Proposed EQS for Water Framework Directive Annex VIII substances: manganese (bioavailable) *(For Consultation)*

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

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

This report is the result of research commissioned and funded by the Environment Agency, International Manganese Institute (IMnI) and Department for Environment, Food and Rural Affairs (Defra)

Author(s):
Peters A, Crane M, Maycock D, Merrington G, Simpson P

Research performed:
2010

Dissemination Status:
Publicly available

Keywords:
Manganese, bioavailable, Water Framework Directive, specific pollutants, predicted no-effect concentrations, freshwater, saltwater

Research Contractor:
wca environment Ltd, Brunel House, Volunteer Way, Faringdon, Oxfordshire, SN7 7YR
01285 712712

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

Collaborators:
International Manganese Institute (IMnI)
Department for Environment, Food and Rural Affairs (Defra)
Environment Agency

Environment Agency Science Project Number:
SC080021/1g

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 by the Environment Agency, and has involved the members and partners of UKTAG. It provides background information to support the ongoing development of the standards and classification methods.

Whilst this document 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.

Note:

This report is an update of Science Report SC040038/SR10 [SNIFFER Report WFD52(x)] 'Proposed EQSs for Water Directive Annex VIII substances: manganese (total dissolved)' produced in 2007 as part of a programme of work commissioned by the UK Technical Advisory Group (UKTAG) to derive Environmental Quality Standards (EQSs) for substances falling under Annex VIII of the Water Framework Directive (WFD). The original report proposed PNECs derived according to the WFD Annex V methodology, but that were much lower than natural background levels of manganese, partly because of a lack of certain data and therefore the use large assessment factors in their derivation. UKTAG were unable to make any recommendations at the time, but noted that standards may be proposed in future. This report represents the inclusion and review of additional new data allowing the data to be assessed, following the EU technical guidance recommended for use in the WFD, in a probabilistic manner to reduce uncertainty.

The additional authors of the 2007 report (N. Sorokin and C. Atkinson, WRc) and the Scotland & Northern Ireland Forum for Environmental Research (SNIFFER) are acknowledged here for their contributions and funding, respectively, toward the original report.

Executive Summary

The UK Technical Advisory Group (UKTAG) has commissioned a programme of work to derive Environmental Quality Standards (EQSs) for substances falling under Annex VIII of the Water Framework Directive (WFD). This report proposes predicted no-effect concentrations (PNECs) for manganese using the methodology described in Annex V of the Directive. There are existing non-statutory EQSs for manganese, 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 manganese, 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. If they were to be adopted as EQSs, the long-term PNECs derived in this report would normally be expressed as an annual average concentration.

For this study, the Environment Agency did not require the derivation of short-term PNECs (maximum acceptable concentrations) for either the freshwater or saltwater environment.

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

Properties and fate in water

Manganese is a naturally occurring and abundant Group VII element. It occurs in the environment as a result of weathering of geological material, but also from point sources arising from its use in steel manufacture and associated with coal mining. The most commonly occurring of 11 possible oxidation states are +2 (e.g. manganese chloride or sulphate), +4 (e.g. manganese dioxide) and +7 (e.g. potassium permanganate), although the latter is unstable in the environment. Most manganese salts, with the exception of phosphates and carbonates, are soluble in water. Manganese oxides are poorly soluble in water.

In anoxic waters, Mn^{2+} is generated by reduction of insoluble Mn^{4+} species and mobilised from sediments from which it diffuses into the water column. Its solubility is controlled by the precipitation of insoluble species. In most oxygenated waters, the thermodynamically stable form of manganese is insoluble manganese oxide. Under reducing conditions it may be present as the free Mn^{2+} ion, as soluble inorganic complexes or, more likely, as insoluble carbonates and oxides. Organic

complexation can also occur.

Uptake of manganese by aquatic invertebrates and fish increases with temperature and decreases with pH.

Availability of data

Freshwater long-term data are available for algae, bacteria, crustaceans (cladocerans, amphipods, and crayfish), fish (both salmonid and cyprinid), insects (mosquito and midge larvae), snails, oligochaete worms, and macrophytes. Crustaceans appear to be the most sensitive to long-term exposures to manganese.

Long-term saltwater data are available only for algae, annelids, crustaceans and molluscs.

Data from experiments using permanganate have not been included in this report because these describe effects following exposure to an oxidation state that is unlikely to occur in the field.

Derivation of PNECs

Manganese is an essential and naturally occurring substance that organisms will have been exposed to over an evolutionary timescale. The consideration of background concentrations may be appropriate as part of the compliance assessment process.

The +7 oxidation state is unstable in water, due to being a strong oxidising agent, so is only environmentally relevant near to permanganate discharge points. An EQS needs to address the presence of the +2 and +4 forms. Water quality conditions such as pH and hardness influence toxicity although the effect varies for different organisms. Bioavailability correction models for manganese have recently been developed by the International Manganese Institute for three species covering three trophic levels. These bioavailability correction models will be used for correcting manganese exposure concentrations for comparison against a single generic EQS for manganese, which relates to conditions of high bioavailability.

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

The lowest reliable long-term toxicity datum is a 28 - 42 day EC10 of 0.096 mg l^{-1} for growth of the crustacean amphipod *Hyalella azteca*. A $PNEC_{freshwater\_lt}$ can be derived using an assessment factor approach. Because data are available for three taxonomic groups, an assessment factor of 10 is recommended. When applied to the lowest reliable EC10 of 96 µg l^{-1} for the crustacean, *Hyalella azteca*, this results in a $PNEC_{freshwater\_lt}$ of 9.6 µg l^{-1} manganese (dissolved). This value is more stringent than that derived by the SSD approach.

As long-term NOEC data are available for a variety of fish, invertebrates, and primary producers (algae and higher plants) however, an SSD approach is considered to be appropriate. Multiple test results are available for several species

which have been tested to assess the effects of various water quality parameters (principally hardness) on the bioavailability of dissolved manganese. In these cases the lowest NOEC or EC10 value has been used in the derivation of the PNEC, instead of using geometric means of all of the available values. By treating the data in this way the resulting PNEC will better represent manganese toxicity under conditions of relatively high bioavailability, rather than under conditions of "average" bioavailability (in the test systems). This is considered to be the most appropriate approach, and takes into account comments on the use of geometric mean values from bioavailability studies (SCHER 2009).

An HC5 of 246 µg l^{-1} (dissolved Mn), with a confidence interval of 62 to 572 µg l^{-1}, can be calculated from an SSD that meets all goodness-of-fit criteria. An analysis of field evidence, in terms of Mn^{2+} exposure, suggests that an assessment factor of at least 2 would be expected to ensure protection of potentially sensitive taxa, and that no changes would be observed in whole community metrics at this level of protection. Based on comparison with assessment factors applied to HC5 values in European risk assessments for metals with similar data profiles, an assessment factor of 2 is considered to be appropriate for the derivation of the PNEC from the HC5.

$PNEC_{freshwater\_lt}$ = 246 ug l^{-1}/AF (2) = 123 µg l^{-1} manganese (bioavailable)

The existing EQS for dissolved manganese is 30 µg l^{-1}. This was derived by applying an assessment factor of 10 to the lowest reliable chronic datum available at that time, a survival LOEC of 320 µg l^{-1} for brown trout fry.

Long-term PNEC for saltwaters

The most sensitive and reliable long-term toxicity values relate manganese exposure over 7–20 days to growth of Pacific oyster, *Crassostrea gigas*, and hatching of yellow crab, *Cancer anthonyi*, both resulting in a lowest observed effect concentration (LOEC) of 10 µg l^{-1}. This is supported by an experiment to assess effects on settlement of oyster spat, where a NOEC of 20 µg l^{-1} was estimated. An assessment factor of 2 is recommended to extrapolate to a NOEC from the LOECs of 10 µg l^{-1} and another factor of 100 is recommended to account for interspecies differences in sensitivity because there are no long-term NOECs for saltwater fish or algae. Although chronic data are not plentiful, indications of a steep dose response in these studies suggest a factor no greater than 100 is required. This results in a $PNEC_{saltwater\_lt}$ of 0.05 µg l^{-1} manganese (dissolved).

There is no existing saltwater EQS for manganese.

PNECs for sediment and secondary poisoning

Although manganese is found in sediments, there is only one study describing its toxicity to sediment-dwelling organisms. The study was not deemed suitable for PNEC derivation. It is therefore not possible to derive a $PNEC_{sediment}$.

Manganese can be significantly bioaccumulated by aquatic biota, especially by lower trophic levels. Given the essentiality of manganese, and the fact that it is accumulated to a greater extent by primary producers than by consumers, a PNEC for secondary poisoning is not considered to be relevant.

Summary of proposed PNECs

Receiving medium/exposure scenario	Proposed PNEC (µg l^{-1} total dissolved manganese)	Existing EQS (µg l^{-1})
Freshwater/long-term	9.6 (AF approach) 123 (SSD approach) (bioavailable)	30
Saltwater/long-term	0.05	No standard
Secondary poisoning	No standard	No standard

Analysis

The data quality requirements are that, at a third of the EQS, total error of measurement should not exceed 50 per cent. Analytical methodologies currently employed by UK environmental regulators are capable of achieving detection limits of below 1 µg l^{-1}. Consequently, the current analytical methods should offer adequate performance to analyse dissolved manganese for compliance purposes in freshwater.

Implementation issues

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

- The proposed freshwater long term PNEC is not subject to excessive uncertainty and analytical techniques are sufficient to assess compliance.
- The freshwater PNEC is derived based on conditions of high bioavailability and therefore bioavailablity needs to be taken into account when assessing compliance.
- The saltwater PNEC of 0.05 µg l^{-1} is an order of magnitude below the low end of concentrations reported in seawater and is therefore not implementable as an EQS.

Contents

1. Introduction

The UK Technical Advisory Group (UKTAG) supporting the implementation of the Water Framework Directive (2000/60/EC)[1] is a partnership of UK environmental and conservation agencies. It also includes partners from the Republic of Ireland. UKTAG has commissioned a programme of work to derive Environmental Quality Standards (EQSs) for substances falling under Annex VIII of the Water Framework Directive (WFD). This report proposes predicted no-effect concentrations (PNECs) for manganese using the methodology described in Annex V of the Directive. There are existing (non-statutory) EQS values for manganese, 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 long-term ecotoxicity data for manganese. The data have been subjected to rigorous quality assessment so 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 in detail at this stage. However, this would be an essential step before a regulatory EQS can be recommended.

For this study, the Environment Agency did not require the derivation of short-term PNECs (maximum acceptable concentrations) for either the freshwater or saltwater environment.

1.1 Properties and fate in water

Manganese is a naturally occurring and abundant Group VII element. It occurs in the environment as a result of weathering of geological material, but also from point sources arising from its use in steel manufacture and associated with coal mining. The most commonly occurring of 11 possible oxidation states are +2 (e.g. manganese chloride or sulphate), +4 (e.g. manganese dioxide) and +7 (e.g. potassium permanganate), although the latter is unstable in the environment. Most manganese salts, with the exception of phosphates and carbonates, are soluble in water. Manganese oxides are poorly soluble in water.

In anoxic waters, Mn^{2+} is generated by reduction of insoluble Mn^{4+} species and mobilised from sediments from which it diffuses into the water column. Its solubility is controlled by the precipitation of insoluble species. In most oxygenated waters the thermodynamically stable form of manganese is insoluble manganese oxide. Under reducing conditions it may be present as the free Mn^{2+} ion, as soluble inorganic complexes or, more likely, as insoluble carbonates and oxides. Organic complexation can also occur.

[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.

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

Substance name	Empirical formula	CAS Number	R-phrases and labelling	Solubility in water	Reference
Manganese	Mn	7439-96-5	-	Insoluble (dissolves in dilute mineral acids)	SRC 2007
Mn^{2+}	Mn^{2+}	16397-91-4	-	Soluble	Hedgecott et al. 1998
Manganese(II) acetate	$Mn(CH_3COO)_2$	638-38-0	-	Soluble	Hedgecott et al. 1998
Manganese(II) borate	MnB_4O_7	12228-91-0	-	Insoluble	Hedgecott et al. 1998
Manganese(II) bromide	$MnBr_2$	13446-03-2	R20, R21, R22	Soluble (acidic)	Oxford University 2007
Manganese(II) carbonate	$MnCO_3$	598-62-9	-	Insoluble	Toxnet 2007
Decacarbonyldi manganese(0)	$Mn_2(CO)_{10}$	10170-69-1	-	Insoluble	Hedgecott et al. 1998
Manganese(II) chloride	$MnCl_2$	7773-01-5	-	Very soluble (723 g l^{-1} at 25^{o}C)	WHO 2004, Toxnet 2007
Manganese(II) fluoride	MnF_2	7782-64-1	-	Soluble	Hedgecott et al. 1998
Manganese(IV) oxide	MnO_2	1313-13-9	R20/22	Insoluble	Chemfinder 2005
Manganese hydroxide	$Mn(OH)_2$	12025-99-9	-	Soluble	Hedgecott et al. 1998
Manganese (II) hypophosphite	$Mn(H_2PO_2)_2$	10043-84-2	-	Soluble	Hedgecott et al. 1998
Manganese(II) iodide	MnI_2	7790-33-2	-	Very soluble	Chemfinder 2005
Manganese(II) nitrate	$Mn(NO_3)_2$	10377-66-9	-	Very soluble	Hedgecott et al. 1998
Manganese oleate	$Mn(C_{18}H_{33}O_2)_2$	23250-73-9	-	Insoluble	Hedgecott et al. 1998
Manganese oxalate	MnC_2O_4	640-67-5	-	Slightly soluble	Hedgecott et al. 1998
Manganese(II,III) oxide	Mn_3O_4	1317-35-7	R36, R37, R38	Insoluble	WHO 2004, Oxford University 2007

Substance name	Empirical formula	CAS Number	R-phrases and labelling	Solubility in water	Reference
Manganese(II) phosphate	$MnHPO_4$	7782-76-5	-	Slightly soluble	Hedgecott et al. 1998
Manganese(II) pyrophosphate	MnP_2O_7	53731-35-4	-	Insoluble	Hedgecott et al. 1998
Manganese(II) selenide	MnSe	1313-22-0	-	Insoluble	Hedgecott et al. 1998
Manganese(III) oxide	Mn_2O_3	1317-34-6	-	Insoluble	Hedgecott et al. 1998
Manganese(II) silicate	$MnSiO_3$	7759-00-4	-	Insoluble	Hedgecott et al. 1998
Manganese(II) sulphate	$MnSO_4$	7785-87-7	R48/20/22, R51/53	Soluble in water (52 g l^{-1} at 5°C)	Hedgecott et al. 1998, WHO 2004
Manganese(II) sulphide	MnS	18820-29-6	-	Insoluble	Hedgecott et al. 1998
Manganese(III) fluoride	MnF_3	7783-53-1	R8, R20, R21, R22, R36, R37, R38	Hydrolysed	Oxford University 2007

2.2 PNECs proposed for derivation of quality standards

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 (European Commission 2003), and existing EQSs obtained from the literature (Hedgecott et al. 1998).

If an added risk approach is adopted, a UK background concentration of 10 µg l^{-1} is recommended for adding to these proposed standards (see Section 2.5).

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

Table 2.2 Proposed overall PNECs as a basis for quality standard setting (as dissolved manganese)†

PNEC	TGD deterministic approach (AFs) (µg l^{-1})	TGD probabilistic approach (SSDs) (µg l^{-1})	Existing or proposed quality standards (µg l^{-1})
Freshwater long-term	9.6 (Section 3.1)	123 (as bioavailable manganese) (Section 3.3)	30 (Hedgecott et al. 1998) 20 (WHO 2004)* Nagpal 2001:‡ 700 at 25 mg l^{-1} $CaCO_3$ 800 at 50 mg l^{-1} $CaCO_3$ 1000 at 100 mg l^{-1} $CaCO_3$ 1300 at 150 mg l^{-1} $CaCO_3$ 1900 at 300 mg l^{-1} $CaCO_3$

PNEC	TGD deterministic approach (AFs) ($\mu g\ l^{-1}$)	TGD probabilistic approach (SSDs) ($\mu g\ l^{-1}$)	Existing or proposed quality standards ($\mu g\ l^{-1}$)
Saltwater long-term	0.05 (Section 3.2)		300 (WHO 2004)*
Freshwater sediment long-term	Insufficient data (Section 3.5)		-
Saltwater sediment long-term	Insufficient data (Section 3.5)	-	-
Freshwater secondary poisoning	No PNEC derived (Section 3.6.2)	-	-
Saltwater secondary poisoning	No PNEC derived (Section 3.6.2)	-	-

***** Time period over which this standard should apply is not stated, but acute data were converted into 'chronic' values during derivation.
† All the values are capable of detection with current chemical analytical methods (Hedgecott et al. 1998, WHO 2004).
‡ 30-day mean concentration based on an average of five weekly measurements.
AF = assessment factor

2.3 Hazard classification

This chemical substance is not classified in Annex I of Directive 67/548/EEC.

2.4 Physical and chemical properties

Table 2.3 summarises the physical and chemical properties of the species of interest.

Table 2.3 Physical and chemical properties of manganese

Property	Value	Reference
Molecular formula	Mn	-
Molecular weight	54.938	Windholz et al. 1983
Appearance	Steel grey, lustrous, hard, brittle metal	Budavari et al. 1996
Melting point (°C)	1244	Budavari et al. 1996
Boiling point (°C)	2095	Budavari et al. 1996
Vapour pressure	The metal is an involatile solid at ambient temperatures.	-
Water solubility ($mg\ l^{-1}$)	8.72×10^4 at 25°C	SRC 2007

Soil–water partition coefficient (log Kp)	5 x 10^6 l kg^{-1}	Nyffler et al. 1984

2.5 Environmental fate and partitioning

Table 2.4 summarises the information obtained from the literature on the environmental fate and partitioning of manganese.

