Proposed Environmental Quality Standards for Bromine in Fresh and Marine Waters

S Lewis, N Mole, R Mascarenhas and H James

Research Contractor:
WRc plc

Environment Agency
Rio House
Waterside Drive
Aztec West
Bristol
BS12 4UD

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Publishing Organisation:
Environment Agency
Rio House
Waterside Drive
Aztec West
Almondsbury
Bristol BS12 4UD

Tel: 01454 624400 Fax: 01454 624409

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Statement of use
This report reviews the available data on the use, fate/behaviour and aquatic toxicity of bromine. Environmental Quality Standards have been proposed, for the protection of aquatic life, which will assist Agency staff in assessing the effect of this substance on water quality.

Research contractor
This document was produced under R&D Project i053 by:

WRc plc
Henley Road
Medmenham
Marlow
Buckinghamshire
SL7 2HD

Tel: 01491 571531 Fax: 01491 579094

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Environment Agency's Project Manager
The Environment Agency's Project Manager for R&D Project i053 was:
S Killeen - Head Office

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EXECUTIVE SUMMARY

This report proposes environmental quality standards (EQSs) for bromine to protect freshwater and marine life.

The data available on the toxicity of bromine to freshwater and marine aquatic life are reviewed in detail in Appendices A and B respectively. Mammalian toxicity is reviewed in Appendix C.

The main text considers the entry to and fate and behaviour of bromine in the aquatic environment. EQSs are then derived, based on this and the toxicity data in Appendices A and B.

For freshwaters, a tentative EQS of 5 $\mu g\, l^{-1}$ TRO (as measured by the DPD or equivalent method) expressed as a maximum allowable concentration (MAC) is proposed. In addition, a tentative EQS of 2 $\mu g\, l^{-1}$ TRO (as measured by the DPD or equivalent method) to be expressed as an annual average is proposed.

For marine waters, a tentative EQS of 10 $\mu g\, l^{-1}$ TRO (as measured by the DPD or equivalent method) expressed as a maximum allowable concentration (MAC) is proposed.

The EQSs will principally be of use to control discharges of cooling water effluents where bromine is used as a biocide and complements the R&D Note proposing EQSs for chlorine (NRA R&D Note 332).

KEY WORDS

Environmental Quality Standards (EQSs), bromine, bromamines, biocides, cooling water.

1. INTRODUCTION

This report aims to propose environmental quality standards (EQSs) for bromine to protect freshwater and marine life. These will principally be of use to control discharges of cooling water effluents where bromine is used as a biocide.

The data available on the toxicity of bromine to freshwater and marine aquatic life are reviewed in detail in Appendices A and B respectively and data on mammalian toxicity are reviewed in Appendix C. The main text considers the entry to and fate and behaviour of bromine in the aquatic environment. Based on this and the toxicity data in Appendices A and B environmental quality standards are proposed for the protection of fresh and marine waters, Section 3 and Table 1.1.

In addition, the toxicity data presented in this report should also help to resolve some of the problems encountered in the derivation of the EQSs for chlorine (NRA R&D Note 332) which are also outlined below.

The data reviewed for the chlorine EQSs were concerned with the toxicity of inputs of chlorine, however, when chlorine is added to water containing bromide, free and combined chlorine react with the bromide present leading to a reaction in the chlorine species and an increase in bromine species overtime.

Thus, for freshwaters, it was unclear as to whether the presence of bromide would affect the toxicity or whether errors in TAC analysis may occur.

For marine waters, the reactions occurring ultimately lead to the removal of detectable free and combined chlorine resulting in the formation of reactive bromine species and hence a variety of oxidants not containing chlorine. The standard was therefore set as total residual oxidants (defined by the appropriate analytical method) assuming that residual chlorine and bromine had similar toxicities. Thus the current review was necessary, not only because of bromine's use as a high usage biocide, but to enable consideration of the problems encountered with chlorine and to investigate the toxicity of bromine in freshwaters as well as to confirm the assumption made for marine waters.

Table 1.1 Proposed environmental quality standards for bromine

Use	Proposed Environmental Quality Standard ($\mu g\ l^{-1}$)[1] MAC	AA
Protection of freshwater life	5 TRO[2]	2 TRO[2]
Protection of marine life	10 TRO[2]	

MAC - Maximum allowable concentration
AA - Annual average
TRO - Total residual oxidant - free and combined bromine and chlorine (see Section 2.3 for definition)
1 - as measured by the DPD or equivalent method
2 - proposed as a tentative EQS

2. BROMINE IN THE ENVIRONMENT

2.1 Physico-chemical properties

The physico-chemical properties of bromine are given in Table 2.1. Bromine is a dense, mobile, reddish-brown liquid which volatilises readily at room temperature to a red vapour with a strong disagreeable odour. Bromine is irritating to the eyes and throat, is very toxic by inhalation (with an occupational exposure standard of 0.7 mg m^{-3}, long term, eight hour time weighted average), and is extremely corrosive to most metals. In contact with skin, liquid bromine produces painful burns which are slow to heal.

