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Preconsultation report: Proposed EQS for Water Framework Directive Annex VIII substances: iron (total dissolved)

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The UK Technical Advisory Group (UKTAG) supporting the implementation of the Water Framework Directive (2000/60/EC) is a partnership of UK environmental and conservation agencies. It also includes partners from the Republic of Ireland. This report is the result of research commissioned and funded on behalf of UKTAG by the Scotland & Northern Ireland Forum for Environmental Research (SNIFFER) and the Environment Agency's Science Programme.

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Steve Killeen Head of Science

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 WFD on behalf of its members and partners.

This technical document has been developed through a collaborative project, managed and facilitated by the Scotland & Northern Ireland Forum for Environmental Research (SNIFFER), the Environment Agency and the Scottish Environment Protection Agency (SEPA) and has involved the members and partners of UKTAG. It provides background information to support the ongoing development of the standards and classification methods.

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

Executive Summary

This document is a **preconsultation report** and was presented as background information during the UK Technical Advisory Group (UKTAG) Stakeholder Review on Specific Pollutants from June to August 2007. The actual standards proposed during the consultation were given in the UKTAG document 'Proposals for Environmental Quality Standards for Annex VIII Substances (SR1 - 2007, June 2007)'. Therefore, this overriding UKTAG document should also be referred to when considering the information given here.

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 iron using the methodology described in Annex V of the Directive. There are existing EQSs for iron, but the method used to derive these is not considered to comply with the requirements of Annex V and so is unsuitable for deriving Annex VIII EQSs.

The PNECs described in this report are based on a technical assessment of the available ecotoxicity data for iron, along with any data that relate impacts under field conditions to exposure concentrations. The data have been subjected to rigorous quality assessment such that decisions are based only on scientifically sound data. Following consultation with an independent peer review group, critical data have been identified and assessment factors selected in accordance with the guidance given in Annex V.

Where possible, PNECs have been derived for freshwater and saltwater environments, and for long-term/continuous exposure and short-term/transient exposure. If they were to be adopted as EQSs, the long-term PNEC would normally be expressed as an annual average concentration and the short-term PNEC as a 95th percentile concentration.

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.

Properties and fate in water
Iron is a naturally occurring element that also enters the environment from industrial sources. It is an essential micronutrient and plays an important role in many life processes. In ionic form, its most common oxidation states are +2 and +3, and both Fe(II) and Fe(III) ions bond with anions or form coordination compounds.

Iron can exist in numerous chemical and physical forms that are dependent on water quality conditions, and ferrous [Fe(II)] ions are oxidised to the ferric [Fe(III)] species under most environmental conditions. Insoluble ferric species are stabilised in colloidal form by adsorption to natural organic compounds. Colloidal or

microparticulate forms of iron are often measured as ‘dissolved’ iron.

The adverse effects of iron are influenced by the chemical form present, pH and dissolved organic concentrations. While the forms of iron responsible for toxicity are difficult to determine, dissolved Fe(II) appears to be more toxic than Fe(III), although data suggest that precipitates of the latter can also contribute to toxicity through ‘smothering’ effects.

Availability of data

Laboratory toxicity data are available for both Fe(II) and Fe(III) compounds. The data for freshwater organisms covers eight taxonomic groups (algae, protozoans, rotifers, crustaceans, molluscs, annelids, insects and fish) in acute toxicity tests. Chronic toxicity data are only available for five taxonomic groups (algae, macrophytes, crustaceans, insects and fish).

Data for acute toxicity to marine species are available for five taxonomic groups (crustaceans, molluscs, annelids, echinoderms and fish). Chronic toxicity data are limited to algae and crustaceans. Field studies with freshwater organisms have also been reported.

From evaluation of the data, large numbers of laboratory-based studies involve exposure to particulate iron with low levels of dissolved iron, making it difficult to separate toxic effects from physical effects.

Derivation of PNECs

The ‘added risk’ approach could be appropriate when setting EQSs for iron. This is because iron is a naturally occurring substance that organisms will have been exposed to over an evolutionary timescale. In this case, the PNEC applies only to the ‘added’ contribution over and above the background level. A practical consequence of this is that compliance assessment would need to consider background levels of iron, at least at a regional scale, if not a local scale. However, natural background concentrations for iron are expected to be very high in comparison to anthropogenic inputs. In this case, a realistic option for implementation would be to set EQSs at background levels rather than on the basis of the PNECs proposed here.

For aquatic organisms, which are mainly exposed via water, dissolved iron species are relevant for toxicity. The dissolved fraction is defined as that which passes through a 0.45 µm filter.

<u>Long-term PNEC for freshwaters</u>

Fish and crustaceans are the most sensitive taxa following chronic exposure.

It is proposed that the $PNEC_{freshwater\_lt}$ is derived on the basis of a 21-day zero equivalence point [no observed effects concentration (NOEC) equivalent] of 0.16 mg l^{-1} for a reproduction study of *Daphnia magna*. The study investigated the effects of waterborne iron in hard reconstituted water. The datum is supported by studies showing similar sensitivities of *Daphnia carinata* and the amphipod *Gammarus pulex*. An assessment factor of 10 is recommended, given the large amount of data for different taxa, to produce a $PNEC_{freshwater\_lt}$ of 0.016 mg l^{-1} iron (dissolved).

This PNEC is approximately 60 times lower than the existing EQS of 1 mg l^{-1}. This reflects new data that have become available since the original EQS was derived, as well as a difference in the derivation approach: the existing EQS was based on field observation – particularly the relationship between iron concentrations and fishery status.

Short-term PNEC for freshwaters
Fish and crustaceans are the most sensitive taxa following acute exposure.

The lowest valid acute toxicity value for iron is a 96-hour LC50 of 0.41 mg l^{-1} for brook trout (*Salvelinus fontinalis*) exposed to iron at pH 5.5. The datum is supported by a study showing similar sensitivities of the fry of carp (*Cyprinus carpio*). The recommended PNEC is based on the brook trout datum and guidance given in the EU Technical Guidance Document (TGD) on effects assessment for intermittent releases. A lower assessment factor of 10 (instead of 100) is recommended in order to extrapolate from the 50 per cent acute effect level to the short-term no effect level given the large quantity of data available. This results in a $PNEC_{freshwater\_lt}$ of 0.041 mg l^{-1} iron (dissolved).

There is no existing short-term EQS for freshwaters. The 1998 update to the EQS report for iron suggested that, as releases of iron were more likely to be long-term or near continuous point discharges and diffuse inputs, the short-term standard seemed inappropriate.

Long-term PNEC for saltwaters
Limited data are available and no chronic data have been found for fish, which were a sensitive group in terms of acute toxicity to freshwater organisms. The saltwater data are based on nominal concentrations of iron and are, therefore, not suitable for PNEC derivation.

The available data do not appear to differ markedly in the range of values obtained for the related freshwater species. Consequently, the proposed $PNEC_{saltwater\_lt}$ is based on the lowest reliable long-term freshwater data point of a 21-day zero equivalence point (NOEC equivalent) of 0.16 mg l^{-1} for a reproduction study of *Daphnia magna* and an increased assessment factor of 100 recommended resulting in a $PNEC_{saltwater\_lt}$ of 0.0016 mg l^{-1} iron (dissolved).

This PNEC is 625 times lower than the existing EQS of 1 mg l^{-1}. This reflects new data that have become available since the original EQS was derived, as well as a difference in the derivation approach. A large assessment factor is recommended due to lack of saltwater toxicity data for the proposed PNEC. The existing EQS was based on field observation, particularly the relationship between iron concentrations and fishery status, and 'read across' from the freshwater EQS.

Short-term PNEC for saltwaters
Fish are the most sensitive taxonomic group with respect to the acute toxicity of iron. Although acute data were available for more taxonomic groups than for the chronic studies, the data were still limited and there were some issues with its validity as no chemical analysis of the exposure concentrations was performed in most cases.

For this reason, the proposed $PNEC_{saltwater\_st}$ is based on the lowest reliable short-term freshwater data point, a 96-hour LC50 of 0.41 mg l^{-1} for brook trout *Salvelinus fontinalis*. An increased assessment factor of 100 is recommended, resulting in a $PNEC_{saltwater\_st}$ of 0.0041 mg l^{-1} iron (dissolved).

There is no existing short-term EQS for saltwater. The 1998 update to the EQS report for iron suggested that, as releases of iron were more likely to be long-term or near continuous point discharges and diffuse inputs, the short-term standard seemed inappropriate.

PNEC for secondary poisoning

Iron is an essential element that has been shown not to bioaccumulate in higher organisms. This is due to the organism's body regulating its requirements for iron and not storing excessive amounts. Therefore, PNECs for secondary poisoning of predators are not proposed.

PNEC for sediment

No sediment toxicity data have been located specifically for iron so a $PNEC_{sediment}$ could not be generated.

Summary of proposed PNECs

Receiving medium/exposure scenario	**Proposed PNEC (mg l^{-1} total dissolved iron)**	**Existing EQS (mg l^{-1})**
Freshwater/long-term	0.016	1
Freshwater/short-term	0.041	No standard
Saltwater/long-term	0.0016	1
Saltwater/short-term	0.0041	No standard

Analysis

The lowest proposed PNEC derived for iron is 1.6 µg l^{-1}. The data quality requirements are that, at a third of the EQS, total error of measurement should not exceed 50 per cent. The limit of detection for atomic emission spectrometry is 0.3 µg l^{-1} (in clean water). This suggests that current analytical methods may not be adequate to analyse iron for compliance with the proposed PNECs.

Implementation issues

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

1. An understanding of the magnitude and variability in background concentrations of iron in surface waters is required in order to inform a decision about the need for an added risk approach. The proposed PNECs are likely to be insignificant compared with backgrounds, in which case it may be appropriate to consider adopting the background (regional or local, depending on spatial variability) as the basis for an EQS.

2. Current analytical sensitivity is inadequate to distinguish the small PNEC from background levels of iron. However, current analytical methods are adequate if

EQSs are based on backgrounds.

3. High uncertainty in the extrapolations to derive saltwater PNECs may be reduced by the generation of additional ecotoxicological data.

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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 iron using the methodology described in Annex V of the Directive. There are existing EQSs for iron, but the method used to derive these is not considered to comply with the requirements of Annex V and so is unsuitable for deriving Annex VIII EQSs.

The PNECs described in this report are based on a technical assessment of the available ecotoxicity data for iron, along with any data that relate impacts under field conditions to exposure concentrations. The data have been subjected to rigorous quality assessment such that decisions are based only on scientifically sound data.[2] Following consultation with an independent peer review group, critical data have been identified and assessment factors selected in accordance with the guidance given in Annex V. 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.

This report provides a data sheet for iron (total dissolved).

1.1 Properties and fate in water

Iron is a naturally occurring element that also enters the environment from industrial sources. It is an essential micronutrient and plays an important role in many life processes. In ionic form, its most common oxidation states are +2 and +3, and both Fe(II) and Fe(III) ions bond with anions or form coordination compounds.

Iron can exist in numerous chemical and physical forms that are dependent on water quality conditions, and ferrous [Fe(II)] ions are oxidised to the ferric [Fe(III)] species under most environmental conditions. Insoluble ferric species are stabilised in colloidal form by adsorption to natural organic compounds. Colloidal or microparticulate forms of iron are often measured as ‘dissolved’ iron.

The adverse effects of iron are influenced by the chemical form present, pH and dissolved organic concentrations. While the forms of iron responsible for toxicity are difficult to determine, dissolved Fe(II) appears to be more toxic than Fe(III), although data

[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

[2] Data quality assessment sheets are provided in Annex 1.

suggest that precipitates of the latter can also contribute to toxicity through ‘smothering’ effects.

2. Results and observations

2.1 Identity of substance

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

Table 2.1 Species covered by this report

Name	CAS Number
Iron	7439-89-6

2.2 PNECs proposed for derivation of quality standards

The PNECs contained in this report refer to the 'added' dissolved concentration of iron to be added to the natural background level.

Table 2.2 lists proposed PNECs, obtained using the methodology described in the Technical Guidance Document (TGD) issued by the European Chemicals Bureau (ECB) on risk assessment of chemical substances [23], and existing EQSs obtained from the literature [1, 2].

Section 2.6 summarises the effects data identified from the literature for iron. The use of these data to derive the values given in Table 2.2 is explained in Sections 3 and 4.

