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Proposed EQS for Water Framework Directive Annex VIII substances: arsenic (total dissolved)

Science Report: SC040038/SR
SNIFFER Report: WFD52(iii)

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

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Author(s):
Lepper P, Sorokin N, Maycock D, Crane M, Atkinson C Hope S-J and Comber S

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

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

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

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Steve Killeen Head of Science

Use of this report

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

This technical document has been developed through a collaborative project managed and facilitated by the Scotland & Northern Ireland Forum for Environmental Research (SNIFFER), the Environment Agency and the Scottish Environment Protection Agency (SEPA) and has involved 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 arsenic using the methodology described in Annex V of the Directive. There are existing EQSs for arsenic, but the method used to derive these is not considered to comply with the requirements of Annex V and so is unsuitable for deriving Annex VIII EQSs.

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

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

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

Properties and fate in water
Arsenic is a naturally occurring element but also enters the environment from anthropogenic sources. Under aerobic conditions, the pentavalent form As(V) predominates over the less thermodynamically stable trivalent form As(III). Arsine (–3) and elemental arsenic occur only under strongly reducing conditions and are rarely found in surface waters.

Whereas As(III) is thought to act by binding to sulfhydryl groups in proteins, As(V) competes with phosphorus to affect oxidative phosphorylation. Indeed, phosphorus can offset the toxicity of arsenic. Arsenic can also occur as organic compounds, but these are less toxic than the inorganic forms.

Availability of data
A substantial number of laboratory toxicity data are available for both As(V) and As(III). The taxonomic spread of data for freshwater organisms is extensive and there is evidence that algae and crustaceans are the most sensitive taxa following acute and chronic exposure.

Although much fewer in number and covering fewer taxa, saltwater data are available for algae, crustaceans, fish, molluscs, annelids and echinoderms. Again, algae are particularly sensitive along with echinoderms and crustaceans. There are no field or mesocosm data available for arsenic.

Water quality factors are known to influence arsenic speciation, availability and toxicity. However, there is not yet an adequate understanding to take these factors into account in deriving PNECs in a quantitative way. Finally, although arsenic is a known genotoxic carcinogen, there is no information on such effects in aquatic organisms.

Derivation of PNECs

The 'added risk' approach is considered appropriate when deriving PNECs for arsenic because arsenic is a naturally occurring substance which organisms will have been exposed to over an evolutionary timescale. This takes account of background concentrations and the PNEC applies only to the 'added' contribution over and above the background level. A practical consequence of this is that compliance assessment would need to consider background levels, at least at a regional scale, if not a local scale.

Neither As(V) nor As(III) is consistently more toxic than the other to aquatic organisms and so there is no compelling justification to have separate PNECs for them. By basing PNECs on the lowest credible data, this uncertainty should be accommodated. The proposed values therefore refer to total arsenic.

Long-term PNEC for freshwaters

The lowest effect concentration was obtained in a study with the water flea, *Daphnia pulex*, where a 20 per cent reduction in reproduction resulted from chronic exposure to 10 µg l^{-1} As(V). Similar concentrations have given rise to such effects in another species of water flea, *Daphnia magna*, and inhibition of algal growth. However, these were not reported sufficiently adequately to form the basis of a PNEC and the algal study used an obsolete protocol, allowing exposure for 14 days. Consequently, they are suitable only as supporting data.

According to the Annex V methodology, a no observed effect concentration (NOEC) can be derived from the lowest observed effect concentration (LOEC) of 10 µg l^{-1} by dividing it by 2. To this, an assessment factor of 10 is justified on the basis that data for other trophic levels are also available. As a result, a $PNEC_{freshwater_lt}$ of 0.5 µg l^{-1} arsenic (dissolved) is recommended.

This PNEC is 100 times lower than the existing statutory EQS of 50 µg l^{-1} developed in 1992. The existing EQS was based on an assessment factor of just 2 applied to a chronic LC10 of 0.14 mg l^{-1} for bluegill sunfish (*Lepomis macrochirus*). The difference reflects data for more sensitive species that have become available since the original EQS was derived and the application of larger assessment factors (as required by the Annex V methodology).

Short-term PNEC for freshwaters

Algae appear to the most sensitive taxonomic group to arsenic and the lowest reliable effect concentration is a 96-hour EC50 (reduction in algal biomass) of 79

µg l^{-1} As(III) for *Scenedesmus acutus*. Studies with other taxa indicate lower sensitivity and so should be protected by a PNEC based on data for algae. The indications that algae are indeed the most sensitive taxonomic group encourages the use of a small assessment factor (10), resulting in a $PNEC_{freshwater_st}$ of 8 µg l^{-1} arsenic (dissolved). This is lower than the lowest validated algal LOEC and should, therefore, protect algal communities in the event of a short-term peak in exposure.

There is no existing short-term EQS for arsenic.

Long-term PNEC for saltwaters

Similar toxicities (expressed as LOECs) are seen in studies with the marine diatom, *Skeletonema costatum*, for As(III) and As(V) (10 and 13 µg l^{-1}, respectively). Although most invertebrates are less sensitive, embryo development in the sea urchin *Strongylocentrosus purpuratus* was impaired after 48-hour exposure to As(V). This gave rise to a LOEC of 11 µg l^{-1}, but it was not possible to estimate a NOEC from this study. Subsequent re-analysis of the study data gave an EC50 of 15 µg l^{-1} and an EC10 (considered equivalent to a NOEC) of 6 µg l^{-1}. Given the availability of data for other trophic levels, an assessment factor of 10 applied to this LOEC is recommended, leading to a $PNEC_{saltwater_lt}$ of 0.6 µg l^{-1} arsenic (dissolved).

The proposed PNEC is 40 times lower than the existing statutory EQS of 25 µg l^{-1}, based on an assessment factor of 10 applied to an acute LC50 of 0.232 mg l^{-1} for the crab, *Cancer magister*. This is entirely a consequence of new data that have become available since the original EQS was derived.

Short-term PNEC for saltwaters

Poorly reported studies indicate effects on crustaceans at concentrations of arsenic as low as 3 µg l^{-1}, but more reliable studies with embryo development in sea urchins give rise to EC50 values of 15 µg l^{-1}. However, the lowest reliable effect concentration is a 96-hour LC50 of 11 µg l^{-1} As(V) for the crustacean *Tigriopus brevicornis*. These concentrations are similar to those giving rise to effects following chronic exposure to arsenic, indicating a low acute:chronic ratio. This justifies the use of an assessment factor of only 10 applied to the *Tigriopus* 96-hour LC50, resulting in a $PNEC_{saltwater_st}$ of 1.1 µg l^{-1} arsenic (dissolved).

There is no existing short-term EQS for arsenic.

PNEC for secondary poisoning

There is no evidence of biomagnification of arsenic in food chains, with the possible exception of algae and higher plants. Secondary poisoning of predators, e.g. mammals and birds, is not considered a significant risk and PNECs for secondary poisoning are not proposed.

PNEC for sediment

There are insufficient data to derive a sediment PNEC for arsenic and the use of equilibrium partitioning to estimate a value based on aquatic toxicity data cannot be justified for metals.

Summary of proposed PNECs

Receiving medium/ exposure scenario	Proposed PNEC (µg l^{-1} total dissolved arsenic)	Existing EQS (µg l^{-1} total dissolved arsenic)
Freshwater/long-term	0.5	50
Freshwater/short-term	8	No standard
Saltwater/long-term	0.6	25
Saltwater/short-term	1.1	No standard

Analysis
The lowest proposed PNEC derived for arsenic is 0.5 µg l^{-1}. The data quality requirements are that, at a third of the EQS, the total error of measurement should not exceed 50 per cent. Current analytical methodologies provide detection limits as low as 3 ng l^{-1}, which suggests that they would be adequate for assessing compliance.

Implementation issues
As an 'added risk' approach is proposed, background concentrations of arsenic would need to be established.

There are no further outstanding issues that need to be addressed before these PNECs can be used as EQSs. The PNECs proposed above are, therefore, recommended for adoption as EQSs.

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1. Introduction

The UK Technical Advisory Group (UKTAG) supporting the implementation of the Water Framework Directive (2000/60/EC)[1] is a partnership of UK environmental and conservation agencies. It also includes partners from the Republic of Ireland. UKTAG has commissioned a programme of work to derive Environmental Quality Standards (EQSs) for substances falling under Annex VIII of the Water Framework Directive (WFD). This report proposes predicted no-effect concentrations (PNECs) for arsenic using the methodology described in Annex V of the Directive. There are existing EQSs for arsenic, but the method used to derive these is not considered to comply with the requirements of Annex V and so is unsuitable for deriving Annex VIII EQSs.

The PNECs described in this report are based on a technical assessment of the available ecotoxicity data for arsenic, 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 arsenic (total dissolved).

1.1 Properties and fate in water

Arsenic is a naturally occurring element but also enters the environment from anthropogenic sources. Under aerobic conditions, the pentavalent form [As(V)] predominates over the less thermodynamically stable trivalent form [As(III)]. Arsine (3–) and elemental arsenic occur only under strongly reducing conditions and are rarely found in surface waters.

Whereas As(III) is thought to act by binding to sulfhydryl groups in proteins, As(V) competes with phosphorus to affect oxidative phosphorylation. Indeed, phosphorus can offset the toxicity of arsenic. Arsenic can also occur as organic compounds but these are less toxic than the inorganic forms.

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

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

2. Results and observations

2.1 Identity of substance

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

Table 2.1 Species covered by this report

Name	CAS Number
Arsenic metal	7440-38-2

2.2 PNECs proposed for derivation of quality standards

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

Arsenic bioavailability and toxicity can be influenced and modified by changes in water or sediment chemistry. Ideally, any PNECs should be derived so that they take into account the effects of physico-chemical parameters such as pH, water hardness and dissolved organic matter. However, although there has been extensive research into the effects of metal bioavailability and the influence of water quality on arsenic toxicity, there is as yet insufficient scientific knowledge to take these parameters into consideration when setting quality standards for arsenic.

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 [24], and existing EQSs obtained from the literature [18, 19].

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

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

$PNEC_{add}$	TDG deterministic approach (AFs)	TGD probabilistic approach (SSDs)	Existing EQS
Freshwater short-term	8 µg l^{-1} (see Section 4.1.1)	–	–
Freshwater long-term	0.5 µg l^{-1} (see Section 4.1.1)	Appropriate data not available	50 µg l^{-1} (AA)
Saltwater short-term	1.1 µg l^{-1} (see Section 4.1.2)	–	–
Saltwater long-term	0.6 µg l^{-1} (see Section 4.1.2)	Appropriate data not available	25 µg l^{-1} (AA)

$PNEC_{add}$	TDG deterministic approach (AFs)	TGD probabilistic approach (SSDs)	Existing EQS
Freshwater sediment short-term	No data available	–	–
Freshwater sediment long-term	No data available	–	–
Saltwater sediment short-term	No data available	–	–
Saltwater sediment long-term	No data available	–	–
Freshwater secondary poisoning	No PNEC derived (trigger criteria not met; see Section 4.5)	–	–
Saltwater secondary poisoning	No PNEC derived (insufficient data; see Section 4.5)	–	–

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

2.3 Hazard classification

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

Table 2.3 Hazard classification

R-phrases and labelling	Reference
Arsenic metal: T; R23/25 - N; R50-53	[1]

2.4 Physical and chemical properties

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

Table 2.4 Physical and chemical properties of arsenic (metal)

Property	Value	Reference
Molecular formula	As	
Vapour pressure	7.5×10^{-3} mmHg at 280°C	[2]
Solubility in water	Insoluble (as metal)	[2]
Relative molecular weight	74.92	[3]

2.5 Environmental fate and partitioning

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

Table 2.5 Environmental fate and partitioning of arsenic

Property	Value	Reference
Abiotic fate	Arsenic and its compounds occur in crystalline, powder, amorphous or vitreous forms. They usually occur in trace quantities in all rock, soil, water and air.	[6, 7]
Speciation	Arsenic can exist in four oxidation states: –3, 0, +3 and +5. Only in strongly reducing environments can elemental arsenic and arsine (–3) exist. Arsenic as a free element (0 oxidation state) is rarely encountered in natural waters. Soluble inorganic arsenate (+5 oxidation state) predominates, but not exclusively, under normal conditions because it is thermodynamically more stable than arsenite (+3 oxidation state). In well-oxygenated water and sediments, nearly all arsenic is present in the thermodynamically stable pentavalent state (arsenate). Some arsenite and arsenate species can interchange oxidation state depending on redox potential (Eh), pH and biological processes. Other forms of dissolved arsenic that may be present in the water column include monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). Both are largely derived from biomethylation of inorganic arsenic by aquatic algae (particularly in transitional waters). Within higher organisms (e.g. fish and crustaceans), the organoarsenical arsenobetaine predominates.	[7]
Hydrolytic stability	Not applicable	[7]
Photostability	The rates of photochemical decomposition of arsenite, DMA and MMA in water have been well studied. All species were found to degrade rapidly in aerated distilled water, with slower degradation occurring in de-aerated solutions and in seawater. Half-lives for the degradation of DMA, MMA and arsenite were 9.2, 11.5 and 0.9 minutes respectively for aerated distilled water, and 25, 19 and 8 minutes for de-aerated distilled water.	[7]
Distribution in water/sediment systems	Transport and partitioning of arsenic in water depends on the chemical form of arsenic and on interactions with other minerals present. The distribution and transport of arsenic in sediment is a complex process, depending on oxidation state, water quality, native bacteria and sediment type. Both adsorption of arsenic on iron-rich oxides on the surface of sediments and incorporation of arsenic into sediments by co-precipitation with hydrous iron oxides are factors controlling mobilisation of arsenic in sediment. In addition, the extent of	[7] [7]

Property	Value	Reference
	adsorption and remobilisation of arsenic compounds during water–sediment interactions varies with the oxidation state of arsenic as well as the temperature, Eh and pH of the water. The extent of uptake and the rate of adsorption of arsenate decrease with increasing temperature from 20 to 40°C. The amount of arsenate adsorbed increases as the pH of the system increases. Leaching does not appear to be a significant route of arsenic loss from soil, since many arsenic species tend to adsorb to soils.	[7] [5]
Degradation in soil	Arsenic compounds may be metabolised by soil bacteria to alkylarsines, MMA and arsenate. Many soil organisms are capable of metabolising arsenic, and the reduced forms (largely methylated arsines such as MMA and DMA) will volatilise from soil. The half-life of DMA in soil is approximately 20 days. Arsenic removal from soil porewater may also occur as a result of precipitation.	[7]
Background concentrations	Concentrations of arsenic in open ocean seawater are typically 1–2 µg l^{-1}. Arsenic is widely distributed in surface freshwaters, and concentrations in rivers and lakes are generally below 10 µg l^{-1}, although individual samples may range up to 5 mg l^{-1} near anthropogenic sources. Arsenic levels in groundwater average about 1–2 µg l^{-1}, except in areas with volcanic rock and sulfide mineral deposits where arsenic levels can range up to 3 mg l^{-1}. Mean sediment arsenic concentrations range from 5 to 3,000 mg/kg, with the higher levels occurring in areas of contamination.	[7]
Biotransformation	Most biotransformation of arsenic species occurs in the soil, in sediments, in plants and animals, and in zones of biological activity in the oceans. Biomethylation and bioreduction are probably the most important pathways. Under aerobic conditions, the mixed microbial cultures of lake sediments were able to reduce arsenate (the predominant from of arsenic in water) to arsenite and a variety of methylated arsenicals, and also to oxidise arsenite to arsenate. Under anaerobic conditions, however, only reduction occurs.	[7] [17]
Partition coefficients	The extent of arsenic adsorption (Kp) depends strongly upon the pH of the water, the arsenic oxidation state and the temperature. In acidic and neutral waters, As(V) is extensively adsorbed, while As(III) is relatively weakly adsorbed. In waters with a high pH, Kp values are considerably lower for both oxidation states: • Log Kp (sediment/water) – estimated 3.82 • Log Kp (particulate matter/water) – estimated 4.00	[16] [19]

Property	Value	Reference
Bioaccumulation BCF	The accumulation of trivalent and pentavalent arsenic by the green alga, *Chlorella vulgaris*, isolated from an arsenic-contaminated environment has been examined. Bioconcentration factors (BCFs) ranged from 1.4 to 330 with exposure concentrations of 0 to 10,000 mg l^{-1}.	[4]
	Mean arsenic concentrations in pooled insect samples from the Red River were 0.12 and 0.5 µg/g dry weight arsenic, corresponding to BCFs of 0.06 and 0.04, respectively. Mean arsenic concentration in the Red River water was 14 µg l^{-1}.	[5]
	The WRc EQS report contains only limited data on bioaccumulation, with the majority coming from field-collected samples. Generally, the BCFs of marine species were considerably higher than for freshwater species. For laboratory data, the BCFs reported for freshwater fish species were below 1. No laboratory data for saltwater fish species were reported.	[18]
	Only one acceptable bioconcentration test with a saltwater species was cited in the US Environmental Protection Agency (US EPA) report on arsenic. This was a BCF of 350, which was obtained with the oyster, *Crassostrea virginica*, after 112 days of exposure.	[20] [131]
	Marine organisms normally contain arsenic residues ranging from <1 to >100 mg/kg, predominantly as organic arsenic species such as arsenosugars (macroalgae) and arsenobetaine (invertebrates and fish). Bioaccumulation of organic arsenic compounds, after their biogenesis from inorganic forms, occurs in aquatic organisms. BCFs in freshwater invertebrates and fish for arsenic compounds are lower than for marine organisms (see below). Biomagnification in aquatic food chains has not been observed.	[7] [91]
	BCF	
	Estuarine phytoplankton, *Thalassiosira pseudomonas, Skeletonema costatum* and *Dunaliella tertiolecta* — 1,462–3,688	[9]
	Green alga, *Chlorella vulgaris* — 200–300	[10]
	Aquatic plant, *Hydrilla verticillata* — 140–1,120	[11]
	Bluegill fish, *Lepomis macrochirus* — 4	[15]
	Snail — 17	[12]

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 systematic approach.