Table 2.1 Physico-chemical properties of bromine

NAME	Bromine
CAS NUMBER	7726-95-6
CHEMICAL FORMULA	Br_2
ATOMIC NUMBER	35
RELATIVE MOLECULAR MASS	159.8
VALENCE	-1, 1, 3, 5, 7
APPEARANCE	Dense, dark red fuming liquid Sharp, penetrating, suffocating odour Poisonous
VAPOUR PRESSURE	175 mm Hg at 21 °C (cf. water 18.65 mm Hg at 21 °C, diethyl ether 400 mm Hg at 18 °C)
MELTING POINT	-7.2 °C
BOILING POINT	58.78 °C
DENSITY	3.119 kg $^{m-}$3 at 20 °C
SOLUBILITY IN WATER	41.7 g l^{-1} at 0 °C 35.8 g l^{-1} at 20 °C 35.2 g l^{-1} at 50 °C

Note: Data obtained from Cotton and Wilkinson (1980), Degrémont (1979), Weast (1973) and White (1986).

2.2 Uses

In recent years there has been an increased interest in the use of bromine in water treatment. Currently, the main use of bromine in water treatment is for cooling water disinfection, however, it is also used in swimming pool disinfection. There has also been a move toward the use of bromine releasing biocides as slimacides in the paper industry, with their use for this application approved in a number of European countries.

Bromine is available for water treatment applications in various forms. These include activated sodium bromide (sodium bromide in conjunction with chlorine); liquid bromine chloride (BrCl), and solid bromo-chloro-dimethyl hydantoin (BCDMH) ($C_5H_6BrClN_2O_2$) (Conley and Puzig 1987).

Bromine was originally applied for use in swimming pools as liquid bromine, but safety concerns meant that a solid form of bromine (bromo-chloro-dimethyl hydantoin) was developed. This releases hypochlorous acid (the biocidal active form of chlorine) in addition to hypobromous acid which is the main active bromine form. For economic reasons the solid form is only used for indoor and small outdoor pools.

In most cooling water applications the bromine is generated *in situ* by the oxidation of bromide by chlorine, although in some cases bromine chloride is used (which decomposes in water to give hypobromous acid and hydrochlorous acid). The use of bromine in cooling water disinfection has gained increased interest due to the possibility of a number of potential benefits outlined below.

With respect to pH, cooling-waters can be divided into two categories: pH controlled (where acid is constantly added to maintain the pH, approximately pH 7.5) and pH uncontrolled or pH free (which have an equilibrium of approximately pH 8.2 - 9.2 due to the build up of salts as the water is recirculated). As can be seen from Table 2.2 and Figure 2.1, in freshwaters in pH controlled systems, (pH 7.5) a large proportion of any added chlorine will be in the biocidal form (i.e. hypochlorous acid), while in uncontrolled pH systems, only a small proportion of any added chlorine will be in the biocidal form. However, under these conditions (pH 8 and 9) approximately 90-50% of bromine will be in the biocidal form (hypobromous acid) and thus will be a more effective biocide than chlorine.

In addition, bromamines have been found to have a greater biocidal potential than chloramines and have the additional advantage of being less persistent. Therefore, in the presence of low concentrations of ammonia in pH uncontrolled cooling waters, bromine provides antimicrobial performance superior to chlorine, not only due to the increased biocidal potential of bromamines, but also because of an increased stability of hypobromous acid compared to hypochlorous acid (Ross 1991).

Bromine is rarely used for potable water disinfection, although bromine-impregnated resins have been used on oil rigs and ocean-going ships for drinking water disinfection.

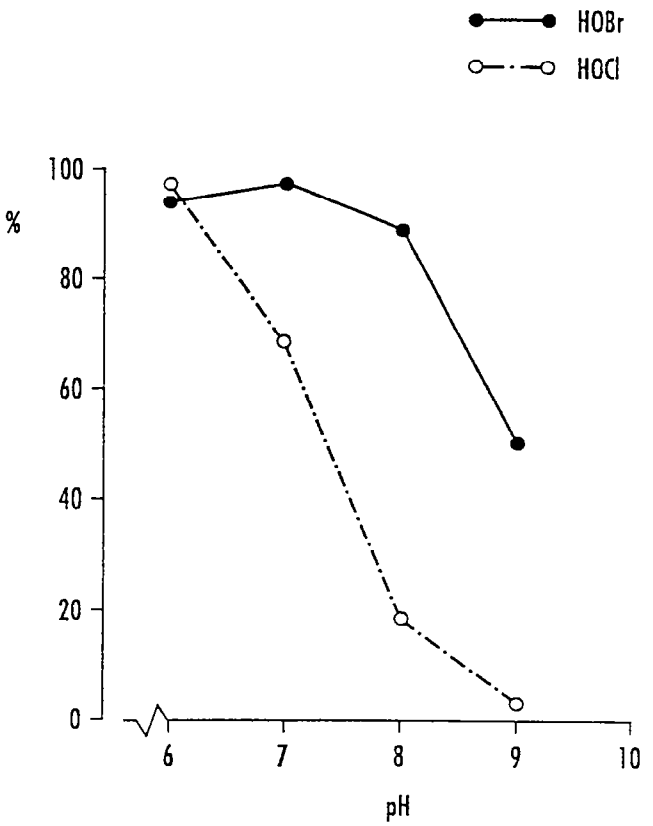

Figure 2.1 Percentage of hypobromous acid and hypochlorous acid present at pH 6-9

Table 2.2 Effect of pH on hypobromous acid and hypochlorous acid dissociation

pH	Bromine	Chlorine
6.0	5% Br_2 95% HOBr	97.5% HOCl 2.5% OCl^-
7.0	99% HOBr 1% OBr^-	70% HOCl 30% OCl^-
8.0	90% HOBr 10% OBr^-	19% HOCl 81% OCl^-
9.0	50% HOBr 50% OBr^-	3% HOCl 97% OCl^-

2.3 Terminology

Bromine can be described in the literature as 'free', 'active', 'available', 'combined', 'residual' or by some combination of these adjectives. The definitions used for expressing the presence of bromine or oxidants in water are given below.