Table 2.2 Proposed overall PNECs as basis for quality standard setting (as total dissolved iron)

PNEC	TGD deterministic approach (AFs)	TGD probabilistic approach (SSDs)	Existing EQS
Freshwater short-term	0.041 mg l^{-1} (Section 4.1.1)	-	-
Freshwater long-term	0.016 mg l^{-1} (Section 4.1.1)	Lack of data (Section 4.2)	1.0 mg l^{-1} (DAA)
Saltwater short-term	0.0041 mg l^{-1} (Section 4.1.3	-	-
Saltwater long-term	0.0016 mg l^{-1} (Section 4.1.3)	Lack of data (Section 4.2)	1.0 mg l^{-1} (DAA)
Freshwater sediment short-term	No requirement (Section 4.4)	-	
Freshwater sediment long-term	No requirement (Section 4.4)	Lack of data (Section 4.2)	
Saltwater sediment short-term	No requirement (Section 4.4)	-	
Saltwater sediment long-term	No requirement (Section 4.4)	Lack of data (Section 4.2)	

PNEC	TGD deterministic approach (AFs)	TGD probabilistic approach (SSDs)	Existing EQS
Freshwater secondary poisoning	No requirement (Section 4.5)	-	
Saltwater secondary poisoning	No requirement (Section 4.5)	-	

AF = assessment factor
DAA = dissolved annual average
SSD = species sensitivity distribution

In 1988, EQSs were proposed for iron [1] and statutory standards of 1.0 mg l^{-1} 'dissolved' (filterable) iron, expressed as annual averages, were subsequently adopted for the protection of freshwater and marine life. The current standard for the protection of freshwater life is based on field observation, particularly the relationship between iron concentrations and fishery status.

In 1998, an update to the EQSs for iron in water [2] recommended retention of the current standard for the protection of freshwater life (annual average of 1.0 mg l^{-1} 'dissolved' iron). However, the update stated that if annual average concentrations consistently exceeded 0.3 mg l^{-1} 'dissolved' iron, it may be necessary to carry out a biological survey to determine whether or not there is evidence of biological degradation. It was recommended that ochreous deposits, particularly if associated with point source discharges, may also trigger such an investigation. The update also recommended that the annual average of 1.0 mg l^{-1} for the protection of saltwater life based on measurements of filterable iron should be retained.

The update stated:

> *Furthermore, given that releases of iron are unlikely to occur in short episodes, a Maximum Allowable Concentration is considered inappropriate. The current approach (expressed as an Annual Average) is more consistent with the likely exposure of iron in water, i.e. long-term and near continuous point discharges and diffuse inputs*.

2.3 Hazard classification

Table 2.3 gives the R-phrases (Risk-phrases) and labelling for the species of interest.

Table 2.3 Hazard classification

R-phrases and labelling	Reference
None listed for iron	-

2.4 Physical and chemical properties

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

Table 2.4 Physical and chemical properties of iron

Property	Value	Reference
Molecular formula	Fe	-
Molecular weight	55.85	[3]
Appearance	Silvery-white or grey, soft, ductile, malleable metal	[3]
Melting point (°C)	1,535	[3]
Boiling point (°C)	3,000	[3]
Vapour pressure	The metal is an involatile solid at normal temperatures	-
Water solubility (mg l^{-1})	Insoluble as metal	[4]
Soil–water partition coefficient (log Kp)	No data	-

2.5 Environmental fate and partitioning

Table 2.5 summarises the information obtained from the literature on the environmental fate and partitioning of iron.

Table 2.5 Environmental fate and partitioning of iron

Property	Value	Reference
Abiotic fate	Hydrous iron(II) oxides [FeO(OH)] are generally red–brown gels and are the major constituents of soil. The major iron ores found in nature are haematite (Fe_2O_3), magnetite (Fe_3O_4), limonite [FeO(OH)] and siderite ($FeCO_3$). Iron in crustal rocks is mobilised by weathering of iron silicates and carbonates. The weathering products are oxidised and hydrolysed to insoluble ferric hydroxides and hydrous oxides that are then transported within the aquatic environment.	[13]
Speciation	Iron exists in numerous forms in water and is commonly bonded or co-ordinated to other species such as water molecules or electron donor partners. In solution, iron(II) ions are expected to hydrolyse or form complexes. At pH <1, the hexaaqua ion ($[Fe(H_2O)_6]^{3+}$) is the predominant species. As the pH increases above 1, a stepwise hydrolysis occurs. Between pH 1–2, various species of hydroxo and oxo iron compounds may be formed. Above pH 2, colloidal gels are formed, giving a precipitate of the red–brown gelatinous hydrous iron oxide. In the presence of complexing anions, such as chloride, the hydrolysis of iron(III) can result in chloro, aqua and hydroxo species. Iron(II) ions may be oxidised to iron(III) under most environmental conditions. The iron(II) ion can be oxidised by common oxidants such as nitrate. Iron(II) and iron(III) ions can form complexes with ligands containing halide, nitrogen, oxygen and sulfur donor groups.	[13]

Property	Value	Reference
Hydrolytic stability	The insoluble ferric species are stabilised in colloidal form by adsorption to natural organic compounds, such as humic and tannic acids and surfactants, and by inorganic anions, such as phosphate and silicate. Colloidal or microparticulate forms of iron are often measured as 'dissolved' iron. In coastal and offshore water, dissolved iron is present as the $Fe(OH)^{2+}$ species. Studies of the rate of hydrolysis of ferric iron in synthetic solutions such as sodium nitrate and sodium chloride at ionic strengths typical of marine waters indicate that the rate of hydrolysis is slow.	[11] [12]
Photostability	Not applicable	-
Distribution in water/sediment systems (active substance)	Chelating agents such as EDTA and NTA are common water pollutants which can strongly affect the behaviour of metal ions in natural waters. Removal of chelated iron is difficult with conventional municipal water treatment processes. Iron(III) is kept in solution by chelation in algal cultures that secrete quantities of the iron-selective hydroxamate chelating agents during periods of heavy algal bloom. Iron is strongly bound to humic substances, a process which keeps it in solution and transports the metal. Iron flocculates rapidly as salinity increases and this can be expected to reduce the bioavailability of iron in marine environments. In oxygenated aquatic systems, dissolved iron occurs principally as the Fe(III) form. In reducing environments such as lakes, some fjords and marine basins with restricted circulation and long deep-water residence times, however, significant concentrations of reduced ferrous iron can be found. In anoxic fresh and marine waters, ferrous iron is mobilised from sediments and diffuses into the water column. Its solubility is controlled by the precipitation of insoluble iron sulfides.	[2] [1] [14] [1]
Distribution in water and sediment systems (metabolites)	Not applicable	-
Degradation in soil	Adsorption of iron depends on soil organic matter and pH, with an increase in either of these factors usually resulting in an increase in adsorption. The mobility of iron ions in soils is also influenced by redox potential, with iron being more mobile under reducing conditions. Iron compounds do not volatilise from moist or dry soil surfaces due to their ionic character.	[13]

Property	Value	Reference
Partition coefficients (log Kow)	No log octanol-water partition coefficient can be determined for iron and it salts	[11]
Bioaccumulation BCF	Most taxa do not appear to bioaccumulate iron to any significant extent, or if accumulation is evident, associated bioconcentration factors (BCFs) are generally less than 100. An exception to this is algae and higher plants (aquatic mosses and flowering plants), which do appear to have the potential to accumulate high concentrations of iron, though BCFs may also be affected by adsorption to cell surfaces. BCF Finfish, *Oreochromis mossambicus* 48 [5] Aquatic algae, *Hydrodictyon reticulatum* 48 [6] Aquatic bryophytes (mosses): *Brachythecium rivulare* 32,391 [7] *Scapania undulate* 93,776 [10] Clam, *Mercenaria mercenaria* 0.004–0.02 [10] Shrimp, *Palaeomonetes pugio* 0.003–0.02 [10] Following accumulation by mussels of iron from water and food in the laboratory, the biological half-life for iron from the slow (long retention) compartment was 140–215 days. A biological half-life of 4–7 days was estimated from the medium compartment.	[2] [8,9]

2.6 Effects data

A summary of the mode of action for this substance can be found in Section 2.6.5.

Data collation followed a tiered approach. First, the critical data for freshwater and marine species from the existing EQS documents [1, 2] were recorded. Further data published after derivation of the current UK EQS were then retrieved from the US Environmental Protection Agency (US EPA) ECOTOX database.[3]

The evaluation of the effects data for iron should be considered in the context of the following statements from the update to proposed EQSs for iron in water [2], which concluded that:

> *Much of the available laboratory data is difficult to interpret because iron concentrations are usually expressed in terms of total iron or 'loading rate' and, in some cases, it is likely that observed effects are due to physical effects of iron precipitates. On balance, more useful information can be gained from field studies, especially where the forms of iron present are reasonably well characterized and ecologically meaningful measures of effect, for example biodiversity and population size, have been made. For these reasons, it is proposed that field data again take precedence over laboratory studies*.

[3] http://www.epa.gov/ecotox/

It was evident from the evaluation of available data that large numbers of laboratory-based studies involve exposure to particulate iron and that dissolved iron levels are low. This can make it difficult to separate toxic effects from physical effects.

In addition, iron is an essential micronutrient for plants (and animals) and, when absent or when availability is limited, primary productivity may be impaired [14]. Iron's role as an essential nutrient arises from the requirement for iron as a cofactor for a number of enzymes (e.g. in cytochromes and iron-sulfur proteins such as ferredoxin) where it plays an important part in respiratory and photosynthetic electron transport. Iron is also involved in the biosynthesis of chlorophyll and in nitrogen fixation. Iron may also play a role in the cycling of certain nutrients, e.g. in reducing sulfide toxicity to plants by forming insoluble iron sulfide and by reducing the availability of phosphorus, thus reducing the risk of eutrophication.

Iron can exert adverse effects on aquatic organisms and these effects are influenced by a number of factors especially the chemical form of iron present, pH and dissolved oxygen concentrations. These factors play a role through effects on the speciation, bioavailability and even the physical form of iron.

Iron can exist in a variety of chemical and physical forms, depending on water quality conditions. The forms of iron responsible for observed toxicity or biological degradation in the field remain difficult to determine, but dissolved ferrous iron [Fe(II)] appears to be more toxic than ferric iron [Fe(III)], which predominates under oxic, near neutral conditions. Nevertheless, laboratory and field data suggest that precipitates of Fe(III) also contribute to biological effects. Organisms particularly at risk appear to be grazing invertebrates and, potentially, fish (through congestion of the gills by iron flocs).

Information about the iron species present in a toxicity test is important in coming to a proper interpretation of effects. Studies where effects have been based on nominal iron concentrations are of limited use. Consequently, studies based on unmeasured iron concentrations/species have been discarded from the PNEC derivation (Klimisch Code 3) and have not undergone further quality assessment. The quality assessment sheets for these studies are not presented in Annex 1.

2.6.1 Toxicity to freshwater organisms

Single species acute toxicity data (i.e. from studies of ≤96 hours duration) are available for eight different taxonomic groups (i.e. algae protozoans, rotifers, crustaceans, molluscs, annelids, insects and fish) including the 'base set' taxa required for use of the assessment factor approach given in the EU Technical Guidance Document [23].

Chronic toxicity data (i.e. from studies of ≥96 hours duration) are available only for five taxonomic groups (i.e. algae, macrophytes, crustaceans, insects and fish). However, the results of a number of field studies with freshwater organisms have been reported.

Tables 2.6 and 2.7 summarise the long-term chronic data and short-term acute data, respectively. Figure 2.1 displays the cumulative distribution function of freshwater long-term data as given in Table 2.6 and Figure 2.2 displays the cumulative distribution f1unction of freshwater short-term data as listed in Table 2.7. These curves do not represent species sensitivity distributions but are simply a means of representing the range of long-term and short-term toxicity data available for freshwater species.

Fish and crustaceans are the most sensitive species with regard to both chronic and acute effects of iron. However, there is overlap between the sensitivities of representatives of different taxonomic groups. The range of acute toxicity values is over approximately two orders of magnitude. Algae appear to be of lower sensitivity, which is consistent with the fact that iron is an essential micronutrient required for growth. Effects of iron on freshwater biota have been reviewed by a number of authors [1, 2] who concluded that it is important, but often difficult, to distinguish between adverse effects due to iron in its dissolved state and the physical effects of particulate iron in conventional laboratory-based toxicity tests.

A number of field studies on the effects of iron on macroinvertebrates and fish are available, but several of these involve exposure of organisms to other metals in mixtures with iron, which clearly causes problems for the interpretation of the data. In addition, there was limited quantification of the actual exposure concentrations of dissolved and total iron in some instances.

