

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

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

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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.

Note:

This report is an update of report Number SCHO0407BLWD-E-E 'Proposed EQSs for Water Directive Annex VIII substances: Cyanide (free)' produced in 2007 as part of a programme of work commissioned by the UK Technical Advisory Group (UKTAG) to derive Environmental Quality Standards (EQSs) for substances falling under Annex VIII of the Water Framework Directive (WFD). The original report proposed PNECs derived according to the Annex V methodology but because of a lack of certain data, large assessment factors were used in their derivation. This led to the UKTAG concluding that the values were unsuitable for use as EQSs since they were subject to excessive uncertainty, but that this uncertainty may be reduced by appropriate additional ecotoxicity testing [45]. Consequently an ecotoxicity study on the alga *Pseudokirchneriella subcapitata* (Environment Agency, 2008) was commissioned with the aim of reducing the data gap, assessment factors and ultimately the uncertainty in the PNEC values [46]. This report incorporates the results of that study and PNECs are re-visited using the more complete dataset. It should be noted that no additional review of any other data/literature that may have been published since the original 2007 report has been made.

Executive Summary

The UK Technical Advisory Group (UKTAG) has commissioned a programme of work to derive Environmental Quality Standards (EQSs) for substances falling under Annex VIII of the Water Framework Directive (WFD). This report proposes predicted no-effect concentrations (PNECs) for cyanide using the methodology described in Annex V of the Directive. There are existing EQSs for cyanide, 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 cyanide, 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.

The PNECs described in this report are based on a technical assessment of the available ecotoxicity data for cyanide, 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. This report is an update of report Number SCHO0407BLWD-E-E 'Proposed EQSs for Water Directive Annex VIII substances: Cyanide' produced in 2007 as part of a programme of work commissioned by the UK Technical Advisory Group (UKTAG) to derive Environmental Quality Standards (EQSs) for substances falling under Annex VIII of the Water Framework Directive (WFD). The original report proposed PNECs derived according to the Annex V methodology but because of a lack of certain data, large assessment factors were used in their derivation. This led to the UKTAG concluding that the values were unsuitable for use as EQSs since they were subject to excessive uncertainty, but that this uncertainty may be reduced by appropriate additional ecotoxicity testing [45]. Consequently an ecotoxicity study on the alga *Pseudokirchneriella subcapitata* (Environment Agency 2008) was commissioned with the aim of reducing the data gap, assessment factors and ultimately the uncertainty in the PNEC values [46]. This report incorporates the results of this study and PNECs are re-visited using the more complete dataset. It should be noted that no additional review of any other data/literature that may have been published since the original 2007 report has been made.

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

Cyanides are extensively used in industry and are also emitted from car exhaust fumes. They also occur ubiquitously in the environment and are found in a range of aquatic organisms such as arthropods, macrophytes, fungi and bacteria.

Volatilisation and biodegradation are important transformation processes for cyanide in ambient waters. Hydrogen cyanide can be biodegraded by acclimated microbial cultures, but is usually toxic to unacclimated microbial systems at high concentrations.

Cyanides are readily soluble in water where they exist in the free state (CN^- and HCN), as simple cyanides (e.g. NaCN), complex cyanides (organic or metal complexes) or total cyanide (all available species). Hydrogen cyanide (HCN) dissociates in water to give the free ion (CN^-) under alkaline conditions (50 per cent of both forms at pH 9.36). The CN^- ion has a half-life of 15 days in water; HCN has a tendency to volatilise from water, with a half-life measured from hours to a few days. Simple cyanides readily dissociate, as do some metal complexes (e.g. zinc and cadmium) releasing free CN^- . Other metal complexes containing cyanide are very stable with limited dissociation.

Cyanide acts as a respiratory depressant and can inhibit aerobic metabolism. Free cyanide ions can also pass through the gill membranes.

Undissociated HCN is primarily used to determine toxicity, with HCN being more toxic than CN^- . However, CN^- contributes to toxicity due to formation of HCN at pH values up to around 8. Simple cyanides readily dissociate and hydrolyse to form HCN and CN^- and, therefore, have the same toxicity as free cyanide. Therefore, only data on free cyanide are used to set the PNECs in this report.

Availability of data

Based on the physical and chemical data for cyanide, there is no evidence to suggest that its toxicity is affected by alkalinity or hardness. However, complexation with metals may reduce bioavailability.

Approximately 50 freshwater data points were available for cyanide. Acute toxicity data were located for algae, annelids, crustaceans, fish, insects, molluscs and protozoans. Chronic exposure data was less extensive and covered toxicity to algae, crustaceans, cnidaria, fish and macrophytes. The majority of the data available related to fish and crustaceans, with the former being the most represented.

A similar number of data points were located for marine organisms. Short-term data were available for algae, annelids, crustaceans, echinoderms, fish and molluscs; the majority of the data related to crustaceans and molluscs. The long-term database was smaller with data available only for algae and fish.

Derivation of PNECs

Long-term PNEC for freshwaters

Fish appear to be the most sensitive taxonomic group, followed by crustaceans.

The lowest reliable long-term data point is a 289-day LOEC value of $5.2 \mu\text{g l}^{-1}$ HCN for total inhibition of spawning in the bluegill *Lepomis macrochirus*. Good quality data are also available for algae and invertebrates. An assessment factor of 10 could be applied to this value, but as it represents a significant effects level, an increased factor of 20 is proposed (composed of the assessment factor of 10 plus an additional factor of 2 to account for the conversion of the LOEC to a NOEC), resulting in a $\text{PNEC}_{\text{freshwater_lt}}$ of $0.26 \mu\text{g l}^{-1}$ HCN.

This PNEC is lower than the existing 1998 EQS of $1 \mu\text{g l}^{-1}$ HCN. This was based on a value of ca. $10 \mu\text{g l}^{-1}$ obtained from a study on the effects of cyanide on salmonid reproduction to which an assessment factor of 10 was applied.

Short-term PNEC for freshwaters

As in the long-term studies, fish and crustaceans were found to be the most sensitive taxonomic groups.

The most sensitive, reliable result was a 96-hour LC50 of $28 \mu\text{g l}^{-1}$ HCN for rainbow trout (*Oncorhynchus mykiss*). Given that good quality data are available for both fish, crustaceans and algae and that fish are the most sensitive organisms to both long- and short-term exposures to cyanide, an assessment factor of 10 is proposed. This results in a $\text{PNEC}_{\text{freshwater_st}}$ of $2.8 \mu\text{g l}^{-1}$ HCN.

In comparison to the current EQS, the proposed PNEC is 2 times lower. The 1998 EQS of $5 \mu\text{g l}^{-1}$ HCN was based on applying an assessment factor of 10 to an LC50 of $43 \mu\text{g l}^{-1}$ HCN obtained in a study on the same species.

Long-term PNEC for saltwaters

The data suggest saltwater organisms to be of similar sensitivity to freshwater organisms with similar effect values. Given that cyanide acts as a respiratory depressant, similar effects would be expected in both environments. Because of this, the freshwater and saltwater datasets were combined.

As there were no high quality long-term saltwater data, the lowest reliable data point of the combined dataset is the 289-day LOEC value of $5.2 \mu\text{g l}^{-1}$ HCN for total inhibition of spawning in the bluegill *Lepomis macrochirus*. An assessment factor of 50 could be applied to this value because there are long-term data for three freshwater trophic levels and short-term data for echinoderms and molluscs. The short-term data indicate that whilst molluscs may be sensitive to cyanide, echinoderms are of reduced sensitivity compared to crustaceans and fish.

Therefore, given the available data in the combined dataset, a total assessment factor of 100 is proposed (including a factor of 2 to convert the LOEC to a NOEC), resulting in a $PNEC_{\text{saltwater}_{\text{lt}}}$ of $0.052 \mu\text{g l}^{-1}$ HCN.

This is considerably lower than the existing EQS of $1 \mu\text{g l}^{-1}$ HCN, which was ‘read across’ from the freshwater EQS.

Short-term PNEC for saltwaters

For the same reasons as outlined for the long-term data, the freshwater and saltwater datasets were combined for the derivation of a short-term PNEC for saltwaters. Based on the available short-term saltwater data, crustaceans appear to be the most sensitive taxonomic group.

The most sensitive and reliable datum is a 96-hour LC50 of $4.2 \mu\text{g l}^{-1}$ for larvae of the rock crab *Cancer irroratus*. This value is supported by a saltwater mollusc and fish study. Given that in the combined dataset reliable data are available for three trophic levels (algae, invertebrates including echinoderms and molluscs and fish) an assessment factor of 10 is proposed resulting in a $PNEC_{\text{saltwater}_{\text{st}}}$ of $0.42 \mu\text{g l}^{-1}$ HCN.

This is lower than the existing EQS of $5 \mu\text{g l}^{-1}$ HCN, which was ‘read across’ from the freshwater EQS.

PNEC for secondary poisoning

The available data suggest that the likelihood of free cyanide to bioaccumulate in aquatic organisms is low. Accumulation through the food chain is also not expected due to the rapid detoxification of cyanide by most organisms. Because the bioconcentration factor data for free cyanide are below 100, there is no requirement to derive a PNEC for secondary poisoning.

PNEC for sediments

Based on the low tendency of free cyanide to adsorb to particulate materials and the knowledge that the toxicity of complex cyanides in sediments is due to the release of free cyanide in the water column, a PNEC for sediments is not relevant.

Summary of proposed PNECs

Receiving medium/exposure scenario	Proposed PNEC ($\mu\text{g l}^{-1}$ HCN)	Existing EQS ($\mu\text{g l}^{-1}$ HCN)
Freshwater/long-term	0.26	1
Freshwater/short-term	2.8	5
Saltwater/long-term	0.052	1
Saltwater/short-term	0.42	5

Analysis

It is customary to distinguish between total and free cyanide because of their differences in toxicity. Free cyanide is operationally defined as those cyanide forms that are readily oxidised to cyanogen chloride by treatment with chlorine. These forms include free cyanide plus any complex forms that readily dissociate. Total cyanide is determined after treatment of the sample to convert all forms into free cyanide. In wastewaters and effluents, separation of cyanide by distillation or micro-diffusion may be required to overcome potential interferences and to enhance limits of detection by preconcentration of the sample.

The lowest proposed PNEC derived for cyanide is $0.052 \mu\text{g l}^{-1}$ HCN. From the literature, it can be seen that analytical methodologies provide detection limits of around $5\text{--}10 \mu\text{g l}^{-1}$, which suggests that they may not be adequate to analyse cyanide for compliance with the proposed PNECs.

Implementation issues

- Analytical capabilities may not be sufficient to enable analysis of free cyanide at the levels required to assess compliance
- Uncertainties associated with the long term saltwater standard could be reduced through availability of additional reliable long term saltwater studies.

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1. Introduction

The UK Technical Advisory Group (UKTAG) supporting the implementation of the Water Framework Directive (2000/60/EC)¹ is a partnership of UK environmental and conservation agencies. It also includes partners from the Republic of Ireland. UKTAG has commissioned a programme of work to derive Environmental Quality Standards (EQSs) for substances falling under Annex VIII of the Water Framework Directive (WFD). This report proposes predicted no-effect concentrations (PNECs) for cyanide using the methodology described in Annex V of the Directive. There are existing EQSs for cyanide, 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 cyanide, along with any data that relate impacts under field conditions to exposure concentrations. The data have been subjected to rigorous quality assessment such that decisions are based only on scientifically sound data.² Following consultation with an independent peer review group, critical data have been identified and assessment factors selected in accordance with the guidance given in Annex V. The feasibility of implementing these PNECs as EQSs has not been considered at this stage. However, this would be an essential step before a regulatory EQS can be recommended.

1.1 Properties and fate in water

Cyanides are used extensively in industry and are also emitted from car exhaust fumes. They also occur ubiquitously in the environment and are found in a range of aquatic organisms such as arthropods, macrophytes, fungi and bacteria. Volatilisation and biodegradation are important transformation processes for cyanide in ambient waters. Hydrogen cyanide can be biodegraded by acclimated microbial cultures, but is usually toxic at high concentrations to unacclimated microbial systems.