- **Free Available Bromine (FAB);**

  When bromine is present in water in a form in which it is available to act as an oxidant it may be described in the literature as 'free', 'available', 'active' or 'residual' bromine or by some combination of these adjectives. These forms can be conveniently classified as free available bromine (FAB), i.e. that present as an equilibrium mixture of hypobromous acid (HOBr) and hypobromite ions (OBr) and Br_2.

- **Combined Available Bromine (CAB);**

  Combined (or bound) available bromine (CAB) is that available as bromamines or other compounds with N-C links. These can also be considered as oxidants.

- **Total Available/Residual Bromine (TAB/TRB);**

  Total available bromine (TAB) is essentially the sum of FAB and CAB. 'Residual' is analogous to 'available'. The use of the word residual serves to emphasise the concept of a pool of oxy-disinfectant capacity remaining after the initial demand has been satisfied. There can be free, combined and total residual bromine.

- **Total Residual Oxidant (TRO);**

- TRO is basically the sum of all the oxidants.

WHAT IS TRO?

Free

Bromine: Br_2, HOBr, OBr^-
Chlorine: Cl_2, HOCl, OCl
Mixed: BrCl, $BrCl_2^-$ $BrCl_3^{2-}$, Br_2Cl^-

Combined:

Inorganic

NH_2Br, $NHBr_2$, NBr_3
NH_2Cl, $NHCl_2$, NCl_3
NHBrCl

- **Organic bromamines and chloramines**

  RNHBr, $RNBr_2$, RNHCl, $RNCl_2$ (R= amine, amide or amino acid)

  Some other oxidation products may contribute to TRO.

  Peroxides, naturally produced by the action of sunlight, can be present at levels of around 5 $\mu g\, l^{-1}$ and will interfere with some analytical techniques. The exact concentrations will vary depending on sunlight intensity, concentration of sensitisers (e.g. humic acid), temperature, dissolved oxygen and the presence of quenching compounds. They are just formed from natural processes and are continually destroyed and reformed. However they will contribute to most analytical techniques that determine "TRO". This effect has not been quantified but in theory some modifications to the analysis may reduce the effect of peroxides or allow them to be quantified separately to active halogens.

2.4 Behaviour and fate in the environment

2.4.1 Chemistry of bromine in water

Many of the reactions of bromine in water are analogous to those of chlorine, i.e. it will react with ammonia, nitrogen-containing organic compounds, TOC (humic and fulvic acids) with a different balance of by-products often being formed due to the different reaction kinetics.

In aqueous solutions bromine hydrolyses to hypobromous acid (HOBr) (free bromine) (Equation 1), which further dissociates to give hypobromite ions (OBr^-) and hydrogen ions (H^+) (Equation 2) with the degree of dissociation being highly pH dependent.

$Br_2 + H_2O$ <--> HOBr + Br^- (Equation 1)

HOBr <--> OBr- + H+ (Equation 2)

A comparison between the dissociation of hypobromous acid and hypochlorous acid is given in Table 2.2. From this it is clear that the major bromine species present, over the typical range of pH found in natural waters, will be hypobromous acid. At a pH of 8.3, typical of many industrial effluents, less than 13% of active hypochlorous acid remains. Conversely 70% of active hypobromous acid is present at this pH (Ross 1991).

In water, bromine reacts rapidly with ammonia to form monobromamine (NH_2Br), dibromamine ($NHBr_2$) and tribromamine (NBr_3) (combined bromine). These reactions are more rapid than the equivalent reactions for chlorine, perhaps by a factor of as much as ten. The bromamines are readily interconvertible by changing the pH of the solution and also undergo a series of decomposition reactions. This is in contrast to the relative stability of the chloramines formed by the reaction of chlorine with ammonia.

The reaction of bromine with ammonia has a breakpoint, analogous to that for chlorine, and this occurs at an ammonia to bromine molar ratio of 0.67. The basis for this phenomenon is the decomposition of dibromamine, to yield nitrogen and bromide, following the equation given below (Equation 3):

$$2NHBr_2 + H_2O \rightarrow N_2 + HOBr + Br^- + H^+ \qquad \text{(Equation 3)}$$

The half-life of a 5 mg l^{-1} solution of dibromamine at pH 8 and 25 °C is 30 minutes (*cf.* a half-life of 19 hours for monobromamine; Jolley and Carpenter 1983).

The proportions of the different bromamines formed are dependent on the pH, the bromine to ammonia ratio and the temperature. At bromine concentrations below the breakpoint, the combined bromine exists as a mixture of monobromamine and dibromamine, i.e. there is no tribromamine present. In the pH range 6-8 and up to an ammonia to bromine molar ratio of 30, dibromamine is the predominant species. In general, at higher ammonia to bromine ratios there will be a greater proportion of monobromamine.