Table 2.4 Environmental fate and partitioning of manganese

Property	Value	Reference
Abiotic fate	Manganese is a naturally occurring metal element found widely in rock, soil, water and food. The most commonly occurring of 11 possible oxidation states are +2 (e.g. manganese chloride or sulphate), +4 (e.g. manganese dioxide) and +7 (e.g. potassium permanganate), although the latter is unstable in the environment. Most manganese salts, with the exception of phosphates and carbonates, are soluble in water. Manganese oxides are poorly soluble in water. Elemental manganese and inorganic manganese compounds have negligible vapour pressures.	Hedgecott et al. 1998
Speciation	In most oxygenated waters, the thermodynamically stable form of manganese is Mn^{4+} present as insoluble manganese oxide. However, owing to kinetics and complexation, Mn may be present under oxic conditions as the free Mn^{2+} ion, as soluble inorganic complexes such as $[Mn(HCO_3)]^+$ (aq) or $Mn(SO_4)$ (aq) or, more likely, as insoluble $MnCO_3$ and MnO_2. Organic complexation may occur, particularly in higher alkalinity waters where organic ligands are present. At pH 8, a major proportion of insoluble Mn will be associated with colloids and particulates such as clay, iron oxides and organisms (bacteria). The kinetics of oxidation of Mn^{2+} are slow at a pH <8.5, with half-lives of oxidation potentially in the order of days, although oxidation may be catalysed by the presence of manganese dioxide.	Hedgecott et al. 1998, WHO 2004 Zaw and Chiswell 1999 Huntsman and Sunda 1980
Photostability	Not applicable	
Distribution in water/sediment systems (active substance)	In anoxic fresh and marine waters, Mn^{2+} is generated from the reduction of insoluble Mn^{4+} species and is mobilised from sediments and diffuses into the water column. Its solubility is controlled by the precipitation of insoluble Mn species.	Hedgecott et al. 1998
Distribution in water and sediment systems (metabolites)	Not applicable	
Fate in soil	Manganese in soil can migrate as particulate matter to air or water. Alternatively, soluble manganese compounds can be leached from the soil. In soil, as in water, its solubility is determined by two major variables, pH and redox potential. An increase in pH usually results in an increase in adsorption. The mobility of manganese ions in soils is also influenced by redox potential, with manganese being more mobile under reducing conditions. Manganese compounds do not volatilise from moist or dry soil surfaces due to their ionic character.	WHO 2004
Partition coefficients (log Kow)	No log octanol–water partition coefficient can be determined for manganese and its salts	
Bioaccumulation BCF	Manganese in water can be significantly bioaccumulated by aquatic biota. Bioaccumulation is greatest at lower trophic levels: BCF	WHO 2004

Property	Value		Reference
	Marine and freshwater plants	2000–20000	
	Phytoplankton	2500–6300	
	Marine macroalgae	300–5500	
	Intertidal mussels	800–830	
	Fish	35–930	

BCF = bioconcentration factor

The properties and hence the availability of manganese in water depend on its speciation, which in turn is controlled by pH and electron activity (pE). Manganese that has precipitated out of solution as insoluble oxides or carbonates may not be as available to aquatic organisms as, for example, loosely bound organic complexes (which are only likely to be of significance at higher pH values). In addition to the insoluble species, manganese may bind reversibly to inorganic anions or to organic compounds as metal complexes or organometallic compounds. In general, it is only at low pH and under anoxic conditions that manganese occurs in true solution (i.e. as Mn^{2+}).

Oxidation–reduction (redox) reactions are those that involve changes in the oxidation state of the reactants. Manganese is oxidised when it loses electrons and changes from the +2 to +4 oxidation state (Equation 1). When the reaction is reversed and the ion gains electrons, it is referred to as reduction (Equation 2).

Equation 1 $Mn^{2+} \rightarrow Mn^{4+} + 2e^-$

Equation 2 $Mn^{4+} + 2e^- \rightarrow Mn^{2+}$

Oxidation–reduction processes may be significant in the environmental chemistry of manganese in natural waters and wastewaters under some conditions.

The redox chemistry of manganese and subsequent dynamic equilibria between dissolved and particulate phases means that partition coefficients for water/sediment and water/soil are difficult to measure. This is because precipitation of manganese from the dissolved phase will increase the apparent partition coefficient above the sorption equilibrium achieved between Mn^{2+} and the particulate surface. Consequently, partition coefficients are rarely reported.

Uptake of manganese by aquatic invertebrates and fish increases significantly with temperature and decreases with pH, whereas dissolved oxygen has no significant effect. Uptake of manganese has been found to increase with decreasing salinity (WHO 2004).

Background concentrations of total manganese in natural freshwaters free of anthropogenic inputs range from 0.01 to >10 mg l^{-1}, but rarely exceed 1 mg l^{-1}. Concentrations are usually less than 0.2 mg l^{-1} (Reimer 1999). Mean dissolved manganese concentrations of 6–117 µg l^{-1} were found in six UK rivers (Neal et al. 2000), while ranges of 2.2–230 µg l^{-1} have been reported in UK acid waters (Reader et al. 1989). Background manganese concentrations in open seawater are considerably lower, ranging from 0.4 to 10 µg l^{-1} (WHO 2004). The lower 10th percentile of dissolved manganese concentrations in surface freshwater samples collected by the Environment Agency since 1998 is approximately 10 µg l^{-1}; this is recommended for use as a

conservative generic background concentration if an added risk approach to deriving an EQS is adopted.

The implications of manganese chemistry in natural waters when setting EQSs are that standards need to be set to cover the +2 and +4 oxidation states. The +7 (permanganate) oxidation state is unstable in water, so is only environmentally relevant near to permanganate discharge points.

There is a case for adopting an added risk approach to standard-setting for ionic manganese in freshwaters, as it is a ubiquitous element found at background concentrations in all natural systems. However, concentrations vary widely depending upon local conditions. The selection of background concentrations may be based on similar approaches for other metals.

2.6 Effects data

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

For freshwaters and saltwaters, sensitive data were collated from the existing UK EQS report for manganese (Hedgecott et al. 1998). Further data on soluble forms of manganese published after derivation of the current UK EQS were retrieved from:

- the US Environmental Protection Agency (US EPA) ECOTOX database (USEPA 2005);
- Web of Science®;[3]
- a World Health Organization (WHO) Concise International Chemical Assessment Document (CICAD) (WHO 2004);
- studies provided by the International Manganese Institute (IMnI 2008, IMnI 2009a-f)

Data on the mammalian toxicity of manganese were taken from US EPA (2003, 2004) reports and an Agency for Toxic Substances and Disease Registry (ATSDR 2000) profile. Avian toxicity data were taken from those cited by Laskey and Edens (1985).

Sensitive data were defined as the most sensitive results for a taxonomic group. If the same species was exposed to different salts of manganese, then data for all of these salts were tabulated. Data on the pesticides Maneb (CAS No. 12427-38-2) and Mancozeb (CAS No. 8018-01-7), which contain manganese, were neither searched for nor assessed.

There are indications that manganese toxicity in freshwater is related to hardness (e.g. Lasier et al. 2000, Reimer 1999, Stubblefield et al. 1997), with higher toxicity in softer water. The 1998 EQS report (Hedgecott et al. 1998) concluded that the relationship between toxicity and hardness is much less pronounced than its relationship with pH, and that hardness need not be taken into account when setting freshwater manganese standards. However, the opposite conclusion was drawn in work that provides the

[3] http://scientific.thomson.com/products/wos/

ecotoxicological basis for manganese standards in British Columbia, Canada (Reimer 1999). As a result, British Columbia's maximum allowable concentrations (MACs) range from 0.8 mg l^{-1} Mn at 25 mg l^{-1} $CaCO_3$ to 3.8 mg l^{-1} Mn at 300 mg l^{-1} $CaCO_3$, a difference of 4.75-fold; 30-day average concentrations range from 0.7 mg l^{-1} Mn at 25 mg l^{-1} $CaCO_3$ to 1.9 mg l^{-1} Mn at 300 mg l^{-1} $CaCO_3$, a difference of 2.7-fold (Nagpal 2001).

There is a regulatory cost in terms of complexity and potential misunderstanding in providing multiple standards for a single substance, but there may be economic benefits if less stringent EQS values can justifiably be set for higher hardness freshwaters. An alternative approach which establishes a single generic EQS for high bioavailability conditions, and applies a bioavailability correction to measured dissolved manganese concentrations is proposed here.

2.6.1 Toxicity to freshwater organisms

Chronic (long-term) freshwater data are available for 11 taxonomic groups, including algae, bacteria, crustaceans (cladocerans, amphipods, and crayfish), fish (both salmonid and cyprinind), insects (mosquito and midge larvae), macrophytes, oligochaete worms, and snails. Crustaceans appear to be the most sensitive to long-term exposures to manganese. The lowest chronic EC10 is for the amphipod *Hyalella azteca*. Table 2.5 summarises the most sensitive long-term freshwater toxicity data found for ionic manganese; these data were also used in the construction of the species sensitivity distribution (SSD). Some studies which have investigated the protective effect of hardness have also been undertaken. The most sensitive results, relating to the conditions of highest bioavailability, have been used in the SSD. No data were found for colloidal, particulate or complexed manganese, although these latter forms are likely to be less available and, therefore, less toxic. Studies undertaken for the development of a biotic ligand model (BLM) for manganese indicate that the free manganese ion (Mn^{2+} (aq)) is likely to be the species which causes toxic effects in aquatic organisms. These studies also show that competition between manganese ions and other cations in the solution (principally H^+ and Ca^{2+}) can reduce the toxicity of manganese to the tested species. This is consistent with the principles of BLMs (Di Toro et al. 2001, Paquin et al. 2002).

Sensitive algae and microbial data are available for sheath-forming bacteria and green algae. The most sensitive value for primary producers is a 72 hour ErC10 of 2.27 mg l^{-1} Mn for *Pseudokirchneriella subcapitata* based on growth rate (IMnI 2008). Endpoints relating to growth (rather than biomass) are preferred for tests on primary producers (ECHA 2008), and therefore results based on growth rates are used for both algal and aquatic plant tests. A number of IC50 values for effects on the population abundance of green algae after prolonged exposure to manganese as both the chloride and the sulphate salt are also available. However, an IC50 represents an effect, rather than a no-effect concentration and these studies were not used in the SSD.

Higher plant data are available for the duckweed *Lemna minor*, with a 7-day growth ErC10 of 41.54 mg l^{-1} Mn based on frond growth (IMnI 2009a). This result was found at a pH of between 6.6 and 7.6. Additional LOEC data are also available for *Lemna perpusilla* although they are not considered to be reliable and were not used in the SSD (Nasu and Kugimoto 1981).

Long-term invertebrate data are available for crustaceans, insects, oligochaetes, and molluscs. The most sensitive reliable result is a study on the amphipod, *Hyalella azteca*, which resulted in an EC10 of 0.096 mg l^{-1} Mn (IMnI 2009b). This is the lowest reliable chronic toxicity result in the entire database. Cladoceran species (*Daphnia magna* and *Ceriodaphnia dubia*) are relatively insensitive to the effects of manganese when compared to *H. azteca*. Tests on insects, oligochaetes, and molluscs indicate that these taxonomic groups are less sensitive than crustaceans to the effects of manganese.

Fish data are available for both salmonid and cyprinid species. The most sensitive fish toxicity value is a 30-day *Salmo trutta* sac fry mortality NOEC of 0.155 mg l^{-1} Mn (Reader et al. 1989). This test was performed at pH 4.4 to 4.5, and testing at higher pH (6.5 to 6.6) resulted in a higher NOEC of 0.35 mg l^{-1} Mn. This was not considered to be a relevant study for PNEC derivation (see Section 3.2). Other fish long-term NOEC and LC10 data range from 0.96 to 4.63 mg l^{-1} Mn. The lowest reliable and relevant fish datum is an EC10 of 1.52 mg l^{-1} Mn for the rainbow trout *Oncorhynchus mykiss* (Davies and Brinkmann 1998).

A study of the acute toxicity of manganese to *H. azteca* (Lasier et al. 2000) also found this species to be more sensitive to the effects of manganese than *Ceriodaphnia dubia*. The study also identified a protective effect of water hardness on manganese toxicity in the acute tests for this species. The chronic toxicity of manganese to *H. azteca* has been studied in 28 day growth tests (Norwood et al. 2007), although as the focus of the study was the bioaccumulation of manganese the results reported differ from those normally reported for chronic toxicity tests. Twenty-eight day LC25 values were reported for tests performed in different types of containers, either glass or high density polyethylene, and tests performed in glass containers appeared to result in greater sensitivity of the test subjects to manganese. No explanation for the apparent difference in sensitivity of *H. azteca* to manganese when tests were undertaken in different types of containers could be provided by the authors. Enhanced growth was observed, relative to controls, at low manganese concentrations for the tests undertaken in high density polyethylene containers. The tests undertaken in glass containers resulted in an EC25 for growth of 116 μg l^{-1}, with a confidence interval of 0.014 μg l^{-1} to 962 mg l^{-1}. This result is comparable to that identified in the recent study (IMnI 2009b). The results of the test undertaken in high density polyethylene containers resulted in an EC25 for growth of 7.04 mg l^{-1}, with a confidence interval of 5.26 mg l^{-1} to 9.46 mg l^{-1}.

A series of chronic bioaccumulation tests of manganese by *H. azteca* have also been reported (Norwood et al. 2006) in which organisms were exposed to manganese at concentrations between approximately 0.05 mg l^{-1} and 10 mg l^{-1} for a period of 28 days in high density polyethylene containers. Any effects on either growth or mortality were not reported. The exposure concentrations were considered to be representative of conditions at relatively heavily contaminated sites. Based on the toxicity data for *H. azteca* used in the SSD, considerable effects on the growth (and survival) of the test subjects would be expected in the bioaccumulation tests conducted at elevated concentrations, although the lack of such reported effects in the Norwood et al. (2006) study may be explained by the use of high density polyethylene containers for these tests. This does raise some uncertainties about the apparently high sensitivity of this

species to manganese when tested in glass containers, as was the case in the recent study (IMnI 2009b). Since there are two studies which have yielded comparable results for this species the recent IMnI data (IMnI 2009b) are considered to be valid and useable for PNEC derivation.

Table 2.5. Summary of reliable and relevant chronic aquatic toxicity data for freshwater organisms exposed to manganese as used in the species sensitivity distribution.

Mn species (test substance)	Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (mg l^{-1})	Expo-sure[1]	Toxicant analysis[2]	Comments	Reference
Algae and microbes											
Mn^{2+} ($MnCl_2$)	*Pseudokirchneriella subcapitata*	Green alga	Algae	EbC10 ErC10	Cell yield Growth	72 hours	0.62 2.27	s	y	RI=1; Hardness 64 mg l^{-1} $CaCO_3$; pH 7.5 – 8.4; 24 °C	IMnI 2008
Higher plants											
Mn^{2+} ($MnCl_2$)	*Lemna minor*	Duckweed	Macrophytes	EbC10 ErC10	Total frond count Frond growth	7 days	23.37 41.54	ss	y	RI=1; Hardness 52 mg l^{-1} $CaCO_3$; pH 6.6 – 7.6; 22 – 24 °C; DOC 0.7 mg l^{-1}	IMnI 2009a
Crustaceans											
Mn^{2+} ($MnCl_2$)	*Ceriodaphnia dubia*	Water flea	Crustaceans	EC10[4]	Reproduction	7 days	3.11	ss	y	RI=1; Hardness 32 mg l^{-1} $CaCO_3$; pH 7.1 – 7.6; 24 – 25 °C; DOC <0.5 mg l^{-1}	ENSR 1992
Mn^{2+} ($MnCl_2$)	*Daphnia magna*	Water flea	Crustaceans	NOEC[3] *estimated*	Reproduction	21 days	2.05	ss	y	RI=2; pH 7.7; 18°C; DO 9 mg l^{-1} O_2; hardness 45.3 mg l^{-1} $CaCO_3$	Biesinger and Christensen 1972
Mn^{2+} ($MnCl_2$)	*Hyalella azteca*	Amphipod	Crustaceans	EC10	Growth (dry weight)	28 – 42 days	0.096	f	y	RI=1; Hardness 104 ± 5 mg l^{-1} $CaCO_3$; pH 7.3 – 8.1; 19 – 24 °C; DOC <0.5 mg l^{-1}	IMnI 2009b
Insects											

Mn species (test substance)	Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. ($mg\ l^{-1}$)	Expo-sure[1]	Toxicant analysis[2]	Comments	Reference
Mn^{2+} ($MnCl_2$)	*Chironomus tentans*	Midge larva	Insect: Diptera	EC10	Survival	54 day	16.34	f	y	RI = 2; Hardness 89 ± 20 mg l^{-1} $CaCO_3$; pH 7.3 - 8.0, 19 – 24 ˚C; DO 6.0 – 9.0 mg l^{-1}	IMnI 2009c
Oligochaetes (Worms)											
Mn^{2+} ($MnCl_2$)	*Aeolosoma sp*	Worm	Oligochaetes	EC10	Population growth	14 days	2.52	ss	y	RI=2; Hardness 48 mg l^{-1} $CaCO_3$, pH 7.4 – 7.9; DO 7.9 – 8.8 mg l^{-1}; 23–25 °C	IMnI 2009d
Molluscs											
Mn^{2+} ($MnCl_2$)	*Lymnaea stagnalis*	Great Pond Snail	Molluscs: Pulmonate	EC10	Growth	30 days	7.70	ss	y	RI=2; Hardness 174 ± 5; pH 7.1 – 8.4; DOC <0.05; 25 ± 2 °C	IMnI 2009e
Vertebrates (fish)											
Mn^{2+} ($MnCl_2$)	*Salmo trutta*	Brown trout	Fish (Salmonidae)	EC10[4]	Growth (ELS)	62 days	3.44	f	y	RI=1; Hardness 151.8 mg l^{-1} $CaCO_3$; pH 7.9; temp 11.8; DO 8.7 mg l^{-1}	Stubblefield et al. 1997
Mn^{2+} ($MnSO_4$)	*Salvelinus fontinalis*	Brook trout	Fish (Salmonidae)	EC10[4]	Growth (ELS)	65 days	1.63	f	y	RI=1; Hardness 31.2 mg l^{-1} $CaCO_3$; pH 7.2; temp 12.2°C	Davies and Brinkman 1998
Mn^{2+} ($MnSO_4$)	*Oncorhynchus mykiss*	Rainbow trout	Fish (Salmonidae)	EC10[4]	Growth (ELS)	65 days	1.52	f	y	RI=1; Hardness 31.3 mg l^{-1} $CaCO_3$; pH 7.2; temp 13.3°C	Davies and Brinkman 1998
Mn^{2+} ($MnCl_2$)	*Danio rerio*	Zebra fish	Fish	EC10	Survival (early	35 days	4.63	f	y	RI=1; Hardness	IMnI 2009f

Mn species (test substance)	Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (mg l^{-1})	Expo-sure[1]	Toxicant analysis[2]	Comments	Reference
			(Cyprinidae)		life stage)					95 ± 3.9 mg l^{-1} $CaCO_3$; pH 7.5 – 8.8 mg l^{-1}; 27 ± 0.5 °C; DO 7.6 – 8.8 mg l^{-1}.	
Mn^{2+} ($MnCl_2$)	*Pimephales promelas*	Fathead minnow	Fish (Cyprinidae)	EC10[4]	Larval survival	28 days	2.07	ss	y	RI=1; 30 mg l^{-1} $CaCO_3$, pH 7. 7; temp 25°C	ENSR 1996

[1] Exposure: s = static; ss = semi-static; f = flow-through.
[2] Toxicant analysis: y = measured; n = not measured.
[3] NOEC calculated as LOEC/2 as size of effect at LOEC estimated as >10 <20 % relative to control.
[4] EC10 value recalculated from originally reported data
ErC10 = concentration that results in a 10% reduction in growth rate
EbC10 = concentration that results in a 10% reduction in biomass
LOEC = lowest observed effect concentration; NOEC = no observed effect concentration
LCx = concentration lethal to X% of the organisms tested; ECx = concentration effective against X% of the organisms tested
IC50 = concentration at which the population effect of the organisms tested is inhibited by 50%
DO = dissolved oxygen
DOC = dissolved organic carbon
RI = reliability index (see Annex I)

2.6.2 Toxicity to saltwater organisms

In comparison with the freshwater dataset, only limited saltwater data were available. Long-term data were available for only four taxonomic groups including algae, annelids, crustaceans and molluscs. Crustaceans and molluscs appear to be most sensitive to long-term exposures.