Cyanides are readily soluble in water where they exist in the free state (CN^- and HCN), as simple cyanides (e.g. NaCN), complex cyanides (organic or metal complexes) or total cyanide (all available species). Hydrogen cyanide (HCN) dissociates in water to give the free ion (CN^-) under alkaline conditions (50 per cent of both forms at pH 9.36). The CN^- ion has a half-life of 15 days in water; HCN has a tendency to volatilise from water, with a half-life measured from hours to a few days. Simple cyanides readily dissociate, as do some metal complexes (e.g. zinc and cadmium) releasing free CN^- . Other metal complexes containing cyanide are very stable with limited dissociation.

Cyanide acts as a respiratory depressant and can inhibit aerobic metabolism. Free cyanide ions can also pass through the gill membranes.

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

² Data quality assessment sheets are provided in Annex 1.

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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
Hydrogen cyanide (HCN)	74-90-8

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 [44], and existing EQSs obtained from the literature [11].

Section 2.6 summarises the effects data identified from the literature for cyanide. 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 [free cyanide (HCN and CN⁻)]

PNEC	TGD deterministic approach (AFs) ($\mu\text{g l}^{-1}$)	TGD probabilistic approach (SSDs) ($\mu\text{g l}^{-1}$)	Existing EQS ($\mu\text{g l}^{-1}$)
Freshwater short-term	2.8	Insufficient data	5.0 (MAC)
Freshwater long-term	0.26	Insufficient data	1.0 (AA)
Saltwater short-term	0.42	Insufficient data	5.0 (MAC)
Saltwater long-term	0.052	Insufficient data	1.0 (AA)
Freshwater sediment short-term	NR	NA	NA
Freshwater sediment long-term	NR	Insufficient data	NA
Saltwater sediment short-term	NR	NA	NA
Saltwater sediment long-term	NR	Insufficient data	NA
Secondary poisoning	NR	NA	NA

AA = annual average

AF = assessment factor

MAC = maximum allowable concentration

NA = not applicable

NR = not required

SSD = species sensitivity distribution

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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 for HCN	Reference
T+; R26/27/28 - N; R50-53	
R26/27/28: Very toxic by inhalation, in contact with skin and if swallowed.	[3]
R50/53: Very toxic to aquatic organisms; may cause long-term adverse effects in the aquatic environment.	

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 hydrogen cyanide

Property	Value	Reference
Molecular formula	HCN	
Molecular structure	$\text{N} \equiv \text{C} - \text{H}$	
Molecular weight	27.03	[1]
Composition	C: 44.44; H: 3.73; N: 51.83	[1]
Appearance	Colourless gas or liquid	[1]
Melting point (°C)	-13.4	[1]
Boiling point (°C)	25.6	[1]
Vapour pressure	620 mmHg at 20°C	[2]
Henry's Law constant	1.33×10^{-4} atm-m ³ /mol at 25°C	[3]
Water solubility (mg l ⁻¹)	1,000,000 at 25°C	[3]
Dissociation constant (pKa)	9.36	[3]
Octanol-water partition coefficient (log Kow)	0.35-1.07	[3]

2.5 Environmental fate and partitioning

Cyanide exists in water in the 'free' state (CN⁻ and HCN), as 'simple' cyanides such as sodium cyanide (that readily dissociate), 'complex' cyanides such as organic or metal complexes (that dissociate at a slower rate) or 'total' cyanide representing all available species. Hydrogen cyanide dissociates in water according to the following equation:



Based on its pKa of 9.36, 50 per cent of the cyanide will be present as CN⁻ and 50 per cent as the undissociated molecule (HCN) at a pH of 9.36. Below pH 7, almost all cyanide will be present in the undissociated form (HCN) and, above a pH value of approximately 11, all of the cyanide will be present as the free ion (CN⁻) [11].

Assuming no chemical reactions occur in water (e.g. complexation with metal ions), at lower pH values the fully associated HCN molecule will show a tendency to volatilise from water, with a half-life measured from hours to a few days (see Table 2.5).

However, the aquatic fate of cyanide is complicated by its tendency to complex with metal ions. The complexes of iron and nickel with cyanide are very stable, with only limited dissociation of the complex to free cyanide in water. Other complexes such as zinc and cadmium readily dissociate, releasing free cyanide ions and possibly increasing the potential for toxicity. The dissociation of cyanide from the iron cyanide complex Fe(CN)₆ can occur via photolysis. The degree to which this occurs depends on the exposure of the complex to sunlight; hence, conditions such as water depth, suspended solid concentration and season may affect the photolytic process considerably.

Toxicity of cyanide is determined primarily by the concentration of undissociated HCN in the water column (even in the case of simple cyanides and metal cyanide complexes) [11]. Hydrocyanic acid (HCN) is also more toxic than the free cyanide (CN⁻). However, as pH increases (up to around pH 8) the cyanide ion increasingly contributes to toxicity due to the formation of HCN [14]. Simple cyanides, such as sodium cyanide, readily dissociate and hydrolyse to form HCN and CN⁻ and consequently have similar toxicities to the free form. The toxicity of metal cyanide complexes depends on the rate of dissociation to form free cyanide [11].

The solubility of cyanide in water, combined with its dissociation at higher pH values, means that the tendency for free cyanide (CN⁻ and HCN) to adsorb to particulate material is low, as is its likelihood to bioaccumulate.

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

Table 2.5 Environmental fate and partitioning of cyanide

Property	Value	Reference
Abiotic fate	If released into water, hydrogen cyanide is not expected to adsorb to suspended solids and sediment.	[4]
	The cyanide ion has a half-life of 15 days in water and is not thought of as persistent in the environment.	[5]
	Oxidation, hydrolysis and photolysis are the three predominant chemical processes that cause loss of simple cyanides in aquatic media.	[6]
Volatilisation	Volatilisation from water surfaces is expected to be an important fate process based upon the Henry's Law constant of 1.33×10^4	[7]

Property	Value	Reference
Photolysis	atm-m ³ /mol.	
	At pH <9.2, most free cyanide should exist as hydrogen cyanide, a volatile form of cyanide. A half-life for this process cannot be determined due to lack of data. Hydrogen cyanide will readily volatilise from water.	[6]
	Estimated volatilisation half-lives for a model river and model lake are 3 hours and 3 days, respectively.	[8]
	Although the significance of hydrolysis in the fate of cyanides in water has not been fully investigated, hydrogen cyanide and cyanide ions have been found to be very resistant to photolysis by natural sunlight except under heterogeneous photocatalytic conditions. However, photocatalytic oxidation may not be significant in natural waters due to light reduction at increasing depth.	[6]
	<p>The extent of photolysis is dependant on several factors including:</p> <ul style="list-style-type: none"> • the degree of illumination • time of day and year • latitude • depth and turbidity of the water. <p>In deep, turbid and shaded waters, photolysis can be assumed to be negligible.</p> <p>Hydrolysis rates of hydrogen cyanide in acidic solution and cyanides under alkaline conditions indicate that hydrolysis is not competitive with volatilisation and biodegradation for removal of free cyanide from ambient waters.</p>	[9]

Property	Value	Reference
Biodegradation	Biodegradation is an important transformation process for cyanide in natural surface waters and is dependent on such factors as cyanide concentrations, pH, temperature, availability of nutrients and acclimation of microbes.	[6]
	Hydrogen cyanide can be biodegraded by acclimated microbial cultures, but is usually toxic at high concentrations to unacclimated microbial systems.	[10]
	Hydrogen cyanide can be biodegraded in the aquatic environment by a variety of organisms, including bacteria and fungi. Cyanide can be biodegraded by bacteria such as <i>Pseudomonas putida</i> , which has been shown to degrade >99% of sodium cyanide (400 mg l ⁻¹ CN ⁻ added) to ammonia and carbon dioxide after 7 days. Fungi also biodegraded a 1.3 mg l ⁻¹ CN ⁻ test solution of potassium cyanide by 45% in 3 days.	[11]
	During biological sewage treatment, volatilisation may account for around 15% of total cyanide removal and is potentially a major removal process.	[10]
Partition coefficients (log Kow)	0.35–1.07	[3]
	0.66	[6]
Bioaccumulation BCF	Estimated bioconcentration factor (BCF) of 3 from log Kow and a regression-derived equation suggests the potential for bioconcentration in aquatic organisms is low.	[8, 12]
	Experimental BCF values for rainbow trout range from 1.69–4.12.	[13]

2.6 Effects data

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

Data collation followed a tiered approach. First, critical freshwater and saltwater data were compiled from existing EQS documents. Further data published after derivation of the current UK EQS were then retrieved from the US Environmental Protection Agency (US EPA) ECOTOX database.³

As data on sediment-dwelling organisms and mammalian or avian chronic oral toxicity are not usually available in ECOTOX, further data were sought from ScienceDirect®⁴ and Web of Science®.⁵

³ <http://www.epa.gov/ecotox/>

⁴ <http://www.sciencedirect.com/>

In addition, data were also sought from:

- World Health Organization (WHO) *Concise International Chemical Assessment Document (CICAD) 61. Hydrogen Cyanide and Cyanides: Human Health Aspects* [41];
- US EPA Integrated Risk Information System (IRIS) database [39];
- Hazardous Substances Data Bank (HSDB®) database of the US National Library of Medicine [43].

Toxicity data for cyanide in sediments were not found.

The toxicity of cyanide in water is largely determined by the concentration of undissociated molecular hydrocyanic acid (HCN) [11]. Although molecular HCN is more toxic than the CN⁻ ion, the latter also contributes to total toxicity, especially as pH increases. Toxic concentrations of cyanide are, therefore, best expressed as free cyanide (HCN and CN⁻). Simple cyanides such as potassium cyanide also readily dissociate and hydrolyse to form HCN and CN⁻, and therefore have the same toxicity as free cyanide. For these reasons only data on free cyanide were reviewed in the UK EQS report [11] and the same approach has been taken in this report.

Based on the physical and chemical data for cyanide, there is no evidence to suggest that cyanide toxicity is affected by alkalinity or hardness [11]. However, complexation with metals may reduce bioavailability [11].

2.6.1 Toxicity to freshwater organisms

Approximately 50 freshwater data points were available for cyanide. Approximately 35 data points were available for short-term exposures, with fish and crustaceans making up the bulk of the short-term database. A smaller long-term database was available, with fish and crustaceans again making up the bulk of the data.

Diagrammatic representations of the available freshwater data (cumulative distribution functions) for cyanide 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 cyanide PNECs. The lowest critical freshwater data for cyanide are presented in Tables 2.6 and 2.7.