However, above the breakpoint the main combined bromine species is tribromamine which, in the pH range of 7-8, decomposes in accordance with Equation 4 (below):

$$2NBr_3 + 3H_2O \rightarrow N_2 + 3HOBr + 3Br^- + 3H^+ \qquad \text{(Equation 4)}$$

Bromamines have greater biocidal properties than chloramines thus adding to the biocidal effect of HOBr.

Bromine will also react readily with nitrogen-containing organic compounds (e.g. amino acids) to produce organic bromamines. There is some evidence that these reactions are faster than the reaction of bromine with ammonia and also that they are significantly faster than their analogous chlorine reactions. There is also evidence that some organo-bromamines are relatively unstable, with the half-life of N-bromoglycine (at pH 8.4 and 25 °C) being 100 minutes (Jolley and Carpenter 1983), although other authors (e.g. White 1986) report evidence of some long-lived, but unspecified, organo-bromamines.

More recent data (Great Lakes Chemicals (Europe) Ltd) on the dissipation and decay of chlorine and bromine oxidants (resulting from the disinfection of secondary treated sewage with chlorine and a mixture of chlorine and sodium bromide) discharged into a freshwater

stream, indicates that the oxidants generated in the presence of sodium bromide decay approximately three times faster than oxidants generated in the absence of sodium bromide. In addition, decay of oxidants generated under such conditions was found to occur in three phases. An initial short phase (lasting six to ten minutes) is characterised by a rapid decay. A second phase (lasting approximately 50 minutes) is characterised by a slower decay and first order decay kinetics. Under controlled conditions the third phase is characterised by very slow decay. However, in this study, because of dilution and possibly other reactions, oxidants dissipated much faster in the stream than could be accounted for by decay alone.

Bromine will react more rapidly than chlorine with organic material in water, but will produce by-products that are broadly analogous to those produced by chlorine, e.g. bromoform, bromophenols, bromoaldehydes, bromoacetonitriles, bromoacetic acids, bromoketones, bromohydrins. There is little or no information available on the levels that would be formed by the addition of bromine alone as an oxidant, with virtually the only identification of brominated organics having been made either for chlorinated or ozonated systems (where the chlorine/ozone oxidises bromide to bromine, e.g. Glaze *et al.* 1993) where the bromine concentration is unknown. Hence the data available on concentrations of by-products formed cannot be readily extrapolated to other situations.

The environmental chemistry of some bromine releasing biocides (bromine chloride, sodium bromide and 1-bromo-3-chloro-5-dimethyl hydantoin) is as follows:

(a) Bromine chloride is an interhalogen compound which is liquid under pressure and gaseous at atmospheric pressure. It exists in equilibrium with bromine and chlorine in both phases (Roberts and Gleeson 1978). When this gas mixture is introduced into freshwater, it produces hypobromous acid through the following reactions (Equations 5, 6, 7, 8):

$BrCl + H_2O \rightarrow HOBr + H^+ + Cl^-$ (Equation 5)

$Br_2 + H_2O \rightarrow HOBr + H^+ + Br^-$ (Equation 6)

$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^-$ (Equation 7)

$HOCl + Br^- \rightarrow HOBr + Cl^-$ (Equation 8)

The chemistry of bromine chloride in seawater is less well known. Bromochlorinated seawater should contain a mixture of the bromine species similar to that found in freshwater systems except that bromate may not be formed in freshwater whereas it is formed in seawater.

(b) Sodium bromide *per se* is a stable salt with no biocidal activity. The salt dissociates in water to sodium and bromide ion which do not undergo any further dissociation. Sodium bromide needs to be activated to obtain hypobromous acid. In order to achieve this, sodium bromide is injected into the water with or after activators such as chlorine or sodium hypochlorite, the resulting hypochlorous acid rapidly oxidise the sodium bromide producing hypobromous acid (Ross 1991) (Equation 9).

$HOCl + NaBr \rightarrow HOBr + NaCl$ (Equation 9)

When hypobromous acid passes through a heat exchange unit some is converted back to bromide ion and water.

(c) 1-bromo-3-chloro-5-dimethyl hydantoin (BCDMH) (Equation 10):

$C_5H_6\ BrClN_2O_2 + 2H_2O \rightarrow C5H_8N_2O_2 + HOBr + HOCl$ (Equation 10)

2.4.2 Fate and by-products of bromine in aquatic environments

Table 2.3 gives a summary of the possible bromine species and by-products that may be found in the aquatic environment as a result of discharging bromine-containing water. The highest by-product concentrations are likely to occur for the bromamines, followed possibly by bromoform and the bromophenols. However, as noted above, there is little firm information available.

Table 2.3 Possible bromine species/by-products resulting from discharging bromine-containing waters to the aquatic environment

Bromine species
Bromine
Hypobromous acid/hypobromite ion
By-products
monobromamine
dibromamine
tribromamine
organo-bromamines
bromoform
bromophenols (monobromo-, tribromo-, dibromo-)
bromoacetic acids
bromoacetonitriles (especially dibromo-)
bromoaldehydes
bromoketones (e.g. bromoacetone)
bromohydrins (e.g. 3-bromo-2-methyl-2-butanol)
BMX (3-bromo-4-(dibromomethyl)-5-hydroxy-2(5H)furanone)
Bromopicrin
Cyanogen bromide

Seawater

In seawater it is likely that the predominant species formed will be inorganic bromamines (especially dibromamine) because of the relatively high ammonia concentrations present. As a result, because of the limited stability of inorganic bromamines, it is likely that the total bromine residual will decay fairly rapidly to bromide. There is likely to be at least some formation of organic bromamines and brominated organics, which may well persist.