Critical data were collected from a WRc report [18] on the toxicity and bioaccumulation of arsenic in freshwater and saltwater life as well as the existing arsenic EQS report [19].

In addition, data were collected from:

- World Health Organization (WHO) *Environmental Health Criteria 224: Arsenic and Arsenic Compounds* [7];
- US EPA Ambient Water Quality Criteria for Arsenic – 1984 [20].

The available arsenic database was further supplemented by collecting data from the US EPA ECOTOX database[3] using the CAS Numbers listed in Table 2.6.

Table 2.6 CAS numbers used for ECOTOX search

CAS Number	Formula	Chemical name
1303-28-2	As_2O_5	Arsenic pentoxide
1327-53-3	As_2O_3	Arsenic trioxide
7440-38-2	As	Arsenic
7631-89-2	AsH_2NaO_4	Sodium arsenate
7778-39-4	AsH_3O_4	Arsenic acid
7778-43-0	$AsHNa_2O_4$	Disodium arsenate
7784-46-5	$AsNaO_2$	Sodium arsenite
13464-38-5 15502-74-6	$AsNa_3O_4$ $AsO_3^{(3-)}$	Arsenic acid, trisodium salt Arsenite

Additional mammalian, avian and sediment toxicity data were collected from Web of Science®[4] and from recent review documents [7, 13, 39, 41].

2.6.1 Toxicity to freshwater organisms

Freshwater long-term (lt) and short-term (st) toxicity data are available for various taxonomic groups including algae, macrophytes, crustaceans, fish, insects, molluscs amphibians, rotifers, protozoans and bacteria. Algae, crustaceans and fish appear to be the most sensitive in relation to long-term exposures. Algae and crustaceans are also the most sensitive to short-term exposures, although amphibians and insects also appear to be sensitive.

Diagrammatic representations of the available freshwater data (cumulative distribution functions) for arsenic are presented in Figures 2.1 and 2.2. These diagrams include all data regardless of quality and provide an overview of the spread of the available data. These diagrams are not species sensitivity distributions and have not been used to set the arsenic PNECs. The lowest critical freshwater data for arsenic are presented in Tables 2.7 and 2.8.

[3] http://www.epa.gov/ecotox/
[4] http://scientific.thomson.com/products/wos/

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

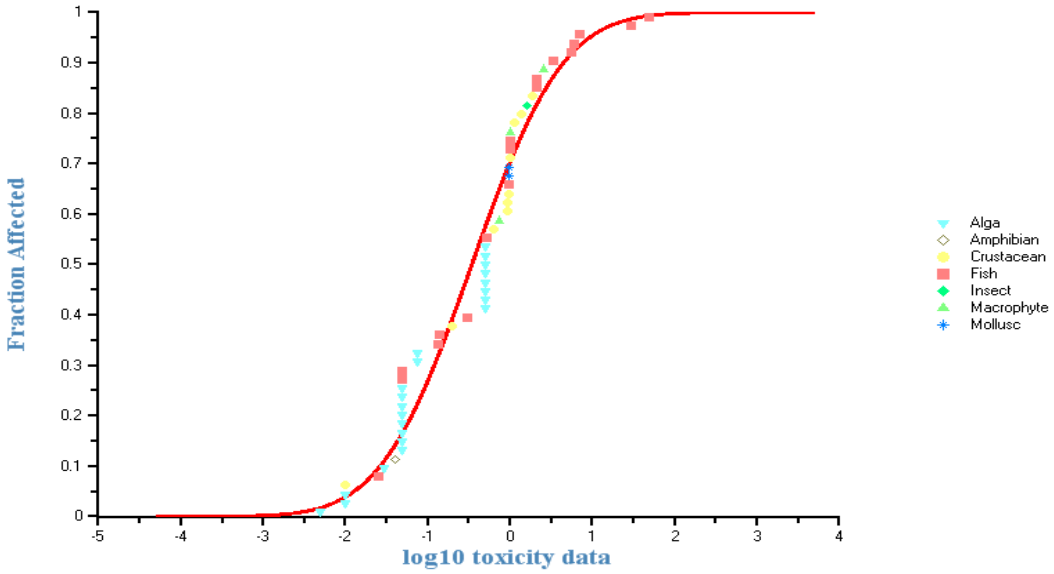

Figure 2.2 Cumulative distribution function of freshwater short-term data (mg l^{-1}) for arsenic

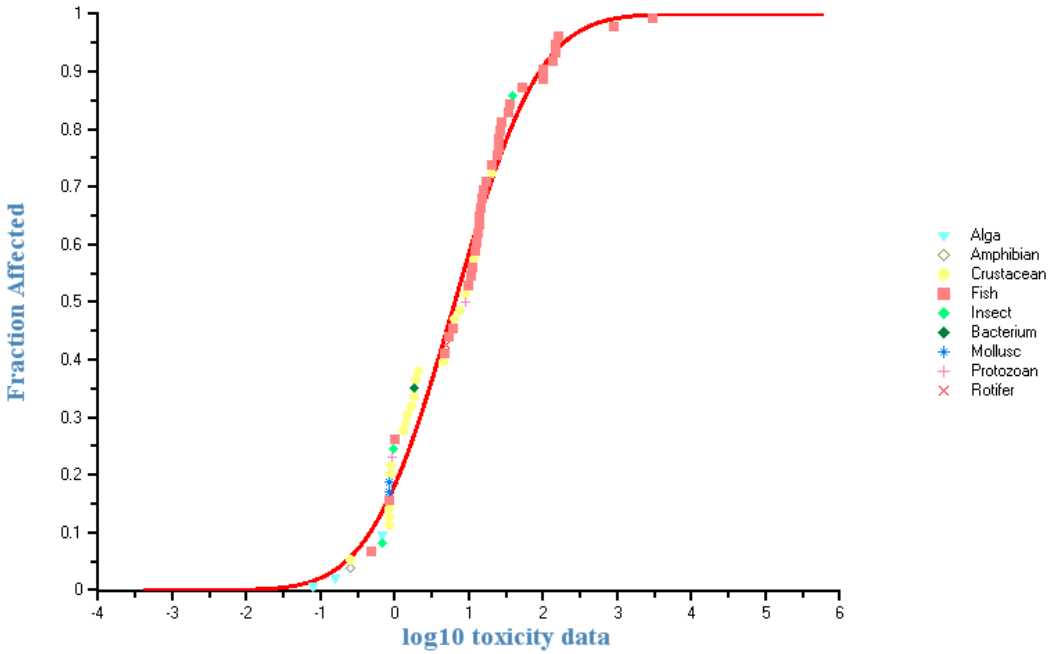

Table 2.7 Most sensitive long-term aquatic toxicity data for freshwater organisms exposed to arsenic

Test substance	Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (mg l^{-1})	Expo-sure[1]	Toxicant analysis[2]	Comments	Refer-ence
Algae and microbes											
$AsNa_3O_4$ (V)	*Stichogloea doederleinii*	'Golden'/brown alga	Algae	LOEC	Growth no. of cells	72–96 hours	0.005	-	-	RI = 4	[55]
$AsNa_3O_4$ (V)	*Chlamydomonas* sp. *Dicityosphaerium elegans* *Monoraphidium griffithii* *Scenedesmus denticulatus* *Asterionella formosa* *Synedra nana* *Tabellaria teilingii* *Cryptompnas* sp.	Green algae Diatoms Cryptophyceae	Algae	LOEC	Growth no. of cells	72–96 hours	0.050	-	-	RI = 4	[55]
$AsNa_3O_4$ (V)	*Monoraphidium conrortum* *Quadrigula pfitzerii* *Closterium acutum* *Cosmarium pygmaeum* *Cyclotella comta*	Green algae Diatom	Algae	LOEC	Growth no. of cells	72–96 hours	0.500	-	-	RI = 4	[55]
$AsNa_3O_4$ (V)	*Monosigna* sp. *Ochromonas* sp. *Kephyrion planctonicum*	'Golden'/brown alga	Algae	NOEC (unbounded)	Growth no. of cells	72–96 hours	0.500	-	-	RI = 4	[55]
$AsHNa_2O_4$ (V)	*Ankistrodesmus falcatus*	Green alga	Algae	EC50 LOEC NOEC	Growth	14 days	0.256 0.100 0.010	s	n	pH 7; 24°C; hardness 16–17 mg l^{-1} $CaCO_3$; RI = 2	[98]
$AsHNa_2O_4$ (V)	*Scenedesmus obliquus*	Green alga	Algae	EC50 LOEC (= lowest conc. tested)	Growth	14 days	0.048 0.010	s	n	pH 7; 24°C; hardness 16–17 mg l^{-1} $CaCO_3$; RI = 2	[98]
$AsHNa_2O_4$ (V)	*Chlorella vulgaris*	Green alga	Algae	NOEC	Population	3–4 months	0.030	-	n	pH 2.2–8 lowest concentration tolerated	[42]

Test substance	Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (mg l^{-1})	Expo-sure[1]	Toxicant analysis[2]	Comments	Refer-ence
$AsH_3O_4 \cdot 3Na$ (V)	*Ochromonas vallesiaca*	Chrysophyte	Algae	Effect	Growth	20 days	0.075	s	n	20°C	[81]
$AsH_3O_4 \cdot 3Na$ (V)	*Melosira granulata*	Diatom	Algae	Effect	Growth	20 days	0.075	s	n	20°C	[81]
Higher plants											
As (III)	*Lemna minor*	Duckweed	Macrophytes	NOEC LC50	Growth	14 days	<0.75 0.63	f	y	23°C; RI = 4	[59]
As (V)	*Myriophyllum spicatum*	Eurasian watermilfoil	Macrophytes	EC50	Biomass	32 days	2.6	f	n	20°C	[93]
$AsHNa_2O_4$ (V)	*Azolla pinnata*	Water velvet	Macrophytes	NOEC unbounded?	Growth/ chlorophyll	28 days	1.0	ss	n	pH 8.5; 21°C	[87]
Invertebrates											
As_2O_5 (V)	*Daphnia magna*	Water flea	Crustaceans	NOEC unbounded	Mortality/ reproduction	14 days	0.932	ss	y	pH 6.9–7.3; 14-16°C; hardness 42–45 mg l^{-1} $CaCO_3$	[91]
As_2O_3 (III)	*Daphnia magna*	Water flea	Crustaceans	NOEC unbounded	Mortality/ reproduction	14 days	0.955	ss	y	pH 6.9–7.3; 14–16°C; hardness 42–45 mg l^{-1} $CaCO_3$	[91]
$AsNaO_2$ (III)	*Daphnia magna*	Water flea	Crustaceans	NOEC LOEC	Mortality/ reproduction	28 days	0.630 1.320	ss	y	pH 7.2–8.1; 20.8°C; hardness 46–50 mg l^{-1} $CaCO_3$; RI = 1	[64]
As_2O_3 (III)	*Daphnia magna*	Water flea	Crustaceans	NOEC	Reproduction	21 days	1.85	ss	y	21°C; RI = 2	[97]
$AsHNa_2O_4$ (V)	*Daphnia magna*	Water flea	Crustaceans	EC50	Reproduction	21 days	1.4	ss	n	pH 7.74; 18°C; hardness 45.3 mg l^{-1} $CaCO_3$	[26]
As_2O_5 (V)	*Daphnia pulex*	Water flea	Crustaceans	LOEC	Reproduction	26 days	0.01	ss	n	Significant decrease in reproduction; RI = 2	[37]
$AsNaO_2$ (III)	*Ceriodaphnia dubia*	Water flea	Crustaceans	MATC	Production of young	7 days	1.14	ss	y	pH 8.2; 25°C; hardness 100 mg l^{-1} $CaCO_3$	[90]
$AsNaO_2$ (III)	*Ceriodaphnia dubia*	Water flea	Crustaceans	Effect	Survival/ brood size	24 days	1.00	ss	y	pH 7.3–9.3; 25°C; hardness EPA moderately hard; RI = 2	[52]
As_2O_3 (III)	*Lymnaea emarginata*	Pond snail	Molluscs	NOEC unbounded	Mortality	28 days	0.961	f	y	pH 6.9–7.3; 14–16°C; hardness 42–45 mg l^{-1} $CaCO_3$	[91]

Test substance	Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (mg l^{-1})	Expo-sure[1]	Toxicant analysis[2]	Comments	Refer-ence
As_2O_3 (III)	*Helisoma campanulatum*	Ramshorn snail	Molluscs	NOEC unbounded	Mortality	28 days	0.961	f	y	pH 6.9–7.3; 14–16°C; hardness 42–45 mg l^{-1} $CaCO_3$	[91]
As_2O_3 (III)	*Gammarus pseudolimnaeus*	Amphipod	Crustaceans	Effect (mortality)	80% 100%	7 days 14 days	0.961	f	y	pH 6.9–7.3; 14–16°C; hardness 42–45 mg l^{-1} $CaCO_3$	[91]
As (III)	*Gammarus fossarum*	Gammarid	Crustaceans	LC50	Immobilisa-tion	10 days	0.200	f	y	12°C; RI = 1	[34]
As (III)	*Heptagenia sulphurea*	Mayfly	Insects	LC50	Immobilisa-tion	10 days	1.600	f	y	12°C; RI = 1	[34]
Vertebrates (fish and amphibians)											
$AsHNa_2O_4$ (V)	*Clarias batrachus*	Walking catfish	Fish	Effect	Change in protein content	14 days	1.0	-	n	pH 8.5; 22°C; hardness 'soft'	[58]
$AsNaO_2$ (III)	*Jordanella floridae*	Flagfish	Fish	NOEC LOEC	Growth	31 days	2.13 4.12	f	y	pH 7.2–8.1; 23–25.8°C; hardness 46–50 mg l^{-1} $CaCO_3$; RI = 1	[64]
As_2O_3 (III)	*Oncorhynchus kisutch*	Coho salmon, silver salmon	Fish	Effect	Significant reduction in migration success	6 months	0.300	f	y	pH 8.2; 3.8–13.8°C; hardness 69 mg l^{-1} $CaCO_3$; RI = 1	[74]
As_2O_3 (III)	*Oncorhynchus mykiss* (parr)	Rainbow trout	Fish	NOEC unbounded	Mortality	28 days	0.961	f	y	pH 6.9–7.3; 14–16°C; hardness 42–45 mg l^{-1} $CaCO_3$	[91]
$AsNaO_2$ (III)	*Oncorhynchus mykiss*	Rainbow trout	Fish	LC10 LC50	Mortality (egg)	28 days	0.134 0.54	ss	y	pH 7.2–7.8; 12–13°C; hardness 93–105 mg l^{-1} $CaCO_3$	[27, 28]
As_2O_3 (III)	*Oryzias latipes*	Japanese medaka	Fish	≈ EC50 LOEC NOEC	Hatching success	7–11 days (expo-sure of 2–3-day-old embryos until hatching)	0.100 0.050 0.025	ss	n	hatching success in controls 85%; EC50 = 54%; LOEC = 75%; NOEC = 86%; RI = 4	[57]

Test substance	Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (mg l^{-1})	Expo-sure[1]	Toxicant analysis[2]	Comments	Refer-ence
As_2O_5 (V)	*Pimephales promelas*	Fathead minnow	Fish	NOEC LOEC	Growth ELS test	30 days	0.53 1.50	f	n	pH 6.9–7.8; 25°C; hardness 45–48 mg l^{-1} $CaCO_3$; RI = 2	[40]
$AsNaO_2$ (III)	*Pimephales promelas*	Fathead minnow	Fish	NOEC LOEC	Growth	29 days	2.13 4.30	f	y	pH 7.2-8.1; 23–25.8°C; hardness 46–50 mg l^{-1} $CaCO_3$; RI = 1	[64]
$AsNaO_2$ (III)	*Pimephales promelas*	Fathead minnow	Fish	MATC	Mortality/ growth/ reproduction	32 days	3.33	f	y	pH 7.4; 25°C; hardness 43.9 mg l^{-1} $CaCO_3$	[90]
$AsNaO_2$ (III)	*Pimephales promelas*	Fathead minnow	Fish	EC50	Weight/no. of young per female	32 days	7.08	f	y	pH 7.4; 25°C; hardness 43.9 mg l^{-1} $CaCO_3$	[90]
As (III)	*Gastrophryne carolinensis*	Narrow-mouthed toad	Amphibians	LC50	Mortality (fertilisation until 4-day post-hatch)	7 days	0.040	ss	n	pH 7–7.8; hardness 195 mg l^{-1} $CaCO_3$	[29]