A study of brown trout (*Salmo trutta*) in four Danish streams showed that normal hatching of caged eggs and survival of alevins occurred at concentrations of 0.11 mg l^{-1} dissolved iron (and 0.31 mg l^{-1} total iron) [24]. At mildly contaminated sites, there was an effect on egg viability at dissolved iron concentrations of 0.23 and 0.58 mg l^{-1} (0.46 and 0.82 mg l^{-1} total iron). This effect on eggs may have resulted from smothering by iron floc, as alevins were unaffected. Normal egg viability was reported at another site where the concentration of dissolved iron was 2.09 mg l^{-1} (3.02 mg l^{-1} total iron), though some alevin mortalities were recorded [24].

In 1988, Rasmussen and Lindegaard [25] published an investigation into the invertebrate fauna in the lowland River Vidaa in Denmark. This river had a pH range of between 6.7 and 8.8 and an annual average concentration of filterable iron up to 32 mg l^{-1}. At this site, intensification of agriculture over pyrite-rich soils and reduced water levels were held responsible for rising iron concentrations in the river. The study included determinations of both total iron and filterable iron. The data provide good evidence for an inverse relationship between filterable iron concentrations and invertebrate diversity. At concentrations below 0.2 mg l^{-1}, the communities were effectively undisturbed, with 67 taxa being represented and *Ephemeroptera* and *Plecoptera* being dominant. Increases in iron concentration from 0.2 to 0.3 mg l^{-1} correlated with reduced abundance of 14 taxa out of the 53 taxa recorded. The taxa lost (*Naididae*, *Ephemeroptera*, *Plecoptera* and certain *Chironomidae*) were predominantly grazers feeding on biofilm, which might be expected to be most sensitive to metalliferous precipitates. At 10 mg l^{-1} iron, only 10 taxa were recorded and the species surviving were those normally found in organically-enriched environments (*Tubificidae*, certain *Chironomidae* and *Tipulidae*). Effects on the numbers of individuals were also correlated with concentrations of filterable iron but only at levels in excess of 1.0 mg l^{-1}.

Another study [26] confirmed adverse effects of iron on invertebrates under field conditions. In the study, *Gammarus pulex* were deployed in the West Okement River in Devon, a site that is subject to metalliferous contamination from quarrying activities. A number of metals, including iron, were detected at elevated concentrations at the contaminated study site. Total iron concentrations ranged between 0.79 and 2.34 mg l^{-1} at the contaminated site compared with concentrations between 0.32 and 0.34 mg l^{-1} at a reference site. The study found that the feeding rate in caged *Gammarus* was reduced

and mortalities increased at the contaminated site relative to corresponding levels at the reference site. Based on measured bioaccumulation data, metals were implicated in these responses and a significant inverse correlation between iron body burdens and feeding rate was found (the only metal for which such a relationship was evident). The sensitivity of feeding rate in *Gammarus* when exposed to iron was subsequently confirmed in a laboratory study (see Table 2.6).

Figure 2.1 Cumulative distribution function of freshwater long-term data (mg l^{-1}) for iron

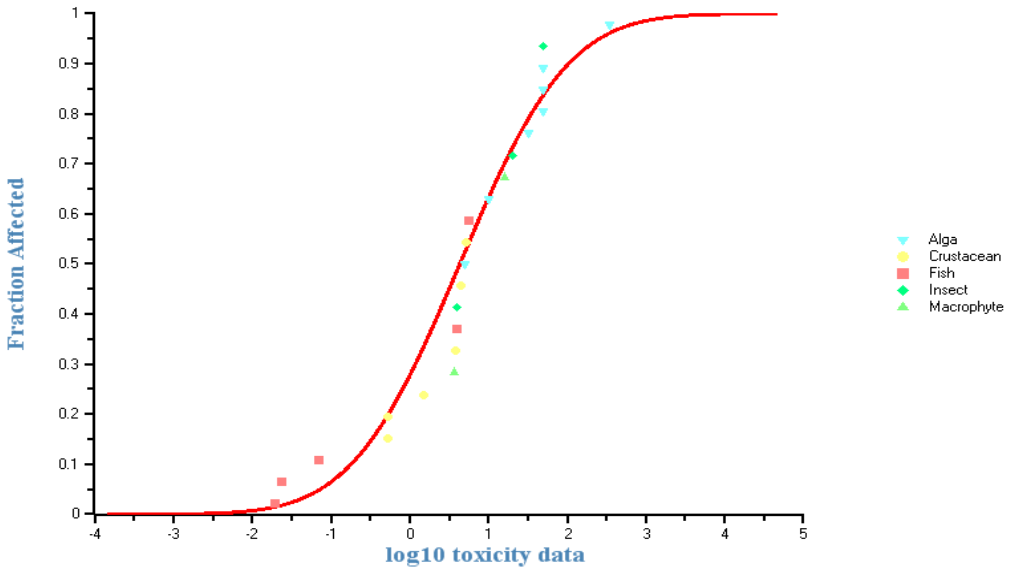

Figure 2.2 Cumulative distribution function of freshwater short-term data (mg l^{-1}) for iron

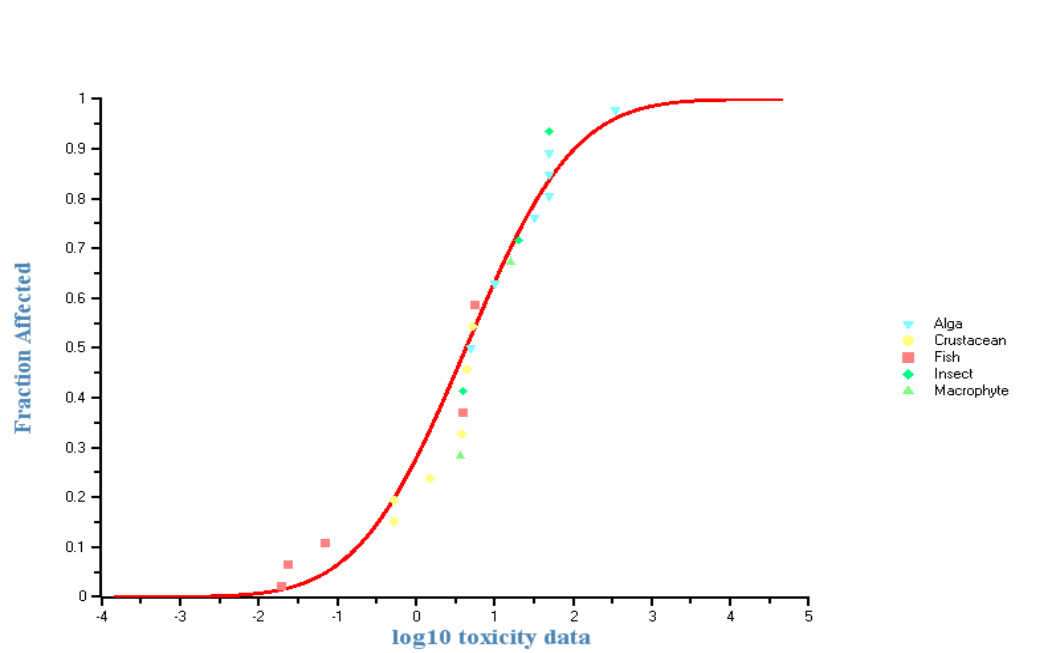

Table 2.6 Lowest available long-term aquatic toxicity data for freshwater organisms exposed to iron

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration (days)	Conc. (mg l^{-1})[1]	Exposure[2]	Toxicant analysis[3]	Comments (reliability index)[4]	Reference
Anabaena doliolum	Blue-green alga	ALG	LOEC	Chlorophyll content	15	10	s	n	As $FeCl_3$ (3)	[27]
Anabaena doliolum	Blue-green alga	ALG	NOEC	Growth	10	50	s	n	– (3)	[58]
Aulosira fertilissima	Blue-green alga	ALG	NOEC	Growth	10	50	s	n	– (3)	[58]
Nostoc punctiforme	Blue-green alga	ALG	NOEC	Growth	10	50	s	n	– (3)	[58]
Chlorella vulgaris	**Green alga**	**ALG**	**LOEC**	**Chlorophyll content**	**15**	**5**	**s**	**n**	**As $FeCl_3$ (3)**	**[27]**
Chlorella vulgaris	Green alga	ALG	NOEC	Growth	28	350	s	n	As $FeCl_3$ (3)	[59]
Chlorella vulgaris	Green alga	ALG	NOEC	Growth – stimulation	28	350	s	n	As $FeCl_4$ (3)	[59]
Chlorella vulgaris	Green alga	ALG	NOEC	Growth – stimulation	28	350	s	n	As $Fe(NO_3)_3$ (3)	[59]
Hydrodictyon reticulatum	Water net	ALG	LOEC	Growth	7	32	s	n	As $FeCl_3$; pH 7.5 (3)	[6]
Lemna minor	Duckweed	MAC	EC50	Growth	4	3.7	s	n	As $FeCl_3$; pH 7.5 (3)	[60]
Spriodela polyrrhiza	Macrophyte	MAC	NOEC	Growth	7	16	s	n	As $FeCl_3$; pH 7.5 (3)	[61]
Daphnia carinata	Water flea	CRU	NOEC	Growth	10	0.53	ss	y	–	[62]
Daphnia carinata	Water flea	CRU	NOEC	Reproduction	10	0.54	ss	y	–	[62]
Daphnia carinata	Water flea	CRU	NOEC	Mortality	10	1.5	ss	y	–	[63]
Daphnia magna	Water flea	CRU	LOEC	Reproduction	21	4.4	ss	y	As $FeCl_3$; pH 7.74	[64]
Daphnia magna	Water flea	CRU	LOEC	Reproduction	21	5.2	ss	y	As $FeCl_3$; pH 7.74	[64]
Daphnia magna	**Water flea**	**CRU**	**NOEC**	**Reproduction**	**21**	**0.16**	**ss**	**y**	**As $FeCl_3$; pH 7.0–8.0 (2)**	**[28]**
Asellus aquaticus	Isopod adult	CRU	LC50	Mortality	9	256–383	s	y	As $FeSO_4$; pH 4.5	[65]
Asellus aquaticus	Isopod adult	CRU	LC50	Mortality	9	431–467	s	y	As $FeSO_4$; pH 6.5	[65]

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration (days)	Conc. (mg l^{-1})[1]	Exposure[2]	Toxicant analysis[3]	Comments (reliability index)[4]	Reference
Gammarus minus	Amphipod coupled adults	CRU	NOEC	Mortality	7	4.2	f	y	As $FeSO_4$; pH 7.2	[66]
Gammarus pulex	Amphipod	CRU	NOEC	Feeding rate	6	1.0	s	y	As $FeSO_4$; pH 6.6–7.9	[26]
Gammarus pulex	Amphipod	CRU	LOEC	Feeding rate	6	2.0	s	n	As $FeSO_4$; pH 6.6–7.9 (3)	[26]
Acroneuria lycorias	Stonefly larvae	INS	LC50	Mortality	9	16	s	n	As $FeSO_4$; pH 7.25 (3)	[32]
Chematopscyche sp.	Caddisfly	INS	NOEC	Emergence	4	4.0	s	n	As $FeSO_4$; pH 7.25 (3)	[32]
Hydropsyche betteni	Caddisfly	INS	LC50	Mortality	7	16	s	n	As $FeSO_4$; pH 7.25 (3)	[32]
Leptophlebia marginata	Mayfly (larvae)	INS	NOEC	Mortality	30	20	ss	y	As $FeSO_4$; pH 4.5	[15]
Leptophlebia marginata	Mayfly (larvae)	INS	LOEC	Mortality	30	50	ss	y	As $FeSO_4$; pH 4.5	[15]
Leptophlebia marginata	Mayfly (larvae)	INS	NOEC	Mortality	30	50	ss	y	As $FeSO_4$; pH 7.0	[15]
Brachydanio rerio	Zebrafish (fertilised eggs)	FIS	LOEC	Hatching	14	4.0	s	n	As $FeCl_3$; pH 4.0 (3)	[67]
Coregonus lavaretus	**Whitefish**	**FIS**	**NOEC**	**Physiology**	**30**	**0.07**	**ss**	**y**	**As $FeSO_4$ (2)**	**[29]**
Onchorynchus mykiss	Rainbow trout eggs	FIS	LOEC	Embryogenesis	28	5.7	f	y	As $FeSO_4$; pH 6.8	[68]
Salmo trutta	**Brown trout**	**FIS**	**NOEC**	**Skeletal effects**	**30**	**0.0235**	**f**	**y**	**As $FeCl_3$; pH 6.5 (3)**	**[30]**
Salmo trutta	**Brown trout**	**FIS**	**LOEC**	**Skeletal effects**	**30**	**0.0196**	**f**	**y**	**As $FeCl_3$; pH 4.5 (3)**	**[30]**