Freshwater

In river waters, typically with relatively low ammonia concentrations and relatively high organic carbon contents, it is likely that far higher concentrations of both organic bromamines and brominated organics will be formed than is the case in sea water, i.e. the persistent portion of the bromine by-products will be higher.

In neither case is there likely to be any free bromine remaining after a very short contact period, certainly not for effluent bromine concentrations up to a few milligrams per litre. However, this may be hard to confirm since the standard analytical procedures are incapable of distinguishing free and combined bromine (i.e. incapable of distinguishing hypobromous acid and the inorganic bromamines) (see Section 2.5). If any free bromine should persist in an open environment then it would be destroyed by photolysis, yielding bromide and bromate.

Sewage disinfection

Although not currently practised in the UK, the disinfection of municipal sewage effluents using bromine or bromine releasing biocides has been investigated by a number of authors. However, the studies have not been considered in the derivation of this EQS since the addition of a powerful oxidant such as bromine to sewage effluents will result in a series of by-products of unknown toxicity and persistence. The individual effects of these would not be possible to assess.

To assess the effect of these substances other approaches such as direct toxicity assessment of the brominated effluent may need to be applied. The use of direct toxicity assessments is an area of research which has recently been undertaken by the Environment Agency (as the NRA, HMIP and SNIFFER).

A pilot study has been carried out to produce a framework for the national implementation of toxicity-based limits for appropriate complex effluents. The procedure derived has been the subject of a consultation exercise with interested parties which lasted from July - October 1996. On the basis of the resulting discussions a joint regulatory/industry 'Demonstration Project' is to be carried out in which the approach will be trialled on a number of discharges. A revised protocol for the demonstration project is being agreed.

2.5 Analysis

2.5.1 Analytical requirements for EQS monitoring

The adequate monitoring of EQSs requires a suitably accurate analytical method.

The accepted approach for the derivation of the accuracy requirements of an analytical system (when monitoring to a particular water quality standard) is described in WRc Report NS30 (Cheeseman *et al.* 1989).

For an EQS of X units, the error on a single analytical result should not be larger than X/10 concentration units or 20% of the concentration in the sample, whichever is the greater. Following the convention of dividing the tolerable error equally between random and systematic sources, this implies:

- a maximum tolerable standard deviation of X/40 concentration units or 5% of the concentration in the sample, whichever is the greater; and
- a maximum tolerable bias of X/20 concentration units or 10% of the concentration in the sample, whichever is the greater.

It is recommended that the target limit of detection should be set at X/10 concentration units.

For example, for a proposed EQS of 1 mg l^{-1}:

- the limit of detection should be 0.1 mg l^{-1} or less;
- the total error should not exceed 0.1 mg l^{-1} or 20% of the determinand concentration (whichever is the greater);
- the systematic error or bias should not exceed 0.05 mg l^{-1} or 10% of the determinand concentration (whichever is the greater); and
- the total standard deviation of individual results should not exceed 0.025 mg l^{-1} or 5% of the determinand concentration (whichever is the greater).

2.5.2 Analytical technique

With respect to this report, analytical techniques of importance relate to total residual oxidants and are analogous to those carried out for chlorine. An ideal method for the determination of TRO would have a detection limit of 0.5 - 1 µg l^{-1}, a working range of 0.5 - 10 000 µg l^{-1}, be able to separate different species, have low operator skill requirements and be portable.

Several methods have low theoretical detection limits, however very few methods have low µg l^{-1} working ranges. Most TRO methods are based on an iodometric technique. To measure TRO, iodide is first added to the sample. Any oxidant should oxidise the iodide to iodine. The iodine is determined either colorimetrically or amperometrically.

Most commonly encountered oxidising species, from disinfection, will oxidise iodide, therefore peroxides will interfere. Also if there is a high level of organic matter present, the iodine can be removed by the organics by either reduction or sorption before the determination can be made. This will therefore lead to an underestimation of the TRO.

Amperometric titration

This method is designed for free chlorine, however, some organic chloramines will interfere (e.g. chlorosuccinimide), most bromine oxidants will also appear as free chlorine.

The sample is titrated against a reducing agent (e.g. PAO, phenylarsine oxide) and the potential monitored using an electrode. The titration should give a "classic titration curve" and the end point can be used to calculate the free chlorine concentration. TRO is measured in a similar way but after the addition of iodide.

Back titration can also be used in a similar way. In this method, a known quantity (x moles) of the reducing agent is added to the sample in excess. The reduced sample is then titrated against an oxidising reagent, normally iodine, to an end-point (y moles). The difference (x-y moles) is the quantity of TRO initially present in the sample. The back titration method reduces the effect of any iodine demand in the sample.

The concentrated PAO reagent is fairly hazardous and its use may be restricted to enclosed systems subject to COSHH requirements.

The method cannot be readily used in the field, however it may be possible to use the back titration method under these conditions. The reducing agent can be added to the sample immediately and the sample titrated upon returning the laboratory.

