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Proposed EQS for Water Framework Directive Annex VIII substances: ammonia (un-ionised)

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

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Steve Killeen Head of Science

Use of this report

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

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

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

Executive Summary

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

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

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

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

Properties and fate in water

Ammonia is a ubiquitous contaminant of surface waters, entering watercourses from a variety of point and diffuse sources. It comprises two principal forms: the ionised ammonium ion (NH_4^+) and un-ionised ammonia (NH_3). The toxicity of ammonia to fish is attributable mainly to the un-ionised NH_3 molecule. The proportion of un-ionised ammonia increases with increasing temperature and pH, but decreases with increasing salinity. At pH 8.5, the proportion of un-ionised ammonia is approximately 10 times that at pH 7.5 and, for every 9°C increase in temperature, the proportion of un-ionised ammonia approximately doubles.

Ammonia is lost from water by volatilisation and, under aerobic conditions, it is oxidised by nitrifying bacteria to nitrite and then to nitrate. Ammonia is not expected to adsorb to soil particulate matter, suspended solids or sediment. Although ammonia is assimilated by aquatic plants for use as a nitrogen source, its bioaccumulation in biota is not important.

Availability of data
Throughout this report, toxicity data are expressed as un-ionised ammonia. This effectively removes the need to account for banding of standards due to pH and temperature. Methods for estimating concentrations of ionised and un-ionised ammonia are well-developed and are based on an understanding of its speciation in water.

Acute toxicity data are available for algae, rotifers, crustaceans, molluscs, annelids, planarians, insects, fish and amphibians; chronic toxicity data are also available for algae, crustaceans, molluscs, annelids, insects, fish and amphibians. Data are particularly plentiful for fish and one semi-field study with freshwater organisms is also available.

Fish are clearly the most sensitive species with regard to both chronic and acute effects of ammonia. However, there is overlap between the sensitivities of representatives of different taxonomic groups and the range of acute toxicity values from all taxa is within approximately two orders of magnitude. Algae appear to be of lower sensitivity, probably because they can utilise ammonia as a nitrogen source.

Fewer acute toxicity data are available for marine organisms but include algae, rotifers, crustaceans, molluscs, echinoderms and fish. Chronic saltwater data are only available for algae, crustaceans (three species) and fish (two species). Echinoderms are the most sensitive taxonomic group with respect to the acute toxicity of un-ionised ammonia. However, the range of acute toxicity values is within approximately two orders of magnitude. There are too few data to draw conclusions about the chronic toxicity of ammonia to different taxonomic groups, although sensitivities of marine crustaceans and fish are in the range obtained for the corresponding freshwater species.

The data suggest that fish exposed under saline conditions may be more at risk if they are larvae or juveniles, if temperature is elevated, if salinity is near the seawater value, or if the pH is low.

Derivation of PNECs

Long-term PNEC for freshwaters
The lowest credible concentration of un-ionised ammonia at which long-term effects were found is 0.022 mg NH_3-N l^{-1} when a cumulative mortality of 71 per cent was observed for eggs, larvae and fry of rainbow trout (*Oncorhynchus mykiss*) over 73 days exposure. No NOEC value was derived in the study since effects were observed at the lowest exposure concentration. However, data on the concentration–response curve for similar effects in other fish have been evaluated, and these indicate 2–3 times difference in exposure concentration for a 50 per cent reduction in survival (from the controls) in such an early life stage test.

The $PNEC_{freshwater\_lt}$ can be derived based on the 73-day LOEC of 0.022 mg NH_3-N l^{-1} to which a factor of 2 is applied to estimate a NOEC and an assessment factor of 10 to extrapolate to a $PNEC_{freshwater\_lt}$ of 1.1 µg l^{-1} un-ionised ammonia. This factor is justified on the basis of a large body of data, including a multigenerational study with a sensitive species and a series of simulated ecosystem studies.

Existing EQSs were developed in 1988 for the protection of freshwater fish (both cyprinids and salmonids) and are appreciably less stringent than those proposed here. Significantly, invertebrate data were not considered at the time due to a lack of data. In European Commission designated waters, the standards are 0.021 and 0.78 mg l^{-1} for un-ionised ammonia and total ammonia, respectively. In non-EC designated waters, an EQS of 15 µg l^{-1} un-ionised ammonia was developed. This was based on the 95th percentile estimated from field data to which a factor of 2 was applied, and would normally be expressed as an annual average.

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

Fish are the most sensitive taxon to acute exposure with the lowest valid acute LC50 value being 0.068 mg NH_3-N l^{-1} for *Oncorhynchus gorbuscha* (pink salmon) alevins exposed to ammonia for a 96-hour period. Similar sensitivity was evident in other studies with the same species, and *Prosopium williamsoni* (mountain whitefish) and the crustacean *Hyalella* were only slightly less sensitive.

The recommended $PNEC_{freshwater\_st}$ of 6.8 µg l^{-1} un-ionised ammonia is based on the 96-hour LC50 for pink salmon and application of a reduced assessment factor of 10 (instead of 100) since this datum is supported by an extensive body of toxicological data for a wide range of taxa.

There is no existing short-term EQS for ammonia.

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

The marine dataset is too small to draw firm conclusions on possible differences between the sensitivities of freshwater and saltwater organisms, particularly with regard to chronic effects. However, marine taxa do not appear to differ markedly from the range of sensitivities obtained for corresponding freshwater species. Consequently, freshwater toxicity data for ammonia have been considered in the derivation of PNEC values for the protection of marine life.

The lowest reliable NOEC for long-term effects of ammonia on marine biota is 0.066 mg NH_3-N l^{-1} for growth of Dover sole (*Solea solea*). Short-term test data are available that indicate this may not be the most sensitive taxonomic group (molluscs and echinoderms are more sensitive following acute exposure) and so an assessment factor of 100 is recommended to protect these taxa, resulting in a $PNEC_{saltwater\_lt}$ of 0.66 µg l^{-1} un-ionised ammonia.

The existing EQS for the protection of saltwater fish and shellfish for un-ionised ammonia is appreciably less stringent at 0.021 mg l^{-1}, expressed as an annual average concentration. This was based on a combination of acute toxicity data to fish and threshold concentrations inferred from field data. A recent report for the Environment Agency on ammonia in estuaries concluded that the proposed Habitats Directive threshold value for un-ionised ammonia of 0.021 mg l^{-1} may be protective in good quality waters but may not be protective in poor quality waters.

<u>Short-term PNEC for saltwaters</u>

The most sensitive species in laboratory studies was the sea urchin, *Strongylocentrotus purpuratus*, for which a reliable 72-hour LC50 of 0.057 mg l^{-1} for larval survival is reported.

A PNEC to protect against short-term exposure to ammonia can be derived on the basis of an assessment factor of 10 applied to this datum, resulting in a $PNEC_{saltwater\_st}$ of 5.7 µg l^{-1} un-ionised ammonia.

There is no existing EQS for short-term exposures to marine life.

PNEC for secondary poisoning and sediments
Ammonia does not accumulate in sediments and the derivation of sediment PNECs for the protection of benthic organisms is not required. Given the high water solubility of ammonia, bioaccumulation is not considered important.

Summary of proposed PNECs

Receiving medium/exposure scenario	**Proposed PNEC (µg l^{-1} un-ionised ammonia)**	**Existing EQS (µg l^{-1} un-ionised ammonia)**
Freshwater/long-term	1.1	15
Freshwater/short-term	6.8	No standard
Saltwater/long-term	0.66	21
Saltwater/short-term	5.7	No standard

Analysis
Total ammonia is typically measured in monitoring programmes along with physico-chemical parameters (pH, temperature and conductivity or salinity) and un-ionised ammonia is then calculated. Current analytical methodologies provide detection limits of around 20 µg l^{-1}, which do not offer adequate sensitivity to analyse ammonia with the proposed PNECs.

Implementation issues
Before PNECs for un-ionised ammonia can be adopted as EQSs, it will be necessary to address the following issues:

1. Current analytical methods are not sensitive enough to assess compliance with proposed PNECs in receiving waters. This will require further investigation.

2. Data that describe effects in the field are available through ecological classification programmes. These could help inform the PNEC derivation and may influence the final outcome.

3. As an interim measure, existing EQSs may be used but these are only relevant to long-term exposure. There are no interim options for short-term exposure to ammonia.

Contents

1. Introduction

The UK Technical Advisory Group (UKTAG) supporting the implementation of the Water Framework Directive (2000/60/EC)[1] is a partnership of UK environmental and conservation agencies. It also includes partners from the Republic of Ireland. UKTAG has commissioned a programme of work to derive Environmental Quality Standards (EQSs) for substances falling under Annex VIII of the Water Framework Directive (WFD). This report proposes predicted no-effect concentrations (PNECs) for ammonia using the methodology described in Annex V of the Directive. There are existing EQSs for ammonia, 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. There is also a separate programme of work aimed at developing ammonia standards for classifying surface waters.

The PNECs described in this report are based on a technical assessment of the available ecotoxicity data for ammonia, along with any data that relate impacts under field conditions to exposure concentrations. The data have been subjected to rigorous quality assessment such that decisions are based only on scientifically sound data.[2] Following consultation with an independent peer review group, critical data have been identified and assessment factors selected in accordance with the guidance given in Annex V. The feasibility of implementing these PNECs as EQSs has not been considered at this stage. However, this would be an essential step before a regulatory EQS can be recommended.

This report provides a data sheet for ammonia.

1.1 Properties and fate in water

Ammonia is a ubiquitous contaminant of surface waters, entering watercourses from a variety of point and diffuse sources. It has two main forms:

- ionised ammonium ion (NH_4^+)
- un-ionised ammonia (NH_3).

The toxicity of ammonia to fish is attributable mainly to the un-ionised NH_3 molecule. The proportion of un-ionised ammonia increases with increasing temperature and pH, but decreases with increasing salinity. At pH 8.5, the proportion of un-ionised ammonia is approximately 10 times that at pH 7.5 and, for every 9°C increase in temperature, the proportion of un-ionised ammonia approximately doubles.

Ammonia is lost from water by volatilisation and, under aerobic conditions, it is oxidised by nitrifying bacteria to nitrite and then to nitrate. Ammonia is not expected to adsorb to soil particulate matter, suspended solids or sediment. Although ammonia is assimilated

[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.

by aquatic plants for use as a nitrogen source, its bioaccumulation in biota is not important.

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
Ammonia	7664-41-7

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 [12], and existing EQSs obtained from the literature [1].

Section 2.6 summarises the effects data identified from the literature for ammonia. 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 (as un-ionised ammonia)

PNEC	TDG deterministic approach (AFs)	TGD probabilistic approach (SSDs)	Existing EQS
Freshwater short-term	6.8 μg l^{-1} (Section 3.1.1)	–	–
Freshwater long-term	1.1 μg l^{-1} (Section 3.1.1)	Lack of data (Section 3.2)	0.015 mg l^{-1} (AA)
Saltwater short-term	5.7 μg l^{-1} (Section 3.1.2)	–	–
Saltwater long-term	0.66 μg l^{-1} (Section 3.1.2)	Lack of data (Section 3.2)	0.021 mg l^{-1} (AA)
Freshwater sediment short-term	No requirement (Section 3.4)	–	–
Freshwater sediment long-term	No requirement (Section 3.4)	Lack of data (Section 3.4)	–
Saltwater sediment short-term	No requirement (Section 3.4)	–	–
Saltwater sediment long-term	No requirement (Section 3.4)	Lack of data (Section 3.4)	–

PNEC	TDG deterministic approach (AFs)	TGD probabilistic approach (SSDs)	Existing EQS
Freshwater secondary poisoning	No requirement	–	–
Saltwater secondary poisoning	No requirement	–	–

AA = annual average
AF = assessment factor
SSD = species sensitivity distribution

In European Commission designated waters, the EQSs for protection of freshwater fish are 0.021 and 0.78 mg l^{-1} for unionised ammonia and total ammonia, respectively. These are based on mandatory values expressed as 95th percentiles. An EQS of 0.015 mg l^{-1} for un-ionised ammonia, expressed as an annual average, has been proposed for non-EC designated waters. The EQS for the protection of saltwater fish and shellfish for un-ionised ammonia is 0.021 mg l^{-1}, expressed as an annual average concentration [1]. A recent report for the Environment Agency on ammonia in estuaries concluded that the proposed Habitats Directive threshold value for un-ionised ammonia of 0.021 mg l^{-1} may be protective in good quality waters but may not be protective in poor quality waters [2].

In the most recent review by the US Environmental Protection Agency (US EPA) of the aquatic life ambient water criteria for ammonia, the acute criterion for freshwater was dependent on pH and fish species and the chronic criterion for freshwater was dependent on pH and temperature. At lower temperatures, the chronic criterion is also dependent on the presence or absence of early life stages of fish [3].

2.3 Hazard classification

Table 2.3 gives the R-phrases (Risk-phrases) and labelling for ammonia.

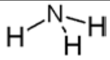
Table 2.3 Hazard classification

R-phrases and labelling	Reference
R10[T;R23][C;R34]N;R50	[4]

2.4 Physical and chemical properties

Table 2.4 summarises the physical and chemical properties of ammonia.