A diagrammatic representation of the available long term saltwater data (cumulative density function) for manganese is presented in Figure 2.1. This diagram includes all data regardless of quality and provides an overview of the spread of the available data. This diagram is not a species sensitivity distribution and has not been used to set the manganese PNEC. The lowest critical saltwater data for manganese is presented in Table 2.6.

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

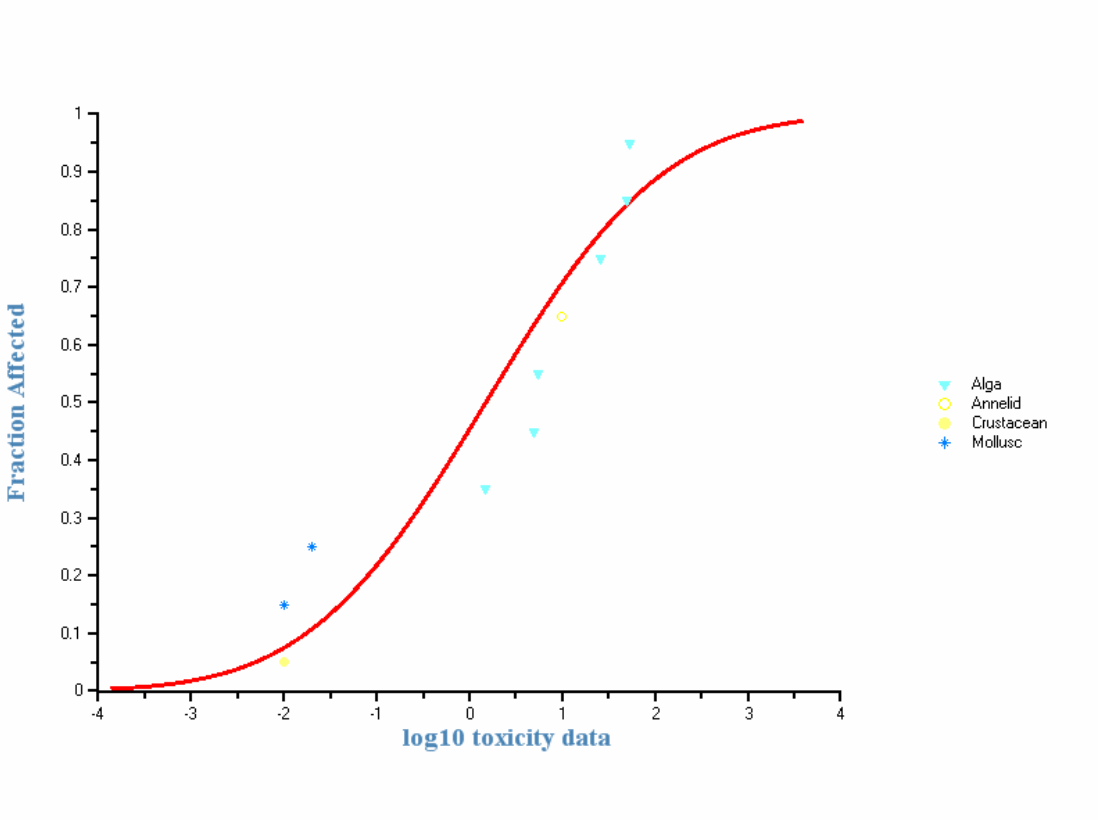


Table 2.6 Most sensitive long-term aquatic toxicity data for saltwater organisms exposed to manganese

Mn species and test substance	Scientific name	Common name	Taxonomic Group	Endpoint	Effect	Test duration (days)	Conc. (mg l^{-1})	Exposure[1]	Toxicant analysis[2]	Comments	Reference
Algae											
Mn^{2+} ($MnCl_2$)	*Ditylum brightwellii*	Diatom	Algae	IC50	Population abundance	5	1.5	s	n	RI = 2	Canterford and Canterford 1980
Mn^{2+} ($MnSO_4$)	*Fucus vesiculosus*	Brown alga (seaweed)	Algae	NOEC	Growth	35	5.0	ss	n	10°C; salinity 31‰	Munda and Hudnik 1986
Mn^{2+} ($MnSO_4$)	*Nitzschia closterium*	Diatom	Algae	IC50	Population abundance	4	25.7	s	n	pH 8.0–8.1; 15–16°C; exposure in nonchelating medium	Rosko and Rachlin 1975
Mn^{2+} ($MnCl_2$)	*Chlorella stigmatophra*	Green alga	Algae	IC50	Population abundance	24	50.0	s	n	Salinity 28‰	Christensen 1979
Mn^{2+} ($MnSO_4$)	*Nitzschia closterium*	Diatom	Algae	IC50	Population abundance	4	53.8	s	n	pH 6.7–6.9; 15–16°C; exposure in chelating medium	Rosko and Rachlin 1975
Invertebrates											
Mn^{2+} ($MnCl_2$)	*Cancer anthonyi*	Yellow crab	Crustaceans	Effect	Hatching rate	7	0.01	ss	n	Hatching rate 38% (91% in control); RI = 2	MacDonald et al. 1988
Mn^{2+}	*Crassostrea gigas*	Pacific oyster	Molluscs	Effect	Growth	14	0.01	s	y	17% reduced growth, but little difference after 14 days depuration; RI = 2	Watling 1983
Mn^{2+}	*Crassostrea gigas*	Pacific oyster	Molluscs	NOEC	Settlement	20	0.02	s	y	0.02 mg l^{-1} was the highest test concentration; RI = 2	Watling 1983
Mn^{2+} ($MnSO_4$)	*Nereis diversicolor*	Ragworm	Annelids	NOEC	Mortality	15	10.0	s	n	13°C; salinity 17.5‰	Bryan and Hummerstone 1973

[1] Exposure: s = static; ss = semi-static.
[2] Toxicant analysis: y = measured; n = not measured.
NOEC = no observed effect concentration

IC50 = concentration at which the population effect of the organisms tested is inhibited by 50%
RI = reliability index (see Annex I)

2.6.3 Toxicity to sediment-dwelling organisms

Toxicity data for manganese concentrations in freshwater sediment (e.g. on a mg kg^{-1} sediment basis) were not found. No effect on survival was found in field saltwater sediments when soldier crabs (*Mictyris longicarpus*) were exposed for 21 days to 4300 mg Mn kg^{-1} dry weight (dw) (Weimin et al. 1994).

2.6.4 Endocrine-disrupting effects

Manganese is not a known endocrine disruptor.

2.6.5 Mode of action of manganese

Studies into the development of a BLM for manganese suggest that it may be possible to predict manganese toxicity as a function of water quality parameters such as pH and Ca concentrations. These studies indicate that assuming the presence of a “biotic ligand" for manganese, where Mn^{2+} ions compete with Ca^{2+} and H^{+} ions to occupy the binding sites, may provide a refined understanding of the toxicity of manganese to freshwater organisms.

In mammals, the various *in vivo* and *in vitro* effects of manganese cannot be explained in terms of basic biochemical mechanisms (WHO 1981). However, effects on the central nervous system may be explained by reductions in dopamine levels and impairment of oxidative enzymes caused by the accumulation of manganese in the brain. Intact oxidative enzyme systems are needed to supply the energy for the degradation and synthesis of catecholamines involved in synaptic transmission. Any changes in these systems may affect behaviour and could be related to the initial psychiatric phase of chronic manganese poisoning.

In addition to its toxic effects, manganese is also an essential nutrient for micro-organisms, plants and aquatic and terrestrial animals. Manganese is thought to serve as either a cofactor involved in the activation of metal-enzyme complexes or as an integral part of metalloenzymes integral to carbohydrate, lipid, and protein metabolism. Deficiencies in dietary manganese have been reported to result in reduced growth and skeletal abnormalities (NRC 1993). Positive correlations have been found between assemblage scores for aquatic species and increasing manganese concentrations of 3–600 µg l^{-1} in upland streams (Hirst et al. 2002). It has also been reported that a diet containing 12–13 mg Mn kg^{-1} is required to maintain normal growth of captive carp (Satoh et al. 1987). The US National Academy of Science National Research Council recommended manganese nutrient requirements for a variety of fish ranging from 2.4 (channel catfish) to 13 mg Mn kg^{-1} of diet (rainbow trout and common carp) (NRC 1993). Consequently, any quality standards derived for manganese must take into account the essential requirements for this metal in certain aquatic organisms.

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

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

3.1.1 PNEC for freshwaters

PNEC accounting for the annual average concentration
Crustaceans appear to be the most sensitive taxonomic group in the freshwater database, followed by fish. The most sensitive reliable result is a study on the amphipod, *Hyalella azteca*, which resulted in an EC10 of 0.096 mg l^{-1} Mn. Using the assessment factor method to derive a $PNEC_{freshwater\_lt}$ requires that an assessment factor of 10 is applied to the lowest NOEC or EC10. Hence, the $PNEC_{freshwater\_lt}$ is as follows:

$PNEC_{freshwater\_lt}$ = 0.096 mg l^{-1}/AF (10) = 9.6 µg l^{-1} manganese (dissolved)

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

3.1.2 PNEC for saltwaters

Table 2.6 summarises the most sensitive long-term saltwater toxicity data found for ionic manganese. No data were found for colloidal, particulate or complexed manganese. The effects database for marine species is considerably smaller than that for freshwater organisms. The TGD states that where saltwater toxicity data are not available freshwater data may be used *in lieu* of data for estuarine/marine species providing that there are no obvious differences in sensitivity between freshwater and saltwater organisms. Invertebrates are sensitive to Mn in both mediums but would appear to be more sensitive in saltwaters. There are also differences in the chemistry of manganese in increasingly saline systems, and as a result of this the use of a combined dataset is not recommended.

PNEC accounting for the annual average concentration
Algal data are available for diatoms, green algae and brown macroalgae. The most sensitive algal value is a 5-day IC50 of 1.5 mg l^{-1} Mn for population abundance of the diatom *Ditylum brightwelli* (Canterford and Canterford 1980). However, an IC50 represents an effect, rather than a no-effect concentration. The lowest long-term algal

NOEC for manganese is a 35-day growth NOEC of 5.0 mg l^{-1} Mn for the brown alga *Fucus vesiculosus* (Munda and Hudnik 1986).

Invertebrate data are available for annelids, crustaceans and molluscs. Crustaceans and molluscs are most sensitive, with substantial effects on yellow crab *Cancer anthonyi* hatching rate at 10 µg l^{-1} Mn (MacDonald et al. 1988) and 17 per cent reduced growth in the Pacific oyster *Crassostrea gigas*, also at 10 µg l^{-1} Mn (Watling 1983). In the latter study, these effects disappeared after a 14-day depuration period and, in the same study, the oyster spat settlement NOEC was $\geq$20 µg l^{-1} Mn. Both studies are regarded as valid with restriction. However, the dose-response curve for yellow crab embryo hatching is unusual because there is a substantial reduction in mean percentage hatch when controls (91.1 per cent hatch $\pm$ standard deviation of 6.6 per cent) are compared with embryos exposed to 10 µg l^{-1} Mn (38.3 $\pm$ 9.7 per cent). There is then a much shallower dose response between effects at this concentration and those at 100 µg l^{-1} Mn (29.3 $\pm$ 9.7 per cent) and 1 mg l^{-1} Mn (23.1 $\pm$ 9.8 per cent), with unusually similar standard deviations. There were no successfully hatched embryos in treatments exposed to 10, 100 or 1000 mg l^{-1} Mn.

No long-term manganese toxicity data could be found for saltwater fish.

The most sensitive and reliable long-term toxicity value for deriving an annual average quality standard is for reduced growth of Pacific oyster *Crassostrea gigas* spat, with a LOEC of 10 µg l^{-1} Mn (Watling 1983). There was a 17 per cent effect at this concentration; thus, division of this value by 2 is required to obtain an estimate of the NOEC. An assessment factor of 100 (Table 25 in the EU TGD; European Commission 2003) should be applied to this value as there are no long-term NOECs for saltwater fish or microalgae:

$PNEC_{saltwater\_lt}$ = 5 µg l^{-1}/AF (100) = 0.05 µg l^{-1} manganese (dissolved)

This value is very low and is an order of magnitude below the low end of the range of concentrations reported in seawater (WHO 2004). The short-term database was reviewed (see Appendix II) for data to support the long-term derivation. The only fish data were results obtained from exposure to manganese permanganate and are not considered relevant for reasons already stated in Section 2.5. As a consequence no chronic to acute ratios can be derived for comparison between saltwater taxa or for comparison with the freshwater database. Data for echinoderms, an exclusive marine species, were available that suggest this taxonomic group is less sensitive than molluscs or crustaceans. The short-term data for molluscs and crustaceans are for different species than those appearing in the chronic database so no definitive conclusions can be drawn.

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

3.2.1 Annual average PNEC for freshwaters

The minimum number of long-term toxicity data (at least 10 NOECs from eight taxonomic groups) is exceeded in the case of chronic freshwater toxicity data for manganese (Table 2.5). The minimum data requirements for the use of an SSD in PNEC derivation are shown in Table 3.1, along with the number of available reliable tests for each taxonomic group.

Table 3.1 Comparison of long-term manganese ecotoxicological dataset against minimum requirements for SSD derivation according to EU Technical Guidance

Minimum SSD Requirement	Number of reliable NOECs in dataset[1]	Species
Fish	3	*Salmo trutta*[2] *Oncorhynchus mykiss* *Salvelinus fontinalis*
2nd chordate family (fish, amphibian etc)	2	*Danio rerio* *Pimephales promelas*
Crustacean (e.g. cladoceran, copepod, ostracod, isopod, amphipod, crayfish etc.)	3	*Hyalella azteca* *Daphnia magna* *Ceriodaphnia dubia*
Insect (e.g. mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc)	1	*Chironomus tentans*
Family in a phylum other than arthropoda/chordate (e.g. Rotifera, Annelida, Mollusca, etc)	1	*Lymnaea stagnalis*
Family in any insect order or phylum not represented	1	*Aeolosoma sp*
Algae	1	*Pseudokirchneriella subcapitata*
Higher plant	1	*Lemna minor*
Total NOECs	13	
Taxa Criteria Met	8/8	

1: Reliability defined as a score of 1 or 2 against Klimisch et al. (1997) criteria
2: NOEC for growth from Early Life Stage test

Four NOEC values are available for *Salmo trutta* (brown trout) and the geometric mean of these four tests is 0.878 mg l^{-1}. Two of the tests are from sac fry tests and the remaining two results are from early life stage tests. The sac fry tests were performed at pH 4.5 and 6.5, in a soft water, and were static tests over 30 days. Furthermore, only a single test concentration was used in the tests so no true dose response was observed, although LOEC and NOEC values were derived for the two tests, respectively. Both of these tests have previously been given a reliability rating of 2 (Environment Agency 2007), although there are some uncertainties about their relevance for use in PNEC

derivation, particularly given the low pH values at which the tests were performed (Reader et al. 1989). The early life stage tests were assigned a reliability rating of 1 and were performed over 62 days in flow-through conditions (Stubblefield et al. 1997). These tests are therefore considered to be the most reliable of all of the available test results for this species. The most sensitive effect was on growth, resulting in a NOEC of 2.78 mg l^{-1} Mn. Effects were also seen on survival at higher concentrations. Current guidance (ECHA 2008) indicates that the greatest weight should be given to the most reliable available test in cases where there are several results for a single species. In this case the NOEC for growth of 2.78 mg l^{-1} Mn is considered to be the most reliable of the four results available for this species. Whilst there are indications that lower NOEC values may be observed under relatively acidic conditions, it is not clear if these results are due to the effects of manganese alone, and it is also unclear whether a true dose response was observed in the tests. The LOEC and NOEC values reported are also higher than the lowest NOEC, or EC10 value, from the toxicity dataset (the reliable EC10 of 0.096 mg l^{-1} for *Hyalella azteca*). The data for growth are therefore the only endpoint used for *Salmo trutta* in the SSD; EC10 values for these tests were recalculated from the original test data for use in the SSD.

The EC10 for the growth of *Hyalella azteca* (IMnI 2009b) has been used in this effects assessment, although there is some uncertainty surrounding this result because of the considerable differences in the toxicity of manganese to this species observed for tests undertaken in different types of containers (Norwood et al. 2007) (see Section 2.6.1). As two equivalent test results are available for experiments undertaken in glass containers, and only a single result is available for an experiment undertaken in high density polyethylene, the result of the recent study (IMnI 2009b) conducted in glass containers is considered to be most reliable for use in the SSD.

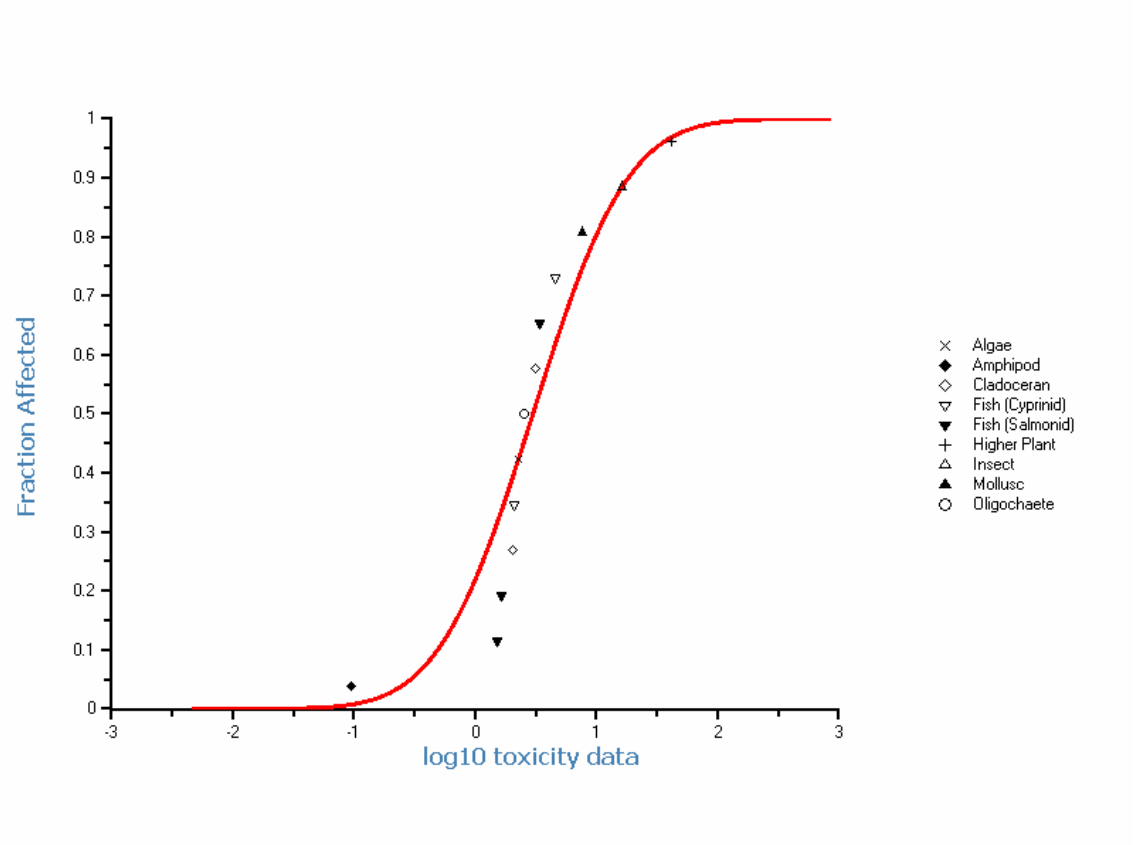
An EC16 value of 4.1 mg l^{-1} is also available for *Daphnia magna* (Biesinger and Christiansen 1972) which is recalculated from the original data for effects on reproduction. This level of effect was the lowest effect concentration which could be reliably calculated from the original data. A NOEC value was extrapolated from this EC16 value (assuming it to be equivalent to a LOEC at 16 per cent effect), in accordance with current guidance (ECHA 2008), by dividing the LOEC value by 2. This resulted in an estimated NOEC value of 2.05 mg l^{-1}.