⁵ <http://scientific.thomson.com/products/wos/>

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Figure 2.1 Cumulative distribution function of freshwater long-term data ($\mu\text{g l}^{-1}$) for cyanide

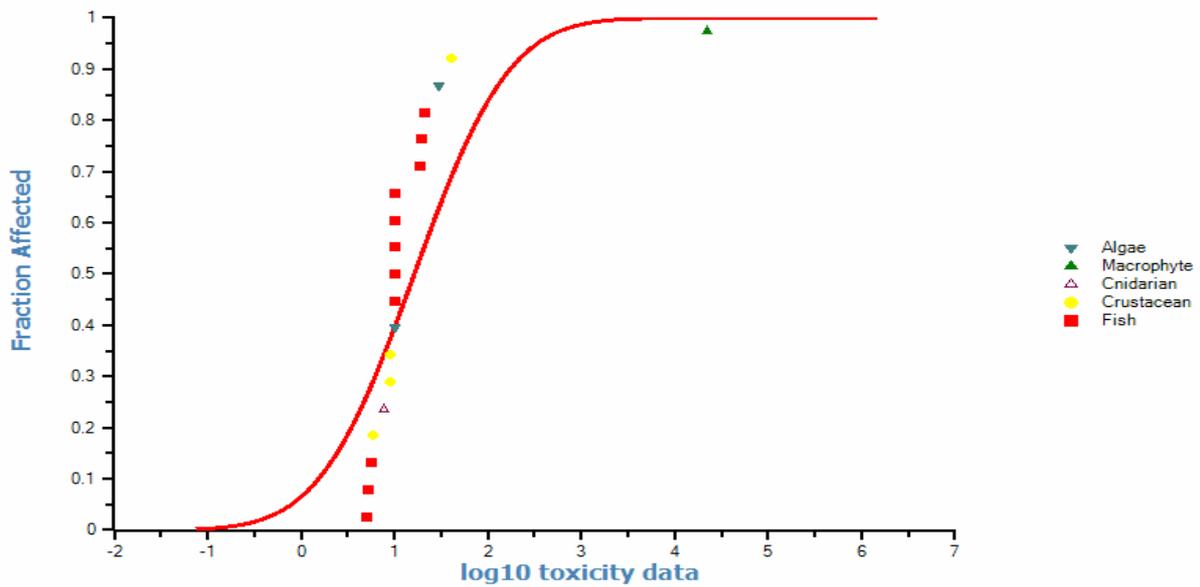


Figure 2.2 Cumulative distribution function of freshwater short-term data ($\mu\text{g l}^{-1}$) for cyanide

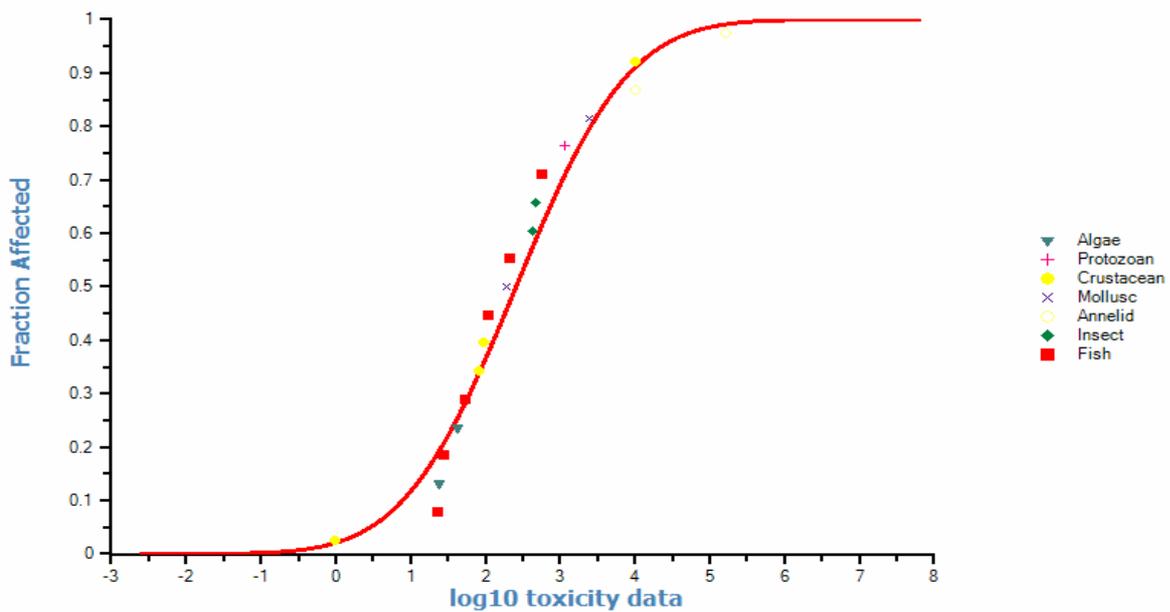


Table 2.6 Most sensitive long-term aquatic toxicity data for freshwater organisms exposed to cyanide

Test substance	Species	Taxonomic group	Endpoint	Effect	Test duration (days)	Conc. ($\mu\text{g l}^{-1}$ total cyanide)	Comments	Reliability index ¹	Reference
Potassium cyanide	<i>Pseudokirchneriella subcapitata</i>	Algae	NOEC	Growth (growth rate and biomass)	3	10 * (measured)	22 ± 1 °C	2	[45]
Potassium cyanide	<i>Scenedesmus quadricauda</i>	Algae	LOEC	Population growth	7	30 (nominal)	ND	3	[15]
Sodium cyanide	<i>Hydra viridissima</i>	Cnidaria	NOEC	Population growth	6	200 (nominal)* 110 (measured)*	30°C	2	[16]
Sodium cyanide	<i>Moinodaphnia macleayi</i>	Crustaceans	NOEC	Reproduction	5	20 (nominal)* 9.6 (measured)*	30°C	2	[16]
Sodium cyanide	<i>Moinodaphnia macleayi</i>	Crustaceans	LOEC	Reproduction	5	67 (nominal)* 34 (measured)*	30°C	2	[16]
Hydrogen cyanide	<i>Asellus communis</i>	Crustaceans	NOEC LOEC	Growth	98	41* (measured) 55* (measured)	pH 7–7.9; 18°C. Exposed along with <i>Gammarus</i>	3	[17]
Hydrogen cyanide	<i>Gammarus pseudolimnaeus</i>	Crustaceans	NOEC LOEC	Growth	98	4* (measured) 9* (measured)	pH 7–7.9; 18°C. Exposed along with <i>Asellus</i>	3	[17]
Hydrogen cyanide	<i>Asellus communis</i>	Crustaceans	NOEC LOEC	Growth	112	29* (measured) 40* (measured)	pH 7–7.9; 18°C. Exposed alone	2	[17]
Hydrogen cyanide	<i>Gammarus pseudolimnaeus</i>	Crustaceans	NOEC LOEC	Growth	83	21* (measured) 32* (measured)	pH 7–7.9; 18°C. Exposed alone	2	[17]
Sodium cyanide	<i>Lepomis macrochirus</i>	Fish	Complete inhibition of spawning	Reproduction	289	5.2* (measured)	pH 8.1; 24.9°C	2	[18]
Hydrogen cyanide	<i>Oncorhynchus mykiss</i>	Fish	LOEC	Growth	20	5 (nominal)	pH 7.9; 6°C; hardness 127 mg l ⁻¹ CaCO ₃	3	[19]

Proposed EQS for Water Framework Directive Annex VIII substances: cyanide (free) (For consultation)

Test substance	Species	Taxonomic group	Endpoint	Effect	Test duration (days)	Conc. ($\mu\text{g l}^{-1}$ total cyanide)	Comments	Reliability index ¹	Reference
Hydrogen cyanide	<i>Oncorhynchus mykiss</i>	Fish	Reduction in growth	Growth rate	18	10 (measured)	pH 7.9; 12.5°C; hardness 127 mg l^{-1} CaCO_3	3	[20]
Potassium cyanide	<i>Oncorhynchus mykiss</i>	Fish	NOEC	Growth rate	20	10* (measured)	6°C; hardness 127 mg l^{-1} CaCO_3	2	[21]
Hydrogen cyanide	<i>Oncorhynchus mykiss</i>	Fish	LOEC	Growth rate	20	15* (measured)	6°C; hardness 127 mg l^{-1} CaCO_3	2	[21]
Hydrogen cyanide	<i>Salvelinus fontinalis</i>	Fish	NOEC	Egg production	234	5.7* (measured)	9-15°C; alkalinity 236-239 mg l^{-1} CaCO_3	2	[48]

¹ See Annex 1. * Expressed as free cyanide (HCN). Free cyanide is operationally defined as those cyanide forms that are readily oxidised to cyanogen chloride by treatment with chlorine. These forms include free cyanide plus any complex forms that readily dissociate. Total cyanide is determined after treatment of the sample to convert all forms into free cyanide. In wastewaters and effluents, separation of cyanide by distillation or micro-diffusion may be required to overcome potential interferences and to enhance limits of detection by pre-concentration of the sample.

LOEC = lowest observed effect concentration; NOEC = no observed effect concentration; ND = no data

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

Test substance	Species	Taxonomic group	Endpoint	Effect	Test duration	Conc. ($\mu\text{g l}^{-1}$ total cyanide)	Comments	Reliability index ¹	Reference
Sodium cyanide	<i>Chlorococcales</i>	Algae	EC10	Physiology	24 hours	24* (ND)	ND	4	[22]
Free cyanide	<i>Ankistrodesmus falcatus</i>	Algae	EC50	ND	10 days	1250* (ND)	ND	4	[23]
Potassium cyanide	<i>Pseudokirchneriella subcapitata</i>	Algae	NOEC	Growth (growth rate) Growth (biomass)	72 hours 72 hours	42 * (measured) 17 * (measured)	22 \pm 1 °C 22 \pm 1 °C	2 2	[45]
Potassium cyanide	<i>Spirostomum ambiguum</i>	Protozoans	EC50	Development	48 hours	1180 (nominal)	pH 7.4; 25°C; hardness 2.8 mg l ⁻¹ CaCO ₃	3	[24]
Potassium cyanide	<i>Daphnia pulex</i>	Crustaceans	LC50	Mortality	48 hours	1 (nominal)	pH 8.5; 25°C	3	[25]
Potassium cyanide	<i>Ceriodaphnia dubia</i>	Crustaceans	EC50	Feeding rate	1 hour	94 (nominal)	25°C	3	[26]
Ammonium thiocyanate	<i>Moina micrura</i>	Crustaceans	LC50	Mortality	96 hours	15,460 (nominal)	pH 7; 20°C; hardness 105 mg l ⁻¹ CaCO ₃	3	[27]
Ammonium thiocyanate	<i>Branchiura sowerbyi</i>	Annelids	LC50	Mortality	96 hours	166,880 (nominal)	pH 7; 28°C; hardness 105 mg l ⁻¹ CaCO ₃	3	[27]
Hydrogen cyanide	<i>Asellus communis</i>	Crustaceans	LC50	Mortality	96 hours	2,295* (measured)	pH 7–7.9; 18°C. Exposed alone	2	[17]
Hydrogen cyanide	<i>Gammarus pseudolimnaeus</i>	Crustaceans	LC50	Mortality	96 hours	169* (measured)	pH 7–7.9; 18°C. Exposed alone	2	[17]
Potassium cyanide	<i>Salmo salar</i>	Fish	LC50	Mortality	24 hours	23 (22*) (measured)	pH 8; 11.8°C; DO = 3.5 mg l ⁻¹	2	[28]
Potassium cyanide	<i>Oncorhynchus mykiss</i>	Fish	LC50	Mortality	96 hours	43* (measured)	pH 7.34; 12°C; hardness 127 mg l ⁻¹ CaCO ₃	2	[29]
Unknown form	<i>Oncorhynchus mykiss</i>	Fish	LC50	Mortality	96 hours	28* (measured)	pH 8.0; 6°C; hardness 127 mg l ⁻¹ CaCO ₃	2	[30]

Proposed EQS for Water Framework Directive Annex VIII substances: cyanide (free) (For consultation)

¹ See Annex 1. * Expressed as free cyanide (HCN) – See Table 2.6.

ECx = concentration effective against X% of the organisms tested; LC50 = concentration lethal to 50% of the organisms tested

ND = no data; DO = dissolved oxygen

2.6.2 Toxicity to saltwater organisms

Approximately 50 saltwater data points were available for cyanide. Approximately 30 data points were available for short-term exposures, with crustaceans and molluscs making up the bulk of the short-term database, though one data point was available for echinoderms. A much smaller long-term database was available with data only for algae and one data point for fish.

A preliminary analysis of the relative sensitivity of the species tested (i.e. not taking into account the quality of the available studies) indicates that crustacean and molluscs are most sensitive to acute exposures.

Diagrammatic representations of the available saltwater data (cumulative distribution functions) for cyanide 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 cyanide PNECs. The lowest critical saltwater data for cyanide are presented in Tables 2.8 and 2.9.