Table 2.4 Physical and chemical properties of ammonia

Property	Value	Reference
Molecular formula	NH_3	
Molecular structure		
Molecular weight	17.03	[5]
Composition	N: 82.24; H: 17.76	[5]
Appearance	Colourless gas	[5]
Melting point (°C)	–77.7	[5]
Boiling point (°C)	–33.35	[5]
Vapour pressure	7,510 mmHg at 25°C	[6]
Henry's Law constant	1.61 x 10^{-5} atm-m^3/mol at 25°C	[6]
Water solubility (mg l^{-1})	531,000 at 20°C	[6]
Dissociation constant (pKa)	9.25 at 25°C	[6]

2.5 Environmental fate and partitioning

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

Table 2.5 Environmental fate and partitioning of ammonia

Property	Value	Reference
Abiotic fate	Ammonia in water may be transferred to the atmosphere by volatilisation from the air–water interface; this process has a measurable effect on ammonia levels in water.	[4]
	The rate of volatilisation of ammonia from water will increase with increasing pH (generally only important for pH values >7) and temperature, and can be influenced by other environmental factors such as flow conditions and wind speed.	[11]
Speciation	Total ammonia in the environment comprises two principal forms: the ionised ammonium ion (NH_4^+) and un-ionised ammonia (NH_3). The proportion of ionised and un-ionised ammonia can be calculated from total ammonia, taking into account salinity, temperature and pH. The proportion of un-ionised ammonia increases with increasing temperature and pH, but decreases with increasing salinity.	[1]
	Of the three main factors affecting ammonia speciation, salinity appears to be relatively unimportant. Temperature and pH, however, have a much more significant effect. At pH 8.5, the proportion of un-ionised ammonia is approximately 10	[6]

Property	Value	Reference
	times that at pH 7.5 and, for every 9°C increase in temperature, the proportion of un-ionised ammonia approximately doubles. At the pH of most biological systems, ammonia exists predominantly in the ionised form (NH_4^+).	 [4]
Hydrolytic stability Photostability Distribution in water/sediment systems Degradation in soil	Concentrations of ammonia in water are controlled directly by volatilisation to and dissolution from the atmosphere. Ammonia dissolves readily in water where it ionises to form the ammonium ion. Under normal atmospheric conditions, ammonia does not undergo any primary photochemical reactions at wavelengths greater than 290 nm. In water, ammonia is not expected to adsorb to suspended solids and sediment to an appreciable degree. Ammonia at natural concentrations in soil is not believed to have a very long half-life. In soil, ammonia may either volatilise to the atmosphere or undergo microbial transformation to nitrate or nitrite anions. Uptake by plants can be a significant fate process. Based on its solubility, ammonia is not expected to adsorb to particulate matter to an appreciable degree.	[1] [4] [4] [1] [11] [1]
Biodegradation	Under aerobic conditions, ammonia in water is rapidly converted into nitrate by nitrification. Bacteria of the genus *Nitrosomonas* oxidise ammonia to nitrite and *Nitrobacter* convert the nitrite into nitrate. The pH in water is increased by the presence of ammonia ions, in the form of hydroxide ions. Temperature, oxygen supply and pH of the water are factors in determining the rate of oxidation. Aerobic biological treatment (as utilised in wastewater treatment works) completely nitrifies ammonia to nitrate. Ammonia is assimilated by aquatic algae and macrophytes for use as a nitrogen source.	[8] [9] [4, 11]
Partition coefficients (log Kow)	Estimated log Kow = 0.23	[10]
Bioaccumulation BCF	Ammonia does not bioaccumulate in aquatic biota.	[7]

BCF = bioconcentration factor

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 data from the existing EQS document for freshwater and marine species were identified [1]. Then data published after the derivation of the current UK EQS were retrieved from the US EPA AQUIRE database.[3]

Finally, other data sources were evaluated including:

- World Health Organization (WHO) *Environmental Health Criteria (EHC) 54: Ammonia* [4];
- information from the 1999 update of the US EPA aquatic life ambient water quality criteria for ammonia [3];
- recent work carried out for the Environment Agency [2].

Different studies on ammonia toxicity with the same species may be available. However, if these are conducted with water samples at different pHs, temperatures and conductivities or salinities, it would not be logical to generate a geometric mean for such a dataset as these parameters affect the toxicity of ammonia. A geometric mean has been generated only for datasets for the same species carried out for consistent durations and under comparable water quality conditions.

2.6.1 Toxicity to freshwater organisms

Single species acute toxicity data (i.e. typically from studies of ≤96-hour duration) are available for nine different taxonomic groups (i.e. algae, rotifers, crustaceans, molluscs, annelids, planarians, insects, fish and amphibians) including the 'base set' taxa required for use of the assessment factor approach in the EU Technical Guidance Document [12].

Chronic toxicity data (i.e. typically from studies of ≥96-hour duration) are available for algae, crustaceans, molluscs, annelids, insects, fish and amphibians. Results of one higher tier semi-field study with freshwater organisms are also available.

Tables 2.6 and 2.7 summarise available long-term chronic data and short-term acute data, respectively. Figure 2.1 displays the cumulative distribution function of freshwater long-term data as given in Table 2.6 and Figure 2.2 displays the cumulative distribution function of freshwater short-term data as listed in Table 2.7. These curves do not represent species sensitivity distributions, but are instead a means of representing the range of long-term and short-term toxicity data available for freshwater species. Figure 2.2 includes data from a series of short-term studies that investigated biochemical endpoints in fish, some of which apparently resulted in low toxicity values [46–50]. However, since the significance of the measured changes to the overall health of individuals and, ultimately, populations is uncertain these values have not been used in the derivation of the PNEC value.

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

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

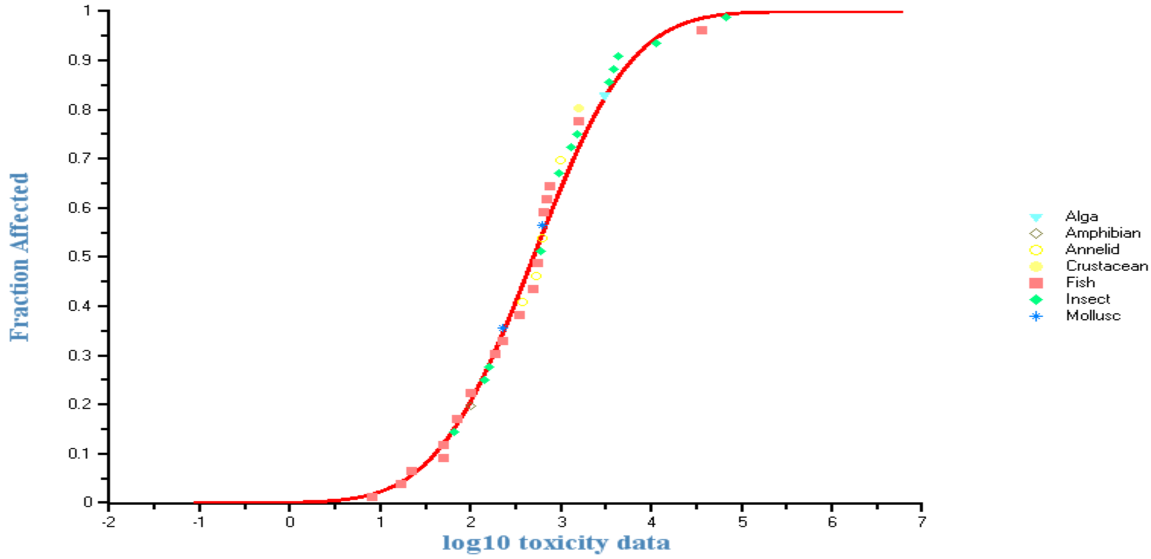
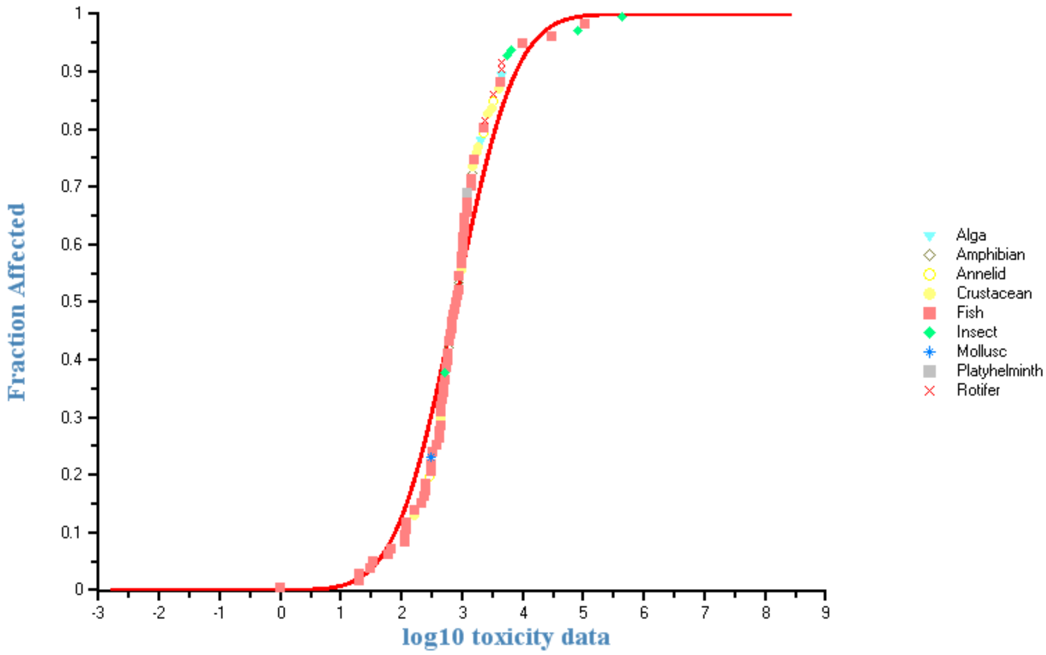


Figure 2.2 Cumulative distribution function of freshwater short-term data (μg l^{-1}) for un-ionised ammonia



It is evident that fish are the most sensitive species with regard to both chronic and acute effects of ammonia. However, there is overlap between the sensitivities of representatives of different taxonomic groups. The range of acute toxicity values was within approximately two orders of magnitude. Algae appear to be of lower sensitivity, consistent with the fact that ammonia represents a nitrogen source for these organisms, which is required for growth.

Effects of ammonia on freshwater biota have been reviewed by a number of authors [1, 3, 13–16]. It is generally accepted that the toxicity of ammonia to fish is attributable to the un-ionised NH_3 molecule. Although there is some evidence that the ionised fraction may have a toxic effect, it has been reported to be considerably lower than that of the un-ionised fraction [1, 11]. Therefore all the effects data in Tables 2.6 and 2.7 are expressed as un-ionised concentrations (as mg NH_3-N l^{-1}). The tables also show comparative data for species when the effects of ammonia have been evaluated at differing pH values. From these data, it is evident that there is a general trend of decreasing toxicity of un-ionised ammonia with increasing pH.

A 76-week study on the effects of ammonia on microinvertebrates and fish was conducted in the 1980s in outdoor experimental streams supplied with water from the Mississippi River [17]. Each stream was 520-metres long and constructed in an alternating pool-riffle design. The study design comprised one control and three treatment streams (dosed at approximately 0.05, 0.15 and 0.45 mg NH_3-N l^{-1}). In the study, the top 215 m of the four steams was used in the period between 8 June 1983 and 18 November 1984. The study reach of each stream included four pools: three complete riffles and the upper portion of a fourth riffle. Total ammonia measurements were made 1–4 times each week and un-ionised ammonia concentrations were calculated from these data and physico-chemical information (pH, temperature and conductivity). Populations of four major microinvertebrate taxonomic groups (cladocerans, copepods, protozoans and rotifers) were monitored during a 4-week period early in the study, and six fish species (bluegills, channel catfish, fathead minnows, rainbow trout, walleyes and white suckers) were tested for various time intervals, from 4 to 26 weeks, throughout the 76-week study period. Based on comparisons with the control stream, copepods and rotifers were unaffected in all three treatment streams. Cladoceran and protozoan populations were reduced in at least two treatment streams but, because of large variability, effects were considered to be inconclusive. However, complete mortality of cladocerans did occur in the ~0.15 and ~0.45 mg NH_3-N l^{-1} treatments when placed in *in situ* biomonitor chambers. All six fish species were affected in at least one or more treatments. Generally, the fish effect values were in agreement with the data from relevant laboratory toxicity studies of the species involved. Of the 12 fish groups tested, one channel catfish group and one white sucker group were affected at the lowest nominal exposure concentration of approximately 0.05 mg NH_3-N l^{-1}.

A study on the effects of ammonia on pond ecology was also carried out at the College Station of Texas A and M University [18]. The pond (surface area = 1.78 hectares) was dosed with anhydrous ammonia and the effects on the resident organisms were followed over a three-month period. Chemical, physical and biological data were taken 1 week prior to, on the day of, and at selected intervals following addition of ammonia. Ammonia nitrogen levels before treatment were 0.2–0.4 mg l^{-1}. The day after treatment the ammonia-nitrogen level stabilised at 37.7 mg l^{-1} and gradually declined to 5.0 mg l^{-1} after 3 months. The pH of the pond water before treatment was 6.9 but increased to 10.3

during treatment and remained above 9.0 for 2 weeks following treatment. Phytoplankton and zooplankton counts were reduced by 96 and 99 per cent, respectively, following treatment and aquatic vegetation was severely affected. The most adversely affected macroinvertebrates were crayfish and freshwater shrimps. A total fish kill was indicated since no live fish were caught by netting. Dead frogs and tadpoles were also observed in the pond.

In another study, freshwater macroinvertebrate communities were exposed to ammonia for 29 days in artificial streams or mesocosms [19]. After establishment, a range of species from the taxa Ephemeroptera, Plecoptera and Tricoptera were exposed at 16^{o}C to three ammonia concentrations based on the US EPA 1985 Chronic Criterion Value (CCV). The criterion units (nominal un-ionised ammonia concentration/US EPA CCV) were 1.9, 5.8 and 12 for the low, medium and high treatments. Only the mayflies *Deleatidium* sp. (Ephemeroptera: Leptophlebiidae) and *Coloburiscus humeralis* (Ephemeroptera: Oligoneuriidae) showed significant reductions in abundance at the high exposure treatment. The abundance of juvenile *Deleatidium* sp. had a negative concentration response that resulted in an 82 per cent decrease in abundance at the high treatment.

