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Proposed EQS for Water Framework Directive Annex VIII substances: chlorine (free available)

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

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Author(s):
Sorokin N, Atkinson C, Aldous E, Rule K and Comber S

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Research Contractor:
WRc plc, Frankland Road, Blagrove, Swindon
Wiltshire, SN5 8YF
Tel: +44 1793 865000

Environment Agency's Project Manager:
Stephanie Cole/Lindsey Sturdy, Chemicals Science

Collaborator(s):
Scottish Environment Protection Agency (SEPA)
Scotland & Northern Ireland Forum for Environmental
Research (SNIFFER)
Environment and Heritage Service (EHS)

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Steve Killeen Head of Science

Use of this report

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

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

Whilst this 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.

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). There are existing EQSs for chlorine, 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 chlorine, 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.

EU Risk Assessment Reports (EU RARs) have been compiled for chlorine and hypochlorite. The UK is committed to the use of PNECs derived through this process as the basis for Water Framework Directive Annex X EQSs. Consequently, this report recommends available RAR PNECs as the corresponding proposed PNECs.

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

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

Properties and fate in water
Chlorine is a highly reactive gas that dissolves readily in water. Following dissolution in water, the main species at pH <2 will be chlorine (Cl_2) but, at higher pH values, hypochlorous acid (HOCl) and hypochlorite (OCl^-) predominate. At 25 C and pH 7, 70 per cent of chlorine is present as HOCl and, at pH 8, 80 per cent of the chlorine is present as OCl^-. Chlorine can also form toxic compounds with amines.

The species of primary concern here are those determined as free available chlorine (FAC), which is the concentration of hypochlorous acid and the hypochlorite ion, excluding other chlorine compounds and chloride.

The main removal pathways for chlorine species in water are abiotic degradation (e.g. reaction with other chemicals present in the water), volatilisation and photolytic reactions. Biodegradation and bioaccumulation of chlorine species are not

important and only chlorinated organic byproducts would be bioaccumulated to any extent.

Hypochlorous acid is more toxic than the hypochlorite ion. Consequently, across the pH range most usually found in freshwaters (6.5–7.2), chlorine is likely to be in its most toxic form. Temperature also plays a role in the speciation of chlorine, although it has a less pronounced impact than pH. Aquatic organisms tend to be more sensitive to chlorine at higher temperatures and so added care may be warranted when chlorine is present in heated water discharges.

Availability of data

Freshwater acute toxicity data are available for algae, crustaceans, fish, annelids, platyhelminths, macrophytes, molluscs, rotifers and insects. However, chronic exposure data could only be located for algae, molluscs, fish, protozoans and bacteria; of these, no observed effect concentrations (NOECs) are available only for algae, bacteria and fish.

Data for marine organisms are available for six different taxonomic groups (algae, crustaceans, fish, molluscs, rotifers and echinoderms) but chronic toxicity data are available only for algae, molluscs, crustaceans and fish, with only fish and mollusc studies yielding long-term NOECs.

Derivation of PNECs

Given the short persistence of chlorine in water, a short-term PNEC is considered to be of greater relevance than a long-term PNEC.

Long-term PNEC for freshwaters

The lowest reliable long-term (lt) value is a 7-day NOEC of 3 µg l^{-1} as total residual chlorine (TRC), corresponding to 2.1 µg l^{-1} FAC, for a reduction in species richness of periphytic communities in a microcosm study. No valid long-term NOEC data are available for crustaceans, but short-term exposures for these organisms and a supporting field mesocosm study indicate that crustaceans may be more sensitive than algae.

Consequently, the 7-day NOEC of 3 µg l^{-1} TRC is recommended as the basis for a PNEC, but with an assessment factor of 50 to account for the uncertainties introduced by the lack of crustacean data. This results in a $PNEC_{freshwater\_lt}$ of 0.06 µg l^{-1} TRC (equivalent to 0.04 µg l^{-1} FAC). The generation of chronic invertebrate data would help reduce uncertainty in this extrapolation and thereby justify the use of a smaller assessment factor.

This PNEC is appreciably lower than the existing 1994 EQS of 2 µg l^{-1}. This was based on a different critical datum in which exposure to 3.4 µg l^{-1} chloramine (as opposed to chlorine) for 15 weeks inhibited reproduction in *Gammarus* without any assessment factor and expressed as total available chlorine.

Short-term PNEC for freshwaters

Good quality short-term (st) freshwater data are available only for crustaceans, with supporting data for fish. Valid data for algae are lacking from both the freshwater and saltwater datasets; consequently, the ‘base set’ of data required by Annex V cannot be fulfilled.

The sodium hypochlorite (NaClO) EU RAR identified the lowest reliable short-term data point as the 24-hour LC50 of 5 µg l^{-1} FAC for *Ceriodaphnia dubia*. In the absence of relevant supporting data, an assessment factor of 100 should be applied to this datum, resulting in a $PNEC_{freshwater\_st}$ of 0.05 µg l^{-1} FAC.

The proposed PNEC is again appreciably lower than the 1994 EQS of 5 µg l^{-1}, expressed as total available chlorine. This was based on an assessment factor of 2 applied to a mayfly (*Isonychia* sp.) acute LC50 of 9.3 µg l^{-1}.

Long-term PNEC for saltwaters

Reliable long-term NOECs are available only for marine fish with some supporting data for molluscs. Good quality data for marine algae are lacking and so the Annex V requirement for a ‘base set’ of data is not met. However, by combining the freshwater and saltwater datasets (as in the hypochlorite EU RAR), the addition of data for algae results in the creation of a saltwater dataset containing all three trophic levels.

Based on this combined dataset, the lowest reliable long-term value was the 7-day NOEC of 2.1 µg l^{-1} FAC for a reduction in species richness of periphytic communities in a freshwater microcosm study used to derive the $PNEC_{freshwater\_lt}$. The same PNEC is proposed for the saltwater environment: $PNEC_{saltwater\_lt}$ of 0.06 µg l^{-1} TRC (equivalent to 0.04 µg l^{-1} FAC).

A long-term EQS for the protection of saltwater life was not derived in the 1994 review.

Short-term PNEC for saltwaters

Good quality short-term data for saltwater species are available only for crustaceans and fish. Even by combining the freshwater and saltwater datasets, it is still not possible to obtain the required ‘base set’ of algae, crustaceans and fish.

The EU RAR for NaClO identified the lowest reliable short-term data point as the 24-hour LC50 of 5 µg l^{-1} FAC for the freshwater crustacean *Ceriodaphnia dubia*. Applying the guidance for the assessment of intermittent releases, a standard assessment factor of 100 should be applied to the LC50 for *Ceriodaphnia dubia* resulting in a $PNEC_{saltwater\_st}$ of 0.05 µg l^{-1} FAC.

The 1994 EQS was based on an assessment factor of ~2 applied to acute LC50s of 28 µg l^{-1} for plaice and sole for total residual oxidants (TRO). This resulted in an EQS of 10 µg l^{-1}, substantially higher than the $PNEC_{saltwater\_st}$ proposed here.

PNEC for secondary poisoning

Given the high water solubility of chlorine and its rapid degradation in the environment, bioaccumulation of chlorine species is not important.

PNEC for sediments

Due to the rapid reaction of chlorine in the sediment compartment, a PNEC for sediments is not relevant.

Summary of proposed PNECs

Receiving medium/exposure scenario	**Proposed PNEC ($\mu g\ l^{-1}$ free available chlorine)**	**Existing EQS ($\mu g\ l^{-1}$)**
Freshwater/long-term	0.04	2 (total available chlorine)
Freshwater/short-term	0.05	5 (total available chlorine)
Saltwater/long-term	0.04	No standard
Saltwater/short-term	0.05	10 (total residual oxidant)

Analysis

Colorimetry is the most common method of determining either free residual chlorine or total residual chlorine. This method can be used in the field as well as in the laboratory. Laboratory methods have lower detection limits, but analysis has to be carried out immediately to avoid loss of analyte. Detection limits are as low as 10 $\mu g\ l^{-1}$, depending on the sophistication of the equipment.

The lowest proposed PNEC derived for chlorine is 0.06 $\mu g\ l^{-1}$ TRC for fresh and salt waters, corresponding to a FAC concentration of 0.04 $\mu g\ l^{-1}$. The data quality requirements are that, at a third of the EQS, total measurement error should not exceed 50 per cent. It is unlikely that current analytical methodologies are sufficiently sensitive to assess compliance in the receiving water with the PNECs proposed here.

Implementation issues

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

1. Monitoring is usually carried out in the field using test kits but these are not sufficiently sensitive to assess compliance in the receiving water. Either monitoring will have to be carried out only in effluent waste streams or analytical methods will have to be improved to deliver greater sensitivity.

2. A lack of ecotoxicological data gives rise to a considerable degree of uncertainty in the extrapolations from the available data. Generation of additional ecotoxicological data would help to reduce uncertainty and may result in different PNECs.

3. In the interim, current EQSs should be adopted until these issues can be addressed.

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 chlorine using the methodology described in Annex V of the Directive. There are existing EQSs for chlorine, 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 chlorine, along with any data that relate impacts under field conditions to exposure concentrations. The data have been subjected to rigorous quality assessment such that decisions are based only on scientifically sound data.[2] Following consultation with an independent peer review group, critical data have been identified and assessment factors selected in accordance with the guidance given in Annex V.

EU Risk Assessment Reports (EU RARs) have been compiled for chlorine and hypochlorite. The UK is committed to the use of PNECs derived through this process as the basis for Water Framework Directive Annex X EQSs. Consequently, this report recommends available RAR PNECs as the corresponding proposed PNECs.

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

This report provides a data sheet for chlorine (free available).

1.1 Properties and fate in water

Chlorine is a highly reactive gas that dissolves readily in water. Following dissolution in water, the main species at pH <2 will be chlorine (Cl_2) but, at higher pH values, hypochlorous acid (HOCl) and hypochlorite (OCl^-) predominate. At 25 C and pH 7, 70 per cent of chlorine will be present as HOCl and, at pH 8, 80 per cent of the chlorine will be present as OCl^-. Chlorine can also form toxic compounds with amines.

The species of primary concern here are those determined as free available chlorine (FAC), which is the concentration of hypochlorous acid and the hypochlorite ion, excluding other chlorine compounds and chloride.

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[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 relating to data in the draft sodium hypochlorite RAR are provided in Annex 1. Annex 2 contains additional data quality sheets.

The main removal pathways for chlorine species in water are abiotic degradation (e.g. reaction with other chemicals present in the water), volatilisation and photolytic reactions. Biodegradation and bioaccumulation of chlorine species are not important and only chlorinated organic byproducts would be bioaccumulated to any extent.

Hypochlorous acid is more toxic than the hypochlorite ion. Consequently, across the pH range most usually found in freshwaters (6.5–7.2), chlorine is likely to be in its most toxic form. Temperature also plays a role in the speciation of chlorine, although it has a less pronounced impact than pH. Aquatic organisms tend to be more sensitive to chlorine at higher temperatures and so added care may be warranted when chlorine is present in heated water discharges.

2. Results and observations

2.1 Identity of substance

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

Table 2.1 Species covered by this report

Name	CAS Number
Chlorine	7782-50-5

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 [49], and existing EQSs obtained from the literature [45].