Figure 2.3 Cumulative distribution function of saltwater long-term data ($\mu\text{g l}^{-1}$) for cyanide

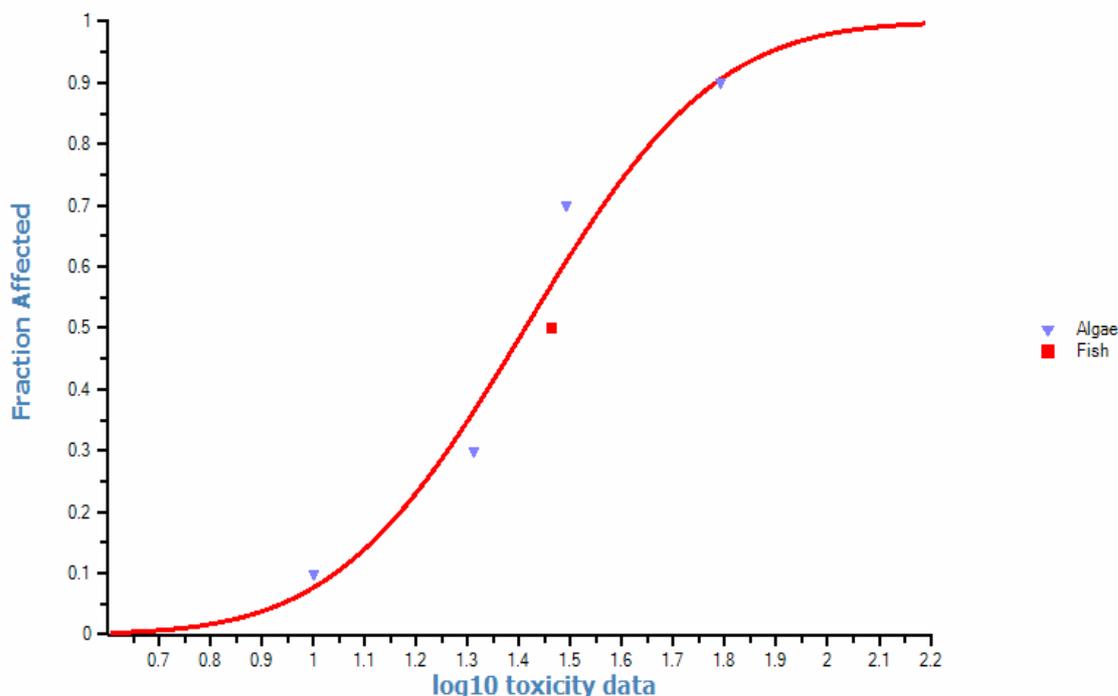


Figure 2.4 Cumulative distribution function of saltwater short-term data ($\mu\text{g l}^{-1}$) for cyanide

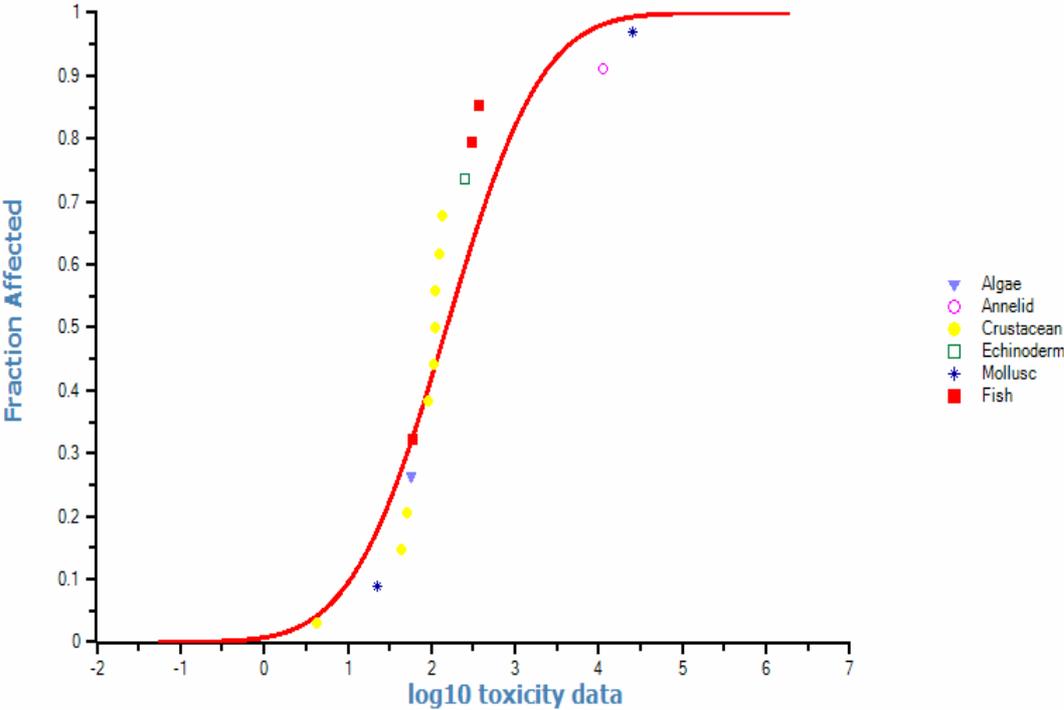


Table 2.8 Most sensitive long-term aquatic toxicity data for saltwater organisms exposed to cyanide

Test substance	Species	Taxonomic group	Endpoint	Effect	Test duration	Conc. ($\mu\text{g l}^{-1}$ total cyanide)	Comments	Reliability index ¹	Reference
Sodium cyanide	<i>Nitzschia closterium</i>	Algae	LOEC	Population growth	72 hours	10 (measured)	pH 8; 18°C	2	[31]
Sodium cyanide	<i>Nitzschia closterium</i>	Algae	LOEC	Population growth	72 hours	62 (measured)	pH 8; 21°C	2	[31]
Sodium cyanide	<i>Nitzschia closterium</i>	Algae	NOEC	Population growth	72 hours	31 (measured)	pH 8; 21°C	2	[31]
Potassium cyanide	<i>Champia parvula</i>	Algae	MATC	Reproduction	14 days	20.5 (measured)	21°C; salinity 30 ppt	2	[32]
Unknown form	<i>Cyprinodon variegatus</i>	Fish	No effect	Survival	Chronic ELS	29* (ND)	ND	4	[33]

¹ See Annex 1.

* Expressed as free cyanide (HCN) – see Table 2.6.

ELS = early life stage

LOEC = lowest observed effect concentration

NOEC = no observed effect concentration

MATC = maximum allowable toxicant concentration

ND = no data

ppt = parts per trillion

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

Test substance	Species	Taxonomic group	Endpoint	Effect	Duration	Conc. ($\mu\text{g l}^{-1}$ total cyanide)	Comments	Reliability index ¹	Reference
Sodium cyanide	<i>Nitzschia closterium</i>	Algae	EC50	Population growth	72 hours	57 (measured)	pH 8; 21°C	2	[31]
Sodium cyanide	<i>Dinophilus gyrociliatus</i>	Annelid	LC50	Mortality	24 hours	11446 (ND)	21°C	4	[49]
Potassium cyanide	<i>Cancer irroratus</i>	Crustaceans	LC50	Mortality	96 hours	4.2* (measured)	20°C; salinity 30 ppt	2	[34]
Unknown form	<i>Cancer irroratus</i>	Crustaceans	LC50	Mortality	96 hours	44* (measured)	ND	3	[37]
Sodium cyanide	<i>Cancer productus</i>	Crustaceans	LC50	Mortality	96 hours	107 (measured)	pH 7.7; 10°C; salinity 28 ppt	1	[35]
Sodium cyanide	<i>Cancer magister</i>	Crustaceans	LC50	Mortality	96 hours	51 (measured)	pH 7.7; 10°C; salinity 28 ppt	1	[35]
Potassium cyanide	<i>Anthocidaris crassispira</i>	Echinoderm	LOEC	Development	12 hours	250 (ND)	28°C	4	[47]
Sodium cyanide	<i>Chlamys asperrima</i>	Molluscs	EC50	Development	48 hours	28 (22.4*) (measured)	pH 8; 18°C; salinity 31.6 ppt	1	[36]
Sodium cyanide	<i>Chlamys asperrima</i>	Molluscs	LOEC	Development	48 hours	10.5 (8.4*) (measured)	pH 8; 18°C; salinity 31.6 ppt	1	[36]
Unknown form	<i>Cyprinodon variegatus</i>	Fish	LC50	Mortality	Acute	300* (measured)	ND	4	[33]
Unknown form	<i>Menidia menidia</i>	Fish	LC50	Mortality	Acute	59* (measured)	ND	4	[33]
Unknown form	<i>Pseudopleuronectes americanus</i>	Fish	LC50	Mortality	Acute	372* (nominal)	ND	4	[33]

¹ See Annex 1. * Expressed as free cyanide (HCN) – see Table 2.6.

EC50 = concentration effective against 50% of the organisms tested; LC50 = concentration lethal to 50% of the organisms tested

LOEC = lowest observed effect concentration; NOEC = no observed effect concentration

ND = no data; ppt = parts per trillion

2.6.3 Toxicity to sediment-dwelling organisms

No sediment toxicity data for cyanide were found.

2.6.4 Endocrine-disrupting effects

No information was found to suggest that cyanide has endocrine-disrupting effects.

2.6.5 Mode of action of cyanide

Cyanide acts as a respiratory depressant and can inhibit aerobic metabolism by irreversibly binding to the haem group of cytochrome oxidase [11]. Free cyanide ions can also pass through the gill membrane causing biochemical disturbances, possibly resulting in tissue damage and nervous system effects [11]. This may result in sublethal effects such as erratic or lethargic behaviour, impairment of swimming and effects on metabolism [11].

Sublethally, cyanide has also been shown to increase brain dopamine levels in fish. This inhibits the production of gonadotrophins, which may result in negative effects on fish reproduction [11].

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

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

3.1.1 PNECs for freshwaters

PNEC for deriving an annual average concentration

Long-term toxicity data were located for algae, cnidaria, crustaceans, fish and macrophytes. Fish appear to be the most sensitive species, followed by crustaceans.

Only one long-term algal data point could be located for cyanide in the original dataset. A LOEC of $30 \mu\text{g l}^{-1}$ total CN was reported for the algae *Scenedesmus quadricauda* after a 7-day exposure [15]. However, this value was based on nominal data for total cyanide. In addition, few data were available with which to assess the quality of the study and so it was not included in the PNEC derivation. Therefore, the Environment Agency commissioned a study of the effects of potassium cyanide on *Pseudokirchneriella subcapitata* [45]. The 72-hour study was carried out to OECD Guideline 201 and involved analytical confirmation of the exposure concentrations. The study reported 72-hour NOEC values of $10 \mu\text{g l}^{-1}$ HCN for effects on growth as measured using both growth rate and biomass endpoints.

One of the lowest reported long-term values for crustaceans was a 5-day NOEC of $9.6 \mu\text{g l}^{-1}$ HCN (LOEC $34 \mu\text{g l}^{-1}$ HCN) for reproduction in the crustacean *Moinodaphnia macleayi* [16]. Effects were based on measured free cyanide concentrations.

The lowest crustacean value was a 98-day NOEC (growth) in *Gammarus pseudolimnaeus* of $4 \mu\text{g l}^{-1}$ HCN (LOEC = $9 \mu\text{g l}^{-1}$ HCN). However, the organisms were exposed in conjunction with *Asellus communis* [17]. Consequently, this low effect concentration could be caused in part by competition and so this value has been used as supporting information only [17]. For the same reason, the 98-day NOEC in *Asellus communis* of $41 \mu\text{g l}^{-1}$ HCN is also used only as supporting information [17]. However, in the same study tests were also carried out on the two species independently. These tests resulted in a 112-day NOEC of $29 \mu\text{g l}^{-1}$ HCN in *Asellus communis* and an 83-day NOEC of $21 \mu\text{g l}^{-1}$ HCN in *Gammarus pseudolimnaeus*. In both tests, good details of the test methodology and concentration measurements were given, making these NOECs suitable for PNEC derivation.

The only other long-term invertebrate data point available was a 6-day NOEC of $110 \mu\text{g l}^{-1}$ HCN for population growth in the cnidarian *Hydra viridissima* [16]. Effects were based on measured free cyanide concentrations.

Three long-term effect values were reported at $\sim 5 \mu\text{g l}^{-1}$ for fish. The first was a 20-day LOEC (growth) in rainbow trout of $5 \mu\text{g l}^{-1}$ total CN [19]. However, this value was based on nominal concentrations and so was not used in the derivation of the PNEC.

The second was a 289-day LOEC value for total inhibition of spawning in the bluegill (*Lepomis macrochirus*) at $5.2 \mu\text{g l}^{-1}$ HCN, which was also the lowest concentration tested [18]. This was a well-documented study with measured concentrations of free cyanide and calculation of molecular cyanide using the measured pH and temperature. As only an unbounded LOEC was available in the study it is necessary to estimate a NOEC from the available data and this requires the use of an appropriate conversion factor. The TGD specifies that a factor of 2 can be used where the observed effects at the LOEC are $\leq 40\%$. However, in the bluegill study the LOEC reflects total inhibition of spawning and on this basis a factor greater than 2 could be applied. In contrast, a 20-day study in the rainbow trout reported a NOEC (growth rate) of $10 \mu\text{g l}^{-1}$ HCN and a corresponding LOEC of $15 \mu\text{g l}^{-1}$ HCN based on measured concentrations of HCN [21]. This study indicates that long-term studies in fish may have a steep concentration-response curve and that a conversion factor of 2 may be appropriate to convert the LOEC in the bluegill *Lepomis macrochirus* study.