Table 2.6 Ammonia long-term aquatic toxicity data for freshwater organisms[1]

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (mg l^{-1})	Exposure[2]	Toxicant analysis[3]	Comments	Reliability index[4]	Reference
Mixed algal populations	**Various**	**ALG**	**LOEC**	**Abundance**	**14 days**	**3.0**	**s**	**m**	**No pH data**	**2**	**[18]**
Daphnia magna	Water flea	CRU	LOEC	Growth, reproduction	ND	1.6	psr	-	No pH data	-	[55]
Musculium transversum	Fingernail clam	MOL	NOEC	Mortality	28 days	0.23	-	-	No pH data	-	[57]
Musculium transversum	Fingernail clam	MOL	LOEC	Mortality	28 days	0.63	-	-	No pH data	-	[57]
Lumbriculus variegatus	Oligochaete worm	ANN	LC50	Mortality	10 days	0.37	s	-	pH 6.5	-	[56]
Lumbriculus variegatus	Oligochaete worm	ANN	LC50	Mortality	10 days	0.54	s	-	pH 7.2	-	[56]
Lumbriculus variegatus	Oligochaete worm	ANN	LC50	Mortality	10 days	0.63	s	-	pH 7.8	-	[56]
Lumbriculus variegatus	Oligochaete worm	ANN	LC50	Mortality	10 days	0.99	s	-	pH 8.6	-	[56]
Chironomus tentans	Midge	INS	LC50	Mortality	10 days	0.60	s	-	pH 6.5	-	[56]
Chironomus tentans	Midge	INS	LC50	Mortality	10 days	1.3	s	-	pH 7.2	-	[56]
Chironomus tentans	Midge	INS	LC50	Mortality	10 days	3.8	s	-	pH 7.8	-	[56]
Chironomus tentans	Midge	INS	LC50	Mortality	10 days	11.4	s	-	pH 8.6	-	[56]
Pteronarcella badia	Stonefly	INS	NOEC	Emergence	ND	3.4	pf	-	No pH data	-	[58]
Pteronarcella badia	Stonefly	INS	LC50	Mortality	24 days	1.5	pf	-	No pH data	-	[58]
Pteronarcella badia	Stonefly	INS	LC50	Mortality	30 days	4.4	pf	-	No pH data	-	[58]
Coloburiscus humeralis	Spiny gilled mayfly	INS	NOEC	Mortality	29 days	0.16	f	-	pH 7.99–8.75	-	[19]
Coloburiscus humeralis	Spiny gilled mayfly	INS	LOEC	Mortality	29 days	0.36	f	-	pH 7.99–8.75	-	[19]
Coloburiscus humeralis	Spiny gilled mayfly	INS	MATC	Mortality	29 days	0.23	f	-	pH 7.99–8.75	-	[19]
***Deleatidium* sp.**	**Mayfly**	**INS**	**NOEC**	**Mortality**	**29 days**	**0.066**	**f**	**m**	**pH 7.99–8.75**	**1**	**[19]**
Deleatidium sp.	Mayfly	INS	LOEC	Mortality	29 days	0.158	f	-	pH 7.99–8.75	-	[19]
Deleatidium sp.	Mayfly	INS	MATC	Mortality	29 days	0.102	f	-	pH 7.99–8.75	-	[19]
Cyprinus carpio	Carp (larvae)	FIS	LC50	Mortality	28 days	0.5	f	-	pH 7.22–7.88	-	[54]

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (mg l^{-1})	Exposure[2]	Toxicant analysis[3]	Comments	Reliability index[4]	Reference
Cyprinus carpio	Carp (embryos)	FIS	NOEC	Hatching	31 days	0.66	f	-	pH 7.66–7.93	-	[54]
Cyprinus carpio	Carp (hatchlings)	FIS	LOEC	Mortality	31 days	0.66	f	-	pH 7.66–7.93	-	[54]
Cyprinus carpio	Carp	FIS	NOEC	Growth	31 days	0.23	f	-	pH 7.66–7.93	-	[54]
Cyprinus carpio	Carp	FIS	LOEC	Growth	31 days	0.35	f	-	pH 7.22–7.88	-	[54]
Oncorhynchus mykiss	**Rainbow trout**	**FIS**	**LOEC**	**Growth**	**6 months**	**0.017**	**ND**	**ND**	**Low DO (5.8 mg l^{-1})**	**3**	**[51]**
Oncorhynchus mykiss	Rainbow trout	FIS	LOEC	Growth, survival	-	0.05	-	-	No pH data	-	[53]
Oncorhynchus mykiss	**Rainbow trout**	**FIS**	**LC50**	**Mortality**	**72 days**	**0.046**	**f**	**m**	**pH 7.4**	**2**	**[20]**
Oncorhynchus mykiss	**Rainbow trout**	**FIS**	**NOEC**	**Growth**	**1,460 days**	**0.07**	**f**	**m**	**pH 7.7**	**1**	**[21]**
Oncorhynchus mykiss	**Rainbow trout**	**FIS**	**LOEC**	**Mortality**	**73 days**	**0.022**	**f**	**m**	**pH 7.47–7.57**	**1**	**[22]**
Rutilus rutilus	Roach (embryos)	FIS	NOEC	Mortality	31 days	0.55	f	-	pH 7.68–7.98	-	[54]
Rutilus rutilus	Roach (embryos)	FIS	NOEC	Mortality	130 days	0.1	f	-	pH 7.77–7.81	-	[54]
Rana clamitans	**Green frog (tadpoles)**	**AMP**	**NOEC**	**Growth**	**114 days**	**1.0**	**f**	**m**	**pH 8.0–8.6**	**1**	**[23]**

[1] The lowest valid data for algal, invertebrate and fish species are highlighted in bold font.
[2] Exposure: s = static; f = flow-through; psr: presumably static replacement; pf = presumably flow-through.
[3] Toxicant analysis: m = measured.
[4] See Annex 1.
ALG = algae; ANN = annelids; CRU = crustaceans; FIS = fish; INS = insects; MOL = molluscs; AMP = amphibians
LOEC = lowest observed effect concentration
MATC = maximum allowable toxicant concentration
NOEC = no observed effect concentration
LC50 = concentration lethal to 50% of the organisms tested
ND = no data

Table 2.7 Ammonia short-term aquatic toxicity data for freshwater organisms[1]

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (mg l^{-1})	Exposure[2]	Toxicant analysis[3]	Comments	Reliability index[4]	Reference
Chlorella vulgaris	**Green alga**	**ALG**	**EC50**	**Abundance**	**5 days**	**2.0**	**s**	**ND**	**No pH data**	**4**	**[24]**
Chlorella vulgaris	Green alga	ALG	EC100	Abundance	5 days	4.5	s	-	No pH data	-	[24]
Brachionus rubens	Rotifer (neonates)	ROT	LC50	Mortality	24 hours	3.2	s	-	pH 7.4–7.8	-	[82]
Brachionus rubens	Rotifer (neonates)	ROT	LC50	Mortality	96 hours	4.6	ps	-	No pH data	-	[83]
Philodina acuticornus	Rotifer	ROT	EC50	Mortality	ND	2.4	s	-	No pH data	-	[63]
Daphnia magna	Water flea (neonates)	CRU	LC10	Mortality	ND	0.45	s	-	No pH data	-	[71]
Daphnia magna	Water flea (neonates)	CRU	LC50	Mortality	48 hours	1.7	s	-	No pH data	-	[73]
Daphnia magna	Water flea (neonates)	CRU	LC50	Mortality	48 hours	4.1	s	-	No pH data	-	[55]
Daphnia pulicaria	Water flea (neonates)	CRU	LC50	Mortality	48 hours	0.95	s	-	No pH data	-	[64]
Hyalella azteca	**Scud**	**CRU**	**LC50**	**Mortality**	**96 hours**	**0.16**	**s**	**m**	**pH 6.47–6.90**	**1**	**[25]**
Hyalella azteca	Scud	CRU	LC50	Mortality	96 hours	1.8	s	-	pH 7.29–7.83	-	[25]
Orconectes nais	Crayfish	CRU	LC50	Mortality	-	2.6	ps	-	No pH data	-	[65]
Orconectes nais	Crayfish	CRU	LC50	Mortality	-	3.1	ps	-	No pH data	-	[67]
Potamopyrgus jenkinsi	Gastropod	MOL	LC50	Mortality	96 hours	0.31	ps	-	No pH data	-	[96]
Tubifex tubifex	Tubificid worm	ANN	LC50	Mortality	-	2.21	ps	-	No pH data	-	[84]
Lumbriculus variegatus	Oligochaete worm	ANN	LC50	Mortality	96 hours	0.29	s	-	pH 6.47–6.9	-	[25]
Lumbriculus variegatus	Oligochaete worm	ANN	LC50	Mortality	96 hours	3.2	s	-	pH 7.29–7.83	-	[25]
Dendrocoelum lacterum	Flatworm	PLA	LC50	Mortality	-	1.2	ps	-	No pH data	-	[84]
Chironomus tentans	Midge	INS	LC50	Mortality	96 hours	0.53	s	-	pH 6.47–6.9	-	[25]
Chironomus tentans	Midge	INS	LC50	Mortality	96 hours	5.6	s	-	pH 7.29–7.83	-	[25]
Stenelmis sexineata	Beetle	INS	LC50	Mortality	-	6.6	ps	-	No pH data	-	[67]

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (mg l^{-1})	Exposure[2]	Toxicant analysis[3]	Comments	Reliability index[4]	Reference
Campostoma anomalum	Stoneroller	FIS	LC50	Mortality	96 hours	1.4	ps	-	No pH data	-	[86]
Catostomus commersoni	White sucker	FIS	LC50	Mortality	96 hours	1.1	ps	-	No pH data	-	[34]
Catostomus commersoni	White sucker	FIS	LC50	Mortality	96 hours	0.65	ps	-	No pH data	-	[86]
Castomus platyrhynchus	Mountain sucker	FIS	LC50	Mortality	96 hours	0.55	ps	-	No pH data	-	[87]
Clarias batrachus	Walking catfish	FIS	LC50	Mortality	96 hours	4.3	ps	-	No pH data	-	[69]
Cottus bairdi	Mottled sculpin	FIS	LC50	Mortality	96 hours	1.1	ps	-	No pH data	-	[88]
Cyprinus carpio	Carp	FIS	LC50	Mortality	96 hours	0.90	ps	-	No pH data	-	[74]
Cyprinus carpio	Carp	FIS	LC50	Mortality	96 hours	0.66	ps	-	No pH data	-	[97]
Cyprinus carpio	Carp	FIS	LC50	Mortality	96 hours	0.44	ps	-	No pH data	-	[97]
Etheostoma spectabile	Orange throat darter	FIS	LC50	Mortality	96 hours	0.74	ps	-	No pH data	-	[67]
Galaxias maculatus	Common jollytail (juveniles)	FIS	LC50	Mortality	96 hours	1.6	ps	-	pH 8.7–9.4	-	[76]
Hypophthalmichthys molitrix	Silver carp (fingerlings)	FIS	LC50	Mortality	96 hours	0.38	ps	-	No pH data	-	[97]
Hypophthalmichthys nobilis	Carp (fingerlings)	FIS	LC50	Mortality	96 hours	0.30	ps	-	No pH data	-	[97]
Lebistes reticulatus	Guppy	FIS	LC50	Mortality	96 hours	0.25	ps	-	pH 7.2–7.8	-	[70]
Lepomis cyanellus	Green sunfish	FIS	LC50	Mortality	96 hours	0.41	ps	-	pH 6.6	-	[76]
Lepomis cyanellus	Green sunfish	FIS	LC50	Mortality	96 hours	1.4	ps	-	pH 8.7	-	[76]
Leuciscus idus	Golden orfe	FIS	LOEC	Behaviour	0.1 days	1.2	ps	-	No pH data	-	[68]
Ictalurus punctatus	Channel catfish	FIS	LC50	Mortality	48 hours	1.0	ps	-	No pH data	-	[95]
Micropterus salmoides	Largemouth bass	FIS	LC50	Mortality	96 hours	0.82	ps	-	No pH data	-	[78]
Morone americana	White perch	FIS	LC50	Mortality	96 hours	0.12	ps	-	pH 6.0	-	[85]
Morone americana	White perch	FIS	LC50	Mortality	96 hours	0.43	ps	-	pH 8.0	-	[85]

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (mg l^{-1})	Exposure[2]	Toxicant analysis[3]	Comments	Reliability index[4]	Reference
Notemigonus cryoleucas	Golden shiner	FIS	LC50	Mortality	96 hours	0.98	ps	-	No pH data	-	[61]
Notemigonus cryoleucas	Golden shiner	FIS	LC50	Mortality	96 hours	0.59	ps	-	No pH data	-	[86]
Nothiobranchius guentheri	Killifish	FIS	LC50	Mortality	24 hours	0.57	ps	-	pH 7.75–7.82	-	[81]
Nothiobranchius guentheri	Killifish	FIS	LC50	Mortality	24 hours	1.2	ps	-	pH 7.75–7.82	-	[81]
Notoropsis spilopterus	Spotfin shiner	FIS	LC50	Mortality	96 hours	0.98	ps	-	No pH data	-	[77]
Notoropsis spilopterus	Spotfin shiner	FIS	LC50	Mortality	96 hours	1.1	ps	-	No pH data	-	[86]
Notropis lutrensis	Red shiner	FIS	LC50	Mortality	96 hours	0.88	ps	-	No pH data	-	[67]
Notropsis whipplei	Steelcolour shiner	FIS	LC50	Mortality	96 hours	1.0	ps	-	No pH data	-	[86]
Oncorhynchus kisutch	Coho salmon (fingerlings)	FIS	LC50	Mortality	96 hours	0.45	ps	-	No pH data	-	[62]
Oncorhynchus mykiss	**Rainbow trout**	**FIS**	**Unspecified Effect**	**Survival**	**48 hours**	**0.03**	**ps**	**n**	**No data**	**4**	**[52]**
Oncorhynchus mykiss	Rainbow trout	FIS	LC50	Mortality	96 hours	0.23	ps	-	pH 7.6–8.1	-	[89]
Oncorhynchus mykiss	Rainbow trout	FIS	LC50	Mortality	96 hours	0.11	ps	-	pH 6.51	-	[93]
Oncorhynchus mykiss	Rainbow trout	FIS	LC50	Mortality	96 hours	0.67	ps	-	pH 7.7–7.9	-	[92]
Oncorhynchus gorbuscha	**Pink salmon (alevins)**	**FIS**	**LC50**	**Mortality**	**96 hours**	**0.068**	**ps**	**m**	**No pH data**	**1**	**[26]**
Oncorhynchus tshawytscha	Chinook salmon	FIS	LC50	Mortality	96 hours	0.45	ps	-	No pH data	-	[80]
Oncorhynchus tshawytscha	Chinook salmon	FIS	LC50	Mortality	24 hours	0.30	ps	-	pH 7.6–7.9	-	[66]
Oncorhynchus tshawytscha	Chinook salmon	FIS	LC50	Mortality	24 hours	0.33	ps	-	No pH data	-	[87]
Pimephales promelas	Fathead minnow	FIS	LC50	Mortality	96 hours	1.0	ps	-	pH 6.0–9.0	-	[94]