Section 2.6 summarises the effects data identified from the literature for chlorine. 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 basis for quality standard setting

PNEC	TDG deterministic approach (AFs)	TGD probabilistic approach (SSDs)	Existing EQS
Freshwater short-term	0.05 µg l^{-1} FAC (Section 3.1.1)	NA	5 µg l^{-1} TAC (MAC)
Freshwater long-term	0.04 µg l^{-1} FAC (Section 3.1.1)	Lack of data	2 µg l^{-1} TAC (AA)
Saltwater short-term	0.05 µg l^{-1} FAC (Section 3.1.2)	NA	10 µg l^{-1} TRO (MAC)
Saltwater long-term	0.04 µg l^{-1} FAC (Section 3.1.2)	Lack of data	ND
Freshwater sediment short-term	NA	NA	NA
Freshwater sediment long-term	NA	Lack of data	NA
Saltwater sediment short-term	NA	NA	NA
Saltwater sediment long-term	NA	Lack of data	NA

PNEC	TDG deterministic approach (AFs)	TGD probabilistic approach (SSDs)	Existing EQS
Freshwater secondary poisoning	NA	NA	NA
Saltwater secondary poisoning	NA	NA	NA

AF = assessment factor; SSD = species sensitivity distribution
FAC = free available chlorine
AA = annual average; MAC = maximum allowable concentration
NA = not applicable; ND = no data
TAC = total available chlorine; TRO = total residual oxidant

2.3 Hazard classification

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

Table 2.3 Hazard classification

R-phrases and labelling	Reference
Chlorine R 23: Toxic by inhalation R 36/37/38: Irritating to the eyes, respiratory tract and skin R 50: Very toxic to aquatic organisms	[1]
Sodium hypochlorite R 31: Contact with acids liberates toxic gas R 34: Causes burns	[2]

2.4 Physical and chemical properties

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

Table 2.4 Physical and chemical properties of chlorine

Property	Value	Reference
Molecular formula	Cl_2	[1]
Molecular structure	Cl–Cl	[1]
Molecular weight	70.9	[1]
Appearance	Gas at room temperature	[1]
Melting point (°C)	–101	[1]
Boiling point (°C)	–34	[1]
Vapour pressure	6,780 hPa at 20°C	[1]
Henry's Law constant	9.83×10^{-3} Pa m^3 mol^{-1} (predicted)	[1]
Water solubility (g l^{-1})	9.78 at 10°C 6.9 at 25°C	[1]
Octanol–water partition coefficient (log Kow)	NA (see Table 2.5)	

2.5 Environmental fate and partitioning

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

Table 2.5 Environmental fate and partitioning of chlorine

Property	**Value**	**Reference**
Abiotic fate	Chlorine is a pale greenish-yellow gas of marked odour, irritating to the eyes and throat, and toxic. It is a halogen, which although not corrosive when pure and dry, becomes highly corrosive in the presence of small amounts of moisture. Chlorine is highly reactive with most elements and may give rise to explosive reactions with ammonia gas, hydrogen, etc. Elemental chlorine is, therefore, unlikely to persist for long in the environment.	[3]
Speciation	Chlorine generally exhibits a valence of –1 in compounds, but it also exists in the formal positive oxidation states of +1 ($NaClO$), +3 ($NaClO_2$), +4 (ClO_2), +5 ($NaClO_3$) and +7 ($NaClO_4$).	[1]
	Following the dissolution of chlorine in water, the main species will be: • Cl_2 at pH <2 • Cl_2 and HOCl at pH 2–5 • HOCl at pH 5.0 • HOCl and OCl^- at pH 5–10 • OCl^- at pH ≥10.	[3]
Hydrolytic stability	Chlorine is soluble in water and in salt solutions, the solubility decreasing with salt strength and temperature (above 10^oC). Chlorine partially hydrolyses in aqueous solution to form hydrochloric acid (HCl) and hypochlorous acid (HOCl). In the presence of ammonia (Cl/N<1), chlorine reacts to produce chloramines (NH_xCl_y where x = 0–2 and y = 1–3).	[4]
	Where bromide is present in natural waters, it will substitute the chlorine atom in hydrochlorous acid to produce hydrobromous acid (HOBr).	
	Chlorine is very reactive and so does not persist. Free chlorine (HOCl and OCl^-) decomposes to chloride and oxygen. The persistence will depend on other chemicals present but typically chlorine is consumed via chemical demand (10–50%), phototransformation (5–30%) and volatilisation (20–80%). The half-life of hypochlorite is estimated to be less than 2 hours. Half-lives of 420 and 9 hours have been calculated for mono- and dichloramine, respectively.	[5] [6] [7]

Property	Value	Reference
Photostability	If chlorine is emitted in the molecular form to the atmosphere, the main reaction is photolysis, which gives atomic chlorine. The estimated half life is 2–4 hours. Degradation of chlorine in water is also influenced by exposure to the ultraviolet (UV) component of sunlight. Chlorine reacts in water to produce hypochlorous acid (HOCl), which may then react photochemically to form Cl^0, then chlorate (ClO_3^-) and oxygen.	[8] [9]
Distribution in water/sediment systems (active substance)	The high water solubility of chlorine will mean that there is no appreciable sorption onto particulate material.	[1]
Distribution in water and sediment systems (metabolites)	When chlorine reacts with ammonia to produce chloramines or with other organic compounds to produce chlorinated byproducts, their greater lipophilicity will result in an increased tendency to sorb to particulate materials.	[1]
Degradation in soil	Mobility of chlorine in soil is assumed to be of little relevance as chlorine in an aqueous solution binds covalently to soil organics within the first few millimetres or centimetres of the soil surface.	[1]
Biodegradation	The rapid chemical reactions associated with chlorine in the environment mean that biodegradation is not a significant pathway compared with abiotic processes. However, for organochlorine byproducts, which are more chemically stable and bioavailable, biodegradation will potentially be a more significant pathway.	[1]
Partition coefficients (log Kow)	An octanol–water distribution coefficient cannot be defined or measured, as both chlorine and hypochlorous acid have oxidising properties and will react with the organic phase in methods used to determine log Kow.	[1]
Bioaccumulation BCF	Bioaccumulation has not been observed in biota and is not expected for molecular chlorine as it is chemically reactive and easily ionised in aqueous solution. Aqueous chlorine species (e.g. hypochlorite) do not bioaccumulate, although more lipophilic organochlorine byproducts would be expected to demonstrate greater bioaccumulation.	[1]

BCF = bioconcentration factor

Chlorine is a highly soluble gas that reacts rapidly in water to generate a range of pH-dependent products, dominated by HOCl and OCl^- at environmental pH values. At pH 8.0, the split between HOCl and OCl^- is 50:50, with OCl^- predominating at greater (more alkaline) pH values and HOCl the main species below pH 8.0 (>90 per cent at pH 6.0 and below).

The main removal pathways for chlorine species in water are via abiotic degradation pathways such as reaction via oxidisation of other chemicals present in the water, whether they are:

- metals, e.g. manganese and iron;
- inorganic in nature, e.g. ammonia, cyanide, hydrogen sulphide;
- organic in nature, e.g. humics, fulvics, organic nitrogen.

Photolytic reactions to form chlorates are another important loss mechanism, as is volatilisation. Biodegradation and bioaccumulation of chlorine species are not considered of importance in the environment and only chlorinated organic byproducts would be expected to be bioaccumulated. However, low molecular weight byproducts of chlorine reactions, such as chloramines and trihalogenated methanes (e.g. chloroform) are not expected to accumulate appreciably in biota.

The high solubility and reactivity of chlorine species means that sorption to soil or sediment is not a significant process in the aquatic fate and behaviour of chlorine.

A number of terms can be used to describe chlorine in water, such as ‘free’, ‘active’, ‘available’, ‘combined’ and ‘residual’ chlorine. The following definitions are used in this report:

- **Free available chlorine (FAC)** is the concentration of chlorine available in a mixture, at equilibrium, of hypochlorous acid and the hypochlorite ion. It is the form in which it is available to act as an oxidant.
- **Combined available chlorine (CAC)** is the available chlorine as chloramines or other N-Cl linked compounds.
- **Total residual/available chlorine (TR/AC)** (the terms total residual and total available chlorine can be used interchangeably) relates to the sum of the FAC and CAC.
- **Total residual oxidant (TRO)** is the sum of all oxidants including non-chlorine species. In water containing bromine, such as seawater, there is displacement of chlorine by bromine resulting in hypobromous acid, hypobromite ions and bromamines.

The reaction of chlorine with ammonia to produce mono-, di- or trichloramines is an important fate process. These reaction products are of concern due to the potentially high toxicity of chloramines and their greater persistence in the environment in comparison with chlorine [2]. However, it is not within the scope of this report to examine the effects of chloramines.

2.6 Effects data

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

Data collation followed a tiered approach.

- The critical freshwater and marine data from existing EQS documents were collated.
- Further data published after derivation of the current UK EQS were retrieved from the US Environmental Protection Agency (US EPA) ECOTOX database.[3]
- Draft European Union Risk Assessment Reports (RARs) were available for chlorine and sodium hypochlorite [1, 2]. Given the comprehensive nature of the EU RARs in terms of data collection and assessment, these reports were used as the primary data source for aquatic, sediment and secondary poisoning data for chlorine.
- Data contained in the existing EQS and ECOTOX databases were compared with the RARs and any supplementary data included in the assessment.
- In addition, data published after the RARs (post-2004) were sought and any new data compiled.

It was not possible to locate any sediment effects data.

A number of environmental factors may affect the toxicity of chlorine to aquatic organisms. Chlorine dissociates in water to form hypochlorous acid (HOCl) and the hypochlorite ion (OCl^-), the ratio of these species being dependent on pH and temperature (see Section 2.5). At 25°C and pH 7, 70 per cent of chlorine will be present as HOCl and, at pH 8, 80 per cent of the chlorine will be present as OCl^- [10]. Hypochlorous acid is more toxic than the hypochlorite ion and, consequently, chlorine is likely to be in its most toxic form across the pH range (6.5–7.2) most usually found in freshwaters.

In addition to pH, temperature also plays a role in the speciation of chlorine, although it has a less pronounced impact than pH. Aquatic organisms tend to be more sensitive to chlorine at higher temperatures, resulting in lower effect concentrations [11]. There is evidence to suggest that the effects of temperature and exposure to chlorine species are synergistic [2].

The effects of pH and temperature on toxicity have been taken into account when assessing the available data. However, given that chlorine is likely to be most toxic around pH 7, standards based on this pH range will be protective of effects at other pH ranges. Consequently, it is not thought that specific PNECs for different pH values are required. Temperature has a less pronounced effect on toxicity, but as chlorine exposure may be associated with localised increases in temperature (e.g. releases from power facilities) this should be borne in mind when using any derived PNECs. However, there are insufficient high quality data for temperature-chlorine interaction effects in aquatic taxa to set temperature dependent PNECs for chlorine.

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

2.6.1 Toxicity to freshwater organisms

Freshwater acute toxicity data are available for various taxonomic groups including algae, crustaceans, fish, annelids, platyhelminthes, macrophytes, molluscs, rotifers and insects. However, chronic exposure data could only be located for algae, molluscs, fish, protozoans and bacteria. The majority of the available data were for fish species, with a reasonably large database of invertebrate data, primarily comprising crustacean and mollusc data. Far fewer algal data are available for chlorine than for the other trophic levels.

Diagrammatic representations of the available freshwater data (cumulative distribution functions) for chlorine are presented in Figures 2.1 and 2.2. These diagrams include all data regardless of quality and provide an overview of the spread of the available data. These diagrams are not species sensitivity distributions and have not been used to set the chlorine PNECs. The lowest critical freshwater data for chlorine are presented in Tables 2.6 and 2.7.

In addition to the above data, there are a number of field studies with chlorine. These are dealt with in Section 3.