[1] Exposure: s = static; ss = semi-static; f = flow-through.
[2] Toxicant analysis: y = measured; n = not measured.
ELS = early life stages
LOEC = lowest observed effect concentration
MATC = maximum allowable toxicant concentration
NOEC = no observed effect concentration
EC50 = concentration effective against 50% of the organisms tested
LCx = concentration lethal to X% of the organisms tested
RI = reliability index (see Annex 1)

Table 2.8 Most sensitive short-term aquatic toxicity data for freshwater organisms exposed to arsenic

Test substance	Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (mg l^{-1})	Expo-sure[1]	Toxicant analysis[2]	Comments	Refer-ence
Algae and microbes											
$AsHNa_2O_4$ (V)	*Vibrio fisheri*	Luminescent bacteria	Bacteria	EC20	Light emission	15 min	1.820	s	y	pH 5; 15°C; RI = 2	[48]
$AsNa_3O_4$ (V)	*Scenedesmus obliquus*	Green alga	Algae	EC50	Population size	96 hours	0.159	ss	n	pH 7; 24°C; RI = 2	[38]
$AsNaO_2$ (III)	*Scenedesmus obliquus*	Green alga	Algae	EC50	Population size	96 hours	0.079	ss	n	pH 7; 24°C; RI = 2	[38]
$AsNa_3O_4$ (V)	*Selenastrum capricornutum*	Green alga	Algae	EC50	Population size	96 hours	0.690	s	y	-	[33]
Invertebrates											
AsH_3O_4 (V)	*Tetrahymena thermophila*	Ciliated protozoan	Protozoans	NOEC	Population abundance	48 hours	0.900	-	n		[80]
$AsNaO_2$ (III)	*Ceriodaphnia reticulata*	Water flea	Crustaceans	EC50	Immobilisation	48 hours	1.27	s	n	pH 8; 23°C; hardness 240 mg l^{-1} $CaCO_3$	[45]
As (III)	*Daphnia pulex*	Water flea	Crustaceans	LC50	Mortality	48 hours	1.9	s	n	pH 7.2–7.4; hardness 45 mg l^{-1} $CaCO_3$	[70]
As (III)	*Simocephalus vetulus*	Water flea	Crustaceans	LC50	Mortality	48 hours	1.7	s	n	pH 7.2–7.4; hardness 45 mg l^{-1} $CaCO_3$	[70]
$AsNaO_2$ (III)	*Simocephalus serrulatus*	Water flea	Crustaceans	EC50	Immobilisation	48 hours	1.4	s	n	pH 7.4; 16°C; hardness 44 mg l^{-1} $CaCO_3$	[69]
$AsHNa_2O_4$ (V)	*Bosmina longirostris*	Water flea	Crustaceans	EC50	Immobilisation	96 hours	0.850	s	y	pH 6.8; 17°C; hardness 120 mg l^{-1} $CaCO_3$; RI = 2	[79]
As_2O_3 (III)	*Bosmina longirostris*	Water flea	Crustaceans	EC50	Immobilisation	96 hours	0.250	-	n	RI = 4	[75]
$AsNaO_2$ (III)	*Ceriodaphnia dubia*	Water flea	Crustaceans	LC50	Mortality	48 hours	1.448	s	y	pH 8.2; 25°C; hardness 100 mg l^{-1} $CaCO_3$	[90]
As_2O_3 (III)	*Chironomus tentans*	Midge	Chironomids	EC50	Immobilisation	48 hours	0.680	s	n	pH 6.3; 14°C; hardness 25 mg l^{-1} $CaCO_3$; RI = 2	[61]
$AsNaO_2$ (III)	*Gammarus pseudolimnaeus*	Amphipod	Crustaceans	EC50	Immobilisation	96 hours	0.874	f	y	pH 7.2–8.1; 18.5°C; hardness 46–50 mg l^{-1} $CaCO_3$; RI = 1	[64]

Test substance	Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (mg l^{-1})	Expo-sure[1]	Toxicant analysis[2]	Comments	Refer-ence
$AsNaO_2$ (III)	*Gammarus pseudolimnaeus*	Amphipod	Crustaceans	LC50	Mortality	96 hours	0.875	f	y	pH 7.7; 18.4°C; hardness 46.3 mg l^{-1} $CaCO_3$	[33]
Fish											
As_2O_5 (V)	*Thymallus arcticus*	Arctic grayling	Fish	LC50	Mortality (juvenile)	96 hours	4.76	s	n	pH 7.1– 8; 12°C; hardness 41.3 mg l^{-1} $CaCO_3$; RI = 2	[31]
As_2O_3 (III)	*Barbus javanicus*	Barb	Fish	LC50	Mortality	96 hours	24.17	s	n	pH 7.1–7.2; 23°C; hardness 230 mg l^{-1} $CaCO_3$	[51]
As (III)	*Carassius auratus*	Goldfish	Fish	LC50	Mortality	7 days	0.490	ss	n	pH 7; hardness 195 mg l^{-1} $CaCO_3$	[29]
As_2O_3 (III)	*Channa punctata*	Snake-head catfish	Fish	LC50	Mortality (fingerling)	96 hours	10.9	s	n		[89]
As_2O_3 (III)	*Oncorhynchus mykiss*	Rainbow trout	Fish	LC10	Mortality	96 hours	12.1	s	y	pH 8.4; 12°C; hardness 250 mg l^{-1} $CaCO_3$; RI = 2	[97]
As_2O_3 (III)	*Colisa fasciatus*	Giant gaurami	Fish	LC50	Mortality (fingerling)	96 hours	6.1			pH 7.1; 30°C	[88]
$AsNaO_2$ (III)	*Pimephales promelas*	Fathead minnow	Fish	LC50	Mortality	96 hours	12.6	f	y	pH 7.4; 25°C; hardness 43.9 mg l^{-1} $CaCO_3$	[90]
As_2O_3 (III)	*Barbus sophore*	Two-spot barb	Fish	LC50	Mortality	48 hours	14	s	n	34.8°C	[77]
$AsNaO_2$ (III)	*Oryzias latipes*	Japanese medaka	Fish	LC50	Mortality	7 days	14.6	ss	n	21°C; RI = 2	[94]
$AsNaO_2$ (III)	*Oreochromis mossambicus*	Tilapia	Fish	LC50	Mortality	144 hours	15.98	ss	y	pH 7.7; 24.7°C; RI = 1	[63]
As_2O_3 (III)	*Rana hexadactyla*	Frog	Amphibians	LC50	Mortality	96 hours	0.249	ss	n	pH 6.1; 15°C; hardness 20 mg l^{-1} $CaCO_3$; RI = 2	[60]

[1] Exposure: s = static; ss = semi-static; f = flow-through.
[2] Toxicant analysis: y = measured; n = not measured.
NOEC = no observed effect concentration
ECx = concentration effective against X% of the organisms tested
LCx = concentration lethal to X% of the organisms tested
RI = reliability index (see Annex 1)

2.6.2 Toxicity to saltwater organisms

Saltwater long-term and short-term toxicity data are available for various taxonomic groups including algae, crustaceans, fish, molluscs, annelids and echinoderms. Echinoderms, molluscs and algae appear to be the most sensitive to long-term exposures to arsenic. Algae and echinoderms are also the most sensitive to short-term exposures, although crustaceans also appear to be sensitive.

Diagrammatic representations of the available saltwater data (cumulative distribution functions) for arsenic are presented in Figures 2.3 and 2.4. These diagrams include all data regardless of quality and provide an overview of the spread of the available data. These diagrams are not species sensitivity distributions and have not been used to set the arsenic PNECs. The lowest critical saltwater data for arsenic are presented in Tables 2.9 and 2.10.

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

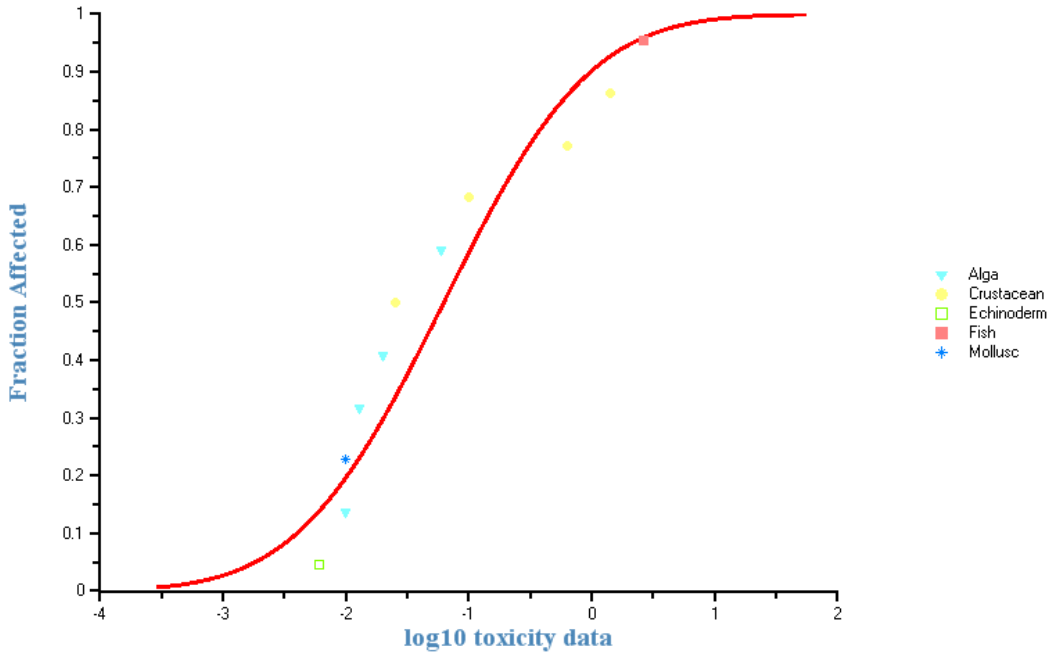

Figure 2.4 Cumulative distribution function of saltwater short-term data (mg l^{-1}) for arsenic

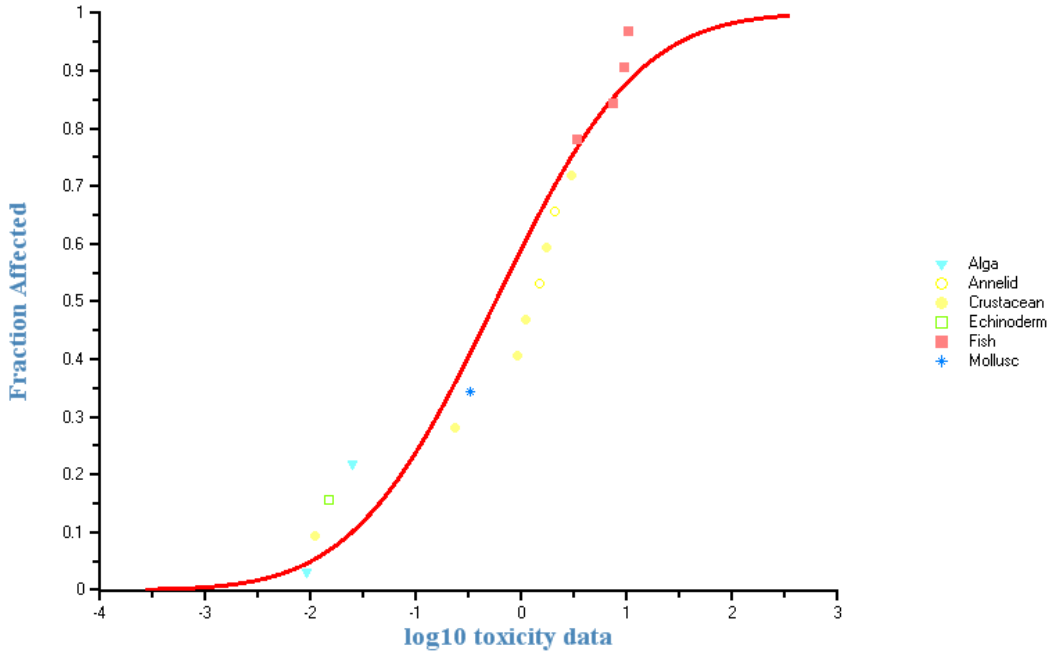

Table 2.9 Most sensitive long-term aquatic toxicity data for saltwater organisms exposed to arsenic

Test substance	Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (mg l^{-1})	Expo-sure[1]	Toxicant analysis[2]	Comments	Reference
Algae											
As (III)	*Skeletonema costatum*	Diatom	Algae	LOEC	Growth	6–8 days	0.010	s	y	20°C; RI = 2	[85]
As (V)	*Skeletonema costatum*	Diatom	Algae	LOEC	Growth	6–8 days	0.013	s	y	20°C; RI = 2	[85]
$AsHNa_2O_4$ (V)	*Fucus serratus*	Brown alga	Algae	NOEC	Growth/ mortality	17 weeks	0.02	f	y	16–20°C; salinity 12.5–22%	[50]
				LOEC	After 3 weeks, first signs of toxicity (brown stains and reduced growth)	17 weeks	0.05			MATC = 31.6 µg l^{-1}; RI = 1	
				LC100	Reduced growth, all test organisms dead after 17 weeks)	17 weeks	0.1				
$AsNaO_2$ (III)	*Champia parvula*	Red alga	Algae	NOEC	Sexual reproduction	14 days	0.060	ss	y	20–22°C; RI = 1	[96]
Invertebrates											
$AsHNa_2O_4$ (V)	*Strongylocentrotus purpuratus*	Purple sea urchin	Echinoderms	LOEC EC10	Embryo development	48 hours	0.011 0.006[3]	s	n	pH 7.8; 15°C; salinity 34 ppt; RI = 2	[49]
AsH_2NaO_4 (V)	*Palaemonetes pugio*	Daggerblade grass shrimp	Crustaceans	NOEC unbounded	Growth	28 days	0.025	s	y	20–25°C; salinity 12.5 ppt; RI = 2	[65]
As (V)	*Eurytemora affinis*	Copepod	Crustaceans	Effect	Mortality	15 days	0.100			Significant increase in juvenile mortality	[84]

Test substance	Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (mg l^{-1})	Expo-sure[1]	Toxicant analysis[2]	Comments	Reference
As (III)	*Americamysis bahia*	Opossum shrimp	Crustaceans	NOEC	Reproduction	29–51 days	0.631	f	y	pH 7.8–8.2; 20–25°C; salinity 30 ppt	[67]
$AsHNa_2O_4$ (V)	*Nitocra spinipes*	Harpacticoid copepod	Crustaceans	EC50	Reproduction	13 days	1.4	f	n	salinity 7 ppt	[25]
AsH_3O_4 (V)	*Crassostrea gigas*	Pacific oyster	Molluscs	NOEC unbounded	Mortality	21 days	0.010	s	y	RI = 3	[46]
Fish											
As_2O_3 (III)	*Oncorhynchus gorbuscha*	Pink salmon	Fish	NOEC	Mortality	10 days	2.65	s	n	pH 7.7; 10.5°C; RI = 2	[54]

[1] Exposure: s = static; ss = semi-static; f = flow-through.
[2] Toxicant analysis: y = measured; n = not measured.
[3] Calculated from data reported in the study [49].
LOEC = lowest observed effect concentration
NOEC = no observed effect concentration
MATC = maximum allowable toxicant concentration
ECx = concentration effective against X% of the organisms tested
LC100 = concentration lethal to 100% of the organisms tested
ppt = parts per trillion
RI = reliability index (see Annex 1)

Table 2.10 Most sensitive short-term aquatic toxicity data for saltwater organisms exposed to arsenic

Test substance	Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (mg l^{-1})	Exposure[1]	Toxicant analysis[2]	Comments	Reference
AsH_3O_4 (V)	*Skeletonema costatum*	Diatom	Algae	EC50	Population abundance	5 days	0.009	f	n	RI = 4	[76]
As (V)	*Skeletonema costatum*	Diatom	Algae	Effect	Biochemical	25 hours	0.025	ss	y	20°C	[86]
Invertebrates											
As (III)	*Acartia clausi*	Calanoid copepod	Crustaceans	LC50	Mortality	96 hours	0.907	s	n	8°C; salinity 30 ppt	[66]
As (III)	*Penaeus chinensis*	Shrimp	Crustaceans	LC50	Mortality	96 hours	0.003				[132]
As_2O_5 (V)	*Cancer magister*	Dungeness or edible crab	Crustaceans	LC50	Mortality	24 hours	0.232	s	n	pH 8.1; 15°C; salinity 34%	[68]
$AsHNa_2O_4$ (V)	*Nitocra spinipes*	Harpacticoid copepod	Crustaceans	LC50	Mortality female	96 hours	3.0	f	n	Salinity 7 ppt	[25]
AsH_2KO_4 (V)	*Tigriopus brevicornis*	Harpacticoid copepod	Crustaceans	LC50	Mortality	96 hours	0.011	s	n	pH 7.7–8.1; 20°C; salinity 35 ppt; RI = 2	[47]
As (III)	*Americamysis bahia*	Opossum shrimp	Crustaceans	LC50	Mortality	96 hours	1.74	f	y	pH 7.8–8.2; 20–25°C; salinity 30 ppt	[67]
As_2O_5 (V)	*Crassostrea gigas*	Pacific oyster	Molluscs	EC50	Development	48 hours	0.326	s	n	pH 8.1; 20°C; salinity 34%	[68]
As_2O_3 (III)	*Capitella capitata*	Worm	Polychaetes	LC50	Mortality	96 hours	2.050	s	n		[83]
As_2O_3 (III)	*Ophryotrocha labronica*	Worm	Polychaetes	LC50	Mortality	96 hours	1.500	s	n		[83]
$AsHNa_2O_4$ (V)	*Strongylocentrotus purpuratus*	Purple sea urchin	Echinoderms	LOEC EC10 EC50	Embryo development	48 hours	0.011 0.006[3] 0.015[3]	s	n	pH 7.8; 15°C; salinity 34 ppt; RI = 2	[49]
As_2O_3 (III)	*Corophium insidiosum*	Amphipod	Crustaceans	LC50	Mortality	96 hours	1.100	-	n	19°C	[82]
Fish											
As_2O_3 (III)	*Therapon jarbua*	Tigerfish	Fish	LC50 LC10	Mortality	96 hours	3.38 1.03	s	n	Salinity 36 g l^{-1}; RI = 2	[100]
As_2O_3 (III)	*Oncorhynchus gorbuscha*	Pink salmon	Fish	NOEC	Mortality	72 hours	9.5	s	n	pH 7.7; 10.5°C; RI = 2	[54]

Test substance	Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (mg l^{-1})	Exposure[1]	Toxicant analysis[2]	Comments	Reference
As_2O_5 (V)	*Morone saxatilis*	Striped bass	Fish	LC50	Mortality	96 hours	10.3	s	y	pH 8.1; 25°C; salinity 22.5 ppt; RI = 2	[43]
AsH_2NaO_4 (V)	*Morone saxatilis*	Striped bass	Fish	LC50	Mortality	96 hours	7.28	f	y	pH 7.6; 16-21.5°C; salinity 3.5–5.2 ppt; RI = 4	[62]

[1] Exposure: s = static; ss = semi-static; f = flow-through.
[2] Toxicant analysis: y = measured; n = not measured.
[3] Calculated from data reported in the study [49].
ppt = parts per trillion
LOEC = lowest observed effect concentration
NOEC = no observed effect concentration
ECx = concentration effective against X% of the organisms tested
LCx = concentration lethal to X% of the organisms tested
RI = reliability index (see Annex 1)

2.6.3 Toxicity to sediment-dwelling organisms

Freshwater sediment
Toxicity data for arsenic concentrations in freshwater sediment (e.g. on a mg/kg sediment basis) were not found.