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (mg l^{-1})	Exposure[2]	Toxicant analysis[3]	Comments	Reliability index[4]	Reference
Poecilia reticulata	Guppy (fry)	FIS	LC50	Mortality	96 hours	1.2	ps	-	No pH data	-	[79]
Prosopium williamsoni	Mountain whitefish	FIS	LC50	Mortality	96 hours	0.11	ps	-	No pH data	-	[87]
Salmo aguabonita	Golden trout	FIS	LC50	Mortality	96 hours	0.62	ps	-	No pH data	-	[88]
Salmo clarki	Cutthroat trout	FIS	LC50	Mortality	96 hours	0.52	ps	-	No pH data	-	[90]
Salmo salar (smolts)	Atlantic salmon	FIS	LC50	Mortality	24 hours	0.12	ps	-	No pH data	-	[59]
Salmo salar	Atlantic salmon	FIS	LC50	Mortality	24 hours	0.16	ps	-	pH 8.12	-	[60]
Salmo trutta	Brown trout	FIS	LC50	Mortality	96 hours	0.47	ps	-	No pH data	-	[72]
Salvelinus fontinalis	Brook trout	FIS	LC50	Mortality	96 hours	0.79	ps	-	No pH data	-	[87]
Stizostedion vitreum	Walleye	FIS	LC50	Mortality	96 hours	0.70	ps	-	No pH data	-	[55]
Tilapia aurea	Blue tilapia	FIS	LC50	Mortality	72 hours	2.3	ps	-	No pH data	-	[75]
Bufo americanus	American toad	AMP	NOEC	Growth, survival	3 days	>0.9	s	-	pH 8.7	-	[23]
Rana clamitans	**Green frog**	**AMP**	**NOEC**	**Growth, survival**	**4 days**	**0.6**	**s**	**-**	**pH 8.7**	1	**[23]**
Rana pipiens	Leopard frog	AMP	NOEC	Growth, survival	5 days	1.5	s	-	pH 8.7	-	[23]

1 The lowest valid L(E)C50s for algae, invertebrates and fish are highlighted in bold font.
2 Exposure: s = static; ps = presumably static.
3 Toxicant analysis: m = measured; n = nominal.
4 See Annex 1.
ALG = algae; AMP = amphibians; ANN = annelids; CRU = crustaceans; FIS = fish; INS = insects; MOL = molluscs; PLA = planarians; ROT = rotifers
LOEC = lowest observed effect concentration
MATC = maximum allowable toxicant concentration
NOEC = no observed effect concentration
ND = no data
ECx = concentration effective against X% of the organisms tested
LCx = concentration lethal to X% of the organisms tested

2.6.2 Toxicity to saltwater organisms

Single species acute toxicity data (i.e. typically from studies of ≤96-hour duration) for marine organisms are available for six different taxonomic groups, i.e. algae, rotifers, crustaceans, molluscs, echinoderms and fish, including the 'base set' taxa required for use of the assessment factor approach in the EU Technical Guidance Document [12].

Chronic toxicity data (i.e. typically from studies of ≥96-hour duration) are only available for algae, crustaceans (three species) and fish (two species). No results from higher tier mesocosm or field studies with marine aquatic organisms have been reported. Chronic and acute toxicity data for marine species are summarised in Tables 2.8 and 2.9, respectively. All the effects data in Tables 2.8 and 2.9 are expressed as un-ionised concentrations (as mg NH_3-N l^{-1}).

Figure 2.3 displays the cumulative distribution function of marine long-term data as given in Table 2.8 and Figure 2.4 displays the cumulative distribution function of marine short-term data as listed in Table 2.9. These curves do not represent species sensitivity distributions, but are instead a means of representing the range of long-term and short-term toxicity data available for marine species. Figure 2.4 contains data for a short-term study to investigate biochemical endpoints in fish, which resulted in a low toxicity value (0.001 mg NH_3-N l^{-1}) [46]. However, since the significance of the measured changes to the overall health of individuals and, ultimately, populations is uncertain this value has not been used in the derivation of the PNEC value.

Echinoderms are the most sensitive taxonomic group with respect to the acute toxicity of un-ionised ammonia, though there is overlap between the sensitivities of representatives of different taxonomic groups. However, the range of acute toxicity values was within approximately two orders of magnitude. A conclusion on the long-term toxicity of ammonia to different taxonomic groups cannot be drawn due to lack of data. The results of the available long-term tests with marine crustaceans and fish are in the range of results obtained for the corresponding freshwater species.

In several acute studies with fish species, the toxicity of un-ionised ammonia has been found to vary with salinity such that the acute effects decrease with increasing salinity to a minimum at a point which corresponds to approximately 10‰, and then increases again as the salinity approaches that of full strength (35‰) seawater [1]. During ammonia exposures in saline waters, whether chronic or episodic, fish may be more at risk if:

- they are larvae or juveniles;
- temperature is elevated;
- salinity is near the seawater value;
- the pH value is decreased.

Fish will further be at risk if they are not feeding, are stressed and if they are active and swimming [2].

The data for marine algae indicate that this taxonomic group is of lower sensitivity than other groups. This finding is consistent with the fact that ammonia represents a nitrogen source for these organisms which is required for growth.

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

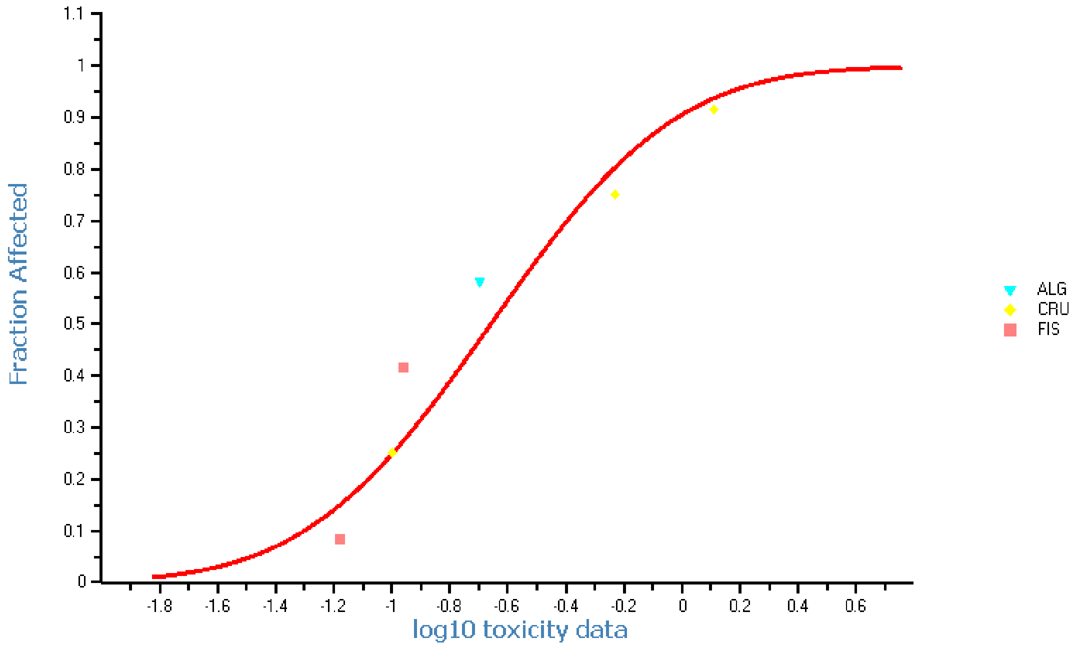


Figure 2.4 Cumulative distribution function of saltwater short-term data (µg l^{-1}) for un-ionised ammonia

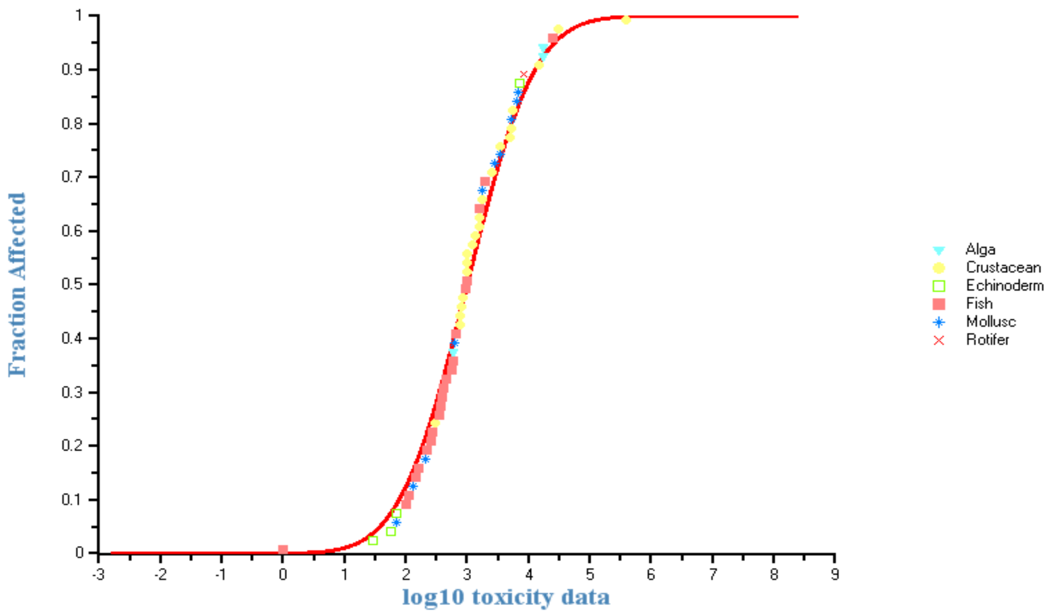


Table 2.8 Ammonia long-term toxicity data for marine organisms[1]

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration (days)	Conc. (mg l^{-1})	Exposure[2]	Toxicant analysis[3]	Comments	Reliability index[4]	Reference
Estuarine benthic diatoms	**Various**	**ALG**	**LOEC**	**Growth**	**10**	**0.2**	**s**	**m**		**1**	**[27]**
Penaeus setiferus	Shrimp	CRU	EC50	Growth	21	0.59	pf	-	No pH data	-	[28]
Penaeus japonica	Kuruma shrimp	CRU	LC50	Mortality	20	1.29	pf	-	No pH data	-	[98]
Macrobrachium rosenbergii	**Prawn**	**CRU**	**LOEC**	**Growth**	**42**	**0.10**	**f**	**m**	**No pH data**	**2**	**[28]**
Scophthalmus maximus	Turbot (juveniles)	FIS	NOEC	Growth	11	0.11	f	m	pH 6.8–7.9	1	[29]
Solea solea	**Sole (juveniles)**	**FIS**	**NOEC**	**Growth**	**42**	**0.066**	**f**	**m**	**pH 6.9–7.9**	**1**	**[29]**

1 The lowest valid toxicity values for algae, invertebrates and fish are highlighted in bold font.
2 Exposure: s = static; f = flow-through; pf = presumably flow-through.
3 Toxicant analysis: m = measured.
4 See Annex 1.
ALG = algae; CRU = crustaceans; FIS = fish
LOEC = lowest observed effect concentration
NOEC = no observed effect concentration
EC50 = concentration effective against 50% of the organisms tested
LC50 = concentration lethal to 50% of the organisms tested

Table 2.9 Ammonia short-term toxicity data for marine organisms[1]

Scientific name	Common name	Taxonomic group	End-point	Effect	Test duration (hours)	Conc. (mg l^{-1})	Expo-sure[2]	Toxicant analysis[3]	Comments	Reliability index[4]	Refer-ence
Dunaliella tertolecta	**Chlorophyte**	**ALG**	**EC50**	**Photosynthetic activity**	**1.5**	**17.0**	**s**	**n**	**pH 8.0–9.5**	**2**	**[30]**
Phaeodactylum tricornutum	**Diatom**	**ALG**	**EC50**	**Photosynthetic activity**	**1.5**	**17.0**	**s**	**n**	**pH 8.0–9.5**	**2**	**[30]**
Brachionus plicatilis	Rotifer	ROT	LC50	Mortality	96	8.6	s	-	pH 7.5–7.9	-	[113]
Niitocra spinipes	Copepod	CRU	LC50	Mortality	96	1.2	s	-	No pH data	-	[112]
Tisbe battagliai	Copepod (nauplii)	CRU	LC50	Mortality	96	0.79	s	-	No pH data	-	[44]
Crangon crangon	Brown shrimp	CRU	LC50	Mortality	24	1.6	s	-	No pH data	-	[32]
Penaeus chinensis	Fleshy prawn (juveniles)	CRU	LC50	Mortality	120	1.4	sr	-	pH 7.94	-	[102]
Penaeus monodon	Tiger prawn (juveniles)	CRU	LC50	Mortality	144	0.77	sr	-	pH 7.57	-	[103]
Penaeus monodon	Tiger prawn (juveniles)	CRU	LC50	Mortality	144	1.0	sr	-	pH 7.70	-	[101]
Penaeus pencillatus	Redtail prawn (juveniles)	CRU	LC50	Mortality	120	0.86	sr	-	pH 8.10	-	[100]
Homarus americanus	Lobster	CRU	LC50	Mortality	96	1.8	ps	-	No pH data	-	[104]
Ampelisca abdita	Amphipod	CRU	LC50	Mortality	96	0.83	s	-	pH 8.0	-	[110]
Eohausterius estuarius	Amphipod	CRU	LC50	Mortality	96	2.5	s	-	pH 8.0	-	[110]
Grandidierella japonica	Amphipod	CRU	LC50	Mortality	96	3.5	s	-	pH 8.0	-	[110]
Rhepoxynius abronius	Amphipod	CRU	LC50	Mortality	96	1.6	s	-	pH 8.0	-	[110]
Crassostrea gigas	Pacific oyster	CRU	LC50	Mortality	48	0.13	s	-	No pH data	-	[45]
Crassostrea virginica	Eastern oyster	MOL	LC50	Mortality	96	6.8	s	-	No pH data	-	[105]
Haliotis rufescens	Red abalone (larvae)	MOL	LC50	Mortality	48	0.07	s	-	ND	-	[99]
Mercenaria mercanaria	Quahog clam	MOL	LC50	Mortality	96	2.9	s	-	No pH data	-	[105]
Anadara granosa	Ark shell	MOL	LC50	Mortality	168	1.8	s	-	pH 7.9	-	[91]
Ruditapes phillipinarum	Ark shell	MOL	LC50	Mortality	168	0.64	s	-	pH 7.9	-	[91]
Argopecten irradians	Bay scallop	MOL	LC50	Mortality	96	5.3	s	-	pH 8.1	-	[111]
Dendaster excentricus	**Sand dollar (larvae)**	**ECH**	**LC50**	**Mortality**	**72**	**0.03**	**s**	**-**	**No pH data**	**4**	**[45]**