Multiple test results are available for several species which have been tested to assess the effects of various water quality parameters (principally hardness) on the bioavailability of dissolved manganese. Tests on *Ceriodaphnia dubia*, *Oncorhynchus mykiss*, and *Salvelinus fontinalis* have been reported from studies undertaken to assess the bioavailability of manganese. In these cases the lowest NOEC or EC10 value has been used in the derivation of the PNEC, instead of using geometric means of all of the available values. By treating the data in this way the resulting PNEC will better represent manganese toxicity under conditions of relatively high bioavailability, rather than under conditions of “average” bioavailability (in the test systems). This is considered to be the most appropriate approach, and takes into account comments on the use of geometric mean values from bioavailability studies (SCHER 2009).

Reliable tests on algae (*Pseudokirchneriella subcapitata*) and higher plants (*Lemna minor*) have been reported for both biomass and growth rates. As is common for tests of this type, biomass data produce lower EC10 values than growth rate data. In Europe the preference is to use test results which are based on growth rate, provided that a minimum growth rate was observed in the control tests. Both of these tests met the required growth rate in the controls for the use of endpoints which are based on growth rate endpoints. Both the growth rate and biomass based endpoints were assessed in different SSDs as they have significant effects on the overall fit of the distributions.

The SSDs were calculated using the E_TX (version 2.0) programme (van Vlardingen et al. 2004). Figure 3.1 shows the SSD using growth endpoints for primary producers. The resulting SSD does not meet all of the goodness-of-fit criteria: the distribution is rejected at the 10% significance level for the Anderson-Darling, Kolmogorov-Smirnov, and Cramer von Mises tests for normality, and at the 5% significance level for the Kolmogorov-Smirnov test. This SSD results in an HC5 of 276 µg l^{-1} (dissolved Mn), with a confidence interval of 69 to 649 µg l^{-1}.

Figure 3.1 Species Sensitivity Distribution of chronic freshwater toxicity data for manganese using growth endpoints for primary producers (mg l^{-1})



Because the SSD which used growth rate endpoints for primary producers failed to meet tests for normality, the SSD was recalculated using the biomass based endpoints for both of the tests on algae (*Pseudokirchneriella subcapitata*) and higher plants (*Lemna minor*). The resulting SSD meets all of the goodness-of-fit criteria and results in an HC5 of 246

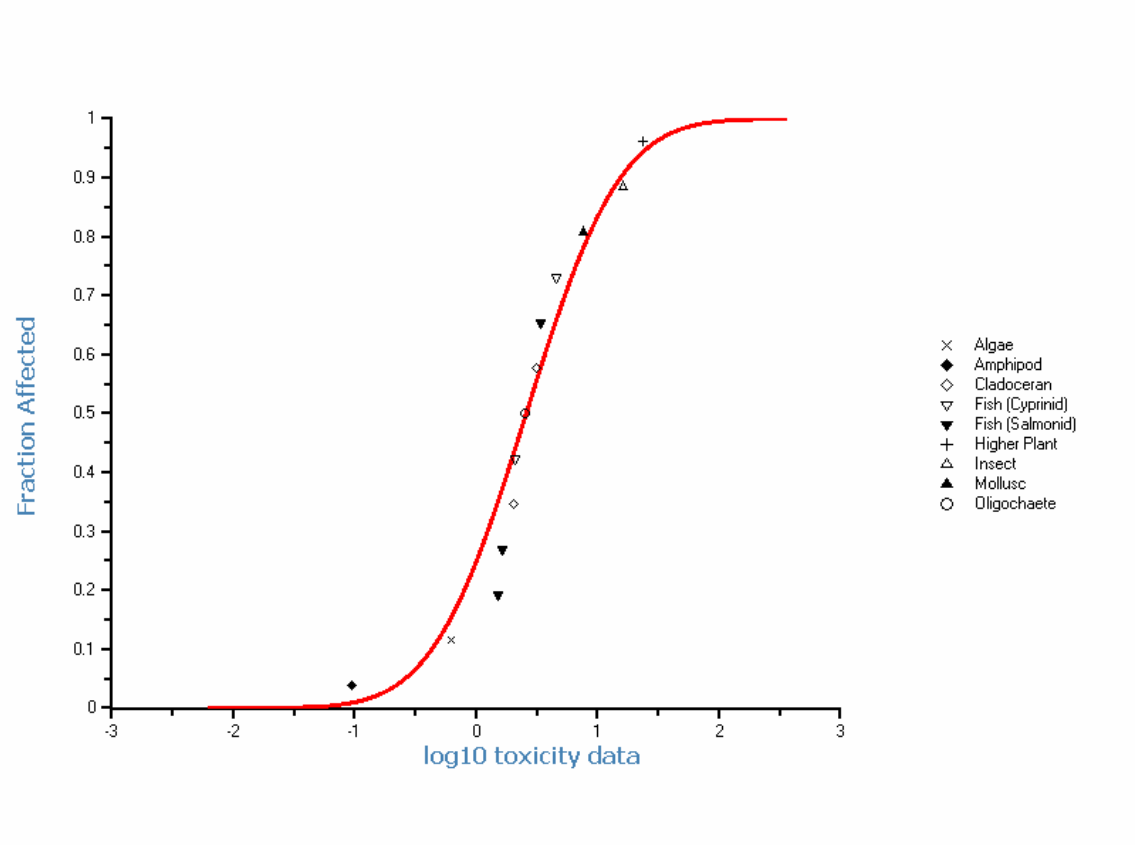
µg l^{-1} (dissolved Mn), with a confidence interval of 57 to 572 µg l^{-1}. This SSD is shown in Figure 3.2.

The same dataset has also been fitted to a Burr type III distribution, which results in an HC5 value of 270 µg l^{-1}. The resulting distribution from this fit is shown in Figure 3.3. The value derived here is comparable to the value of 246 µg l^{-1} derived using a log-normal distribution.

It is proposed to use the SSD which is derived using biomass based endpoints for primary producers and assumes a log-normal distribution (Figure 3.2) for the derivation of a generic PNEC for manganese. Whilst it would be more usual to select the higher value (i.e. using growth, rather than biomass, data for primary producers) it should be noted that the difference between the two HC5 values is small (approximately 10%) and satisfying the model assumptions for normality are considered to be of greater importance. The PNEC is derived from the HC5 by applying an assessment factor of between 1 and 5. The assessment factor should be selected on a case by case basis (ECHA 2008) taking into account the following issues:

- The overall quality of the database
- The representivity of taxonomic groups
- Knowledge of the mode of action
- Uncertainties around the HC5
- Comparison with field and mesocosm studies

Figure 3.2 Species Sensitivity Distribution of chronic freshwater toxicity data for manganese using biomass endpoints for primary producers (mg l-1)



The majority of the studies included in the SSD are high quality studies with reliability indices of 1. Most of the studies which have received a reliability rating of 2 are studies on invertebrates for which there is no internationally agreed test method available. This is not unexpected considering the range of different species and taxonomic groups which must be included in an SSD in order to meet the criteria for minimum coverage of taxonomic groups. A summary of the different datasets considered and their resulting HC5 values is provided in Table 3.2.

Figure 3.3 Species Sensitivity Distribution of chronic freshwater toxicity data for manganese using biomass endpoints for primary producers (mg l^{-1}), fitted to a Burr type III distribution

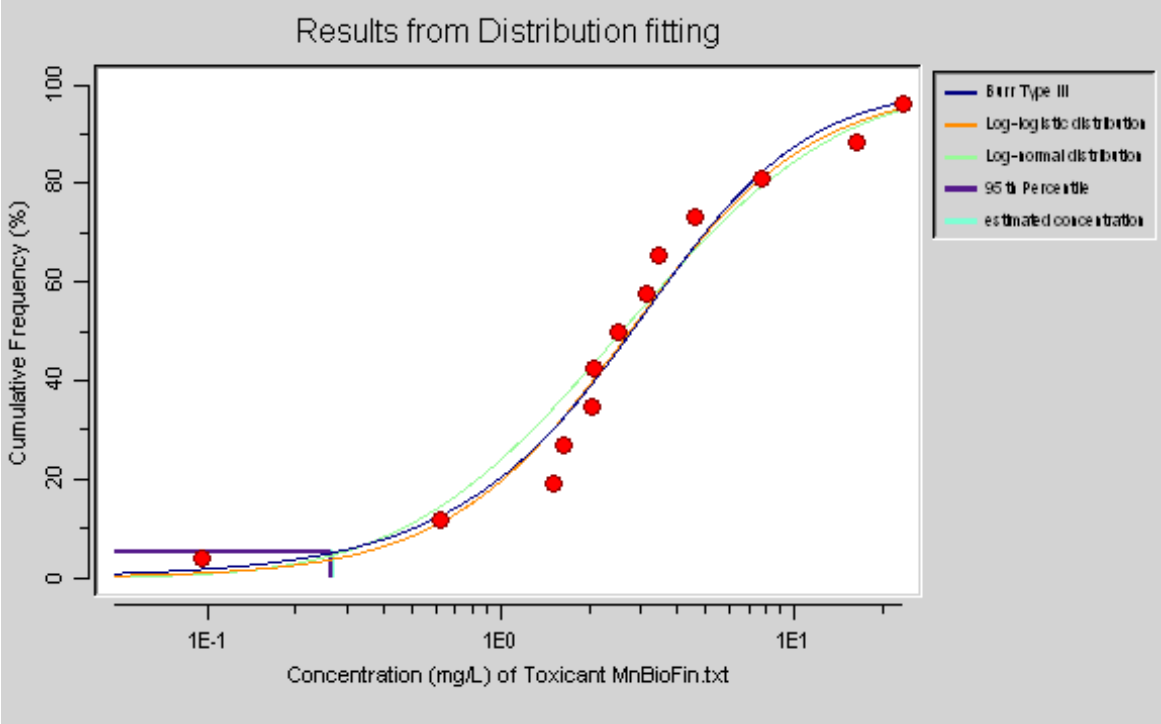


Table 3.2 Goodness of fit and HC5 values for different SSD options considered

Data used	Distribution	Goodness of Fit	HC5 (µg l^{-1})
All data, plant growth	Log Normal	Rejected	276
All data, plant biomass	Log Normal	Accepted	246
All data, plant biomass	Burr Type III		270

The different approaches for deriving the SSD all result in very similar values for the HC5, ranging from 246 to 276 µg l^{-1}. Data are available which meet all of the requirements of SSD construction under the TGD. There is possible over-representation of fish, and under representation of insects and molluscs, relative to the proportions of species found in typical European freshwater ecosystems. This potential limitation is common to all SSDs, and this is expected to be at least partly due to the very limited

number of standard test methods available for insects and the fact that historically toxicity testing tended to focus on fish species. The available test data do not indicate that molluscs, insects or higher plants are likely to be especially sensitive to the effects of manganese, although considerable apparent differences in sensitivity between different species of the same taxonomic group have been observed for some other metals, e.g. the toxicity of nickel to snail species (Denmark 2008).

The numbers of insect and mollusc species included in the databases of previous metals risk assessments are shown in Table 3.3. This information suggests that the chronic aquatic toxicity database for manganese is no less well represented for these taxonomic groups than in previous metals risk assessments, with a comparable level of data being available particularly for insects.

Table 3.3 Number of insect and mollusc species included in databases from previous metals risk assessments

Metal	Insects	Molluscs
CrO_4^{2-}	1	1
Cd	1	2
Zn	1	2
Cu	3	4
Ni	2	2
Pb	1	1
Mn	1	1

There is some evidence from recent studies into the development of a BLM for manganese to suggest that toxic effects are likely to be due to the free manganese ion (Mn^{2+}), and that the toxicity of this species may be reduced by competition for binding sites on biological surfaces by other cations. Similar modes of action have also been observed for other metals for which BLMs have been developed. Manganese is also an essential element for micro-organisms, plants, and aquatic and terrestrial animals.

The difference between the median HC5 and the 90% lower confidence limit is a factor of 3.9, and the difference between the median HC5 and the 90% upper confidence limit is a factor of 2.3. The overall spread of the HC5 estimate is a factor of 9.2.

There is a single EC10 value which is below the HC5. This is the EC10 for growth of *Hyalella azteca* and is a factor of 2.5 times lower than the HC5 value. This EC10 value is above the lower 95% confidence limit of the HC5. The next lowest EC10 value is for the alga *Pseudokirchneriella subcapitata*, based on biomass, and is higher than the upper 95% confidence limit of the HC5.

No field or mesocosm studies of manganese toxicity have been identified. Some studies have reported levels of accumulation of manganese by various different organisms, but these studies have generally not related the observed accumulation to effects. A notable exception is the study on *Hyalella azteca* toxicity and body burdens (Norwood et al. 2007). Information from ecological monitoring of benthic invertebrates and chemical monitoring of manganese concentrations may allow an assessment of the potential impacts of manganese on indigenous freshwater macroinvertebrates.

It is appropriate to consider the assessment factors which have previously been applied to risk assessments of other metals under the Existing Substances Regulations. In discussions on the size of assessment factor to be applied to the HC5 to derive the aquatic PNEC for Cu, attention was drawn to NOEC values that fell below the HC5 (ECI 2007). For Cu the difference between the NOEC for the most sensitive species (*Brachionus calyciflorus*) and the HC5 was a maximum factor of 1.4. The relative uncertainties associated with how protective a likely PNEC set at the HC5 would be to the NOECs that fell below the HC5 was considered to be low because of the relatively large data set (n = 139 NOECs; 27 species-specific NOEC values) and the fact that not all the NOECs for *B. calyciflorus* fell below the HC5. NOECs below the HC5 are thought not generally to occur when the sample size is below 10-20; the availability of more data points increases the probability of NOECs below the value of the HC5 (Denmark 2007). For Ni, uncertainties associated with the NOECs from the chronic tests on the snail *Lymnea stagnalis* (which were up to a factor of 1.8 lower than the HC5) contributed to the application of an assessment factor of 2 to the HC5.

The number of species included in each of the metal databases, and the availability of bioavailability corrections and mesocosm or field studies are summarised in Table 3.4, along with the selected (or proposed) assessment factors for chromates, cadmium, zinc, copper, nickel, lead, and manganese.

Table 3.4 Summary of data availability and assessment factors applied in previous metals risk assessments

Metal	Number of Species	Bioavailability correction	BLM Extrapolation	Field Data	Assessment Factor
CrO_4^{2-}	28	No	No	No	3
Cd	28	Hardness	No	Mesocosm	2
Zn	18	Yes	No	Yes	2
Cu	27	Yes	Yes	Yes	1
Ni	31	Yes	Yes	No	2
Pb	16	No	No	No	2 or 3
Mn[1]	13	In progress	No	In progress	2

1: Based on information which is currently available

For manganese, the most comparable previous risk assessments of metals are those for chromates and lead. Both of these metals have a larger number of tested species than manganese, but had no reliable bioavailability corrections or field data, whereas bioavailability correction is currently under development for manganese. No agreement has yet been reached on the assessment factor which should be applied in deriving the PNEC for lead. Metals for which an assessment factor of 2 has been agreed had bioavailability corrections, and either a BLM extrapolation or reliable field data available, in addition to larger chronic toxicity databases. An assessment of the effects of manganese exposure on invertebrates in the field will also be undertaken once a bioavailability correction tool is available. As stated previously preliminary analysis of the available field data suggests that an assessment factor of 2 will be sufficiently protective.

Based on the considerations above an assessment factor of 2 is considered to be appropriate for the derivation of the PNEC from the HC5:

$PNEC_{freshwater\_lt}$ = 246 ug l^{-1}/AF (2) = 123 µg l^{-1} manganese (bioavailable)

The PNEC value derived using an assessment factor of 2 is higher than the lowest EC10 in the database (*Hyalella azteca*) (IMnI 2009b). The growth endpoint in this study was analyzed as the mean dry weight per original organism (biomass) and the dry weight per surviving organism. The most sensitive endpoint of these two has been used in the SSD derivation but it is worth noting that the alternative measurement (biomass) produced a EC10 of 1063.1 µg l^{-1} Mn. It is unclear why heavier organisms should have been more susceptible to manganese toxicity. The survival EC10 was 188 µg l^{-1} Mn. No reproduction EC10 could be derived but the NOEC for reproduction would appear to be greater than 286 µg l^{-1} Mn. It is, therefore, reasonable to consider that the EC10 based on the dry weight per surviving organism is only one part of the growth picture and that the effects seen at 96 µg l^{-1} Mn are unlikely to be significant at the population level.

These values are lower than those from an earlier SSD approach reported using short-term freshwater toxicity data (*n* = 21) (WHO 2004). An acute-to-chronic extrapolation factor of 2 was applied to the result in that report to derive an overall guidance value of 200 µg l^{-1} Mn for the protection of 95% of freshwater species with 50% confidence. An SSD approach was also applied to short-term saltwater toxicity data (*n* = 9), again with an acute-to-chronic extrapolation factor of 2, to produce an overall guidance value of 300 µg l^{-1} Mn for the protection of 95% of saltwater species with 50% confidence (WHO 2004). The approach taken by WHO (2004) is not consistent with European guidance on SSDs (EC 2003, ECHA 2008) in which acute-to-chronic extrapolations are not used and an assessment factor is applied to the HC5. However, the HC5 of 200 µg l^{-1} Mn derived by WHO is remarkably similar to the value of 246 µg l^{-1} Mn derived in this report.

3.2.2 Annual average PNEC for marine water bodies (saltwater)

There are insufficient data to derive a TGD compliant long-term saltwater PNEC using a species sensitivity distribution.

3.3 Derivation of existing EQSs

The 1998 EQS report (Hedgecott et al. 1998) described standards for ‘dissolved’ manganese in water, i.e. that portion passing through a 0.45-µm filter.

The long-term freshwater standard was extrapolated from laboratory data for salmonid fish. The data indicated that, while manganese toxicity was related to hardness, with higher toxicity in softer water, the effect of hardness was less significant than that of pH.

The lowest concentration reported to be significantly lethal was 320 µg l^{-1} of added manganese, causing an increase in mortality of 15% in brown trout fry in a 30-day study at pH 4.5 (with no increase in mortality at pH 6.6). An assessment factor of 10 was

applied to this value to give an EQS of 30 µg l^{-1} "dissolved" manganese expressed as an annual average for the protection of freshwater aquatic life against toxic effects of manganese, independently of pH. Field data were judged to confirm the validity of this standard.