Saltwater sediment
Only one study [46] could be located for saltwater sediment. Only one concentration was used in this study (20.5 mg/kg). At this concentration, no effects were observed on survival and only limited cytological alterations were noted.

2.6.4 Endocrine-disrupting effects

There is no evidence that arsenic compounds are endocrine disrupters.

2.6.5 Mode of action of relevant arsenic species

Both pentavalent and trivalent soluble arsenic compounds are rapidly and extensively absorbed from the gastrointestinal tract. In many species, arsenic metabolism is characterised by two main types of reactions:

- reduction reactions of pentavalent to trivalent arsenic;
- oxidative methylation reactions in which trivalent forms of arsenic are sequentially methylated to form mono-, di- and trimethylated products.

Methylation of inorganic arsenic facilitates the excretion of inorganic arsenic from the body in urine. There are major qualitative and quantitative interspecies differences in methylation to the extent that some species exhibit minimal or no arsenic methylation (e.g. marmoset monkey, guinea pig, chimpanzee). In humans, however, inorganic arsenic is extensively methylated and the metabolites are excreted primarily in the urine [7].

Both inorganic and organic forms of arsenic may cause adverse effects in laboratory animals as well as in aquatic and terrestrial biota. The effects induced by arsenic range from acute lethality to chronic effects such as cancer. The degree of toxicity of arsenic is basically dependent on the form (e.g. inorganic or organic) and the oxidation state of the arsenical. It is generally considered that inorganic arsenicals are more toxic than organic arsenicals. Within these two classes, the trivalent forms are more toxic than the pentavalent forms, at least at high doses. Several different organ systems are affected by arsenic, including skin, respiratory, cardiovascular, immune, genitourinary, reproductive, gastrointestinal and nervous systems [7].

Aquatic and terrestrial biota exhibit a wide range of sensitivities to different arsenic species. Their sensitivity is modified by biological and abiotic factors. The mode of toxicity and mechanism of uptake of arsenate by organisms differ considerably. This may explain why there are interspecies differences in organism response to arsenate and arsenite.

The primary mechanism of arsenite toxicity is considered to result from its binding to protein sulfhydryl groups. Arsenate is known to affect oxidative phosphorylation by

competition with phosphate. In environments where phosphate concentrations are high, arsenate toxicity to biota is generally reduced. As arsenate is a phosphate analogue, organisms living in elevated arsenate environments must acquire the nutrient phosphorous to avoid or reduce arsenic toxicity [7].

Arsenic and its compounds were evaluated by the International Agency for Research on Cancer (IARC) in 1973 [32] and the evaluation was updated in 1987 [101]. There was *sufficient evidence* for carcinogenicity to humans and *limited evidence* for carcinogenicity to animals. The overall evaluation was that arsenic and arsenic compounds are carcinogenic to humans (Group 1). This evaluation applies to the group of chemicals (i.e. arsenic and arsenic compounds) as a whole and not necessarily to all individual chemicals within the group [7].

3. Derivation of quality standards for arsenic

3.1 Use of the added risk approach

The EU Technical Guidance Document [24] does not provide specific guidance on dealing with (essential) elements such as arsenic that have natural background concentrations in the environment. However, according to Struijs *et al.* [23] and Crommentuijn *et al.* [16], the added risk approach may be used to deal with such substances.

In this approach, both the predicted environmental concentration (PEC) and the predicted no effect concentration (PNEC) are determined on the basis of the added amount of arsenic, resulting in an 'added PEC' (PEC_{add}) and 'added PNEC' ($PNEC_{add}$), respectively.

The use of the added risk approach (a method that in principle can be used for all naturally occurring substances) implies that only the anthropogenic additions of a substance (i.e. the amount added to the natural background concentration) are considered to be relevant for the effect assessment of that substance.[5] Thus, the contribution of the natural background concentration to toxic effects is ignored.

The maximum permissible concentration (MPC) in a water body or in sediment is the sum of the local natural background concentration ($C_{backgrnd}$) and the $PNEC_{add}$. The $PNEC_{add}$ is equivalent to the EQS:

$MPC = PNEC_{add} + C_{backgrnd}$ (with $PNEC_{add} \approx EQS$)

$PEC_{add} = EC - C_{backgrnd}$ (with EC = actual environmental concentration at site X)

Two assumptions underlie this approach:

1. The extent to which the background concentration of a metal has an impact on ecosystem structure and function is not relevant. Any potential adverse or positive effect of the background concentration can be considered as effects contributing to the natural biodiversity of ecosystems.

2. As species in an ecosystem are adapted to the prevailing background level, it is assumed that the same amount of a metal added by human activities, in principle, causes the same effect. In such circumstances, however, all environmental

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

parameters determining metal toxicity must be equal apart from the background level of the metal concerned (i.e. it is not the ‘absolute’ level of a metal that is decisive for the occurrence/extent of adverse effects, only the added amount).

The background concentration and the $PNEC_{add}$ are independently derived values. Real world background concentrations may be derived on the basis of monitoring data for relatively pristine areas or be based on calculations using geological and hydrological data.

In addition, the use of the added risk approach implies that there is no risk of deficiency of essential metals at the level of the calculated quality standard. By definition, the background concentration in a given ecosystem provides the resident organisms with the required essential metals.

3.2 Consideration of factors determining arsenic bioavailability and toxicity in the water column

Arsenic exists in the environment in various chemical forms. The presence of one arsenic species over another and the availability of each species is dependent on physico-chemical processes, such as pH, hardness and dissolved organic matter (DOM). Ideally, the influence of water quality parameters on the bioavailability and toxicity of arsenic should be taken into consideration in setting quality standards. However, there is currently insufficient scientific knowledge for arsenic to consider these issues quantitatively.

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

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

4.1.1 PNECs for freshwaters

A number of low effect concentrations are available for fish and amphibian species in studies carried out by Birge, Black and colleagues [27–29]. As the effect concentrations found in these studies are usually very low compared with those observed by other authors, the entire information provided by them was examined carefully, but no plausible reason for the large discrepancies in their data could be found. Nevertheless, it was decided by the EU Member State Experts of the Technical Meeting on Existing Substances that these data are not suitable for the derivation of a $PNEC_{aqua}$.

Furthermore, in the derivation of quality standards for WFD Annex X priority substances, the data produced by Birge, Black and colleagues were not deemed reliable for standard setting [the subject was discussed again by an expert group consisting of experts from Member States, industry and 'green' non-governmental organisations (NGOs)]. This report follows the decision by the Experts of the Technical Meeting on Existing Substances and the data provided by these authors [27–29] are not considered for PNEC and EQS derivation.

PNEC accounting for the annual average concentration

Algae with no observed effect concentration (NOEC) or lowest observed effect concentration (LOEC) values in the range of 5 to 50 µg l^{-1} As(V) appear, on face value, to be the most sensitive taxonomic group in the freshwater database (Table 2.7). However, the reliability of the study by Hörnström [55] with the lowest reported LOEC of 5 µg l^{-1} As for the alga, *Stichogloea doederleinii*, is not assignable due to insufficient reporting of study details. The alga study by Vocke *et al.* [98] resulted in the next lowest algae LOEC of 10 µg l^{-1}. Although this study is considered reliable, it was conducted in line with a now obsolete test protocol allowing 14 days of exposure. Consequently, this study is used only in a supporting capacity.

Crustaceans also appear to be sensitive to the effects of arsenic. The water quality guidelines for arsenic issued by the Government of British Columbia [21] state that:

> 'A review of the available literature revealed that the invertebrate *Daphnia magna* (common throughout British Columbia) was the most sensitive freshwater organism to arsenic. US pesticide regulatory tests yielded a lowest observed effect concentration (LOEC) for growth of 38 µg l^{-1} for arsenic acid (H_3AsO_4), which is equivalent to 20 µg l^{-1} as arsenic, in a 21-d flow-through

chronic bioassay. The no observed effect concentration (NOEC) was determined to be 20 µg l^{-1} arsenic acid, which is equivalent to 10.5 µg l^{-1} arsenic.'

The full report was not available and no other study reporting results at this level of sensitivity of *Daphnia magna* could be found. However, a lower test result was reported for the related species, *Daphnia pulex*, with a LOEC of 10 µg l^{-1} As for significant effects on reproduction [37]. This study was based on nominal concentrations, but used a semi-static exposure regime. Given the fate of arsenic in water, it is unlikely that this chemical would have undergone significant degradation and therefore this study has been treated as reliable with restriction. This assumption is supported by studies where nominal concentrations of arsenic have been found to be almost identical to measured concentrations [52, 65, 79, 94, 97].

Fish appear to be slightly less sensitive to arsenic than algae and crustaceans. The lowest reported long-term data point was a NOEC of 25 µg l^{-1} As(III) (embryos of Japanese medaka) [57]. This study was carried out to a standard protocol, but reported few experimental details. Consequently the reliability of this study was difficult to assign. The lowest reliable study for fish was a significant effect on migration of coho salmon exposed to 300 µg l^{-1} arsenic trioxide for 6 months [74]. This was a well-documented study based on a flow-through system with measured exposure concentrations and is regarded as valid for PNEC derivation.

It is recommended that the $PNEC_{add}$ is based on the LOEC of 10 µg l^{-1} for significant effects on the reproduction of *Daphnia pulex* reported by Chen *et al.* [37]. The total number of neonates per female at the LOEC of 10 µg l^{-1} As(V) is approximately 20 per cent lower than the control. Hence, according to the TGD [24], a NOEC can be calculated by dividing the LOEC by 2. Because long-term NOEC data for at least three trophic levels are available, the appropriate assessment factor to be applied to the lowest NOEC is 10. Hence, the $PNEC_{add,freshwater_lt}$ is calculated as follows:

$PNEC_{add,freshwater_lt}$ = 10 µg l^{-1}/(2 × AF 10) = 0.5 µg l^{-1} arsenic (dissolved)

PNEC accounting for transient concentration peaks
Algae appear to be the most acutely sensitive taxonomic group. The lowest reported acute effects value was reported by Chen *et al.* [38] and refers to the algal species *Scenedesmus acutus* (96-hour EC50 of 79 µg l^{-1}; Table 2.8). This study was based on nominal concentrations, but used a semi-static exposure regime (24-hour renewal). Given the fate of arsenic in water, it is unlikely that this chemical would have undergone significant degradation and therefore this study has been treated as reliable with restriction. This assumption is supported by studies where nominal concentrations of arsenic have been found to be almost identical to measured concentrations [52, 65, 79, 94, 97].

Crustaceans appear to be less sensitive to the short-term effects of arsenic. The lowest available LC50 is 250 µg l^{-1} for the cladoceran *Bosmina longirostris* [75]. However, this study was generated by the US Fisheries and Wildlife Service and it was not possible to locate the original report. Consequently, there were few details with which to assess the quality of this study. A slightly higher EC50 (96-hour EC50 immobilisation) of 850 µg l^{-1} As(V) was reported for the same species [79]. This study was based on measured

exposure concentrations and a static regime and is therefore suitable for PNEC derivation. A slightly lower EC50 (immobilisation) of 680 µg l^{-1} has been reported for the midge *Chironomus tentans* [61]. This was a static study based on nominal exposure concentrations and is used as supporting information.

Fish appear to be less sensitive to acute exposures to arsenic. The lowest reasonable quality study for fish was a 96-hour LC50 of 4.76 mg l^{-1} for juveniles of the Arctic grayling (*Thymallus arcticus*) [31]. This study was based on nominal concentrations and has been used as supporting information only. All other fish data indicate lower sensitivity (apart from the data of Birge et al. [29], which are regarded as unreliable).

Amphibians also appear to be sensitive to arsenic, with a 96-hour LC50 of 249 µg l^{-1} for the frog *Rana hexadactyla* [60]. This was a semi-static study with nominal exposure concentrations and has been used as supporting information.

It is recommended that the $PNEC_{add,freshwater_st}$ for effects following short-term exposure to arsenic is based on the lowest EC50 of 79 µg l^{-1} As reported for *Scenedesmus acutus* and guidance in the TGD on effects assessment for intermittent releases (Section 3.3.2 of Part II of the TGD [24]). Algae appear to be the most sensitive species to acute exposures. In addition, supporting information from the long-term data base indicate that they are also one of the most sensitive organisms to long-term exposures [*Scenedesmus obliquus* 14-day NOEC of 10 µg l^{-1} [98] (Table 2.7)]. Therefore, only a reduced assessment factor of 10 (instead of 100) is required to extrapolate from the 50 per cent acute effect level to a short-term no effect level. As the resulting short-term PNEC is then slightly lower than the lowest validated long-term NOEC for *Scenedesmus obliquus* [98], the proposed PNEC should prevent any significant impact on the algae community in the event of exposure peaks.

$PNEC_{add,freshwater_st}$ = 79 µg l^{-1}/AF (10) = 8 µg l^{-1} arsenic (dissolved)

4.1.2 PNECs for saltwaters

Freshwaters and saltwaters differ in various abiotic physico-chemical factors including natural background concentrations of essential and other elements. For metals/metalloids, it was decided not to combine the freshwater and saltwater effects databases, but to derive PNECs for freshwaters and saltwaters on the basis of their respective effects data.

PNEC accounting for the annual average concentration
The lowest reliable chronic data in the saltwater database are for the marine diatom *Skeletonema costatum*, with LOECs of 10 µg l^{-1} for As(III) and 13 µg l^{-1} for As (V) [85] (it was not possible to derive a NOEC or an LC10 from the data reported in the study references). This study was based on measured exposure concentrations in a static system and was regarded as suitable for PNEC derivation. In addition, data for the macroalga *Fucus serratus* indicate a growth and mortality related NOEC of 20 µg l^{-1} As(V) [50]. This datapoint was generated in a flow-through study with measured exposure concentrations and is reliable for PNEC derivation.

Reliable chronic effects values for marine invertebrates are much higher than those reported for algae (Table 2.9). However, there is one 48-hour test on sea urchin embryo development that may be considered as an early life stage (ELS) test rather than an

acute test [49]; this reported a LOEC of 11 µg l^{-1} As(V) for development of embryos of the sea urchin *Strongylocentrosus purpuratus.* On the basis of the data reported, it was possible to derive an EC10 of 6 µg l^{-1} and an EC50 of 15 µg l^{-1}.[6] This study was based on nominal concentrations, but was regarded as valid for PNEC derivation because in studies where nominal concentrations of arsenic have been compared with measured concentrations they have been found to be almost identical [52, 65, 79, 94, 97].

Only limited fish data were available. Only one long-term study was available that reported a 10-day NOEC (mortality) of 2,650 µg l^{-1} As(III) [54]. However, this study was based on nominal concentrations in a static system and is therefore used as supporting data only.