Figure 2.1 **Cumulative distribution function of freshwater long-term data (µg l^{-1}) for chlorine**

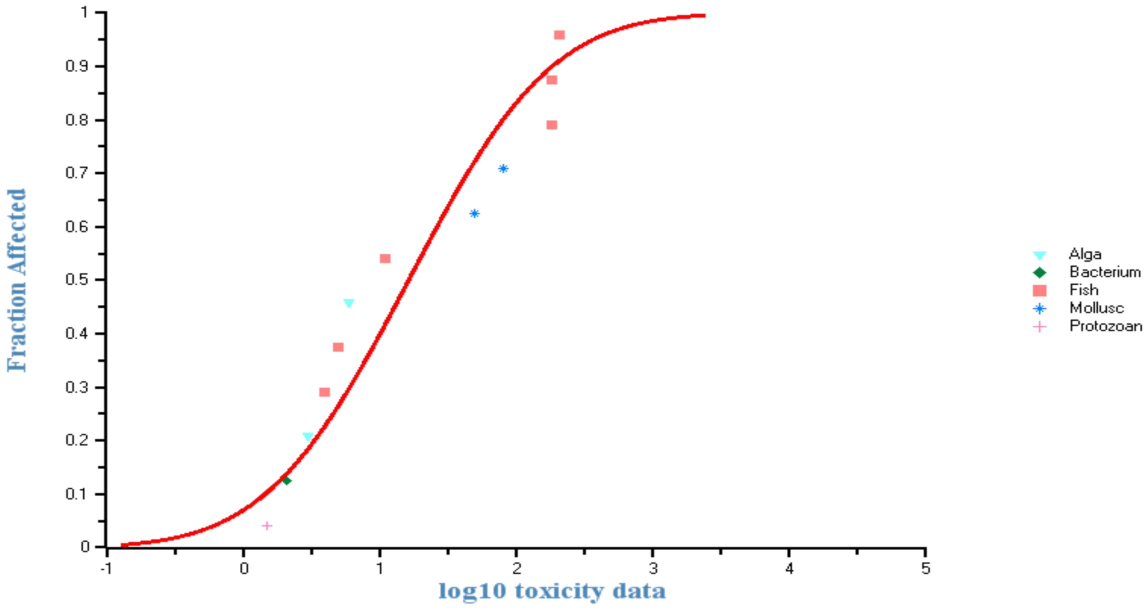

Figure 2.2 Cumulative distribution function of freshwater short-term data (µg l^{-1}) for chlorine

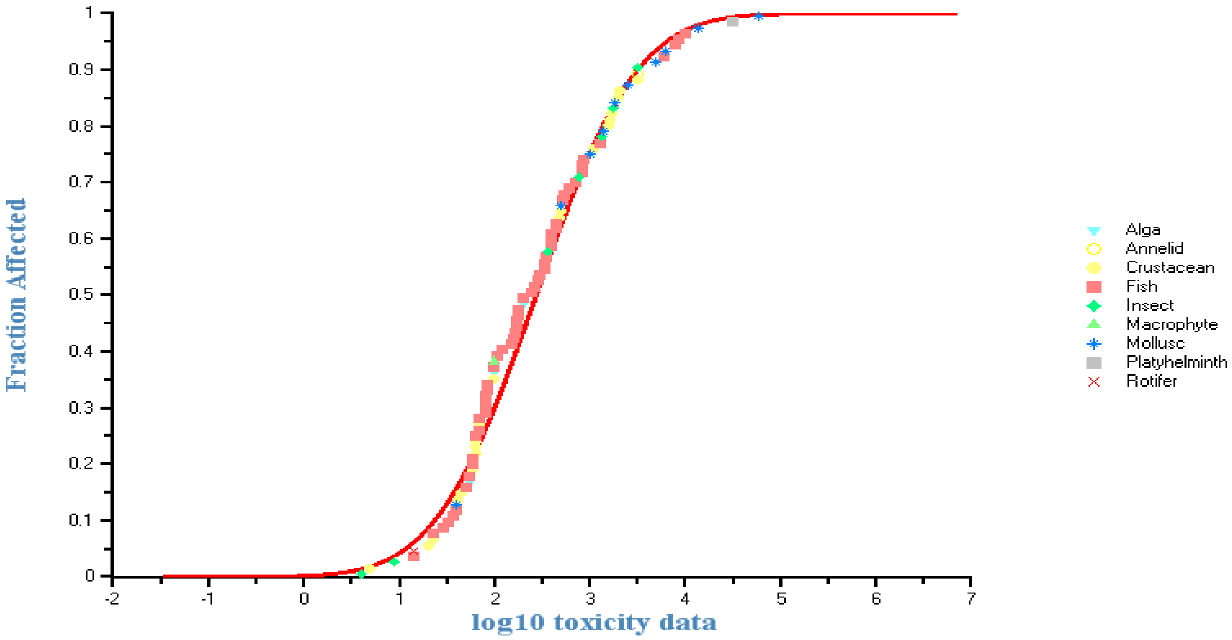

Table 2.6 Most sensitive long-term aquatic toxicity data for freshwater organisms exposed to chlorine

Scientific name	Common name	Endpoint, effect	Test duration (days)	Conc. (µg l^{-1})	Exposure[1]	Analysis/ analyte[2]	Comments	Reliability index[3]	Reference
Microcosm	Periphytic community	Algal biomass/LOEC	7	6	NR	y; TRC	pH 8.1; 19.4°C	2	[12]
Microcosm	Periphytic community	Algal biomass/NOEC	7	3	NR	y; TRC	pH 8.1; 19.4°C	2	[12]
Microcosm	Periphytic community	Algal biomass/EC20	7	2.7	NR	y; TRC	pH 8.1; 19.4°C	2	[12]
Microcosm	Microbial population	~50% reduction in chlorophyll a	28	2.1	NR	y; TRC	pH 7.7; 9.6–17°C; 60 mg l^{-1} $CaCO_3$	2	[13]
Microcosm	Microbial population	NOEC (alkaline phosphatase activity)	28	2.1	NR	y; TRC	pH 7.7; 9.6–17°C; 60 mg l^{-1} $CaCO_3$	2	[13]
Mesocosm (field test)	Protozoa and algal communities	NOEC zooplankton density	24	1.5	NR	y; TRC	pH 8.0; 22.5°C; 197 mg l^{-1} $CaCO_3$	2	[13]
Corbicula fluminea	Clam	100% mortality	36	50	f	y; TRC	pH 7.7; 25°C	2	[14]
Dreissena polymorpha	Zebra mussel	55–100% mortality	56	80–1350	f	y; Cl_2	pH 7.9–8.5; 7.2–17.5°C; 142 mg l^{-1} $CaCO_3$	2	[15]
Dreissena polymorpha	Zebra mussel	LC50	9	500	s, ss	y; TRO	pH 8.2; 20°C; 137 mg l^{-1} $CaCO_3$	2	[16]
Catostomus commersoni (field test)	White sucker	No mortality	134	>183	f	y; TRC	NR	2	[17]
Catostomus commersoni (field test)	White sucker	No growth reduction	134	>183	f	y; TRC	NR	2	[17]
Oncorhynchus mykiss (field test)	Rainbow trout	No growth reduction	49	>206	f	y; TRC	NR	2	[17]
Oncorhynchus mykiss (field test)	Rainbow trout	No mortality	49	>206	f	y; TRC	NR	2	[17]
Ictalurus punctatus (field test)	Cannel catfish	No mortality	134	>183	f	y; TRC	NR	2	[17]

Scientific name	**Common name**	**Endpoint, effect**	**Test duration (days)**	**Conc. ($\mu g\ l^{-1}$)**	**Exposure[1]**	**Analysis/ analyte[2]**	**Comments**	**Reliability index[3]**	**Reference**
Ictalurus punctatus (field test)	Cannel catfish	0–25% growth reduction	134	5/52	f	y; TRC	NR	2	[17]
Ictalurus punctatus (field test)	Cannel catfish	34% growth reduction	134	162	f	y; TRC	NR	2	[17]
Oncorhynchus kisutch	Coho salmon	Decrease in growth	21	11	NR	y; TAC	NR	4	[18]
Salmo fontinalis	Brook trout	Depressed activity	7	4	NR	y; HOCl	NR	4	[19]

[1] Exposure: s = static; f = flow-through; ss = semi-static.
[2] Analysis: y = measured.
[3] See Annex 1.
LOEC = lowest observed effect concentration
NOEC = no observed effect concentration
EC20 = concentration effective against 20% of the organisms tested
LC50 = concentration lethal to 50% of the organisms tested
TAC = total available chlorine
TRC = total residual chlorine
TRO = total residual oxidant
NR = not reported

Table 2.7 Most sensitive short-term aquatic toxicity data for freshwater organisms exposed to chlorine

Scientific name	Common name	Endpoint, effect	Test duration	Conc. (µg l^{-1})	Exposure[1]	Analysis/ analyte[2]	Comments	Reliability index[3]	Reference
Chlorella sorokiniana	Alga	43% reduction in cell numbers	20 hours	600	ss	y; Cl_2	pH 7.0; 28–32°C	3	[20]
Chlorella sorokiniana	Alga	27% reduction in cell numbers	20 hours	200	ss	y; Cl_2	pH 7.0; 28–32°C	3	[20]
Aphanizomenon flos-aquae	Blue-green alga	50% inhibition nitrification	22 hours	54	s	NR; FAC	NR	3	[21]
Phytoplankton		Reduced chlorophyll a	30 min	100	s	y; TRC	–	3	[22]
Ceriodaphnia dubia	Water flea	LC50	24 hours	6	f	y; OCl^-	pH 8.0; 25°C	2	[10]
Ceriodaphnia dubia	Water flea	LC50	24 hours	5	f	y; HOCl	pH 7.0; 25°C	2	[10]
Daphnia magna	Water flea	LC50	30 min	97	f	y; TRC	pH 7.4; 21°C	3	[23]
Daphnia magna	Water flea	LC50	60 min	63	f	y; TRC	pH 7.4; 21°C	3	[23]
Daphnia magna	Water flea	EC50	48 hours	20	s	n	pH 8.4; 17.5–19°C	2	[24]
Baetis harrisoni (field test)	Mayfly	LC50	96 hours	4.1	f	y; TRC	13–23°C	2	[25]
Baetis harrisoni (field test)	Mayfly	LC50	96 hours	4.8	f	y; TRC	13–23°C	2	[25]
Oncorhynchus mykiss	Rainbow trout	120-hour LC50	40 min x 3/day	50	int f	y; TRC	pH 6.5–7.6; 12°C	2	[26]
Oncorhynchus kisutch	Coho salmon	24–168-hour LC50	40 min x 3/day	84–297	int f	y; TRC	pH 6.5–7.6; 6–12°C	2	[26]
Cyprinus carpio	Carp	96–166-hour LC50	40 min x 3/day	245	int f	y; TRC	pH 6.5–7.6; 6°C	2	[26]
Ictalurus punctatus	Catfish	120-hour LC50	40 min x 3/day	33	int f	y; TRC	pH 6.5–7.6; 24°C	2	[26]
Oncorhynchus mykiss	Rainbow trout	24-hour LC50	40 min x 3/day	260–290	int f	y; TRC	pH 6.5–7.7; 5, 12, 17°C	2	[11]
Oncorhynchus mykiss	Rainbow trout	48-hour LC50	40 min x 3/day	90–160	int f	y; TRC	pH 6.5–7.7; 5, 12, 17°C	2	[11]

Scientific name	Common name	Endpoint, effect	Test duration	Conc. ($\mu g\ l^{-1}$)	Exposure[1]	Analysis/ analyte[2]	Comments	Reliability index[3]	Reference
Oncorhynchus mykiss	Rainbow trout	72-hour LC50	40 min x 3/day	70–100	int f	y; TRC	pH 6.5–7.7; 5, 12, 17°C	2	[11]
Oncorhynchus mykiss	Rainbow trout	96-hour LC50	40 min x 3/day	60	int f	y; TRC	pH 6.5–7.7; 5°C	2	[11]
Ictalurus punctatus	Catfish	72-hour LC50	40 min x 3/day	90–120	int f	y; TRC	pH 6.5–7.7; 5–24°C	2	[11]
Ictalurus punctatus	Catfish	96-hour LC50	40 min x 3/day	64	int f	y; TRC	pH 6.5–7.7; 24°C	2	[11]
Pimephales promelas	Fathead minnow	96-hour LC50	1 hour/day	80	int f	y; TRC	pH 7.0; 27°C	2	[27]

[1] Exposure: s = static; f = flow-through; ss = semi-static; int = internal.

[2] Analysis: y = measured; n = nominal.

[3] See Annex 1.

EC50 = concentration effective against 50% of the organisms tested
LC50 = concentration lethal to 50% of the organisms tested
FAC = free available chlorine
TRC = total residual chlorine
NR = not reported

2.6.2 Toxicity to saltwater organisms

Single species test toxicity data for marine organisms are available for six different taxonomic groups, i.e. algae, crustaceans, fish, molluscs (bivalves), rotifers and echinoderms (sea urchins). Chronic toxicity data are available only for algae, molluscs, crustaceans and fish.

Diagrammatic representations of the available saltwater data (cumulative distribution functions) for chlorine are presented in Figures 2.3 and 2.4. These diagrams include all data regardless of quality and provide an overview of the spread of the available data. These diagrams are not species sensitivity distributions and have not been used to set the chlorine PNECs. The lowest critical saltwater data for chlorine are presented in Tables 2.8 and 2.9.