It has been reported that residuals down to 1 $\mu g\, l^{-1}$ have been measured by this method after modification to "super-sensitise" it with a signal converter (White 1986). However this was carried out in treated drinking water. It is subject to interferences and the reagents must be regularly standardised, so it is doubtful if meaningful measurements could be made at this low level. Without investigating this method in the laboratory, it is impossible to assess it fully, but a detection limit of 50-100 $\mu g\, l^{-1}$ is likely.

Electrode methods

Membrane electrodes have been used to measure free chlorine and also particular chloramines. The membranes allow only the species of interest to cross into the electrode. The potential of the oxidant is then measured and compared with calibration. This method is species specific and so cannot be used for TRO. By adding iodide, an iodine electrode can be used to measure the iodine oxidised by the TRO. This is subject to the problems discussed above (i.e. iodine demand from organics and the fact that most oxidants can oxidise iodide e.g. nitrite, peroxides). In addition, the electrode response is very temperature dependent and requires simultaneous temperature measurement and compensation.

This method can be used in the laboratory or field if a suitable portable electrode system is available. Some models (e.g. Orion electrode) state a range between 0.01 - 5 $mg\, l^{-1}$. An

electrode signal may well be possible at levels of 10 $\mu g\ l^{-1}$. However, whether this signal will represent the TRO concentration is not known, bearing in mind the possible problems with this method (i.e. temperature dependence, iodine demand, oxidant losses across the electrode membrane).

Iodometric methods

Potassium iodide is added to the sample to produce elemental iodine as described above. The iodine is then titrated with sodium thiosulphate with starch as an indicator. This method is only accurate at concentrations above 1 $mg\ l^{-1}$ TRO. It is not really a field technique but is commonly used in laboratories to test and calibrate other techniques.

DPD

The N-N-diethyl-p-phenylenediamine (DPD) method is a colorimetric method. The oxidant reacts with the DPD to form a coloured product which is measured photometrically and compared with a calibration from standards or with a colour chart. By using various reagents and buffers, the reaction can be made species specific or TRO can be measured by the addition of iodide. However, again the use of iodide is subject to the same problems discussed above. The coloured product can decolourise with time, and excess oxidant can result in the formation of a colourless quinoid product which can lead to significant underestimation of residuals.

This method is portable and has a quoted working range of 1 $\mu g\ l^{-1}$ to 5 $mg\ l^{-1}$. However, in natural waters, there are problems with the background absorbance of the sample, particularly if suspended solids are present. The lowest confident detection limit is approximately 0.1 $mg\ l^{-1}$. Apparent residuals of 10 $\mu g\ l^{-1}$ can be quantified, however, variations in the background absorbance or peroxide concentrations of the sample can account for this. The upper limit is 1 $mg\ l^{-1}$. Above this, decolourisation can occur due to excess oxidant and poor mixing.

DPD can also be used with ferrous ammonium sulphate (FAS) in titrations. This has a theoretical limit of detection of below 10 $\mu g\ l^{-1}$, however, in practice it will be subject to interferences from peroxides and background absorption. The detection limit will be nearer 0.1 $mg\ l^{-1}$. It is not a field technique.

There is current research into using DPD techniques in conjunction with flow injection analysis to improve detection limits and separate different species (Gordon *et al.* 1991). However, at this stage this is not a routine technique and is not designed for field applications.

Others

There are currently a number of novel techniques being investigated to measure residual oxidants. These include chemiluminescence, polarography and mass spectrometry measurements and separation techniques such as ion and liquid chromatography. Some of these show promise but are not yet validated for routine use.

Modification of methods to determine bromine residuals

While some methods for the determination of TRO (e.g. those relying on oxidation of iodide to iodine) will not distinguish between chlorine and bromine, it is possible to use a modification of the DPD method to determine "residual bromine", which corresponds to free bromine plus bromamines (Palin 1974). This modification was based on the addition of glycine, which removes free chlorine by converting it to combined chlorine. Problems were reported with the application of this original modification when chloride and bromide are present at seawater concentrations (Madec and Courtot-Coupez 1981); these were overcome by iodide addition to the phosphate buffer/DPD mixture prior to sample addition. However, this method has been found to be of limited use in high ammonia systems (Davis *et al.* 1993).

A further modification to the DPD method, to allow the separate determination of free bromamines (Palin 1980) involves addition of sodium nitrite to destroy free bromine. The use of this later modification for the analysis of residual bromine in the presence of ammonia nitrogen (e.g. when bromine is used as a disinfectant in natural water swimming pools) has been investigated (Strupler 1987). It was noted that that results produced by the DPD method with a 100 ml test portion of a sample containing bromamines, with varying additions of iodide (0, one crystal and 1 g), were identical so far as mono- and dibromamine were concerned and were closely comparable to those given by the acid iodometric method. However the behaviour of tribromamine was different. Tribromamine responds only partially in the absence of iodide, and the response after addition of 1 g of iodide is of the order of 70%. Also, a large proportion of the tribromamine (about 85%) is eliminated by the nitrite reagent. The application of correction factors is therefore necessary; the factors applied depend on whether the samples contain free bromine and all of the bromamines, free bromine and mono- and dibromamine, or free bromine and tribromamine (which is the case when an excess of bromine is present). Use of these factors allows the estimation of three different fractions *viz.* free bromine, mono- plus dibromamine, and tribromamine.