For some acute effects, the lowest reported values are in the same range as the lowest chronic data, i.e. the 120-hour EC50 for the alga *Skeletonema costatum* of 9 µg l^{-1} As(V) and the 96-hour LC50 of the crustacean *Tigriopus brevicornis* of 11 µg l^{-1} As(V). Following the given in the TGD [24] for marine risk assessment, this situation (in which the lowest acute effect values are as low as the lowest chronic values) requires the use of an assessment factor of 1,000 on the lowest acute value. However, as it is not possible to gain access to the background documentation for the *Skeletonema* acute EC50 of 9 µg l^{-1} in the original reference [76], the validity of this test result is not assignable. The other references have been evaluated and are considered reliable.

Based on the data in Tables 2.9 and 2.10, the saltwater acute to chronic ratios for arsenic appear to be low, suggesting that only a small assessment factor would be required to extrapolate from an effects concentration to a no-effect concentration. In addition, long-term NOECs for more than three marine species representing three trophic levels (i.e. algae, crustaceans, echinoderms and molluscs) plus acute data for a further marine group (annelids) are available. Consequently, it is recommended that the $PNEC_{add,saltwater_lt}$ is based on the lowest available EC10 of 6 µg l^{-1} reported by Garman *et al.* [49] and an assessment factor of 10:

$PNEC_{add,saltwater_lt}$ = 6 µg l^{-1}/AF (10) = 0.6 µg l^{-1} arsenic (dissolved)

PNEC accounting for transient concentration peaks
The lowest acute data point for algae was a 120-hour EC50 of 9 µg l^{-1} for growth of the alga *Skeletonema costatum* [76]. However, it was not possible to gain access to the background documentation of this study in the original reference by the US Office of Pesticide Programs. Consequently, the validity of this test result is not assignable.

The lowest invertebrate value, cited in the ECOTOX database, was a report of a 96-hour LC50 of 3 µg l^{-1} As(III) for the prawn, *Penaeus chinensis* [132]. However, this value was taken from an English abstract of a Chinese publication and it has not been possible to verify its reliability. The next lowest value was a 96-hour EC50 of 11 µg l^{-1} for the mortality of the crustacean *Tigriopus brevicornis* [47]. This study was based on nominal concentrations but was regarded as valid for PNEC derivation because, in studies where nominal concentrations of arsenic have been compared with measured concentrations, they have been found to be almost identical [52, 65, 79, 94, 97].

[6] Data for calculations were taken from Figure 2A of the study by Garman *et al.* 1997 [49] and the LC10 and LC50 values were calculated using the software program ToxRat® [22]. Input data were 0 (control), 2.3, 11, 23, 46, 93 µg l^{-1} As and respectively 88, 78, 62, 23, 2, 0 per cent of normal embryo development.

In addition to the crustacean data a 48-hour EC50 of 15 µg l^{-1} was reported for larval development of the echinoderm species *Strongylocentrosus purpuratus* (Table 2.10) [49]. This study was also based on nominal exposure concentrations, but has been used as supporting data.

Fish appear to be of lower sensitivity to arsenic than algae, crustaceans or echinoderms. The lowest reported effect concentration was a 96-hour LC10 of 1,030 µg l^{-1} reported for the tiger fish (*Therapon jarbua)* [100]. This study was also based on nominal exposure concentrations, but has been used as supporting data.

The TGD does not provide specific guidance for assessment of the acute effects of intermittent releases to marine water bodies. Therefore, it is recommended that a PNEC for effects following short-term exposure to arsenic is derived on the basis of general guidance in the TGD on effects assessment for intermittent releases (Section 3.3.2 of Part II of the TGD [24]) and the lowest valid EC50 of 11 µg l^{-1} for mortality of the copepod *Tigriopus brevicornis*. As the acute effects values of these most sensitive species are nearly in the range of the lowest chronic effects values (i.e. very low acute to chronic effects ratios), it is recommended that only a reduced assessment factor of 10 (instead of 100) is used to extrapolate from the 50 per cent acute effect level to a short-term no effect level.

$PNEC_{add,saltwater_st}$ = 11 µg l^{-1}/AF (10) = 1.1 µg l^{-1} arsenic (dissolved)

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

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

4.3 Derivation of existing EQSs

WRc's 1992 report to the Department of Environment [18] supported both the freshwater and saltwater EQSs proposed previously by Mance *et al.* [19].

The freshwater EQS was based on an LC10 of 0.14 mg l^{-1} As obtained in a 16-week study on bluegill sunfish (*Lepomis macrochirus*). An assessment factor of 2 was applied to this value resulting in a rounded EQS of **50 µg l^{-1} total dissolved arsenic** expressed as an annual average concentration.

For the protection of saltwater life, the lowest acute effect values were a 96-hour LC50 of 508 µg l^{-1} for copepod (*Acartia clausi*) and a concentration of 577 µg l^{-1} observed for arrested spore development in an 18-hour study on red alga *Plumaria elegans*. An assessment factor of 20 was applied to these values because the available data covered an extremely small range of biota. This resulted in an EQS of **25 µg l^{-1} total dissolved arsenic** expressed as an annual average concentration.

4.4 Derivation of PNECs for sediment

4.4.1 PNEC derivation by the TGD deterministic approach

Reliable experimental data on sediment toxicity are not available. Therefore, it was not possible to derive a sediment standard.

4.4.2 PNEC derivation by the TGD probabilistic approach

Because no experimental effects data for benthic organisms are available, statistical extrapolation cannot be applied to derive a $PNEC_{add,sediment.}$

4.5 Derivation of PNECs for secondary poisoning of predators

4.5.1 Mammalian and avian toxicity data

Recent reviews have been published on arsenic toxicity to mammals and birds [7, 13, 39, 41]. Additional literature searches were performed to locate any lower effect data published since the above reviews. Table 4.1 summarises the information obtained from these sources.

Table 4.1 Most sensitive mammalian and bird oral toxicity data relevant for the assessment of secondary poisoning

Study and result	Details
Sub-chronic toxicity to mammals	
Hughes and Thompson 1996 [56] Cited in WHO 2001 [7] **Sub-chronic LOAEL = 3 µg arsenic/kg bw/day**	Mice received sodium arsenate in their drinking water for 28 days at concentrations of either 0.025 mg arsenate l^{-1} (3 µg arsenic/kg bw/day) or 2.5 mg arsenate l^{-1} (300 µg arsenic/kg bw/day). Clinical chemistry showed effects on the kidney at both doses. However, this was not supported by any histopathological changes. Thus a LOAEL of 3 µg arsenic/kg bw/day was set.
Blakley *et al.* 1980 [30] Cited in WHO 2001 [7] **Sub-chronic LOAEL = 0.5 mg arsenic l^{-1}**	Mice received sodium arsenite in their drinking water at a concentration of 0.5, 2 or 10 mg arsenic l^{-1} for 3 weeks. At all levels, an immunosuppression of the humoral response was observed. No other toxicity effects were discussed.
Chronic toxicity to mammals	
WHO 2002 [39] **Chronic carcinogenicity LOEL = 500 µg sodium arsenate l^{-1} water**	C57B1/6J mice received sodium arsenate at a concentration of 500 µg l^{-1} via their drinking water for 26 months. Based on an increased incidence of tumours in the intestinal tract, lungs, liver and, to a smaller extent, other organs, a LOAEL at this level can be set.

Study and result	Details
Carmignani *et al.* 1983 [36] Cited in WHO 2001 [7] **Chronic LOEL = 50 µg arsenic ml^{-1} water**	Rats received sodium arsenate in their drinking water at a concentration of 50 µg arsenic ml^{-1} for 360 days. Focal changes in the kidney glomerulus and tubules and swollen hepatocytes near the centrilobular vein in the liver were the effects noted. Thus this level can be set as a LOAEL. No effects were noted on baseline cardiovascular parameters such as heart rate, blood pressure, electrocardiogram patterns and cardiovascular responses to several neurohumoral agonists. This LOEL is also applicable to rats and rabbits administered this concentration for 18 and 10 months, respectively, on the basis of the occurrence of changes in baseline cardiovascular parameters [35].
Thorgeirsson *et al.* 1994 [95] Cited in WHO 2001 [7] **Chronic NOEL = 0.1 mg sodium arsenate/kg bw/day for 5 days/week**	Cynomolgus monkeys (*Macaca fascicularis*) received sodium arsenate orally at a concentration of 0.1 mg/kg bw/day for 5 days a week for at least 15 years. No malignant tumours occurred. However, details are lacking as to other toxic effects observed.
Ng *et al.* 1998, 1999 [72, 73] Cited in WHO 2001 [7] **Chronic LOAEL = 0.07–0.08 mg arsenic/kg bw/day**	C57BL/6J mice received sodium arsenate in their drinking water at a concentration of 500 mg arsenic l^{-1}, which equates to approximately 0.07–0.08 mg arsenic/kg bw/day, for 26 months. Decreased survival and increased incidence of tumours in the gastrointestinal tract, lung, liver, spleen, skin, eye and reproductive system were observed. A NOEC could not be calculated from these data.
Effects on reproduction of mammals	
Pant *et al.* 2004 [78] **Reproductive LOAEL = 4 mg arsenic l^{-1} water**	Male mice received sodium arsenite in their drinking water at a concentration of 53.39 µmol l^{-1} (4 mg arsenic l^{-1}) for 365 days. Effects observed included decreased absolute and relative testicular weight, decreased testicular marker enzymes, decreased sperm count and sperm motility, increased abnormal sperm, and arsenic accumulation in testes, epididymis, seminal vesicle and prostate gland. A NOEC could not be calculated from these data.
Embryotoxicity and teratogenicity	
Nemec *et al.* 1973 [71] Cited in WHO 2001 [7] **Developmental NOAEL = 7.5 mg arsenic acid/kg bw/day**	Female CD1 mice received daily doses of 0, 7.5, 24 or 48 mg arsenic acid/kg bw during days 6–15 of gestation via oral gavage. Developmental toxicity effects were also seen at doses that cause maternal toxicity; thus a NOAEL was set at 7.5 mg/kg bw/day. No teratogenic effects were observed at any dose.

Study and result	Details
Nemec *et al.* 1973 [71] Cited in WHO 2001 [7] **Developmental NOAEL = 0.75 mg arsenic acid/kg bw/day**	Female New Zealand white rabbits received daily doses of 0, 0.19, 0.75 or 3 mg arsenic acid/kg bw during days 6–18 of gestation via oral gavage. Developmental toxicity effects (i.e. increased foetal resorptions and decreased foetal weight) were also seen at doses that caused maternal toxicity (i.e. decreased weight gain and mortality). Thus a NOAEL was set at 0.75 mg/kg bw/day. No teratogenic effects were observed at any dose.
Sub-chronic toxicity to birds	
Office of Pesticide Programs 2000 [76] Cited in ECOTOX 2005 [44] **Sub-chronic LD50 = 46 mg arsenic acid/kg bw**	18-week-old Northern Bobwhite (*Colinus virginianus*) received arsenic via oral capsules for 14 days. Based on mortality, an LD50 of 46 mg/kg bw was derived.
Office of Pesticide Programs 2000 [76] Cited in ECOTOX 2005 [44] **Sub-chronic LD50 = 28.9 mg arsenic acid/kg bw**	27-week-old Northern Bobwhite (*Colinus virginianus*) received arsenic acid via oral capsules for 21 days. Based on mortality, an LD50 of 28.9 mg/kg bw was derived.
Stanley *et al.* 1994 [92] Cited in ECOTOX 2005 [44] **Sub-chronic NOEL = 93.3 µg disodium salt of arsenic acid/g**	1-year-old Mallard ducks (*Anas platyrhynchos*) received the disodium salt of arsenic acid via the diet for 53 days. Based on effects on the onset of egg production, a NOEL of 93 µg/g was derived.
Holcman and Stibilj 1997 [53] Cited in ECOTOX 2005 [44] **Sub-chronic NOEL = 30 mg As(III)/kg diet**	Rhode Island Red hens received arsenic trioxide in their diet at levels of up to 30 mg As(III)/kg for 19 days. No effects were observed on food consumption, number of eggs per hen, body weight or average egg weight.
Whitworth *et al.* 1991 [99] Cited in ECOTOX 2005 [44] **Chronic NOAEL = 100 mg arsenic/kg bw**	1-day-old Mallard ducks (*Anas platyrhynchos*) received arsenic via oral capsules for 9 weeks. No behavioural changes or general toxicity was observed at this concentration.
Long-term toxicity to birds	
Stanley *et al.* 1994 [92] Cited in ECOTOX 2005 [44] **Chronic LOEL = 22 µg disodium salt of arsenic acid/g**	1-year-old Mallard ducks (*Anas platyrhynchos*) received the disodium salt of arsenic acid via the diet for approximately 173 days. At 22 µg/g, residues were detected in the liver, at 403 µg/g the body weight was affected and so was the liver/body weight ratio. An overall LOEL was set at 22 µg/g.

bw = body weight
LOAEL = lowest observed adverse effect level
NOAEL = no observed adverse effect level

4.5.2 PNECs for secondary poisoning of predators

Biomagnification of arsenic has not been observed in aquatic food chains [7]. With the exception of algae and higher plants, bioaccumulation of arsenic in organisms appears to be very low (normally well below BCF 100; see Section 2.5). In saline environments, however, arsenic BCFs are reported to be generally higher.

Based on the available information on bioaccumulation, biotransformation and metabolisation, secondary poisoning of predators appears not to be a realistic scenario. Therefore, it is not considered necessary to derive a quality standard for the protection of predators from secondary poisoning.

5. Analysis and monitoring

Standard methods published by the US EPA for the measurement of total arsenic in water and wastewater, solid wastes, soil and sediments include:

- inductively coupled plasma-mass spectrometry (ICP-MS) [118, 119, 125]
- ICP-atomic emission spectrometry (ICP-AES) [121]
- atomic absorption spectrometry (AAS) [120]
- graphite furnace atomic absorption spectrometry (GFAAS) [120]
- quartz furnace hydride generation [122]
- an electrochemical method using anodic stripping voltammetry (ASV) [123].

Methods using AAS typically provide a limit of detection of 0.5 µg l^{-1} in aqueous samples. A modification to EPA Method 1632 hydride generation [122] using cryogenic gas chromatography (GC) allows the technique to be adopted for As(III), As(V), MMA and DMA to the 3 ng l^{-1} level [124].

Similar methods are recommended by the American Public Health Association (APHA) for water using:

- AAS/hydride generation [103]
- AAS/graphite furnace technique [104]
- ICP-AES [105]
- silver diethyldithiocarbamate (SDDC) spectrophotometry [106].

The AAS/hydride generation method is generally resistant to matrix and chemical interferences [103]. Techniques to compensate for these interferences have been described by the US EPA [115].

Speciation of inorganic arsenic in environmental samples is usually accomplished by chromatographic separation, chelation–extraction or elution of As(III) and then reduction of As(V) with subsequent similar treatment [e.g. 109, 127].

Samples may be prepared for AAS in a variety of ways. Most often, the gaseous hydride procedure is employed [112, 126]. This takes the form of reduction of arsenic species by sodium borohydride to gaseous arsine (AsH_3), which is then trapped and introduced into the flame or heated quartz cell. This approach measures total inorganic arsenic, but may not detect all organic forms unless preceded by a digestion step using nitric, sulfuric and/or perchloric acids [129].

Speciation of organoarsenicals, rather than total arsenic, is usually accomplished by employing separation procedures prior to introduction of the sample material into a detection system. Limits of detection in such systems are generally less than 1 µg l^{-1}. Various types of high-pressure liquid chromatography (HPLC) or chelation–extraction techniques are most commonly used in combination with AAS, ICP-AES or ICP-MS detection methods [e.g. 107, 110, 113, 130]. Another approach involves selective reduction of As(V) to As(III) (permitting quantification of individual inorganic arsenic

species) and selective distillation of methylarsines to quantify MMA and DMA [102, 108, 111]. For arsenic compounds such as arsenobetaine and arsenocholine, HPLC-ICP-MS has been employed to determine arsenic speciation in blood plasma that was entirely arsenobetaine [114].

Arsenic in environmental samples other than water is generally determined by AAS techniques (limits of detection around 0.1 mg/kg), with samples prepared by digestion with nitric, sulfuric and/or perchloric acids [120, 121]. Other methods include a spectrophotometric technique in which a soluble red complex of arsine and SDDC is formed [117] and analysed using ICP-AES [116, 121], GFAAS, ICP-MS [125] and X-ray fluorescence [128].

Atomic absorption spectrophotometry is also the most common analytical procedure for measuring arsenic in biological materials [112, 126] with limits of detection of around 0.05 mg/kg being readily achievable. Inductively coupled plasma atomic emission spectrometry and ICP-MS are increasingly common techniques for the analysis of arsenic. Both methods can generally provide lower detection limits than absorbance detection methods.

The lowest proposed PNEC derived for fresh and salt waters for arsenic is 0.5 µg l^{-1}. To provide adequate precision and accuracy, the data quality requirements are that, at a third of the EQS, the total error of measurement should not exceed 50 per cent. From the literature, it can be seen that analytical methodologies provide detection limits as low as 3 ng l^{-1}, which suggests that they offer adequate performance to analyse for compliance.

6. Conclusions

6.1 Availability of data

A substantial number of laboratory toxicity data are available for both As(V) and As(III). The taxonomic spread of data for freshwater organisms is extensive and there is evidence that algae and crustaceans are the most sensitive taxa following acute and chronic exposure.