[1] The lowest NOECs for algae, invertebrates and fish are highlighted in bold font.
[2] Exposure: s = static; ss = semi-static; f = flow-through.
[3] Toxicant analysis: y = measured; n = not measured.
[4] See Annex 1.
ALG = algae; CRU = crustaceans; FIS = fish; INS = insects
LOEC = lowest observed effect concentration; NOEC = no observed effect concentration; LC50 = concentration lethal to 50% of the organisms tested

Table 2.7 Lowest available short-term aquatic toxicity data for freshwater organisms exposed to iron

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration (hours)	Conc. (mg l^{-1})[1]	Exposure[2]	Toxicant analysis[3]	Comments (reliability index)[4]	Reference
Anabaena circinalis	**Blue green alga**	**ALG**	**EC50**	**Growth**	**24**	**15**	**s**	**n**	**–** (3)	**[31]**
Microcystis aeruginosa	**Blue green alga**	**ALG**	**EC50**	**Growth**	**24**	**15**	**s**	**n**	**–** (3)	**[31]**
Tetrahymena pyriformis	Protozoa	PRO	EC50	Growth	9	105	s	n	As $FeCl_3$ (3)	[69]
Brachionus calyciflorus	Rotifer	ROT	LC50	Mortality	24	35	s	n	As $FeCl_4$ (3)	[70]
Streptocephalus proboscideus	Rotifer	ROT	LC50	Mortality	24	216	s	n	As $FeCl_3$ (3)	[70]
Asellus aquaticus	Isopod	CRU	LC50	Mortality	96	124	ss	n	– (3)	[71]
Daphnia magna	Water flea (<24 hours old)	CRU	EC50	Immobilisation	48	7.2	s	n	As $FeSO_4$; pH 7.2–7.8 (3)	[72]
Daphnia magna	Water flea (<24 hours old)	CRU	LC50	Mortality	24	14.3	s	n	As $FeSO_4$; pH 7.4 (3)	[73]
Daphnia magna	Water flea (<24 hours old)	CRU	LC50	Mortality	48	9.6	s	y	As $FeCl_3$; pH 7.74	[64]
Daphnia pulex	Water flea (<24 hours old)	CRU	EC50	Mortality	24	100	s	n	As $FeSO_4$; pH 7.4 (3)	[73]
Crangonyx pseudogracilis	Amphipod adult (4 mm)	CRU	LC50	Mortality	96	120	ss	n	As $FeCl_3$; pH 6.75 (3)	[71]
Crangonyx pseudogracilis	Amphipod adult (4 mm)	CRU	LC50	Mortality	96	95	ss	n	As $FeSO_4$; pH 6.75 (3)	[71]
Bulinus globosus	Snail	MOL	LC50	Mortality	96	24.5	s	n	As $FeCl_3$; pH 7.15 (3)	[74]
Tubifex tubifex	Blood worm	ANN	LC50	Mortality	96	102	ss	n	As $FeCl_3$; pH 7.6 (3)	[75]
Ephemerella subvaria	**Mayfly**	**INS**	**LC50**	**Mortality**	**96**	**0.32**	**s**	**n**	**As $FeSO_4$; pH 7.25 (3)**	**[32]**
Leptophlebia marginata	Mayfly (larvae)	INS	LC50	Mortality	96	90	ss	y	As $FeSO_4$; pH 4.5 (2)	[15]
Leptophlebia marginata	Mayfly (larvae)	INS	LC50	Mortality	96	106	ss	y	As $FeSO_4$; pH 6.5 (2)	[15]

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration (hours)	Conc. (mg l^{-1})[1]	Exposure[2]	Toxicant analysis[3]	Comments (reliability index)[4]	Reference
Brachydanio rerio	Zebrafish (larvae)	FIS	NOEC	Mortality	48	32	s	n	As $FeCl_3$ (3)	[67]
Cyprinus carpio	Carp 3.2 cm fry	FIS	LC50	Mortality	96	0.96	ss	n	As $FeSO_4$; pH 7.1 (3)	[56]
Cyprinus carpio	Carp 6.9 cm fry	FIS	LC50	Mortality	96	1.74	ss	n	As $FeSO_4$; pH 7.1 (3)	[56]
Oncorhynchus mykiss	Rainbow trout juveniles	FIS	LC50	Mortality	96	2.9	f	y	As $Fe_2(SO_4)_3$; pH 7.2–7.5	[1]
Oryzias latipes	Medaka (8 day fry)	FIS	LC50	Mortality	24	1.0–10	s	n	As $NH_4Fe(SO_4)_2$; pH 6.9 (3)	[76]
Salmo trutta	Brown trout juveniles	FIS	LC50	Mortality	96	8.5	f	y	As $Fe_2(SO_4)_3$; pH 7.2–7.5	[1]
Salvelinus fontinalis	**Brook trout 14 months old**	**FIS**	**LC50**	**Mortality**	**96**	**0.41 (d)**	**f**	**y**	**As $FeSO_4$; pH 5.5 (2)**	**[33]**
Salvelinus fontinalis	Brook trout 14 months old	FIS	LC50	Mortality	96	0.48 (d)	f	y	As $FeSO_4$; pH 6.0 (2)	[33]
Salvelinus fontinalis	Brook trout 14 months old	FIS	LC50	Mortality	96	1.75 (d)	f	y	As $FeSO_4$; pH 7.2 (2)	[33]

[1] The lowest L(E)C50s for algae, invertebrates and fish are highlighted in bold font; d = dissolved.
[2] Exposure: s = static; ss = semi-static; f = flow-through.
[3] Toxicant analysis: y = measured; n = not measured.
[4] See Annex 1.
ALG = algae; ANN = annelids; CRU = crustaceans; FIS = fish; INS = insects; MOL = molluscs; PRO = protozoans; ROT = rotifers
NOEC = no observed effect concentration
EC50 = concentration effective against 50% of the organisms tested
LC50 = concentration lethal to 50% of the organisms tested

2.6.2 Toxicity to saltwater organisms

Single species acute toxicity data are available for five different taxonomic groups (i.e. crustaceans, molluscs, annelids, echinoderms and fish), but do not include all necessary data for the 'base set' taxa required for use of the assessment factor approach given in the EU Technical Guidance Document [23]. This is because no acute toxicity data have been found for effects on marine algae.

Chronic toxicity data are available only for two taxonomic groups (i.e. algae and crustaceans). No chronic data have been found for fish.

Tables 2.8 and 2.9 summarise the long-term chronic data and short-term acute data, respectively. Figure 2.3 displays the cumulative distribution function of marine long-term data as given in Table 2.8 and Figure 2.4 displays the cumulative distribution function of marine short-term data as listed in Table 2.9. These curves do not represent species sensitivity distributions but are instead a means of representing the range of long-term and short-term toxicity data available for marine species.

As for freshwater organisms, fish are apparently the most sensitive taxonomic group with respect to the acute toxicity of iron, although again there is overlap between the sensitivities of representatives of different taxonomic groups. However, the range of acute toxicity values is within approximately two orders of magnitude. Conclusions about the long-term toxicity of different taxonomic groups cannot be drawn due to lack of reliable data. The results of the available long-term tests with marine crustaceans and fish are in the range of results obtained for the related freshwater species.

The short-term data for marine algae indicate that this taxonomic group is of lower sensitivity than other groups. This finding is consistent with the fact that iron is an essential micronutrient for these organisms (required for growth).

Figure 2.3 Cumulative distribution function of saltwater long-term data (mg l^{-1}) for iron

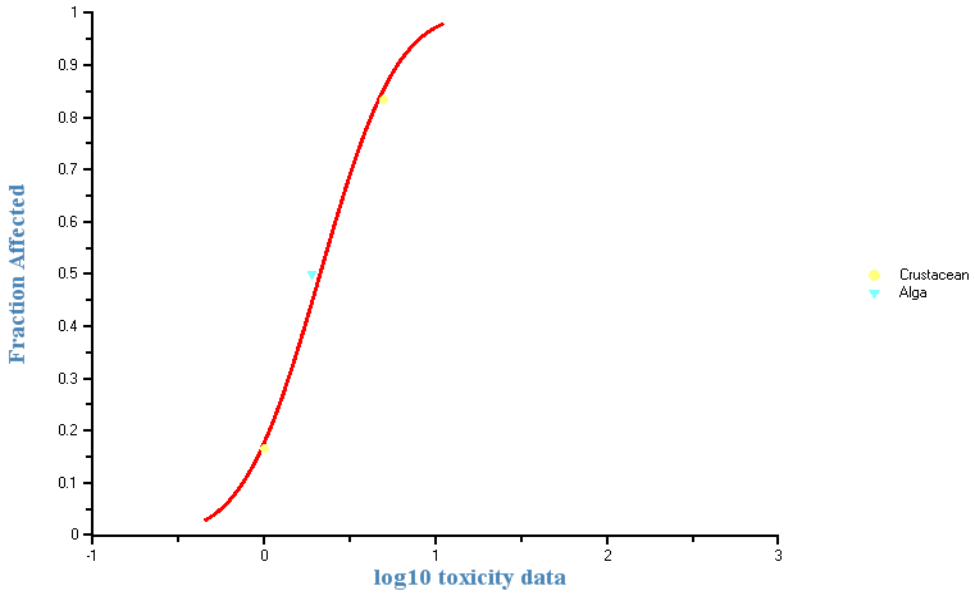

Figure 2.4 Cumulative distribution function of saltwater short-term data (mg l^{-1}) for iron

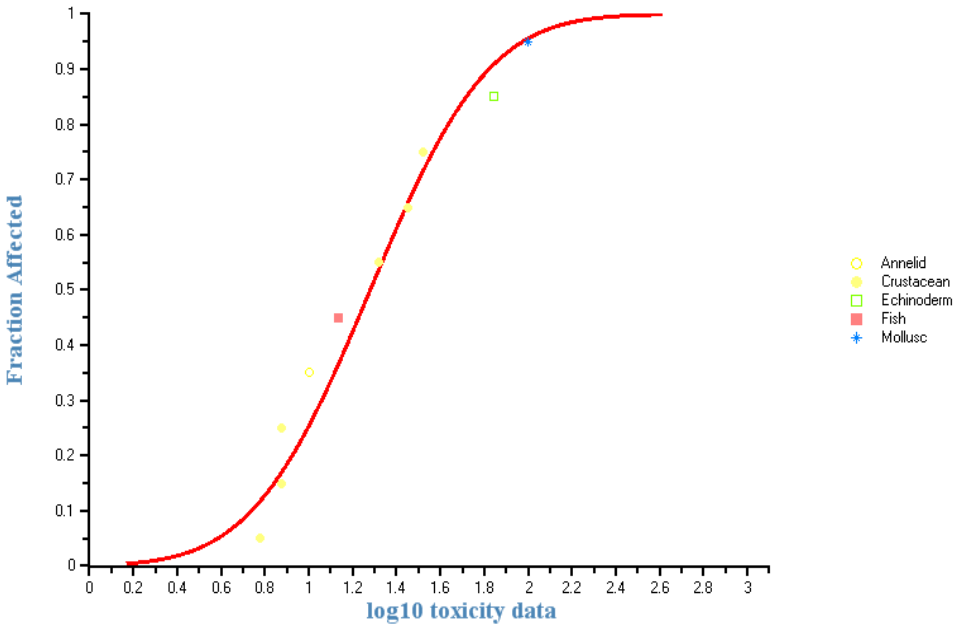

Table 2.8 Lowest available long-term aquatic toxicity data for saltwater organisms exposed to iron

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration (days)	Conc. (mg l^{-1})[1]	Exposure[2]	Toxicant analysis[3]	Comments (reliability index)[4]	Reference
Haslea ostrearia	**Algae**	**ALG**	**NOEC**	**Growth**	**10**	**1.9**	**s**	**n**	**As $FeCl_3$**	**[34]**
Temora longicornis	Copepod	CRU	NOEC	Reproduction	18	5	ps	n	Iron waste; pH 6.9 (3)	[36]
Temora longicornis	Copepod	CRU	LOEC	Reproduction	18	6	ps	n	Iron waste; pH 6.9 (3)	[36]
Cancer anthonyi	**Yellow crab eggs**	**CRU**	**NOEC**	**Hatching**	**7**	**1.0**	**s**	**n**	**As $FeCl_3$; pH 7.8 (3)**	**[35]**
Cancer anthonyi	Yellow crab eggs	CRU	LOEC	Hatching	7	100	s	n	As $FeCl_3$; pH 7.8 (3)	[35]
Cancer anthonyi	Yellow crab eggs	CRU	NOEC	Hatching	7	100	s	n	As $FeCl_3$; pH 7.8 (3)	[35]
Cancer anthonyi	Yellow crab eggs	CRU	LOEC	Hatching	7	1,000	s	n	As $FeCl_3$; pH 7.8 (3)	[35]

