fungus, all embryos were treated with metal-free malachite green (4 mg l^{-1}) for 10 min during the first 3 days of incubation. The malachite green concentration is just below 5 mg l^{-1}, the concentration that has been reported to increase the zinc permeability 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.
Principles of method if other than guideline	No details reported in RAR.
Details on results (CI, statistics, etc.):	Statistics: p = 0.05. Test 1: at 51 µg l^{-1}, growth of female fish was significantly reduced. Reproductive parameters (mean spawnings per female and embryo production appeared to be reduced at 85 µg l^{-1}, although not statistically significant (because of the high variation among all groups). Test 2: at 139 µg l^{-1}, growth of male fish was significantly reduced. Reproductive parameters (mean spawnings per female and embryo production appeared to be reduced at 139 µg l^{-1}, although not statistically significant (because of the high variation among all groups).

Authors	VAN GINNEKEN, I. 1994a.
Year	1994
Title	The Effect of Zinc Oxide on the Growth of the Unicellular alga *Selenastrum capricornutum*.
Bibliographic Source	Report No. AASc/0022 (year of test: 1993/1994), Janssen Pharmaceutica N.V., Beerse, Belgium (Sponsor: International Lead and Zinc Research Association Inc. (ILZRO), North Carolina, U.S.A.)
Test material	Zinc oxide (ZnO) EPM-grade
Test species	*Pseudokirchneriella subcapitata*
Taxonomic group	Algae
Exposure duration	72 hours
Endpoint	NOEC
Effect parameter	Growth
Effect concentration (µg l^{-1})	24
Nominal/Measured	Measured
Test media type	Culture medium: No data. Test medium according to OECD-guideline No. 201 (nominal background zinc concentration: 1.4 µg l^{-1}; hardness 24 mg l^{-1} (as $CaCO_3$)), but EDTA was omitted. Test medium sterile-filtered (0.45 µm filter) before use in test.
Klimisch code	
Free text phrase	
Principles of method if other than guideline	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. In the test, a control, a filtrate (0.45 µm filter) of a 100 mg ZnO l^{-1} 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^{-1}, equivalent to 0.1 mg ZnO l^{-1}), 22% inhibition of the specific growth rate was observed. Reported measured dissolved-zinc concentrations in test water: based on analyses zinc in 0.45 µm filtered test waters. Actual dissolved background zinc concentration in test medium after 72 hours: 0.024 mg Zn l^{-1} (equivalent to 0.03 mg ZnO l^{-1}). It is noted that after 72 hours, the 0.95% and 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^{-1} (0.02 to 0.03 mg ZnO l^{-1}). Actual dissolved concentrations: based on measurements of dissolved zinc (0.45 µm filter); the values listed in Table 2.7 are based on measurements after 72 hours. Dissolution procedure for preparing the stock solution (100 mg ZnO l^{-1} dispersion): no data. 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, B. 1997: Letter, dated 13 February 1997, with an overview of ecotoxicity data of zinc oxide submitted by the lead company, Grillo zincoxid GMBH, Goslar). 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^{-1}): The data for Red Seal-grade ZnO show that nominal concentrations of 1 to 500 mg ZnO l^{-1} "modified algal medium" resulted in dissolved (0.2 µm filter) zinc concentrations of 0.3 to 0.4 mg Zn l^{-1} 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^{-1} "modified algal medium" resulted in dissolved zinc concentrations of 0.4 to 0.9 mg Zn l^{-1} in 4 days and dissolved zinc concentrations of 0.7 to 1.8 mg Zn l^{-1} 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^{-1}) a slow but steady further increase after day 4.
Details on results (CI, statistics, etc.):	No statistics reported.

Authors	VAN DE VYVER, G.
Year	2001
Title	Chronic toxicity of zinc to freshwater sponges – Report Phase 3: determination of dose-response (April 2001).
Bibliographic Source	Laboratoire de Physiologie Cellulaire et Génétique de levures, Université Libre de Bruxelles, Belgium.
Test material	Zinc chloride ($ZnCl_2$)
Test species	(1) *Ephydatia fluviatilis,* (2) *Ephydatia muelleri,* (3) *Spongilla lacustris,* and (4) *Eunapius fragilis.*
Taxonomic group	Porifera
Exposure duration	7 days
Endpoint	NOEC
Effect parameter	Development
Effect concentration (µg l^{-1})	(1), (2) and (4) – 43 (3) - 65
Nominal/Measured	Nominal test concentrations in first set of tests: 0, 3.3 x 10^{-7}, 6.6 x 10^{-7}, 10^{-6}, 3.3 x 10^{-6}, 6.6 x 10^{-6}, 10^{-5}, 10^{-4} Mol l^{-1}, corresponding to 0, 6.5, 21, 43, 65, 215, 430, 650 and 6,500 µg Zn l^{-1} (range-finding, based on results from the study by Richelle *et al*. '95. Arch Hydrobiol. 135, 209-231). Background zinc concentration in Elendt M4 control medium: 6.5 µg l^{-1} (added as $ZnCl_2$: 13 µg l^{-1}). Nominal test concentrations in second set of tests: 0, 3.3 x 10^{-7}, 6.6 x 10^{-7}, 1 x 10^{-6}, 3.3 x 10^{-6}, 5 x 10^{-6}, 6.6 x 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} Mol l^{-1}, corresponding to 0, 6.5, 21, 43, 65, 215, 430, 650 and 6,500 µg Zn l^{-1}.
Test media type	<u>All tests were performed in two different artificial media</u>: 1. 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^{-1} (added as $ZnCl_2$: 13 µg l^{-1}). The pH is not given in the test report or in OECD 211, but based on that of similar Elendt M7 medium. No data on acclimation of the sponges to Elendt M4 medium prior to testing. 2. In "M" medium as also used by Richelle et al. '95; this medium is used as culture medium in the laboratory that performed the tests. The background zinc concentration in this medium is <1 µg l^{-1} (no zinc added to the artificial medium prepared from distilled water; therefore 6.5 µg Zn/l was added to "M" medium to give the same control background concentration as in Elendt M4 medium. Note: Elendt M4 contains EDTA (6.8 µMol l^{-1}) and is because of the presence of this chelating agent <u>not</u> recommended in OECD 211 for toxicity testing of metals.

	However, the EDTA concentration is below the maximum value (10 µMol l^{-1}) used in the RAR as selection criterion for algal studies and moreover, the results of the sponges tests in Elendt M4 (with EDTA) and "M" medium (without EDTA) are identical or very similar, with for two of the sponge species the lowest NOEC in Elendt M4. Thus, for sponges, the EDTA concentration in Elendt M4 does not affect the zinc toxicity.
Klimisch code	
Free text phrase	
Principles of method if other than guideline	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 7 days. All experiments were carried out in triplicate with the same strains which were used for sponge cultures. A (zinc) concentration was considered by the study author 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 toxix (++++) when there was no aggregation at all and the cells died within 24 h. One additional toxicity category, 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 +). Thus the NOEC was set at the highest concentration of category – (non toxic).
Details on results (CI, statistics, etc.):	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. The NOEC values given in Table 2.7 are based on the tests performed in Elendt M4 medium. The tests in “M” medium were rejected because this medium does not meet the relevance criteria: the hardness value (300 mg l^{-1}) is higher than the maximum value used as criterion for hardness (250 mg l^{-1}). It is noted. however, that the results of the tests in Elendt M4 medium and “M” medium were identical or very similar in this study: the tests in “M” medium resulted in NOEC values of 65 µg l^{-1} for *E.fluviatilis, E. muelleri and S. lacustris* and 43 µg l^{-1} for *E. fragilis*. 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.

Authors	VAN WOENSEL, M.
Year	1994
Title	The Effect of Zinc Powder on the Growth of the Unicellular Green Alga *Selenastrum capricornutum,*
Bibliographic Source	Report No. AASc/0021, Jansssen Pharmaceutica N.V., Beerse, Belgium (Sponsor: International Lead and Zinc Research Organization Inc. (ILZRO), North Carolina, U.S.A.
Test material	Zn powder
Test species	*Pseudokirchneriella subcapitata*
Taxonomic group	Algae
Exposure duration	72 hours
Endpoint	NOEC
Effect parameter	Growth
Effect concentration (µg l^{-1})	50
Nominal/Measured	Measured.
Test media type	Culture medium: Bold's Basal Medium**.** Test medium according to OECD-guideline No. 201 (nominal background zinc concentration: 1.4 µg l^{-1}; hardness 24 mg l^{-1} (as $CaCO_3$)), but EDTA was omitted. The actual background concentration of zinc in the test medium was $\leq$ 10 µg l^{-1}.
Klimisch code	
Free text phrase	
Principles of method if other than guideline	Test conducted according to OECD-guideline 201 and under GLP. Test compound: zinc powder (median diameter 13.4 µm; 0.5% residue on 45 µm filter).
Details on results (CI, statistics, etc.):	No statistics reported. $NOEC_{growth}$ based on the 72-h average specific growth rate (µ). Growth parameter: cell number (specific growth rate and biomass). In the test, a control, a filtrate of a 100 mg Zn l^{-1} 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^{-1} 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 $\leq$ 10, 50, 50, 90, 230 and 760 µg l^{-1}, espectively, 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^{-1}. The nominal 72-h NOEC for both growth rate and biomass was 3.05% of the filtrate (actual dissolved-Zn concentration: 50 µg l^{-1}); at the next higher concentration (9.76% of the filtrate; actual dissolved-Zn concentration 90 µg l^{-1}), growth rate and biomass were reduced 27% and 69%, respectively. Note that according to the data reported in Coleman *et al*. (1971, Botanical Gazette. 132, 102-109), Bold's Basal

	Medium contains a background zinc concentration of 1,880 µg l^{-1}, 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^{-1} and the test is accepted for PNEC derivation.

Authors	WHITTON, B.A.
Year	1967
Title	Studies on the growth of riverain Cladophora in culture.
Bibliographic Source	Archives of Microbiology, 58, 21-29.
Test material	Zinc – form not stated
Test species	*Cladophora glomerata* (1 cm fragments)
Taxonomic group	Algae
Exposure duration	72 hours
Endpoint	NOEC
Effect parameter	Growth
Effect concentration (µg l^{-1})	60
Nominal/Measured	nominal
Test media type	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^{-1}, as $CaCO_3$) was calculated from the data on the modified No. 10 medium of Chu reported in Hargraves and Whitton (1976, British Phycological Journal. 11, 215-223); the total hardness of the test medium will be somewhat higher than 35 mg l^{-1} because of the addition of 10% river water.
Klimisch code	
Free text phrase	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.
Principles of method if other than guideline	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**.**
Details on results (CI, statistics, etc.):	No statistics reported. Growth parameter: weight. The results for zinc in this EDTA-free medium were reported as “no obvious inhibition at 60 µg l^{-1}, “obvious inhibition” at 80 µg l^{-1} and “killed” at 100 µg l^{-1}. The results for zinc in the same medium containing 3.2 mg l^{-1} Na.EDTA (10 x 10^{-3} mMol l^{-1}, equal to the upper limit of EDTA in test medium used in the RAR as selection criterion) were reported as “no

	obvious inhibition at 300 µg l^{-1}, “obvious inhibition” at 400 µg l^{-1} and “killed” at 500 µg l^{-1}).

Appendix II: Additional freshwater Zn ecotoxicity data

Authors	AZUARA-GARCÍA, R., SARMA, S.S.S. AND NANDINI, S.
Year	2006
Title	The combined effects of zinc and alga on the life table demography of *Anuraeopsis fissa* and *Brachionus rubens* (Rotifera).
Bibliographic Source	Journal of Environmental Science and Health Part A. 41, 559-572.
Test material	Zinc chloride ($ZnCl_2$) analytical grade (Sigma Chemicals, USA)
Test species	(1) *Anuraeopsis fissa* Gosse (2) *Brachionus rubens* Ehrenberg
Taxonomic group	Rotifera
Exposure duration	≤ 25 days (experiments were terminated when each individual from every cohort had died)
Endpoint	Rate of population increase
Effect parameter	NOEC
Effect concentration (µg l^{-1})	48 (*A. fissa*) 24 (*B. rubens*)
Nominal/Measured	Measured - ~ 35% decrease in Zn concentration after 24 h (renewal period). Geomean used to calculate exposure concentration.
Test media type	EPA medium (96 mg $NaHCO_3$, 60 mg $CaSO_4$, 60 mg $MgSO_4$ and 4 mg KCl l^{-1} distilled water)
Klimisch code	2
Free text phrase	
Principles of method if other than guideline	Test organisms: isolated from university pond at Benamerita Autonomous University of Puebla (Mexico). Clonal populations of the rotifer species were separately raised using *Chlorella vulagaris* as food and moderately hard water (80 – 100 mg $CaCO_3$ l^{-1}) as the medium (EPA medium. EPA/600/4-85/013. 1985). Environmental conditions in culture and tests: 23 ± 1°C; pH 7.1 – 7.3; continuous illumination. Test system: semi-static, renewal every 24 h. Test details: 4 replicates, 50 ml jars containing 20 ml test solution, 20 neonates (< 3 h following hatching). Tested at 2 food levels. Zinc chloride concentrations: control, 125, 250 and 500 µg l^{-1} (= 60, 120, 240 µg Zn l^{-1})
Details on results (CI, statistics, etc.):	NOEC values were not reported in the study but the raw data, as means and standard deviations, was presented in tabular form. *t*-tests were used to compare Zn treatments with controls. The NOECs were the same irrespective of food level. For *B. rubens* the lowest test concentration

	produced a significant effect, therefore the NOEC = LOEC/2. The data did not provide a good enough fit to derive LC10 values.