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

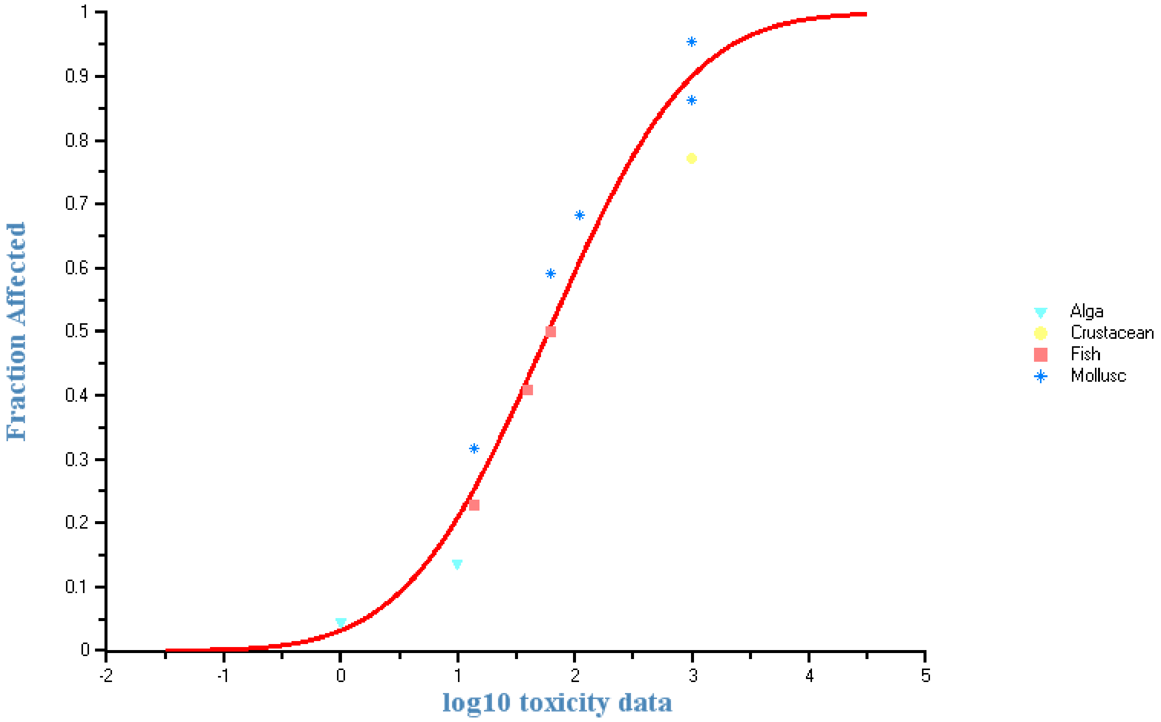

Figure 2.4 Cumulative distribution function of saltwater short-term data (µg l^{-1}) for chlorine

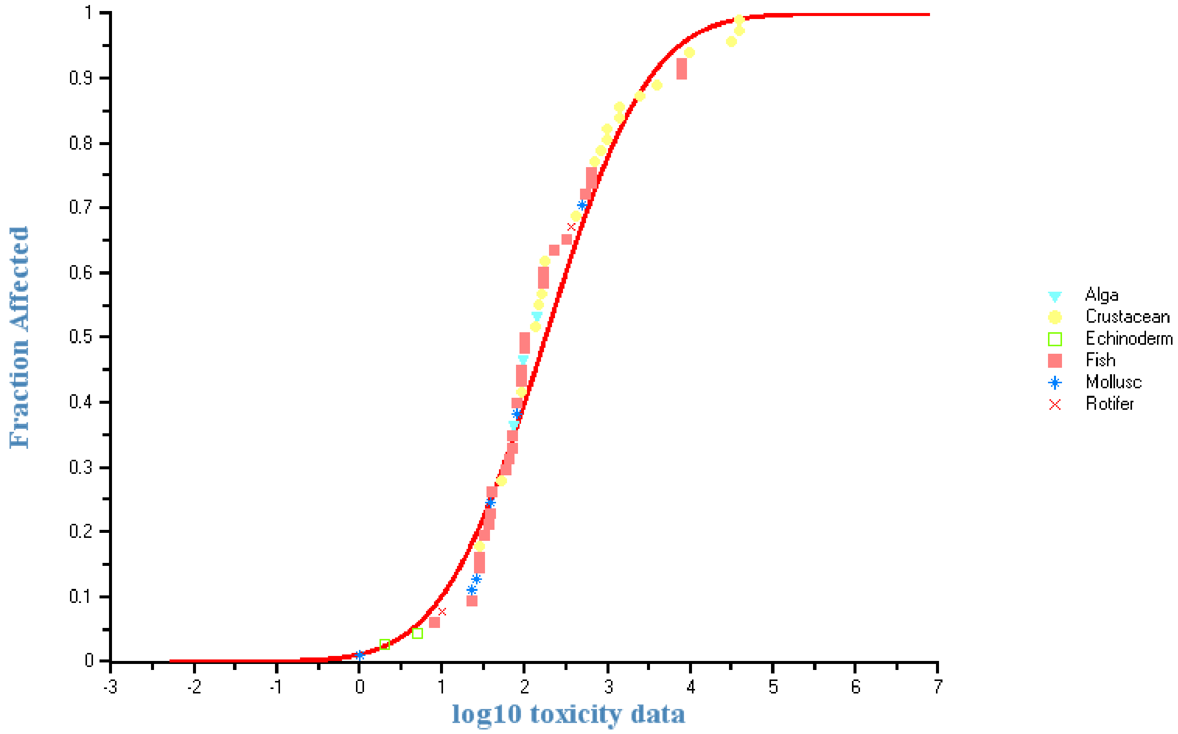

Table 2.8 Most sensitive long-term toxicity data for saltwater organisms exposed to chlorine

Scientific name	Common name	Endpoint, effect	Test duration	Conc. (µg l^{-1})	Exposure[1]	Analysis/ analyte[2]	Comments	Reliability index[3]	Reference
Phytoplankton (microcosm)		50% cell density reduction	21 days	1–10	f, DA	y; TRC	pH 7.7–7.9; 6–8°C	2	[28]
Phytoplankton (microcosm)		13–58% ATP reduction	1 year	10	f	y; TRC	10.1–30.8°C	2	[29]
Crassostrea virginica	Eastern oyster	NOEC (inferred) effect on shell deposition	15 days	7	f (field)	y; TRO	pH 7.1; 31.2°C	2	[30]
Crassostrea virginica	Eastern oyster	14% effect on shell deposition (LOEC)	15 days	14	f (field)	y; TRO	pH 7.1; 31.2°C	2	[30]
Brachidontes striatulus	Mussel	100% mortality	20 days	1,000	f	n; Cl_2	pH 8.0; 29.2–29.8°C	2	[31]
Brachidontes striatulus	Mussel	100% mortality	24 days	1,000	f	n; Cl_2	pH 8.0; 29.2–29.8°C	2	[31]
Brachidontes striatulus	Mussel	100% mortality	102 hours	5,000	f	n; Cl_2	pH 8.0; 29.2–29.8°C	2	[31]
Brachidontes striatulus	Mussel	100% mortality	156 hours	5,000	f	n; Cl_2	pH 8.0; 29.2–29.8°C	2	[31]
Acartia tonsa	Copepod	LC100	15 days	1,000	NR	NR	NR	3	[23]
Perna viridis	Mussel	100% mortality	21days	1,000	f	n; Cl_2	pH 8.0; 28.9–29.4°C	2	[32]
Perna viridis	Mussel	100% mortality	33 days	1,000	f	n; Cl_2	pH 8.0; 28.9–29.4°C	2	[32]
Brevoortia tyrannus	White fish	0% mortality	19 days	62	f	y; TRO	pH 7.1; 31.2°C	2	[30]
Leiostomus xanthurus	Spot	26% mortality	20 days	14	f	y; TRO	pH 7.1; 31.2°C	2	[30]
Anguilla anguilla	Eel	100% mortality	7 days	102	f	y; TRO	22°C	2	[33]
Dicentrarchus labrax	Sea bass	100% mortality	7 days	59	f	y; TRO	21°C	2	[33]
Menidia peninsulae	Tidewater silverside	NOEC	28 days	40	f	y; CPO	pH 7.9–8.0; 25°C	1	[34]

[1] Exposure: f = flow-through. [2] Analysis: y = measured; n = nominal. [3] See Annex 1.

ATP = adenosine triphosphate; TRC = total residual chlorine; TRO = total residual oxidant; CPO = chlorine-produced oxidants

LOEC = lowest observed effect concentration; NOEC = no observed effect concentration; LC100 = concentration lethal to 100% of the organisms tested

DA = daily chemical addition; NR = not reported

Table 2.9 Most sensitive short-term toxicity data for saltwater organisms exposed to chlorine

Scientific name	Common name	Endpoint, effect	Test duration	Conc. (µg l^{-1})	Exposure[1]	Analysis/ analyte[2]	Comments	Reliability index[3]	Reference
Thalassiosira pseudonana	Diatom	LC50	24 hours	75	NR	y	20°C	3	[35]
Brachionus plicatilis	Rotifer	LC50	30 min	10–180	f	y; TRO	pH 7.9–8.1; 20, 25, 27.5°C	2	[36]
Brachionus plicatilis	Rotifer	LC100	30 min	460–1,760	f	y; TRO	pH 7.9–8.1; 20, 25, 27.5°C	2	[36]
Mercenaria mercenaria	Clam	TL50	48 hours	1	s, f	y; TRC	17–28°C	2	[37]
Crassostrea virginica	Eastern oyster	EC50	48 hours	26	NR	y; Cl_2	no pH provided; 20, 25°C	2	[38]
Strongylocentrotus droesbachiensis	Sea urchin	EC50 fertilisation	5 min	5–6	NR	y; TRO	NR	4	[39]
Dendrater excentricus	Sea urchin	EC50 fertilisation	5 min	2–20	NR	y; TRO	NR	4	[39]
Acartia tonsa	Copepod	LC50	48 hours	29	f	y; Cl_2	20–25°C	2	[38]
Pandalus goniurus	Shrimp	LC50	96 hours	90	f	y; TRO	pH 8.0; 14.8°C	2	[40]
Morone saxatilis	Bass	incipient LC50		70	f	y; TRC	pH 6.8; 18°C	3	[41]
Morone saxatilis	Bass	incipient LC50		40	f	y; TRC	pH 6.8; 18°C	3	[41]
Morone saxatilis	Bass	LC50	48 hours	8	f	y; TRC	pH 6.8; 18°C	3	[41]
Oncorhynchus kisutch	Coho salmon	LC50	96 hours	32	f	y; TRO	pH 8.0; 14.8°C	2	[40]
Clupea harengus	Herring	LC50	96 hours	65	f	y; TRO	pH 8.0; 14.8°C	2	[40]
Solea solea	Sole	LC50	96 hours	28	NR	y; TRO	NR	4	[42]
Pleuronectes platessa	Plaice	LC50	96 hours	28	NR	y; TRO	NR	4	[43]
Cymatogaster aggregata	Shiner perch	LC50	96 hours	71	f	y; TRO	pH 8.0; 14.8°C	2	[40]
Leiostomus xanthurus	Spot	TLm (= LC50)	96 hours	90	f	y; TRC	pH 7.5; 14.2–16°C	1	[44]

[1] Exposure: s = static; f = flow-through. [2] Analysis: y = measured. [3] See Annex 1.

LCx = concentration lethal to X% of the organisms tested; EC50 = concentration effective against 50% of the organisms tested; TLm = threshold limit, median
TRC = total residual chlorine; TRO = total residual oxidant; NR = not reported

2.6.3 Toxicity to sediment-dwelling organisms

The EU RARs [1, 2] state that the sediment compartment is not relevant for assessment due to the rapid reaction of chlorine with organic matter, forming products such as chloramines, and eventual reduction to chloride. Consequently, sediment toxicity was not assessed.

2.6.4 Endocrine-disrupting effects

No data could be located on the endocrine effects of chlorine.

2.6.5 Mode of action of chlorine and the occurrence of metabolites in the aquatic environment

Chlorine, or more specifically hypochlorous acid (HOCl) and the hypochlorite ion (OCl^-), are oxidative and highly reactive compounds. HOCl reacts by chlorination of the nitrogen containing compounds in organisms such as amino acids [2]. Consequently, biological processes such as enzyme function become impaired resulting in negative effects and possibly death [45].