In the third study a NOEC of $5.7 \mu\text{g l}^{-1}$ HCN was reported for egg production in brook trout *Salvelinus fontinalis* [48]. The study evaluated the effects of continuous exposure on survival, growth and reproduction. The studies were started with adults 144 days prior to spawning and were continued through a 90-day growth period of the second generation. Temperature, pH and free cyanide concentrations were measured.

The most sensitive and reliable long-term toxicity value for deriving an annual average quality standard is, therefore, a 289-day LOEC value of $5.2 \mu\text{g l}^{-1}$ HCN for total inhibition of spawning in the bluegill *Lepomis macrochirus* [18]. Good quality long-term data were also available for algae and invertebrates. An assessment factor of 10 (as defined in the TGD [44]) could be applied to this value, but because the datum represents a significant effects level, an increased factor of 20 (composed of the assessment factor of 10 plus an additional factor of 2 to account for the conversion of the LOEC to a NOEC) is proposed:

$$\text{PNEC}_{\text{freshwater_It}} = 5.2 \mu\text{g l}^{-1} \text{ HCN/AF (20)} = 0.26 \mu\text{g l}^{-1} \text{ HCN}$$

PNEC for deriving a maximum allowable concentration

Short-term toxicity data were located for algae, annelids, crustaceans, fish, insects, molluscs and protozoans. Fish and crustaceans appear to be the most sensitive species.

Only two data points were available for algae exposed to free cyanide (HCN) in the original dataset. An EC10 of $24 \mu\text{g l}^{-1}$ HCN was reported for *Chlorococcales* algae after a 24-hour exposure [22]. This value was derived from a static, open test with no details of chemical analysis. These data are deemed unreliable and were not used in the PNEC derivation. In addition, a 10-day EC50 of $1,250 \mu\text{g l}^{-1}$ HCN was reported for the algae *Ankistrodesmus falcatus* [23]. However, the limited information available raised questions

concerning the quality of this study. Therefore, the Environment Agency commissioned a study of the effects of potassium cyanide on *Pseudokirchneriella subcapitata* [45]. The 72-hour study was carried out to OECD Guideline 201 and involved analytical confirmation of the exposure concentrations. The study reported 72-hour EC50 values of 42 µg l⁻¹ HCH for effects on growth as measured using the growth rate endpoint and 17 µg l⁻¹ HCH for effects on growth as measured using the biomass endpoint. In keeping with the TGD the EC50 based on growth rate rather than biomass has been used in the derivation of the PNEC.

On first examination, crustaceans appear to be the most sensitive organisms to acute exposures of cyanide. The lowest short-term invertebrate value is a 48-hour LC50 of 1.0 µg l⁻¹ total CN for the water flea *Daphnia pulex* [25]. However, in this study test organisms were less sensitive at higher cyanide concentrations. Consequently, this value is likely to be an anomaly, which is an assumption also made in the cyanide UK EQS report [11]. The next most sensitive invertebrate result was a 1-hour EC50 (feeding rate) of 94 µg l⁻¹ total CN in *Ceriodaphnia dubia* [26]. However, this value was based on nominal concentrations and so was not used in the PNEC derivation.

The lowest good quality data point for crustaceans was a 96-hour LC50 of 169 µg l⁻¹ HCN in *Gammarus pseudolimnaeus* [17]. In addition, a 96-hour LC50 of 2,295 µg l⁻¹ HCN for *Asellus communis* was reported in the same study. This was a well-documented study with measured exposure concentrations and, as such, would be regarded as suitable for PNEC derivation.

The most sensitive short-term fish value is a 24-hour LC50 of 23 µg l⁻¹ total CN (22 µg l⁻¹ HCN) for salmon, *Salmo salar*, though this was generated at a low dissolved oxygen (DO) concentration (3.5 mg l⁻¹) [28]. Tests run at higher DO levels indicated lower toxicity with 24-hour LC50s of 36, 52 and 73 µg l⁻¹ total CN at DO levels of 5, 7 and 10 mg l⁻¹, respectively. Consequently, the lowest LC50 of 23 µg l⁻¹ total CN is likely to have been the result of the combined effects of cyanide and oxygen stress, and so it has not been used to generate the PNEC. The next most sensitive result is a 96-hour LC50 for rainbow trout *Oncorhynchus mykiss* of 28 µg l⁻¹ HCN [30]. This was a well-documented study with measured concentrations of HCN.

The most sensitive and reliable short-term toxicity value for deriving a maximum allowable concentration is therefore a 96-hour LC50 of 28.0 µg l⁻¹ HCN for rainbow trout *Oncorhynchus mykiss* [21]. Good quality data are available for algae, crustaceans and fish. In addition, fish are the most sensitive organisms to both long-term and short-term exposures of cyanide. Consequently, a reduced assessment factor of 10 is proposed, with the lowest LC50 resulting in the following short-term PNEC:

$$\text{PNEC}_{\text{freshwater_st}} = 28.0 \mu\text{g l}^{-1} \text{ HCN/AF (10)} = 2.8 \mu\text{g l}^{-1} \text{ HCN}$$

3.1.2 PNECs for saltwaters

The database for saltwater toxicity was smaller than that for freshwater, but all relevant trophic levels were represented. The available data suggest that saltwater organisms are of similar sensitivity to freshwater organisms, with similar effects values in both environments (Tables 2.6–2.9). Given the mode of action of cyanide as a respiratory

depressant, similar effects on organisms would be expected in both environments. Based on these factors, the freshwater and saltwater data were combined.

PNEC for deriving an annual average concentration

Long-term saltwater data were available for algae and fish only.

The most sensitive long-term algal value is an unbounded 72-hour population growth LOEC of $10 \mu\text{g l}^{-1}$ total CN for the diatom *Nitzschia closterium* [31]. This is a well-documented study with daily measurement of total cyanide concentrations. This value is also supported by a 14-day maximum allowable toxicant concentration (MATC) of $20.5 \mu\text{g total CN l}^{-1}$ for the red alga *Champia parvula* [32]. The results of this study were also based on measured concentrations and the study was conducted in a semi-static regime. These data have been used as supporting information in the derivation of the PNEC.

No long-term data were found for saltwater invertebrates but short-term data are available for echinoderms and molluscs.

Only one long-term study could be located for a saltwater fish. Sheepshead minnow *Cyprinodon variegatus* early life stages exposed to cyanide suffered no mortality at $29 \mu\text{g l}^{-1}$ HCN [33]. However, there was very limited information with which to assess the quality of this study. Therefore, it can be used only to provide an indication of the sensitivity of marine fish to chronic exposures of cyanide.

Because there are no high quality long-term saltwater data for free cyanide, the saltwater PNEC is based on the combined saltwater and freshwater datasets. The lowest reliable data point in the combined dataset is a 289-day LOEC value of $5.2 \mu\text{g l}^{-1}$ HCN for total inhibition of spawning in the bluegill *Lepomis macrochirus* [18]. As only an unbounded LOEC was available in the study it is necessary to estimate a NOEC from the available data and this requires the use of an appropriate conversion factor. The TGD specifies that a factor of 2 can be used where the observed effects at the LOEC are $\leq 40\%$. However, in the bluegill study the LOEC reflects total inhibition of spawning and on this basis a factor greater than 2 could be applied. However, the available evidence from another chronic fish study (see Section 3.1.1) is that there is a steep concentration-response curve so that a factor of 2 should be appropriate to convert the LOEC to a NOEC.

An assessment factor of 50 could be applied to this value because there are long-term data for three freshwater trophic levels and short-term data for echinoderms and molluscs. The short-term data indicate that whilst molluscs may be sensitive to cyanide, echinoderms (at a sensitive life stage) are of reduced sensitivity compared to crustaceans and fish.

Therefore, given the available data in the combined dataset, a total assessment factor of 100 is proposed (including a factor of 2 to convert the LOEC to a NOEC)

$$\text{PNEC}_{\text{saltwater_It}} = 5.2 \mu\text{g l}^{-1} \text{ HCN}/\text{AF (100)} = 0.052 \mu\text{g l}^{-1} \text{ HCN}$$

PNEC for deriving a maximum allowable concentration

Short-term saltwater data were available for algae, annelids, crustaceans, echinoderms, fish and molluscs. Based on the available data, crustaceans appear to be the most sensitive marine organisms.

The most sensitive short-term algal value is a 72-hour population growth EC50 of 57 $\mu\text{g l}^{-1}$ total CN for the diatom *Nitzschia closterium* [31]. This is a well-documented study with daily measurement of total cyanide concentrations. No other short-term saltwater algal data were available.

The most sensitive short-term invertebrate value is a 96-hour LC50 of 4.2 $\mu\text{g l}^{-1}$ HCN for larvae of the rock crab (*Cancer irroratus*) [34]. This was a flow-through test with measured HCH concentrations. This value was also used as the critical data point in the US EPA ambient water quality criteria for cyanide in marine waters [33]. However, the value has recently been scrutinised because of the unusually high sensitivity of *C. irroratus* in comparison with other *Cancer* species [37]. Caldwell *et al.* [37] found LC50 values 10-times higher than this value (72-hour LC50 of 44 $\mu\text{g l}^{-1}$ HCN) for the same species, but state that this result requires further testing and confirmation.

Despite these reservations, the *C. irroratus* value is supported by 48-hour development LOEC and EC50 values of 8.4 and 22.4 $\mu\text{g l}^{-1}$ respectively for the mollusc *Chlamys asperimma* [36]. This study was based on measured total cyanide concentrations. Free cyanide was reported to be within 80 per cent of total concentrations. This study is used as supporting information in the derivation of the PNEC.

The most sensitive short-term fish toxicity value is an LC50 of 59 $\mu\text{g l}^{-1}$ HCN for the silverside *Menidia menidia* [33]. There was very little information with which to assess this study, but it was a flow-through study with measured concentrations (expressed as HCN). Consequently, it has been used to provide supporting information in the derivation of the PNEC.

The most sensitive and reliable short-term toxicity value for deriving a maximum allowable concentration is, therefore, a 96-hour LC50 of 4.2 $\mu\text{g l}^{-1}$ HCN for the rock crab *Cancer irroratus* [34]. In the combined dataset (freshwater and saltwater) L(E)C50 values are available from three trophic levels (algae, invertebrates, including, molluscs and echinoderms, and fish), an assessment factor of 10 is appropriate.

$$\text{PNEC}_{\text{saltwater_st}} = 4.2 \mu\text{g l}^{-1} \text{ HCN/AF (10)} = 0.42 \mu\text{g l}^{-1} \text{ HCN}$$

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

The EQS values proposed in the 1998 report [11] were expressed as free cyanide.

For the long-term protection of freshwater species, chronic toxicity data were limited to algae, crustaceans and fish with significant sublethal effects, particularly effects on the reproductive physiology and growth of fish. A reasonably extensive and reliable dataset indicated effects of cyanide on salmonid reproduction at around $10 \mu\text{g l}^{-1}$. However, effect concentrations as low as $5 \mu\text{g l}^{-1}$ were also reported in two other studies, though the effects observed were difficult to interpret. An assessment factor of 10 was applied to the salmonid reproduction value resulting in a freshwater EQS of $1 \mu\text{g l}^{-1}$ expressed as an annual average. The assessment factor was considered appropriate to provide protection against possible reproductive effects of cyanide caused by prolonged exposure to lower concentrations, such as those indicated by the other two studies.

The short-term freshwater standard was based on fish data, as fish appeared to be the most sensitive group of organisms. The lowest credible acute effects concentration for free cyanide was a 96-hour LC50 of $43 \mu\text{g l}^{-1}$ for the rainbow trout *Oncorhynchus mykiss*. To this an assessment factor of approximately 10 was applied to give a maximum allowable concentration of $5.0 \mu\text{g l}^{-1}$.