Authors	BRODEUR, J.C., ASOREY, C.M., SZTRUM, A. AND HERKOVITS, J.
Year	2009
Title	Acute and subchronic toxicity of arsenite and zinc to tadpoles of *Rhinella arenarum* both alone and in combination.
Bibliographic Source	Journal of Toxicology and Environmental Health, Part A, 72, 884-890.
Test material	Zinc chloride ($ZnCl_2$) (Mallinckrodt, Phillipsburg, NJ)
Test species	*Rhinella arenarum*
Taxonomic group	Amphibian
Exposure duration	21 days
Endpoint	Survival
Effect parameter	LC10
Effect concentration (µg l^{-1})	840 µg l^{-1}
Nominal/Measured	Nominal
Test media type	Artificial medium – AMPITOX solution (ASL) – 36 µg l^{-1} NaCl + 0.5 µg l^{-1} KCl, 1 µg l^{-1} $CaCl_2$+ 0.2 µg l^{-1} $NaHCO_3$ prepared using distilled water (additional information from authors).
Klimisch code	2
Free text phrase	No measured concentrations, background Zn < 1 µg l^{-1} no pH.
Principles of method if other than guideline	Test organisms: Adult *R. arenarum*, weighing 200 – 250 g, captured in the wild in Lobos county fields, Buenos Aires Province, Argentina. Ovulation of female toad induced by intrperitoneal injection of homologous hypophysis suspended in 1 ml ASL. Oocytes were fertilized in vitro using fresh sperm suspended in ASL. The resulting embryos were maintained in ASL at 20 ± 2°C until reaching Gosner stage 25. Tadpoles fed boiled Swiss chard *ad libitum* at stage 24-25. Environmental conditions in culture and tests: 20 ± 2°C; hardness ~ 90 mg $CaCO_3$ l^{-1}. Test system: static renewal every 48 h Test details: 3 replicates, 10 tadpoles per replicate. Exposure repeated twice with tadpoles from a unique but distinct pair of parents on each occasion. Zinc chloride concentrations: contol, 0.01, 0.1, 0.5, 1, 2, 5, 10 and 50 mg l^{-1} (nominal)
Details on results (CI, statistics, etc.):	LC10, LC50 and LC90 calculated by fitting a 4-parameter logistic regression equation to the survival data using the GraphPad Prism software version 3.02. 21-day LC10 840 µg l^{-1} (95% CI:840 – 850); LC50 1300 µg l^{-1} (95% CI: 1120 – 1510). 100% survival in controls (additional information from authors).

Authors	BRINKMAN, S. AND WOODLING J.
Year	2005
Title	Zinc toxicity to the mottled sculpin (*Cottus bairdi)* in high-hardness water.
Bibliographic Source	Environmental Toxicology and Chemistry, 24, 1515-1517.
Test material	Zinc sulphate heptahydrate ($ZnSO_4 \cdot 7H_2O$)
Test species	*Cottus bairdi*
Taxonomic group	Fish
Exposure duration	30 days
Endpoint	Survival
Effect parameter	NOEC
Effect concentration (µg l^{-1})	169
Nominal/Measured	Measured
Test media type	Dechlorinated Fort Collins municipal tap water and on-site well water mixed to create a nominal hardness of 150 mg $CaCO_3$ l^{-1}.
Klimisch code	1
Free text phrase	
Principles of method if other than guideline	Test organisms: recently emerged *C. bairdi* were collected from White River, ~ 5 km E of Meeker, Coloado, USA. Hardness and dissolved Zn at site were 240 mg $CaCO_3$ l^{-1} and < 10 µg l^{-1}. Environmental conditions in culture and tests: Fish mean length at start of test 27 mm and considered to be “young-of-the-year”. Fish held for 26 d in mixture of dechlorinated tap water and on-site well water of similar hardness value to collection site. After this period hardness decreased to 150 and fish acclimated for a further 18 d. 12:12 light/dark photoperiod. Test system: Flow-through Test details: 4 replicates, 7 fish per replicate Zinc chloride concentrations: control, 50, 100, 200, 400 and 800 µg l^{-1} (nominal). Mean measured: <5, 50, 94,172,379 and 778 µg l^{-1}.
Details on results (CI, statistics, etc.):	All fish exposed to highest test concentration died by day 9 and 85% mortality at 379 at day 13. No difference in length or weight were seen in fish surviving the 30-d exposure among the different exposure levels.

Authors	KÄLLQVIST, T., ROSSELAND, B.O., HYTTERØD, S. AND KRISTIANSEN, T.
Year	2003
Title	Effects of zinc on the early life stages of brown trout (*Salmo trutta*) at different levels of water hardness.
Bibliographic Source	Norwegian Institute for Water Research, Oslo, Norway. Report No. 4687 -2003.
Test material	Zinc sulphate heptahydrate ($ZnSO_4 \cdot 7H_2O$)
Test species	*Salmo trutta*
Taxonomic group	Fish
Exposure duration	~ 120 days
Endpoint	Hatching success
Effect parameter	NOEC
Effect concentration (µg l^{-1})	Species geomean = 112 [250 - 6(Cb) and 57 – 6 (Cb)]
Nominal/Measured	Measured (98 – 114% of nominal, background subtracted)
Test media type	Natural lake waters from Lake Maridalsvann (hardness 8.6 mg $CaCO_3$ l^{-1}) and Lake Store Sandungen (hardness 6.7 mg $CaCO_3$ l^{-1}). High-hardness waters were prepared by adding calcium to achieve a hardness level of 100 mg $CaCO_3$ l^{-1}. Mean background Zn concentration 6 µg l^{-1} in both lakes.
Klimisch code	1
Free text phrase	
Principles of method if other than guideline	OECD 210: Fish, Early-life stage toxicity test (1992). Test organisms: Local hatchery, Setesdalen Settefisk, located at the outlet of Lake Byglkandsfjorden, provided the egg and milt from their brood stock. The brood stock represents a 1st generation from wild brown trout. Tests started with fertilized eggs. Environmental conditions in culture and tests: 3.5°C at start of test and gradually increased to 5.7°C after one month, thereafter temperature held at 5.7 – 6.3°C; pH 6.2 – 6.9; DO 70 – 95% saturation. Test system: flow-through Test details: 2 replicates. Zinc concentrations: control, 10, 25, 50, 100 and 250 µg l^{-1} (soft waters) and control, 50, 100, 250, 500 and 1000 µg l^{-1} (hard waters).
Details on results (CI, statistics, etc.):	“positive” stimulatory effects of zinc exposure, i.e. shorter time to hatching and increased length were ignored when determining the NOEC values. There are no obvious reasons for the differences in the results in the two lake waters. The differences in pH and TOC between the two lakes were minor and not expected to have influenced the toxicity of Zn. Test results from hardwaters used for PNEC derivation. Results for soft water exposure 55 and 51 µg l^{-1}, Maridalsvann and Sandungen, respectively.

Authors	MUYSSEN, B., BOSSUYT, B. AND JANSSEN, C.R. 2003.
Year	2003
Title	Ecotoxicity of zinc to algae and daphnids tested in natural soft surface waters (Final report).
Bibliographic Source	Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University, Belgium (Sponsor: International Lead and Zinc Research Organization, ILZRO, United States).
Test material	Zinc chloride ($ZnCl_2$)
Test species	*Pseudokirchneriella subcapitata* (Strain CCAP 278/4)
Taxonomic group	Algae
Exposure duration	72 hours
Endpoint	Growth rate
Effect parameter	NOEC
Effect concentration (µg l^{-1})	67 µg l^{-1} L. Maridalsvann 86 µg l^{-1} L. Sandungen
Nominal/Measured	
Test media type	Natural lake waters from Lake Maridalsvann (hardness 8.04 mg $CaCO_3$ l^{-1}) and Lake Store Sandungen (hardness 6.1 mg $CaCO_3$ l^{-1}). High-hardness waters were prepared by adding calcium to achieve a hardness level of 100 mg $CaCO_3$ l^{-1}. Mean background Zn concentration 10 µg l^{-1} in both lakes.
Klimisch code	1
Free text phrase	
Principles of method if other than guideline	OECD 201: Alga, growth inhibition test (1984). Test organisms: obtained from Culture Collection of Algae and Protozoa, CEH, Windermere, UK and gradually (10-weeks) acclimated from standard ISO medium (ISO 1987; OECD 1984) with a hardness of 24 mg l-1 (as $CaCO_3$) to ISO medium with an adjusted hardness of 5 mg l^{-1} and of 100 mg l^{-1}. EDTA replaced by Aldrich humic acid. Tests initiated with exponentially growing algae - 10^4 cells ml^{-1}. Environmental conditions in tests: 22 ± 1°C; pH 6.7 (L. Maridalsvann) and 6.4 (L. Sandungen); continuous illumination. Test system: static, manually shaken 3 x a day Test details: 3 replicates (6 for control). Zinc concentrations: control + 6 concentrations (logarithmic series 32 – 560 µg l^{-1} (soft waters) and 100 - 1800 µg l^{-1} (hard waters).
Details on results (CI, statistics, etc.):	Test results from hard- and soft-waters used for PNEC derivation. Results for soft water exposure 28 and 65 µg l^{-1}, Maridalsvann and Sandungen, respectively.

Authors	MUYSSEN, B., BOSSUYT, B. AND JANSSEN, C.R. 2003.
Year	2003
Title	Ecotoxicity of zinc to algae and daphnids tested in natural soft surface waters (Final report).
Bibliographic Source	Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University, Belgium (Sponsor: International Lead and Zinc Research Organization, ILZRO, United States).
Test material	Zinc chloride ($ZnCl_2$)
Test species	*Daphnia longispina*
Taxonomic group	Crustacean
Exposure duration	21 days
Endpoint	Reproduction
Effect parameter	NOEC
Effect concentration (µg l^{-1})	82 µg l^{-1} L. Maridalsvann 199 µg l^{-1} L. Sandungen
Nominal/Measured	Measured
Test media type	Natural lake waters from Lake Maridalsvann (hardness 8.04 mg $CaCO_3$ l^{-1}) and Lake Store Sandungen (hardness 6.1 mg $CaCO_3$ l^{-1}). High-hardness waters were prepared by adding calcium to achieve a hardness level of 100 mg $CaCO_3$ l^{-1}. Mean background Zn concentration 10 µg l^{-1} in both lakes.
Klimisch code	1
Free text phrase	
Principles of method if other than guideline	OECD 211: *Daphnia magna* reproduction test (1998). Test organisms: originally collected in oligotrophic pond in Oberkirchen, Germany and cultured in its natural water at hardness of 20 – 40 mg $CaCO_3$ l^{-1}. Acclimation to experimental hardness levels took place over 7 weeks and spanned 2 generations. Age at start of test < 24 h old Environmental conditions in tests: 20°C; 16:8 light/dark photoperiod; fed 3:1 mix of *P. subcapitata* and *C. reinhardtii*. Test system: static renewal, 3 x a week Test details: 10 replicates, 1 organism per replicate. Zinc concentrations: control + 5 concentrations (logarithmic series 10 - 220 µg l^{-1} (soft waters) and 22 - 440 µg l^{-1} (hard waters).
Details on results (CI, statistics, etc.):	Test results from hard- and soft-waters used for PNEC derivation. Results for soft water exposure 37 and 41 µg l^{-1}, Maridalsvann and Sandungen, respectively.

Authors	WILDE, K.L., STAUBER, J.L., MARKICH, S.J., FRANKLIN, M. AND BROWN, P.L.
Year	2006
Title	The effect of pH on the uptake and toxicity of copper and zinc in a tropical freshwater alga (*Chlorella* sp.)
Bibliographic Source	Archives of Environmental contamination and Toxicology, 51, 174-185.
Test material	Zinc sulphate heptahydrate ($ZnSO_4 \cdot 7H_2O$)
Test species	*Chlorella* sp. (isolate 12)
Taxonomic group	Algae
Exposure duration	48 hours
Endpoint	Growth rate
Effect parameter	EC10 (minimum detectable effect concentration)
Effect concentration (µg l^{-1})	48 (species geomean based on measured concentrations less background, excluding result at pH 5.5 which is below the relevancy criteria)
Nominal/Measured	Measured
Test media type	Synthetic freshwater (Zn background 0.8 ± 0.1 µg l^{-1})
Klimisch code	1
Free text phrase	
Principles of method if other than guideline	Test organisms: isolated from Lake Aesake, Strickland River, Papua New Guinea. Alga maintained in JM/5 media (Thompson et al. 1988. Culture collection of algae and protozoa: catalogue of strains. NERC, UK) at 27°C; 12:12 light/dark photoperiod. Cells in exponential growth phase (5-7 days) were used for all experiments, initial cell density 2-4 x 10^3 cells ml^{-1}. Environmental conditions in culture and tests: 27°C; pH adjusted using dilute HCL or NaOH to give six test pH levels (5.5, 6.0, 6.5, 7.0, 7.5 and 8.0); hardness 43 mg $CaCO_3$ l^{-1}; 12:12 light/dark photoperiod. Test system: static, manual shaking twice daily by hand. Test details: 3 replicates. Zinc concentrations: control, and minimum of 5 Zn concentrations.
Details on results (CI, statistics, etc.):	The minimum detectable effect concentration (MDEC), similar to the EC10, an alternative measure to the LOEC was estimated using the approach described by Ahsanullah and Williams (1991). The MDEC was calculated from a regression model and is defined as the metal concentration at which the growth rate became significantly ($p \leq 0.05$) lower than the control treatments.