The short-term freshwater standard was derived from the lowest acute effects concentration, which was a 96-hour LC50 of 3.68 mg l^{-1} for a salmonid species. An assessment factor of 10 was applied to this value to give a rounded EQS of 300 µg l^{-1} "dissolved" manganese as a maximum allowable concentration.

Standards for the protection of marine life were not proposed as the data were inadequate for derivation. Freshwater standards could not be read across because of differences in the chemistry of manganese in increasingly saline systems. The large margin between actual monitored concentrations and the majority of the effects concentrations described in the available toxicity data indicated that the absence of any marine standards was unlikely to be problematic.

3.4 Derivation of PNECs for sediment

No data on the toxicity of manganese in sediments could be found for algal, invertebrate or fish species, except for one saltwater sediment value for crabs. No effect on survival was found when soldier crabs (*Mictyris longicarpus*) were exposed for 21 days to 4300 mg Mn kg^{-1} dw in field sediments (Weimin et al. 1994). Because of this paucity of empirical toxicity data, no PNEC has been derived for sediments.

3.5 Derivation of PNECs for secondary poisoning of predators

3.6.1 Mammalian and avian toxicity data

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

Table 3.5 Most sensitive mammalian and avian oral toxicity data relevant for the assessment of secondary poisoning

Study and result	Details
Long-term toxicity to mammals	
NTP 1993 **Chronic NOAEL = 90 mg kg^{-1} bw day^{-1}**	The chronic toxicity of manganese was investigated in a two-year oral exposure study. Concentrations of 0, 1500, 5000 or 15000 mg manganese sulphate monohydrate per kg body weight (bw) were fed in the diet to male and female F344 rats (70 per sex). These dietary concentrations resulted in doses ranging from 30–331 mg kg^{-1} per day for males and 26–270 mg kg^{-1} per day for females. Ten rats per group were sacrificed at 9 and 15 months. Survival of males in the high dose group significantly decreased starting at week 93 of the study, and death was attributed to advanced renal disease

Study and result	Details
	associated with manganese exposure. Food consumption was similar for all groups. However, by the end of the study, high-dose males exhibited a mean body weight that was 10% lower than controls. No clinical findings or effects on haematological or clinical chemistry parameters were attributed to manganese exposure in any group. Tissue concentrations of manganese were elevated in the livers of mid- and high-dose males, concurrent with a decrease in hepatic iron concentrations. Renal disease in high-dose males was the only pathological effect noted. No increases in tumour incidence were attributed to manganese exposure.
Effects on reproduction of mammals	
Laskey et al. 1982 **NOAEL = 55 mg kg^{-1} bw day^{-1}**	Long-Evans rats were exposed to 0, 400, 1100 or 3550 mg Mn kg^{-1} bw (as Mn_3O_4) in the diet from day 2 of mother's gestation to 224 days of age. Assuming a food consumption factor of 0.05 (USEPA 1986), the average daily dose at the termination of the study was 0, 20, 55, or 177 mg kg^{-1} d^{-1}. The investigators observed a dose-related decrease in serum testosterone concentration (without a concomitant increase in serum luteinising hormone concentration) and reduced fertility at the highest dose. Testes weight, number of ovulations, resorption and pre-implantation deaths, litter size and foetal weights were unaffected by manganese exposure. However, studies also exist that report no adverse reproductive effects in female rats following oral manganese exposure. For example, Pappas et al. (1997) dosed pregnant rats with up to 620 mg Mn kg^{-1} d^{-1} (as $MnCl_2$) throughout gestation. No treatment-related effects were reported in dam health, litter size or sex ratios of the pups. The study did not include more extensive analysis of female reproductive organs.
Embryotoxicity and teratogenicity	
Sánchez et al. 1993 **Maternal toxicity NOAEL = 1.1 mg kg^{-1} bw day^{-1}** **Embryo/foetal NOEAL = 0.56 mg kg^{-1} bw day^{-1}**	The embryotoxic and teratogenic potential of manganese during organogenesis was investigated. Pregnant Swiss mice received daily subcutaneous injections of 0, 2, 4, 8 or 16 mg Mn kg^{-1} d^{-1} of $MnCl_2 \cdot 4H_2O$ on days 6–15 of gestation. These doses correspond to 0, 0.56, 1.1, 2.2, or 4.4 mg Mn kg^{-1} d^{-1}, respectively. Dams were sacrificed on gestational day 18. Significant reductions in weight gain and food consumption were reported in dams receiving ≥8 mg kg^{-1} d^{-1} and treatment-related deaths were reported at 16 mg kg^{-1} d^{-1}. A significant increase in the number of late resorptions was observed at doses of 4 mg kg^{-1} d^{-1} and higher, and reduced foetal body weight and an increased incidence of morphological defects were reported at doses of ≥2.2 mg kg^{-1} d^{-1}. No difference was seen in the incidence of individual or total malformations in treated groups when compared with controls.
Long-term toxicity to birds	
No data could be found on the effects of Mn on avian	Japanese quail (*Corturnix coturnix japonica*) were fed Mn as manganese oxide (Mn_3O_4) in the diet at 5000 mg kg^{-1} food

Study and result	Details
reproduction. One long-term growth and behaviour study by Laskey and Edens (1985) was found. **Chronic NOAEL = 977 mg kg^{-1} bw day^{-1}**	over a 75-day period. Dosing began when chicks were 1 day old. Daily Mn consumption ranged from 575 mg Mn kg^{-1} d^{-1} for adults at the end of the study and 977 mg Mn kg^{-1} d^{-1} for 20-day-old birds. There was no reduction in growth, but male aggressive behaviour was 25–50% reduced relative to controls. Reduced aggressive behaviour was not considered to be an important adverse effect.

NOAEL = no observed adverse effect level

3.6.2 PNECs for secondary poisoning of predators

Manganese is an essential element and can be bioconcentrated by lower trophic levels in aquatic systems. It is involved in photosynthesis (Gerretsen 1949, Gavalas and Clark 1971) and bioconcentration factors are consequently higher for primary producers than for consumers. Reported bioconcentration factors for a variety of aquatic organisms are given below:

- 2000–20000 for aquatic plants
- 2500–6300 for phytoplankton
- 300–5500 for seaweeds
- 800–830 for intertidal mussels
- 35-930 for fish species.

Uptake of manganese by aquatic invertebrates and fish increases with temperature and decreasing salinity. Uptake decreases with increasing pH because the less soluble Mn^{4+} is favoured at higher pH values. Biomagnification up the food chain is either weak, or does not occur (WHO 2004). Kwasnik et al. (1978) found that there was no biomagnifications in a simple freshwater food-chain, with maximum BCFs of 911, 65 and 23 for algae, *Daphnia magna* and fathead minnows (*Pimephales promelas*), respectively. In contrast, other authors have found weak biomagnification (Stokes et al. 1988).

Given the essentiality of manganese, and the fact that it is accumulated to a greater extent by primary producers than by consumers, a PNEC for secondary poisoning is not considered to be relevant.

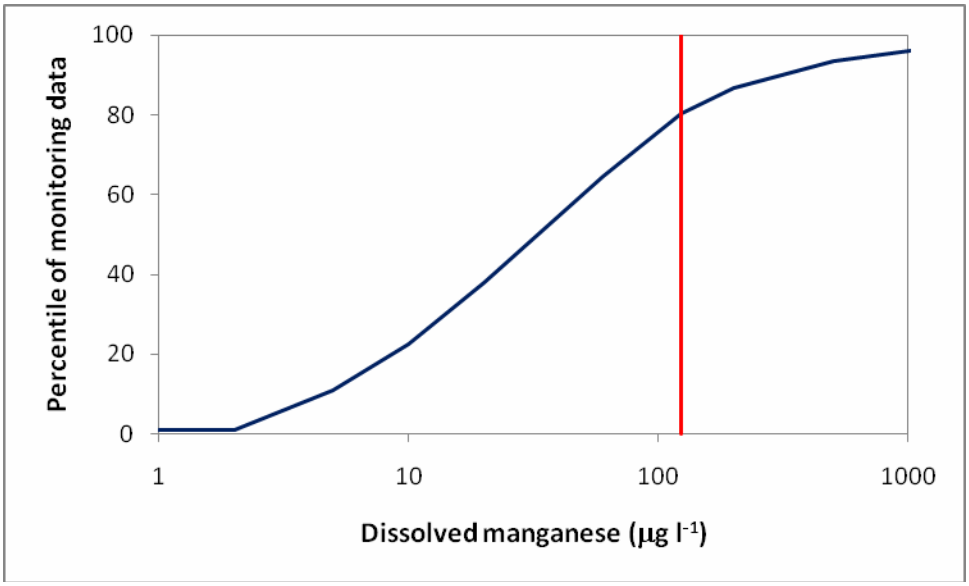
4. Analysis, monitoring and compliance assessment

Analysis of manganese in freshwater samples is typically performed by ICP-OES, with limits of detection of <1 µg l^{-1} being achievable in many routine regulatory analyses of freshwaters (pers. comm. S. Brown, SEPA, Dec 2009).

The proposed PNECs derived for manganese range from 0.05 to 123 µg l^{-1}. To provide adequate precision and accuracy, the data quality requirements are that, at a third of the EQS, total error of measurement should not exceed 50 per cent. From the literature, it can be seen that analytical methodologies employing on-line concentration analysis or flow-injection preconcentration coupled with electrothermal AAS extraction/ preconcentration GC-MS are capable of achieving detection limits as low as 1 ng l^{-1}. Consequently, the current analytical methods should offer adequate performance to analyse manganese for compliance purposes in water.

It is also necessary to consider the potential implications of any proposed standards before they can be implemented to provide an indication of whether or not they are likely to be useful in driving environmental improvements. A provisional compliance assessment of the PNEC for manganese which has been derived in this report has therefore been undertaken against monitoring data for dissolved manganese collected by the Environment Agency. A total of 36068 individual measurements for dissolved manganese were available and these have been compared against the recommended PNEC value of 123 µg l^{-1} which is derived in this report. This provisional comparison has been made against individual monitoring data, rather than against annual average data, as would be the case for compliance assessment against a long term quality standard.

Figure 4.1 Provisional compliance assessment for manganese in England and Wales (n=36068)



The overall situation for all of the data for England and Wales is shown in Figure 4.1, which suggests that 80% would be expected to pass the proposed PNEC value of 123 µg l^{-1} (solid red line) derived in this report. The potential compliance situation has also been estimated for Wales and Anglian Region. These two regions represent two different situations in terms of both exposure and water quality conditions. Wales has a long history of mining for both coal and minerals which could result in increased exposure to manganese, and its water quality is typified by relatively low pH, low hardness, and low DOC concentrations, which may result in high bioavailability of manganese to some organisms. Anglian Region is predominantly agricultural land and the water quality is typified by high pH, high hardness, and high DOC concentrations, which is likely to reduce bioavailability to some organisms.

There is considerably more monitoring data available for Wales than for each of the Environment Agency English regions. Almost half of the available monitoring data for dissolved manganese have been collected in Wales, whereas none of the English regions have more than 4000 data points. This situation may reflect a higher expected risk from manganese in Wales because of the history of mining activity. Approximately 83 per cent of samples from Wales had dissolved manganese concentrations below 123 µg l^{-1}. Conversely, approximately 95 per cent of samples from Anglian Region had dissolved manganese concentrations which are below the proposed PNEC. Similarly, more than 99% of samples reported in Thames Region had dissolved manganese concentrations which were below the proposed PNEC value.

The biotic ligand model for manganese will likely be used in the compliance assessment, and this is described in detail in a separate report (IMnI, 2010).

5. Conclusions

5.1 Availability of data

Freshwater long-term (lt) data are available for algae, bacteria, crustaceans (cladocerans, amphipods, and crayfish), fish (both salmonid and cyprinind), insects (mosquito and midge larvae), macrophytes, oligochaete worms, and snails. Crustaceans appear to be the most sensitive to long-term exposures to manganese.

Long-term saltwater data are available only for algae, annelids, crustaceans and molluscs.

Data from experiments using permanganate have not been included in this report because these describe effects following exposure to an oxidation state that is unlikely to occur in the field.

5.2 Derivation of PNECs

The +7 oxidation state is unstable in water, so is only environmentally relevant near to permanganate discharge points. EQSs therefore need to address the presence of the +2 and +4 forms. In addition, pH and hardness influence toxicity, and bioavailability corrections to take account of these effects are currently under development.

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

5.2.1 Long-term PNEC for freshwaters

Crustaceans appear to be the most sensitive taxonomic group, followed by fish.

Using the assessment factor method to derive a $PNEC_{freshwater}$ requires that an assessment factor of 10 is applied to the lowest reliable NOEC or EC10. The lowest reliable long-term toxicity datum is a 28 - 42 day EC10 of 0.096 mg l^{-1} for growth of the crustacean amphipod *Hyalella azteca*. This results in:

$PNEC_{freshwater\_lt}$ = 96 µg l^{-1}/AF (10) = 9.6 µg l^{-1} manganese (dissolved)

As long-term NOEC data are available for a variety of fish, invertebrates, and primary producers (algae and higher plants), an SSD approach is considered to be appropriate. An HC5 of 246 µg l^{-1} (dissolved Mn), with a confidence interval of 62 to 572 µg l^{-1}, can be calculated from an SSD that meets all goodness-of-fit criteria. An analysis of field evidence suggests that an assessment factor of at least 2 would be expected to ensure protection of potentially sensitive taxa, and that no changes would be observed in whole community metrics at this level of protection. Based on comparison with assessment factors applied to HC5 values in European risk

assessments for metals with similar data profiles, an assessment factor of 2 is considered to be appropriate for the derivation of the PNEC from the HC5.

$PNEC_{freshwater\_lt}$ = 246 ug l^{-1}/AF (2) = 123 µg l^{-1} manganese (bioavailable)

The PNEC value derived using an assessment factor of 2 is higher than the lowest EC10 in the database (*Hyalella azteca*). Two growth data endpoints were available from this study together with an EC10 for survival and an indicative NOEC value for reproduction. The results, when considered as a whole picture, indicate that the PNEC derived in this report is likely to be adequately protective of sensitive freshwater organisms at the population level.

5.2.2 Long-term PNEC for saltwaters

The most sensitive and reliable long-term toxicity values relate manganese exposure over 7–20 days to growth of Pacific oyster, *Crassostrea gigas*, and hatching of yellow crab, *Cancer anthonyi*, both resulting in a lowest observed effect concentration (LOEC) of 10 µg l^{-1}. This is supported by an experiment to assess effects on settlement of oyster spat, where a NOEC of 20 µg l^{-1} was estimated. An assessment factor of 2 is recommended to extrapolate to a NOEC from the LOECs of 10 µg l^{-1}, and another factor of 100 is recommended to account for interspecies differences in sensitivity because there are no long-term NOECs for saltwater fish or algae. Although chronic data are not plentiful, indications of a steep dose response in these studies suggest a factor no greater than 100 is required. This results in a $PNEC_{saltwater\_lt}$ of 0.05 µg l^{-1} manganese (dissolved). There is no existing saltwater EQS for manganese.

5.2.3 PNECs for sediment and secondary poisoning

Although manganese is found in sediments, there is only one study describing its toxicity to sediment-dwelling organisms. The study was not deemed suitable for PNEC derivation. It is therefore not possible to derive a $PNEC_{sediment}$.

Manganese is an essential element and can be significantly bioaccumulated by aquatic primary producers, but is accumulated to a lesser extent by consumers. As a result of this an EQS for secondary poisoning of manganese is not considered to be relevant

Table 5.1 Summary of proposed PNECs

Receiving medium/exposure scenario	Proposed PNEC (µg l^{-1} manganese)	Existing EQS (µg l^{-1})
Freshwater/long-term	9.6 (AF approach) 123 (SSD approach) (bioavailable)	30
Saltwater/long-term	0.05	No standard
Secondary poisoning	none proposed	No standard

5.3 Analysis

The data quality requirements are that, at a third of the EQS, total error of measurement should not exceed 50 per cent. Analytical methodologies currently employed by UK environmental regulators are capable of achieving detection limits below 1 µg l^{-1}. Consequently, the current analytical methods should offer adequate performance to analyse manganese for compliance purposes in freshwater.

5.4 Implementation issues

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

- The proposed freshwater long term PNEC is not subject to excessive uncertainty and analytical techniques are sufficient to assess compliance.
- The freshwater PNEC is derived based on conditions of high bioavailability and therefore bioavailablity needs to be taken into account when assessing compliance.
- The saltwater PNEC of 0.05 µg l^{-1} is an order of magnitude below the low end of concentrations reported in seawater and is therefore not implementable as an EQS.

References & Bibliography

ABBASI, S.A. 1988. Synergistic extraction and atomic absorption spectrometric determination of manganese in environmental samples using N-p-aminophenyl-2-furyl-acrylohydroxamic acid and trioctylmethyl ammonium cation. Analytical Letters, 21, 1935–1944.

Agency for Toxic Substances and Disease Registry (ATSDR), 2000. Toxicological profile for manganese. Atlanta, GA: ATSDR, US Department of Health and Human Services.

BEKLEMISHEV, M.K., STOYAN, T.A. AND DOLMANOVA, I.F. 1997. Sorptioncatalytic determination of manganese directly on a paper-based chelating sorbent. Analyst, 122, 1161–1165.

BIESINGER, K.E. AND CHRISTENSEN, G .M. 1972. Effects of various metals on survival, growth, reproduction, and metabolism of *Daphnia magna.* Journal of the Fisheries Research Board of Canada, 29, 1691–1700.

BRYAN, G.W. AND HUMMERSTONE, L.G. 1973. Adaptation of the polychaete *Nereis diversicolor* to manganese in estuarine sediments. Journal of the Marine Biological Association of the UK, 53, 859–872.

BUDAVARI, S., O'NEIL, M.J., SMITH, A., HECKELMAN, P.E. AND KINNEARY, J.F. 1996. Editors The Merck Index: An Encyclopaedia of Chemicals, Drugs, and Biologicals (12th edn.). Rahway, NJ: Merck & Co., Inc.

CANTERFORD, G.S. AND CANTERFORD, D.R. 1980. Toxicity of heavy metals to the marine diatom *Ditylum brightwellii* (West) Grunow: correlation between toxicity and metal speciation. Journal of the Marine Biological Association of the UK, 60, 227–242.

CHEMFINDER 2005. ChemFinder database. Cambridge, MA: CambridgeSoft Corp. Available from http://chemfinder.cambridgesoft.com/ [Accessed 12 February 2007]

CHIN, C.S., JOHNSON, K.S. AND COALE, K.H. 1992. Spectrophotometric determination of dissolved manganese in natural waters with 1-(2-pyridylazo)-2-naphthol: application to analysis in situ in hydrothermal plumes. Marine Chemistry, 37, No. 1–2, 65–82.

CHRISTENSEN, E.R., SCHERFIG, J. AND DIXON, P.S. 1979. Effects of manganese, copper and lead on *Selenastrum capricornutum* and *Chlorella stigmatophora.* Water Research, 13, 79–92.