Scientific name	Common name	Taxonomic group	End-point	Effect	Test duration (hours)	Conc. (mg l^{-1})	Expo-sure[2]	Toxicant analysis[3]	Comments	Reliability index[4]	Refer-ence
Strongylocentrotus purpuratus	**Purple sea urchin (larvae)**	**ECH**	**LC50**	**Mortality**	**72**	**0.057**	**s**	**m**	**pH 8.0**	**1**	**[31]**
Agonus cataphractus	**Armed bullhead**	**FIS**	**LC50**	**Mortality**	**24**	**0.26**	**s**	**m[5]**	**No pH data**	**1**	**[32]**
Diplodus sargus	**Sea bream (larvae)**	**FIS**	**EC50**	**Feeding**	**24**	**0.10**	**s**	**m**	**No pH data**	**2**	**[33]**
Diplodus sargus	Sea bream (larvae)	FIS	LC50	Mortality	24	0.36	s	-	No pH data	2	[33]
Gaidropsarus carpensis	Rockling (larvae)	FIS	EC50	Feeding	24	0.11	s	-	No pH data	2	[33]
Gaidropsarus carpensis	Rockling (larvae)	FIS	LC50	Mortality	24	0.46	s	-	No pH data	2	[33]
Gasterosteus aculeatus	Stickleback	FIS	LC50	Mortality	96	1.6	s	-	No pH data		[106]
Lithognathus mormyrus	Sea bream (larvae)	FIS	EC50	Feeding	24	0.16	s	-	No pH data	2	[33]
Lithognathus mormyrus	Sea bream (larvae)	FIS	LC50	Mortality	24	0.38	s	-	No pH data	2	[33]
Morone saxatilus	Striped bass	FIS	LC50	Mortality	96	0.66	s	-	No pH data	-	[106]
Monacanthus hispidus	Planehead filefish	FIS	LC50	Mortality	96	0.57	s	-	No pH data	-	[114]
Mugil caphalaus	Striped mullet	FIS	LC50	Mortality	96	0.98	s	-	No pH data	-	[114]
Oncorhynchus mykiss	Rainbow trout (yearlings)	FIS	LC50	Mortality	24	0.59	s	-	pH 7.45	-	[107]
Oncorhynchus tschawytscha	Chinook salmon (parr)	FIS	LC50	Mortality	24	0.95	s	-	No pH data	-	[66]
Pachymetopa blochi	Sea bream (larvae)	FIS	LC50	Feeding	24	0.22	s	-	No pH data	2	[33]
Pachymetopa blochi	Sea bream (larvae)	FIS	LC50	Mortality	24	0.42	s	-	No pH data	2	[33]
Pagrus major	Red sea bream	FIS	LC50	Mortality	24	0.15	s	-	pH 8.11–8.97	-	[109]
Salmo salar	Atlantic salmon (smolts)	FIS	LC50	Mortality	24	0.27	s	-	No pH data	-	[107]
Sciaenops ocellatus	Red drum	FIS	LC50	Mortality	96	0.39	s	-	No pH data	-	[108]

1 The lowest valid L(E)C50s values for algae, invertebrates and fish are highlighted in bold font.
2 Exposure: s = static; sr = static replacement; ps = presumably static.
3 Toxicant analysis: m = measured; n = nominal.
4 See Annex 1.
5 Stock solution only measured.
ALG = algae; CRU = crustaceans; ECH = echinoderms; FIS = fish; MOL = molluscs; ROT = rotifers
EC50 = concentration effective against 50% of the organisms tested
LC50 = concentration lethal to 50% of the organisms tested; ND = no data

2.6.3 Toxicity to sediment-dwelling organisms

Ammonia does not accumulate in sediments. However, sediment-dwelling organisms (e.g. crustaceans, molluscs and most annelids) that use water in the boundary layer between the sediments and the water column for feeding or reproduction may be exposed to ammonia in the water column. Toxicity data on ammonia concentrations in sediment (e.g. on a mg/kg sediment basis) were not found.

2.6.4 Endocrine-disrupting effects

No information could be located on potential endocrine-disrupting effects of ammonia in relation to aquatic organisms.

2.6.5 Mode of action of ammonia

A number of mechanisms for the effect of un-ionised ammonia on the growth of fish have been proposed [22] including:

- gill damage eventually causing suffocation;
- alteration of biochemical mechanisms including stimulation of glycolysis and suppression of the Krebs cycle leading to a build-up of acidic metabolites causing death by acidosis;
- alteration of biochemical mechanisms leading to death from a depletion of ATP (adenosine 5′-triphosphate) in the basilar region of the brain;
- osmoregulatory disturbance;
- severe electrolyte imbalance;
- reduction in cellular K^+ levels and hyperexcitability;
- inhibition of ATP production by uncoupling oxidative phosphorylation;
- increase in cerebral glutamine levels leading to a decrease in the neuro-inhibitor GABA (γ-aminobutyric acid) causing hyperexcitability.

At present, no single definitive hypothesis for the toxicity of un-ionised ammonia to fish has been established.

2.6.6 Fate and occurrence of relevant metabolites in the aquatic environment

Ammonia is present in all natural waters, even if at only very low concentrations, and is derived either from the breakdown of organic nitrogen (mineralisation) or by the reduction of nitrate (denitrification). Although ammonia is produced in the natural nitrogen cycle, anthropogenic sources such as sewage treatment and industrial effluents and run-off from agricultural land are major contributors to the ammonia present in the aquatic environment.

Concentrations of ammonia in the aquatic environment are dependent on the direct inputs, oxidation and reduction within receiving waters and by volatilisation to and dissolution from the atmosphere. Total ammonia is generally measured in water and the concentration of un-ionised ammonia is subsequently calculated using information on the physico-chemical parameters of the receiving water [1]. In water, the important equilibrium is the acid-base equilibrium:

$$NH_{4(aq)}^{+} \rightleftharpoons NH_{3(aq)} + H^{+}_{(aq)}$$

This relationship is dependent on pH, as well as on temperature and ionic strength; thus, these factors must be taken into account when determining the relative proportions of ionised and (more toxic) un-ionised ammonia. At pH 8.5, the proportion of un-ionised ammonia is approximately 10 times that at pH 7.5 and, for every 9ºC increase in temperature, the proportion of un-ionised ammonia approximately doubles.

The main removal pathway for ammonia in water is via biological nitrification. Bacteria convert the ammonia into nitrate, creating an oxygen demand (BOD). The bacteria that oxidise ammonia to nitrite are largely of the genus *Nitrosomonas*, while conversion of nitrite into nitrate is carried out primarily by the genus *Nitrobacter*. Temperature, oxygen supply and pH of the water are key factors in determining the rate of oxidation.

In water, volatilisation is the most important abiotic fate pathway. Volatilisation is also an important process in the removal of ammonia from soils. Uptake by plants both in water and soil can also be a significant fate process. Photolysis is negligible under normal atmospheric conditions at wavelengths greater than 290 nm.

Based on its solubility, ammonia is not expected to adsorb to soil particulate matter, suspended solids or sediment to an appreciable degree. In addition, bioaccumulation of ammonia in biota is not considered of importance in the environment as it does not accumulate in lipid-rich tissues in the same manner as organic chemicals, even though levels of ammonia in the blood of exposed animals may increase.

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 accounting for the annual average concentration
For the freshwater environment, data are available for the 'base set' of toxicity tests (i.e. tests with algae, crustaceans and fish) and thus the EU Technical Guidance Document assessment factor method can be applied [12]. Among the taxonomic groups for which long-term toxicity tests are available (algae, crustaceans, molluscs, annelids, insects, fish and amphibians), fish are the most sensitive species.

The only long-term (lt) algal study involved mixed algal populations and a 14-day lowest observed effect concentration (LOEC) of 3.0 mg NH_3-N l^{-1} was derived [18]. The mesocosm study was carried out using a static regime and incorporated measurement of exposure concentrations. However, the study was unreplicated and only used one exposure concentration, which limits the reliability of the information. The data indicate lower sensitivity of algal species relative to invertebrates and fish; this is not unexpected given that ammonia represents a nitrogen source that is required for the growth of species from this taxon.

The lowest valid long-term toxicity data for freshwater invertebrates is a 29-day NOEC of 0.066 mg NH_3-N l^{-1} for lethality in the mayfly *Deleatidium* sp. [19]. The data from a simulated field study using artificial streams can be considered valid since it was carried out using a flow-though regime and incorporated measurement of exposure concentrations.

In hazard and risk assessments, the availability of data from multigenerational studies is considered to be particularly important for the derivation of robust longer-term PNEC values since such studies cover all potential sensitive life stages of the organisms tested. Thurston *et al.* [21] exposed rainbow trout fry over a 5-year period to five concentrations of un-ionised ammonia within the range 0.01 to 0.07 mg NH_3-N l^{-1}. The study examined the spawning ability, egg numbers and viability, growth of progeny, and mortality of parents and progeny, and histological examinations of the parental and F1 fish were performed. No effects were seen except for the common appearance of histopathological lesions in parental and F1 fish at concentrations as low as 0.016 mg NH_3-N l^{-1}. On exposure of the F1 generation from eggs to 10-months old, no effects on mortality or growth were seen up to and including the highest exposure concentration of 0.07 mg

NH_3-N l^{-1}. It was suggested that any early growth retardation that may have occurred as a result of ammonia exposure was compensated for during the subsequent months.

A number of other studies have also investigated the effects of long-term ammonia exposure on the early life stages of fish. In the development of proposed EQSs for ammonia [1], the lowest credible concentration of un-ionised ammonia at which long-term lethal effects were reported was 0.022 mg NH_3-N l^{-1} [22]. At this concentration, a cumulative mortality of 71 per cent was observed for eggs, larvae and fry of rainbow trout (*Oncorhynchus mykiss*) over 73-days exposure. The study can be considered valid since the method was defined in detail, a flow-through procedure was used and there was regular analysis of the exposure concentrations. No NOEC value was derived in the study since effects were observed at the lowest exposure concentration (0.022 mg NH_3-N l^{-1}). In addition, it was not possible to use the EU TGD approach to generate a NOEC from the LOEC because the level of effects at the LOEC (71 per cent) is too high for this approach to be valid. Instead, data on the steepness of the concentration–response curve for similar effects in fish have been evaluated. These indicate that there would normally only be in the region of 2–3 times difference in exposure concentration for a 50 per cent reduction in survival (from the controls) in an early life stage test [34, 35].

Calamari *et al.* [20] carried out tests with rainbow trout exposing the fertilised eggs and fry to low levels of un-ionised ammonia over a period of 72 days. The calculated LC50 was 0.046 mg NH_3-N l^{-1}, with experimental concentrations of 0.008 and 0.021 mg NH_3-N l^{-1} resulting in corrected mortalities of 15 and 23 per cent, respectively. However, the authors recognised there was uncertainty over these data because of the elevated concentrations of free carbon dioxide (CO_2) in the water and the high control mortality (30 per cent).

In a 6-month study, Smith and Piper [51] reported lesions and reduced growth in the gills and liver of rainbow trout exposed to 0.017 mg NH_3-N l^{-1}. However, the dissolved oxygen concentrations in the study were very low, averaging 5.8 mg l^{-1}. Consequently, this study is not regarded as of sufficient quality for the derivation of the PNEC.

A long-term (114 day) study initiated with tadpoles of the green frog (*Rana clamitans*) reported a NOEC for growth and survival of 1.0 mg NH_3-N l^{-1} [23]. The study can be considered valid since the test procedure is well-described, was carried out using a flow-through regime and incorporated measurement of exposure concentrations.

The $PNEC_{freshwater\_lt}$ should be derived on the basis of the 73-day LOEC of 0.022 mg NH_3-N l^{-1} and an assessment factor of 20 (2 for conversion of LOEC into NOEC and additional factor of 10), given the steep concentration–response curves for long-term effects of un-ionised ammonia, and the large body of data, including a multigenerational study with a sensitive species and a series of simulated ecosystem studies:

$PNEC_{freshwater\_lt}$ = 0.022 mg l^{-1}/AF (20) = 1.1 µg l^{-1} un-ionised ammonia

PNEC accounting for transient concentration peaks
Single species acute toxicity data are available for nine different taxonomic groups (i.e. algae, rotifers, crustaceans, molluscs, annelids, planarians, insects, fish and amphibians), with fish being the most sensitive group. A series of short-term (st) studies to investigate biochemical endpoints in fish are available and some of these resulted in

low toxicity values [46–50]. However, since the significance of the measured changes to the overall health of individuals and, ultimately, populations is uncertain these values have not been used in the derivation of the PNEC value.
The only short-term algal study was for the green alga *Chlorella vulgaris* for which a 5-day EC50 of 2.0 mg NH_3-N l^{-1} was derived [24]. There are issues with the validity of this study since it is not known whether it incorporated measurement of exposure concentrations. The data indicate lower sensitivity of algal species relative to invertebrates and fish; this is not unexpected given that ammonia represents a nitrogen source, which is required for the growth of species in this taxon.