Appendix III: Saltwater Zn ecotoxicity data

Authors	AHSANULLAH M AND WILLIAMS AR
Year	1991
Title	Sublethal effects and bioaccumulation of cadmium, chromium, copper, and zinc in the marine amphipod *Allorchestes compressa*
Bibliographic Source	Mar Biol 108:59-65
Test material	$ZnSO_4$
Test species	*Allorchestes compressa* Dana
Taxonomic group	Crustacea
Exposure duration	28 d
Endpoint	LC10
Effect parameter	Survival
Effect concentration ($\mu g\ l^{-1}$)	61.5 (63 less background)
Nominal/Measured	M
Test media type	SW
Klimisch code	2
Free text phrase	Comparable to guideline study with acceptable restrictions
Principles of method if other than guideline	Origin of test organism: lab-culture Lifestage: first instar juveniles Test system: flow-through Test details: 50 juveniles and 50 g of seagrass / 8l-tank - Surviving amphipods were counted and weighed. - Metal concentrations and water parameters were monitored. Dose-response: observed Control mortality: < 20% Zinc concentrations: semi-duplicates, semi-repeated experiments with different zinc levels: Control (1.5), 30, 30, 103; Control (1.5), 70, 135, 264 T=19°C; pH=8.0; Salinity= 31 $\%_o$
Details on results (CI, statistics, etc.):	- MECs were estimated by interpolation from regression model - MECs: survival < biomass < weight y=95.6 (1-1/(1+EXP(2.88-0.0185x) if x =0, then y= 90.52 if x=63, then y = 81.01 (90 % of control response = LC10)

Authors	ANDERSON BS AND HUNT JW
Year	1988
Title	Bioassay Methods for Evaluating the Toxicity of Heavy Metals, Biocides and Sewage Effluent Using Microscopic Stages of Giant Kelp *Macrocystis pyrifera* (Agardh): A Preliminary Report.
Bibliographic Source	Mar Environ Res 26(2):113-134
Test material	$ZnSO_4$
Test species	*Macrocystis pyrifera*
Taxonomic group	Algae
Exposure duration	48 h
Endpoint	NOEC
Effect parameter	Germination tube growth
Effect concentration (µg l^{-1})	190.2
Nominal/Measured	M
Test media type	UV treated FSW
Klimisch code	1
Free text phrase	Comparable to guideline study
Principles of method if other than guideline	Origin of test organism: Monterey, California, zoospore released in lab Lifestage: zoospore Test system: static Test details: 450000 spores/250mL Dose-response: yes Control data: Germination: 80% Zinc concentrations: 6 treatments : control-10000; toxicant dilutions in logarithmic order; 5 replicates, 3 tests T=11.5-17°C; pH=7.75-7.95 / 8.4-8.6; Salinity=33-37 / 34-36‰
Details on results (CI, statistics, etc.):	Arcsine-transformed data; Anova/Dunett's The germination of zoospores was less sensitive than growth of germination tubes. Reported NOEC values for germination are 2030, 5500, 1730 ug/L Zn. The reported LOEC values for germination tube growth were 589, 553, and 1090 ug/L, and the toxic responses at LOECs were <30, <30, and >30% compared to the control response, respectively. These LOECs are also the lowest tested concentrations, respectively. Therefore, the NOEC was derived as the geomean of (589/3,553/3), and the LOEC of 1090ug/L with a toxic response > 30% compared with the control response was excluded from the NOEc calculation.

Authors	BEIRAS R AND ALBENTOSA M
Year	2004
Title	Inhibition of embryo development of the commercial bivalves *Ruditapes decussatus* and *Mytilus galloprovincialis* by trace metals; implications for the implementation of seawater quality criteria
Bibliographic Source	Aquaculture 230: 205-213
Test material	$ZnCl_2$
Test species	***1)*** *Mytilus galloprovincialis* ***2)*** *Ruditapes decussatus*
Taxonomic group	Mollusca
Exposure duration	48 h
Endpoint	Embryogenesis
Effect parameter	NOEC
Effect concentration ($\mu g\ l^{-1}$)	1) 80.0 [*M. galloprovincialis*] 2) 55.0 [*R. decussatus*]
Nominal/Measured	N
Test media type	1) FSW 2) ASW
Klimisch code	2
Free text phrase	Comparable to guideline study with acceptable restrictions
Principles of method if other than guideline	Origin of test organism: Adults collected by local fishermen in Galicia. Spawning induced in lab. Lifestage: fertilized eggs Test system: static Test details: After fertilization and before first cleavage, fertilized eggs were exposed (20 eggs ml^{-1} in 20 ml-vials). Endpoint = percentage of normal D-larvae in samples of a minimum of 100 individuals per vial. Normal larva: shell was D-shaped (straight hinge) and mantle did not protrude out of the shell. Dose-response: observed Control mortality: NR, but control included Zinc concentrations: - 5 exposure concentrations (0-160), 5 replicates [*M. galloprovinincialis*] T=20°C; pH=NR; Salinity=NR [*M. galloprovinincialis*] Zinc concentrations: - 4 exposure concentrations (0-250), 5 replicates [*R. decussatus*] T=20°C; pH=8; Salinity=34‰ [*R. decussatus*]
Details on results (CI, statistics, etc.):	***M. galloprovinincialis*** - EC10 value could not be calculated, LOEC was calculated by using ANOVA and a posteriori Student–Newman–Keuls test. Homoscedasticity was checked by the Levene test. NOEC= highest EC<LOEC, NOEC estimated from graph≈EC10 ***R. decussatus*** - EC10 and 95% confidence intervals (3.9-80.4) were calculated by probit method using SPSS statistical software.

	In order to pool together different experiments, percentages of normal D-larvae in each vial (P) were previously corrected by the control response: P'= Px100/Pc, where P' is the corrected percentage of normal D-larvae, and Pc is the average percentage of normal D-larvae in the control.

Authors	CESAR A, MARÍN-GUIRAO L, VITA R AND MARÍN A
Year	2002
Title	Sensitivity of Mediterranean amphipods and sea urchins to reference toxicants
Bibliographic Source	*Ciencias Marinas* 28(4): 407–417
Test guideline (according / similar to; deviations: yes/no)	similar to EPA/600/ R-95-136, Environment Canada EPS 1/RM/27 and CETESB L5.250 with slight adaptations
Test material	$ZnSO_4$
Test species	1) *Arbacia lixula* 2) *Paracentrotus lividus* 3) *Sphaerechinus granularis*
Taxonomic group	Echinodermata
Exposure duration	1) 38 h 2) 28 h 3) 28h
Endpoint	NOEC
Effect parameter	Embryogenesis
Effect concentration ($\mu g\ l^{-1}$)	10
Nominal/Measured	N
Test media type	FSW from clean site in Spain
Klimisch code	2
Free text phrase	Comparable to guideline study with acceptable restrictions
Principles of method if other than guideline	Origin of test organism: Adults collected at clean site in Spain. Spawning induced in lab. Lifestage: fertilized eggs Test system: static Test details: -10 ml solution with 400 embryos in 15 ml vials - First 100 embryos were examined for normal development. - At the beginning and at the end of every test, temperature, salinity, dissolved oxygen and pH were measured, parameter were constant. Dose-response: IC25 and IC 50s were calculated Control data:> 90% larvae reached normal pluteus larvae stage Zinc concentrations: - 6 concentrations, 10 SW controls - 3 replicates/ treatment T=20°C; pH=NR but measured; Salinity= 38‰
Details on results (CI, statistics, etc.):	NOEC=IC25/3 IC25 = 30±1; IC50 = 50±2 [*A. lixula*]; 50±1 [*P. lividus*]; 60±1 [*S. granularis*] IC25 calculated using the Linear Interpolation Method (US EPA, 1993)

Authors	CONROY PT, HUNT JW AND ANDERSON BS
Year	1996
Title	Validation of a short-term toxicity test endpoint by comparison with longer-term effects on larval Red abalone *Haliotis rufescens*
Bibliographic Source	Environmental Toxicology and Chemistry 15 (7): 1245–1250
Test guideline (according / similar to; deviations: yes/no)	According to Anderson BS, Hunt JW, Turpen SL, Coulon AR, Martin M, McKeown DL & Palmer FH (1990) Procedures manual for conducting toxicity tests developed by the Marine Bioassay Project. 90-10WQ. State Water Resources Control Board, Sacramento, CA, USA.
Test material	$ZnSO_4$
Test species	*Haliotis rufescens*
Taxonomic group	Mollusca
Exposure duration	10 days
Endpoint	NOEC
Effect parameter	Development (Metamorphosis)
Effect concentration (µg l^{-1})	7.48
Nominal/Measured	Results based on nominal concentrations. Analytical verification reports concentrations within 17% of nominal
Test media type	Not reported – assume NSW
Klimisch code	2
Free text phrase	Comparable to guideline study
Principles of method if other than guideline	Origin of test organism: lab culture at Granite Canyon, CA Lifestage: veliger larvae Test system: flow-through Test details: a proportional diluter system introduced 125 ml of fresh toxicant solution into each test container approx. every 17 min; 200 larvae from each replicate were scored metamorphosed or non-metamorphosed. Dose-response: EC50 reported Control mortality: met acceptability criteria Zinc concentrations: control, 5.6, 10, 18, 32, 56, 100; 4 replicates T=15°C; Salinity=NR (water criteria met acceptability criteria)
Details on results (CI, statistics, etc.):	Normality: Shapiro-Wilk's test, homogeneity: *F* test; Arcsine transformation, ANOVA and Dunett's NOEC 10-d continuous exposure 10 µg l^{-1} NOEC 10-d exposure-recovery 5.6 µg l^{-1}GEOMEAN 7.48 µg l^{-1}

Authors	EKLUND B
Year	2005
Title	Development of a growth inhibition test with the marine and brackish water red alga *Ceramium tenuicorne*.
Bibliographic Source	Marine Pollution Bulletin 50: 921–930
Test guideline (according / similar to; deviations: yes/no)	According to: Eklund B. 2004. Growth inhibition test with the marine and brackish water macroalga *Ceramium tenuicorne. ITM-rapport 131.* Test will become an international standard within ISO.
Test material	Zn
Test species	*Ceramium tenuicorne*
Taxonomic group	Algae
Exposure duration	7d
Endpoint	EC10
Effect parameter	length
Effect concentration (µg l^{-1})	11.9
Nominal/Measured	N
Test media type	NSW, enriched with nutrients;
Klimisch code	2
Free text phrase	Comparable to guideline study with acceptable restrictions.
Principles of method if other than guideline	Origin of test organism: acclimated for > 10 years Lifestage:2.5 mm long algae Test system: static Test details: 2 plants / 10 ml Dose-response: EC50 reported Control growth: 35mm/11d Zinc concentrations: 6 replicates per treatment; repeated test 5x T=22°C; pH=; Salinity= 20‰
Details on results (CI, statistics, etc.):	Calculations performed using RegTox 6.3 EC10 = GEOMEAN(16,7.7,18,15,7.2)

Note : Britta Eklund confirmed to IZA absence of EDTA and background in test media

Authors	FISHER NS, JONES GJ AND NELSON DM
Year	1981
Title	Effects of Copper and Zinc on Growth, Morphology, and Metabolism of *Asterionella japonica* (Cleve)
Bibliographic Source	J Exper Marine Biol & Ecol 51/1: 37-56
Test material	ZnSO4
Test species	*Asterionella japonica*
Taxonomic group	Algae
Exposure duration	4 days
Endpoint	Growth
Effect parameter	EC10 (LI)
Effect concentration (µg l^{-1})	20.6
Nominal/Measured	Nominal
Test media type	NSW (Bass Strait, near Melbourne, AU), zinc background concentration is 1.5 µg l^{-1}
Klimisch code	2
Free text phrase	Acceptable, well-documented publication/study which meets basic scientific principles
Principles of method if other than guideline	Origin of test organism: clonal culture established from a cell isolated from Bass Strait (clean waters, near Melbourne, AU) 4 months prior to the experiment Lifestage: 3000 cells ml^{-1} Test system: static Test details: / Dose-response: yes Control data: graphed; Growth rate > 3: Day0:3000-Day4: 600000 cells ml^{-1}; Specific growth rate per day=ln(600000-3000)/4=3.32 Zinc concentrations: control, 20, 40, 60 µg Zn/L; 3 replicates T= 17°C; pH= NR; Salinity= 35‰
Details on results (CI, statistics, etc.):	NOEC with 9.5% inhibition =20 µg Zn l^{-1} IZA recalculated EC10 using linear interpolation (not verified) but is consistent with NOEC.