In fish, chlorine damages gills resulting in increased mucous production and impaired respiratory exchange at the gill surface [45]. Oxidation of haemoglobin may also occur resulting in reduced oxygen transport within the organism [45].

An important fate process for chlorine is the reaction with ammonia to produce mono-, di- or trichloramines. This is of concern to environmental regulators because of the potentially high toxicity of chloramines and their higher persistence in comparison with chlorine [2]. However, this report is concerned with the effects of chlorine alone and does not include its reaction products.

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

Draft EU RARs are available for both chlorine and sodium hypochlorite [1, 2]. The data used and outcomes of these reports have been subjected to extensive peer review and the UK is committed to the use of these data for chemical risk assessment purposes. Risk Assessment Reports have also been adopted for the derivation of the Water Framework Directive Annex X EQSs. Consequently, where RAR PNECs are available, they have been adopted in this report as the proposed PNECs as derived using the TGD approach.

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

3.1.1 PNECs for freshwaters

PNEC accounting for an annual average concentration
High quality long-term (lt) toxicity data were available only for algae, molluscs and fish. Since long-term crustacean data are lacking, strictly speaking, the 'base set' of toxicity data (i.e. tests with algae, *Daphnia*, and fish) is not met.

Long-term data for algae are limited to a number of laboratory and field mesocosm/microcosm studies with periphytic communities. Cairns *et al.* [12] studied the effects of two concentrations of sodium hypochlorite (6.3 and 57 µg l^{-1} TRC) on naturally derived periphytic communities. Tests were flow-through and ran for seven days. A lowest observed effect concentration (LOEC) of 6 µg l^{-1} TRC was reported based on a reduction in species richness. An associated EC20 of 2.7 µg l^{-1} TRC was also reported [12]. The sodium hypochlorite RAR regarded this as significant and calculated a no observed effect concentration (NOEC) of 3 µg l^{-1} TRC based on guidance in the TGD [49] (NOEC = LOEC/2). This study was regarded by the RAR as valid with restriction since a non-standard test system was used [2].

In another laboratory microcosm study, Pratt *et al.* [13] investigated the effects of continuous exposure to chlorine on microbial population endpoints over 28 days. A 28-day NOEC of 2.1 µg l^{-1} TRC was reported for a number of species and alkaline phosphatase activity. This study was regarded as valid with restriction due to the use of a non standard test system [2]. In addition, a 50 per cent reduction in non-taxonomic specific chlorophyll a was reported at 2.1 µg l^{-1} TRC. However, a NOEC could not be calculated for this endpoint and so the RAR regarded the chlorophyll data as supporting information only [2].

Pratt *et al.* [13] also studied the effects of chlorine on protozoan and algal populations in field mesocosms. Polythene bags with lake water and littoral sediment were provided for colonisation. The mesocosms were subjected to a pulse of chlorine once a day and decay curves used to estimate average chlorine concentrations. The most sensitive endpoint was a 24-day NOEC of 1.5 µg l^{-1} TRC for zooplankton density [13]. However, this endpoint was based on number of zooplankton per ml of water and no data were given on the taxonomic composition or actual elimination of taxa. The use of a pulse system of exposure also leads to some doubt about the reliability of this value and so it was considered as supporting information only in the RAR [2].

There are only limited long-term data for invertebrates, with reliable data available only for molluscs. In addition, no long-term NOEC data were available. The lowest reliable data for molluscs was a report of 100 per cent mortality in clam (*Corbicula fluminea)* exposed to 50 µg l^{-1} TRC for 36 days [14]. The authors of the draft sodium hypochlorite RAR [2] estimate that 50 per cent mortality would have occurred after an 8-day exposure to this concentration. The only other reliable data located for molluscs were for the zebra mussel (*Dreissena polymorpha*), which appears to be less sensitive than the clam. In flow-through tests, a 56-day exposure to 80 µg l^{-1} free chlorine resulted in 55 per cent mortality [15]. A 9-day LC50 of 500 µg l^{-1} TRO has also been reported for this species [16].

Only limited long-term toxicity data were available for fish, with only one study of reasonable quality identified in the sodium hypochlorite RAR [2] and some additional long-term data located in the UK EQS [45]. The sodium hypochlorite RAR identifies a field study in which several fish species (rainbow trout, white fish and channel catfish) were exposed for up to 134 days under flow-through conditions. In this study, fish were exposed to three chlorine concentrations with one or two replicates. The channel catfish (*Ictalurus punctatus*) showed a decrease in weight of 25 and 34 per cent at 52 and 162 µg l^{-1} TRC, respectively [17]. The next lowest concentration tested (5 µg l^{-1} TRC) produced no significant difference from the controls. Although the test was not run under standard conditions, the RAR suggests that the 25 per cent effect was significant and thus the 5 µg l^{-1} TRC no effect concentration could be treated as a NOEC. This data point was used as supporting information in the sodium hypochlorite RAR [2].

The UK EQS report [45] also identified few long-term studies for fish. The lowest reported effect was decreased activity in brook trout after a 7-day exposure to 4 µg l^{-1} HOCl [19]. The extent of the effect was not reported and few experimental details were available with which to assess the quality of the study. A concentration of 11 µg l^{-1} TAC has also been reported to affect growth rates in juvenile coho salmon [18]. However, once again few experimental details were available with which to assess the quality of the study. All other long-term effect and no effect concentrations for fish were higher than the values reported above.

There are few high quality long-term NOECs available for freshwater organisms. Valid NOEC data were only available for algae, with some supporting data available for fish and zooplankton. Good quality data for invertebrates were lacking and so the 'base set' of data required by the TGD was not fulfilled.

The draft sodium hypochlorite RAR [2] used the following approach to derive the long-term freshwater PNEC. Based on the non-specific mode of action of chlorine (oxidation), the freshwater and saltwater datasets were combined. As the long-term marine dataset contains good quality data for molluscs (see Section 3.1.2), the addition of this value results in the creation of a base set containing algae, molluscs and fish.

The lowest reliable long-term value is a 7-day NOEC of 3 µg l^{-1} TRC (corresponding to 2.1 µg l^{-1} FAC) for a reduction in species richness of periphytic communities in a microcosm study. This value was calculated, based on guidance in the TGD [49], from a LOEC of 6 µg l^{-1} TRC. However, as no valid long-term NOEC data were available for crustaceans, the algal data may not be protective of all species. Information on short-term exposures for these organisms (24-hour LC50 of 5 µg l^{-1} FAC in *Ceriodaphnia*) and a supporting study reporting a 28-day NOEC of only 1.5 µg l^{-1} TRC for zooplankton density in a field mesocosm, indicate that crustaceans may be more sensitive than algae. Consequently, the long-term effects assessment was based on the 7-day NOEC of 3 µg l^{-1} TRC with an increased assessment factor of 50.

$\text{PNEC}_{\text{freshwater\_lt}}$ = 3 µg l^{-1} TRC/AF (50) = 0.06 µg l^{-1} TRC (equivalent to 0.04 µg l^{-1} FAC)

PNEC accounting for transient concentration peaks
Acute toxicity data are available for various taxonomic groups including algae, crustaceans, fish, annelids, platyhelminths, molluscs and insects. Crustaceans are the most sensitive organisms with a range of reported effect concentrations one order of magnitude lower than that reported for fish.

Only limited short-term (st) algal data could be located and all were of unsuitable quality to be included in the assessment.

One study tested the effects of two concentrations of chlorine (200 and 600 µg l^{-1}) on *Chlorella sorokiniana* in the dark at 28–32°C for 20 hours [20]. The 600 µg l^{-1} exposure resulted in a 43 per cent reduction in cell numbers, whereas the 200 µg l^{-1} exposure resulted in only a 27 per cent reduction. The test methodology was not reported clearly in this study and so it is not regarded as reliable [2]. Another paper reported the effects of short-term exposures to chlorine on blue-green algae (*Aphanizomenon flos-aquae)*. Algae were exposed for 22 hours in a static system with various concentrations of chlorine. A concentration of 54 µg l^{-1} FAC resulted in 50 per cent inhibition of nitrogen fixation [21]. However, there is no mention of replication or chemical analysis in this study and so it would not be regarded as suitable for PNEC derivation. In addition to the two studies above, Brooks and Liptak reported that 100 µg l^{-1} TRC caused chlorophyll a depletion in phytoplankton after a static exposure for 30 minutes [22]. However, insufficient description of the methodology resulted in this paper being excluded from the RAR assessment [2]. All other short-term algal data were of lower sensitivity and poor quality.

Although short-term data were available for various invertebrate taxa (crustaceans, annelids, platyhelminths, molluscs and insects) only a few data, for crustaceans, were suitable for inclusion in the PNEC derivation [2].

The lowest data point available was a value located in the open literature, but not included in the draft sodium hypochlorite RAR. In a field study using artificial streams, mayfly nymphs (*Baetis harrisoni*) collected from the wild (one stream and one river location) were exposed to various chlorine concentrations in a flow-through system [25]. A 96-hour LC50 of 4.1 µg l^{-1} TRC was reported for mayflies collected from the stream location and an LC50 of 4.8 µg l^{-1} TRC reported in mayflies collected from the river location [25]. The study does not report the system pH, but states that the TRC measured in the tests was equivalent to free available chlorine. The study was also carried out in replicate under flow-through conditions with chemical analysis.

The next lowest reported data points were 24-hour LC50 values of 6 and 5 µg l^{-1} of OCl^- and HOCl, respectively, reported for the copepod *Ceriodaphnia dubia* in a flow-through test without food [10]. These data are considered 'valid with restriction' due to a lack of information on replicates and control performance, and because the concentrations were based on measurement of the stock solution and dilution ratios [2].

The next lowest reliable data point, according to the draft sodium hypochlorite RAR, was a 48-hour EC50 in juvenile *Daphnia magna* of 20 µg l^{-1} [24].

Much of the short-term toxicity data for fish were produced in the 1970s using intermittent chlorine exposures. The lowest acute effect concentration for fish was reported in a study where fish were exposed to 40-minute bursts of chlorine three times a day. The lowest effect was a 120-hour LC50 of 33 µg l^{-1} TRC for the channel catfish (*Ictalurus punctatus*) exposed at 24°C [26]. The next most sensitive fish species was the trout (*Oncorynchus mykiss*) with a 120-hour LC50 of 50 µg l^{-1} TRC following exposure at 12°C.

In a subsequent study, the same authors reported a 96-hour LC50 for the trout of 60 µg l^{-1} TRC exposed at 5°C and a 96-hour LC50 of 64 µg l^{-1} TRC in the catfish exposed at 24°C [11].

In addition, similar effects concentrations have been reported in fathead minnow when exposed to chlorine for 1 hour per day for 4 days with a 96-hour LC50 of 80 µg l^{-1} TRC [27].

All the exposures in the fish studies were intermittent. Consequently, it is difficult to obtain a suitable continuous exposure effect concentration for use in the PNEC derivation. As a result, the fish data reported above have been used as supporting data in the PNEC derivation.

Good quality short-term freshwater EC/LC50 data were only available for crustaceans, with supporting data for fish. Valid data for algae were lacking and so the 'base set' of data required by the TGD was not fulfilled. Given the non-specific mode of action of chlorine (oxidation), it is appropriate to combine the freshwater and saltwater datasets. However, the saltwater dataset is also lacking in good quality algal data. Consequently, the TGD 'base set' of algae, crustaceans and fish cannot be fulfilled.

The draft sodium hypochlorite RAR identified the lowest reliable short-term data point as the 24-hour LC50 of 5 µg l^{-1} FAC for *Ceriodaphnia dubia*. Based on guidance in the TGD for the assessment of intermittent releases [49], a standard assessment factor of 100 should be applied to the LC50 for *Ceriodaphnia dubia* resulting in the following $PNEC_{freshwater\_st}$:

$PNEC_{freshwater\_st}$ = 5 µg l^{-1} FAC/AF (100) = 0.05 µg l^{-1} FAC

3.1.2 PNECs for saltwaters

Saltwater toxicity data are available for various taxonomic groups including algae, crustaceans, fish, rotifers, molluscs and echinoderms. However, chronic exposure data could be located only for algae, molluscs, fish and crustaceans.