The lowest short-term toxicity datum for freshwater invertebrates is a 96-hour LC50 of 0.16 mg NH_3-N l^{-1} for lethality in the scud *Hyalella azteca* [25]. This study can be considered valid since it is well-described and incorporated measurement of the exposure concentrations.

The reported acute (short-term) toxicity test data for fish covers a range of species, including salmonids and cyprinids. The lowest valid acute LC50 value is 0.068 mg NH_3-N l^{-1} for *Oncorhynchus gorbuscha* (pink salmon) alevins exposed to ammonia under static conditions for a 96-hour period [26]. Other test species found to be sensitive to ammonia over short exposure periods were *Oncorhynchus mykiss* (rainbow trout) and *Prosopium williamsoni* (mountain whitefish), with 96-hour LC50 values of 0.11 mg NH_3-N l^{-1} for both species (see Table 2.7).

A short-term PNEC for ammonia should be derived from the 96-hour LC50 value for pink salmon (*Oncorhynchus gorbuscha)* and guidance given in the TGD on effects assessment for intermittent releases (Section 3.3.2 of Part II of the TGD [12]). Given the large body of available data, only a reduced assessment factor of 10 (instead of 100) need be used in order to extrapolate from the 50 per cent acute effect level to the short-term no effect level.

$PNEC_{freshwater\_st}$ = 0.068 mg l^{-1}/AF (10) = 6.8 µg l^{-1} un-ionised ammonia

PNEC based on outdoor simulated ecosystem studies
In a 76-week exposure study of the effects of ammonia on microinvertebrates and fish in outdoor experimental streams, it was found that the lowest un-ionised ammonia concentrations that affected the growth of two fish species (channel catfish and white sucker respectively) were 0.011–0.026 mg NH_3-N l^{-1} and 0.040–0.074 mg NH_3-N l^{-1} [17]. However, the data from this study (see Section 2.6.1 for experimental details) cannot be used alone for the derivation of PNECs since effects were observed at the lowest exposure concentration (i.e. some NOECs were unbounded). The results of the study can be used to confirm the appropriateness of PNECs derived on the basis of other information. On this basis, the data from this simulated ecosystem study are consistent with the data from the chronic laboratory studies in which the lowest LOEC was 0.022 mg NH_3-N l^{-1} (see above).

3.1.2 PNECs for saltwaters

The effects database for marine species is smaller than that for freshwater organisms. Acute (short-term) toxicity data are available for six different taxonomic groups (i.e. algae, rotifers, crustaceans, molluscs, echinoderms and fish), while chronic (longer-term) data are only available for algae, crustaceans and fish.

The marine database is too small to draw firm conclusions on possible differences, particularly with regard to chronic effects. However, the toxicity data that are available for marine taxa do not appear to differ markedly from the range of values obtained for corresponding freshwater species (see Tables 2.6–2.9).

Since there are no obvious differences in the sensitivity of freshwater or saltwater species from the same taxonomic group, the TGD approach suggests that freshwater data can be used for the derivation of the marine PNEC, where appropriate. Therefore, the suggested freshwater PNECs for ammonia have been considered in the derivation of corresponding PNECs values for the protection of marine water bodies.

PNEC accounting for the annual average concentration
Long-term single species toxicity data for marine organisms are only available for algae, crustaceans (three species) and fish (two species) (Table 2.8).

The only long-term algal study was for mixed estuarine benthic diatom populations with a 10-day LOEC of 0.2 mg NH_3-N l^{-1} derived [27]. The study was carried out using a static regime and incorporated measurement of the exposure concentrations. The data indicate lower sensitivity of algal species relative to invertebrates and fish; this is not unexpected given that ammonia represents a nitrogen source, which is required for the growth of species in this taxon.

The lowest long-term toxicity data for marine invertebrates is a 42-day LOEC of 0.1 mg NH_3-N l^{-1} for growth in the prawn *Macrobrachium rosenbergii* [28]. The study was carried out using a flow-through regime and incorporated measurement of exposure concentrations.

The lowest available NOEC value for long-term effects of ammonia on marine biota is 0.066 mg NH_3-N l^{-1} for reduced growth of Dover sole *(Solea solea)* a flatfish species [29]. This study is considered valid since the test procedure used is well-defined, a flow-through procedure was used and there was regular analysis of the exposure concentrations.

The NOEC of 0.066 mg l^{-1} would normally be divided by an assessment factor of 100 according to the TGD provisions for marine effects assessment, applicable when three long-term tests of freshwater or saltwater species representing three trophic levels are available. However, this standard assessment factor can be reduced to 10 if short-term tests with marine species (e.g. molluscs or echinoderms) are available and the studies indicate that:

- these species do not belong to the most sensitive group;
- it can be determined with high probability that long-term NOECs generated for these marine groups would not be lower than those already obtained.

Short term tests with marine molluscs and echinoderms are available and the data indicate that echinoderms are of higher sensitivity than marine fish species (see Table 2.9).

On the basis of the limited current dataset, it is proposed to use the maximum assessment factor of 100 to obtain the $PNEC_{saltwater\_lt}$ on the basis of the NOEC of 0.066 mg NH_3-N l^{-1} calculated for absence of effects on the growth of the sole *Solea solea*.

$PNEC_{saltwater\_lt}$ = 0.066 mg l^{-1}/AF (100) = 0.66 µg l^{-1} un-ionised ammonia

The assessment factor of 100 could be reduced if longer-term data was available for echinoderms which are the most sensitive species in short-term tests (see below).

PNEC accounting for transient concentration peaks
Single species acute toxicity data for marine organisms are available for six different taxonomic groups (i.e. algae, rotifers, crustaceans, molluscs, echinoderms and fish), with echinoderms being the most sensitive group. A short-term study to investigate biochemical endpoints in fish is available that resulted in a low toxicity value for ammonia (0.001 mg NH_3-N l^{-1}) [46]. However, since the significance of the measured changes to the overall health of individuals and, ultimately, populations is uncertain this value has not been used in the derivation of the PNEC value.

The only short-term algal study was for the chlorophtye *Dunaliella tertolecta* and the diatom *Phaeodactylum tricornutum* with 1.5-hour EC50 values (for effects on photosynthetic activity) of 17.0 mg NH_3-N l^{-1} [30]. The study was carried out using a static regime but did not incorporate measurement of exposure concentrations. The data indicate lower sensitivity of algal species relative to invertebrates and fish; this is not unexpected given that ammonia represents a nitrogen source, which is required for the growth of species in this taxon.

The lowest reported short-term toxicity data for marine invertebrates is a 72-hour LC50 of 0.03 mg NH_3-N l^{-1} for lethality in larvae of the sand dollar [45]. However this in-house US EPA data could not be verified and does not appear on the US EPA ECOTOX database.[4] A study carried out as part of the Southern California Bight Pilot Project reported a 72-hour LC50 value of 0.057 mg NH_3-N l^{-1} for the mortality of larvae of the purple sea urchin *Strongylocentrotus purpuratus* [31]. The study was well-described and incorporated measurement of exposure concentrations.

Acute (short-term) toxicity test data are available for a range of marine fish species. The lowest recorded acute toxicity value for fish is a 24-hour EC50 of 0.1 mg NH_3-N l^{-1} for inhibition of first feeding in larvae of the sea bream (*Diplodus sargus*) [33]. In this study, the short-term sublethal endpoint was measured in the larvae of a number of marine species (*Gaidropsarus capensis*, *Diplodus sargus*, *Lithognathus mormyrus* and *Pachymetopon blochi*). However, there are issues with the experimental design used to measure feeding rate in this study. The next lowest short-term toxicity value was a 96-hour LC50 value of 0.26 mg NH_3-N l^{-1} for mortality of the armed bullhead *Agonus cataphractus* [32]. The study can be considered valid as there were measured data for the ammonia stock solution and the experimental concentrations were changed on a daily basis.

A PNEC to protect against short-term exposure to ammonia can be derived on the basis of general guidance given in the TGD on effects assessment for intermittent releases

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

(Section 3.3.2 of Part II of the TGD [12]) and the lowest reported acute toxicity value, the 72-hour LC50 of 0.057 mg l^{-1} for mortality of the larvae of the purple sea urchin *Strongylocentrotus purpuratus*. A reduced assessment factor of 10 can be used (instead of 100 which is considered overprotective) based on data for key marine species (molluscs and echinoderms) in order to extrapolate from the 50 per cent acute sublethal effect level to the short-term no effect level.

$PNEC_{saltwater\_st}$ = 0.057 mg l^{-1}/AF (10) = 5.7 µg l^{-1} un-ionised ammonia

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 species sensitivity distribution (SSD) approach cannot be used for PNEC derivation.

3.3 Derivation of existing EQSs

In the 1988 report [1], the European Inland Fisheries Advisory Commission (EIFAC) standard of 0.021 mg NH_3-N l^{-1} expressed as a 95th percentile for the protection of freshwater fish was considered too stringent, particularly for cyprinid fisheries. A separate standard for nondesignated waters would, therefore, be justified.

As a result, it was proposed that, as much of the data was from chronic exposure tests, the EQS should be expressed as an annual average concentration. Analysis of distributions for un-ionised ammonia concentrations suggested that the ratio between the 95th percentile and average concentrations was generally between 2 and 4. A conservative ratio of 2 was initially applied to the EIFAC standard to express it as an annual average of 0.01 mg NH_3-N l^{-1}. However, since this standard was already considered to be too protective, **15 µg NH_3-N l^{-1}** (equivalent to 0.018 mg NH_3 l^{-1}) expressed as an annual average was tentatively suggested as a suitable EQS for the protection of freshwater fish in nondesignated waters.

On the basis of laboratory and field data, the report assumed that this long-term freshwater standard for protecting fish populations would also safeguard other freshwater life and so no additional standard was proposed.

For the long-term protection of marine species, reliance was placed on data from laboratory tests. The standard was based on EC10 values in the range of 0.03–0.13 mg NH_3-N l^{-1} obtained for five species, this being the nearest approximation to a ‘no observed effect level’, and on the results of other toxicity tests. There was also evidence that, at intermediate salinities, the acute toxicity of ammonia was reduced somewhat relative to that in freshwater. At salinities approaching full-strength seawater, however, the LC50 values were found to be similar to those obtained for freshwater. Therefore, for the protection of marine fish, an EQS of **21 µg NH_3-N l^{-1}** expressed as an annual average concentration of un-ionised ammonia was proposed.

The few data on the toxicity of ammonia to algae and invertebrates suggested that the tested species would be relatively unaffected at the concentrations present in tidal waters. In view of the limited data, it was not considered appropriate to propose an EQS for the protection of other saltwater life.

No short-term standards were proposed.

3.4 Derivation of PNECs for sediment

Given the chemical structure of ammonia, the octanol–water partition coefficient (log Kow) is likely to be low. This is confirmed by the measured log Kow value of 0.23 (see Section 2.5). Ammonia does not accumulate in sediments (see Section 2.6) and the derivation of sediment PNECs for the protection of benthic organisms is not required.

3.5 Derivation of PNECs for secondary poisoning of predators

3.5.1 Mammalian and avian toxicity data

There have not been any formal studies on the effects of ammonia on reproduction, embryotoxicity or teratogenicity in mammals using modern internationally recognised test procedures. No systematic conclusions were drawn from studies undertaken to investigate the effects of ammonia in hen houses on the egg-laying performance of intensively reared poultry [4].

There is also no evidence that ammonia is mutagenic or carcinogenic in mammals. However, there is evidence that ammonia can produce inflammatory lesions of the colon and cellular proliferation, which could increase susceptibility to malignant change. Evidence was found that ammonia was responsible for the increased incidence of tumours with increased dietary protein intake. Ammonia neither caused tumours nor increased the spontaneous incidence of tumours in life-time studies on mice [4].

3.5.2 PNECs for secondary poisoning of predators

There is no evidence that ammonia bioaccumulates in marine biota, as the log Kow value is 0.23. Since the trigger of BCF >100 (log Kow >3) is not met, the derivation of PNECs to protect against secondary poisoning of predators is not required.

4. Analysis and compliance

Water and wastewater samples can be analysed for ammonia by US EPA Test Methods 1689 [36], 1690 [37], and 349.0 [38]. Analogous procedures have also been approved by the American Public Health Association (APHA), the American Water Works Association (AWWA) and the Water Environment Federation (WEF), e.g. Method APHA 4500 [39]. These methods are suitable for drinking and surface freshwaters, saline waters, domestic and industrial effluents, and biosolids. Ammonia is reported as ammonia nitrogen and includes the sum of NH_3 and NH_4^+.

Two methods that are suitable for water employ colorimetric techniques: Nesslerization and phenate methods. Nessler's reagent, an alkaline mixture of mercuric and potassium iodide, produces a yellow to brown colour with ammonia, whereas the phenate reagent (alkaline phenol and hypochlorite) produces a blue colour [36, 37, 39].

In the titrimetric method, the distillate is titrated with standard sulfuric acid and an appropriate indicator. The ammonia electrode employs a hydrophobic gas-permeable membrane to separate the sample solution from an internal ammonium chloride solution. Ammonia diffusing through the membrane changes the pH of the internal solution and is sensed by a pH electrode.

For determining NH_3-N concentrations above 5 mg l^{-1}, the titrimetric and ammonia-selective electrode methods are preferred. In contrast, gas chromatography/mass spectrometry methods have been developed that permit NH_3 detection at concentrations near 20 µg l^{-1} for environmental waters [40].

When ammonia is found in biological materials at physiological pH (7.2), most of it (99 per cent) will be found as the ammonium ion due to its pKa of 9.2.