Authors	FISHER NS AND FROOD D
Year	1980
Title	Heavy Metals and Marine Diatoms: Influence of Dissolved Organic Compounds on Toxicity and Selection for Metal Tolerance Among Four Species
Bibliographic Source	Marine Biology 59, 85-93
Test material	ZnSO4
Test species	***1)*** *Asterionella japonica* ***2)*** *Chaetoceros compressum* ***3)*** *Nitschia closterium* ***4)*** *Skeletonema costatum*
Taxonomic group	Algae
Exposure duration	3 days
Endpoint	Growth
Effect parameter	EC10
Effect concentration (µg l^{-1})	1) 2.15 – 46.95 [*A. japonica*] 2) 7.13 – 56.51 [*C. compressum*] 3) 12.33 – 53.48 [*N. closterium*] 4) 1.43 – 70.24 [*S.costatum*]
Nominal/Measured	Nominal
Test media type	F/2 medium without added EDTA prepared from FSW (Bass Strait - zinc background concentration ~ 1.5 µg l^{-1} or Corio Bay – zinc background ~ 5.2 µg l^{-1} both near Melbourne, AU)
Klimisch code	2
Free text phrase	Acceptable, well-documented publication/study which meets basic scientific principles
Principles of method if other than guideline	Origin of test organism: for each species, at least 2 clones were aseptically cultured, from either Corio Bay or Hobsons's Bay and one from Bass Strait. Cultures established several months prior to experimentation and maintained in Bass Strait waters. Lifestage: 3 x 10^3 cell ml^{-1} [*A. japonica*]; 5 x 10^3 cell ml^{-1} [*C. compressum*]; 3 x 10^3 cell ml^{-1} [*N. closterium*]; 10^4 cell ml^{-1} [*S.costatum*] Test system: static Test details: / Dose-response: no Control data: Specific growth rate per day=variable Zinc concentrations: control, 20, 40, 60 µg Zn l^{-1}; 3 replicates T= 17°C; pH= NR; Salinity= 35‰
Details on results (CI, statistics, etc.):	EC10 recalculated using the Toxicity Relationship Analysis Program (TRAP) from the U.S. EPA National Health and Environmental Effects Research Laboratory (NHEERL).

Authors	GORSKI J AND NUGEGODA. D
Year	2006
Title	Sublethal toxicity of trace metals to larvae of the blacklip abalone, *Haliotis rubra*
Bibliographic Source	Environmental Toxicology and Chemistry 25: 1360-1367
Test guideline (according / similar to; deviations: yes/no)	Similar to: Hunt JW, Anderson BS. 1990. Abalone larval development: Short-term toxicity test protocol. In: Anderson BW, Hunt JW, Turpen SL, Coulon AR, Martin M, McKeown DL, Palmer FH, eds, *Procedures Manual for Conducting Toxicity Tests Developed by the Marine Bioassay Project.* 90-10WQ. California State Water Resources Control Board, Sacramento, CA, USA, pp 17–48.
Test material	$ZnCl_2$
Test species	*Haliotis rubra*
Taxonomic group	Mollusca
Exposure duration	48 h
Endpoint	EC10
Effect parameter	development
Effect concentration (µg l^{-1})	20.4
Nominal/Measured	N
Test media type	FSW
Klimisch code	2
Free text phrase	Comparable to guideline study with acceptable restrictions
Principles of method if other than guideline	Origin of test organism: Lara, Victoria, Australia Lifestage: fertilized egg, 1-h old Test system: static Test details: 20 eggs ml^{-1}; 400 ml jars Dose-response: yes Control mortality: < 13% Zinc concentrations: Control, 8, 16, 32, 64, 128; 4 replicates T=20°C; pH=7.3; Salinity= NR
Details on results (CI, statistics, etc.):	EC10-95% CI (18.5-21.9) calculated using Toxcalc statistical software (Tidepool Scientific, McKinleyville, CA, USA) NOECs calculated using Dunnett's test; NOEC=8

Authors	HAN T AND CHOI G-W
Year	2005
Title	A novel marine algal toxicity bioassay based on sporulation inhibition in the green macroalga *Ulva pertusa* (Chlorophyta)
Bibliographic Source	Aquatic Toxicology 75:202–212
Test material	$ZnNO_3$
Test species	*Ulva pertusa*
Taxonomic group	Algae
Exposure duration	5 d
Endpoint	NOEC
Effect parameter	Reproduction, sporulation
Effect concentration (µg l^{-1})	313
Nominal/Measured	N
Test media type	ASW (Coralife, Energy Savers, CA, USA)
Klimisch code	2
Free text phrase	Comparable to guideline study with acceptable restrictions
Principles of method if other than guideline	Origin of test organism: Ahmin, Korea Lifestage: disk from marginal thallus Test system: static Test details: Percentage sporulation assessed from coloured proportion of the surface area Dose-response: yes, EC50 reported Control data: >75% sporulation Zinc concentrations: control, 313-5000; 8 replicates T=15°C; pH=; Salinity= 35 $‰_o$
Details on results (CI, statistics, etc.):	Arcsine-transformed data, Anova/LSD at $p<0.05$ EC50 with 95%CI =738 (554-880); NOEC=EC<10

Authors	HARMON VL AND LANGDON CJ
Year	1996
Title	A 7-d toxicity test for marine pollutants using the pacific mysid *Mysidopsis intii.* 2. Protocol evaluation
Bibliographic Source	Environmental Toxicology and Chemistry, Vol. 15, No. 10, pp. 1824–1830
Test guideline (according / similar to; deviations: yes/no)	EPA/600/4-87/028
Test material	$ZnSO_4$
Test species	1) *Americamysis bahia* 2) *Mysidopsis intii*
Taxonomic group	Crustacea
Exposure duration	7 d
Endpoint	NOEC
Effect parameter	Survival
Effect concentration ($\mu g\ l^{-1}$)	101
Nominal/Measured	N
Test media type	FSW
Klimisch code	2
Free text phrase	Comparable to guideline study with acceptable restrictions.
Principles of method if other than guideline	Origin of test organism: laboratory culture Lifestage: 7-d Test system: static renewal Test details: 5 organisms/beaker, 150 ml test medium, 8 replicates [A. bahia]; 15/beaker, 1 l test medium, 3 replicates [M. intii] Dose-response: yes Control mortality: < 20% Zinc concentrations: control, 37, 50, 70, 101, 230 ; [*A. bahia*] -T=26-27°C; pH=NR; Salinity= 30±1‰ [*M. intii*] T=20±2°C; pH=NR; Salinity= 34±2‰
Details on results (CI, statistics, etc.):	MATC= Geomean(NOEC,LOEC)=Geomean (101,230); Survival<dry weight / reproduction Normality and homogeneity of variance tested with Shapiro–Wilks and Bartlett's tests, respectively. Steel's Many-One Rank test used for analysis of non-normal data distributions. If acceptable normality and homogeneity of variance, ANOVA performed with significance at 0.05 level. Dunnett's multiple-range or Bonferroni test used to determine the significance of differences among treatments at 0.05 level when equal or unequal numbers of replicates in treatments, respectively. NOEC = greatest concentration that mysids tolerated without showing statistically significant adverse survival, growth, or developmental effects compared with controls. MATC = geometric mean of the NOEC concentration and the lowest toxicant concentration tested that resulted in a significant (0.05 level) adverse effect.

Authors	HUNT JW, ANDERSON BS, TURPEN SL, ENGLUND MA AND PIEKARSKI W
Year	1997
Title	Precision and Sensitivity of a Seven-Day Growth and Survival Toxicity Test Using the West Coast Marine Mysid Crustacean *Holmesimysis costata*
Bibliographic Source	Environ.Toxicol.Chem. 16(4)(4):824-834
Test guideline (according / similar to; deviations: yes/no)	According to EPA/600/R-95-136
Test material	$ZnSO_4$
Test species	*Holmemysis costata*
Taxonomic group	Crustacea
Exposure duration	24 days
Endpoint	NOEC
Effect parameter	Survival
Effect concentration (µg l^{-1})	5.6
Nominal/Measured	N (chemically verified, 14% variation)
Test media type	NSW, background zinc not reported
Klimisch code	1
Free text phrase	Comparable to guideline study
Principles of method if other than guideline	Origin of test organism: Adults from Granite Canyon, CA Lifestage: 3- day old juveniles Test system: static renewal Test details: 5 organisms/200 ml Dose-response: yes (mortality according to zinc concentration) Control mortality: < 20 % mysid, mortality recorded daily Zinc concentrations: control, 5.6, 10, 18, 32, 56, 100 µg l^{-1}, 8 replicates T=12±2°C; pH=recorded but not presented; Salinity=33±2‰
Details on results (CI, statistics, etc.):	NOEC calculated for survival and growth data (Arcsine-squareroot transformed data) using ANOVA and Dunett's NOEC : growth>survival

Authors	JOHNSON HL, STAUBER JL, ADAMS MS AND JOLLEY DF
Year	2007
Title	Copper and Zinc Tolerance of Two Tropical Microalgae After Copper Acclimation.
Bibliographic Source	Environmental Toxicology 22 (3) 234 – 244.
Test material	$ZnCl_2$
Test species	*Nitzschia closterium*
Taxonomic group	Algae
Exposure duration	72 h
Endpoint	IC10
Effect parameter	Growth (cell division rate)
Effect concentration (µg l^{-1})	84
Nominal/Measured	M
Test media type	FSW; dissolved zinc <10ug/L
Klimisch code	2
Free text phrase	Comparable to guideline study
Principles of method if other than guideline	Origin of test organism: Coral Sea, Australia; long-term lab culture in G medium with 79ug l^{-1} Zn Lifestage: exp growing cells Test system: static Test details: initial cell density=20000-40000 cells ml^{-1} Dose-response: yes Control data: growth rate > 1 doubling/day Zinc concentrations: control, 5 concentrations (50-600), triplicates T=27°C; pH=8; Salinity=34‰
Details on results (CI, statistics, etc.):	IC10 calculated using Linear Interpolation (ToxCalc 5.0.23) IC10=84±64

Authors	KARBE L
Year	1972
Title	Marine Hydroiden als Test Organismen zur Priifung der Toxizit/it yon Abwasserstoffen. Die Wirkung von Schwermetallen auf Kolonien yon *Eirene viridula*
Bibliographic Source	Marine Biology 12, 316-328
Test material	$ZnSO_4$
Test species	*Eirene viridula*
Taxonomic group	Cnidaria
Exposure duration	3 mo
Endpoint	NOEC
Effect parameter	development
Effect concentration (µg l^{-1})	300
Nominal/Measured	N
Test media type	FSW; North sea
Klimisch code	2
Free text phrase	Acceptable, well-documented publication/study which meets basic scientific principles
Principles of method if other than guideline	Origin of test organism: lab culture, Lifestage: stolones with polyps Test system: static renewal Test details: 200 ml Dose-response: yes Control mortality: assumed to be none Zinc concentrations: 100, 300, 1000, 1500, 3000; duplicate tests T=20°C; pH=7.9-8.2; Salinity= 30‰
Details on results (CI, statistics, etc.):	Test statistics NR; based on visual assessment and measurements

Authors	KING CK AND RIDDLE MJ
Year	2001
Title	Effects of metal contaminants on the development of the common antarctic sea urchin *Sterechinus neumayeri* and comparisons of sensitivity with tropical and temperate echinoids
Bibliographic Source	Marine Ecology-Progress Series 215: 143-154
Test material	$ZnSO_4$
Test species	*Sterechinus neumayeri* Meissner
Taxonomic group	Echinodermata
Exposure duration	20-23d
Endpoint	NOEC
Effect parameter	Embryogenesis
Effect concentration ($\mu g\ l^{-1}$)	80
Nominal/Measured	N
Test media type	FSW from O'Brien Bay, BG<5ug/L
Klimisch code	2
Free text phrase	Comparable to guideline study with acceptable restrictions.
Principles of method if other than guideline	Origin of test organism: Adults collected from O'Brien Bay, Antarctica. Spawning induced in lab. Lifestage: fertilized eggs (3h-old) Test system: static Test details: 1ml of embryo solution were added to 7 ml of test solution (6-8 embryos ml^{-1}); at the end of test , mortality, abnormalities, and slower development relative to controls were test criteria Dose-response: EC 50 reported Control data: graphed, above 65% of normal development Zinc concentrations: 4 replicates/treatment (3 tests) T=0°C; pH=8-8.2; Salinity= $34\%_o$
Details on results (CI, statistics, etc.):	NOEC and LOEC calculated using Dunnett's multiple comparison test. 20–23 d 2-arm pluteus: EC50=326.8 (CV=24.6) ; LOEC=320, NOEC=160 6–8 d hatched blastula: EC50=2230 (CV=20.5); NOEC=800; LOEC=1200

Authors	LEE CH, RYU TK, CHANG M AND CHOI JW
Year	2004
Title	Effect of Silver, Cadmium, Chromium, Copper, and Zinc on the Fertilization of the Northern Pacific Asteroid, *Asterias amurensis*
Bibliographic Source	Bull. Environ. Contam. Toxicol. 73:613–619
Test guideline (according / similar to; deviations: yes/no)	similar to EPA/600/ R-95-136 with slight adaptations
Test material	$ZnCl_2$
Test species	*Asterias amurensis*
Taxonomic group	Echinodermata
Exposure duration	80 min
Endpoint	Sperm cell toxicity
Effect parameter	NOEC
Effect concentration (µg l^{-1})	50
Nominal/Measured	N
Test media type	FSW
Klimisch code	2
Free text phrase	Comparable to guideline study with acceptable restrictions.
Principles of method if other than guideline	Origin of test organism: Adults were collected in Korea. Spawning induced in lab. Lifestage: sperm, eggs Test system: static Test details: 1ml of test solution, approx. 10^6sperms in 50 µl FSW were exposed for 20 min, then 300 eggs in 100 µl FSW were added. After 60min, 100 eggs were examined for presence of fertilization membrane. Dose-response: EC 50s reported Control mortality: fertilization> 80% Zinc concentrations: - 5 or 7 concentrations plus control (50-1600) - Treatments in triplicates; Experiments were repeated three times with different pairs of males and females. T=15°C; Salinity = 32‰
Details on results (CI, statistics, etc.):	LOEC was calculated using Dunnett's Test LOEC= 250 NOEC < LOEC, actual NOEC might be higher, NOEC = lowest tested concentration EC50 (mean of 3 tests) = 550 ±140