[1] The lowest NOECs for algae, invertebrates and fish are highlighted in bold font.
[2] Exposure: s = static; ps = presumably static.
[3] Toxicant analysis: n = not measured.
[4] See Annex 1.
ALG = algae; CRU = crustaceans
LOEC = lowest observed effect concentration
NOEC = no observed effect concentration

Table 2.9 Lowest available short-term aquatic toxicity data for marine organisms exposed to iron

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration (hours)	Conc. (mg l^{-1})[1]	Exposure[2]	Toxicant analysis[3]	Comments (reliability index)[4]	Reference
Artemia salina	Brine shrimp cysts	CRU	LC50	Mortality	24	28.3	s	n	As $FeCl_4$ (3)	[70]
Calanus finmarchicus	Copepod	CRU	NOEC	Mortality	24	7.5	ps	n	Iron waste, pH 6.9 (3)	[36]
Nitocra spinipes	Copepod	CRU	LC50	Mortality	96	21	s	n	As $FeCl_3$; pH 8.0 (3)	[77]
***Pseudocalanus* sp.**	**Copepod**	**CRU**	**LOEC**	**Mortality**	**24**	**6**	**ps**	**n**	**Iron waste; pH 6.9 (3)**	**[36]**
Temora longicornis	Copepod	CRU	NOEC	Mortality	48	7.5	ps	n	Iron waste; pH 6.9 (3)	[36]
Crangon crangon	Shrimp adult	CRU	LC50	Mortality	48	33–100	ps	n	As Fe^{3+} (3)	[78]
Cardium edule	Cockle adult	MOL	LC50	Mortality	48	100–330	ps	n	As Fe^{3+} (3)	[78]
Ophryotrocha diadema	Adult	ANN	LC50	Mortality	48	10–33	ps	y	As $FeCl_3$	[57]
Arbacia punctulata	Plutei larvae	ECH	NOEC	Development	17.5	70	ps	n	As $FeCl_3$ (3)	[79]
Morone saxatilis	**Striped bass**	**FIS**	**LC50**	**Mortality**	**96**	**13.6**	**s**	**n**	**As $FeCl_2$ (3)**	**[37]**

[1] The lowest L(E)C50s for invertebrates and fish are highlighted in bold font.
[2] Exposure: s = static; ps = presumably static.
[3] Toxicant analysis: y = measured; n = not measured.
[4] See Annex 1.
ANN = annelids; CRU = crustaceans; ECH = echinoderms; FIS = fish; MOL = molluscs
NOEC = no observed effect concentration
LOEC = lowest observed effect concentration
LC50 = concentration lethal to 50% of the organisms tested

2.6.3 Toxicity to sediment-dwelling organisms

No sediment toxicity data specifically for iron have been found. Iron exerts toxic effects in the water column, but can also precipitate from it under appropriate water quality conditions resulting in a smothering effect on benthic organisms.

2.6.4 Endocrine-disrupting effects

There is no evidence to suggest that iron is an endocrine-disrupting substance.

2.6.5 Mode of action of iron

Under typical conditions for laboratory aquatic toxicity tests (i.e. test solutions that are well-aerated and at near neutral pH), rapid oxidation of Fe(II) to Fe(III) would be expected, subsequently precipitating as hydroxides in the test vessels. The period over which test organisms would actually be exposed to Fe(II) is unknown because the kinetics of oxidation are not well understood. However, it is possible that test animals in such tests are exposed to Fe(III) for much longer than they are exposed to Fe(II) [1, 2].

Iron toxicity in the aquatic environment may arise via a number of possible routes:

- toxicity of Fe(II) or Fe(III) in their dissolved states;
- pH effects caused by the fact that certain iron salts (e.g. $FeCl_3$) are strong acids;
- destabilisation of the substrate by flocs of ferric hydroxide.
- 'smothering' effects of iron precipitates, particularly the ferric oxides and hydroxides, which predominate under neutral and oxic conditions. This could impair gill function, prevent algal growth and impede movement of affected organisms [15].

2.6.6 Fate and occurrence of relevant metabolites in the aquatic environment

The properties and hence the bioavailability of iron in water depend on its speciation. However, the speciation of iron in natural water is dependent upon pH and electron activity (pE). Iron that has precipitated out of solution as $FeOH_3$ may not be as bioavailable to aquatic organisms as, for example, loosely bound organic complexes. In addition to the hydrated and hydroxyl species, iron may bind reversibly to inorganic anions or to organic compounds as metal complexes or organometallic compounds. Generally speaking, it is only at low pH and under anoxic conditions that iron occurs in true solution [as Fe(II)].

Iron exists in numerous forms in water and undergoes chemical reactions such as acid–base (Equation 1), precipitation (Equation 2) and oxidation (Equation 3) reactions.

Equation 1 $Fe(H_2O)_6^{3+} \rightarrow FeOH(H_2O)_5^{2+} + H^+$

Equation 2 $Fe(H_2O)_6^{3+} \rightarrow Fe(OH)_3(s) + 3H_2O + 3H^+$

Equation 3 $Fe(H_2O)_6^{2+} \rightarrow Fe(OH)_3(s) + 3H_2O + e^- + 3H^+$

These reactions transform the metal ion in water to more stable forms.

The hydrated Fe(III) ion is a relatively strong acid and, therefore, at neutral pH values or above hydrated Fe(III) is minus at least one hydrogen ion. This means that some discharges, such as acid mine water, will derive part of their acidic character from the hydrated Fe(III) as shown in Equation 4.

Equation 4 $Fe(H_2O)_6^{3+} \rightarrow Fe(OH)_3(s) + 3H_2O + 3H^+$

Oxidation–reduction (redox) reactions are those that involve changes in the oxidation state of the reactants. Iron is oxidised when it loses an electron and passes from the +2 to +3 valence state (Equation 5). When the reaction is reversed with the ion gaining an electron, it is referred to as reduction (Equation 6).

Equation 5 $Fe^{2+} \rightarrow Fe^{3+} + e^-$

Equation 6 $Fe^{3+} + e^- \rightarrow Fe^{2+}$

Oxidation–reduction processes are significant in the environmental chemistry of iron in natural waters and wastewaters.

The transfer of electrons in a redox reaction is accompanied by H^+ ion transfer and there is a close relationship between redox and acid–base processes. For example, if Fe(II) loses an electron at pH 7, three hydrogen ions are also lost to form highly insoluble ferric hydroxide.

In the previous EQS reports for iron [1, 2], it was concluded that most taxa do not appear to bioaccumulate iron to any significant extent, or if accumulation is evident, associated bioconcentration factors are usually less than 100. Tissue concentrations can vary seasonally and for marine organisms may vary with salinity. In addition, although iron may accumulate in aquatic organisms, it is rapidly excreted once the organism is transferred to clean water [1].

An exception to this statement is the data for algae and higher plants, some of which do appear to have the potential to accumulate high concentrations of iron [7, 16]. However, much of this may occur as precipitates on the cell surface, rather than actual absorption.

Only limited data have been published on bioaccumulation of iron since the previous review [2]. Data for aquatic invertebrates such as the bivalve *Ruditapes decussates* [17] and the brown shrimp *Penaeus aztecus* [18] indicate that there was limited accumulation of iron in the exposed organisms.

For algae and higher plants, the additional data support the previous view that bioaccumulation of iron can occur. For example, Gosavi *et al.* [19] demonstrated that iron accumulated in four genera of macroalgae (*Ulva* sp. *Enteromorpha* sp., *Cladophora* sp. and *Chaetomorpha* sp.). However, iron bioaccumulation in plants can be influenced by environmental factors. For example, Pinto *et al.* [20] found that organic matter decreased the uptake of iron by sorghum plants.

Previously, Wong and Tam [16] studied the bioconcentration of iron in artificial food chains and found no evidence for biomagnification of iron. Indeed, the contrary was evident with the data indicating a ‘dilution’ of iron as it was consumed by higher

organisms. Furthermore, previous studies with invertebrates and fish also support the view that bioconcentration factors for these taxa are low (e.g. [21]) and that iron uptake is regulated [22].

3. Derivation of quality standards for iron

3.1 Use of the added risk approach

The EU Technical Guidance Document (TGD) [23] does not provide specific guidance on dealing with (essential) elements such as iron that have a natural background concentration in the environment. However, according to Struijs *et al.* [53] and Crommentuijn *et al.* [54], 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 iron, resulting in an 'added' PEC (PEC_{add}) and an '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.[4] 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, causes the same effect. In such circumstances, however, all environmental

[4] For aquatic organisms, which are mainly exposed via water, the dissolved iron species are especially relevant for toxicity. Therefore, the dissolved iron concentration in water is a better indicator of toxicity than the total iron concentration. In practice, the dissolved fraction is defined as the fraction that passes through a 0.45 µm filter. All waterborne iron concentrations mentioned in this report refer to the dissolved iron concentration, whereas in the case of sediment they refer to the total iron concentration.

parameters determining metal toxicity must be equal except 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 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.

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

4.1 Derivation of PNECs by the TGD deterministic approach (AF method)

4.1.1 PNECs for freshwaters

PNEC accounting for the annual average concentration
For the freshwater environment, data are available for the 'base set' of toxicity tests (i.e. tests with algae, crustaceans and fish) and thus the TGD assessment factor method can be applied. Among the taxonomic groups for which long-term toxicity tests are available (algae, macrophytes, crustaceans, insects and fish), fish and crustaceans are evidently the most sensitive species (see Table 2.6).

The lowest long-term (lt) toxicity value found was from a study which examined the effects of various trace metals under acidic conditions (either at pH 4.5 or 6.5) on yolk-sac fry of brown trout *Salmo trutta* [30]. Iron was administered as $FeCl_3$ at a single concentration, regarded as typical of the concentration that may be encountered in soft acidic waters in the UK. However, it was observed that only 50–60 per cent of the iron administered remained in solution. At pH 4.5, the measured concentration of iron was 0.0196 mg l^{-1} whereas at pH 6.5 it was 0.0235 mg l^{-1}. At both pH values, no significant increase in mortalities was found relative to the controls. However, at pH 4.5, skeletal abnormalities (impaired calcification) were observed in a high proportion of surviving fry. Although the study involved the use of a flow-through system and the measurement of exposure concentrations, there are a number of concerns regarding its quality. First, it is possible that pH itself could be a contributing factor to the observed effects at pH 4.5. Secondly, the threshold concentration for effects appears to be extremely low and substantially less than background concentrations. Given these concerns and also the lack of a concentration–response relationship, this study is not considered suitable for use in the derivation of the PNEC.

A study using 1-year-old whitefish *Coregonus lavaretus* investigated the effects of exposure to three types of iron-rich water (natural iron-rich humic water, humic-free water with added inorganic iron concentrations, and natural iron-rich humic water with added inorganic iron) on the bioaccumulation of iron and resulting physiological effects [29]. The 30-day studies were performed semi-statically by replacing 30 per cent of the water and 30 per cent of the original iron every other day; the total and dissolved iron concentrations in each test vessel were confirmed by chemical analysis. The iron was added as a 1:1 solution of $FeSO_4/FeCl_2$. In natural iron-rich humic water, both the bioaccumulation and physiological effects of iron exposure (at 0.1 and 0.35 mg l^{-1} dissolved iron) were negligible. In the humic-free water with iron added at 2 and 8 mg l^{-1}

(0.07 and 0.2 mg l^{-1} dissolved iron), no accumulation of iron or physiological effects were found at the 2 mg l^{-1} added iron (0.07 mg l^{-1} dissolved iron) concentration. In the humic-free water with 8 mg l^{-1} added iron (0.2 mg l^{-1} dissolved iron), accumulation was accompanied by decreased glycogen phosphorylase activities and micrososmal EROD[5] activity in the liver, and decreased plasma sodium and potassium concentrations. The overall significance of the physiological endpoints measured in this study on the long-term health of fish is unclear, so it is not considered appropriate to use these data directly for the derivation of the PNEC. However, the derived PNEC should be set to ensure that it protects against these effects.