Due to insufficient saltwater toxicity data, the EQSs derived for the protection of freshwater life were proposed as tentative values for the protection of saltwater organisms until further data were available.

3.4 Derivation of PNECs for sediment

Based on calculated log Kow values of 0.35–1.07 [3], the TGD criterion for setting sediment standards is not met, although, as cyanide dissociates in water, log Kow may not provide a true indication of the partitioning of cyanide in waters.

The fate data for cyanide (see Section 2.5) suggest that free cyanide will volatilise from the water column and will not partition to sediments or biota [11]. However, cyanide may complex with transition metals in the water column, particularly iron [38]. Hexacyanoferrate complexes are more likely to partition to and be persistent in sediments [11]. Such complexes are of lower toxicity than free cyanide [31, 36] and the associated toxicity is typically related to the capacity of the complex to release free cyanide into solution. This dissociation process will vary in rate from almost zero for iron to complete dissociation for cadmium and zinc [11].

Therefore, based on the fate of free cyanide and the knowledge that the toxicity of complex cyanides in sediments is due to the release of free cyanide to the water column, a sediment quality standard has not been set.

3.5 Derivation of PNECs for secondary poisoning of predators

3.5.1 Mammalian and avian toxicity data

The TGD [44] 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 observed 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. Table 3.1 summarises the no-effect values used by international organisations to set human health standards for cyanide.

Table 3.1 Mammalian and avian toxicity data for cyanide

Chemical	Endpoint	Value	Species	Duration	Effect	Regulatory Body*	Reference
KCN	NOAEL	10.8 mg CN/kg bw/day	Weanling albino rat (in diet)	2 years	No effects at any dose	US EPA IRIS	[39]
NaCN	NOAEL	10.8 mg CN/kg bw/day	Rat (in diet)	2 years	No effects at any dose	US EPA IRIS	[39]
HCN	NOAEL	10.8 mg CN/kg bw/day	Rat (in diet)	2 years	No effects at any dose	US EPA IRIS	[39]
Free cyanide	NOAEL	10.8 mg CN/kg bw/day	Rat (in diet)	2 years	No effects at any dose	US EPA IRIS	[39]
HCN	NOAEL	100 ppm diet (5 mg CN/kg bw/day)	Rat (in diet; no details given)	2 years	Increased thiocyanate levels	JECFA	[40]
Cyanide	LOAEL	1.2 mg CN/kg bw/day	Pig	6 months	Behavioural patterns and serum biochemistry	WHO	[41]
NaCN	LOAEL	12.5 mg CN/kg bw/day	Male rat	13 weeks	Reproductive effects	ATSDR	[6]
NaCN	NOAEL	4.5 mg CN/kg bw/day	Male rat	13 weeks	Reproductive effects	ATSDR	[6]

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

ATSDR = Agency for Toxic Substances and Disease Registry

JECFA = Joint Expert Committee on Food Additives

LOAEL = lowest observed adverse effect level

bw = body weight

ppm =parts per million

3.5.2 PNECs for secondary poisoning of predators

There are no data to indicate that simple metal cyanides and hydrogen cyanide bioaccumulate in aquatic organisms. Studies on the uptake of hydrogen cyanide in

rainbow trout report BCFs of 1.79 when exposed to $10 \mu\text{g l}^{-1}$ and BCFs of 1.69–4.12 when exposed to $20 \mu\text{g l}^{-1}$, respectively, for 15 days [13].

Accumulation through the food web is not expected due to the rapid detoxification of cyanide by most organisms or the lethal effect of large doses. Sublethal doses of cyanide are readily metabolised due to the reaction of cyanide with thiosulfate, in the presence of rhodanese, to produce thiocyanate (SCN^-), which is much less toxic [42]. Calculated BCFs of 170 and 80 for SCN^- in the plasma of juvenile rainbow trout exposed to $0.32 \mu\text{mol l}^{-1}$ CN and $0.98 \mu\text{mol l}^{-1}$ CN (20.8 and $63.8 \mu\text{g l}^{-1}$), respectively, suggest that metabolic conversion may be concentration dependent [42].

The available BCF data for (free) cyanide are all below the TGD trigger value of 100. Consequently, there is no requirement to derive a quality standard for the protection of top predators.

4. Analysis and monitoring

Because of the differences in toxicity between free and complex cyanides, it is customary to differentiate between total cyanide and free cyanide.

Free cyanide is operationally defined as the cyanide forms that are readily oxidised to cyanogen chloride by treatment with chlorine. These forms include free cyanide plus any complex forms that are readily dissociable and, therefore, potential sources of the toxic HCN species.

Total cyanide is determined after treatment of the sample to convert all forms into free cyanide. Note that in wastewaters and effluents, separation of cyanide by distillation or micro-diffusion may be required to overcome potential interferences and to enhance limits of detection by preconcentration of the sample.

Two analytical techniques may be used for the determination of cyanide. These are:

- colorimetry using chloramine T oxidation and reaction with pyridine/barbituric acid;
- potentiometry using an ion selective electrode [11].

The limit of detection achieved by the former is typically in the range 5–10 $\mu\text{g l}^{-1}$ and by the latter 20–50 $\mu\text{g l}^{-1}$. The standard deviation of analysis at concentrations several times larger than the limit of detection is around 5 per cent.

The lowest proposed PNEC derived for fresh- and saltwaters for cyanide is 0.052 $\mu\text{g l}^{-1}$ HCN. The data quality requirements are that, at a third of the EQS, total error of measurement should not exceed 50 per cent. From the literature, it can be seen that analytical methodologies provide detection limits of around 5–10 $\mu\text{g l}^{-1}$, which suggests that current analytical methodologies do not offer adequate performance to analyse for compliance with the TGD-derived PNECs for water.

5. Conclusions

5.1 Availability of data

Undissociated HCN is primarily used to determine toxicity, with HCN being more toxic than CN^- . However, CN^- contributes to toxicity due to formation of HCN at pH values up to around 8. Simple cyanides readily dissociate and hydrolyse to form HCN and CN^- , and, therefore, have the same toxicity as free cyanide. Therefore, only data on free cyanide are used to set the PNECs in this report.

Based on the physical and chemical data for cyanide, there is no evidence to suggest that its toxicity is affected by alkalinity or hardness. However, complexation with metals may reduce bioavailability.

Approximately 50 freshwater data points were available for cyanide. Acute toxicity data were located for algae, annelids, crustaceans, fish, insects, molluscs and protozoans. Chronic exposure data was less extensive and covered toxicity to algae, cnidaria crustaceans, fish and macrophytes. The majority of the data available related to fish and crustaceans, with the former being the best represented.

A similar number of data points were located for marine organisms. Short-term data were available for algae, annelids, crustaceans, echinoderms, molluscs and fish; the majority of the data related to crustaceans and molluscs. The long-term database was smaller with data available only for algae and fish.

5.2 Derivation of PNECs

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

5.2.1 Long-term PNEC for freshwaters

Fish appear to be the most sensitive taxonomic group, followed by crustaceans.

The lowest reliable long-term data point is a 289-day LOEC value of $5.2 \mu\text{g l}^{-1}$ HCN for total inhibition of spawning in the bluegill *Lepomis macrochirus*. Good quality data are also available for algae and invertebrates. An assessment factor of 10 could be applied to this value, but as it represents a significant effects level, an increased factor of 20 is proposed (including a factor of 2 to convert the LOEC to a NOEC), resulting in a $\text{PNEC}_{\text{freshwater_lt}}$ of $0.26 \mu\text{g l}^{-1}$ HCN.

This PNEC is lower than the existing 1998 EQS of $1 \mu\text{g l}^{-1}$ HCN. This was based on a value of ca. $10 \mu\text{g l}^{-1}$ obtained from a study on the effects of cyanide on salmonid reproduction to which an assessment factor of 10 was applied.

5.2.2 Short-term PNEC for freshwaters

As in the long-term studies, fish and crustaceans were found to be the most sensitive taxonomic groups.

The most sensitive, reliable result was a 96-hour LC50 of 28 $\mu\text{g l}^{-1}$ HCN for rainbow trout (*Oncorhynchus mykiss*). Given that good quality data are available for both fish, crustaceans and algae and that fish are the most sensitive organisms to both long- and short-term exposures to cyanide, an assessment factor of 10 is proposed. This results in a $\text{PNEC}_{\text{freshwater_st}}$ of 2.8 $\mu\text{g l}^{-1}$ HCN.

In comparison to the current EQS, the proposed PNEC is 2 times lower. The 1998 EQS of 5 $\mu\text{g l}^{-1}$ HCN was based on applying an assessment factor of 10 to an LC50 of 43 $\mu\text{g l}^{-1}$ HCN obtained in a study on the same species.

5.2.3 Long-term PNEC for saltwaters

The data suggests saltwater organisms to be of similar sensitivity to freshwater organisms with similar effect values. Given that cyanide acts as a respiratory depressant, similar effects would be expected in both environments. Because of this, the freshwater and saltwater datasets were combined.

As there were no high quality long-term saltwater data, the lowest reliable data point of the combined dataset is the 289-day value of 5.2 $\mu\text{g l}^{-1}$ HCN for total inhibition of spawning in the bluegill *Lepomis macrochirus*. An assessment factor of 50 could be applied to this value because there are long-term data for three freshwater trophic levels and short-term data for echinoderms and molluscs. The short-term data indicate that whilst molluscs may be sensitive to cyanide, echinoderms are of reduced sensitivity compared to crustaceans and fish.

Therefore, given the available data in the combined dataset a total assessment factor of 100 is proposed (including a factor of 2 to convert the LOEC to a NOEC), resulting in a $\text{PNEC}_{\text{saltwater_lt}}$ of 0.052 $\mu\text{g l}^{-1}$ HCN.

This is considerably lower than the existing EQS of 1 $\mu\text{g l}^{-1}$ HCN, which was 'read across' from the freshwater EQS.

5.2.4 Short-term PNEC for saltwaters

For the same reasons as outlined for the long-term data, the freshwater and saltwater datasets were combined for the derivation of a short-term PNEC for saltwaters. Based on the available short-term saltwater data, crustaceans appear to be the most sensitive taxonomic group.

The most sensitive and reliable datum is a 96-hour LC50 of 4.2 $\mu\text{g l}^{-1}$ for larvae of the rock crab *Cancer irroratus*. This value is supported by a mollusc and fish study. Given that in the combined dataset reliable data are available for three trophic levels (algae, invertebrates including echinoderms and molluscs and fish) an assessment factor of 10 is proposed resulting in a $\text{PNEC}_{\text{saltwater_st}}$ of 0.42 $\mu\text{g l}^{-1}$ HCN.

This is lower than the existing EQS of 5 µg l⁻¹ HCN, which was ‘read across’ from the freshwater EQS.

5.2.5 PNEC for secondary poisoning

The available data suggest that the likelihood of free cyanide to bioaccumulate in aquatic organisms is low. Accumulation through the food chain is also not expected due to the rapid detoxification of cyanide by most organisms. Because the BCF data for free cyanide are below 100, there is no requirement to derive a PNEC for secondary poisoning.

5.2.6 PNEC for sediments

Based on the low tendency of free cyanide to adsorb to particulate materials and the knowledge that the toxicity of complex cyanides in sediments is due to the release of free cyanide in the water column, a PNEC for sediments is not relevant.

Table 5.1 Summary of proposed PNECs

Receiving medium/exposure scenario	Proposed PNEC (µg l ⁻¹ HCN)	Existing EQS (µg l ⁻¹ HCN)
Freshwater long-term	0.26	1
Freshwater/short-term	2.8	5
Saltwater long-term	0.052	1
Saltwater/short-term	0.42	5

5.3 Analysis

It is customary to distinguish between total and free cyanide because of their differences in toxicity. Free cyanide is operationally defined as those cyanide forms that are readily oxidised to cyanogen chloride by treatment with chlorine. These forms include free cyanide plus any complex forms that readily dissociate. Total cyanide is determined after treatment of the sample to convert all forms into free cyanide. In wastewaters and effluents, separation of cyanide by distillation or micro-diffusion may be required to overcome potential interferences and to enhance limits of detection by preconcentration of the sample. The lowest proposed PNEC derived for cyanide is 0.052 µg l⁻¹ HCN. From the literature, it can be seen that analytical methodologies provide detection limits of around 5–10 µg l⁻¹, which suggests that they may not be adequate to analyse cyanide for compliance with the proposed PNECs.