Authors	LE DEAN, L. AND DEVINEAU, J.
Year	1985 (1987)
Title	In search of standardisation: A comparison of toxicity bioassays on two marine crustaceans (*Palaemon serratus* and *Tigriopus brevicornis*)
Bibliographic Source	Rev. Trav. Inst. Peches marit. 49 (3 et 4):187-198
Test material	ZnSO4
Test species	*Tigriopus brevicornis*
Taxonomic group	Crustacea
Exposure duration	10 d
Endpoint	NOEC
Effect parameter	Reproduction and larval development
Effect concentration ($\mu g\ l^{-1}$)	297
Nominal/Measured	N
Test media type	ASW
Klimisch code	2
Free text phrase	
Principles of method if other than guideline	Origin of test organism: North Atlantic, acclimated for 3 weeks Lifestage: ovigerous females Test system: static Test details: 1 female / 20 ml Dose-response: yes Control mortality: < 10 % Zinc concentrations: control, 270, 297, 324, 351, 378, 405; 30 replicates T=20°C; pH=7.8-8; Salinity =34-36‰
Details on results (CI, statistics, etc.):	NOEC=EC3; EC50=325 ±2 NOEC-larval development: 297

Authors	NOVELLI, A.A., LOSSO, C., GHETTI, P.F. AND VOLPI GHIRARDINI, A.
Year	2003
Title	Toxicity of heavy metals using sperm cell and embryo toxicity bioassays with *Paracentrotus lividus* (Echinodermata: Echinoidea): comparisons with exposure concentrations in the lagoon of Venice, Italy
Bibliographic Source	Environmental Toxicology and Chemistry, 22, 1295–1301,
Test guideline (according / similar to; deviations: yes/no)	Similar to EPA 600/ R-95/136 with deviations
Test material	$Zn(NO_3)_2$
Test species	*Paracentrotus lividus*
Taxonomic group	Echinodermata
Exposure duration	3 d
Endpoint	EC 10
Effect parameter	Embryogenesis
Effect concentration ($\mu g\ l^{-1}$)	23
Nominal/Measured	N
Test media type	ASW (Ocean Fish, Prodac International Padua, Italy)
Klimisch code	2
Free text phrase	Comparable to guideline study with acceptable restrictions
Principles of method if other than guideline	Origin of test organism: Adults collected in northern Adriatic Sea, near the barrier island of Lido (Italy); spawning induced in lab. Lifestage: fertilized eggs Test details: Test system: sperm:egg ratio 10:1; 1 ml of fertilized eggs added to 10 ml of test solution; 100 larvae examined for normal plutei Dose-response: EC 50 reported Control mortality: > 80% fertilization & normal plutei Zinc concentrations: 6 concentrations & control in triplicates T=18°C; pH=8; Salinity = 35‰
Details on results (CI, statistics, etc.):	EC: Embryogenesis < sperm cell toxicity Authors recalculated EC10 and CI: 23 (17-29) NOEC calculated with the EPA Probit Analysis program

Authors	PAVICIC, J., SKREBLIN, M., KREGAR, I., TUSEKZNIDARIC, M. AND STEGNAR, P.
Year	1994
Title	Embryolarval tolerance of *Mytilus galloprovincialis*, exposed to the elevated sea-water metal concentrations.1. Toxic effects of Cd, Zn and Hg in relation to the metallothionein level
Bibliographic Source	Comparative Biochemistry and Physiology C Pharmacology Toxicology & Endocrinology 107, 249-257
Test material	$ZnSO_4$
Test species	*Mytilus galloprovincialis*
Taxonomic group	Mollusca
Exposure duration	48 h
Endpoint	Embryogenesis, development
Effect parameter	NOEC
Effect concentration (µg l^{-1})	90
Nominal/Measured	N
Test media type	FSW
Klimisch code	2
Free text phrase	Acceptable, well-documented publication/study which meets basic scientific principles
Principles of method if other than guideline	Origin of test organism: Lifestage: fertilized egg Test system: static Test details: 1L; 30-40 eggs ml^{-1} Dose-response: yes, EC50 reported Control mortality: NR but control included Zinc concentrations: control, 40, 90, 140, 180; triplicate T=20°C; pH=; Salinity=37-38‰
Details on results (CI, statistics, etc.):	LOEC (p=0.05) =90 ; Note : LOEC=EC8.2, NOEC=40 development < growth

Authors	RADENAC, G., FICHET, D. AND MIRAMAND, P.
Year	2001
Title	Bioaccumulation and toxicity of four dissolved metals in *Paracentrotus lividus* sea-urchin embryo.
Bibliographic Source	Marine Environmental Research 51, 151-166
Test material	$Zn(NO_3)_2$
Test species	*Paracentrotus lividus*
Taxonomic group	Echinodermata
Exposure duration	48 h
Endpoint	Embryogenesis
Effect parameter	EC10 (LI)
Effect concentration (µg l^{-1})	17.7
Nominal/Measured	N
Test media type	ASW (Sigma Aldrich)
Klimisch code	2
Free text phrase	
Principles of method if other than guideline	Origin of test organism: Adults collected in Bay of Biscay, France; spawning induced in lab. Lifestage: fertilized eggs Test system: static Test details: 500 larvae ml^{-1} in 1-l tanks, 100 larvae from 3x 5-ml aliquots were examined for anomalies of skeleton, blockage at gastrula or blastula stages, delayed larvae and dead embryos. Mortality was assessed in 3 aliquots of 0.2 ml from each subsample (100 larvae=100% survivals). Dose-response: Control mortality: Zinc concentrations: 0, 5, 25, 50, 250, 500, triplicates T=22°C; pH=8.3; Salinity= 34‰
Details on results (CI, statistics, etc.):	Differences between abnormality frequencies were tested with non-parametric Kruskall-Wallis test. LOEC(EC<10)=10. All treatments were different. LC10 re-calculated via linear interpolation from percentage of control response =17.7. Test abnormalities corrected using Abbot's formula.

Authors	REISH, D.J., GERLINGER, T.V., PHILLIPS, C.A. AND SCHMIDTBAUER, P.D.
Year	1977
Title	Toxicity of Formulated Mine Tailings on Marine Polychaete
Bibliographic Source	Marine Biological Consultants, Costa Mesa, CA:133.
Test material	$ZnCl_2$
Test species	*Capitella capitata*
Taxonomic group	Annelida
Exposure duration	2 months
Endpoint	NOEC
Effect parameter	Reproduction
Effect concentration ($\mu g\ l^{-1}$)	100
Nominal/Measured	N but verified
Test media type	FSW; BG:8 (Coastal Californian SW)
Klimisch code	2
Free text phrase	Acceptable, well-documented publication/study which meets basic scientific principles
Principles of method if other than guideline	Origin of test organism: laboratory culture originated from LA, California Lifestage: 10-12d old young adults Test system: static-renewal Test details: 25 organism/2.5L; number of tubes with eggs examined Dose-response: yes Control mortality: <20% Zinc concentrations: control, 100, 320, 560, 1000, 3200; 4 replicates T=15 & 20 C°C; pH=; Salinity=32‰
Details on results (CI, statistics, etc.):	NOEC'= from two tests at different temperatures (320, 560) NOEC=EC10< in tests at both temperatures Statistical significance using Mann-Whitney U & Wilcoxon test

Authors	REISH, D.J., GERLINGER, T.V., PHILLIPS, C.A. AND SCHMIDTBAUER, P.D.
Year	1977
Title	Toxicity of Formulated Mine Tailings on Marine Polychaete
Bibliographic Source	Marine Biological Consultants, Costa Mesa, CA:133.
Test material	$ZnCl_2$
Test species	*Neanthes arenaceodentata*
Taxonomic group	Annelida
Exposure duration	7 months
Endpoint	NOEC
Effect parameter	Reproduction
Effect concentration (µg l^{-1})	33.3
Nominal/Measured	N but verified
Test media type	FSW; BG:8 (Coastal Californian SW)
Klimisch code	2
Free text phrase	Acceptable, well-documented publication/study which meets basic scientific principles
Principles of method if other than guideline	Origin of test organism: laboratory culture originated from LA, California Lifestage: 2-months old young adults Test system: static renewal Test details: 1 organism/50 ml; 20 organisms / concentration Dose-response: yes Control mortality: <20% Zinc concentrations: control, 100, 320, 560, 1000, 1800 (3200) T=15 & 20°C; pH=; Salinity=32‰
Details on results (CI, statistics, etc.):	Statistical significance using Mann-Whitney U & Wilcoxon test NOEC' at 15 & 20C (100,100) NOEC' at 15C =EC30; NOEC' at 20C =EC20; NOEC=NOEC'/3;

Authors	REISH, D.J. AND CARR, R.S.
Year	1978
Title	The effect of heavy metals on the survival, reproduction, development and life cycles of two species of polychaetous annelids
Bibliographic Source	Mar Pollut Bull 9:24-29
Test material	$ZnCl_2$
Test species	1) *Ctenodrilus serratus* 2) *Ophryotrocha diadema*
Taxonomic group	Annelida
Exposure duration	1) 21 d 2) 28 d
Endpoint	NOEC
Effect parameter	Reproduction
Effect concentration (µg l^{-1})	100
Nominal/Measured	N
Test media type	FSW with sodium citrate added as chelator
Klimisch code	2
Free text phrase	Acceptable, well-documented publication/study which meets basic scientific principles
Principles of method if other than guideline	Origin of test organism: laboratory culture for 3 years Lifestage: adults Test system: static Test details: 4 organisms/20mL replicate Dose-response: yes Control mortality: 0 Zinc concentrations: Control, chelator control, 50,100, 500, 1000, 1750, 2500 µg l^{-1}; 10 replicates/treatment T=°C; pH=7.8; Salinity= not reported
Details on results (CI, statistics, etc.):	LOEC=500, NOEC< LOEC Non-parametric Mann-Whitney U-Test with 0.05-level of significance No effect of sodium citrate on results

Authors	SOMASUNDARAM, B., KING, P.E. AND SHACKLEY, S.E.
Year	1984
Title	Some morphological effects of zinc upon the yolk- sac larvae of *Clupea harengus* L.
Bibliographic Source	Journal of Fish Biology, 25:333-343
Test material	$ZnSO_4$
Test species	*Clupea harengus* L.
Taxonomic group	Fish
Exposure duration	27-d
Endpoint	NOEC
Effect parameter	Development
Effect concentration (µg l^{-1})	25
Nominal/Measured	N
Test media type	ASW (Tropical Marine Salts)
Klimisch code	2
Free text phrase	Acceptable, well-documented publication/study which meets basic scientific principles
Principles of method if other than guideline	Origin of test organism: Adults collected in Milford Haven estuary, South West Wales, UK; eggs artificially fertilized Lifestage: fertilized egg Test system: static renewal Test details: eggs in 2L, At intervals of 13, 14, 15, 16, 17, 18, 21, 23, 25 and 27 days after fertilization, samples of live larvae were examined. The frequencies of mouth/branchial and vertebral column abnormalities were determined from random samples of 20-30 larvae. The incubation period (time from fertilization to 50% hatching), body length and eye and otic capsule diameter at hatching were also measured. Dose-response: yes Control abnormality: 10% Zinc concentrations: control, 50, 100, 500, 2000, 6000, 12000 µg l^{-1} T=8°C; pH=7.5; Salinity= 21‰
Details on results (CI, statistics, etc.):	Reported significance level for testing differences: $p<0.05$ LOEC=50ug/L; toxic response at LOEC <20%, NOEC=LOEC/2

Authors	STRÖMGREN, T.
Year	1979
Title	The effects of zinc on the increase in length of five species of intertidal fucales
Bibliographic Source	J Exp Mar Biol Ecol 40:95-102
Test material	ZnCl2
Test species	*1) Ascophyllum nodosum* *2) Fucus serratus* *3) Fucus spiralis* *4) Fucus vesiculosus* 5) *Pelvetia canaliculata*
Taxonomic group	Algae
Exposure duration	10 d
Endpoint	EC10 (LI)
Effect parameter	growth
Effect concentration ($\mu g\ l^{-1}$)	1) 69.4 [*A.nodosum*] 2) 409.9 [*F. serratus*] 3) 100.6 [*F. spiralis*] 4) 71.0 [*F.vesiculosus*] 5) 719.8 [*P.canaliculata*]
Nominal/Measured	N
Test media type	SW from moderately contaminated site (sewage sludge), BG:7-9
Klimisch code	2
Free text phrase	Acceptable, well-documented publication/study which meets basic scientific principles
Principles of method if other than guideline	Origin of test organism: moderately exposed and unpolluted site in Trondheimsfjorden Lifestage: 20mm long apices Test system: flow-through Test details: Dose-response: yes Zn concentrations: control, 25, 100, 1400, 2900, 7000, and 14000 $\mu g\ l^{-1}$ (additional concentrations for test with *A. nodosum* : 250, 1000, 10000 and 100000 $\mu g\ l^{-1}$) 10 replicates T=6.4-6.8°C; Salinity= 33.4-33.5‰
Details on results (CI, statistics, etc.):	EC10 recalculated using the Toxicity Relationship Analysis Program (TRAP) from the U.S. EPA National Health and Environmental Effects Research Laboratory (NHEERL).