Although much fewer in number and covering fewer taxa, saltwater data are available for algae, crustaceans, fish, molluscs, annelids and echinoderms. Again, algae are particularly sensitive along with echinoderms and crustaceans. There are no field or mesocosm data available for arsenic.

Although water quality factors are known to influence arsenic speciation, bioavailability and toxicity, there is not yet an adequate understanding to take these factors into account in deriving PNECs in a quantitative way. Finally, although arsenic is a known genotoxic carcinogen, there is no information on such effects in aquatic organisms.

6.2 Derivation of PNECs

The ‘added risk’ approach is considered appropriate when deriving PNECs for arsenic because arsenic is a naturally occurring substance which organisms will have been exposed to over an evolutionary timescale. This takes account of background concentrations and the PNEC applies only to the ‘added’ contribution over and above the background level. A practical consequence of this is that compliance assessment would need to consider background levels, at least at a regional scale, if not a local scale.

Neither As(V) nor As(III) is consistently more toxic than the other to aquatic organisms and so there is no compelling justification for separate PNECs for them. However, by basing PNECs on the lowest credible data, this uncertainty should be accommodated. The proposed values, therefore, refer to total arsenic.

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

6.2.1 Long-term PNEC for freshwaters

The lowest effect concentration was obtained in a study with the water flea, *Daphnia pulex*, where a 20 per cent reduction in reproduction resulted from chronic exposure to 10 µg l^{-1} As(V). Similar concentrations have given rise to such effects in another species of water flea, *Daphnia magna*, and inhibition of algal growth. However, these were not reported adequately to form the basis of a PNEC and the algal study used an obsolete protocol, allowing exposure for 14 days. Consequently, they are suitable only as supporting data.

According to the Annex V methodology, a NOEC can be derived from the LOEC of 10 µg l^{-1} by dividing it by 2. To this, an assessment factor of 10 is justified on the basis that

data for other trophic levels are also available. As a result, a $PNEC_{freshwater_lt}$ of 0.5 µg l^{-1} arsenic (dissolved) is recommended.

This PNEC is 100 times lower than the existing statutory EQS of 50 µg l^{-1} developed in 1992. The existing EQS was based on an assessment factor of just 2 applied to a chronic LC10 of 0.14 mg l^{-1} for bluegill sunfish (*Lepomis macrochirus*). The difference reflects data for more sensitive species that have become available since the original EQS was derived and the application of larger assessment factors, as required by the Annex V methodology.

6.2.2 Short-term PNEC for freshwaters

Algae appear to the most sensitive taxonomic group to arsenic and the lowest reliable effect concentration is a 96-hour EC50 (reduction in algal biomass) of 79 µg l^{-1} As(III) for *Scenedesmus acutus*. Studies with other taxa indicate lower sensitivity and so should be protected by a PNEC based on data for algae. The indications that algae are indeed the most sensitive taxonomic group encourages the use of a small assessment factor (10), resulting in a $PNEC_{freshwater_st}$ of 8 µg l^{-1} arsenic (dissolved). This is lower than the lowest validated algal LOEC, and should, therefore, protect algal communities in the event of a short-term peak in exposure.

There is no existing short-term EQS for arsenic.

6.2.3 Long-term PNECs for saltwaters

Similar toxicities (expressed as LOECs) are seen in studies with the marine diatom, *Skeletonema costatum*, for As(III) and As(V) (10 and 13 µg l^{-1}, respectively). Although most invertebrates are less sensitive, embryo development in the sea urchin *Strongylocentrosus purpuratus* was impaired after 48-hour exposure to As(V). This gave rise to a LOEC of 11 µg l^{-1}, but it was not possible to estimate a NOEC from this study. Subsequent re-analysis of the study data gave an EC50 of 15 µg l^{-1} and an EC10 (considered equivalent to a NOEC) of 6 µg l^{-1}. Given the availability of data for other trophic levels, an assessment factor of 10 applied to this LOEC is recommended, leading to a $PNEC_{saltwater_lt}$ of 0.6 µg l^{-1} arsenic (dissolved).

The proposed PNEC is 40 times lower than the existing statutory EQS of 25 µg l^{-1}, based on an assessment factor of 10 applied to an acute LC50 of 0.232 mg l^{-1} for the crab, *Cancer magister*. This is entirely a consequence of new data that have become available since the original EQS was derived.

6.2.4 Short-term PNECs for saltwaters

Poorly reported studies indicate effects on crustaceans at concentrations of arsenic as low as 3 µg l^{-1}, but more reliable studies with embryo development in sea urchins give rise to EC50 values of 15 µg l^{-1}. However, the lowest reliable effect concentration is a 96-hour LC50 of 11 µg l^{-1} As(V) for the crustacean, *Tigriopus brevicornis*. These concentrations are similar to those giving rise to effects following chronic exposure to arsenic, indicating a low acute:chronic ratio. This justifies the use of an assessment factor of only 10 applied to the *T. brevicornis* 96-hour LC50, resulting in a $PNEC_{saltwater_st}$ of 1.1 µg l^{-1} arsenic (dissolved).

There is no existing short-term EQS for arsenic.

6.2.5 PNEC for secondary poisoning

There is no evidence of biomagnification of arsenic in food chains with the possible exception of algae and higher plants. Secondary poisoning of predators, e.g. mammals and birds, is not considered a significant risk and PNECs for secondary poisoning are not proposed.

6.2.6 PNEC for sediments

There are insufficient data to derive a sediment PNEC for arsenic and the use of equilibrium partitioning to estimate a value based on aquatic toxicity data cannot be justified for metals.

Table 6.1 Summary of proposed PNECs

Receiving medium/exposure scenario	Proposed PNEC (µg l^{-1} total dissolved arsenic)	Existing EQS (µg l^{-1} total dissolved arsenic)
Freshwater/long-term	0.5	50
Freshwater/short-term	8	No standard
Saltwater/long-term	0.6	25
Saltwater/short-term	1.1	No standard

6.3 Analysis

The lowest proposed PNEC derived for arsenic is 0.5 µg l^{-1}. The data quality requirements are that, at a third of the EQS, the total error of measurement should not exceed 50 per cent. Current analytical methodologies provide detection limits as low as 3 ng l^{-1}, which suggests that they would be adequate for assessing compliance.

6.4 Implementation issues

As an ‘added risk’ approach is proposed, background concentrations of arsenic would need to be established.

There are no further outstanding issues that need to be addressed before these PNECs can be used as EQSs. The PNECs proposed above are, therefore, recommended for adoption as EQSs.

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List of abbreviations

AA	annual average
AAS	atomic absorption spectroscopy
AF	assessment factor
APHA	American Public Health Association
ASV	anodic stripping voltammetry
BCF	bioconcentration factor
bw	body weight
CAS	Chemical Abstracts Service
DMA	dimethylarsinic acid
DPC	days postcoitum
EC50	concentration effective against 50% of the organisms tested
ECB	European Chemicals Bureau
ECx	concentration effective against X% of the organisms tested
ELS	early life stages
EQS	Environmental Quality Standard
GFAAS	graphite furnace atomic absorption spectrometry
IARC	International Agency for Research on Cancer
ICP-AES	inductively coupled atomic emission spectrometry
ICP-MS	inductively coupled plasma mass spectrometry
IUPAC	International Union of Pure and Applied Chemistry
LC50	concentration lethal to 50% of the organisms tested
LCx	concentration lethal to X% of the organisms tested
LOAEL	lowest observed adverse effect level
LOEC	lowest observed effect concentration
lt	long term
MATC	maximum allowable toxicant concentration
MMA	monomethylarsonic acid
MPC	maximum permissible concentration
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
OECD	Organisation for Economic Co-operation and Development
PEC	predicted environmental concentration
PNEC	predicted no-effect concentration

ppt	parts per trillion
SDDC	silver diethyldithiocarbamate
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
wf	without feeding
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	**31**
Chemical	Arsenate oxide
Species (common name)	Arctic grayling
Species (scientific name)	*Thymallus arcticus*
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	juvenile ~20 g
Exposure regime (e.g. static, flow-through, feeding, etc.)	Static
Test method	ASTM 1988*
Test concentrations used	Minimum six + control
Number of replicates per concentration	Not reported
Number of organisms per replicate	10
Measurement of exposure concentrations	No
Temperature	12°C
Hardness	41.3 mg l^{-1} $CaCO_3$
pH/salinity	pH 7.1–8
Exposure duration	96 hours
Endpoint (e.g. NOEC, EC50)	EC50
Effect (e.g. reproduction, survival, growth)	Mortality – absence of heartbeat
Concentration	4.76 mg l^{-1}
Initial quality assessment	Moderate – some methodology details not reported, no measured concentrations
Relevance of study	**Relevant**
Klimisch Code	**2**
Comments	Some limited details to assess quality, but based on a standardised method.

* American Society for Testing Materials (ASTM), 1988 *Standard guide for conducting early life-stage toxicity tests with fishes*. E 1241-88. 26 pp. West Conshohocken, PA: ASTM.

Reference number	**34**
Chemical	Sodium arsenite [As(III)]
Species (common name)	1. Snail 2. Isopod 3. Gammarid - epigean 4. Gammarid – hypogean 5. Caddisfly 6. Mayfly
Species (scientific name)	1. *Physa fontinalis* 2. *Asellus aguaticus* 3. *Gammarus fossarum* 4. *Niphargus rhenorhodanensis* 5. *Hydropsyche pellucidula* 6. *Heptagenia sulphurea*
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	1–4. Adult 5 and 6. Last-instar larvae
Exposure regime (e.g. static, flow-through, feeding, etc.)	Flow-through – fed every 48 hours with Tetramin
Test method	Subacute toxicity test. Tests carried out with filtered river water.
Test concentrations used	Three plus control - highest 4.3 mg l^{-1}
Number of replicates per concentration	3
Number of organisms per replicate	5
Measurement of exposure concentrations	Water samples collected every 24 hours for analysis
Temperature	12±2°C
Hardness	Not reported
pH/salinity	Measured but not reported
Exposure duration	240 hours
Endpoint (e.g. NOEC, EC50)	LC50
Effect (e.g. reproduction, survival, growth)	Mortality – taken as immobilisation, determined by gentle probing.
Concentration	1. 2.20 mg l^{-1} 2. 2.31 mg l^{-1} 3. 0.20 mg l^{-1} 4. 3.97 mg l^{-1} 5. 2.40 mg l^{-1} 6. 1.60 mg l^{-1}
Initial quality assessment	Good – reliable and relevant

Relevance of study	**Relevant**
Klimisch Code	**1**
Comments	Field collected: Ain River, France – April 1997. River weakly contaminated but used regularly as test station. Spearman–Karber method used to calculate LC50.

Reference number	37
Chemical	Arsenic trioxide Arsenic pentoxide
Species (common name)	Water flea
Species (scientific name)	*Daphnia pulex*
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	Adults and neonates <24 hours
Exposure regime (e.g. static, flow-through, feeding, etc.)	Static renewal organisms fed
Test method	
Test concentrations used	0, 10, 100, 1000 and 3000 µg l^{-1} As(III) or As(V)
Number of replicates per concentration	Not reported
Number of organisms per replicate	1
Measurement of exposure concentrations	No
Temperature	Not reported
Hardness	Not reported
pH/salinity	Not reported
Exposure duration	26 days – juvenile experiment 22 days – adult experiment
Endpoint (e.g. NOEC, EC50)	Not reported
Effect (e.g. reproduction, survival, growth)	Survival/development/reproduction
Concentration	100% mortality within 24 hours – neonates and 3 days adults 3,000 µg l^{-1} As(III) or As(V). No mortality in other concentrations for adults. For juveniles, significant decline in reproduction at 1,000 µg l^{-1} As(III) and 10, 100 and 1,000 µg l^{-1} As(V) relative to controls. Ephippial egg production was significantly higher at 100 and 1,000 µg l^{-1} As(V) than controls. No significant difference for As(III).
Initial quality assessment	Moderate – details missing for methodology and no measured concentrations

Relevance of study	**Relevant**
Klimisch Code	**2**
Comments	Juvenile experiments – time to reproductive maturity and subsequent reproduction were measured for individuals first exposed to arsenic as <24-hour neonates. For adult experiments, survival and reproduction were measured for individuals first exposed to arsenic after they produced a first clutch.

Reference number	**38**
Chemical	Arsenic trioxide Arsenic pentoxide
Species (common name)	Algae
Species (scientific name)	*Scenedesmus obliguus*
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	Exponential growth phase
Exposure regime (e.g. static, flow-through, feeding, etc.)	Static renewal (24 hours)
Test method	Not stated
Test concentrations used	0, 50, 100, 200, 300 µg l^{-1}
Number of replicates per concentration	2
Number of organisms per replicate	Exponential growth phase
Measurement of exposure concentrations	No
Temperature	24°C
Hardness	–
pH/salinity	pH 7
Exposure duration	96 hours
Endpoint (e.g. NOEC, EC50)	EC50
Effect (e.g. reproduction, survival, growth)	Growth
Concentration	78 µg l^{-1}
Initial quality assessment	Good – few details missing for methodology, but based on semi-static regime
Relevance of study	**Relevant**
Klimisch Code	**2**
Comments	Good – few details missing for methodology, but based on semi-static regime without chemical analysis - relevant

Reference number	**40**
Chemical	Arsenic pentoxide
Species (common name)	Fathead minnow
Species (scientific name)	*Pimephales promelas*
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	ELS
Exposure regime (e.g. static, flow-through, feeding, etc.)	Flow-through
Test method	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Measurement of exposure concentrations	No
Temperature	25°C
Hardness	45–48 mg l^{-1} $CaCO_3$
pH/salinity	pH 6.7–7.8
Exposure duration	30 days
Endpoint (e.g. NOEC, EC50)	NOEC, LOEC
Effect (e.g. reproduction, survival, growth)	Growth
Concentration	0.53 (NOEC), 1.5 (LOEC)
Initial quality assessment	Moderate – details missing for methodology and no measured concentrations
Relevance of study	**Relevant**
Klimisch Code	**2**
Comments	US EPA study so likely to be of reasonable quality. Not possible to obtain original report.

Reference number	**43**
Chemical	Arsenic pentoxide
Species (common name)	Striped bass
Species (scientific name)	*Morone saxatilis*
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	1.8 g
Exposure regime (e.g. static, flow-through, feeding, etc.)	Static
Test method	ASTM 1988* EPA 1975**
Test concentrations used	Highest concentration 100 mg l^{-1}, tested in a 60% serial dilution
Number of replicates per concentration	2
Number of organisms per replicate	5
Measurement of exposure concentrations	Some – LC50 calculated from nominal values as not all concentrations measured. For those that were, As was 102–104% of nominal
Temperature	20°C
Hardness	4,430 mg l^{-1} $CaCO_3$
pH/salinity	pH 8.12/22 ppt
Exposure duration	96 hours
Endpoint (e.g. NOEC, EC50)	LC50
Effect (e.g. reproduction, survival, growth)	Mortality
Concentration	10.3 mg l^{-1}
Initial quality assessment	Good – reliable and relevant
Relevance of study	**Relevant**
Klimisch Code	**2**
Comments	

* American Society for Testing Materials (ASTM), 1988 *Standard guide for conducting early life-stage toxicity tests with fishes*. E 1241-88. 26 pp. West Conshohocken, PA: ASTM.

** US Environmental Protection Agency (US EPA), 1975 *Methods for the acute toxicity tests with fish, macroinvertebrates and amphibians*. The Committee on Methods for Toxicity Tests with Aquatic Organisms, Ecological Research Series EPA-660-75-009. Washington, DC: US EPA.

Reference number	**46**
Chemical	Arsenic acid
Species (common name)	Pacific oyster
Species (scientific name)	*Crassostrea gigas*
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	5 months
Exposure regime (e.g. static, flow-through, feeding, etc.)	Static renewal every 24 hours
Test method	
Test concentrations used	One: 10 µg l^{-1}
Number of replicates per concentration	6
Number of organisms per replicate	10
Measurement of exposure concentrations	Yes
Temperature	Not reported
Hardness	
pH/salinity	Not reported
Exposure duration	21 days
Endpoint (e.g. NOEC, EC50)	Not reported
Effect (e.g. reproduction, survival, growth)	Mortality/cytological effects
Concentration	Only one test concentration – no effect on mortality. Organelle abnormalities, especially of mitochondria and nuclei observed in gill epithelium
Initial quality assessment	Only one test concentration
Relevance of study	
Klimisch Code	**3**
Comments	Oysters also exposed to sediment bound arsenic at 20.5 mg/kg – this had no effect on mortality

Reference number	**47**
Chemical	Arsenic acid (potassium salt)
Species (common name)	Copepod
Species (scientific name)	*Tigriopus brevicornis* (Muller)
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	1. Ovigerous female 2. Copepodid 3. Nauplius
Exposure regime (e.g. static, flow-through, feeding, etc.)	Static
Test method	
Test concentrations used	5–6 test concentrations
Number of replicates per concentration	3
Number of organisms per replicate	1. 30 2. 30 3. 20
Measurement of exposure concentrations	No
Temperature	20°C
Hardness	
pH/salinity	pH 7.7–8.14 /membrane-filtered (0.45 µm) 35% NaCl seawater.
Exposure duration	96 hours
Endpoint (e.g. NOEC, EC50)	LC50
Effect (e.g. reproduction, survival, growth)	Mortality
Concentration	1. 27.5 µg l^{-1} 2. 19.8 µg l^{-1} 3. 10.9 µg l^{-1}
Initial quality assessment	Moderate – no chemical analysis. Sensitive result
Relevance of study	**Relevant**
Klimisch Code	**2**
Comments	Field collected: French North Atlantic coast near the Loire Estuary (Le Croisic) acclimated in the laboratory for 96 hours before testing.