DAVIES, P.H. AND BRINKMAN, S.F. 1994. Acute and chronic toxicity of manganese to exposed and unexposed rainbow and brown trout. Fort Collins, CO: Colorado Division of Wildlife.

DAVIES, P.H., BRINKMAN, S.F. AND MCINTYRE, M. 1998. Toxicity of manganese to early-life stage and fry of brook trout (*Salvelinus fontinalis*) and Rainbow trout

(*Oncorhynchus mykiss*) in water hardness of 30 and 150 mg/l. Colorado Division of Wildlife, Fort Collins, Colorado, USA.

DENMARK, 2007. European Union Risk Assessment Report on nickel, nickel sulphate, nickel carbonate, nickel chloride, nickel dinitrate, Denmark, Draft report October 2007. Prepared by Denmark, Danish Environmental Protection Agency on behalf of the European Union.

DITORO, D.M., ALLEN, H.E., BERGMAN, H.L., MEYER, J.S., PAQUIN, P.R. AND SANTORE, R.C. 2001. Biotic Ligand Model of the acute toxicity of metals. 1. Technical basis. Environmental Toxicology and Chemistry, 20, 2383-2396.

DIVISION OF SPECIALIZED INFORMATION SERVICES (SIS) OF THE US NATIONAL LIBRARY OF MEDICINE (NLM), 2005. Toxicology Data Network (TOXNET®): Hazardous Substances Data Bank (HSDB®) [online]. Bethesda, MD: SIS. http://toxnet.nlm.nih.gov/ [Accessed 12 February 2007]

ECHA, 2008. Guidance on information requirements and chemical safety assessment. Chapter R10: Characterisation of dose [concentration]-response for environment. European Chemicals Agency, Helsinki, Finland.

ECI , 2007. European Union Risk Assessment Report on Copper, copper(ii)sulphate pentahydrate, copper(i)oxide, copper(ii)oxide, dicopper chloride trihydroxide. Voluntary risk assessment, draft February 2007. European Copper Institute.

ENSR, 1992. Chronic toxicity of manganese to *Ceriodaphnia dubia* under static renewal conditions at four levels of water hardness. Doc. 8505-092-047. ENSR, Colorado, USA.

ENSR 1996. Early life stage toxicity of manganese to the fathead minnow (*Pimephales promelas*) under flow-through test conditions. Doc 7076-001-302. ENSR, Colorado, USA.

ENVIRONMENT AGENCY, 2007. Proposed EQS for Water Framework Directive Annex VIII substances: manganese (total dissolved). SC040038/SR10. Environment Agency, Bristol, UK.

EUROPEAN COMMISSION JOINT RESEARCH CENTRE (JRC), 2003. Technical Guidance Document on risk assessment in support of Commission Directive 93/67/EEC on risk assessment for new notified substances and Commission Regulation (EC) No. 1488/94 on risk assessment for existing substances and Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. Part II. EUR 20418 EN/2. Luxembourg: Office for Official Publications of the European Communities. Available from: http://ecb.jrc.it/tgdoc [Accessed 12 February 2007]

GAVALAS, N. AND CLARKE, H. 1971. On the role of manganese in photosynthesis. Plant Physiology, 47,139-143.

GERRETSEN, F. 1949. Manganese in relation to photosynthesis. Plant and Soil 1, 346-358.

HEDGECOTT, S., DIXON, E., BAILEY, K.L. AND JILLIANS, S. 1998. Proposed Environmental Quality Standards for manganese in water. DETR 4497/1. London: Department of the Environment, Transport and the Regions (DETR).

HIRST, H., JUTTNER, I. AND ORMEROD, S. J. 2002. Comparing the responses of diatoms and macroinvertebrates to metals in upland streams of Wales and Cornwall. Freshwater Biology, 47, 1752–1765.

HUNTSMAN, S. A. AND SUNDA, W.G. 1980. The role of trace metals in regulating phytoplankton. In The Physiological Ecology of Phytoplankton (ed. I. Morris), pp. 285–328. Berkeley, CA: University of California.

IMnI, 2008. Toxicity of manganese to *Pseudokirchneriella subcapitata* under static test conditions. Prepared by Parametrix, Albany, Oregon, USA for the International Manganese Institute.

IMnI, 2009a. Short-term chronic toxicity of manganese to duckweed (*Lemna minor*) under semi-static renewal exposure conditions. Prepared by Parametrix, Albany, Oregon, USA for the International Manganese Institute.

IMnI, 2009b. The chronic toxicity of manganese to the amphipod crustacean *Hyalella azteca* using a standardised flow-through experiment. Prepared by Parametrix, Albany, Oregon, USA for the International Manganese Institute.

IMnI, 2009c. Lifecycle toxicity of manganese to the midge *Chironomus tentans.* Prepared by Parametrix, Albany, Oregon, USA for the International Manganese Institute.

IMnI, 2009d. Evaluation of chronic toxicity of manganese to the aquatic Oligochaete *Aeolosoma* sp. Prepared by Parametrix, Albany, Oregon, USA for the International Manganese Institute.

IMnI, 2009e. Chronic toxicity of manganese to the great pond snail*, Lymnaea stagnalis*. Prepared by Parametrix, Albany, Oregon, USA for the International Manganese Institute.

IMnI, 2009f. Early life-stage toxicity of manganese to the zebrafish (*Danio rerio*) under flow-through conditions. Prepared by Parametrix, Albany, Oregon, USA for the International Manganese Institute.

IMnI. 2010 Development of a biotic ligand model for manganese. Prepared by wca environment, Faringdon, Oxfordshire, UK for the International Manganese Institute (in press).

KLIMISCH, H.J., ANDREAE, E. AND TILLMANN, U. 1997. A systematic approach for evaluating the quality of experimental and ecotoxicological data. RegulatoryToxicology and Pharmacology, 25, 1-5.

KUCERA, J., SOUKAL, L. AND FALTEJSEK, J. 1986. Low level determination of manganese in biological reference materials by neutron activation analysis. Journal of Radioanalytical Nuclear Chemistry, 107, 361–369.

KWASNIK, G.M., VETTER, R.J., ATCHISON, G.J.1978. The uptake of manganese-54 by green algae (*Protococcoidal chlorella*), *Daphnia magna*, and fathead minnows (*Pimephales promelas*). *Hydrobiologia*, 59,181–185.

LASIER, P.J., WINGER, P.V. AND BOGENRIEDER, K.J. 2000. Toxicity of manganese to *Ceriodaphnia dubia* and *Hyalella azteca*. Archives of Environmental Contamination and Toxicology, 38, 298–304.

LASKEY, J.W. AND EDENS, F.W. 1985. Effects of chronic high-level manganese exposure on male behaviour in the Japanese quail (*Coturnix coturnix japonica)*. Poultry Science, **64**, 579–584.

LASKEY, J.W., REHNBERG, G.L. AND HEIN, J.F. 1982. Effects of chronic manganese (Mn_3O_4) exposure on selected reproductive parameters in rats. Journal of Toxicology and Environmental Health, 9, 677–687.

LAVI, N., LUX, F. AND AL-FASSI , Z.B. 1989. Determination of magnesium, aluminium, phosphorus, copper and manganese in biological fluids by neutron activation analysis. Journal of Radioanalytical and Nuclear Chemistry, 129, 93–102.

MACDONALD, J.M., SHIELDS, J.D. AND ZIMMER-FAUST, R. K. 1988. Acute toxicities of eleven metals to early life-history stages of the yellow crab *Cancer anthonyi.* Marine Biology, 98, 201–207.

MORI, I., FUJITA, Y., IKUTA, K., NAKAHASHI, Y. AND KATO, K. 1989. Highly sensitive and selective fluorimetric methods for the determination of iron (III) and manganese (II) using fluorescein–hydrogen peroxide–triethylenetetramine and fluorescein–hydrogen peroxide–triethylenetetramine/iron, respectively. Fresenius Journal of Analytical Chemistry, 334, 252–255.

MUNDA, I.M. AND HUDNIK, V. 1986. Growth response of *Fucus vesiculosus* to heavy metals, singly and in dual combinations, as related to accumulation. Botanica Marina, 29, 401–412.

NAGPAL, N.K. 2001. Ambient water quality guidelines for manganese. Overview report. Victoria, British Columbia: Government of British Columbia, Ministry of Environment, Water, Air and Climate Change Branch. Available from: http://www.env.gov.bc.ca/wat/wq/BCguidelines/manganese/manganese.html [Accessed 12 December 2009]

NASU, Y. AND KUGIMOTO, M.1981 *Lemna* (duckweed) as an indicator of water pollution. I. The sensitivity of *Lemna paucicostata* to heavy metals. Archives of Environmental Contamination and Toxicology, 10, 159–169.

NRC, 1993. Nutrient requirements of fish. National Research Council Committee on Animal Nutrition. Board on Agriculture, Washington, DC, USA. pp. 113.

NATIONAL TOXICOLOGY PROGRAM (NTP), 1993. Toxicology and carcinogenesis studies of manganese (II) sulfate monohydrate (CAS no. 10034-96-5) in F344/N rats and

B6C3F1 mice (feed studies). NTP Technical Report Series 428. Research Triangle Park, NC, USA.

NEAL,C., JARVIE, H.P., WHITTON, B.A. AND GEMMELL, J. 2000. The water quality of the River Wear, north-east England. Science of the Total Environment, 251–252, 153–172.

NERC. 2001. The Windermere Humic Aqueous Model (WHAM). Natural Environment Research Council, Swindon, UK.

NORWOOD, W.P., BORGMANN, U. AND DIXON, D.G. 2006. Saturation models of arsenic, cobalt, chromium and manganese bioaccumulation by *Hyalella azteca.* Environmental Pollution, 143, 519-528.

NORWOOD, W.P., BORGMANN, U. AND DIXON, D.G. 2007. Chronic toxicity of arsenic, cobalt, chromium and manganese to *Hyalella azteca* in relation to exposure and bioaccumulation. Environmental Pollution, 147, 262-272.

NYFFLER, U.P., LI ,Y.H. AND SANTSCHI, P.H. 1984. Kinetic approach to describe trace-element distribution between particles and solution in natural aquatic systems. Geochimica and Cosmochimica Acta, 48, 1513–1522.

OXFORD UNIVERSITY, 2007. The Physical and Theoretical Chemistry Laboratory: Chemical and other safety information [online]. Available from: http://physchem.ox.ac.uk/MSDS/ [Accessed 12 February 2007]

PAPPAS, B. A., ZHANG, D. AND DAVIDSON, C.M. 1997. Perinatal manganese exposure: behavioral, neurochemical, and histopathological effects in the rat. Neurotoxicology and Teratology, 19, 17–25.

PAQUIN, P.R., GORSUCH, J .W., APTE, S., BATLEY, G.E., BOWLES , K.C., CAMPBELL, P.G C., DELOS, C.G., DITORO, D.M., DWYER, R.L., GALVEZ. F., GENSEMER, R.W., GOSS, G.G., HOGSTRAND, C., JANSSEN, C.R., MCGEER, J.C., NADDY, R.B., PLAYLE, R.C., SANTORE, R.C., SCHNEIDER, U., STUBBLEFIELD, W.A., WOOD, C.M. AND WU, K. 2002. The biotic ligand model: a historical overview: Special issue: The biotic ligand model for metals – current research, future directions, regulatory implications. Comparative Biochemistry and Physiology, Part C 133, 3-35.

PETERS, A., MERRINGTON, G., DE SCHAMPHELAERE, K. AND DELBEKE, K. 2010. Regulatory considerations of bioavailability for metals: Simplification of input parameters for the chronic copper Biotic Ligand Model. Integrated Environmental Assessment and Management (submitted)

READER, J.P., EVERALL, N.C., SAYER, M.D.J. AND MORRIS, R. 1989. The effects of eight trace metals in acid soft water on survival, mineral uptake and skeletal calcium deposition in yolk-sac fry of brown trout, *Salmo trutta* L. Journal of Fish Biology, 35, 187–198.

REIMER, P.S. 1999. Environmental effects of manganese and proposed freshwater guidelines to protect aquatic life in British Columbia. MSc Thesis. Vancouver, BC: University of British Columbia.

RESING, J.A. AND MOTTL, M.J. 1992. Determination of manganese in seawater using flow injection analysis with on-line preconcentration and spectrophotometric detection. Analytical Chemistry, 64, 2682–2687.

ROSKO, J.J.AND RACHLIN, J.W. 1975. The effect of copper, zinc, cobalt and manganese on the growth of the marine diatom *Nitzschia closterium*. Bulletin of the Torrey Botanical Club, 102, 100–106.

SÁNCHEZ, D.J., DOMINGO, J.L., LLOBET, J.M. AND KEEN, C.L. 1993. Maternal and developmental toxicity of manganese in the mouse. Toxicology Letters, 69, 45–52.

SATOH, H., TAKEUCHI, T. AND WATANABE, T. 1987. Availability to carp of manganese in white-fish meal and of various manganese compounds. Nippon Suisan Gakkaishi, 53, 825–832.

SCHER, 2009. Voluntary risk assessment report on lead and its compounds: Environmental part. Scientific Committee on Health and Environmental Risks, European Commission Health and Consumer Protection Directorate General, Brussels, Belgium.

STOKES, P.M., CAMPBELL, P.G.C., SCHROEDER, W.H., TRICK, C., FRANCE, R.L., PUCKETT, K.J., LAZERTE, B., SPEYER, M., HANNA, J.E., DONALDSON, J. 1988. Manganese in the Canadian environment. Ottawa, Ontario, National Research Council of Canada, Associate Committee on Scientific Criteria for Environmental Quality (NRCC No. 26193).

STUBBLEFIELD, W.A., BRINKMAN, S.F., DAVIES, P.H., GARRISON, T. D., HOCKETT, J.R. AND MCINTYRE, W. 1997. Effects of water hardness on the toxicity of manganese to developing brown trout (*Salmo trutta*). Environmental Toxicology and Chemistry, 16, 2082–2089.

SYRACUSE RESEARCH CORPORATION (SRC), 2007. [online] PHYSPROP physical properties database. Syracuse, NY: SRC. Available from: http://www.syrres.com/eSc/physprop.htm [Accessed 12 February 2007]

US ENVIRONMENTAL PROTECTION AGENCY (US EPA), 1986. Reference values for risk assessment. Cincinnati, OH: US EPA, Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office.

US ENVIRONMENTAL PROTECTION AGENCY (US EPA), 2003. Health effects support document for manganese. EPA-822-R-03-003. Washington, DC: US EPA, Office of Water, Health and Ecological Criteria Division.

US ENVIRONMENTAL PROTECTION AGENCY (US EPA), 2004. Drinking water health advisory for manganese. EPA-822-R-04-003. Washington, DC: US EPA, Office of Water, Health and Ecological Criteria Division.

US ENVIRONMENTAL PROTECTION AGENCY (US EPA), 2005 ECOTOX database [online]. Washington, DC: US EPA. Available from http://www.epa.gov/ecotox/ [Accessed 12 February 2007].

Van Vlaardingen, P.L.A., Traas, T.P., Wintersen, A.M., Aldenberg, T., 2004. E_TX 2.0. A program to calculate hazardous concentrations and fraction affected, based on normally distributed toxicity data. Bilthoven, the Netherlands: National Institute for Public Health and the Environment (RIVM). Report no. 601501028/2004, 68 pp.

WATLING, H.R.1983. Comparative study of the effects of metals on the settlement of *Crassostrea gigas*. Bulletin of Environmental Contamination and Toxicology, 31, 344–351.

WEIMIN, Y., BATLEY, G.E. AND ASHANULLAH, M. 1994. Metal bioavailability to the soldier crab *Mictyris longicarpus*. Science of the Total Environment, 141, 27–44.

WINDHOLZ, M., BUDAVARI, S., BLUMETTI, R.F. AND OTTERBEIN, E.S. 1983. Editors The Merck Index: An Encyclopaedia of Chemicals, Drugs, and Biologicals (10th ed.). Rahway, NJ: Merck & Co., Inc.

WORLD HEALTH ORGANIZATION (WHO), 1981. Environmental Health Criteria 17: Manganese. International Programme on Chemical Safety (IPCS). Geneva: WHO. Available from: http://www.who.int/ipcs/publications/ehc/en/ [Accessed 3 February 2009].

WORLD HEALTH ORGANIZATION (WHO), 2004. Concise International Chemical Assessment Document 63. Manganese and its compounds: environmental aspects. Geneva: WHO. Available from: http://www.who.int/ipcs/publications/cicad/en/ [Accessed 12 February 2007]

ZAW, M. AND CHISWELL, B. 1999. Iron and manganese dynamics in lake water. Water Research, 33, 1900–1910.

List of abbreviations

AAS	atomic absorption spectroscopy
AF	assessment factor
aq	aqueous
ATSDR	Agency for Toxic Substances and Disease Registry
BCF	bioconcentration factor
bw	body weight
CAS	Chemical Abstracts Service
CICAD	Concise International Chemical Assessment Document
DAA	dissolved annual average
DO	dissolved oxygen
dw	dry weight
EC50	concentration effective against 50% of the organisms tested
ECx	concentration effective against X% of the organisms tested
EQS	Environmental Quality Standard
GLP	Good Laboratory Practice (OECD)
IC50	concentration at which the population effect of the organisms tested is inhibited by 50%
ICP-MS	inductively coupled plasma mass spectrometry
IUPAC	International Union of Pure and Applied Chemistry
LC50	concentration lethal to 50% of the organisms tested
LCx	concentration lethal to X% of the organisms tested
LOD	limit of detection
LOEC	lowest observed effect concentration
lt	long term
MAC	maximum allowable concentration
NOAEL	no observed adverse effect level
NOEC	no observable effect concentration
OECD	Organisation for Economic Co-operation and Development
PNEC	predicted no-effect concentration
secpois	secondary poisoning
SEPA	Scottish Environment Protection Agency
SNIFFER	Scotland & Northern Ireland Forum for Environmental Research

SSD	species sensitivity distribution
st	short term
TGD	Technical Guidance Document
UKTAG	UK Technical Advisory Group
US EPA	US Environmental Protection Agency
WFD	Water Framework Directive
WHO	World Health Organization

ANNEX 1 Data quality assessment sheets

Identified and ordered alphabetically.

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	BIESINGER, K.E. AND CHRISTENSEN, G .M. 1972. Effects of various metals on survival, growth, reproduction, and metabolism of *Daphnia magna.* Journal of the Fisheries Research Board of Canada, 29, 1691–1700.

Information on the test species	
Test species used	*Daphnia magna*
Source of the test organisms	In-house laboratory culture originally obtained from the University of Michigan.
Holding conditions prior to test	Same source for test medium and culture water.
Life stage of the test species used	< 24 h old

Information on the test design	
Methodology used	No standard methodology cited but description sufficient for evaluation
Form of the test substance	$MnCl_2 \cdot H_2O$
Source of the test substance	American Chemical Society reagent-grade chemical
Type and source of the exposure medium	Lake Superior water, strained though #20 bolting cloth. Some bacteria, algae and detritus in water.
Test concentrations used	Geometric series actual concentration values not stated.
Number of replicates per concentration	4
Number of organisms per replicate	20
Nature of test system	Semi-static with renewal weekly
Measurement of exposure concentrations	Yes but results are based on nominal concentrations
Measurement of other quality parameters	Yes
Test validity criteria satisfied	Not stated
Quality criteria satisfied	Yes
Study conducted to GLP	No
Overall comment on quality	Reproductive parameter: total number of young. A 16% reproductive impairment concentration representing “the minimal reproducible value below which the variability in reproduction could not be detected from controls”.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	CANTERFORD G S AND CANTERFORD D R, 1980 Toxicity of heavy metals to the marine diatom *Ditylum brightwellii* (West) Grunow: correlation between toxicity and metal speciation. Journal of the Marine Biological Association of the UK, 60, 227–242.