Authors	VRANKEN, G., VANDERHAEGHEN, R. AND HEIP, C.
Year	1991
Title	Effects of Pollutants on Life-History Parameters of the Marine Nematode *Monhystera disjuncta*
Bibliographic Source	ICES Journal of Marine Science, 48:325-334
Test material	$ZnCl_2$
Test species	*Monhystera disjuncta*
Taxonomic group	Nematoda
Exposure duration	96 h
Endpoint	Reproduction
Effect parameter	NOEC
Effect concentration (µg l^{-1})	250
Nominal/Measured	N
Test media type	ASW with agar (0.5% agar made with SW from sluice dock of Ostend, BE)
Klimisch code	2
Free text phrase	
Principles of method if other than guideline	Origin of test organism: Adults from sluice dock of Ostend, BE Lifestage: juvenile larvae, 4.5 days old Test system: static Test details: 5 ml metal stock solution mixed with 42.5 ml 0.6% sterile (buffered) bacto-agar (60°C) and 2.5 ml of a sterol mixture; 4 replicates with 30 organisms each; egg deposition of at least 10 females determined Dose-response: yes Control mortality: 0 Zinc concentrations: Control,750,1000,5000 T=17°C;; Salinity= 30‰
Details on results (CI, statistics, etc.):	- MEC =750 = EC24; recalculated NOEC = MEC/3 - EC50 (reproduction): 1900 (800-4300) - MEC: Development:5000; Mortality: 20000 - LC50: 24600 (22700-26600) MECs = lowest concentrations tested which gave a significantly different response compared with blank estimated by log-likelihood test (G-test, Sokal and Rohlf, 1981); significance of reduction in fecundity examined by ANOVA; MECs based on reduction of fecundity determined by comparisons between means by calculating 95% comparison intervals around the means at different concentrations (Sokal and Rholf, 1981)

Authors	WATLING HR
Year	1982
Title	Comparative study of the effects of zinc, cadmium and copper on the larval growth of three oyster species
Bibliographic Source	Bulletin of Environmental Contamination and Toxicology, 28:195-201
Test material	ZnCl2
Test species	1) *Crassostrea cucullata* 2) *Crassostrea gigas* 3) *Crassostrea margaritacea*
Taxonomic group	Mollusca
Exposure duration	4 d
Endpoint	EC10
Effect parameter	Growth (valve width)
Effect concentration ($\mu g\ l^{-1}$)	1 22.9 [*C. cucullata*] 2 57.6 [*C. gigas*] 3 13.3 [*C. margaritacea*]
Nominal/Measured	N
Test media type	FSW
Klimisch code	2
Free text phrase	Acceptable, well-documented publication/study which meets basic scientific principles
Principles of method if other than guideline	Origin of test organism: most likely South Africa, from hatchery stock culture Lifestage: 3-d old larvae, 6-d old larvae for *C. gigas* Test system: static renewal Test details: 2500 larvae/1L Dose-response: yes Control: growth data reported Zinc concentrations: control, 10, 20, 50, 100; 2 replicates T=22-23°C; Salinity=34‰
Details on results (CI, statistics, etc.):	EC10 recalculated using the Toxicity Relationship Analysis Program (TRAP) from the U.S. EPA National Health and Environmental Effects Research Laboratory (NHEERL).

Appendix IV: Bioavailability corrections for zinc

A4.1 Use of the added risk approach

The EU Technical Guidance Document (EC 2003) and the recent Draft EU EQS Guidance (EC 2009) do not provide specific guidance on dealing with (essential) elements such as zinc that have natural background concentrations in the environment. However, according to Struijs et al. (1997) and Crommentuijn et al. (1997), the added risk approach may be used to deal with such substances.

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" PEC (PEC_{add}) and "added" PNEC ($PNEC_{add}$), 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 additions of a substance (i.e. the amount added to the natural background concentration) are considered to be relevant for the effect assessment of that substance.[6] Thus, the contribution of the natural background concentration to toxic effects is ignored.

The maximum permissible concentration (MPC) in a water body or in sediment is the sum of the local natural background concentration ($C_{backgrnd}$) and the $PNEC_{add}$. The $PNEC_{add}$ is equivalent to the EQS:

$MPC = PNEC_{add} + C_{backgrnd}$ (with $PNEC_{add} \approx EQS$)

$PEC_{add} = EC - C_{backgrnd}$ (with EC = actual environmental concentration at site X)

Two assumptions underlie this approach:

1. The extent to which the background concentration of a metal has an impact on ecosystem structure and function is not relevant. Any potential adverse or positive effect of the background concentration can be considered as effects contributing to the natural biodiversity of ecosystems.

2. As species in an ecosystem are adapted to the prevailing background level, it is assumed that the same amount of a metal added by human activities, in principle,

[6] For aquatic organisms, which are mainly exposed via water, the free zinc ions and other dissolved zinc species are especially relevant for toxicity. Therefore, the dissolved zinc concentration in water is a better indicator of toxicity than the total zinc concentration, although the dissolved fraction may contain forms of zinc that are not bioavailable. In practice, the dissolved fraction is defined as the fraction that passes through a 0.45 µm filter. All waterborne zinc concentrations mentioned in this report refer to the dissolved zinc concentration, whereas in the case of sediment, they refer to the total zinc concentration.

causes the same effect. However, in such circumstances all environmental parameters determining metal toxicity must be equal, apart from the background level of the metal concerned (i.e. it is not the absolute level of a metal that is decisive for the occurrence/extent of adverse effects, only the added amount).

The background concentration and the $PNEC_{add}$ are independently derived values. Real world background concentrations may be derived on the basis of monitoring data for relatively pristine areas or may be based on calculations using geological and hydrological data.

In addition, the use of the added risk approach implies that there is no risk of deficiency of essential metals at the level of the calculated quality standard. By definition, the background concentration in a given ecosystem provides the resident organisms with the required essential metals.

A4.2 Factors determining zinc bioavailability and toxicity in the water column

Zinc exists in the environment in various chemical forms. The presence of one zinc species over another and the bioavailability of each species depend on several physicochemical processes, such as the pH and hardness of water and the concentration of dissolved organic matter (DOM).

Ideally, the influence of water quality parameters on bioavailability and toxicity of zinc should be considered when setting quality standards. However, until recently, there was a lack of adequate information with which to determine or estimate the bioavailable fraction quantitatively in either laboratory tests or the environment. The results of a recent extensive research programme conducted as part of the work on the EU RAR on zinc have led to quantitative ways of taking the bioavailability of zinc in water and sediment into account.

A4.3 Validation and use of the Biotic Ligand Model

This section contains an abridged and modified adaptation of the text in Section 3.3.2.1.1 (abiotic factors influencing the aquatic toxicity of zinc) of the EU RAR (2008).

A4.3.1 Use of the Biotic Ligand Model

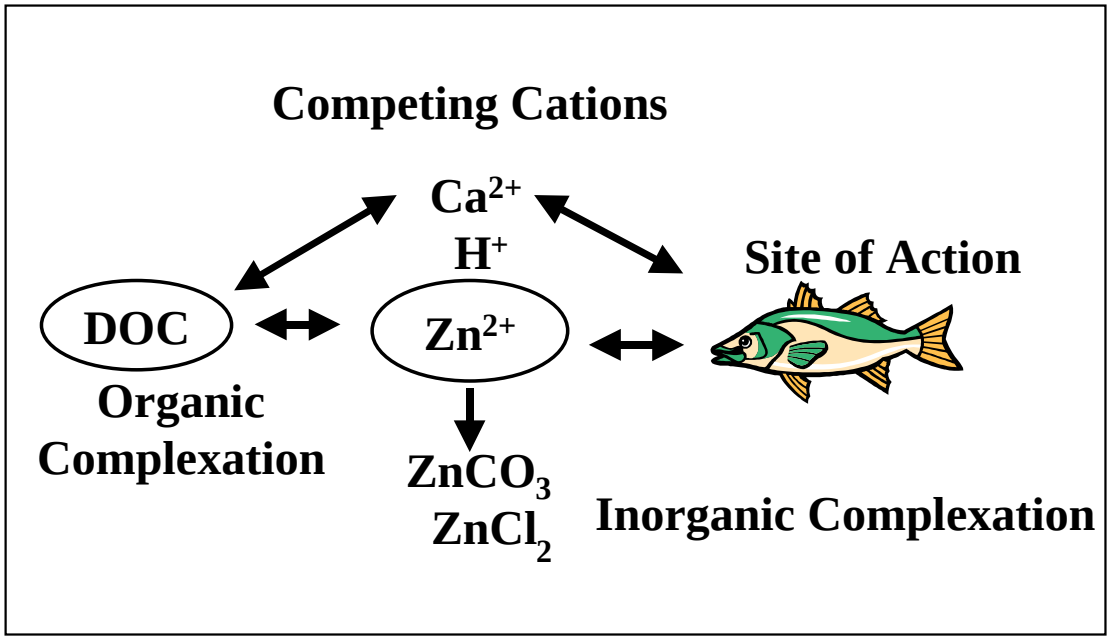
The Biotic Ligand Model (BLM) has been proposed as a tool with which to evaluate quantitatively the manner in which water chemistry affects the speciation and biological availability of metals in aquatic systems. This is an important consideration because the bioavailability and bioreactivity of metals control their potential to cause adverse effects. The BLM approach has gained widespread interest among the scientific and regulatory communities because of its potential use in developing water quality standards and performing aquatic risk assessments for metals.

The BLM performs in a way that considers the important influences of site-specific water quality (Paquin et al. 2002; Figure A4.1). Free zinc ions (Zn^{2+}) bind to the biotic ligand of

organisms; these may be transport or toxic action sites. The concentration of zinc bound to the biotic ligand is directly proportional to the toxic effect and independent of the physicochemical characteristics of the test medium.

However, the chemical activity of Zn^{2+} is reduced by binding to organic (DOC) and inorganic ligands that reduce bioavailability and, thus, effective toxicity. Inorganic ligands include OH^- and CO_3^{2-}. The concentrations of these ligands increase as the pH and alkalinity of the test medium increase. Cations in solution can compete with zinc for the biotic ligand, which also reduces bioavailability to the biotic ligand and thus reduces toxicity. The speciation of Zn^{2+} is calculated by the WHAM V-model (Tipping 1994), 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).

Figure A4.1 Summary of the BLM concept



A4.3.2 Development and validation of Biotic Ligand Models for European surface waters

The overall objectives of the recent research programme (De Schamphelaere et al. 2003, Heijerick et al. 2003) were to:

- develop a Biotic Ligand Model to predict chronic zinc toxicity to three standard test organisms, i.e. the rainbow trout *Oncorhynchus mykiss*, the invertebrate *Daphnia magna*, and the green alga *Pseudokirchneriella subcapitata*;
- validate the developed BLM with different surface waters representative of the observed variation in physicochemistry in EU surface waters.

The BLM development was based on a series of (univariate) chronic toxicity experiments in standard test media in which the major water quality parameters 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 zinc speciation, zinc complexation and chronic zinc toxicity over a relevant range of water chemistry parameters.

The methodology described by De Schamphelaere et al. (2003) was followed to develop the BLMs. This 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 per cent of effect

$f_{ZnBL}^{x\%}$ = the fraction of binding sites that is occupied by Zn when x per cent 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

Mortality was shown to be 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 illustrated the importance of bioavailability modifying factors for chronic zinc toxicity to juvenile rainbow trout. Observed values were:

- 30-day EC50: 108–1,970 µg Zn l^{-1};
- EC10: 38.4–902 µg Zn l^{-1};
- NOEC: 31.5–885 µg Zn l^{-1}.

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 3–4) > pH (H^{+}, factor 2–3) > Na (factor 2). Hence, none 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 laboratory waters but also for natural surface waters. Hence, use of the BLM reduced the variation from a factor of 20 observed in all toxicity tests to a factor 2. This indicates 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 A4.1.

Table A4.1 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 K_{ZnBL} [a]	5.31	5.31	5.31	5.31
Log K_{CaBL}	3.76	3.35	3.70	4.01
Log K_{MgBL}	3.51	3.04	3.15	3.31
Log K_{NaBL}	2.88	2.33	2.45	2.61
Log K_{HBL}	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

[a] Log K_{ZnBL} set to the same value as reported in Heijerick et al. (2002) for the acute zinc BLM for *D. magna*.
[b] Mean ± one-sided 95% confidence limit.
NA = not applicable

The results of the ecotoxicity tests with the invertebrate *Daphnia magna* also illustrated the importance of bioavailability modifying factors for chronic zinc toxicity to this species.