In the determination of ammonia or ammonium ion in biological samples, ammonia is first liberated by distillation, aeration, ion-exchange chromatography, microdiffusion or deproteinisation [41]. The protein-free supernatant can be assayed colorimetrically for ammonia. Traditionally, Kjeldahl distillation methods have been used to determine ammonia levels in biological tissue, but other methods (e.g. colorimetric or ion-specific electrodes) are also available. In the Kjeldahl distillation, ammonia is distilled and subsequently trapped in acid and analysed titrametrically or colorimetrically. Other techniques use the ammonia-selective electrode and enzymatic assays. Discrepancies have been reported between results using electrodes and those using more specific enzymatic procedures because the ammonia electrode responds to both ammonia and volatile amines [42]. Chromatographic separation of ammonia and volatile amines after derivatisation has also been used to obtain specificity [41, 43].

The lowest proposed PNEC derived for un-ionised ammonia is 0.66 µg l^{-1}. However, total ammonia is typically measured in monitoring programmes along with physico-chemical parameters (pH, temperature and conductivity or salinity) and un-ionised ammonia is then calculated. To provide adequate precision and accuracy, the data quality requirements are that, at a third of the EQS, total error of measurement should not exceed 50 per cent. From the literature, it can be seen that analytical methodologies provide detection limits of around 20 µg l^{-1}, which suggests that current analytical methodologies do not offer adequate performance to analyse ammonia for compliance with the TGD-derived PNECs for water.

5. Conclusions

5.1 Availability of data

Throughout this report, toxicity data are expressed as un-ionised ammonia. This effectively removes the need to account for banding of standards due to pH and temperature. Methods for estimating concentrations of ionised and un-ionised ammonia are well developed and are based on an understanding of its speciation in water.

Acute toxicity data are available for algae, rotifers, crustaceans, molluscs, annelids, planarians, insects, fish and amphibians; chronic toxicity data are also available for algae, crustaceans, molluscs, annelids, insects, fish and amphibians. Data are particularly plentiful for fish and one semi-field study with freshwater organisms is also available.

Fish are clearly the most sensitive species with regard to both chronic and acute effects of ammonia. However, there is overlap between the sensitivities of representatives of different taxonomic groups and the range of acute toxicity values from all taxa is within approximately two orders of magnitude. Algae appear to be of lower sensitivity, probably because they can utilise ammonia as a nitrogen source.

Fewer acute toxicity data are available for marine organisms but include algae, rotifers, crustaceans, molluscs, echinoderms and fish. Chronic saltwater data are available only for algae, crustaceans (three species) and fish (two species). Echinoderms are the most sensitive taxonomic group with respect to the acute toxicity of un-ionised ammonia. However, the range of acute toxicity values is within approximately two orders of magnitude. There are too few data to draw conclusions about the chronic toxicity of ammonia to different taxonomic groups, although the sensitivities of marine crustaceans and fish are in the range obtained for the corresponding freshwater species.

The data suggest that fish exposed under saline conditions may be more at risk if:

- they are larvae or juveniles;
- temperature is elevated;
- salinity is near the seawater value;
- the pH is low.

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

The lowest credible concentration of un-ionised ammonia at which long-term effects were found is 0.022 mg NH_3-N l^{-1} when a cumulative mortality of 71 per cent was observed for eggs, larvae and fry of rainbow trout (*Oncorhynchus mykiss*) over 73 days exposure. A NOEC value was not derived in the study since effects were observed at the lowest exposure concentration. However, data on the concentration–response curve for similar effects in other fish have been evaluated and these indicate 2–3 times difference in exposure concentration for a 50 per cent reduction in survival (from the controls) in such an early life stage test.

The $PNEC_{freshwater\_lt}$ can be derived based on the 73-day LOEC of 0.022 mg NH_3-N l^{-1} to which a factor of 2 is applied to estimate a NOEC and an assessment factor of 10 to extrapolate to a $PNEC_{freshwater\_lt}$ of 1.1 µg l^{-1} un-ionised ammonia. This factor is justified on the basis of a large body of data, including a multigenerational study with a sensitive species and a series of simulated ecosystem studies.

Existing EQSs were developed in 1988 for the protection of freshwater fish (both cyprinids and salmonids) and are appreciably less stringent than those proposed here. Significantly, invertebrate data were not considered at the time due to a lack of data. In European Commission designated waters, the standards are 0.021 and 0.78 mg l^{-1} for un-ionised ammonia and total ammonia, respectively. In non-EC designated waters, an EQS of 15 µg l^{-1} un-ionised ammonia was developed. This was based on the 95th percentile estimated from field data to which a factor of 2 was applied, and would normally be expressed as an annual average.

5.2.2 Short-term PNEC for freshwaters

Fish are the most sensitive taxon to acute exposure with the lowest valid acute LC50 value being 0.068 mg NH_3-N l^{-1} for *Oncorhynchus gorbuscha* (pink salmon) alevins exposed to ammonia for a 96-hour period. Similar sensitivity was evident in other studies with the same species and *Prosopium williamsoni* (mountain whitefish) and the crustacean *Hyalella* were only slightly less sensitive.

The recommended $PNEC_{freshwater\_st}$ of 6.8 µg l^{-1} un-ionised ammonia is based on the 96-hour LC50 for pink salmon and application of a reduced assessment factor of 10 (instead of 100) since this datum is supported by an extensive body of toxicological data for a wide range of taxa.

There is no existing short-term EQS for ammonia.

5.2.3 Long-term PNEC for saltwaters

The marine dataset is too small to draw firm conclusions on possible differences between the sensitivities of freshwater and saltwater organisms, particularly with regard to chronic effects. However, marine taxa do not appear to differ markedly from the range of sensitivities obtained for corresponding freshwater species. Consequently, freshwater toxicity data for ammonia have been considered in the derivation of PNEC values for the protection of marine life.

The lowest reliable NOEC for long-term effects of ammonia on marine biota is 0.066 mg NH_3-N l^{-1} for growth of Dover sole (*Solea solea*). Short-term test data are available that indicate this may not be the most sensitive taxonomic group (molluscs and echinoderms are more sensitive following acute exposure) and so an assessment factor of 100 is recommended to protect these taxa, resulting in a PNECsaltwater_lt of 0.66 µg l^{-1} un-ionised ammonia.

The existing EQS for the protection of saltwater fish and shellfish for un-ionised ammonia is appreciably less stringent at 0.021 mg l^{-1}, expressed as an annual average concentration. This was based on a combination of acute toxicity data to fish and threshold concentrations inferred from field data. A recent report [2] for the Environment Agency on ammonia in estuaries concluded that the proposed Habitats Directive threshold value for un-ionised ammonia of 0.021 mg l^{-1}may be protective in good quality waters but may not be protective in poor quality waters.

5.2.4 Short-term PNEC for saltwaters

The most sensitive species in laboratory studies was the sea urchin, *Strongylocentrotus purpuratus*, for which a reliable 72-hour LC50 of 0.057 mg l^{-1} for larval survival is reported.

A PNEC to protect against short-term exposure to ammonia can be derived on the basis of an assessment factor of 10 applied to this datum, resulting in a $PNEC_{saltwater\_st}$ of 5.7 µg l^{-1} un-ionised ammonia.

There is no existing EQS for short-term exposures to marine life.

5.2.5 PNEC for secondary poisoning and sediments

Ammonia does not accumulate in sediments and the derivation of sediment PNECs for the protection of benthic organisms is not required. Given the high water solubility of ammonia, bioaccumulation is not considered important.

Table 5.1 Summary of proposed PNECs

Receiving medium/exposure scenario	Proposed PNEC (µg l^{-1} un-ionised ammonia)	Existing EQS (µg l^{-1} un-ionised ammonia)
Freshwater/long-term	1.1	15
Freshwater/short-term	6.8	No standard
Saltwater/long-term	0.66	21
Saltwater/short-term	5.7	No standard

5.3 Analysis

Total ammonia is typically measured in monitoring programmes along with physico-chemical parameters (pH, temperature and conductivity or salinity) and un-ionised ammonia is then calculated. Current analytical methodologies provide detection limits of around 20 µg l^{-1}, which do not offer adequate sensitivity to analyse ammonia with the proposed PNECs.

5.4 Implementation issues

Before PNECs for un-ionised ammonia can be adopted as EQSs, it will be necessary to address the following issues:

1. Current analytical methods are not sensitive enough to assess compliance with proposed PNECs in receiving waters. This will require further investigation.

2. Data that describe effects in the field are available through ecological classification programmes. These could help inform the PNEC derivation and may influence the final outcome.

3. As an interim measure, existing EQSs may be used but these are relevant only to long-term exposure. There are no interim options for short-term exposure to ammonia.

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List of abbreviations

AA	annual average
AF	assessment factor
APHA	American Public Health Association
AWWA	American Water Works Association
BCF	bioconcentration factor
CAS	Chemical Abstracts Service
CCV	Chronic Criterion Value
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
EHC	Environmental Health Criteria
EIFAC	European Inland Fisheries Advisory Commission
EQS	Environmental Quality Standard
GLP	Good Laboratory Practice (OECD)
IUPAC	International Union of Pure and Applied Chemistry
LC50	concentration lethal to 50% of the organisms tested
LCx	concentration lethal to X% of the organisms tested
LOEC	lowest observed effect concentration
lt	long term
MATC	maximum allowable toxicant concentration
NA	not applicable
ND	no data
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
TGD	Technical Guidance Document
UKTAG	UK Technical Advisory Group
US EPA	US Environmental Protection Agency

WEF	Water Environment Federation
WFD	Water Framework Directive
WHO	World Health Organization

ANNEX 1 Data quality assessment sheets

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

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

Table A1 Klimisch Criteria*

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

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

Reference number	18

Information on the test species	
Test species used	Range of phytoplankton, zooplankton, invertebrate, fish and amphibian species
Source of the test organisms	Resident organisms in pond at Texas A and M University College Station
Holding conditions prior to test	Not stated
Life stage of the test species used	Range of life stages present at start of the study

Information on the test design	
Methodology used	The test method is described.
Form of the test substance	Anhydrous ammonia
Source of the test substance	Not stated
Type and source of the exposure medium	Pond water
Test concentrations used	Single exposure concentration of 37.7 mg NH_3 l^{-1} at start of the test
Number of replicates per concentration	1
Number of organisms per replicate	Not relevant
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static, 3 months, no feeding
Measurement of exposure concentrations	Yes (at beginning of study and throughout the exposure period)
Measurement of water quality parameters	pH and temperature
Test validity criteria satisfied	Not relevant
Water quality criteria satisfied	Not relevant
Study conducted to GLP	Not stated
Overall comment on quality	Moderate

Reliability of study	The limited study as performed is reliable.
Relevance of study	The endpoints measured (species abundance and diversity) are relevant to the assessment of effects on pond ecology.
Klimisch Code	**2**

Reference number	19

Information on the test species	
Test species used	Macroinvertebrate representatives of Ephemeroptera, Plecoptera and Tricoptera
Source of the test organisms	Local source
Holding conditions prior to test	Artificial streams at the National Institute of Water and Atmospheric Research, Hamilton
Life stage of the test species used	Range of life stages

Information on the test design	
Methodology used	The test procedure is described.
Form of the test substance	Not known
Source of the test substance	Not known
Type and source of the exposure medium	Clean freshwater
Test concentrations used	Control, low, medium and high based on the ratio of nominal ammonia concentrations against US EPA Chronic Criterion Values: 0, 1.9, 5.8 and 12 Criterion Units
Number of replicates per concentration	Three streams per treatment
Number of organisms per replicate	Not relevant
Nature of test system (static, semi-static or flow-through, duration, feeding)	Flow-through, 29 days, no additional feeding
Measurement of exposure concentrations	Yes
Measurement of water quality parameters	pH and temperature
Test validity criteria satisfied	Not relevant
Water quality criteria satisfied	Not relevant
Study conducted to GLP	Not relevant
Overall comment on quality	Good

Reliability of study	The study is reliable.
Relevance of study	The endpoints measured (species abundance and diversity) are relevant to the assessment of effects on macroinvertebrate communities.
Klimisch Code	**1**

Reference number	20

Information on the test species	
Test species used	*Oncorhynchus mykiss* (formerly *Salmo gairdneri)*
Source of the test organisms	Obtained from hatchery
Holding conditions prior to test	Flow-through tanks
Life stage of the test species used	Test initiated with eggs one day after fertilisation

Information on the test design	
Methodology used	The test procedure is described.
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Uncontaminated hard water (310 mg l^{-1} $CaCO_3$)
Test concentrations used	0, 0.008, 0.021, 0.05, 0.08, 0.15 mg l^{-1} un-ionised ammonia
Number of replicates per concentration	1
Number of organisms per replicate	>250 in all tanks
Nature of test system (static, semi-static or flow-through, duration, feeding)	Flow-through, 72 day, feeding post yolk-sac stage
Measurement of exposure concentrations	Yes (every 3 days)
Measurement of water quality parameters	pH, temperature, dissolved oxygen, hardness and alkalinity, nitrate and nitrite
Test validity criteria satisfied	Not stated but control mortality over the 72-hour period was 30%.
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	Good

Reliability of study	The study is reliable but there are issues regarding the elevated control mortality.
Relevance of study	The endpoints measured (growth and survival) are relevant to the assessment of effects on fish populations.
Klimisch Code	**2**

Reference number	**21**

Information on the test species	
Test species used	*Oncorhynchus mykiss* (formerly *Salmo gairdneri)*
Source of the test organisms	Obtained from Ennis (Montana) National Fish Hatchery
Holding conditions prior to test	Flow-through tanks
Life stage of the test species used	All life stages in 5-year multigenerational study

Information on the test design	
Methodology used	The test procedure is described.
Form of the test substance	Reagent grade ammonium chloride
Source of the test substance	Not stated
Type and source of the exposure medium	Natural groundwater from a spring
Test concentrations used	0 (control), five concentrations in the range 0.01–0.07 mg l^{-1} un-ionised ammonia
Number of replicates per concentration	1
Number of organisms per replicate	10 males and 20 females in each test trough at the start of the test
Nature of test system (static, semi-static or flow-through, duration, feeding)	Flow-through system, feeding where required for life stage, 1,460 days duration
Measurement of exposure concentrations	Yes (every 4–7 days)
Measurement of water quality parameters	pH, temperature, dissolved oxygen, hardness and alkalinity, nitrate and nitrite
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	High