Reference number	48
Chemical	1. Sodium arsenate 2. Arsenite oxide
Species (common name)	luminescent bacteria
Species (scientific name)	*Vibrio fischeri*
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	
Exposure regime (e.g. static, flow-through, feeding, etc.)	
Test method	Microtox basic test protocol
Test concentrations used	14 dilutions within 0.31–3,600 mg l^{-1}
Number of replicates per concentration	
Number of organisms per replicate	
Measurement of exposure concentrations	Yes
Temperature	15°C
Hardness	
pH/salinity	1. pH 5–8 2. pH 6–9
Exposure duration	15 minutes
Endpoint (e.g. NOEC, EC50)	EC50; EC20 (threshold)
Effect (e.g. reproduction, survival, growth)	Light emission
Concentration	pH 5: 305.5 mg l^{-1}; 1.82 mg l^{-1} pH 6: >3,600.0 mg l^{-1}: 1.86 mg l^{-1} pH 7: 20.3 mg l^{-1}; 2.54 mg l^{-1} pH 8: 5.7 mg l^{-1}; 2.50 mg l^{-1} 2. pH 6: 25.9 mg l^{-1}; 7.47 mg l^{-1} pH 7: 25.9 mg l^{-1}; 6.56 mg l^{-1} pH 8: 24.5 mg l^{-1}; 6.96 mg l^{-1} pH 9: 20.0 mg l^{-1}: 7.56 mg l^{-1}
Initial quality assessment	Good – reliable and relevant

Relevance of study	**Relevant**
Klimisch Code	**2**
Comments	Difference in effect seen at different pH values explained by speciation. $HAsO_4^{2-}$ and $H_2AsO_3^-$ were found to be the most toxic species. At low concentrations, As(V) was found to be more toxic than As(III), independent of pH. At high concentrations, toxicity of As(III) and As(V) was dependent on pH due to its strong influence on chemical speciation. Same results for pH 6 and 7 reported by the same authors in: Fulladosa E, Murat J C, Martinez M and Villaescusa I, 2005 *Patterns of metals and arsenic poisoning in* Vibrio fischeri *bacteria*. Chemosphere*,* **60**, 43–48.

Reference number	**49**
Chemical	Sodium arsenate ($Na_2HAs0_4.7H_20$)
Species (common name)	Purple sea urchin
Species (scientific name)	*Strongylocentrotus purpuratus*
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	Embryo
Exposure regime (e.g. static, flow-through, feeding, etc.)	Static
Test method	
Test concentrations used	0.0023–0.091 mg l^{-1} (0.001–0.040 mM) AsO_4^{3-}
Number of replicates per concentration	10–4 for development 6 for DPC (days postcoitum) Experiment replicated three times
Number of organisms per replicate	50 embryos /ml
Measurement of exposure concentrations	No due to small test volumes
Temperature	15°C
Hardness	Not reported
pH/salinity	pH 7.8/34 ppt
Exposure duration	48 hours
Endpoint (e.g. NOEC, EC50)	LOEC
Effect (e.g. reproduction, survival, growth)	Developmental success data were expressed as the percentage of normal embryos out of 100 counted for each replicate: DPC data were expressed as the percentage of free DNA per replicate for purposes of data analysis.
Concentration	0.011 mg l^{-1} development, 0.023 mg l^{-1} DPC
Initial quality assessment	No measurement of exposure concentration
Relevance of study	**Relevant**
Klimisch Code	**2**
Comments	Field collected: Point Arena, California, USA. September 1993

Reference number	**50**
Chemical	Arsenate (as Na_2HAsO_4)
Species (common name)	Brown alga
Species (scientific name)	*Fucus serratus*
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	Individual fronds 30–50 cm
Exposure regime (e.g. static, flow-through, feeding, etc.)	Flow-through
Test method	
Test concentrations used	0, 20,50 and 100 µg l^{-1} nominal ~1, 18.7, 49.6 and 96.6 µg l^{-1} mean measured
Number of replicates per concentration	1
Number of organisms per replicate	4
Measurement of exposure concentrations	Yes, daily for first seven days, weekly thereafter
Temperature	16–20°C
Hardness	Not reported
pH/salinity	12.5–22%
Exposure duration	1 day to 19 weeks
Endpoint (e.g. NOEC, EC50)	Not reported
Effect (e.g. reproduction, survival, growth)	Growth/survival
Concentration	*Fucus* exposed at 100 µg l^{-1}As showed signs of toxicity after 1 week and started to die from week 3: experiment terminated for this group at week 13. *Fucus* exposed at 50 µg l^{-1}As began to show signs of toxic effect at week 3: experiment terminated at week 17. Control and 20 µg l^{-1}As remained healthy until week 16 at which time it is thought they outgrew their containers.
Initial quality assessment	Moderate – non-specific endpoint but measured concentrations, NOEC 20–50 µg l^{-1} As
Relevance of study	
Klimisch Code	**1**
Comments	Field collected: Fyns Hoved, Denmark, in May 1999

Reference number	**52**
Chemical	Sodium arsenite
Species (common name)	Water flea
Species (scientific name)	*Ceriodaphnia dubia*
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	<24 hours
Exposure regime (e.g. static, flow-through, feeding, etc.)	Static renewal every 48 hours
Test method	
Test concentrations used	0, 1.0, 1.25 and 1.5 mg l^{-1} – nominal (ND, 0.35, 1.0 and 1.4 mg l^{-1} measured)
Number of replicates per concentration	10
Number of organisms per replicate	1
Measurement of exposure concentrations	Yes
Temperature	25°C
Hardness	US EPA moderately hard water
pH/salinity	pH 7.3–9.3
Exposure duration	24 days to third generation
Endpoint (e.g. NOEC, EC50)	Not reported
Effect (e.g. reproduction, survival, growth)	Survival and brood size
Concentration	Decreasing survival for all three generations only under high ultraviolet (UV) irradiance at concentrations ≥1.0 mg l^{-1}. No survival at 1.4 mg l^{-1} second generation. Variable effects on brood size over generations at lowest concentration tested – only slight UV effect.
Initial quality assessment	Moderate – some question as to effect of UV exposure alone although authors state that levels used are below that which are independently lethal.
Relevance of study	
Klimisch Code	**2**
Comments	Tests carried out at two UV treatment levels – 'low' and 'high'.

Reference number	**54**
Chemical	Arsenite oxide
Species (common name)	Pink salmon
Species (scientific name)	*Oncorhynchus gorbuscha*
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	Not stated
Exposure regime (e.g. static, flow-through, feeding, etc.)	Static
Test method	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Measurement of exposure concentrations	No
Temperature	10°C
Hardness	–
pH/salinity	pH 7.7
Exposure duration	10 days
Endpoint (e.g. NOEC, EC50)	NOEC
Effect (e.g. reproduction, survival, growth)	Mortality
Concentration	2.65 $\mu g\ l^{-1}$
Initial quality assessment	Only very limited data available to assess study. Static exposure with nominal exposure concentrations
Relevance of study	
Klimisch Code	**2**
Comments	Not possible to obtain original reference

Reference number	**55**
Chemical	Sodium arsenate
Species (common name)	Various algal species (20)
Species (scientific name)	For example: 1. *Scenedesmus denticulatus* – Chlorophyceae 2. *Monosigna* sp. *Ochromonas* sp. – Chrysophyceae 3. *Stichogloea doederleinii* – Chrysophyceae
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	Not reported
Exposure regime (e.g. static, flow-through, feeding, etc.)	Not reported
Test method	Not reported
Test concentrations used	Not reported
Number of replicates per concentration	Not reported
Number of organisms per replicate	Not reported
Measurement of exposure concentrations	Not reported
Temperature	Not reported
Hardness	Not reported
pH/salinity	Not reported
Exposure duration	72–96 hours
Endpoint (e.g. NOEC, EC50)	LOEC/NOEC
Effect (e.g. reproduction, survival, growth)	Growth – number of cells
Concentration	1. LOEC 0.050 mg l^{-1} 2. NOEC 0.500 mg l^{-1} 3. LOEC 0.005 mg l^{-1}
Initial quality assessment	Insufficient information to assess quality of study
Relevance of study	**Relevant as an indicator of possible sensitivity**
Klimisch Code	**4**
Comments	This paper was written in part as a critical examination of EPA, OECD and ISO standard methods and it is assumed that the study was carried out to similar standards. However, a major departure would appear to be that 'family' species were tested together.

Reference number	**57**
Chemical	Arsenic trioxide
Species (common name)	Japanese medaka
Species (scientific name)	*Oryzias latipes*
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	Embryo 2–3 days
Exposure regime (e.g. static, flow-through, feeding, etc.)	Static renewal
Test method	Standard protocol (Helmstetter *et al.* 1996)*
Test concentrations used	0, 0.025, 0.05 and 0.1 mg l^{-1}
Number of replicates per concentration	2
Number of organisms per replicate	100
Measurement of exposure concentrations	Not reported
Temperature	Not reported
Hardness	Not reported
pH/salinity	Not reported
Exposure duration	Until hatching
Endpoint (e.g. NOEC, EC50)	NA
Effect (e.g. reproduction, survival, growth)	Hatching success/developmental abnormalities
Concentration	54% hatching success at 0.1 mg l^{-1} 75% at 0.05 mg l^{-1} and 86% at 0.025 mg l^{-1} Control ~85%
Initial quality assessment	Difficult to assess. Carried out to standard protocol but water quality parameters not reported. No chemical analysis.
Relevance of study	
Klimisch Code	**4**
Comments	No developmental abnormalities observed. Hatching period reduced in treatments compared with controls.

* Helmstetter M F, Maccubbin A F and Alden R W III, 1996 *The medaka embryo-larval assay: an* in vivo *assay for toxicity, teratogenicity and carcinogenicity*. In Techniques in Aquatic Toxicology (ed. G Ostrander), Chapter 6. pp. 93–124. New York, NY: CRC Lewis.

Reference number	**59**
Chemical	1. As(III) 2. As(V)
Species (common name)	Duckweed
Species (scientific name)	*Lemna minor*
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	Not reported
Exposure regime (e.g. static, flow-through, feeding, etc.)	Flow-through
Test method	
Test concentrations used	Not reported
Number of replicates per concentration	3
Number of organisms per replicate	10 fronds
Measurement of exposure concentrations	Measurements were taken in order to work out BCF. It is not clear whether EC50 values have been derived using measured or nominal concentrations.
Temperature	23°C
Hardness	
pH/salinity	
Exposure duration	14 days
Endpoint (e.g. NOEC, EC50)	EC50; NOEC
Effect (e.g. reproduction, survival, growth)	Growth as leaf coverage expressed as percentage of control
Concentration	1. EC50 0.63 mg l^{-1}; NOEC <0.75 mg l^{-1} 2. EC50 22.2 mg l^{-1}; NOEC <4 mg l^{-1}
Initial quality assessment	Insufficient experimental detail on which to base an assessment
Relevance of study	
Klimisch Code	**4**
Comments	Sterilised growth medium used – pH 5

Reference number	**60**
Chemical	Arsenic trioxide
Species (common name)	Frog
Species (scientific name)	*Rana hexadactyla*
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	Tadpole
Exposure regime (e.g. static, flow-through, feeding, etc.)	Semi-static
Test method	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Measurement of exposure concentrations	No
Temperature	15°C
Hardness	20 mg l^{-1} $CaCO_3$
pH/salinity	pH 6.1
Exposure duration	96 hours
Endpoint (e.g. NOEC, EC50)	LC50
Effect (e.g. reproduction, survival, growth)	Mortality
Concentration	0.249
Initial quality assessment	Moderate and relevant endpoint. No mention of chemical analysis, but based on a semi-static system
Relevance of study	
Klimisch Code	**2**
Comments	

Reference number	**61**
Chemical	Arsenic trioxide
Species (common name)	Midge
Species (scientific name)	*Chironomus tentans* (field collected)
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	Larvae
Exposure regime (e.g. static, flow-through, feeding, etc.)	Static
Test method	Not stated
Test concentrations used	Seven concentrations
Number of replicates per concentration	2
Number of organisms per replicate	10
Measurement of exposure concentrations	Not stated
Temperature	14°C
Hardness	25 mg l^{-1} $CaCO_3$
pH/salinity	pH 6.3
Exposure duration	48 hours
Endpoint (e.g. NOEC, EC50)	EC50
Effect (e.g. reproduction, survival, growth)	Immobilisation
Concentration	680 µg l^{-1}
Initial quality assessment	Moderate and relevant endpoint, but no mention of chemical analysis. Also a static system
Relevance of study	
Klimisch Code	**2**
Comments	Moderate and relevant endpoint. No mention of chemical analysis and a static system.

Reference number	**62**
Chemical	Arsenic pentoxide
Species (common name)	Bass
Species (scientific name)	*Morone saxatilis*
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	ELS
Exposure regime (e.g. static, flow-through, feeding, etc.)	Flow-through
Test method	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Measurement of exposure concentrations	Yes
Temperature	16–21°C
Hardness	3.5–5.2‰
pH/salinity	pH 7.6
Exposure duration	96 hours
Endpoint (e.g. NOEC, EC50)	LC50
Effect (e.g. reproduction, survival, growth)	Mortality
Concentration	7280 µg l^{-1}
Initial quality assessment	Few details with which to assess study. Endpoints were based on flow-through conditions with chemical analysis.
Relevance of study	
Klimisch Code	**4**
Comments	Moderate and relevant endpoint. It was not possible to obtain the original study.

Reference number	**63**
Chemical	Sodium arsenite
Species (common name)	Tilapia
Species (scientific name)	*Oreochromis mossambicus*
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	Mean body length 12.6 cm and body weight 31.7 g wet weight
Exposure regime (e.g. static, flow-through, feeding, etc.)	Static renewal every 24 hours – not fed
Test method	
Test concentrations used	0,1,2,4,10,30,50 and 80 mg l^{-1}
Number of replicates per concentration	2
Number of organisms per replicate	6
Measurement of exposure concentrations	Yes
Temperature	24.7°C
Hardness	Not reported
pH/salinity	pH 7.7
Exposure duration	24, 48, 72, 96, 120 and 144 hours
Endpoint (e.g. NOEC, EC50)	LC50
Effect (e.g. reproduction, survival, growth)	Mortality
Concentration	24 hours: 69.06 mg l^{-1} 48 hours: 51.52 mg l^{-1} 72 hours: 38.44 mg l^{-1} 96 hours: 28.68 mg l^{-1} 120 hours: 21.41 mg l^{-1} 144 hours: 15.98 mg l^{-1}
Initial quality assessment	Good – reliable and relevant
Relevance of study	**Relevant**
Klimisch Code	**1**
Comments	

Reference number	**64**
Chemical	Sodium arsenite
Species (common name)	1. Water flea 2. Fathead minnow 3. Flagfish 4. Amphipod
Species (scientific name)	1. *Daphnia magna* 2. *Pimephales promelas* 3. *Jordanella floridae* 4. *Gammarus pseudolimnaeus*
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	1. <24 hours 2. <24-hour embryo 3. <24-hour embryo 4. selected for uniform size
Exposure regime (e.g. static, flow-through, feeding, etc.)	1. semi-static 2. flow-through 3. flow-through 4. flow-through
Test method	1. acute tests one with/one without feeding (wf) and chronic 2. acute (not fed) and chronic 3. acute (not fed) and chronic 4. acute (not fed) and chronic
Test concentrations used	1. <2 (control) 1,040, 1540, 2210, 4220, 7850, 13,300 µg l^{-1} As 1. <2 (control) 815, 1080,1860,2440, 4190, 6460 µg l^{-1} As (wf) 1. <2 (control) 72.8, 132, 270, 633, 1320, 2670 µg l^{-1} As chronic 2. <2 (control) 1060, 2130,4300,7400,16,500 µg l^{-1} As 3. <2 (control) 1240, 2130, 4120, 7600, 16,300 µg l^{-1} As 4. <2 (control) 303, 583, 1340, 2400 and 5250 µg l^{-1} As
Number of replicates per concentration	1. 2 acute 10 chronic 2. 2 3. 2 4. 2
Number of organisms per replicate	1. Five acute tests; one (seven repeats for reproduction); five (three repeats for survival) 2. started with 100 embryos, 20 fry selected for continuation 3. started with 68 embryos, 20 fry selected for continuation 4. 10
Measurement of exposure concentrations	Yes
Temperature	1. 15.6°C (acute) 20.8°C (chronic) 2. 23–25.8°C 3. 23–25.8°C 4. 18.5°C

Hardness	46.3–49.9 mg l^{-1} $CaCO_3$
pH/salinity	pH 7.2–8.1
Exposure duration	96-hour acute tests ~6 days hatching time + 29 days for fatheads ~7 days + 31 days for flagfish
Endpoint (e.g. NOEC, EC50)	LC50 chronic tests, NOEC, LOEC
Effect (e.g. reproduction, survival, growth)	Mortality/immobilisation Chronic daphnid – survival and offspring production Chronic – fish tests – growth – length and wet weight
Concentration	1. LC50 1.5 mg l^{-1} (wf) 4.34 mg l^{-1}; NOEC 0.633 mg l^{-1}; LOEC 1.32 mg l^{-1} 2. LC50 14.1 mg l^{-1}; NOEC 2.13 mg l^{-1}; LOEC 4.3 mg l^{-1} 3. LC50 14.4 mg l^{-1}; NOEC 2.13 mg l^{-1}; LOEC 4.12 mg l^{-1} 4. LC50 0.874 mg l^{-1}
Initial quality assessment	Good – reliable and relevant
Relevance of study	**Relevant**
Klimisch Code	**1**
Comments	Field collected amphipods – Eau Claire River near Gordon, WI – acclimatised for 1 month prior to testing Temperature failures in fish chronic tests by up to 12°C over 4–17 hours over several days – no apparent effect on mortality or feeding.