Observed 21-day EC50 and NOEC values were 107 – 372 µg Zn l^{-1} and 47.9 – 168 µg Zn l^{-1}, 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 Ca^{2+} (factor 3–4) = pH (factor 3–4) > Mg^{2+} (factor 2 to 3) > Na^{+} (factor 1.5). A concentration of 5 mg DOC l^{-1} resulted in a decrease in toxicity of about a factor of 1.3–1.5, which is comparable to the factor 5 decrease observed with rainbow trout in a test with a DOC concentration four times higher.

Thus, a similar importance of DOC for rainbow trout and *D. magna* is suggested. In general, it can be concluded that the daphnid BLM was able to predict all 21-day 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 *D. magna* are shown in Table A4.2.

Table A4.2 BLM constants for acute (Heijerick et al. 2002) and chronic (De Schamphelaere et al. 2003) zinc toxicity to *Daphnia magna*

	Acute	Chronic
Log K_{ZnBL} [a]	5.31	5.31
Log K_{CaBL}	3.34	3.25
Log K_{MgBL}	3.12	2.71
Log K_{NaBL}	2.37	1.92
Log K_{HBL}	-	5.91
$f_{ZnBL}^{50\%}$ [b]	0.417	0.117±0.13
f_{ZnBL}^{NOEC} [b]	NA	0.077±0.015

[a] Data from Heijerick et al. (2002).
[b] Mean ± one-sided 95% confidence limit.
NA = not applicable

For the green alga *P. subcapitata*, it was demonstrated that the observed 72-hour ErC50[7] and 72-hour ErC10 values were 25.8 – 1630 µg Zn l^{-1} and 4.8 – 608 µg Zn l^{-1}, respectively. These results indicate 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, as for the other organisms, taken into account by the speciation model WHAM V (Tipping 1994). The alga BLM demonstrated a good predictive capacity for the field waters tested and 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 algal species. The relevant BLM constants for the algae are shown in Table A4.3.

Table A4.3 BLM constants for chronic zinc toxicity to *P. subcapitata* (De Schamphelaere et al. 2003)

	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

[a] Since critical biotic ligand concentrations of zinc for *P. subcapitata* are pH-dependent and covered by the stability constant for ZnBL, the constants for the other competing cations were of negligible importance.
[b] Mean ± one-sided 95% confidence limit.

The research consistently illustrated the importance of bioavailability parameters for chronic toxicity of zinc to rainbow trout, daphnids and algae. It also demonstrated that changes in zinc bioavailability to aquatic organisms can be quantified and predicted with a reasonably high degree of precision, as long as the taxon-specific BLM is used.

Quantitative differences were noted with regard to the effect of the individual parameters on chronic toxicity across the three organisms.

- 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 these differences, the BLMs for all three organisms were able to take them into account. The BLMs were able to reduce significantly the variation associated with the effect concentrations, i.e. chronic effect concentrations were generally predicted within a

[7] ErC50 = EC50 in terms of reduction of growth rate

factor of 2 from the observed values for all organisms studied, for both laboratory (artificial) waters and field waters.

In summary, the studies that developed the BLMs 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 to run the BLMs. All binding constants were found to be independent of other variables, with the exception of the binding constant for zinc with the alga, 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 2 of the experimental values. Thus, good performance of the model was apparent. However, experimental values could also vary within a factor of 2.

In the validation studies, a number of field waters from several European sites were tested and the 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 covered a major part of European freshwaters (de Schamphelaere et al. 2003) and were as follows:

- DOC: 4.8 – 27.4 mg l^{-1}
- pH: 5.2 – 8.4
- hardness: 2.5 – 238 mg l^{-1} as $CaCO_3$.

The field waters contained different compositions of the cations studied (i.e. H^+, Ca^{2+}, Mg^{2+}, Na^+) in the series of tests used to develop the BLMs. In addition, the field waters contained dissolved organic matter.

The researchers assumed that the DOC measured in the field almost completely consisted of fulvic acids (i.e. 99.9 per cent) and took the binding constant of zinc to fulvic acids from the literature. Thus, only 0.1 per cent of the DOC was assumed to consist of humic acids. Again, predictions were within a factor of 2 of the experimental values for the algae and fish studies, thus demonstrating good performance by the model.

Koukal et al. (2003) postulated several explanations to account for their observed results in which Suwannee River fulvic acids (SWFA) did not affect toxicity of zinc to *P. subcapitata*, but 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 the fulvic acids coagulated thus altering metal complexing behaviour, or that fulvic acid has a lower ability to adsorb to cell membranes at pH >7. The stronger reduction in toxicity of the humic acids was explained by a reduced bioavailability because of the zinc–humic acid complexes and because of adsorption of the humic acid to the algal surfaces, shielding the cells from free zinc ions. However, a different complexing ability may arise depending on the origin of fulvic acids and the pH of the water.

A4.3.3 Use of BLMs to reduce the variation in zinc toxicity because of site-specific zinc bioavailability

The following stepwise approach was devised in the EU RAR for using the BLMs to correct the bioavailability of zinc. The approach was used in relation to those sites or regions that have a $PEC_{add}/PNEC_{add}$ >1 (Figure A4.2). The bioavailability correction is applied to the PEC_{add},[8] and not to the $PNEC_{add}$. One of the main reasons for correcting the PEC_{add} is that BLMs for each individual organism are not available from the ecotoxicity database.

First, the chronic NOEC values for the three 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. This results in $NOEC_x$ for that site or region. If no sufficient site- or region-specific information on the abiotic parameters (i.e. the chemical activity of the cations Ca^{2+}, Mg^{2+}, Na^{+} and H^{+}) is available, no bioavailability correction is possible.

Next, the chronic $NOEC_x$ values need to be compared with a reference NOEC value ($NOEC_{ref}$). These $NOEC_{ref}$ values (Table A4.4) have been calculated using the BLMs under reference water chemistry conditions.

Table A4.4 $NOEC_{ref}$ values for the three aquatic species for which BLMs have been developed

Species	**$NOEC_{ref}$**
O. mykiss	184
D. magna	86
P. subcapitata	21

These $NOECs_{ref}$ reflect a reasonable worst-case situation that mimics the situation where bioavailability of zinc is very high. Thus, these can be regarded as reference values for the bioavailability at the site or region X.

The $NOEC_X$ is calculated with the site-specific water quality parameters and the BLMs for algae, daphnids and fish. It is then regarded as a surrogate for the actual bioavailable concentration of zinc[9] at that site or region X. The bioavailability factors (BioF) are then derived for each of the three BLM species as follows:

$$BioF_{water,X} = \frac{NOEC_{ref}}{NOEC_x}$$

[8] The measured environmental concentration (EC) of dissolved zinc at site X minus the natural background concentration ($C_{backgrnd}$): $PEC_{add} = EC - C_{backgrnd}$.

[9] The BLM values generally overestimate zinc toxicity, i.e. the NOECs predicted by the BLMs are lower than the experimental values observed at the same water quality as assumed for the BLM prediction.

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 is taken, i.e. the smallest correction for bioavailability.

The bioavailability correction to the PEC_{add} at the site or region X can now be made. The first step is to subtract the zinc background concentration from the measured zinc monitoring data:

$$PEC_{add} = PEC_{dissolved} - C_{backgrnd\_dissolved}$$

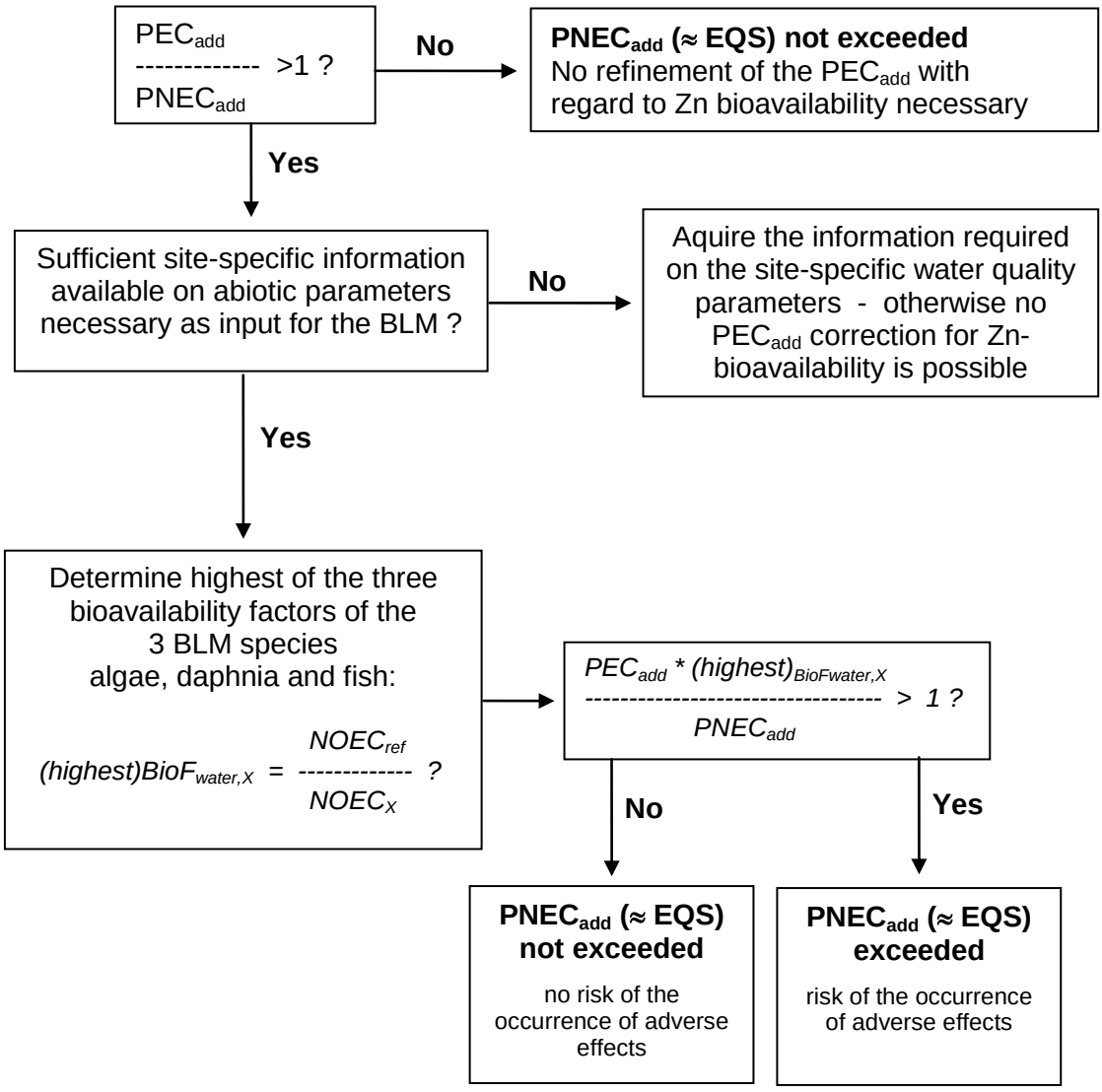
The bioavailable concentration of the added zinc concentration in the water at the site or region X can be calculated from:

$$PEC_{add,bioavailable} = PEC_{add} \times BioF_{water,X}$$

Finally, the $PEC_{add,bioavailable}$ can be compared directly with the corresponding $PNEC_{add}$ (i.e. the EQS).

The derivation of $PNEC_{add}$ proposals is described in Section 3 of this report.

Figure A4.2 Decision tree for correcting the site- or region-specific PEC_{add} for reduced zinc bioavailability in water using Biotic Ligand Models



A4.4 Consideration of factors determining zinc toxicity in sediments

This section is an abridged and modified adaptation of the text in Section 3.3.2.2.1 (Abiotic factors influencing the sediment toxicity of zinc) of the EU RAR (2008).

Dry or wet weight normalised concentrations are not a good expression of zinc toxicity in sediments because zinc can be present in a variety of ways and as various chemical

species with different bioavailabilities and toxicities (EU RAR 2008). These approaches may therefore overestimate the risk of zinc in the sediment.

The limitations of the conventional weight normalised approaches to express concentrations that may affect benthic communities can be overcome by applying the so-called acid volatile sulphide (AVS) approach.

The EU RAR concludes that there is sufficient scientific evidence to adopt the simultaneously extracted metals/acid volatile sulfide (SEM/AVS) (or SEM-AVS) model for correcting for zinc bioavailability and toxicity in sediment (EU RAR 2008).

A4.4.1 The acid volatile sulphide approach

In the early 1990s, the 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+} and Pb^{2+}) in sediment. These metals are referred to as Simultaneously Extracted Metals (SEM), i.e. the metals that are liberated from the sediment together with AVS by the cold extraction of sediment in approximately 1M 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 to 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:

- the total SEM concentration;
- the metals present and the relative solubility of their metal sulphides (Ni > Zn > Cd > Pb > Cu);
- 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^{-1} dry weight (dw)]
[AVS] is the molar concentration of acid volatile sulphide in the sediment (µmol g^{-1} dw).

In theory, no effects are expected when the molar amount of SEM is lower than that of AVS, i.e. SEM/AVS ratio of <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. This is 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 mmol kg^{-1} at an AVS concentration of 1 mmol/kg and 11 mmol kg^{-1} at an AVS concentration of 10 mmol kg^{-1}; the latter SEM concentration is 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 of <1, no effects are expected. At a molar SEM-AVS difference of >0 that corresponds to a molar SEM/AVS ratio >1, effects may occur.