Information on the test species	
Test species used	*Dityllum brightwellii*
Source of the test organisms	Culture
Holding conditions prior to test	Culture
Life stage of the test species used	Exponential growth

Information on the test design	
Methodology used	Static 5-day exposure
Form of the test substance	$MnCl_2$
Source of the test substance	AnalaR
Type and source of the exposure medium	Seawater
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not relevant
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	No
Measurement of water quality parameters	No
Test validity criteria satisfied	-
Water quality criteria satisfied	-
Study conducted to GLP	Not stated
Overall comment on quality	A standard algal test system

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	DAVIES, P.H., BRINKMAN, S.F. AND MCINTYRE, M. 1998. Toxicity of manganese to early-life stage and fry of brook trout (*Salvelinus fontinalis*) and Rainbow trout (*Oncorhynchus mykiss*) in water hardnesses of 30 and 150 mg/l. Colorado Division of Wildlife, Fort Collins, Colorado, USA.

Information on the test species	
Test species used	*Salvelinus fontinalis and Oncorhynchus mykiss*
Source of the test organisms	*S. fontinalis* – 'eyed' eggs from Dubois State Fish Hatchery, Dubois, Wyoming. *O. mykiss* – eggs from Colorado Division of Wildlife Crystal River Hatchery brood stock.
Holding conditions prior to test	Eggs were placed on submerged egg trays and acclimated to each of the water qualities for 2 days prior to initiation of the ELS tests.
Life stage of the test species used	Eggs

Information on the test design	
Methodology used	ASTM standardised ELS test undertaken at two hardness regimes (30 and 150 mg l^{-1} $CaCO_3$). 65 days total exposure.
Form of the test substance	$MnSO_4 \cdot H_2O$
Source of the test substance	Mallinckrodt, Inc., Paris, Kentucky
Type and source of the exposure medium	30 mg $CaCO_3$ l^{-1} hardness: mixture of dechlorinated Fort Collins municipal water and water deionized by reverse osmosis. 150 mg $CaCO_3$ l^{-1} hardness: mixture dechlorinated Fort Collins municipal water and well water from a well located at the Colorado Division of Wildlife Research Facility in Fort Collins.
Test concentrations used	Measured: *S. fontinalis* – (30 mg $CaCO_3$ l^{-1} hardness) 0.03 (control), 0.36, 0.55, 0.85, 2.18, 4.48, 8.9 and 19.9 mg Mn l^{-1}(total). *S. fontinalis* – (150 mg $CaCO_3$ l^{-1} hardness) <0.02 (control), 0.78, 1.68, 3.53, 7.53, 15.65, 34.34 and 73.72 mg Mn l^{-1}(total). *O. mykiss* – (30 mg $CaCO_3$ l^{-1} hardness) <0.02 (control), 0.42, 0.76, 1.47, 2.85, 5.14, 8.4 and 18.7 mg Mn l^{-1}(total). *O. mykiss* – (150 mg $CaCO_3$ l^{-1} hardness) <0.02 (control), 1.25, 2.08, 3.39, 6.48, 18.3, 37.7 and 77.2 mg Mn l^{-1}(total).
Number of replicates per concentration	4

Number of organisms per replicate	10
Nature of test system (static, semi-static or flow-through, duration, feeding)	Flow-through = ~ 1 l of test solution per 0.5 g of fish over a 24 h period.
Measurement of exposure concentrations	Yes
Measurement of water quality parameters	Yes, weekly in alternate replicates.
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to GLP	Yes
Overall comment on quality	Standard Guideline Study.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	ENSR 1996. Early life stage toxicity of manganese to the fathead minnow (*Pimephales promelas*) under flow-through test conditions. Doc 7076-001-302. ENSR, Colorado, USA.
Information on the test species	
Test species used	*Pimephales promelas*
Source of the test organisms	In-house laboratory culture
Holding conditions prior to test	
Life stage of the test species used	Eggs, within 24 h of fertilization
Information on the test design	
Methodology used	ASTM Method E1241-92, Standard Guide for Conducting Early Life-stage Tests with Fishes (ASTM 1992)
Form of the test substance	$MnCl_2 \cdot 4H_2O$; purity 98.8%
Source of the test substance	Mallinckrodt, Inc., Paris, Kentucky.
Type and source of the exposure medium	Fort Collins Environmental Toxicology Laboratory process water obtained from Horsetooth Reservoir. Hardness and alkalinity were not adjusted during the study.
Test concentrations used	Nominal: control (0), 0.09, 0.19, 0.38, 0.75, 1.5, 3 and 6 mg Mn l^{-1}. Measured: 0, 0.19, 0.26, 0.45, 0.82, 1.41, 2.77 and 5.73 mg Mn l^{-1}.
Number of replicates per concentration	4
Number of organisms per replicate	20
Nature of test system (static, semi-static or flow-through, duration, feeding)	Flow-through system. Flow rate of test solution adjusted to deliver 20 ml of test solution per minute to each test chamber ~ flow rate minimum of 1 l of test solution per 0.5 g of fish over a 24 h period.
Measurement of exposure concentrations	Yes
Measurement of water quality parameters	Yes
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to GLP	Yes
Overall comment on quality	Standard Guideline Study.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	ENSR, 1992. Chronic toxicity of manganese to *Ceriodaphnia dubia* under static renewal conditions at four levels of water hardness. Doc. 8505-092-047. ENSR, Colorado, USA.

Information on the test species	
Test species used	*Ceriodaphnia dubia*
Source of the test organisms	In-house laboratory culture
Holding conditions prior to test	On day prior to test initiation, gravid females were isolated in culture water at test temperature.
Life stage of the test species used	< 24 h old

Information on the test design	
Methodology used	*Ceriodaphnia dubia* Survival and Reproduction Test (US EPA Method 1002.0)
Form of the test substance	$MnCl_2 \cdot 4H_2O$
Source of the test substance	Mallinckrodt, Inc., Paris, Kentucky.
Type and source of the exposure medium	Reconstituted water prepared to produce 4 nominal hardness levels: 25, 50, 100 and 200 mg $CaCO_3$ l^{-1}.
Test concentrations used	Nominal (hardness 25 mg $CaCO_3$ l^{-1}): control (0), 0.625, 1.25, 2.5, 5 and 10 mg Mn l^{-1}. Measured (hardness 25 mg $CaCO_3$ l^{-1}): 0.02, 0.38, 0.94, 2.04, 4.41, and 8.57 mg Mn l^{-1}. Nominal (hardness 50 mg $CaCO_3$ l^{-1}): control (0), 0.625, 1.25, 2.5, 5 and 10 mg Mn l^{-1}. Measured (hardness 50 mg $CaCO_3$ l^{-1}): 0.02, 0.48, 0.91, 2.06, 4.55, and 9.44 mg Mn l^{-1}. Nominal (hardness 100 mg $CaCO_3$ l^{-1}): control (0), 1.25, 2.5, 5, 10 and 20 mg Mn l^{-1}. Measured (hardness 100 mg $CaCO_3$ l^{-1}): 0.04, 0.98, 2.12, 4.8, 9.3 and 18.2 mg Mn l^{-1}. Nominal (hardness 200 mg $CaCO_3$ l^{-1}): control (0), 2.5, 5, 10, 20 and 40 mg Mn l^{-1}. Measured (hardness 200 mg $CaCO_3$ l^{-1}): 0.02, 1.94, 4.34, 7.82, 20.4 and 37.6 mg Mn l^{-1}.
Number of replicates per concentration	10
Number of organisms per replicate	1
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static renewal every 24 h. *C. dubiai* fed 0.1 ml each of Yeast-Trout chow-Cerophyl food suspension and *P. subcapitata* suspension.
Measurement of exposure concentrations	Yes

Measurement of water quality parameters	DO, temperature and pH measured in each treatment at the beginning and end of each 24 h exposure period.
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to GLP	Yes
Overall comment on quality	Guideline study. Multiple tests performed to assess the effects of hardness on the bioavailability of dissolved Mn. EC10 values were calculated using the data reported in the study report. NOECs (reproduction) 2.04, 2.06, 4.8 and 7.82 mg Mn l^{-1} at 25, 50, 100 and 200 mg $CaCO_3$ l^{-1}, respectively.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	IMnI, 2008. Toxicity of manganese to *Pseudokirchneriella subcapitata* under static test conditions. Prepared by Parametrix, Albany, Oregon, USA for the International Manganese Institute.

Information on the test species	
Test species used	*Pseudokirchneriella subcapitata*
Source of the test organisms	In-house culture
Holding conditions prior to test	Cultured in US-EPA media. 21 ± 2 °C. Continuous lighting and aeration
Life stage of the test species used	3-day-old culture

Information on the test design	
Methodology used	In-house protocol designed to be compliant with relevant OECD algal testing guideline measuring growth rate and cell yield.
Form of the test substance	$MnCl_2$
Source of the test substance	Alfa Aesar (Ward Hill, MA, USA). Purity ≥ 97%
Type and source of the exposure medium	Filtered well water diluted with de-ionised water. Amended with US-EPA nutrient stocks without EDTA, pH adjusted to 7.5 ± 0.1. MOPS buffer (750 mg l^{-1}) used to control rate of pH change. Mn added to algal stocks at 0.115 mg l^{-1}.
Test concentrations used	Nominal: Control (0), 500, 1000, 2000, 4000, 8000 µg Mn l^{-1}. Measured: 146.3, 627.8, 1106.8, 2178.4, 4071.0, 7808.1 µg Mn l^{-1}
Number of replicates per concentration	3 per test concentration, 6 control
Number of organisms per replicate	5 x 10^3 cells ml^{-1} at test initiation
Nature of test system	Static
Measurement of exposure concentrations	Yes. At initiation (total and dissolved Mn) and termination (dissolved Mn)
Measurement of other quality parameters	Initiation: hardness, alkalinity, residual chlorine, ammonia, pH, temperature. Daily: pH and temperature.
Test validity criteria satisfied	Yes
Quality criteria satisfied	Yes
Study conducted to GLP	Yes, although no signatures in report
Overall comment on quality	The toxicity of manganese to *P. subcapitata* was determined using a standard 72 hour algal toxicity test according to OECD guideline methodology. Growth rate and cell yield were the test endpoints. EDTA (which can affect the bioavailability of metals) was omitted from the growth media, although Manganese was present as trace element at a concentration of 0.115 mg Mn l^{-1}. A dose-response relationship was apparent with the most sensitive endpoint being cell yield with

	an EC_{10} of 0.62 mg Mn l^{-1} based on measured concentrations (including the background Mn present in the media). Associated confidence interval of 0.28 – 1.40 mg Mn l^{-1}.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	IMnI, 2009a. Short-term chronic toxicity of manganese to duckweed (*Lemna minor*) under semi-static renewal exposure conditions. Prepared by Parametrix, Albany, Oregon, USA for the International Manganese Institute.

Information on the test species	
Test species used	*Lemna minor* Duckweed
Source of the test organisms	University of Toronta Culture and Collection
Holding conditions prior to test	Held for 9 days prior to testing
Life stage of the test species used	Plants with 3 fronds

Information on the test design	
Methodology used	7 day plant growth test designed to comply with requirements of OECD guideline 221
Form of the test substance	$MnCl_2$
Source of the test substance	Alfa Aesar (Ward Hill, MA, USA). Purity ≥ 97%
Type and source of the exposure medium	Swedish Standard Lemna Growth Media
Test concentrations used	Nominal: Control (0), 3.88, 7.75, 15.5, 31, 62 mg Mn^{-1} Measured: <0.05, 3.34, 7.01, 15.57, 30.72, 64.94
Number of replicates per concentration	3
Number of organisms per replicate	3
Nature of test system	Static renewal
Measurement of exposure concentrations	Yes. Total and dissolved Mn on initiation. Dissolved Mn on renewal.
Measurement of other quality parameters	Hardness, alkalinity, conductivity, total residual chlorine, ammonia, pH (initiation). pH (renewal), temperature (daily)
Test validity criteria satisfied	Yes
Quality criteria satisfied	Yes
Study conducted to GLP	Yes
Overall comment on quality	Guideline study. The toxicity of Mn to aquatic higher plants was determined in a standardised 7 day growth test using *L. minor*. An EC_{10} of 23.37 mg Mn l^{-1} was derived from total frond counts with an associated 95% confidence interval of 11.67 – 46.78 mg Mn l^{-1}. Growth rate, based on frond counts, was also affected with an EC_{10} of 41.54 mg l^{-1}. Both these endpoints were based on the measured concentrations of Mn in the test.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	IMnI, 2009b. The chronic toxicity of manganese to the amphipod crustacean *Hyalella azteca* using a standardised flow-through experiment. Prepared by Parametrix, Albany, Oregon, USA for the International Manganese Institute.

Information on the test species	
Test species used	Hyalella azteca
Source of the test organisms	Aquatic Biosystems (Fort Collins, CO, USA)
Holding conditions prior to test	Held for 2 days prior to testing with appropriate feeding and water renewal
Life stage of the test species used	Juveniles 7 – 9 days old

Information on the test design	
Methodology used	42 day test measuring survival, growth and reproduction using a protocol based on Environment Canada and ASTM methods for assessing contaminated sediments. Growth endpoint determined after 28 days.
Form of the test substance	$MnCl_2$
Source of the test substance	Alfa Aesar (Ward Hill, MA, USA). Purity ≥ 97%
Type and source of the exposure medium	Well water blended with reverse osmosis water to achieve target water hardness of 80 – 120 mg l^{-1} $CaCO_3$
Test concentrations used	Nominal: Control (0), 250, 500, 1000, 2000, 4000, 8000 µg Mn l^{-1} Measured: <62.5, 136.0, 285.9, 627.2, 1402.6, 3351.1, 6663.6 µg Mn l^{-1}
Number of replicates per concentration	12 (4 for weight determination after 28 days)
Number of organisms per replicate	10
Nature of test system	Flow through (24 volume changes day^{-1}, with 2-3 hour static period for feeding each day)
Measurement of exposure concentrations	Yes
Measurement of other quality parameters	Temperature, DO, pH, conductivity, hardness and alkalinity measured at initiation and weekly thereafter
Test validity criteria satisfied	Yes
Quality criteria satisfied	Yes
Study conducted to GLP	Yes
Overall comment on quality	Guideline study, although reproduction endpoint too variable to derive an effect concentration. The chronic toxicity of Manganese to the amphipod crustacean *Hyalella azteca* was investigated using a standardised flow-through experiment. Test chambers containing formulated sediment were inoculated with juvenile *H. azteca* and exposed to measured concentrations of Manganese in overlying water for up to 42

	days. Effects on survival, growth and biomass were determined after 28 days, whilst effects on survival and reproduction were also determined after additional exposure to 35 and 42 days. The most sensitive endpoint determined was growth after 28 days, with an EC_{10} of 0.096 mg Mn^{-1} and an associated 95% confidence interval of 0.013 – 0.72 mg Mn l^{-1}. The NOEC for growth after 28 days was 0.136 mg Mn^{-1}. Variability in reproduction across the treatment groups, independent on Mn concentration, prevented the derivation of a point estimate based on a reproductive endpoint.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	IMnI, 2009c. Lifecycle toxicity of manganese to the midge *Chironomus tentans.* Prepared by Parametrix, Albany, Oregon, USA for the International Manganese Institute.

Information on the test species	
Test species used	*Chironomus tentans*
Source of the test organisms	Aquatic biosystems (Fort Collins, CO, USA)
Holding conditions prior to test	Held in test water
Life stage of the test species used	<24 hours old

Information on the test design	
Methodology used	Midge life-cycle test encompassing effects on survival, adult emergence and reproduction. 54 days exposure. The standardised guideline the protocol is based on is not detailed in the test report.
Form of the test substance	$MnCl_2$
Source of the test substance	Alfa Aesar (Ward Hill, MA, USA). Purity ≥ 97%
Type and source of the exposure medium	Well water blended with reverse osmosis water to achieve target hardness of 80 – 120 mg l^{-1}.
Test concentrations used	Nominal: Control (0), 2.5, 5, 10, 20, 40, 80 mg Mn l^{-1}. Measured: 0.01, 2.40, 4.66, 9.14, 18.12, 35.41, 71.20
Number of replicates per concentration	8
Number of organisms per replicate	15
Nature of test system	Flow through, static for 2-3 hours per day during feeding
Measurement of exposure concentrations	Yes. Total and dissolved Mn on initiation. Dissolved twice weekly for the remainder of the test
Measurement of other quality parameters	Temperature, DO, pH, conductivity (daily). Hardness, alkalinity, total chlorine, total ammonia (weekly)
Test validity criteria satisfied	Yes
Quality criteria satisfied	Yes
Study conducted to GLP	Yes
Overall comment on quality	Test procedure in accordance with generally accepted scientific standards described in sufficient detail. Life-cycle test using the aquatic midge *Chironomus tentans* measuring effects on larval survival, adult emergence and reproduction. Test chambers containing formulated sediment were inoculated with newly hatched 1st instar larvae (<24 old) which were then exposed to Manganese in the overlying water via a flow-through system for 54 days. The most sensitive endpoint was the EC10 for survival

	of 16.34 mg Mn l^{-1} based on measured concentrations. Emergence was also affected at around the same concentration (EC10 of 16.44 mg Mn l^{-1}). Reproduction, however, was not affected at any of the test concentrations.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	IMnI, 2009d. Evaluation of chronic toxicity of manganese to the aquatic Oligochaete *Aeolosoma* sp. Prepared by Parametrix, Albany, Oregon, USA for the International Manganese Institute.

Information on the test species	
Test species used	*Aeolosoma sp*. (Oligochaeta)
Source of the test organisms	Carolina Biological Supply (Burlington, NC, USA)
Holding conditions prior to test	In-house culture
Life stage of the test species used	≤24 hours old

Information on the test design	
Methodology used	14 day test to measure population growth following in-house protocol. No standardised test procedure available.
Form of the test substance	$MnCl_2$
Source of the test substance	Alfa Aesar (Ward Hill, MA, USA). Purity ≥ 97%
Type and source of the exposure medium	Standard synthetic freshwater (soft)
Test concentrations used	Nominal: Control (0), 1.5, 3, 6, 12, 24 mg Mn l^{-1} Measured: 0.01, 1.66, 3.13, 6.31, 13.47, 26.70 mg Mn l^{-1}
Number of replicates per concentration	4
Number of organisms per replicate	5
Nature of test system	Static-renewal with feeding
Measurement of exposure concentrations	Yes, total and dissolved Mn (0.45-µm filter)
Measurement of other quality parameters	Temperature, pH, DO, conductivity
Test validity criteria satisfied	Yes
Quality criteria satisfied	-
Study conducted to GLP	Yes
Overall comment on quality	Test procedure in accordance with generally accepted scientific standards described in sufficient detail. 14 day static renewal test used to derive a population growth endpoint after exposure to Mn in synthetic soft water. Derived an EC_{10} of 2.52 mg Mn l^{-1} with an associated 95% confidence interval of 1.36 – 4.68 mg Mn l^{-1}. Results were calculated based on measured concentrations.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	IMnI, 2009e. Chronic toxicity of manganese to the great pond snail, *Lymnaea stagnalis*. Prepared by Parametrix, Albany, Oregon, USA for the International Manganese Institute.