The lowest chronic toxicity value for freshwater invertebrates is a 21-day zero equivalent point (ZEP) for the cladoceran *Daphnia magna*, which can be regarded as being equivalent to a NOEC [28]. The study investigated the effects of waterborne iron (added as $FeCl_3$) on the growth, reproduction and survival and haemoglobin content in the test organisms over a range of iron concentrations in hard reconstituted water (250 mg l^{-1} $CaCO_3$). The study was carried out using a semi-static design and exposure concentrations were analysed throughout the test. The study found that in the hard water at a pH range of 7.0 to 8.0 the ZEP for reproduction was 0.16 mg l^{-1} Fe and concluded that continuous exposure to higher concentrations would lead to extinction of *Daphnia magna* populations. This data is supported by a similar study using a related species *Daphnia carinata* which found that the 10-d NOEC values for growth and reproduction were 0.53 and 0.54 mg l^{-1} dissolved iron (see Table 6.1). These studies also incorporated a semi-static design and chemical analysis of exposure concentrations. In addition, a 6-day NOEC of 1.0 mg l^{-1} for feeding rate was reported for a study on two populations of the freshwater amphipod *Gammarus pulex* (see Table 2.6) [26]. The study was conducted at neutral pH and, although iron was added as ferrous sulfate, it is likely that the insoluble form of iron would have predominated under the experimental regime. In the semi-static exposure study, test concentrations were measured daily.

Data are available for the effects of iron on a range of freshwater algal (blue-green and green) and macrophyte species (see Table 2.6). These studies showed that the NOEC values were reported at higher concentrations than for crustaceans and fish (i.e. 3.7–350 mg l^{-1} dissolved iron). However, there are issues with the validity of the algal data since none of the studies involved analytical confirmation of the exposure concentrations.

Given the available data (and an assessment of its validity and relevance), it is proposed that the $PNEC_{freshwater\_lt}$ is derived below on the basis of the 21-day ZEP (NOEC equivalent) of 0.16 mg l^{-1} for reproduction of the cladoceran *Daphnia magna* [28] and an assessment factor of 10 given the large body of data for different taxa:

$PNEC_{add,freshwater\_lt}$ = 0.16 mg l^{-1}/AF (10) = 0.016 mg l^{-1} iron (dissolved)

PNEC accounting for transient concentration peaks
Single species acute toxicity data are available for eight different taxonomic groups, i.e. algae, protozoans, rotifers, crustaceans, molluscs, annelids, insects and fish, including the 'base set' taxa (see Table 2.7).

[5] 7-ethoxyresorufin-*O*-deethylase

Data are available for the short-term (st) effects of iron on blue-green algae species (see Table 2.7). These studies showed that EC50 values were reported at higher concentrations for these species than for crustaceans and fish. In addition, there are issues with the validity of the data since the study did not involve analytical confirmation of the exposure concentrations.

The lowest reported acute toxicity value for iron is a 96-hour LC50 value of 0.32 mg l^{-1} for larvae of the mayfly *Ephemerella subvaria* [32]. The static test was conducted at pH 7.25 in soft water (hardness 44 mg l^{-1} $CaCO_3$) at a temperature of 18°C. However, this result is at variance with results for similar species and there is uncertainty because exposure concentrations and the predominant iron species were not measured. The report proposing EQSs for iron [1] considered the result for *Ephemerella subvaria* to be an outlier. Given the marked differences in this value from other relevant data and the absence of confirmatory analysis, it has not been used to derive a PNEC accounting for transient concentration peaks.

A later study, which assessed the short-term effects of iron ($FeSO_4$) on the mortality of the mayfly *Leptophlebia marginata*, found 96-hour LC50 values of 90 mg l^{-1} at pH 4.5 and 106 mg l^{-1} at pH 6.5 [15]. The semi-static exposure was conducted in water hardness of 90–106 mg l^{-1} $CaCO_3$ at a temperature of 10 ± 1°C. Detailed analysis of test solutions showed that, at both pH 4.5 and 6.5, most of the iron (90–97 per cent) was present as Fe(II). This is to be expected at the lower pH but is surprising at the higher pH where oxidation to Fe(III) would be expected. However, it is likely that fresh test solutions were analysed before the ion was oxidised to Fe(III) in the test vessels. Given the low sensitivity of this species compared with other taxa, this study has been used as supporting information in the derivation of the PNEC.

The lowest valid acute toxicity value for iron is a 96-hour LC50 of 0.41 mg l^{-1} for 14-month-old brook trout (*Salvelinus fontinalis*) exposed to $FeSO_4$ at a pH of 5.5 [33]. In this study the exposure concentrations were analysed and the levels of dissolved iron were measured. For fish exposed to iron at pH values of 6.0 and 7.0, 96-hour LC50 values of 0.48 and 1.75 mg l^{-1}, respectively, were obtained. The data for *Salvelinus fontinalis* are supported by a 96-hour LC50 of 0.96 mg l^{-1} for effects on the fry of carp (*Cyprinus carpio*) in a semi-static regime at pH 7.1 (see Table 2.7) [56]. However, exposure concentrations in that study were not confirmed by chemical analysis.

A PNEC for effects following short-term exposure to iron is derived below on the basis of the short-term LC50 data for brook trout *Salvelinus fontinalis* and guidance given in the TGD on effects assessment for intermittent releases (Section 3.3.2 of Part II of the TGD [23]). Given the large body of available data, a lower assessment factor of 10 (instead of 100) is used in order to extrapolate from the 50 per cent acute effect level to the short-term no-effect level:

$PNEC_{add,freshwater\_st}$ = 0.41 mg l^{-1}/AF (10) = 0.041 mg l^{-1} iron (dissolved)

4.1.2 PNEC based on outdoor simulated ecosystem studies or field studies

Data are available from a series of field studies (see Section 2.6.1) which have evaluated the relationship between receiving water iron concentrations and resulting effects on either macroinvertebrates [25, 26] or fish [24].

The lowest reliable value reported for the field was from a study by Rasmussen and Lindegaard [25] which showed good evidence for an inverse relationship between filterable iron concentrations and invertebrate diversity. At concentrations below 0.2 mg l^{-1}, the communities were effectively undisturbed, with 67 taxa represented and *Ephemeroptera* and *Plecoptera* being dominant. Increases in iron concentration from 0.2 to 0.3 mg l^{-1} correlated with reduced abundance of 14 taxa out of the 53 taxa recorded. The taxa lost (*Naididae*, *Ephemeroptera*, *Plecoptera* and certain *Chironomidae*) were predominantly grazers feeding on biofilm and were thus expected to be the ones most sensitive to metalliferous precipitates. At 10 mg l^{-1} iron, only 10 taxa were recorded and the species surviving were those normally found in organically enriched environments (*Tubificidae*, certain *Chironomidae* and *Tipulidae*). Effects on the numbers of individuals also correlated with concentrations of filterable iron but only at levels >1 mg l^{-1}. The study was well conducted and the data have been subjected to appropriate analysis. A clear relationship was obtained between measured filterable iron and biological diversity. The only source of uncertainty stems from the possibility that other contaminants present at the study site could have contributed to the observed effects.

Data from field studies has not been used in establishing PNECs because of the greater perceived uncertainty associated with the measurements of iron exposure concentrations compared with laboratory studies.

4.1.3 PNECs for saltwaters

The toxicity database for marine species is smaller than that for freshwater organisms. Acute (short-term) toxicity data are available for five different taxonomic groups, i.e. crustaceans, molluscs, annelids, echinoderms and fish. No acute toxicity data have been found for algae, though on the basis of freshwater data, algae may not be expected to be the most sensitive group.

Chronic toxicity data are only available for algae (one species) and crustaceans (two species). No chronic data have been found for fish, which were a sensitive group in terms of acute toxicity to freshwater organisms.

The available toxicity data for marine taxa do not appear to differ markedly from the range of values obtained for related freshwater species (see Tables 2.6–2.9). Since there are no differences in the sensitivity of freshwater and saltwater species of the same taxonomic group, the TGD approach of using freshwater data for the marine effect assessment can be used. Therefore, the suggested freshwater PNECs for setting of quality standards have been incorporated into the derivation of corresponding PNEC values for marine water bodies.

PNEC accounting for annual average concentration

Long-term single species toxicity data for marine organisms are available only for algae and crustaceans (see Table 2.8). No chronic toxicity data are available for fish, which were a sensitive freshwater taxonomic group.

In addition, all the available chronic saltwater data are based on nominal concentrations. Consequently, none would be suitable for PNEC derivation.

Given the absence of chronic toxicity data for fish, it is not considered appropriate to derive a $PNEC_{saltwater\_lt}$ based on only marine toxicity data. Instead the $PNEC_{saltwater\_lt}$

should be based on the lowest reliable long-term freshwater data point (21-day ZEP (NOEC equivalent) of 0.16 mg l^{-1} for reproduction of *Daphnia magna* [28]) and an increased assessment factor of 100, to account for the lack of good quality saltwater long-term data:

$PNEC_{add,saltwater\_lt}$ = 0.16 mg l^{-1}/AF (100) = 0.0016 mg l^{-1} iron (dissolved)

PNEC accounting for transient concentration peaks
Single species acute toxicity data for marine organisms are available for five different taxonomic groups, i.e. crustaceans, annelids, molluscs, echinoderms and fish (see Table 2.9). No acute toxicity data are available for algae, though on the basis of freshwater data, algae may not be expected to be the most sensitive group.

Although data are available for a range of taxa, there are issues with the validity of most of these data since there was no chemical analysis of the exposure concentrations. The only study for which there were measured exposure concentrations was the short-term study on the mortality of adults of the polychaete worm *Ophryotrocha diadema* (see Table 2.9) [57]. However, this study was carried out under static conditions and indicates lower sensitivity in this species than found in freshwater species.

Given the limited acute toxicity data set for marine taxa and the uncertainty associated with the available data, it is not considered appropriate to derive a $PNEC_{saltwater\_st}$ based on marine toxicity data. Instead the $PNEC_{saltwater\_st}$ should be based on the lowest reliable short-term freshwater data point (LC50 of 0.41 mg l^{-1} for brook trout *Salvelinus fontinalis*) and an increased assessment factor of 100, to account for the lack of good quality saltwater short-term data:

$PNEC_{add,saltwater\_st}$ = 0.41 mg l^{-1}/AF (100) = 0.0041 mg l^{-1} iron (dissolved)

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

The minimum number of long-term toxicity data (at least 10 NOECs from eight taxonomic groups) is not available. Therefore, the species sensitivity distribution approach cannot be used for PNEC derivation.

4.3 Derivation of existing EQSs

In 1988, EQSs were proposed for iron [1] and statutory standards for dissolved (filterable) iron were subsequently adopted for the protection of freshwater and marine life. These standards were reviewed in a 1998 update [2] (see Section 2.2).

As in the original report, the update placed emphasis on the use of field data for the derivation of standards. Freshwater field studies reported evidence for biological effects at concentrations of filterable iron around or even below the statutory EQS, although it was possible that other substances may have contributed to the effects seen. Therefore, the statutory standard of 1.0 mg l^{-1} filterable iron expressed as an annual average was retained for the protection of freshwater life.

There were no new saltwater field data available for use in the update, and laboratory toxicity data published since the 1988 report showed similar effect concentrations to those reported previously. Therefore, an annual average of 1.0 mg l^{-1} filterable iron for the protection of saltwater life was retained.

4.4 Derivation of PNECs for sediment

Since iron is a metal, the application of the log Kow cut-off for the derivation of a PNEC for the sediment is not relevant. However, iron will sorb to organic matter, to an extent that depends on a number of factors (see Section 2.5). However, no sediment toxicity data have been located specifically for iron and a separate sediment standard cannot, therefore, be derived.

4.5 Derivation of PNECs for secondary poisoning of predators

4.5.1 Mammalian and avian toxicity data

Mammalian data
Iron is an essential trace element required by all forms of life. In mammals, it is required for the synthesis of haem proteins and is largely present as haemoglobin, myoglobin and haem-containing enzymes. The normal human body contains 4.5 g iron. Of this, haemoglobin (which is almost entirely in the blood), comprises 72.9 per cent of total iron, myoglobin 3.3 per cent, oxidative enzymes 0.2 per cent and storage iron (ferritin, hemosiderin and unaccounted iron) 23.5 per cent. Most of the storage iron is found in the liver, bone marrow and spleen [38].

Estimates of the minimum daily requirement for iron in humans depend on age, sex, physiological status and iron bioavailability, and range from about 10 to 50 mg/day. Iron deficiency is of more concern than iron overload. The latter situation is a rare condition that only occurs in certain disease states which cause a breakdown of the normal control of iron absorption and result in excessive levels of iron being stored [39].

In humans, absorption of iron depends on the individual's iron status, i.e. the body's need for iron. Although regulation of intestinal iron absorption is not completely understood, it appears to depend on body stores and requirements such that excessive amounts of iron are not stored in the body [39].