The AVS hypothesis was confirmed in individual single-species acute lethality tests (exposure up to 10 days) (Shine et al. 2003) using:

- different benthic freshwater and saltwater organisms, including amphipods, oligochaetes and snails;
- different divalent metals (cadmium, copper, nickel and zinc, as well as metal mixtures) added to the sediment.

The results of these tests, which were all conducted in the laboratory, consistently showed no toxicity when the molar SEM/AVS ratios were ≤1. Sediments having a ratio of >1 were frequently toxic, but nearly as frequently nontoxic.

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 toxicologically relevant metal concentrations in the porewater.

As well as some long-term studies conducted in the USA, additional validation studies with European freshwater sediments have been performed (DiToro et al. 2002; Shine et al. 2003). These were evaluated in the EU RAR alongside other available long-term studies. In general, the results of the studies confirm the AVS hypothesis.

The long-term field study conducted to validate the concentrations of zinc in European freshwater sediments to determine whether there is a relationship with the AVS approach (Burton et al. 2003) revealed that there is no toxicity to benthic invertebrates if the SEM/AVS ratio is <1 (in fact, the NOEC appeared to be near a SEM/AVS ratio of 2 as no sites with a SEM/AVS ratio <2 showed any adverse effects (with slight effects in the range of 2.34–2.94)). Moreover, Burton et al. (2003) claimed that no long-term effects

could be found if the carbon-normalised AVS fraction ([SEM-AVS]/f_{oc}) is below 100 µmol/g_{oc}. However, the zinc EU RAR rapporteurs are of the opinion that no validation for the carbon-normalised AVS approach was found in the study by Burton et al. (2003)

A4.4.2 Summary and conclusions on the evaluation of the acid volatile sulphide approach

In general, the AVS approach is capable of reducing uncertainty in the prediction of metal toxicity in sediments[10] as shown and explained by DiToro et al. (2002) and Shine et al. (2003). The approach is supported by additional validation studies in Europe, which imply that it is better able to take account of bound and less available metals in the sediment than the conventional wet or dry weight normalised PNEC approach. The AVS approach offers a better explanation of why no effects are observed in studies with relatively high metal concentrations in the sediment by accounting for non-available or less available metal in those anaerobic sediments (DiToro et al. 2002, Shine et al. 2003).

Furthermore, there is a preponderance of evidence showing that the SEM/AVS model is also applicable in dynamic, bioturbated and oxidising field conditions. This is due to the enhanced stability of sulphide complexes of copper, cadmium, zinc, nickel and lead relative to the stability of 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 a high affinity for free metal ions. As such, the concern of remobilisation under oxidised conditions is minimal.

However, the remaining uncertainties (e.g. on the dietary contribution) mean that the AVS approach should be used with some caution. Furthermore, PEC/PNEC ratios should always be evaluated in addition to the AVS-corrected zinc concentrations in sediment. This is illustrated in the study by Van Sprang (2003), who showed that in sediment where zinc levels were as high as 8000 mg kg^{-1} dry weight, AVS-corrected zinc concentrations expressed as SEM/AVS and SEM-AVS were below 1 and 0, respectively. However, biological monitoring did show significant effects on sediment organisms. Thus at high zinc concentrations, the AVS-corrected zinc concentration should not be used exclusively 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 from routes other than the (pore) water, such as via the dietary route of uptake.

All arguments taken together provide sufficient scientific evidence to adopt the SEM/AVS or SEM-AVS model. Moreover, the proposed correction for AVS can be considered a conservative approach since:

- adsorption on organic carbon and complexation with carbonates is not taken into account;

[10] However, some studies show deviations from the SEM/AVS or SEM-AVS model, while other studies challenge the entire concept (e.g. Ankley et al. 1996, Griscom et al. 2000, Lee et al. 2000).

- other bioavailability mediators such as co-precipitation of zinc with, for example, iron/manganese oxyhydroxides are not being considered;
- the mitigating effects of porewater composition are ignored.

A4.4.3 Using the acid volatile sulphide approach to reduce the variation in zinc toxicity because of site-specific zinc bioavailability

Since both the weight-normalised PNEC approach and the AVS approach seem to have merits as well as limitations, the EU RAR suggests the following two-tiered approach (Figure A4.3) to compare a regional or local added sediment concentration of zinc ($\approx PEC_{add} = EC - C_{backgrnd}$) with the $PNEC_{add,sediment}$ (i.e. the $QS_{add,sediment}$).

Tier 1: Assess the regional or site-specific risk of zinc in the sediment, based on the ratio of the PEC_{add} and the $PNEC_{add}$.
- If the ratio is <1, the $QS_{add,sediment}$ is not exceeded and no potential risk can be assumed.
- If the ratio is >1 and the PEC_{add} is ≥900 mg kg^{-1} dw (or the environmental concentration ≥1040 mg kg^{-1} dw), assume exceedence of the $QS_{add,sediment}$ and potential risk.
- If the ratio is >1 and the PEC_{add} is <900 mg kg^{-1} dw (or the environmental concentration <1040 mg kg^{-1} dw), then go to Tier 2.

Tier 2: Assess the regional or site-specific risk, taking into account AVS, by measuring SEM-AVS and decide as follows:
- If SEM-AVS is <0, no exceedence of the $QS_{add,sediment}$ and no potential risk is assumed.
- If SEM-AVS is >0, the excess zinc concentration must be lower than the $QS_{add,sediment}$ (i.e. $[Zn_{excess}]/[QS_{add,sediment}] < 1$). If the ratio is >1, an exceedence of the quality standard and, hence, potential risk can be assumed.

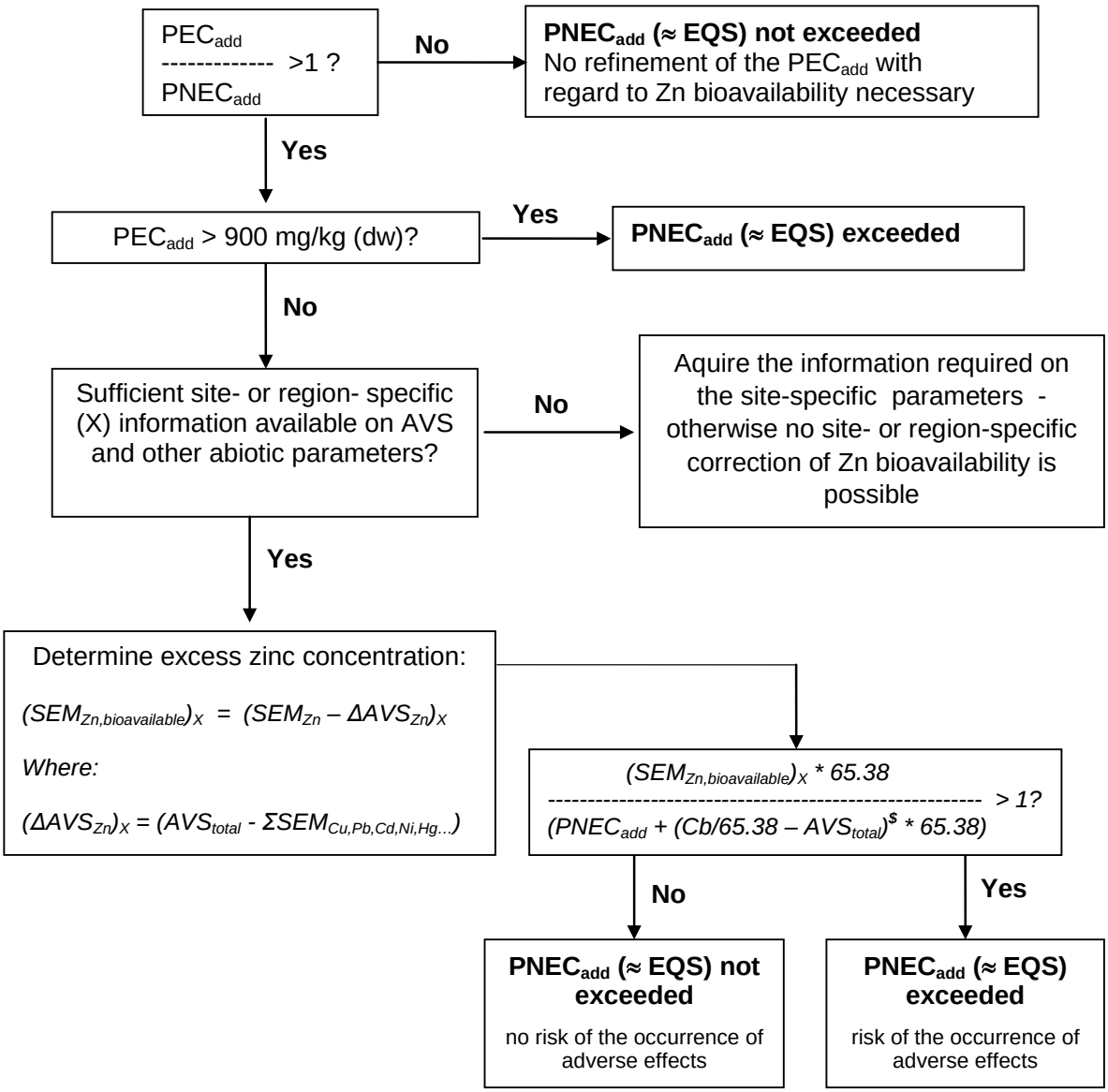
The current proposal for the maximum critical concentration of 900 mg Zn kg^{-1} dw is based on the assumption that effects will always be observed above the value of 140 µmol Zn g^{-1} (DiToro et al. 2002). When translating this value to zinc (molecular weight = 65.38 µg $µmol^{-1}$) and using an arbitrary safety factor of 10 to take into account acute-to-chronic toxicity, this results in a value of 900 mg kg^{-1} dw.

The factor of 10 to take into account acute-to-chronic toxicity is based on acute-to-chronic ratios for zinc from the literature for aquatic species. The average value and 90th-percentile values for the acute-to-chronic ratio from those studies are 6.1 and 12.5, respectively.

The factor of 10 is suggested to take account of:

- the various factors found in the literature;
- the fact that the acute-to-chronic ratio for sediment organisms may be different to that for aquatic organisms.

Figure A4.3 Decision tree for correcting the site- or region-specific PEC_{add} for reduced zinc bioavailability in sediment using the AVS approach



Under Tier 2, the following stepwise approach is proposed to integrate AVS and thus incorporate bioavailability in calculating the PEC.

First, the site- or region-specific bioavailable PEC_{add} should be derived for each site or region X. When based on monitoring data, information is needed on:

- the concentrations of a series of metals in the sediment;
- the AVS content in that sediment.

The AVS-corrected zinc concentration in the sediment is determined as follows:

$(SEM_{Zn,bioavailable})_X = (SEM_{Zn} – \Delta AVS_{Zn})_X$

where $(SEM_{Zn,bioavailable})_X$ represents the bioavailable zinc in the sediment expressed on a molar basis (mol/kg sediment) and corrected for the excess (acid volatile) sulphide in the sediment.

SEM_{Zn} will include the total zinc concentration in the sediment and, thus, the background concentration.

The excess AVS, which is also expressed on a molar basis (mol kg^{-1} sediment), is the total AVS in the sediment minus the AVS that is bound by metals that are more strongly bound to AVS than zinc, i.e.

$\Delta AVS_{Zn} = AVS_{total} – (SEM_{Cu} + SEM_{Pb} + SEM_{Cd} + SEM_{Ni} + SEM_{Hg} + …)$

No bioavailability correction is possible if there is no sufficient site- or region-specific information on the abiotic parameters.

The ratio of the $(SEM_{Zn,bioavailable})_X$ and the $PNEC_{add,sediment}$ is used for the risk characterisation (the derivation of $PNEC_{add}$ proposals is described in Section 3 of this report):

$$\frac{(SEM_{Zn,bioavailable})_X \cdot MW_{Zn}}{PNEC_{add} + \left(\frac{Cb}{MW_{Zn}} - AVS_{total}\right) \cdot MW_{Zn}} > 1?$$

In this equation, the molar-based $(SEM_{Zn,bioavailable})_X$ is transformed to a mg kg^{-1}-based zinc concentration by multiplying with the molecular weight of zinc. Both the nominator and denominator (i.e. the PNEC) should represent the excess bioavailable zinc.

The $PNEC_{add}$ is already assumed to be bioavailable. However, the background concentration (Cb) may not be completely bioavailable; this will depend on how much of it is bound or sequestered to sulphides (AVS). The bioavailable background concentration is, therefore, added to the $PNEC_{add}$ in the denominator.

Thus, the total AVS is subtracted from the background concentration of zinc and converted into a molar basis by dividing it by the molecular weight of zinc. This difference cannot be less than zero, i.e. the case when AVS >>Cb. This difference can also not be higher than the background concentration, i.e. the case when AVS = 0.

The resulting difference is then converted into a dry weight concentration by multiplying by the molecular weight of zinc.

The background concentration may not just be sequestered to AVS. Part of the background zinc may, for example, be sequestered by other minerals, thus, making the estimate of bioavailable background zinc sufficiently conservative.

There will be no risk (i.e. compliance with the $QS_{add,sediment}$) if this ratio ≤ 1, but there will be a risk (i.e. exceedence of the $QS_{add,sediment}$) if this ratio >1.