2.6 Environmental concentrations

No data are available on environmental concentrations of bromine. It has been reported that bromine is not routinely analysed for by the Environment Agency nor by SEPA (Mr S. Killeen, Pers. Comm. 1994: Prof. D. Mackay, Chairman, Association of Directors and River Inspectors of Scotland, Pers. Comm. 1994).

Consents for discharges containing bromine have been reported from some Environment Agency regions. In Welsh region, the discharges are consented as total free halogen. Two (each of 2 mg l^{-1} total free halogen) are from an aluminium production plant which uses bromine to disinfect the cooling system. The majority of the cooling water is discharged to a sea outfall but in emergencies it discharges to the site drainage system. The third (15 mg l^{-1} total free halogen) is from a company which produces brominated anti-knocking compounds and bromine occurs as a by-product in the effluent. It has been reported that there is currently no evidence of environmental effects caused by either of these discharges (K. Cameron, Welsh Region, Pers. Comm., 1994).

In the North East (formerly NRA Yorkshire region) a discharge consent for bromine of 0.06 mg l^{-1} as total residual bromine for a cooling water effluent has been reported. However, no further data are available (S. Killeen, Pers. Comm., 1994).

3. DERIVATION OF EQSs

3.1 Standards in other Countries

No standards for bromine for the protection of the aquatic environment in other countries have been found.

3.2 Protection of freshwater life

Data on the toxicity of bromine to freshwater organisms are considered in detail in Appendix A. The data most relevant to the derivation of the EQSs are further outlined below.

Compared to chlorine, the available data on the toxicity of bromine to freshwater organisms are limited.

For algae, toxicity data of relevance to the derivation of the EQS is limited to one test, a static 72 hour LC50 of 2.0 mg BCDMH l^{-1} for the green algae (*Scenedesmus subspicatus*) (Great Lakes Chemicals (Europe) Ltd.). No further data are available.

While a number of authors have investigated the efficiency of bromine and bromine releasing biocides in removing algal biomass, these studies are not included in the report since they were conducted at the high doses usually applied in power stations and have only reported the presence/absence of biofouling organisms rather than specific effect values.

As may be expected, due to the short persistence of HOBr, data for both invertebrates and fish show that when studies are conducted under flow-through conditions, organisms are more sensitive than when static conditions are used. Sensitivity also decreases in comparison to flow through tests when intermittent exposure is used.

Data on the toxicity of bromine to freshwater invertebrates are available for molluscs, crustaceans and insects (those species found to be most sensitive to chlorine). For bromine, the most sensitive invertebrate species reported are the amphipod, *Hyalella azteca* with a 96 hour LC50 <0.032 mg l^{-1} TRB (only 1 survivor out of 20 at this test concentration) and *Daphnia magna* with an estimated 48 hour LC50 of <0.038 mg l^{-1} TRB (six survivors out of 20 at this test concentration) both for exposure under flow-through conditions (Great Lakes Chemicals (Europe) Ltd.).

Comparative toxicity data for the mayfly, the most sensitive species to chlorine show that chlorine and bromine appear to have similar toxicities if allowance is made for the difference in loss of chlorine and bromine observed in the toxicity tests. The one bromine study available for insects compared the toxicity of HOBr and HOCl to the larvae of the mayfly, *Cloeon sp.*. Based on nominal concentrations the larvae appeared more sensitive to HOCl (96 hour LC50s of 0.46 mg l^{-1} and 0.12 mg l^{-1} for HOBr and HOCl respectively) , however, if the higher losses of Br_2 during the test are taken into account, indications are that actual exposure concentrations for Br_2 and Cl_2 were likely to be in the same range (Bromine Biocides Group).

For fish, only 96 hour LC50 values have been reported. From the available data, the most sensitive fish species is rainbow trout (*Oncorhynchus mykiss*) with a 96 hour LC50 of 0.068 mg l^{-1} TRB under flow-through conditions (Great Lakes Chemicals (Europe) Ltd.). This indicates a sensitivity similar to that exhibited by invertebrates.

In river waters, HOBr will rapidly react with ammonia or organics to form bromamines and brominated organics. The only available data on the toxicity of bromamines are for *Daphnia magna* and rainbow trout. For *Daphnia magna* a 48 hour LC50 of <0.032 mg l^{-1} TRB (assumed to be as bromamines) (8 survivors out of 20 at this test concentration) was reported (Great Lakes Chemicals (Europe) Ltd.). For rainbow trout a 96 hour LC50 of 0.40 mg l^{-1} was determined. The highest test concentration causing no mortality after 96 hours was 0.32 mg l^{-1} and the lowest test concentration causing 100% mortality was 1.0 mg l^{-1}. However, values were expressed as nominals even though mean losses over 12 hours were in the range 81-94% (Bromine Biocides Group).

There are no available data on the bioaccumulation of bromine or bromamines in freshwater organisms. Since chlorine and chloramines do not appear to have any potential for bioaccumulation or bioconcentration (CCREM 1987), it is reasonable to assume that the same is probably true for bromine and bromamines, although additional data are needed to confirm this. The reaction of residual oxidants with organic substances may yield brominated organic compounds which may bioaccumulate, however, this is outside the scope of this report.