Reference number	**65**
Chemical	Sodium arsenate
Species (common name)	Daggerblade grass shrimp
Species (scientific name)	*Palaemonetes pugio*
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	Juvenile
Exposure regime (e.g. static, flow-through, feeding, etc.)	Static renewal
Test method	Yes
Test concentrations used	0, 10 and 25 µg l^{-1} nominal 0.71, 9.67, 24.6 µg l^{-1} measured
Number of replicates per concentration	Not reported
Number of organisms per replicate	Not reported
Measurement of exposure concentrations	Yes
Temperature	20–25°C
Hardness	Not reported
pH/salinity	12.5 ppt
Exposure duration	28 days
Endpoint (e.g. NOEC, EC50)	Not reported
Effect (e.g. reproduction, survival, growth)	Growth
Concentration	No effects seen at concentrations tested
Initial quality assessment	Unbounded NOEC
Relevance of study	
Klimisch Code	**2**
Comments	

Reference number	**74**
Chemical	Arsenic trioxide
Species (common name)	Coho salmon
Species (scientific name)	*Oncorhynchus kisutch* (hatchery bought)
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	Fry
Exposure regime (e.g. static, flow-through, feeding, etc.)	Flow-through
Test method	Not stated
Test concentrations used	0, 10, 30, 100, 300 µg l^{-1}
Number of replicates per concentration	4
Number of organisms per replicate	Not stated (10,000 used in total)
Measurement of exposure concentrations	Yes
Temperature	3.8–13.8°C
Hardness	69 mg l^{-1} $CaCO_3$
pH/salinity	pH 8.2
Exposure duration	6 months (500 tagged smolt from each group were then released to a stream and monitored for migration for 32 days)
Endpoint (e.g. NOEC, EC50)	Significant effect on migration
Effect (e.g. reproduction, survival, growth)	Significant effect on migration, plasma T_4 levels and gill Na^+, K^+-ATPase activity
Concentration	300 µg l^{-1}
Initial quality assessment	Well-documented study with ecologically relevant endpoint (migration)
Relevance of study	
Klimisch Code	**1**
Comments	The study questions the relevance of the plasma T_4 levels and gill Na^+, K^+-ATPase activity.

Reference number	**75**
Chemical	Arsenic trioxide
Species (common name)	Water flea
Species (scientific name)	*Bosmina longirostris*
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	Not stated
Exposure regime (e.g. static, flow-through, feeding, etc.)	Not stated
Test method	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Measurement of exposure concentrations	No
Temperature	Not stated
Hardness	Not stated
pH/salinity	Not stated
Exposure duration	Not stated
Endpoint (e.g. NOEC, EC50)	LC50
Effect (e.g. reproduction, survival, growth)	Immobilisation
Concentration	0.250 µg l^{-1}
Initial quality assessment	US Fisheries and Wildlife Service internal report. Very few details to assess quality.
Relevance of study	**Relevant**
Klimisch Code	**4**
Comments	US Fisheries and Wildlife Service internal report. Very few details to assess quality.

Reference number	**76**
Chemical	Arsenic pentoxide
Species (common name)	Diatom
Species (scientific name)	*Skeletonema costatum*
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	Not stated
Exposure regime (e.g. static, flow-through, feeding, etc.)	Flow-through
Test method	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Measurement of exposure concentrations	No
Temperature	Not stated
Hardness	Not stated
pH/salinity	Not stated
Exposure duration	5 days
Endpoint (e.g. NOEC, EC50)	EC50
Effect (e.g. reproduction, survival, growth)	Population abundance
Concentration	0.009 µg l^{-1}
Initial quality assessment	Office of Pesticide Programs report. Very few details to assess quality.
Relevance of study	**Relevant**
Klimisch Code	**4**
Comments	Given the source of the data and the fact that it was a flow-through test, the data may be regarded as supporting information only.

Reference number	**79**
Chemical	Arsenic pentoxide
Species (common name)	Water flea
Species (scientific name)	*Bosmina longirostris* (field collected)
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	<24-hours old
Exposure regime (e.g. static, flow-through, feeding, etc.)	Static
Test method	Not stated
Test concentrations used	5
Number of replicates per concentration	Not stated
Number of organisms per replicate	10
Measurement of exposure concentrations	Yes (101% of nominal)
Temperature	17°C
Hardness	120 mg l^{-1} $CaCO_3$
pH/salinity	pH 6.8
Exposure duration	48 hours
Endpoint (e.g. NOEC, EC50)	EC50
Effect (e.g. reproduction, survival, growth)	Immobilisation
Concentration	0.850 µg l^{-1}
Initial quality assessment	Good and relevant
Relevance of study	**Relevant**
Klimisch Code	**2**
Comments	No mention of replication, but study looks good and is based on chemical analysis.

Reference number	**85**
Chemical	As(III) and As(V) compound not specified
Species (common name)	Diatom
Species (scientific name)	*Skeletonema costatum*
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	Axenic week old culture
Exposure regime (e.g. static, flow-through, feeding, etc.)	Static
Test method	
Test concentrations used	Control + 2 As(III) 13, 270 nM (1 and 20 µg l^{-1}) Control + 3 As(V) 80, 167 and 340 nM (6, 13, 25 µg l^{-1})
Number of replicates per concentration	Not reported
Number of organisms per replicate	10^6 cells l^{-1}
Measurement of exposure concentrations	Yes
Temperature	20°C
Hardness	
pH/salinity	
Exposure duration	6–8 days (maintained until population reached stationary phase)
Endpoint (e.g. NOEC, EC50)	Not reported
Effect (e.g. reproduction, survival, growth)	Growth inhibition measured as *in vivo* fluorescence
Concentration	As(III) 20 µg l^{-1} As(V) 13 µg l^{-1}
Initial quality assessment	Moderate – limited concentrations tested, but measurement of test concentration and speciation
Relevance of study	**Relevant**
Klimisch Code	**2**
Comments	Cultured and tested in filtered seawater with As concentration ~1 µg l^{-1}, 80% as As(V). ≥6 µg l^{-1} As(V) significantly inhibited ^{14}C uptake over 4 hours during both log and stationary phases. The results of As(V) phosphate test showed that As(V) toxicity reduced by increased concentrations of phosphate.

Reference number	**94**
Chemical	Sodium arsenite [$NaAsO_2$; As(III)] Sodium arsenate [$Na_2HAsO_4 \cdot 7H_20$; As(V)]
Species (common name)	Japanese medaka
Species (scientific name)	*Oryzias latipes*
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	Adult
Exposure regime (e.g. static, flow-through, feeding, etc.)	Semi-static renewal every 24 hours
Test method	OECD 1998*
Test concentrations used	0,1, 5,10,15, and 20 mg l^{-1} As(III) 0,10, 20, 30 and 35 mg l^{-1} As(V)
Number of replicates per concentration	Not reported
Number of organisms per replicate	5
Measurement of exposure concentrations	No
Temperature	21°C
Hardness	Not reported
pH/salinity	Not reported
Exposure duration	7 days
Endpoint (e.g. NOEC, EC50)	LC50
Effect (e.g. reproduction, survival, growth)	Mortality
Concentration	14.6 mg l^{-1} As(III) 30.3 mg l^{-1} As(V)
Initial quality assessment	No measurement of test concentrations
Relevance of study	**Relevant**
Klimisch Code	**2**
Comments	

* Organisation for Economic Co-operation and Development (OECD), 1998 *OECD guidelines for testing of chemicals. No. 212. Fish: short-term toxicity test on embryo and sac-fry stages*. Paris: OECD.

Reference number	**96**
Chemical	Sodium arsenite
Species (common name)	Red alga
Species (scientific name)	*Champia parvula*
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	Mature
Exposure regime (e.g. static, flow-through, feeding, etc.)	Static renewal
Test method	ASTM 1993*
Test concentrations used	5–7 concentrations + control
Number of replicates per concentration	2
Number of organisms per replicate	5 female tips + 1 male tip
Measurement of exposure concentrations	Yes
Temperature	20–22°C
Hardness	
pH/salinity	Filtered seawater
Exposure duration	14 days
Endpoint (e.g. NOEC, EC50)	LOEC; NOEC
Effect (e.g. reproduction, survival, growth)	Growth – dry weight females Sexual reproduction – presence of cystocarps
Concentration	LOEC growth 145 µg l^{-1} NOEC reproduction 60 µg l^{-1}
Initial quality assessment	Good – reliable and relevant
Relevance of study	**Relevance**
Klimisch Code	**1**
Comments	All individuals died at concentrations ≥300 µg l^{-1} phosphate 4.5 µM for above exposure. However, in additional tests, phosphate had no effect on As(III) toxicity over the range tested. This series of test results gives a growth NOEC of 113 µg l^{-1}. A concentration of 10 mg l^{-1} As(V) had no effect on growth, but reproduction was eliminated at this level. In the absence of phosphate, As(V) toxicity was similar to that of As(III), except plants were still alive at 1,076 µg l^{-1} As(V), although they did not grow.

* American Society for Testing Materials (ASTM), 1993 *Annual book of ASTM standards*. Volume 11.01: Water. West Conshohocken, PA: ASTM.

Reference number	97
Chemical	Arsenic oxide
Species (common name)	1. Luminescent bacteria 2. Mixed bacterial community 3. Green alga 4. Water flea 5. Rainbow trout 6. Zebra fish
Species (scientific name)	1. *Vibrio fischeri* 2. Mixed bacterial community 3. *Scenedesmus subspicatus* 4. *Daphnia magna* Straus 1820 5. *Oncorhynchus mykiss* Walbaum 1990 6. *Brachydanio rerio* Hamilton Buchanan
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	4. Neonate – 24 hours 5. Juvenile – 6-cm length trout 6. Not reported
Exposure regime (e.g. static, flow-through, feeding, etc.)	4. Static – acute; semi-static – chronic 5. Static 6. Static
Test method	4. Chronic – OECD Guideline 1993*
Test concentrations used	
Number of replicates per concentration	Two Daphnid chronic exposure – 10
Number of organisms per replicate	1. NA 2. 150 mg l^{-1} of suspended solids 3. Not reported 4. Daphnid chronic exposure 1
Measurement of exposure concentrations	Yes for fish tests and chronic tests with daphnids – measured values did not fall below 90% of nominal concentrations.
Temperature	1. 15°C 2. Not reported 3. 21°C 4. 21°C 5. 12°C 6. 21°C
Hardness	5 and 6. 250 mg l^{-1} $CaCO_3$
pH/salinity	4. pH chronic test measured but not reported 5 and 6. pH 8.4
Exposure duration	1. 30 minutes – acute; 24 hours – chronic 2. 120 minutes 3. 72 hours 4. 48 hours and 21 days 5. 96 hours 6. 96 hours

Endpoint (e.g. NOEC, EC50)	1 and 2. EC50; EC20 3. EC50; EC10 for growth (g) and biomass (b) 4. EC50; EC10 acute : NOEC chronic 5. LC50; LC10 6. LC50; LC10
Effect (e.g. reproduction, survival, growth)	1. Per cent inhibition relative to control 2. O_2 consumption rate 3. Growth measured as cell density/number of cells 4. Acute – immobility; chronic – reproduction 5. Mortality 6. Mortality
Concentration	1. Acute: EC50 72.4 mg l^{-1}; EC20 13.4 mg l^{-1}. Chronic: EC50 20.4 mg l^{-1}; EC20 3.7 mg l^{-1}. 2. EC50 41.7 mg l^{-1}; EC20 28.8 mg l^{-1} 3. $EC50_{(g)}$ 60.3 mg l^{-1}; $EC50_{(b)}$ 34.7 mg l^{-1}; $EC10_{(g)}$ 34.7 mg l^{-1}; $EC10_{(b)}$ 9.4 mg l^{-1} 4. Acute: EC50 2.5 mg l^{-1}; EC10 1.9 mg l^{-1}. Chronic: NOEC 1.85 mg l^{-1} 5. LC50 15.3 mg l^{-1}; LC10 12.1 mg l^{-1} 6. LC50 28.1 mg l^{-1}; LC10 21.9 mg l^{-1}
Initial quality assessment	Moderate to good, some methodology details missing. Measured concentrations not available for all tests.
Relevance of study	
Klimisch Code	**2**
Comments	Two trials for each test species Micro-organisms of activated sludge from aeration tank of municipal laboratory wastewater treatment plant used as bacterial community.

* Organisation for Economic Co-operation and Development (OECD), 1993 *Guidance Document for the Development of OECD Guidelines for Testing of Chemicals*. Environment Monograph No. 76. Paris: OECD.

Reference number	98
Chemical	Disodium arsenate
Species (common name)	1–3. Green algae (chlorophytes) 4. Blue-green alga (cyanophyte)
Species (scientific name)	1. *Ankistrodesmus falcatus* (Corda) Ralfs 2. *Scenedesmus obliquus* (Turp) Kütz 3. *Selenastrum capricornutum* Printz 4. *Macrocoleus vaginatus* (Vauch) Gom
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	1–3. 10–14 day axenic cultures 4. 10–14 day culture
Exposure regime (e.g. static, flow-through, feeding, etc.)	Static
Test method	Algal Assay Procedure Bottle Test (US EPA 1971)* APHA 1975**
Test concentrations used	0, 0.01, 0.10, 1.0, 10, 25, 50, 75 and 100 mg l^{-1}
Number of replicates per concentration	3
Number of organisms per replicate	1–3. 1 x 10^4 /ml 4. 1 ml stock culture reading 30% transmission at 450 nm on spectrophotometer
Measurement of exposure concentrations	No
Temperature	24±2°C
Hardness	
pH/salinity	pH 7
Exposure duration	14 days
Endpoint (e.g. NOEC, EC50)	EC50
Effect (e.g. reproduction, survival, growth)	Growth as chlorophyll a as percentage of the control
Concentration	1. 0.256 mg l^{-1} 2. 0.048 mg l^{-1} 3. 30.76 mg l^{-1} 4. -
Initial quality assessment	Good – reliable and relevant

Relevance of study	**Relevant**
Klimisch Code	**2**
Comments	The experiments were repeated (2–5) times until the dose–response relationship was well defined. However, an old test method run for 14 days was employed. Inferred from the results: *Selenastrum* NOEC 10 mg l^{-1} *Ankistrodesmus* NOEC 0.01 mg l^{-1} *Scenedesmus* LOEC 0.01 mg l^{-1} *Macrocoleus* only statistically significant response at 75 mg l^{-1} – increase in chlorophyll a

* US Environmental Protection Agency (US EPA), 1971 *Algal assay procedure: bottle test*. National Eutrophication Research Program. Corvallis, OR: US EPA National Environmental Research Center.
** American Public Health Association (APHA), 1975 *Standard methods for the examination of water and waste water* (14th edn.). Washington, DC: APHA.

Reference number	**100**
Chemical	Arsenic trioxide
Species (common name)	Tigerfish
Species (scientific name)	*Therapon jarbua* (Forsskal 1775)
Life stage (e.g. egg, embryo, ELS, juvenile, adult)	Juveniles
Exposure regime (e.g. static, flow-through, feeding, etc.)	Static
Test method	APHA 1971* FAO 1977
Test concentrations used	Range 1–8 mg l^{-1}
Number of replicates per concentration	Three (in addition experiment run three times)
Number of organisms per replicate	10?
Measurement of exposure concentrations	No
Temperature	Monitored but not reported
Hardness	
pH/salinity	35.8–36.4 ppt
Exposure duration	96 hours
Endpoint (e.g. NOEC, EC50)	LC50; LC10
Effect (e.g. reproduction, survival, growth)	Mortality
Concentration	3.38 mg l^{-1}; 1.03 mg l^{-1}
Initial quality assessment	Moderate – not native species
Relevance of study	
Klimisch Code	**2**
Comments	Field collected organisms:– latitude 18°42' N and longitude 72°49' off Thal, south of Bombay, January–May 1981.

* American Public Health Association (APHA), American Waterworks Association and Water Pollution Control Federation, 1971 *Standard methods for the examination of water and wastewater.* Washington, DC: APHA.

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

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Environment Agency
Rio House
Waterside Drive, Aztec West
Almondsbury, Bristol BS32 4UD
Tel: 0870 8506506
Email: enquiries@environment-agency.gov.uk
www.environment-agency.gov.uk

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