PNEC accounting for the annual average concentration

Two long-term studies were available on the effects of chlorine on phytoplankton. In a semi-field experiment using tanks under flow-through conditions, cell densities of phytoplankton were reduced by up to 50 per cent at exposure concentrations of 1–10 µg l^{-1} TRC after a 21-day test [28]. HOCl was added to the tanks daily, but was degraded within 2 hours. Consequently, these data relate to intermittent exposures and so are regarded by the sodium hypochlorite RAR as supporting information only [2].

In indoor and outdoor field microcosms, exposure of estuarine phytoplankton to continuous chlorine concentrations resulted in up to a 58 per cent reduction in ATP [29]. Although the added concentration of chlorine ranged from 125 to 1,441 µg l^{-1}, the measured concentration in the system was continually below the detection limit (10 µg l^{-1}). Therefore, this study was regarded as supporting information by the sodium hypochlorite RAR [2]. No other reliable algal data were available.

For invertebrates, the chronic toxicity of chlorine has been investigated only in molluscs and crustaceans. In molluscs, effect concentrations range from approximately 10 to 5,000 µg l^{-1}. The lowest reliable datum identified by the sodium hypochlorite RAR was a 14 per cent reduction in shell deposition in the oyster (*Crassostrea virginica*) at 14 µg l^{-1} TRO [30]. This data point was treated as a LOEC by the RAR and, as the effect was between 10 and 20 per cent, was converted into a NOEC of 7 µg l^{-1} TRO (LOEC/2) in accordance with the EU TGD [2]. The datum was regarded as valid with restriction as it was generated in a non-standard test system.

The only other data, with moderate quality, identified by the draft RAR were a number of LC100 values in the mussels (*Brachidontes striatulus* and *Perna viridis*) of 1,000–5,000 µg l^{-1} Cl_2 generated in a flow-through study [31, 32]. These values were regarded as valid with restriction because of a lack of suitable analysis [2].

In addition to the data in the RAR, a single long-term value for a copepod was identified in the UK EQS document [45]. A 15-day LC100 of 1,000 µg l^{-1} TRO in *Acartia tonsa* was reported [23]. However, there were no details on the experimental procedure and so the quality of the data cannot be assessed.

Only very limited data were available for marine fish, with only one high quality value identified by the sodium hypochlorite RAR [2]. Goodman *et al.* [34] investigated the effect of chlorine on the eggs and early life stages of the tidewater silverside (*Menidia peninsulae*) in a flow-through system for 28 days. Fry of the fish were most sensitive, with a 28-day NOEC of 40 µg l^{-1} chlorine-produced oxidants (CPO) (equivalent to TRC) reported [34]. No sublethal effects were observed and this study was classified as fully valid by the draft RAR.

The draft RAR [2] also identified a field study using troughs through which chlorinated effluents at three levels of chlorination were pumped continuously. The effects of a 20-day exposure to the effluents in white fish (*Brevoortia tyrannus*) and spot (*Leiostomus* *xanthurus*) were investigated. At the lowest concentration (14 µg l^{-1} TRO), 26 per cent mortality occurred in the spot, whereas a concentration of 60 µg l^{-1} TRO had no effect on the survival of the white fish [30]. A clear concentration-effect relationship could not be identified and so it was not possible to calculate a NOEC.

In addition to the fish data reported in the RAR [2], two further long-term studies were identified in the UK EQS [45]. In seven-day experiments with the eel (*Anguilla anguilla*) and sea bass (*Dicentrarchus* labrax), the lowest LC50s were reported as 102 and 59 µg l^{-1} TRO, respectively [33]. The study was conducted under flow-through conditions with measured concentrations.

There are few high quality long-term NOECs available for marine organisms. Good quality NOEC data were available only for fish, with some supporting data available for molluscs. Good quality data for algae were lacking and so the 'base set' of data required by the TGD was not met.

The draft sodium hypochlorite RAR used the following approach to derive the long-term saltwater PNEC. Based on the non-specific mode of action of chlorine (oxidation), the freshwater and saltwater datasets were combined. As the long-term freshwater dataset contains good quality data for algae (see Section 3.1.1), the addition of this value results in the creation of a base set containing algae, molluscs and fish.

Based on the combined freshwater and saltwater datasets, the lowest reliable long-term value was a 7-day NOEC of 3 µg l^{-1} TRC (corresponding to 2.1 µg l^{-1} FAC) for a reduction in species richness of periphytic communities in a freshwater microcosm study. This value was calculated, based on guidance in the TGD, from a LOEC of 6 µg l^{-1} TRC. Based on the available data, the sodium hypochlorite RAR [2] proposed the use of the 7-day NOEC of 3 µg l^{-1} TRC with an assessment factor of 50.

$\text{PNEC}_{\text{saltwater\_lt}}$ = 3 µg l^{-1}TRC/AF (50) = 0.06 µg l^{-1} TRC (equivalent to 0.04 µg l^{-1} FAC)

PNEC accounting for transient concentration peaks
Short-term toxicity data were available for various marine algae. However, none of the data are regarded as suitable for PNEC derivation [2]. The lowest effect concentration was reported for the diatom *Thalassiosira pseudonana* with a 24-hour LC50 of 75 µg l^{-1} in a static test system. However, only the concentrated stock solution was analysed in this study [35]. The majority of the other short-term algal toxicity data were an order of magnitude higher than this value, but all were regarded as of poor quality [2].

The acute toxicity of chlorine has been tested on various marine invertebrates including molluscs, echinoderms, worms and crustaceans. However, few of the data would be regarded as of suitable quality for PNEC derivation, as discussed below.

The lowest reported effect concentration was a 48-hour TL50 of 1 µg l^{-1} TRC for the hard clam (*Mercenaria mercenaria*) in a static test system [37]. However, this value is based on average concentrations, which were extrapolated. Consequently, it was regarded as supporting information in the draft RAR [2].

The next lowest value was a 30-minute LC50 value of 10 µg l^{-1} TRO for the rotifer (*Brachionus plicatilis*) [36]. However, this value was generated under thermal stress (5°C) and so was regarded as supporting information in the draft RAR [2].

Additional data are available for the copepod *Acartia tonsa* and shrimp *Pandalus goniurus* with LC50 values of 29 µg l^{-1} Cl_2 (48-hour exposure) and 90 µg l^{-1} TRO (96-hour exposure), respectively [38, 40]. However, the data were regarded by the sodium hypochlorite RAR as 'valid with restriction' only, as the values were generated by pooling data [2].

In addition to the data in the draft sodium hypochlorite RAR, low effect concentrations have been reported in the literature for the sea urchins *Strongylocentrotus droebachiensis* and *Dendraster excentricus*. Five-minute EC50 values of 5–6 and 2–20 µg l^{-1} TRO, respectively, were reported for effects on fertilisation success [39]. Chemical concentrations were analysed. However, there were very few experimental details so this study has been used as supporting information only.

The lowest reliable data point for marine invertebrates that could be identified was a 48-hour LC50 of 26 µg l^{-1} CPO for the larvae of the oyster (*Crassostrea virginica*) exposed to calcium hypochlorite in a flow-through test [38].

Various short-term studies have been conducted with chlorine and marine fish. The sodium hypochlorite RAR identifies a small number of studies suitable for PNEC derivation, with additional data identified in the UK EQS [45].

The lowest data identified by the draft sodium hypochlorite RAR was a 48-hour LC50 of 8 µg l^{-1} TRC following exposure of striped bass (*Morone saxatilis*) eggs to chlorine in a flow-through test system [41]. However, this value was estimated by the RAR authors from raw data in the report and so should only be used as an indication of effects in fish eggs [2].

The next lowest data point was a 96-hour LC50 of 32 µg l^{-1} TRO for coho salmon exposed in a flow-through system under 5°C thermal stress [40]. This value was regarded as valid with restriction because the LC50 was calculated by pooling data from different tests [2].

The lowest high quality data point (Klimisch code 1) identified by the RAR was a 96-hour TLm (threshold limit, median) (equivalent to an LC50) of 90 µg l^{-1} TRC for the ocean spot (*Leiostomus xanthurus*) in a flow-through system with a continuous flow serial diluter [44].

In addition to the data located in the RAR [2], the UK EQS report [45] also identified two low short-term effect concentrations with the larvae of plaice (*Pleuronectes platessa*) and sole (*Solea solea*), which were used to set the EQS value for marine waters. The plaice study reports a 96-hour LC50 of 28 µg l^{-1} TRO and the sole study a 24-hour LC50 of 28 µg l^{-1} TRO [42, 43]. Both values were based on measured data. However, neither study provided information on the test set-up, such as environmental conditions and exposure type (static or flow-through). For these reasons, the data on plaice and sole are used as supporting information only.

Good quality short-term saltwater EC/LC50 data were only available for crustaceans and fish. Valid data for algae were lacking and so the ‘base set’ of data required by the TGD was not fulfilled. Given the non-specific mode of action of chlorine (oxidation), it would be appropriate to combine the freshwater and saltwater datasets. However, the freshwater dataset is also lacking in good quality algal data. Consequently, the TGD ‘base set’ of algae, crustaceans and fish still cannot be fulfilled.

The sodium hypochlorite RAR identified the lowest reliable short-term data point as the 24-hour LC50 of 5 µg l^{-1} FAC for *Ceriodaphnia dubia*. Based on guidance in the TGD for the assessment of intermittent releases, a standard assessment factor of 100 should be applied to the LC50 for *Ceriodaphnia dubia* resulting in the following $PNEC_{saltwater\_st}$:

$PNEC_{saltwater\_st}$ = 5 µg l^{-1} FAC/AF (100) = 0.05 µg l^{-1} FAC

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

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

3.3 Derivation of existing EQSs

In the 1994 UK EQS report [45], the freshwater standards were expressed as total available chlorine (TAC). The long-term standard for freshwater was derived from data relating to the toxicity of chloramine, since it was found to be more persistent in the environment than free chlorine (HOCl).

Chloramine is a product resulting from the reaction between chlorine and naturally occurring amines. A 15-week study reported that exposure to 3.4 µg l^{-1} total chloramine significantly reduced the number of offspring produced by the shrimp *Gammarus* sp. and, therefore, an EQS of 2 µg l^{-1} TAC expressed as an annual average concentration was proposed to protect freshwater aquatic life from continuous exposure to chlorine.

The short-term standard was based on a 48-hour LC_{50} of 9.3 µg l^{-1} TAC for mayfly (*Isonychia* sp.). An assessment factor of 2 was considered adequate because there was a large dataset on the acute effects of chlorine (decreasing the uncertainty in ensuring protection of the most sensitive species); the available data also indicated a relatively small acute to chronic ratio, and free chlorine (HOCl) is not persistent. This resulted in an EQS of 5 µg l^{-1} TAC expressed as a maximum allowable concentration. However, data for fish suggested that some avoidance behaviour and depressed activity might occur at this concentration.

The potential toxic species present below a chlorine discharge into saltwater will be a mixture of chlorine and bromine species, and a standard expressed as TAC would exclude the effect of bromine species. The standard derived for marine waters was, therefore, expressed as total residual oxidant (TRO), i.e. the sum of both ‘free’ and ‘combined’ residual oxidant including both chlorine and bromine compounds. The limited toxicity data indicated that ‘free’ oxidant is more toxic than ‘combined’ oxidant, but that the toxicity difference may be small. The data also showed that ‘combined’ oxidant is more persistent than ‘free’ oxidant.

The early life stages of fish were found to be particularly sensitive to chlorine. For the larvae of both plaice (*Pleuronectes platessa*) and sole (*Solea solea*), an LC50 of 28 µg l^{-1} TRO was reported. Based on the relatively large dataset on acute effects combined with the relatively short persistence time of free residual oxidants in marine water, an assessment factor of 2 was applied to this value to give an EQS of 10 µg l^{-1} TRO, expressed as a maximum allowable concentration (MAC).

A saltwater annual average concentration was not proposed due to lack of chronic exposure data for chloramines and bromamines.

3.4 Derivation of PNECs for sediment

Due to the rapid reaction of chlorine in sediments, the EU RARs [1, 2] state that the sediment compartment is not relevant for risk assessment.