Information on the test species	
Test species used	*Lymnaea stagnalis* (Mollusca) – Great Pond Snail
Source of the test organisms	Dr Martin Crosell, University of Miami (Florida)
Holding conditions prior to test	In-house culture
Life stage of the test species used	Newly-hatched (<24 hours old)

Information on the test design	
Methodology used	30 day test to measure growth and survival following in-house protocol. No standardised test procedure available.
Form of the test substance	$MnCl_2$
Source of the test substance	Alfa Aesar (Ward Hill, MA, USA). Purity ≥ 97%
Type and source of the exposure medium	Standard Synthetic Freshwater (hard)
Test concentrations used	Nominal: Control (0), 1.88, 3.75, 7.5, 15, 30, 60 mg Mn l^{-1} Measured: <0.01, 1.45, 2.84, 5.75, 12.67, 25.43, 50.89 mg Mn l^{-1}
Number of replicates per concentration	10
Number of organisms per replicate	1
Nature of test system	Static-renewal (80% replacement 3 days per week)
Measurement of exposure concentrations	Yes, total and dissolved Mn (0.45 µm filter) concentrations
Measurement of other quality parameters	Initiation and renewal: temperature, DO, pH, conductivity. Initiation and periodically: hardness, alkalinity, total chlorine, total ammonia.
Test validity criteria satisfied	Yes
Quality criteria satisfied	-
Study conducted to GLP	Yes
Overall comment on quality	Test procedure in accordance with generally accepted scientific standards described in sufficient detail. Most sensitive endpoint is an EC_{10} for growth of 7.70 mg Mn l^{-1} based on measured concentrations over a 30 day static-renewal exposure. Associated 95% confidence interval of 2.71 – 21.86 mg Mn l^{-1}.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	IMnI, 2009f. Early life-stage toxicity of manganese to the zebrafish (*Danio rerio*) under flow-through conditions. Prepared by Parametrix, Albany, Oregon, USA for the International Manganese Institute.

Information on the test species	
Test species used	*Danio rerio* (Zebra fish)
Source of the test organisms	Oregon State University's Sinnhuber Aquatic Research Laboratory
Holding conditions prior to test	-
Life stage of the test species used	Embryos. Fertilised <48 before test initiation

Information on the test design	
Methodology used	Early life-stage survival and growth test designed to comply with the requirements of relevant OECD and ASTM guidelines.
Form of the test substance	$MnCl_2$
Source of the test substance	Alfa Aesar (Ward Hill, MA, USA). Purity ≥ 97%
Type and source of the exposure medium	Well water blended with reverse osmosis water to achieve hardness of 80 – 120 mg l^{-1}
Test concentrations used	Nominal: Control (0), 1000, 2000, 4000, 8000, 16000 µg Mn l^{-1} Measured: 23.09, 513.38, 1034.96, 2102.96, 4496.80, 9334.70
Number of replicates per concentration	4
Number of organisms per replicate	35, thinned to 25 after 48 hours
Nature of test system	Flow through
Measurement of exposure concentrations	Yes. Total and dissolved Mn at each treatment at initiation. Dissolved Mn twice a week thereafter.
Measurement of other quality parameters	Temperature, DO, pH conductivity, hardness, alkalinity, chlorine, ammonia
Test validity criteria satisfied	Yes
Quality criteria satisfied	Yes
Study conducted to GLP	Yes
Overall comment on quality	Guideline study. The long-term toxicity of Mn to cyprinid fish was investigated in a standardised early life-stage toxicity test with the zebrafish (*Danio rerio*) measuring effects on embryo survival and growth up to 30 days post hatch. The most sensitive endpoint was survival with an EC_{10} of 4.63 mg Mn l^{-1} and associated 95% confidence interval of 4.30 – 4.99 mg Mn l^{-1} based on measured concentrations.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	LASKEY J W, REHNBERG G L AND HEIN J F, 1982 Effects of chronic manganese (Mn_3O_4) exposure on selected reproductive parameters in rats. Journal of Toxicology and Environmental Health, 9, 677–687.

Information on the test species	
Test species used	Long-Evans F344 rats
Source of the test organisms	Blue Spruce Farms, Altmont, NY
Holding conditions prior to test	Laboratory
Life stage of the test species used	Juvenile through to adult

Information on the test design	
Methodology used	Standard rodent reproduction test
Form of the test substance	Mn_3O_4
Source of the test substance	Alfa Products, Danvers, MS
Type and source of the exposure medium	Diet
Test concentrations used	0, 1,500, 5,000 or 15,000 mg/kg
Number of replicates per concentration	5–8 males and 5–8 females
Number of organisms per replicate	5–8 males and 5–8 females
Nature of test system	Cage
Measurement of exposure concentrations	Yes
Measurement of other quality parameters	Not applicable
Test validity criteria satisfied	Yes
Quality criteria satisfied	Yes
Study conducted to GLP	Yes
Overall comment on quality	Good quality study cited by US regulatory authorities.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	MACDONALD J M, SHIELDS J D AND ZIMMER-FAUST R K, 1988 Acute toxicities of eleven metals to early life-history stages of the yellow crab *Cancer anthonyi.* Marine Biology, 98, 201–207.

Information on the test species	
Test species used	Yellow crab *Cancer anthonyi*
Source of the test organisms	Trapped from wild at 25–40 m depth
Holding conditions prior to test	One to two weeks in fibreglass tanks with sandy substrates and continuously flowing seawater
Life stage of the test species used	Setae-bearing embryos in the yolk, four-lobed stage

Information on the test design	
Methodology used	Embryo mortality and hatching observed over 7 days
Form of the test substance	$MnCl_2$
Source of the test substance	Not stated
Type and source of the exposure medium	Filtered seawater
Test concentrations used	0.01, 0.1, 1, 10, 100 and 1,000 mg l^{-1}
Number of replicates per concentration	5
Number of organisms per replicate	50–100
Nature of test system (static, semi-static or flow-through, duration, feeding)	Semi-static
Measurement of exposure concentrations	No
Measurement of water quality parameters	Yes
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to GLP	Not stated
Overall comment on quality	An apparently well-performed study, but with no chemical analysis of exposure concentrations because of the small volumes used per replicate (5 ml).

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	NASU Y AND KUGIMOTO M,1981 *Lemna* (duckweed) as an indicator of water pollution. I. The sensitivity of *Lemna paucicostata* to heavy metals. Archives of Environmental Contamination and Toxicology, 10, 159–169.

Information on the test species	
Test species used	*Lemna paucicostata* (synonomous with *Lemna aequinoltials*)
Source of the test organisms	Laboratory of applied botany, University of Kyoto.
Holding conditions prior to test	Cultured in ½ strength Hunter's media
Life stage of the test species used	3 frond colony

Information on the test design	
Methodology used	7 day plant growth test measuring number of fronds and wet weight. Each experiment repeated at least twice
Form of the test substance	$MnCl_2$
Source of the test substance	-
Type and source of the exposure medium	Hoagland Type M-medium (pH 4.1 and 5.1), Bonner-Devirian's Medium (pH 6.1 or 7.1)
Test concentrations used	Control (0), 1, 10, 100 ppm
Number of replicates per concentration	6
Number of organisms per replicate	1
Nature of test system	static
Measurement of exposure concentrations	No
Measurement of other quality parameters	pH
Test validity criteria satisfied	-
Quality criteria satisfied	-
Study conducted to GLP	No
Overall comment on quality	Non standardised test with no analytical verification, limited test concentrations, no statistical analysis of results, non-standard media and physico-chemistry during testing

Reliability of study	**Not reliable**
Relevance of study	**Relevant**
Klimisch Code	**3**

Reference	READER J P, EVERALL N C, SAYER M D J AND MORRIS R, 1989 The effects of eight trace metals in acid soft water on survival, mineral uptake and skeletal calcium deposition in yolk-sac fry of brown trout, *Salmo trutta* L. Journal of Fish Biology, 35, 187–198.

Information on the test species	
Test species used	*Salmo trutta*
Source of the test organisms	Eyed ova from Trent Fish Culture, Mercaston, Derbyshire
Holding conditions prior to test	Hatched in continuously flowing, dechlorinated tapwater
Life stage of the test species used	Alevins 3–6 days post-hatch

Information on the test design	
Methodology used	30-day exposure at pH 4.5 or 6.5
Form of the test substance	$MnCl_2$
Source of the test substance	Not stated (analytical grade)
Type and source of the exposure medium	Filtered, deionised, aerated, pH-adjusted and reconstituted by metered addition of inorganic salt solutions.
Test concentrations used	One nominal concentration of 0.275 mg l^{-1}, measured concentrations of 0.308 mg l^{-1} at pH 4.5 and 0.352 mg l^{-1} at pH 6.5.
Number of replicates per concentration	1
Number of organisms per replicate	30
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	Yes
Measurement of water quality parameters	Yes
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to GLP	Not stated
Overall comment on quality	This was a well performed and reported study, but not designed to estimate a NOEC. 30-day *Salmo trutta* lethality tests with sac fry undertaken under two pH

	regimes yield a NOEC of 0.35 mg l^{-1} Mn at pH 6.5 and a LOEC of 0.31 at pH 4.5. As the effect on survival at pH 4.5 was determined from the original paper to be <20% the technical guidance allows a NOEC for this test to be calculated as 0.31/2 = 0.115 mg Mn l^{-1}.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	SÁNCHEZ D J, DOMINGO J L, LLOBET J M AND KEEN C L, 1993 Maternal and developmental toxicity of manganese in the mouse. Toxicology Letters, 69, 45–52.

Information on the test species	
Test species used	Swiss albino mice
Source of the test organisms	Laboratory culture (Interfauna Iberica)
Holding conditions prior to test	Laboratory
Life stage of the test species used	Pregnant females

Information on the test design	
Methodology used	Daily subcutaneous injections on days 6–15 of gestation
Form of the test substance	$MnCl_2$
Source of the test substance	Not stated
Type and source of the exposure medium	Subcutaneous injection
Test concentrations used	0, 2, 4, 8 and 16 mg Mn/kg per day
Number of replicates per concentration	20 animals per group
Number of organisms per replicate	20 animals per group
Nature of test system (static, semi-static or flow-through, duration, feeding)	Injection of caged animals
Measurement of exposure concentrations	No
Measurement of other quality parameters	Not applicable
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Not applicable
Study conducted to GLP	Yes
Overall comment on quality	This is a good quality study, but the means of exposure via injection is not environmentally relevant.

Reliability of study	**Reliable**
Relevance of study	**Relevant endpoints; irrelevant exposure route**
Klimisch Code	**1**

Reference	STUBBLEFIELD W A, BRINKMAN S F, DAVIES P H, GARRISON T D, HOCKETT J R AND MCINTYRE W, 1997 Effects of water hardness on the toxicity of manganese to developing brown trout (*Salmo trutta*). Environmental Toxicology and Chemistry, 16, 2082–2089.

Information on the test species	
Test species used	*Salmo trutta* (Brown trout)
Source of the test organisms	Colorado Division of Wildlife's Bellvue-Watson Fish Hatchery
Holding conditions prior to test	According to ASTM guideline
Life stage of the test species used	"eyed" eggs

Information on the test design	
Methodology used	ASTM standardised ELS test undertaken at three hardness regimes (30, 150 and 450 mg l^{-1} $CaCO_3$). 62 days total exposure.
Form of the test substance	$MnCl_2$, 98.8% purity
Source of the test substance	VWR Scientific, Denver, Co, USA
Type and source of the exposure medium	Water from Horsetooth reservoir (Colorado, USA) and well water from Colorado Division of Wildlife's Fort Collins research facility blended to achieve target hardness concentrations.
Test concentrations used	30 mg l^{-1} $CaCO_3$ hardness regime: Control (<0.02), 0.51, 0.76, 1.20, 2.19, 3.94, 7.38, 15.5 mg Mn l^{-1}. 150 mg l^{-1} $CaCO_3$ hardness regime: Control (<0.02), 2.78, 4.41, 8.81, 13.86, 28.29, 54.58, 74.90.450 mg l^{-1} $CaCO_3$ hardness regime: Control (<0.02), 2.54, 4.55, 8.68, 16.21 29.88, 55.74, 100.82 mg Mn l^{-1}
Number of replicates per concentration	4
Number of organisms per replicate	15
Nature of test system	Flow through
Measurement of exposure concentrations	Yes
Measurement of other quality parameters	Yes
Test validity criteria satisfied	Yes
Quality criteria satisfied	-
Study conducted to GLP	No
Overall comment on quality	After review, the study by Stubblefield et al. was found to contain two data points that could be used in an SSD, both derived from ELS tests with brown trout under different hardness regimes. It was not considered appropriate to combine these data into a single point. NOECs of 3.94 mg Mn l^{-1} for survival and 2.78 mg Mn l^{-1} for growth were derived from 30 mg l^{-1} $CaCO_3$ and 150 mg L^{-1} $CaCO_3$ hardness regimes respectively. Data

	from a third hardness regime (450 mg l^{-1} $CaCO_3$) was found to be unreliable, and not acceptable for use within an SSD, as a consequence of high control mortality.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	WATLING H R, 1983 Comparative study of the effects of metals on the settlement of *Crassostrea gigas*. Bulletin of Environmental Contamination and Toxicology, 31, 344–351.

Information on the test species	
Test species used	Pacific oyster *Crassostrea gigas*
Source of the test organisms	Hatchery stock cultures
Holding conditions prior to test	Not stated
Life stage of the test species used	Larvae and spat

Information on the test design	
Methodology used	Larvae allowed to settle on black PVC discs at bottom of beakers
Form of the test substance	Mn^{2+}
Source of the test substance	Not stated
Type and source of the exposure medium	Sea water
Test concentrations used	10 and 20 µg l^{-1}
Number of replicates per concentration	3
Number of organisms per replicate	Several hundred
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	Yes
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	This appears to be a well-conducted study, with chemical measurements to determine the stability of metals under test conditions, although actual exposure concentrations were not measured.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

ANNEX II Saltwater short-term toxicity data

Table A2.1 Most sensitive short-term aquatic toxicity data for saltwater organisms exposed to manganese

Mn species (test substance)	Scientific name	Common name	Taxonomic group	End-point	Effect	Test duration	Conc. (mg l^{-1})	Expo-sure[1]	Toxicant analysis[2]	Comments	Reference
Algae and microbes											
Mn^{2+} ($MnCl_2$)	*Asterionella japonica*	Diatom	Algae	IC50	Population abundance	3 days	4.9	-	-	RI = 2	Fisher and Jones 1981
Mn^{2+} [$Mn(NO_3)_2$]	*Vibrio fischeri*	Microbe	Microbes	EC50	Luminescence	15 mins	86.0	-	-	pH 5.33; 15°C	McLoskey et al. 1996
Invertebrates											
Mn^{2+}	*Heliocidaris tuberculata*	Sea urchin	Echinoderms	NOEC	Abnormal larvae	3 days	1.3	s	n	No analysis due to small size of exposure vessels (5–10 ml); RI = 2	Doyle et al. 2003
Mn^{2+} ($MnCl_2$)	*Hemicentrotus pulcherrimus*	Sea urchin	Echinoderms	NOEC	Abnormal larvae	2 days	2.0	s	n	18°C	Kobayashi 1990
Mn^{2+} ($MnCl_2$)	*Anthocidaris crassispina*	Sea urchin	Echinoderms	NOEC	Abnormal egg development	>12 hours	6.6	s	n	28°C	Kobayashi 1971
Mn^{2+} ($MnCl_2$)	*Crassostrea virginica*	American oyster	Molluscs	LC50	Mortality	2 days	16.0	s	n	pH 7.2–8.5; 26°C; salinity 25‰	Calabrese et al. 1973
Mn^{2+} ($MnSO_4$)	*Mytilus edulis*	Mussel	Molluscs	EC50	Abnormal larvae	2 days	30.0	s	y	pH 8.2–8.6; 19°C; DO >70%; salinity 26‰	Morgan et al. 1986
Mn^{2+} ($MnCl_2$)	*Artemia salina*	Brine shrimp	Crustaceans	LC50	Mortality	2 days	51.8	-	-	-	Gajbhiye and Hirota 1990
Mn^{2+} ($MnCl_2$)	*Nitocra spinipes*	Copepod	Crustaceans	LC50	Mortality	4 days	70.0	s	n	pH 8.0; 19.5–20.5°C; salinity 7‰	Bengtsson 1978

[1] Exposure: s = static. [2] Toxicant analysis: y = measured; n = not measured.
NOEC = no observed effect concentration; IC50 = concentration at which the population effect of the organisms tested is inhibited by 50%

EC50 = concentration effective against 50% of the organisms tested; LC50 = concentration lethal to 50% of the organisms tested
DO = dissolved oxygen; RI = reliability index

BENGTSSON B E, 1978 Use of a harpacticoid copepod in toxicity tests. Marine Pollution Bulletin, 9, 238–241.

CALABRESE A, COLLIER R S, NELSON D A AND MACINNES J R, 1973 The toxicity of heavy metals to embryos of the American oyster *Crassostrea virginica*. Marine Biology, 18,162–166.

DOYLE C J, PABLO F, LIM R P AND HYNE R V, 2003 Assessment of metal toxicity in sediment pore water from Lake Macquarie, Australia. Archives of Environmental Contamination and Toxicology, 44, 343–350.

FISHER N S AND JONES G J, 1981 Heavy metals and marine phytoplankton: correlation of toxicity and sulfhydryl-binding. Journal of Phycology, 17, 108–111.

GAJBHIYE S N AND HIROTA R, 1990 Toxicity of heavy metals to brine shrimp *Artemia*. Journal of the Indian Fisheries Association, 20, 43–50.

KOBAYASHI N, 1971 Fertilized sea urchin eggs as an indicatory material for marine pollution bioassay, preliminary experiments. Publications of the Seto Marine Biology Laboratory, 18, 379–406.

KOBAYASHI N, 1990 Marine pollution bioassay by sea urchin eggs: an attempt to enhance sensitivity. Publications of the Seto Marine Biology Laboratory, 34, 225–237.

MCLOSKEY J T, NEWMAN M C AND CLARK S B, 1996 Predicting the relative toxicity of metal ions using ion characteristics: Microtox bioluminescence assay. Environmental Toxicology and Chemistry, 15, 1730–1737.

MORGAN J D, MITCHELL D G AND CHAPMAN P M, 1986 Individual and combined toxicity of manganese and molybdenum to mussel, *Mytilus edulis*, larvae. Bulletin of Environmental Contamination and Toxicology, 37, 303–307.