The chemical form of iron is important in assessing its biological availability [39]. Ferrous iron (Fe^{2+}) is generally more readily absorbed than ferric iron (Fe^{3+}), which is thought to be a result of the greater solubility of ferrous compounds [39]. The amount of iron absorbed is inversely proportional to the intake, with a number of factors influencing absorption, such as form in the diet. In food, iron occurs in three forms: iron oxides, inorganic and organic salts and organic complexes [39].

Most of the available oral toxicological data have been derived from studies involving humans. In other mammalian species, long-term feeding studies are not available; intramuscular injection is used instead as an exposure route, but this is not relevant in

the context of oral exposure. In its derivation of a drinking water guideline value, the World Health Organization [40] did not base its tolerable daily intake (TDI) on the use of no observed adverse effect levels (NOAELs). Instead, because iron absorption is regulated, the TDI was based on preventing excessive storage of iron in the body. As a precaution against storage of excessive iron in the body, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) provisional maximum (TDI) is 0.8 mg/kg body weight. This applies to iron from all sources, except for iron oxides used as colouring agents and iron supplements taken during pregnancy and lactation or for specific chemical requirements [39].

Avian data
Only limited toxicity data were located for birds. Where available, these relate to the oral ingestion of tungsten–iron shot pellets by mallard ducks (*Anas platyrhynchos*) [41–44]. These studies, which have limited relevance, are outlined below.

Brewer *et al.* [45] dosed 20 mallards of both sexes by oral gavage for 30 days with size 4 Hevi Shot (iron–tungsten–nickel) pellets. No mortality was seen in the dosed group nor were there any significant morphological or histopathological abnormalities of the liver or kidneys. Similarly, Kelly *et al.* [41] dosed adult mallards with eight tungsten iron shot pellets and found no adverse effects during a 30-day trial.

Mitchell and colleagues [43, 44] presented results on the effects of tungsten–iron shot pellets (55 per cent tungsten, 45 per cent iron) on haematological parameters and other health effects in male and female mallard ducks following oral dosing of eight pellets on days 30, 60, 90 and 120 of a 150-day trial. Mallards had occasional significant differences in haematocrit and plasma chemistry values compared with control mallards over the 150-day period, but these changes were not considered to be indicative of deleterious effects [43]. No deleterious effects in terms of mortality, body weight, organs weights and histology of the liver and kidneys were seen [44].

In the third study, 16 male and 16 female adult mallards were orally dosed with eight tungsten–iron shot pellets (55 per cent tungsten, 45 per cent iron) on days 0, 30, 60, 90 and 120 of a 150-day trial and reproductive performance was assessed during the last 90 days of the trial. There were no significant differences in egg production and fertility, and hatchability of eggs from tungsten–iron ducks compared with control ducks. In addition, there was no evidence of differences in percentage survivability and body weight of ducklings from tungsten–iron mallards compared with ducklings from control ducks [42].

4.5.2 PNECs for secondary poisoning of predators

Iron is an essential element that has been shown not to bioaccumulate in higher organisms. This is because absorption of iron depends on an organism's requirements for iron and this is regulated so that excessive amounts of iron are not stored in the body. It is, therefore, considered unnecessary to derive a PNEC addressing secondary poisoning of predators.

5. Analysis and monitoring

The most common method for the determination of iron in environmental matrices is inductively coupled plasma (ICP) with atomic emission spectrometry (AES) [50] or mass spectrometry (MS). Limits of detection are typically <10 µg l^{-1} using ICP-AES and as low as 0.3 µg l^{-1} for clean waters [51]. Other methods used include:

- neutron activation analysis (NAA) [46];
- flame atomic absorption spectrometry (FAAS) [47];
- graphite furnace atomic absorption spectrometry (GFAAS) [55];
- X-ray fluorescence [48];
- colorimetry [49].

A colorimetric method using 2,4,6-tripyridyl-s-triazine (TPTZ) [52] can be used to determine iron in water samples down to a limit of detection (LOD) of ~5 µg l^{-1}. Hydroxylamine hydrochloride is added in order to reduce Fe^{3+} to Fe^{2+}, which then reacts with the TPTZ to form a blue-purple complex. A degree of speciation analysis can be obtained by omitting the hydroxylamine hydrochloride, when the TPTZ will only react with Fe^{2+} present in the sample.

In general, iron is released from biological samples, soils and sediments by digestion using a variety of acids including concentrated nitric acid, aqua regia and hydrofluoric acid. The high concentrations of iron typically present in these matrices means that detection may be carried out using methods such as FAAS and ICP-AES.

The lowest proposed PNEC derived for iron is 1.6 µg l^{-1}. The data quality requirements are that, at a third of the EQS, total error of measurement should not exceed 50 per cent. From the literature, the limit of detection achieved by AES is 0.3 µg l^{-1} (in clean water), which suggests that current analytical methodologies may not be adequate to analyse iron for compliance with the derived PNECs for water.

6. Conclusions

6.1 Availability of data

Laboratory toxicity data are available for both Fe(II) and Fe(III) compounds. The data for freshwater organisms covers eight taxonomic groups (algae, protozoans, rotifers, crustaceans, molluscs, annelids, insects and fish) in acute toxicity tests. Chronic toxicity data are only available for five taxonomic groups (algae, macrophytes, crustaceans, insects and fish).

Data for acute toxicity to marine species are available for five taxonomic groups (crustaceans, molluscs, annelids, echinoderms and fish). Chronic toxicity data are limited to algae and crustaceans. Field studies with freshwater organisms have also been reported.

From evaluation of the data, large numbers of laboratory-based studies involve exposure to particulate iron with low levels.

6.2 Derivation of PNECs

The 'added risk' approach could be appropriate when setting EQSs for iron. This is because iron is a naturally occurring substance that organisms will have been exposed to over an evolutionary timescale. In this case, the PNEC applies only to the 'added' contribution over and above the background level. A practical consequence of this is that compliance assessment would need to consider background levels of iron, at least at a regional scale, if not a local scale. However, natural background concentrations for iron are expected to be very high in comparison to anthropogenic inputs. In this case, a realistic option for implementation would be to set EQSs at background levels rather than on the basis of the PNECs proposed here.

For aquatic organisms, which are mainly exposed via water, dissolved iron species are relevant for toxicity. The dissolved fraction is defined as that which passes through a 0.45 µm filter.

The proposed PNECs are described below and summarised in Table 6.1.

6.2.1 Long-term PNEC for freshwaters

Fish and crustaceans are the most sensitive taxa following chronic exposure.

It is proposed that the $PNEC_{freshwater\_lt}$ is derived on the basis of a 21-day ZEP (NOEC equivalent) of 0.16 mg l^{-1} for a reproduction study of *Daphnia magna*. The study investigated the effects of waterborne iron in hard reconstituted water. The datum is supported by studies showing similar sensitivities of *Daphnia carinata* and the amphipod *Gammarus pulex*. An assessment factor of 10 is recommended, given the large amount of data for different taxa, to produce a $PNEC_{freshwater\_lt}$ of 0.016 mg l^{-1} iron (dissolved).

This PNEC is approximately 60 times lower than the existing EQS of 1 mg l^{-1}. This reflects new data that have become available since the original EQS was derived, as well as a difference in the derivation approach: the existing EQS was based on field observation – particularly the relationship between iron concentrations and fishery status.

6.2.2 Short-term PNEC for freshwaters

Fish and crustaceans are the most sensitive taxa following acute exposure.

The lowest valid acute toxicity value for iron is a 96-hour LC50 of 0.41 mg l^{-1} for brook trout (*Salvelinus fontinalis*) exposed to iron at pH 5.5. The datum is supported by a study showing similar sensitivities of the fry of carp (*Cyprinus carpio*). The recommended PNEC is based on the brook trout datum and guidance given in the EU Technical Guidance Document (TGD) on effects assessment for intermittent releases. A lower assessment factor of 10 (instead of 100) is recommended in order to extrapolate from the 50 per cent acute effect level to the short-term no effect level given the large quantity of data available. This results in a $PNEC_{freshwater\_lt}$ of 0.041 mg l^{-1} iron (dissolved).

There is no existing short-term EQS for freshwater. The 1998 update to the EQS report for iron suggested that, as releases of iron were more likely to be long-term or near continuous point discharges and diffuse inputs, the short-term standard seemed inappropriate.

6.2.3 Long-term PNEC for saltwaters

Limited data are available and no chronic data have been found for fish, which were a sensitive group in terms of acute toxicity to freshwater organisms. The saltwater data are based on nominal concentrations of iron and are, therefore, not suitable for PNEC derivation.

The available data do not appear to differ markedly in the range of values obtained for the related freshwater species. Consequently, the proposed $PNEC_{saltwater\_lt}$ is based on the lowest reliable long-term freshwater data point of a 21-day zero equivalence point (NOEC equivalent) of 0.16 mg l^{-1} for a reproduction study of *Daphnia magna* and an increased assessment factor of 100 recommended resulting in a $PNEC_{saltwater\_lt}$ of 0.0016 mg l^{-1} iron (dissolved).

This PNEC is 625 times lower than the existing EQS of 1 mg l^{-1}. This reflects new data that have become available since the original EQS was derived, as well as a difference in the derivation approach. A large assessment factor is recommended due to lack of saltwater toxicity data for the proposed PNEC. The existing EQS was based on field observation, particularly the relationship between iron concentrations and fishery status, and 'read across' from the freshwater EQS.

6.2.4 Short-term PNEC for saltwaters

Fish are the most sensitive taxonomic group with respect to the acute toxicity of iron. Although acute data were available for more taxonomic groups than for the chronic studies, the data were still limited and there were some issues with its validity as no chemical analysis of the exposure concentrations was performed in most cases.

For this reason, the proposed $PNEC_{saltwater\_st}$ is based on the lowest reliable short-term freshwater data point, a 96-hour LC50 of 0.41 mg l^{-1} for brook trout *Salvelinus fontinalis*.

An increased assessment factor of 100 is recommended, resulting in a $PNEC_{saltwater\_st}$ of 0.0041 mg l^{-1} iron (dissolved).

There is no existing short-term EQS for saltwater. The 1998 update to the EQS report for iron suggested that, as releases of iron were more likely to be long-term or near continuous point discharges and diffuse inputs, the short-term standard seemed inappropriate.

6.2.5 PNEC for secondary poisoning

Iron is an essential element that has been shown not to bioaccumulate in higher organisms. This is due to the organism's body regulating its requirements for iron and not storing excessive amounts. Therefore, PNECs for secondary poisoning of predators are not proposed.

6.2.6 PNEC for sediments

No sediment toxicity data have been located specifically for iron so a $PNEC_{sediment}$ could not be generated.

Table 6.1 Summary of proposed PNECs

Receiving medium/exposure scenario	Proposed PNEC (mg l^{-1} total dissolved iron)	Existing EQS (mg l^{-1})
Freshwater/long-term	0.016	1
Freshwater/short-term	0.041	No standard
Saltwater/long-term	0.0016	1
Saltwater/short-term	0.0041	No standard

6.3 Analysis

The lowest proposed PNEC derived for iron is 1.6 µg l^{-1}. The data quality requirements are that, at a third of the EQS, total error of measurement should not exceed 50 per cent. The limit of detection for atomic emission spectrometry is 0.3 µg l^{-1} (in clean water). This suggests that current analytical methods may not be adequate to analyse iron for compliance with the proposed PNECs.

6.4 Implementation issues

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

1. An understanding of the magnitude and variability in background concentrations of iron in surface waters is required in order to inform a decision about the need for an added risk approach. The proposed PNECs are likely to be insignificant compared with backgrounds, in which case it may be appropriate to consider adopting the background (regional or local, depending on spatial variability) as the basis for an EQS.

2. Current analytical sensitivity is inadequate to distinguish the small PNEC from background levels of iron. However, current analytical methods are adequate if EQSs are based on backgrounds.

3. High uncertainty in the extrapolations to derive saltwater PNECs may be reduced by the generation of additional ecotoxicological data.