3.5 Derivation of PNECs for secondary poisoning of predators

3.5.1 Mammalian and avian toxicity data

The EU TGD [49] requires the use of mammalian/avian no-effect data for the assessment of secondary poisoning. Such data are widely used by regulatory bodies, such as the World Health Organization (WHO), to set standards for the protection of human health.

In setting such standards, the available mammalian no observable adverse effect levels (NOAELs) undergo a strict quality assessment, with the lowest high quality data point being used to set the standard. Consequently, a NOAEL used to set a tolerable daily intake (TDI) value or reference dose should be of suitable quality for use in the assessment of secondary poisoning. A summary of the no-effect values used by international organisations to set human health standards for chlorine is given in Table 3.1.

Table 3.1 Mammalian and avian oral toxicity data relevant for the assessment of non-compartment specific secondary poisoning

Chemical	Endpoint	Value	Species	Duration	Effect	Regulatory body*
Chlorine	NOAEL	15 mg/kg bw/day	rat	2 years	None seen	WHO [46]
Chlorine	NOAEL	14.4 mg/kg bw/day	rat	2 years	None seen	US EPA IRIS [47]

* Regulatory body using the respective NOAEL to set a human health standard.
bw = body weight

3.5.2 PNECs for secondary poisoning of predators

Given the high water solubility of chlorine and its rapid degradation in the environment, bioaccumulation of chlorine species is not considered of importance in the environment [2].

4. Analysis and monitoring

The most common method of determining either free residual chlorine or total residual chlorine (i.e. free available plus combined available chlorine such as chloramines) is by colorimetry. Chlorine concentrations can be measured either in the field using simple field kits or in the laboratory using more sophisticated procedures and equipment. Most field kits use the simple addition of a reagent (i.e. an indicator) to a water sample and the colour of the solution is compared with a set of coloured (calibrated) standards. The limitation of field kits is their detection limits, which are typically 0.1 mg l^{-1}, which is well above environmentally safe levels.

Laboratory methods have lower detection limits, but analysis has to be carried out immediately to avoid loss of analyte (unless it is in the combined chlorine form) via chemical reactions such as hydrolysis, oxidation and photolysis. The US Standard Method 4500 [48] outlines different methods to detect residual chlorine, to be undertaken immediately after sample collection. A selection is listed in Table 4.1.

Table 4.1 Approved methods for determination of residual chlorine

Methodology	**Method number***
Iodometric	4500-Cl B and 4500-Cl C
Amperometric titration	4500-Cl D
Low-level amperometric titration	4500-Cl E
DPD ferrous titrimetric	4500-Cl F
DPD colorimetric	4500-Cl G
Syringaldazine (FACTS)	4500-Cl H
Iodometric electrode	4500-Cl I

* See http://standardmethods.org/
DPD = *N*,*N*-diethyl-*p*-phenylenediamine

The DPD colorimetric method (Palin test) is one of the most widely used methods (for field and laboratory applications. It utilises a colorimetric reaction between residual chlorine and DPD, measured at a wavelength of 515 nm, with a limit of detection of 10 µg l^{-1}.

The advantage of these methods is that the detection limits are as low as 10 µg l^{-1}, depending on the sophistication of the equipment. The US method does, however, recommend that sampling for total residual chlorine be completed in the field using a field testing kit to obtain the best results. In special cases, lower detection limit methods could be used by setting up titration equipment in the field.

The lowest proposed PNEC derived for chlorine is 0.06 µg l^{-1} total residual chlorine for fresh and salt waters, corresponding to a freely available chlorine level of 0.04 µg l^{-1}. The data quality requirements are that, at a third of the EQS, total measurement error should not exceed 50 per cent. From the literature, it can be seen that analytical methodologies provide detection limits of around 10 µg l^{-1}, which suggests that they do not offer adequate performance to analyse free chlorine for compliance in the receiving water.

5. Conclusions

5.1 Availability of data

Freshwater acute toxicity data are available for algae, crustaceans, fish, annelids, platyhelminths, macrophytes, molluscs, rotifers and insects. However, chronic exposure data could only be located for algae, molluscs, fish, protozoans and bacteria and, of these, NOECs are available only for algae, bacteria and fish.

Data for marine organisms are available for six different taxonomic groups (algae, crustaceans, fish, molluscs, rotifers and echinoderms). However, chronic toxicity data are available only for algae, molluscs, crustaceans and fish, with only fish and mollusc studies yielding long-term NOECs.

5.2 Derivation of PNECs

Given the short persistence of chlorine in water, a short-term PNEC is considered to be of greater relevance than a long-term PNEC.

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

5.2.1 Long-term PNEC for freshwaters

The lowest reliable long-term value is a 7-day NOEC of 3 µg l^{-1} as total residual chlorine (corresponding to 2.1 µg l^{-1} FAC) for a reduction in species richness of periphytic communities in a microcosm study. No valid long-term NOEC data are available for crustaceans, but short-term exposures for these organisms and a supporting field mesocosm study indicate that crustaceans may be more sensitive than algae.

Consequently, the 7-day NOEC of 3 µg l^{-1} TRC is recommended as the basis for a PNEC, but with an assessment factor of 50 to account for the uncertainties introduced by the lack of crustacean data. This results in a $PNEC_{freshwater\_lt}$ of 0.06 µg l^{-1} TRC (equivalent to 0.04 µg l^{-1} FAC). The generation of chronic invertebrate data would help reduce uncertainty in this extrapolation and thereby justify the use of a smaller assessment factor.

This PNEC is appreciably lower than the existing EQS of 2 µg l^{-1} determined in 1994 [45]. This value was based on a different critical datum, in which exposure to 3.4 µg l^{-1} chloramine (as opposed to chlorine) for 15 weeks inhibited reproduction in *Gammarus*, and was calculated without any assessment factor and expressed as total available chlorine.

5.2.2 Short-term PNEC for freshwaters

Good quality short-term freshwater data are available only for crustaceans, with supporting data for fish. Valid data for algae are lacking from both the freshwater and saltwater datasets; consequently, the ‘base set’ of data required by Annex V cannot be fulfilled.

The sodium hypochlorite RAR [2] identified the lowest reliable short-term data point as the 24-hour LC50 of 5 µg l^{-1} FAC for *Ceriodaphnia dubia*. In the absence of relevant supporting data, an assessment factor of 100 should be applied to this datum, resulting in a $PNEC_{freshwater\_st}$ of 0.05 µg l^{-1} FAC.

The proposed PNEC is again appreciably lower than the 1994 EQS of 5 µg l^{-1}, expressed as total available chlorine. This was based on an assessment factor of 2 applied to a mayfly (*Isonychia* sp.) acute LC50 of 9.3 µg l^{-1}.

5.2.3 Long-term PNEC for saltwaters

Reliable long-term NOECs are available only for marine fish with some supporting data for molluscs. Good quality data for marine algae are lacking and so the Annex V requirement for a 'base set' of data is not met. However, by combining the freshwater and saltwater datasets (as in the hypochlorite RAR), the addition of data for algae results in the creation of a saltwater dataset containing all three trophic levels.

Based on this combined dataset, the lowest reliable long-term value was the 7-day NOEC of 2.1 µg l^{-1} FAC for a reduction in species richness of periphytic communities in a freshwater microcosm study, used to derive the $PNEC_{freshwater\_lt}$. The same PNEC is proposed for the saltwater environment: $PNEC_{saltwater\_lt}$ of 0.06 µg l^{-1} TRC (equivalent to 0.04 µg l^{-1} FAC).

A long-term EQS for the protection of saltwater life was not derived during the 1994 review [45].

5.2.4 Short-term PNEC for saltwaters

Good quality short-term data for saltwater species are available only for crustaceans and fish. Even by combining the freshwater and saltwater datasets, the requirement for a 'base set' of algae, crustaceans and fish still cannot be fulfilled.

The EU RAR for sodium hypochlorite [2] identified the lowest reliable short-term data point as the 24-hour LC50 of 5 µg l^{-1} FAC for the freshwater crustacean *Ceriodaphnia dubia*. Applying the guidance for the assessment of intermittent releases, a standard assessment factor of 100 should be applied to the LC50 for *Ceriodaphnia dubia* resulting in a $PNEC_{saltwater\_st}$ of 0.05 µg l^{-1} FAC.

The 1994 EQS was based on an assessment factor of ~2 applied to acute LC50s of 28 µg l^{-1} for plaice and sole for total residual oxidant, resulting in an EQS of 10 µg l^{-1}, substantially higher than the $PNEC_{saltwater\_st}$ proposed here.

5.2.5 PNEC for secondary poisoning

Given the high water solubility of chlorine and its rapid degradation in the environment, bioaccumulation of chlorine species is not important.

5.2.6 PNEC for sediments

Due to the rapid reaction of chlorine in the sediment compartment, a PNEC for sediments is not relevant.

Table 5.1 Summary of proposed PNECs

Receiving medium/exposure scenario	**Proposed PNEC ($\mu g\ l^{-1}$ free available chlorine)**	**Existing EQS ($\mu g\ l^{-1}$)**
Freshwater/long-term	0.04	2 (total available chlorine)
Freshwater/short-term	0.05	5 (total available chlorine)
Saltwater/long-term	0.04	No standard
Saltwater/short-term	0.05	10 (total residual oxidant)

5.3 Analysis

Colorimetry is the most common method of determining either free residual chlorine or total residual chlorine. This can be used in the field as well as in the laboratory. Laboratory methods have lower detection limits, but analysis has to be carried out immediately to avoid loss of analyte. Detection limits are as low as 10 $\mu g\ l^{-1}$, depending on the sophistication of the equipment.

The lowest proposed PNEC derived for chlorine is 0.06 $\mu g\ l^{-1}$ total residual chlorine for fresh and salt waters, corresponding to a free available chlorine concentration of 0.04 $\mu g\ l^{-1}$. The data quality requirements are that, at a third of the EQS, total measurement error should not exceed 50 per cent. It is unlikely that current analytical methodologies are sufficiently sensitive to assess compliance in the receiving water with the PNECs proposed here.

5.4 Implementation issues

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

1. Monitoring is usually carried out using test kits in the field, but these are not sufficiently sensitive to assess compliance in the receiving water. Either monitoring will have to be carried out only in effluent waste streams or analytical methods will have to be improved to deliver greater sensitivity.

2. A lack of ecotoxicological data gives rise to a considerable degree of uncertainty in the extrapolations from the available data. Generation of additional ecotoxicological data would help reduce uncertainty and may result in different PNECs.

3. In the interim, current EQSs should be adopted until these issues can be addressed.

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[Accessed 5 February 2007]

List of abbreviations

AA	annual average
AF	assessment factor
ATP	adenosine triphosphate
BCF	bioconcentration factor
bw	body weight
CAC	combined available chlorine
CAS	Chemical Abstracts Service
CPO	chlorine-produced oxidants
EC50	concentration effective against 50% of the organisms tested
ECB	European Chemicals Bureau
ECx	concentration effective against X% of the organisms tested
EQS	Environmental Quality Standard
FAC	free available chlorine
GLP	Good Laboratory Practice (OECD)
IRIS	Integrated Risk Information System
LC50	concentration lethal to 50% of the organisms tested
LCx	concentration lethal to X% of the organisms tested
LOEC	lowest observed effect concentration
lt	long term
MAC	maximum allowable concentration
NA	not applicable
ND	no data
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
OECD	Organisation for Economic Co-operation and Development
PNEC	predicted no-effect concentration
RAR	risk assessment report
SEPA	Scottish Environment Protection Agency
SNIFFER	Scotland & Northern Ireland Forum for Environmental Research
SSD	species sensitivity distribution
st	short term
TAC	total available chlorine
TDI	tolerable daily intake

TGD	Technical Guidance Document
TLm	threshold limit, median
TRC	total residual chlorine
TRO	total residual oxidant
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 relating to the sodium hypochlorite Risk Assessment Report

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

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

Table A1 Klimisch Criteria*

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

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

Reference number	**10**

Information on the test species	
Test species used	*Ceriodaphnia dubia*
Life stage of the test species used	

Information on the test design	
NaClO RAR endpoint comment	Test method incomplete

NaClO RAR relevance criteria	
Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference number	**11**

Information on the test species	
Test species used	*Oncorhynchus mykiss* and *Ictalurus punctatus*
Life stage of the test species used	Juvenile