Reliability of study	The study is reliable.
Relevance of study	The endpoints measured (growth, reproduction and survival) are relevant to the assessment of effects on fish populations.
Klimisch Code	**1**

Reference number	22

Information on the test species	
Test species used	*Oncorhynchus mykiss* (formerly *Salmo gairdneri)*
Source of the test organisms	Not stated
Holding conditions prior to test	Flow-through tanks
Life stage of the test species used	Eggs after fertilisation

Information on the test design	
Methodology used	The test procedure is described.
Form of the test substance	Analytical grade ammonium chloride
Source of the test substance	Not stated
Type and source of the exposure medium	Natural, hard, unchlorinated borehole water
Test concentrations used	0 (control), 2.55, 4.52, 8.00, 15.50 and 25.77 mg l^{-1} total ammonia
Number of replicates per concentration	1
Number of organisms per replicate	591–737 eggs in each test vessel at start of test
Nature of test system (static, semi-static or flow-through, duration, feeding)	Flow-through system, no feeding, 73 days
Measurement of exposure concentrations	Yes (frequency not stated)
Measurement of water quality parameters	pH, temperature, dissolved oxygen, hardness
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	High

Reliability of study	The study is reliable but no NOEC could be calculated as effects were evident at the lowest exposure concentration.
Relevance of study	The endpoints measured (growth and survival) are relevant to the assessment of effects on fish populations.
Klimisch Code	**1**

Reference number	23

Information on the test species	
Test species used	*Bufo americanus (*American toad*), Rana clamitans (green frog)* and *Rana pipens* (leopard frog)
Source of the test organisms	*Bufo americanus* from a pond in Washburn County, Wisconsin, USA; *Rana clamitans* from a pond in Dane County, Wisconsin, USA; *Rana pipens* from a supplier Nasco (Fort Atkinson, Wisconsin, USA)
Holding conditions prior to test	Used immediately on receipt for the studies
Life stage of the test species used	Tadpoles

Information on the test design	
Methodology used	The test procedure is described.
Form of the test substance	Ammonium chloride
Source of the test substance	Sigma Chemical Company
Type and source of the exposure medium	Hard dechlorinated, charcoal filtered water (324 mg l^{-1} $CaCO_3$)
Test concentrations used	*B. americanus*: 0, 0.055, 0.11, 0.26, 0.69 and 0.86 mg l^{-1} un-ionised ammonia *R. clamitans*: 0, 0.098, 0.17, 0.35, 0.67, 0.85 mg l^{-1} un-ionised ammonia *R. pipiens*: 0, 0.16, 0.52, 1.37 and 2.22 mg l^{-1} un-ionised ammonia
Number of replicates per concentration	Three (*B. americanus* and *R. clamitans*) and five (*R. pipiens*)
Number of organisms per replicate	30 in each test vessel
Nature of test system (static, semi-static or flow-through, duration, feeding)	Semi-static (daily renewal of solutions), 3-5 days, no feeding
Measurement of exposure concentrations	Yes (immediately before (initial) and after (final) test solution was renewed)
Measurement of water quality parameters	pH and temperature
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	Good

Reliability of study	The study is reliable.
Relevance of study	The endpoint measured (survival) is relevant to the assessment of effects on fish populations.
Klimisch Code	**1**

Reference number	**24**

Information on the test species	
Test species used	*Chlorella vulgaris*
Source of the test organisms	Not known
Holding conditions prior to test	Not known
Life stage of the test species used	Not known

Information on the test design	
Methodology used	Not known
Form of the test substance	Not known
Source of the test substance	Not known
Type and source of the exposure medium	Not known
Test concentrations used	Not known
Number of replicates per concentration	Not known
Number of organisms per replicate	Not known
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Not known
Measurement of exposure concentrations	Not known
Measurement of water quality parameters	Not known
Test validity criteria satisfied	Not known
Water quality criteria satisfied	Not known
Study conducted to GLP	Not known
Overall comment on quality	-

Reliability of study	Not known
Relevance of study	The endpoints (growth and survival) measured are relevant to the assessment of effects on algal populations.
Klimisch Code	**4**

Reference number	25

Information on the test species	
Test species used	*Hyalella azteca* (Scud) freshwater amphipod
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	The test procedure is described.
Form of the test substance	Ammonium chloride solution
Source of the test substance	Not stated
Type and source of the exposure medium	Spike formulated and natural sediments
Test concentrations used	Spiked formulated sediment: 0, 500 and 1000 mg N l^{-1} Spiked natural sediment: 0, 750, 2000 and 3000 mg N l^{-1}
Number of replicates per concentration	Four
Number of organisms per replicate	10
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static, 96 hours, feeding
Measurement of exposure concentrations	Yes (in sediment pore water on days 0 and 4 and in overlying water on day 2)
Measurement of water quality parameters	pH, temperature and hardness
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	Good

Reliability of study	The study is reliable.
Relevance of study	The endpoint measured (survival) is relevant to the assessment of effects on invertebrate populations.
Klimisch Code	**1**

Reference number	26

Information on the test species	
Test species used	*Oncorhynchus gorbuscha (Pink salmon)*
Source of the test organisms	Fertilised eggs obtained from Lovers Cove Creek, Southern Baranof Island, Alaska
Holding conditions prior to test	Incubators supplied with flowing freshwater
Life stage of the test species used	Eyed eggs, alevins and fry

Information on the test design	
Methodology used	The test procedure is well-described.
Form of the test substance	Ammonium sulfate
Source of the test substance	Not stated
Type and source of the exposure medium	'Clean' freshwater
Test concentrations used	Range of un-ionised concentrations
Number of replicates per concentration	1
Number of organisms per replicate	25 animals per test vessel
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static, no feeding, 96-hour duration
Measurement of exposure concentrations	Yes (total ammonia)
Measurement of water quality parameters	pH, temperature and conductivity
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	Good

Reliability of study	The study is reliable.
Relevance of study	The endpoint measured (survival) is relevant to the assessment of effects on invertebrate populations.
Klimisch Code	**1**

Reference number	27

Information on the test species	
Test species used	Ten species of benthic diatoms
Source of the test organisms	Isolated from field samples from the Eems-Dollard estuary
Holding conditions prior to test	Maintained as stock cultures at 15 or 30‰ depending on the salinity of the original habitat
Life stage of the test species used	Growth phase

Information on the test design	
Methodology used	The test procedure is described.
Form of the test substance	Ammonium chloride
Source of the test substance	Not stated
Type and source of the exposure medium	Synthetic growth media
Test concentrations used	0, 3.1, 16.5, 31, 77.5 and 481 mg NH_3-N l^{-1}
Number of replicates per concentration	5
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static, 10 days, no feeding
Measurement of exposure concentrations	Yes (at end of the test)
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	Good

Reliability of study	The study is reliable.
Relevance of study	The endpoint measured (growth as chlorophyll content) is relevant to the assessment of effects on algal populations.
Klimisch Code	**1**

Reference number	28

Information on the test species	
Test species used	*Macrobrachium rosenbergii* (prawn)
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	The test procedure is described.
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	6 weeks 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
Study conducted to GLP	Not stated
Overall comment on quality	Limited data with which to assess study, but appears valid.

Reliability of study	Appears reliable.
Relevance of study	Growth endpoint
Klimisch Code	**2**

Reference number	29

Information on the test species	
Test species used	Dover sole, *Solea solea* (L.) and turbot, *Scophthalmus maxiums* (L.)
Source of the test organisms	Fish reared at Port Erin laboratory from eggs produced by captive spawning stocks of wild origin
Holding conditions prior to test	Flow-through holding tanks
Life stage of the test species used	Juveniles

Information on the test design	
Methodology used	The test procedure is well described.
Form of the test substance	Ammonium chloride
Source of the test substance	Not stated
Type and source of the exposure medium	Seawater
Test concentrations used	Range of concentration from 0.0.5 to 0.8 mg NH_3-N l^{-1}
Number of replicates per concentration	1
Number of organisms per replicate	8 fish per tank
Nature of test system (static, semi-static or flow-through, duration, feeding)	Flow-through, feeding, 11 days duration for turbot, 42 days for sole
Measurement of exposure concentrations	Yes, throughout the study
Measurement of water quality parameters	pH, temperature, dissolved oxygen, salinity
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	High

Reliability of study	The study is reliable.
Relevance of study	The endpoints measured (growth and survival) are relevant to the assessment of effects on algal populations.
Klimisch Code	**1**

Reference number	30

Information on the test species	
Test species used	*Dunaliella tertiolecta* (chlorophyte) *and Phaeodactylum tricornutum* (diatom)
Source of the test organisms	Cultures from the Woods Hole Oceanographic Institution
Holding conditions prior to test	Continuous cultures in appropriate seawater growth media under continuous illumination at 20^{o}C
Life stage of the test species used	Growth phase

Information on the test design	
Methodology used	The test procedure is described.
Form of the test substance	Ammonium chloride
Source of the test substance	Not stated
Type and source of the exposure medium	Appropriate algal growth media for each species
Test concentrations used	0, 9, 17, 28, 34 and 43 mg NH_3 l^{-1}
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, 1.5 hours, no feeding
Measurement of exposure concentrations	No
Measurement of water quality parameters	No
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	Moderate

Reliability of study	The study is reliable given the short exposure regime but absence of measured exposure concentrations.
Relevance of study	The endpoint used (inhibition of photosynthetic activity) is only an indirect measure of growth in algal populations.
Klimisch Code	**2**

Reference number	31

Information on the test species	
Test species used	Purple sea urchin (*Strongylocentrotus purpuratus*)
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Embryos

Information on the test design	
Methodology used	The test procedure is described.
Form of the test substance	Ammonium chloride
Source of the test substance	Not stated
Type and source of the exposure medium	Seawater filtered through a 0.45 µm filter
Test concentrations used	0, 1.0 to 5.6 mg l^{-1} total ammonia
Number of replicates per concentration	As per US EPA (1995) standard methodology*
Number of organisms per replicate	As per US EPA (1995) standard methodology*
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static, 72 hours, no feeding
Measurement of exposure concentrations	Yes
Measurement of water quality parameters	Yes
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	Good

Reliability of study	The study is reliable.
Relevance of study	The endpoint measured (survival) is relevant to the effects on sea urchin populations.
Klimisch Code	**1**

*US Environmental Protection Agency (US EPA), 1995 *Short-term methods for estimating the chronic toxicity of effluents and receiving waters to West Coast marine and estuarine organisms.* Report EPA/600/R-95/136. Washington, DC: US EPA Office of Research and Development.

Reference number	32

Information on the test species	
Test species used	*Agonus cataphractus* (armed bullhead)
Source of the test organisms	River Crouch, Essex
Holding conditions prior to test	Flow-through conditions
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	The test procedure is described.
Form of the test substance	Analytical grade aqueous ammonia
Source of the test substance	Not stated
Type and source of the exposure medium	Filtered seawater
Test concentrations used	0 (control), 7.3, 22.0 and 72.6 mg NH_3 l^{-1}
Number of replicates per concentration	1
Number of organisms per replicate	10
Nature of test system (static, semi-static or flow-through, duration, feeding)	Semi-static (daily renewal of test concentration), 96 hours, no feeding
Measurement of exposure concentrations	No (certified concentration of ammonia in stock solution)
Measurement of water quality parameters	pH, temperature and salinity
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	Good

Reliability of study	The study is reliable.
Relevance of study	The endpoint measured (survival) is relevant to the assessment of effects on algal populations.
Klimisch Code	**1**

Reference number	33

Information on the test species	
Test species used	*Diplodes sargus, Gadropsarus carpensis, Lithognathus mormyrus, Pachmetopa blochi*
Source of the test organisms	From sea-spawned pelagic eggs collected off the Cape of Good Hope
Holding conditions prior to test	Held in incubation baskets in 850-litre closed circulating system
Life stage of the test species used	Larvae of known age

Information on the test design	
Methodology used	The test procedure is generally well-described.
Form of the test substance	Ammonium chloride
Source of the test substance	Not stated
Type and source of the exposure medium	Filtered natural seawater (chemically characterised)
Test concentrations used	Range from 0.1 to 0.6 mg l^{-1} un-ionised ammonia
Number of replicates per concentration	1
Number of organisms per replicate	Not clear from paper
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static, feeding, 24-hour duration
Measurement of exposure concentrations	Not measured
Measurement of water quality parameters	Source water chemically characterised for pH, temperature and salinity
Test validity criteria satisfied	Yes (feeding in control groups >50%)
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	Good quality data

Reliability of study	The study is reliable but there are issues with the approach used to measure feeding rate.
Relevance of study	The endpoints measured (feeding rate and survival) are relevant to the assessment of effects on fish populations.
Klimisch Code	**2**

Reference number	45

Information on the test species	
Test species used	Sand dollar *Dendaster excentricus*
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	US EPA
Form of the test substance	Un-ionised ammonia
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
Study conducted to GLP	Not stated
Overall comment on quality	Insufficient data to assess

Reliability of study	Insufficient data to assess
Relevance of study	Mortality endpoint so relevant
Klimisch Code	**4**

Reference number	51

Information on the test species	
Test species used	*Oncorhynchus mykiss*
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	Un-ionised ammonia
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	Dissolved oxygen very low, averaging 5.8 mg l^{-1}
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	No
Study conducted to GLP	Not stated
Overall comment on quality	Due to the low dissolved oxygen in this study it is not regarded as being of good quality.

Reliability of study	Unreliable
Relevance of study	Growth endpoint so relevant
Klimisch Code	**3**

Reference number	52

Information on the test species	
Test species used	*Oncorhynchus mykiss*
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	Un-ionised ammonia
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	Nominal
Measurement of water quality parameters	No data
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	No
Study conducted to GLP	Not stated
Overall comment on quality	Insufficient data to assess quality

Reliability of study	Insufficient data to assess
Relevance of study	Mortality endpoint so relevant
Klimisch Code	**4**

We are The Environment Agency. It's our job to look after your environment and make it **a better place** – for you, and for future generations.

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

The Environment Agency. Out there, making your environment a better place.

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