5.4 Implementation issues

- Analytical capabilities may not be sufficient to enable analysis of free cyanide at the levels required to assess compliance
- Uncertainty with the long term saltwater PNEC could be reduced with further long term studies on marine species

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List of abbreviations

AA	annual average
AF	assessment factor
BCF	bioconcentration factor
bw	body weight
CAS	Chemical Abstracts Service
CICAD	Concise International Chemical Assessment Document
DO	dissolved oxygen
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
GLP	Good Laboratory Practice (OECD)
HSDB	Hazardous Substances Data Bank
IRIS	Integrated Risk Information System
IUPAC	International Union of Pure and Applied Chemistry
LC50	concentration lethal to 50% of the organisms tested
LOAEL	lowest observed adverse effect level
LOEC	lowest observed effect concentration
lt	long term
MAC	maximum allowable concentration
MATC	maximum allowable toxicant 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
SEPA	Scottish Environment Protection Agency
SNIFFER	Scotland & Northern Ireland Forum for Environmental Research
SSD	species sensitivity distribution
st	short term
TDI	tolerable daily intake

TGD	Technical Guidance Document
UKTAG	UK Technical Advisory Group
US EPA	US Environmental Protection Agency
WFD	Water Framework Directive
WHO	World Health Organization

ANNEX 1 Data quality assessment sheets

Identified and ordered by reference (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	15
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Information on the test species	
Test species used	<i>Scenedesmus quadricauda</i>
Source of the test organisms	Laboratory culture
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Not stated
Form of the test substance	Potassium cyanide (expressed as total CN)
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)	Static
Measurement of exposure concentrations	Nominal
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Endpoint comment	Only limited data available. Effects based on nominal concentrations.
Study conducted to GLP	Not stated

Reliability of study	Unreliable
Relevance of study	Relevant
Klimisch Code	3

Reference	16
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Information on the test species	
Test species used	<i>Moinodaphnia macleayi</i> and <i>Hydra viridissima</i>
Source of the test organisms	In-house cultures
Holding conditions prior to test	South Alligator River water
Life stage of the test species used	<i>Moinodaphnia macleayi</i> - <6 hours old <i>Hydra viridissima</i> – Budding (asexually reproducing)

Information on the test design	
Methodology used	Information provided in paper
Form of the test substance	Not stated (expressed as free CN)
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	0 (Control), 2, 6.7, 20, 67 and 200 µg nominal l ⁻¹
Number of replicates per concentration	<i>Moinodaphnia macleayi</i> – 10 <i>Hydra viridissima</i> - 3
Number of organisms per replicate	<i>Moinodaphnia macleayi</i> – 1 <i>Hydra viridissima</i> - 5
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	Measured
Measurement of water quality parameters	Temperature
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Endpoint comment	Test run at elevated temperature (30°C).
Study conducted to GLP	Not stated

Reliability of study	Reliable
Relevance of study	Relevant
Klimisch Code	2

Reference	17 – two species exposed simultaneously (competition effect)
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Information on the test species	
Test species used	<i>Gammarus pseudolimnaeus</i> and <i>Asellus communis</i>
Source of the test organisms	Collected from wild
Holding conditions prior to test	Held for 12 days prior to testing at 18°C
Life stage of the test species used	Adults

Information on the test design	
Methodology used	Not carried out to a standardised methodology. Method followed that of Smith <i>et al.</i> (1977).* Experimental details outlined below are based on information in both papers. <i>Gammarus pseudolimnaeus</i> exposed alongside <i>Asellus communis</i> .
Form of the test substance	Sodium cyanide (expressed as HCN)
Source of the test substance	Not stated
Type and source of the exposure medium	Deep well water
Test concentrations used	8 concentrations (5–76 µg l ⁻¹)
Number of replicates per concentration	Numbers of replicates not stated
Number of organisms per replicate	40
Nature of test system (static, semi-static or flow-through, duration, feeding)	Semi-static
Measurement of exposure concentrations	Measured
Measurement of water quality parameters	pH, DO, alkalinity and temperature
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Yes
Endpoint comment	Two species exposed simultaneously (competition effect).
Study conducted to GLP	Not stated

Reliability of study	Reliable (but supporting information)
Relevance of study	Relevant
Klimisch Code	2

* Citation taken from the original paper [17]: Smith L L Jr, Broderius S J, Oseid D M, Kimball G L and Koenst W M, 1977 Archives of Environmental Contamination and Toxicology, in press.

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Reference	17 – Species exposed independently
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Information on the test species	
Test species used	<i>Gammarus pseudolimnaeus</i> and <i>Asellus communis</i>
Source of the test organisms	Collected from wild
Holding conditions prior to test	Held for 12 days prior to testing at 18°C
Life stage of the test species used	Adults

Information on the test design	
Methodology used	Not carried out to a standardised methodology. Method followed that of Smith <i>et al.</i> (1977).* Experimental details outlined below are based on information in both papers.
Form of the test substance	Sodium cyanide (expressed as HCN)
Source of the test substance	Not stated
Type and source of the exposure medium	Deep well water.
Test concentrations used	8 concentrations (5–76 µg l ⁻¹)
Number of replicates per concentration	Numbers of reps not stated
Number of organisms per replicate	40
Nature of test system (static, semi-static or flow-through, duration, feeding)	Semi-static
Measurement of exposure concentrations	Measured
Measurement of water quality parameters	pH, DO, alkalinity and temperature
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	yes
Endpoint comment	Species exposed independently of each other in both acute and chronic exposures. Data appear valid. Only issue is that the experiment was not run to a standard method, but is a good study nevertheless.
Study conducted to GLP	Not stated

Reliability of study	Unreliable
Relevance of study	Relevant
Klimisch Code	2

* Citation taken from the original paper [17]: Smith L L Jr, Broderius S J, Oseid D M, Kimball G L and Koenst W M, 1977 Archives of Environmental Contamination and Toxicology, in press.

Reference	18
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Information on the test species	
Test species used	<i>Lepomis macrochirus</i>
Source of the test organisms	Collected from wild
Holding conditions prior to test	Not stated
Life stage of the test species used	Adults

Information on the test design	
Methodology used	Not carried out to a standardised methodology, but a well-documented study.
Form of the test substance	Hydrogen cyanide
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	8 concentrations (5.2–80 µg l ⁻¹)
Number of replicates per concentration	Replicates used (but numbers of replicates not stated)
Number of organisms per replicate	15, reduced to 10 after 140 days
Nature of test system (static, semi-static or flow-through, duration, feeding)	Flow-through
Measurement of exposure concentrations	Measured
Measurement of water quality parameters	pH, DO, alkalinity and temperature
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Yes
Endpoint comment	Complete inhibition of spawning at lowest concentration, but a very long exposure period. Also some spawning recorded at 80 µg l ⁻¹ , but at no other concentration.
Study conducted to GLP	Not stated

Reliability of study	Reliable
Relevance of study	Relevant
Klimisch Code	2

Reference	19
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Information on the test species	
Test species used	<i>Oncorhynchus mykiss</i>
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Not stated.
Form of the test substance	Not stated (expressed as total CN)
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)	Flow-through
Measurement of exposure concentrations	Unmeasured
Measurement of water quality parameters	Hardness, pH and temperature
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Endpoint comment	Few details available with which to assess data
Study conducted to GLP	Not stated

Reliability of study	Unreliable
Relevance of study	Relevant
Klimisch Code	3

Reference	20
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Information on the test species	
Test species used	<i>Oncorhynchus mykiss</i>
Source of the test organisms	Commercial fishery
Holding conditions prior to test	100 fish in 80-litre tanks containing dechlorinated city water at 12.5°C.
Life stage of the test species used	3–12 g

Information on the test design	
Methodology used	Not carried out to a standardised methodology, but a reasonably well-documented study.
Form of the test substance	Not stated (expressed as HCN)
Source of the test substance	Not stated
Type and source of the exposure medium	Dechlorinated city water
Test concentrations used	3 concentrations (0.01, 0.02 and 0.03 mg l ⁻¹)
Number of replicates per concentration	Not clear (does not appear to be replicates as only five test groups of fish mentioned)
Number of organisms per replicate	30
Nature of test system (static, semi-static or flow-through, duration, feeding)	Flow-through
Measurement of exposure concentrations	Measured and found to be within 1% of nominal
Measurement of water quality parameters	pH, DO, salinity and temperature
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	yes
Endpoint comment	Reduction in growth of 3 g fish at 0.01 mg l ⁻¹ . The fish did recover, but not to the level of the controls. There appears to be no replication. Also no effect with larger fish (12 g) at this concentration.
Study conducted to GLP	Not stated

Reliability of study	Unreliable
Relevance of study	Relevant
Klimisch Code	3

Reference	21
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Information on the test species	
Test species used	<i>Oncorhynchus mykiss</i>
Source of the test organisms	Purchased (La pisciculture Quebec)
Holding conditions prior to test	Held in dechlorinated city water at 12°C
Life stage of the test species used	20 g fish

Information on the test design	
Methodology used	Not carried out to a standardised methodology, but a well-documented study.
Form of the test substance	Not stated (expressed as HCN)
Source of the test substance	Not stated
Type and source of the exposure medium	Dechlorinated city water
Test concentrations used	4 concentrations
Number of replicates per concentration	4 replicates at each of three different temperatures 6, 12 and 18°C (fed at 5 x maintenance levels)
Number of organisms per replicate	20
Nature of test system (static, semi-static or flow-through, duration, feeding)	Flow-through
Measurement of exposure concentrations	Measured and found to be within 5% of nominal
Measurement of water quality parameters	pH, DO, alkalinity and temperature
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	yes
Endpoint comment	Greatest effect at lowest temperature
Study conducted to GLP	Not stated
Reliability of study	Reliable
Relevance of study	Relevant
Klimisch Code	2

Reference	22
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Information on the test species	
Test species used	<i>Chlorococcales</i>
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Not stated.
Form of the test substance	Not stated (expressed as free CN)
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)	Static
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	Few details available with which to assess data
Study conducted to GLP	Not stated

Reliability of study	Unreliable
Relevance of study	Unknown
Klimisch Code	4

Reference	23
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Information on the test species	
Test species used	<i>Ankistrodesmus falcatus</i>
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Not stated
Form of the test substance	Not stated (expressed as free CN)
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
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Endpoint comment	Few details available with which to assess data
Study conducted to GLP	Not stated

Reliability of study	Unknown
Relevance of study	Relevant
Klimisch Code	4

Reference	24
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Information on the test species	
Test species used	<i>Spirostomum ambiguum</i>
Source of the test organisms	Laboratory culture
Holding conditions prior to test	Held in deep well natural water
Life stage of the test species used	Cells

Information on the test design	
Methodology used	Not stated.
Form of the test substance	Analytical grade potassium cyanide (expressed as free cyanide)
Source of the test substance	Not stated
Type and source of the exposure medium	Natural unpolluted water
Test concentrations used	5
Number of replicates per concentration	3
Number of organisms per replicate	10–20 cells
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	No
Measurement of water quality parameters	Temperature
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Endpoint comment	A static test with no analysis or mention of sealed vessels to prevent volatilisation.
Study conducted to GLP	Not stated

Reliability of study	Unreliable
Relevance of study	Relevant
Klimisch Code	3

Reference	25
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Information on the test species	
Test species used	<i>Daphnia pulex</i>
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Not carried out to a standardised methodology
Form of the test substance	Potassium cyanide
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)	Static (48-hour duration)
Measurement of exposure concentrations	Nominal
Measurement of water quality parameters	pH and temperature
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Ends point comment	Same species less sensitive to higher concentrations at lower temperatures in same study.
Study conducted to GLP	Not stated

Reliability of study	Unreliable
Relevance of study	Relevant
Klimisch Code	3

Reference	26
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Information on the test species	
Test species used	<i>Ceriodaphnia dubia</i>
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Not stated
Form of the test substance	Potassium cyanide (expressed as total CN)
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)	Static
Measurement of exposure concentrations	Nominal
Measurement of water quality parameters	pH, DO and temperature
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Endpoint comment	No chemical analysis
Study conducted to GLP	Not stated