Information on the test design	
NaClO RAR endpoint comment	Intermittent exposure – study treated as supporting information

NaClO RAR relevance criteria	
Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference number	**12**

Information on the test species	
Test species used	Microcosm
Life stage of the test species used	

Information on the test design	
NaClO RAR endpoint comment	Non standard test method used

NaClO RAR relevance criteria	
Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference number	**13**

Information on the test species	
Test species used	Microbial populations
Life stage of the test species used	

Information on the test design	
NaClO RAR endpoint comment	Non standard test method used

NaClO RAR relevance criteria	
Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference number	**14**

Information on the test species	
Test species used	*Corbicula fluminea*
Life stage of the test species used	Adult

Information on the test design	
NaClO RAR endpoint comment	Data used as supporting information

NaClO RAR relevance criteria	
Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference number	**15**

Information on the test species	
Test species used	*Dreissena polymorpha*
Life stage of the test species used	Adult

Information on the test design	
NaClO RAR endpoint comment	Data used as supporting information

NaClO RAR relevance criteria	
Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference number	**16**

Information on the test species	
Test species used	*Dreissena polymorpha*
Life stage of the test species used	Adult

Information on the test design	
NaClO RAR endpoint comment	Data used as supporting information

NaClO RAR relevance criteria	
Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference number	**17**

Information on the test species	
Test species used	*Catostomus commersoni*, *Oncorhynchus mykiss* and *Icatalurus punctatus*
Life stage of the test species used	Juveniles

Information on the test design	
NaClO RAR endpoint comment	Data used as supporting information

NaClO RAR relevance criteria	
Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference number	**20**

Information on the test species	
Test species used	*Chlorella sorokiniana*
Life stage of the test species used	

Information on the test design	
NaClO RAR endpoint comment	Test method incomplete

NaClO RAR relevance criteria	
Reliability of study	**Unreliable**
Relevance of study	**Relevant**
Klimisch Code	**3**

Reference number	**22**

Information on the test species	
Test species used	Phytoplankton
Life stage of the test species used	

Information on the test design	
NaClO RAR endpoint comment	Test method incomplete

NaClO RAR relevance criteria	
Reliability of study	**Unreliable**
Relevance of study	**Relevant**
Klimisch Code	**3**

Reference number	**23**

Information on the test species	
Test species used	*Daphnia magna* and *Acartia tonsa*
Life stage of the test species used	

Information on the test design	
NaClO RAR endpoint comment	Very short exposures

NaClO RAR relevance criteria	
Reliability of study	**Unreliable**
Relevance of study	**Relevant**
Klimisch Code	**3**

Reference number	**(24)**

Information on the test species	
Test species used	*Daphnia magna*
Life stage of the test species used	Juvenile

Information on the test design	
NaClO RAR endpoint comment	No information

NaClO RAR relevance criteria	
Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference number	**26**

Information on the test species	
Test species used	*Oncorhynchus mykiss, O. kisutch, Cyprinus carpio* and *Ictalurus punctatus*
Life stage of the test species used	Juvenile

Information on the test design	
NaClO RAR endpoint comment	Intermittent exposure – study treated as supporting information

NaClO RAR relevance criteria	
Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference number	**28**

Information on the test species	
Test species used	Phytoplankton
Life stage of the test species used	

Information on the test design	
NaClO RAR endpoint comment	Intermittent exposure – study treated as supporting information

NaClO RAR relevance criteria	
Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference number	**29**

Information on the test species	
Test species used	Plankton
Life stage of the test species used	

Information on the test design	
NaClO RAR endpoint comment	Rapid reduction in exposure concentration – study treated as supporting information

NaClO RAR relevance criteria	
Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference number	**30**

Information on the test species	
Test species used	*Crassostrea virginica, Brevoortia tyrannua* and *Leiostomus xanthurus*
Life stage of the test species used	Adult

Information on the test design	
NaClO RAR endpoint comment	Study treated as supporting information

NaClO RAR relevance criteria	
Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference number	**31**

Information on the test species	
Test species used	*Brachidontes striatulus*
Life stage of the test species used	Juvenile

Information on the test design	
NaClO RAR endpoint comment	No analysis system – study treated as supporting information

NaClO RAR relevance criteria	
Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference number	**32**

Information on the test species	
Test species used	*Perna viridis*
Life stage of the test species used	Juvenile

Information on the test design	
NaClO RAR endpoint comment	Lack of suitable analysis - study treated as supporting information

NaClO RAR relevance criteria	
Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference number	**34**

Information on the test species	
Test species used	*Menidia peninsulae*
Life stage of the test species used	Eggs and fry

Information on the test design	
NaClO RAR endpoint comment	Fully valid

NaClO RAR relevance criteria	
Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference number	**35**

Information on the test species	
Test species used	*Thalassiosira pseudonana*
Life stage of the test species used	

Information on the test design	
NaClO RAR endpoint comment	Lack of suitable analysis - study treated as supporting information

NaClO RAR relevance criteria	
Reliability of study	**Unreliable**
Relevance of study	**Relevant**
Klimisch Code	**3**

Reference number	**36**

Information on the test species	
Test species used	*Brachionus plicatilis*
Life stage of the test species used	ND

Information on the test design	
NaClO RAR endpoint comment	Test carried out under thermal stress – study used as supporting information

NaClO RAR relevance criteria	
Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference number	**37**

Information on the test species	
Test species used	*Mercenaria mercenaria*
Life stage of the test species used	Larvae

Information on the test design	
NaClO RAR endpoint comment	Data based on average extrapolated concentrations – study used as supporting information

NaClO RAR relevance criteria	
Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference number	**38**

Information on the test species	
Test species used	*Arcartia tonsa* and *Crassostrea virginica*
Life stage of the test species used	ND

Information on the test design	
NaClO RAR endpoint comment	Study used as supporting information

NaClO RAR relevance criteria	
Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference number	**40**

Information on the test species	
Test species used	*Pandalus goniurus Oncorhynchus kisutch* and *Clupea harengus*
Life stage of the test species used	Adults

Information on the test design	
NaClO RAR endpoint comment	Endpoint generated under thermal stress - study used as supporting information

NaClO RAR relevance criteria	
Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference number	**41**

Information on the test species	
Test species used	*Morone saxtilis*
Life stage of the test species used	Larvae, juveniles and eggs

Information on the test design	
NaClO RAR endpoint comment	Endpoints estimated from raw data

NaClO RAR relevance criteria	
Reliability of study	**Unreliable**
Relevance of study	**Relevant**
Klimisch Code	**3**

Reference number	**44**

Information on the test species	
Test species used	*Leiostomus xanthurus*
Life stage of the test species used	Juvenile

Information on the test design	
NaClO RAR endpoint comment	Valid study

NaClO RAR relevance criteria	
Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**1**

ANNEX 2 Data quality assessment sheets relating to data additional to those in the sodium hypochlorite Risk Assessment Report

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

Reference number	**18**

Information on the test species	
Test species used	*Oncorhynchus kisutch*
Life stage of the test species used	Adult
Holding conditions prior to test	Not stated
Source of the test organisms	Not stated

Information on the test design	
Methodology used	Not stated
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Not stated
Measurement of exposure concentrations	Not stated expressed as TAC
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Endpoint comment	Due to a lack of experimental data, it is not possible to assess the quality of the data point.
Study conducted to GLP	Not stated

Reliability of study	**Unknown reliability**
Relevance of study	**Relevant**
Klimisch Code	**4**

Reference number	**19**

Information on the test species	
Test species used	*Salmo fontinalis*
Life stage of the test species used	Not stated
Holding conditions prior to test	Not stated
Source of the test organisms	Not stated

Information on the test design	
Methodology used	Not stated
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Not stated
Measurement of exposure concentrations	Not stated expressed as TAC
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Endpoint comment	Due to a lack of experimental data, it is not possible to assess the quality of the data point.
Study conducted to GLP	Not stated

Reliability of study	**Unknown reliability**
Relevance of study	**Relevant**
Klimisch Code	**4**

Reference number	**21**

Information on the test species	
Test species used	*Aphanizomenon flos-aquae*
Life stage of the test species used	-
Holding conditions prior to test	Grown in continuous culture
Source of the test organisms	Laboratory culture

Information on the test design	
Methodology used	Non standard method
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Growth media
Test concentrations used	4–6 concentrations
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated (0.4 ml of algae in 4 ml media)
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static (sealed vessels)
Measurement of exposure concentrations	Not stated
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Endpoint comment	Lack of information on methods and analysis make these data questionable.
Study conducted to GLP	Not stated

Reliability of study	**Unreliable**
Relevance of study	**Relevant**
Klimisch Code	**3**

Reference number	**25**

Information on the test species	
Test species used	*Baetis harisoni*
Life stage of the test species used	Nymphs
Holding conditions prior to test	Held in filtered lake water at 20°C.
Source of the test organisms	Laboratory culture

Information on the test design	
Methodology used	Non standard method
Form of the test substance	Sodium hypochlorite
Source of the test substance	Not stated
Type and source of the exposure medium	Stream water
Test concentrations used	3–5 concentrations
Number of replicates per concentration	3-6
Number of organisms per replicate	35–90
Nature of test system (static, semi-static or flow-through, duration, feeding)	Open flow-through field exposures in artificial streams
Measurement of exposure concentrations	Measured
Measurement of water quality parameters	Dissolved oxygen and temperature
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Not stated
Endpoint comment	Endpoint appears valid but a non-standard method was used.
Study conducted to GLP	Not stated

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

Reference number	**33**

Information on the test species	
Test species used	*Anguilla anguilla* and *Dicentrarchus labrax*
Life stage of the test species used	Not stated
Holding conditions prior to test	Not stated
Source of the test organisms	Not stated

Information on the test design	
Methodology used	Not stated
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Sea water
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Flow-through
Measurement of exposure concentrations	Yes – expressed as TRO
Measurement of water quality parameters	Temperature
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Endpoint comment	Due to a lack of experimental data, this study was regarded valid with restriction.
Study conducted to GLP	Not stated

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

Reference number	**39**

Information on the test species	
Test species used	*Strongylocenttrotus droesbachiensis* and *Dendrater excentricus*
Life stage of the test species used	Eggs
Holding conditions prior to test	Seawater
Source of the test organisms	Not stated

Information on the test design	
Methodology used	Non standard
Form of the test substance	Sodium hypochlorite
Source of the test substance	Not stated
Type and source of the exposure medium	Sea water
Test concentrations used	Various
Number of replicates per concentration	Not stated
Number of organisms per replicate	5,000 eggs
Nature of test system (static, semi-static or flow-through, duration, feeding)	Not stated, but assumed static due to 5 minute exposure time
Measurement of exposure concentrations	Measured – TRO
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Yes, but some failed and were discarded.
Water quality criteria satisfied	Not stated
Endpoint comment	Due to a lack of experimental data, the validity of this study could not be assessed.
Study conducted to GLP	Not stated

Reliability of study	**Unknown**
Relevance of study	**Relevant**
Klimisch Code	**4**

Reference number	**42**

Information on the test species	
Test species used	*Solea solea*
Life stage of the test species used	Larvae
Holding conditions prior to test	Seawater
Source of the test organisms	Not stated

Information on the test design	
Methodology used	Not stated
Form of the test substance	Not stated
Source of the test substance	Not stated
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 stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Not stated
Measurement of exposure concentrations	Measured – TRO
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Endpoint comment	Due to a lack of experimental data, the validity of this study could not be assessed.
Study conducted to GLP	Not stated

Reliability of study	**Unknown**
Relevance of study	**Relevant**
Klimisch Code	**4**

Reference number	**43**

Information on the test species	
Test species used	*Pleuronectes platessa*
Life stage of the test species used	Larvae
Holding conditions prior to test	Seawater
Source of the test organisms	Not stated

Information on the test design	
Methodology used	Not stated
Form of the test substance	Not stated
Source of the test substance	Not stated
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 stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Not stated
Measurement of exposure concentrations	Measured – TRO
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Endpoint comment	Due to a lack of experimental data, the validity of this study could not be assessed.
Study conducted to GLP	Not stated

Reliability of study	**Unknown**
Relevance of study	**Relevant**
Klimisch Code	**4**

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Environment Agency
Rio House
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Almondsbury, Bristol BS32 4UD
Tel: 0870 8506506
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