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List of abbreviations

AES	atomic emission spectrometry
AF	assessment factor
BCF	bioconcentration factor
CAS	Chemical Abstracts Service
DAA	dissolved annual average
EC50	concentration effective against 50% of the organisms tested
EQS	Environmental Quality Standard
FAAS	flame atomic absorption spectrometry
FAO	Food and Agriculture Organization of the United Nations
GFAAS	graphite furnace atomic absorption spectrometry
GLP	Good Laboratory Practice (OECD)
ICP-AES	inductively coupled plasma/atomic emission spectrometry
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC50	concentration lethal to 50% of the organisms tested
LOD	limit of detection
LOEC	lowest observed effect concentration
lt	long term
MAC	maximum allowable concentration
MS	mass spectrometry
NAA	neutron activation analysis
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
OECD	Organisation for Economic Co-operation and Development
PNEC	predicted no-effect concentration
SEPA	Scottish Environment Protection Agency
SNIFFER	Scotland & Northern Ireland Forum for Environmental Research
SSD	species sensitivity distribution
st	short term
TDI	tolerable daily intake
TGD	Technical Guidance Document
TPTZ	2,4,6-tripyridyl-s-triazine
UKTAG	UK Technical Advisory Group

US EPA	US Environmental Protection Agency
WFD	Water Framework Directive
WHO	World Health Organization
ZEP	zero equivalence point

ANNEX 1 Data quality assessment sheets

Identified and ordered by reference number (see References & Bibliography).

Data relevant for PNEC derivation were quality assessed in accordance with the so-called Klimisch Criteria (Table A1).

Table A1 Klimisch Criteria*

Code	Category	Description
1	Reliable without restrictions	Refers to studies/data carried out or generated according to internationally accepted testing-guidelines (preferably GLP**) or in which the test parameters documented are based on a specific (national) testing guideline (preferably GLP), or in which all parameters described are closely related/comparable to a guideline method.
2	Reliable with restrictions	Studies or data (mostly not performed according to GLP) in which the test parameters documented do not comply totally with the specific testing guideline, but are sufficient to accept the data or in which investigations are described that cannot be subsumed under a testing guideline, but which are nevertheless well-documented and scientifically acceptable.
3	Not reliable	Studies/data in which there are interferences between the measuring system and the test substance, or in which organisms/test systems were used that are not relevant in relation to exposure, or which were carried out or generated according to a method which is not acceptable, the documentation of which is not sufficient for an assessment and which is not convincing for an expert assessment.
4	Not assignable	Studies or data which do not give sufficient experimental details and which are only listed in short abstracts or secondary literature.

* Klimisch H-J, Andreae M and Tillmann U, 1997 *A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data.* Regulatory Toxicology and Pharmacology, **25**, 1–5.
** OECD Principles of Good Laboratory Practice (GLP). See:
http://www.oecd.org/department/0,2688,en_2649_34381_1_1_1_1_1,00.html

Reference number	15

Information on the test species	
Test species used	*Leptophlebia marginata*
Source of the test organisms	Field collected
Holding conditions prior to test	Not known
Life stage of the test species used	Adults

Information on the test design	
Methodology used	Limited test method is described
Form of the test substance	Iron sulfate ($FeSO_4$)
Source of the test substance	Not known
Type and source of the exposure medium	Not known
Test concentrations used	0 (control), 10, 20 and 50 mg l^{-1} total iron
Number of replicates per concentration	Not known
Number of organisms per replicate	60
Nature of test system (static, semi-static or flow-through, duration, feeding)	Semi-static
Measurement of exposure concentrations	Measured
Measurement of water quality parameters	Temperature, pH and dissolved oxygen
Test validity criteria satisfied	Not known
Water quality criteria satisfied	Not known
Study conducted to GLP	No
Overall comment on quality	Good

Reliability of study	Well-conducted study with measurement of water concentrations. However, analysis showed that, at both pH 4.5 and 6.5, most of the iron (90–97%) was present as Fe(II). This is to be expected at the lower pH but is surprising at the higher pH where oxidation to Fe(III) would be expected.
Relevance of study	Endpoint (mortality) is a key indicators of population effects
Klimisch Code	**2**

Reference number	24

Information on the test species	
Test species used	*Salmo trutta* (brown trout)
Source of the test organisms	Hatchery
Holding conditions prior to test	Not relevant
Life stage of the test species used	Eyed eggs and alevins

Information on the test design	
Methodology used	The test method is described
Form of the test substance	Not relevant
Source of the test substance	Contamination of receiving waters
Type and source of the exposure medium	Four Danish streams (one clean stream and three contaminated streams)
Test concentrations used	Not relevant
Number of replicates per concentration	Not relevant
Number of organisms per replicate	Not relevant
Nature of test system (static, semi-static or flow-through, duration, feeding)	Organisms deployed in appropriate cages for 45–60 days
Measurement of exposure concentrations	Receiving water iron concentrations measured (dissolved and total iron)
Measurement of water quality parameters	Temperature, pH and hardness
Test validity criteria satisfied	Not relevant
Water quality criteria satisfied	Not relevant
Study conducted to GLP	Not relevant
Overall comment on quality	Good

Reliability of study	Well-conducted study with measurement of water concentrations
Relevance of study	Field study of effects of iron on fish at key life stages
Klimisch Code	**1**

Reference number	25

Information on the test species	
Test species used	Macroinvertebrate communities
Source of the test organisms	Organisms from River Vidaa
Holding conditions prior to test	Not relevant
Life stage of the test species used	Various

Information on the test design	
Methodology used	The test method is well described
Form of the test substance	Natural iron species
Source of the test substance	Natural iron source (pyrite-rich soils)
Type and source of the exposure medium	Natural receiving water
Test concentrations used	Not relevant
Number of replicates per concentration	Not relevant
Number of organisms per replicate	Not relevant
Nature of test system (static, semi-static or flow-through, duration, feeding)	Not relevant
Measurement of exposure concentrations	Receiving water iron concentrations measured (dissolved and total iron)
Measurement of water quality parameters	Temperature, pH and hardness
Test validity criteria satisfied	Not relevant
Water quality criteria satisfied	Not relevant
Study conducted to GLP	Not relevant
Overall comment on quality	Good quality

Reliability of study	Well-conducted study with measurement of water concentrations
Relevance of study	Field study of effects of iron on macroinvertebrate communities
Klimisch Code	**2**

Reference number	26

Information on the test species	
Test species used	*Gammarus pulex* freshwater amphipod
Source of the test organisms	Crags Stream, Derbyshire (population 1) and Haseley Brook, Oxfordshire (population 2)
Holding conditions prior to test	Not relevant
Life stage of the test species used	Various

Information on the test design	
Methodology used	The test method is well described.
Form of the test substance	Metalliferous discharge containing iron
Source of the test substance	Contamination from quarrying activities
Type and source of the exposure medium	Natural receiving water of the West Okemont River
Test concentrations used	Not relevant
Number of replicates per concentration	Not relevant
Number of organisms per replicate	Six holding baskets each holding 22 field cages with equal numbers of populations 1 and 2
Nature of test system (static, semi-static or flow-through, duration, feeding)	Organisms deployed in cages for 6 days and contained fungal conditioned alder leaf discs (four 17 mm discs)
Measurement of exposure concentrations	Receiving water iron concentrations measured (dissolved and total iron) and body burdens
Measurement of water quality parameters	Temperature, pH, conductivity and alkalinity
Test validity criteria satisfied	Not relevant
Water quality criteria satisfied	Not relevant
Study conducted to GLP	Not relevant
Overall comment on quality	Good

Reliability of study	Well-conducted study with measurement of water concentrations and body burdens in exposed organisms
Relevance of study	Field study of effects of metalliferous discharge (including iron) on a key freshwater species
Klimisch Code	**1**

Reference number	**28**

Information on the test species	
Test species used	*Daphnia magna*
Source of the test organisms	In-house cultures
Holding conditions prior to test	Beakers of culture medium with algal feeding
Life stage of the test species used	<24 hour old neonates at start of the test

Information on the test design	
Methodology used	The test methods are described
Form of the test substance	Iron chloride ($FeCl_3.6H_2O$)
Source of the test substance	Commercial supplier
Type and source of the exposure medium	Standard test medium
Test concentrations used	0 and iron concentrations from 0.0001 to 4.1 mg l^{-1}
Number of replicates per concentration	Not known
Number of organisms per replicate	Not known
Nature of test system (static, semi-static or flow-through, duration, feeding)	Semi-static, 21 days, feeding
Measurement of exposure concentrations	Exposure concentrations measured throughout test
Measurement of water quality parameters	Temperature, pH, dissolved oxygen and hardness
Test validity criteria satisfied	Not known
Water quality criteria satisfied	Not known
Study conducted to GLP	No
Overall comment on quality	Good

Reliability of study	Well-conducted study with measurement of exposure concentrations
Relevance of study	Endpoints (growth, reproduction and survival) are key indicators of population effects
Klimisch Code	**2**

Reference number	29

Information on the test species	
Test species used	*Coregonus lavaretus* (whitefish)
Source of the test organisms	Hatchery of the Laukaa Fish Culture Research Station of the Finnish Game and Fisheries Institute
Holding conditions prior to test	Indoor steel tanks containing 500 litres of oxygenated, denitrified and carbon-filtered tap water
Life stage of the test species used	One-year-old juveniles

Information on the test design	
Methodology used	The test methods are well described.
Form of the test substance	1:1 $FeSO_4/FeCl_2$ solution
Source of the test substance	Not stated
Type and source of the exposure medium	Humic-free water Water from the run-off pond of the peat production area
Test concentrations used	0, 0.1 and 0.35 mg l^{-1} dissolved iron (as natural iron-rich humic water) 0, 0.15 and 0.4 mg l^{-1} dissolved iron (as natural iron-rich humic water with added iron) 0, 0.07 and 0.2 mg l^{-1} dissolved iron (humic free water with added iron)
Number of replicates per concentration	One tank per exposure concentration
Number of organisms per replicate	15 fish per tank
Nature of test system (static, semi-static or flow-through, duration, feeding)	Semi-static, replacement of 30% of the water (~150 l) every other day, 30 days, feeding
Measurement of exposure concentrations	Exposure concentrations were measured throughout the test (after 14 and 28 days)
Measurement of water quality parameters	Temperature, pH and dissolved oxygen daily
Test validity criteria satisfied	Yes (<10% mortality in control tank)
Water quality criteria satisfied	Yes
Study conducted to GLP	No
Overall comment on quality	Good

Reliability of study	Well-conducted study with measurement of exposure concentrations
Relevance of study	Endpoints (changes in tissue function and blood parameters) are not key indicators of population effects
Klimisch Code	**2**

Reference number	30

Information on the test species	
Test species used	*Salmo trutta* (Brown trout)
Source of the test organisms	Not known
Holding conditions prior to test	Not known
Life stage of the test species used	Yolk-sac fry

Information on the test design	
Methodology used	The test methods used are well described.
Form of the test substance	Iron chloride ($FeCl_3$)
Source of the test substance	Not known
Type and source of the exposure medium	Not known
Test concentrations used	0 and 0.0196 mg l^{-1} dissolved iron at pH 4.5 or 0.0235 mg l^{-1} dissolved iron at pH 6.5
Number of replicates per concentration	Not known
Number of organisms per replicate	Not known
Nature of test system (static, semi-static or flow-through, duration, feeding)	Flow-through, 30 days, no feeding
Measurement of exposure concentrations	Exposure concentrations were measured
Measurement of water quality parameters	Not known
Test validity criteria satisfied	Not known
Water quality criteria satisfied	Not known
Study conducted to GLP	Not known
Overall comment on quality	Good

Reliability of study	Limited study with measurement of water concentrations but only one exposure concentration per pH level
Relevance of study	Endpoints (development and survival) are key indicators of population effects
Klimisch Code	**3**

Reference number	**33**

Information on the test species	
Test species used	*Salvelinus fontianlis* (Brook trout)
Source of the test organisms	Not known
Holding conditions prior to test	Not known
Life stage of the test species used	14-month-old organisms

Information on the test design	
Methodology used	The test method is described.
Form of the test substance	Iron sulfate ($FeSO_4$)
Source of the test substance	Not stated
Type and source of the exposure medium	Not known
Test concentrations used	0 (control), 0.25, 0.49, 1.1 and 1.7 mg l^{-1} (dissolved iron)
Number of replicates per concentration	Not known
Number of organisms per replicate	Not known
Nature of test system (static, semi-static or flow-through, duration, feeding)	Flow-through
Measurement of exposure concentrations	Exposure concentrations were measured
Measurement of water quality parameters	Temperature, pH and dissolved oxygen
Test validity criteria satisfied	Not known
Water quality criteria satisfied	Not known
Study conducted to GLP	No
Overall comment on quality	Good

Reliability of study	Well-conducted study with measurement of water concentrations
Relevance of study	Endpoint (mortality) is a key indicators of population effects
Klimisch Code	**2**

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Your environment is the air you breathe, the water you drink and the ground you walk on. Working with business, Government and society as a whole, we are making your environment cleaner and healthier.

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