Reliability of study	Questionable
Relevance of study	Relevant
Klimisch Code	3

Reference	27
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Information on the test species	
Test species used	<i>Branchiura sowerbyi</i> and <i>Moina micrura</i>
Source of the test organisms	Not stated
Holding conditions prior to test	Acclimated for 96–192 hours
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Not stated.
Form of the test substance	Analytical grade ammonium thiocyanate
Source of the test substance	Commercial (Darmstadt)
Type and source of the exposure medium	Not stated
Test concentrations used	7
Number of replicates per concentration	5
Number of organisms per replicate	10
Nature of test system (static, semi-static or flow-through, duration, feeding)	Semi-static
Measurement of exposure concentrations	No
Measurement of water quality parameters	pH, DO, alkalinity and hardness
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Yes
Endpoint comment	Although a semi-static test, there was no analysis or mention of sealed vessels to prevent volatilisation.
Study conducted to GLP	Not stated

Reliability of study	Unreliable
Relevance of study	Relevant
Klimisch Code	3

Reference	28
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Information on the test species	
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Test species used	<i>Salmo salar</i>
Source of the test organisms	Cynrig Hatchery (River Usk 1980)
Holding conditions prior to test	160 fish in 400-litre tanks. Continuous flow of borehole/seawater water at 11°C and pH 7.7. DO at saturation.
Life stage of the test species used	2 years

Information on the test design	
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Methodology used	Not carried out to a standardised methodology, but a well-documented study.
Form of the test substance	Potassium cyanide (expressed as total CN)
Source of the test substance	Not stated
Type and source of the exposure medium	Water exposure at varying salinities and DO concentrations
Test concentrations used	0.01–0.11 mg l ⁻¹ at varying DO and salinity
Number of replicates per concentration	Not clear (8–16 test tanks reported in method)
Number of organisms per replicate	5
Nature of test system (static, semi-static or flow-through, duration, feeding)	Semi-static (daily)
Measurement of exposure concentrations	Measured and found to be within 5% of nominal
Measurement of water quality parameters	pH, DO, salinity and temperature
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Yes
Endpoint comment	Valid endpoint but at low dissolved oxygen (3.5 mg l ⁻¹).
Study conducted to GLP	Not stated

Reliability of study	Reliable
Relevance of study	Relevant
Klimisch Code	2

Reference	29
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Information on the test species	
Test species used	<i>Oncorhynchus mykiss</i>
Source of the test organisms	Purchased (La pisciculture Quebec)
Holding conditions prior to test	160 fish in 200-litre tanks. Continuous flow of dechlorinated water at 12°C. Held for 10–14 days prior to testing.
Life stage of the test species used	Fingerlings

Information on the test design	
Methodology used	Not carried out to a standardised methodology, but a well-documented study.
Form of the test substance	Potassium cyanide (expressed as HCN)
Source of the test substance	Not stated
Type and source of the exposure medium	Dechlorinated tap water
Test concentrations used	5 concentrations (0.036–0.1 mg l ⁻¹)
Number of replicates per concentration	Not stated
Number of organisms per replicate	10
Nature of test system (static, semi-static or flow-through, duration, feeding)	Flow-through
Measurement of exposure concentrations	Measured and found to be within 5% of nominal
Measurement of water quality parameters	pH, DO, hardness and temperature
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Yes
Endpoint comment	Valid endpoint
Study conducted to GLP	Not stated

Reliability of study	Reliable
Relevance of study	Relevant
Klimisch Code	2

Reference	30
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Information on the test species	
Test species used	<i>Oncorhynchus mykiss</i>
Source of the test organisms	Purchased (La pisciculture Quebec)
Holding conditions prior to test	Held in dechlorinated city water at 12°C for 2 weeks
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Not carried out to a standardised methodology, but a well-documented study.
Form of the test substance	Not stated (expressed as HCN)
Source of the test substance	Not stated
Type and source of the exposure medium	Dechlorinated city water
Test concentrations used	Various concentrations (0.018–0.087 mg l ⁻¹)
Number of replicates per concentration	Not stated but test run at three different temperatures 6, 12 and 18°C.
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	Measured and found to be within 1% of nominal
Measurement of water quality parameters	pH, DO and temperature
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Yes
Endpoint comment	Greatest effect at lowest temperature
Study conducted to GLP	Not stated

Reliability of study	Reliable
Relevance of study	Relevant
Klimisch Code	2

Reference	31
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Information on the test species	
Test species used	<i>Nitzschia closterium</i>
Source of the test organisms	Obtained from CSIRO algal culture collection (Australia)
Holding conditions prior to test	Stored at 21°C in filtered seawater
Life stage of the test species used	Population

Information on the test design	
Methodology used	Not carried out to a standardised methodology, but a well-documented study.
Form of the test substance	Sodium cyanide (analytical grade) (expressed as total CN)
Source of the test substance	Not stated
Type and source of the exposure medium	Filtered natural seawater
Test concentrations used	6 concentrations (10–200 µg l ⁻¹)
Number of replicates per concentration	4 replicates
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static and semi static (static sealed vessels finally used)
Measurement of exposure concentrations	Measured daily
Measurement of water quality parameters	pH, DO, salinity, temperature
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Endpoint comment	LOEC was lowest concentration tested
Study conducted to GLP	Not stated

Reliability of study	Reliable
Relevance of study	Relevant
Klimisch Code	2

Reference	32
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Information on the test species	
Test species used	<i>Champia parvula</i>
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	NA

Information on the test design	
Methodology used	Not stated
Form of the test substance	Potassium cyanide (expressed as total CN)
Source of the test substance	Not stated
Type and source of the exposure medium	Marine 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)	Static-renewal
Measurement of exposure concentrations	Measured
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Endpoint comment	Measured data with reasonable amount of data available on test.
Study conducted to GLP	Not stated

Reliability of study	Reliable
Relevance of study	Relevant
Klimisch Code	2

Reference	33
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Information on the test species	
Test species used	<i>Pseudopleuronectes americanus</i> and <i>Cyprinodon variegatus</i>
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Not stated.
Form of the test substance	Not stated (expressed as free CN)
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)	Static
Measurement of exposure concentrations	Nominal for <i>Pseudopleuronectes americanus</i> and measured for <i>Cyprinodon variegatus</i>
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Endpoint comment	Few details available with which to assess data
Study conducted to GLP	Not stated

Reliability of study	Unreliable
Relevance of study	Relevant
Klimisch Code	4

Reference	33
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Information on the test species	
Test species used	<i>Cyprinodon variegatus, Menidia menidia,</i>
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Not stated
Form of the test substance	Not stated (expressed as free CN)
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)	Flow-through
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	Few details available with which to assess data
Study conducted to GLP	Not stated

Reliability of study	Unknown
Relevance of study	Relevant
Klimisch Code	4

Reference	34
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Information on the test species	
Test species used	<i>Cancer irroratus</i>
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Larvae

Information on the test design	
Methodology used	Not stated
Form of the test substance	Not stated (expressed as free CN)
Source of the test substance	Not stated
Type and source of the exposure medium	Seawater
Test concentrations used	5 (3–50 µg l ⁻¹)
Number of replicates per concentration	3
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	Measured
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Endpoint comment	The results of this study were used to derive the US EPA water quality criteria for cyanide in saltwater.
Study conducted to GLP	Not stated

Reliability of study	Reliable
Relevance of study	Relevant
Klimisch Code	2

Reference	35
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Information on the test species	
Test species used	<i>Cancer magister</i> and <i>Cancer productus</i>
Source of the test organisms	Collected from wild
Holding conditions prior to test	Filtered natural seawater at 8–10°C until zoeae release.
Life stage of the test species used	Newly hatched (<24 hours)

Information on the test design	
Methodology used	Not carried out to a standardised methodology, but a well-documented study.
Form of the test substance	Sodium cyanide (reagent grade) (expressed as total CN)
Source of the test substance	Purchased from Mallinckrodt Inc.
Type and source of the exposure medium	Aged filtered seawater from national marine fishery service (USA)
Test concentrations used	6 concentrations
Number of replicates per concentration	5
Number of organisms per replicate	10
Nature of test system (static, semi-static or flow-through, duration, feeding)	Semi static
Measurement of exposure concentrations	Measured at 0, 48 and 96 hours and within 73% of nominal
Measurement of water quality parameters	pH, DO, salinity, temperature
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Endpoint comment	Valid endpoint
Study conducted to GLP	Not stated

Reliability of study	Reliable
Relevance of study	Relevant
Klimisch Code	1

Reference	36
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Information on the test species	
Test species used	<i>Chlamys asperrimus</i>
Source of the test organisms	Adults collected from wild
Holding conditions prior to test	Adults stored in cages in Sydney harbours for 5 days
Life stage of the test species used	Fertilised eggs

Information on the test design	
Methodology used	Not carried out to a standardised methodology, but a well-documented study.
Form of the test substance	Sodium cyanide (expressed as total CN)
Source of the test substance	Not stated
Type and source of the exposure medium	Filtered natural seawater
Test concentrations used	6 concentrations
Number of replicates per concentration	7
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	Measured (20% loss of free CN after 48 hours)
Measurement of water quality parameters	pH, DO, salinity, temperature
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Endpoint comment	Valid endpoint
Study conducted to GLP	Not stated

Reliability of study	Reliable
Relevance of study	Relevant
Klimisch Code	1

Reference	45
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Information on the test species	
Test species used	<i>Pseudokirchneriella subcapitata</i>
Source of the test organisms	In house cultures
Holding conditions prior to test	Nutrient media (ATCC 22662)
Life stage of the test species used	Growth phase

Information on the test design	
Methodology used	The method is well described in the report.
Form of the test substance	Analytical material (>96% purity)
Source of the test substance	Sigma-Aldrich, Dorset, UK
Type and source of the exposure medium	Nutrient media
Test concentrations used	0 (control), 10, 20, 40, 80, 160, 320, 640 and 1280 µg l ⁻¹ (nominal KCN concentrations) 0 (control), 4, 8, 16, 32, 64, 130, 260 and 510 µg l ⁻¹ (nominal cyanide concentrations)
Number of replicates per concentration	Six (for controls) and three (for treatments)
Number of organisms per replicate	Initial starting density = 5 x 10 ³ cells/ml
Nature of test system (Static, semi-static or flow through, duration, feeding)	Static, 72 hours, no feeding
Measurement of exposure concentrations	The test concentrations were analysed at the beginning and end of the test (measured values were 68 to 88% of nominal concentrations).
Measurement of water quality parameters	Yes (pH and temperature)
Test validity criteria satisfied	Yes (171 times increase in controls)
Water quality criteria satisfied	Yes
Study conducted to GLP	The study was carried out to the principles of GLP
Comments	The study was well conducted, is of good quality and the exposure concentrations used were measured.
Reliability of study	Reliable
Relevance of study	Relevant
Klimisch Code	2

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Reference	48
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Information on the test species	
Test species used	<i>Salvelinus fontinalis</i>
Source of the test organisms	Wisconsin State Hatchery, Osceola, Wisconsin
Holding conditions prior to test	Well water at 14°C, with fish fed at 1.3% body weight per day
Life stage of the test species used	Adults (nineteen months old)

Information on the test design	
Methodology used	The method is well described in the report.
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Well water, (pH = 7.94 – 8.01 and total alkalinity = 236-239 mg CaCO ₃ l ⁻¹)
Test concentrations used	0 (control), 5.7, 11, 33, 44 55, 66 and 77 µg l ⁻¹
Number of replicates per concentration	Spawning: 1 Growth and survival: 5 (controls) and 3-4 (treatments)
Number of organisms per replicate	Spawning: 6 (2 males and four females) Growth and survival: 25
Nature of test system (Static, semi-static or flow through, duration, feeding)	Continuous flow, 234 days, feeding
Measurement of exposure concentrations	The test concentrations were determined three times per week
Measurement of water quality parameters	Yes (pH, temperature and dissolved oxygen)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Comments	The study was well conducted, is of good quality and the exposure concentrations used were determined.

Reliability of study	Reliable
Relevance of study	Relevant
Klimisch Code	2