Since no data are available on the effect of bromine on aquatic organisms under field conditions, it is not possible to compare the available laboratory data with field data. While, hypobromous acid, like hypochlorous acid, will fairly rapidly dissipate in the aquatic environment, bromamines have been found to persist longer than hypobromous acid although less than chloramines but there are limited data available to confirm this.

Compared to chlorine, only limited data are available on the toxicity of bromine or bromamines to freshwater aquatic life (although the range of available values indicate toxic effects occur at concentrations similar to those found for chlorine). In addition, the majority of available data are from acute studies. Longer studies have only been carried out with the mollusc *Corbicula fluminea* although the concentrations used were fairly high and therefore do not really give an indication of longer term exposure at the lower levels at which some LC50s have been reported. To assess the effects of long term exposure of these chemicals to aquatic organisms, additional chronic data are needed both for HOBr and bromamines. It is fairly likely that the majority of discharges of bromine, for reason of economics, will be intermittent, but, no data are available to assess long term exposure to intermittent discharges.

For the derivation of a standard for the protection of the freshwater environment for bromine it is proposed that a safety factor of approximately 5 is applied to the lowest acute value for hypobromous acid, a 96 hour LC50 <0.032 mg l^{-1} TRB reported for the amphipod, *Hyalella azteca*. This results in an EQS value numerically comparable to that proposed for chlorine. However, a higher safety factor (5) compared to that used for the derivation of the chlorine EQS (2) has been applied because there are fewer toxicity data available for bromine and as at the concentration used for the derivation of the standard more than 50% of the test species were effected (12 out of 20).

In addition, because the use of bromine in cooling water disinfection often includes the use of chlorine in some form, either to activate the bromine or as part of the biocide substance added, it is possible that chlorine induced oxidants may form part of the oxidants present in these tests. It is therefore recommended that any standards be expressed as total residual oxidants, (as measured by the DPD or equivalent method).

Therefore a tentative EQS of 5 $\mu g\ l^{-1}$ TRO (as measured by the DPD or equivalent method) to be expressed as a maximum allowable concentration (MAC) is proposed.

Only limited data are available of the toxicity of bromamines and long term exposure. However, due to the similarity of the toxicity range of bromine with chlorine and in the absence of additional data, a tentative EQS of 2 $\mu g\ l^{-1}$ TRO (as measured by the DPD or equivalent method) to be expressed as an annual average, (equivalent to that proposed for chlorine) is tentatively recommended to protect against chronic exposure.

Because of the potentially different range of oxidants that could be classed under the term TRO, the EQSs have been expressed along with an analytical method, so as to partially define the range of oxidants. Working ranges for this method have been quoted in the range 10 $\mu g\ l^{-1}$ to 5 $mg\ l^{-1}$. However, in natural waters there may be problems with the background absorbance of the sample. Therefore, the standards proposed may be just below the limit of detection and there is a need to improve the analytical method for the determination of TRO.

3.3 Protection of saltwater life

Data on the toxicity of bromine to saltwater organisms are given in Appendix B. Those data of most relevance to the derivation of a saltwater standard for bromine are further outlined below.

No data are available on the toxicity of bromine to saltwater algae or macrophytes. For saltwater invertebrates only acute toxicity data are available. The most sensitive invertebrate species reported is the mysid shrimp (*Mysidopsis bahia*) with flow-through 96 hour LC50s reported in the range 0.092 - 0.17 $mg\ l^{-1}$ TRB (Great Lakes Chemicals (Europe) Ltd.). As with freshwater species sensitivity decreased when exposure was intermittent.

The only available data on the toxicity of bromamines to saltwater invertebrates is again for the mysid shrimp (*Mysidopsis bahia*), with a flow-through 96 hour LC50 of <0.05 $mg\ l^{-1}$ TRB (as bromamines) (Great Lakes Chemicals (Europe) Ltd.).

Burton and Margrey (1978) investigated the acute toxicity of bromine chloride (believed to produce HOBr and HOCl in saltwater, see Section 2.4.1) to two estuarine macroinvertebrates, the grass shrimp (*Palaeomonetes sp.*) and juvenile Blue crab (*Callinectes sapidus*). They concluded that while salinity appears to affect the toxicity of chlorine, it does not appear to affect the toxicity of bromine chloride, however, age and size of organisms tested may play a role in bromine chloride toxicity.

As for invertebrates, only acute data are available for fish. The most sensitive species reported is the estuarine fish, Silverside, *Menidia beryllina*, with a 96 hour LC50 of 0.065 $mg\ l^{-1}$ TRB (Great Lakes Chemicals (Europe) Ltd.). Again, under intermittent exposure sensitivity decreased.

There are no available data on the bioaccumulation of bromine or bromamines in saltwater organisms. However, as suggested for freshwater organisms since chlorine and chloramines do not appear to have any potential for bioaccumulation or bioconcentration (CCREM 1987), it is reasonable to assume that this is probably the same for bromine and bromamines. However, additional data are needed to confirm this. In addition, the reaction of residual oxidants with organic substances may yield brominated organic compounds which may well bioaccumulate (however, this is outside the scope of the report).

Based on the chemistry of bromine in seawater, it is likely that the predominant species formed will be bromamines (especially dibromamine) because of the relatively high ammonia concentrations present. However, because of the limited stability of inorganic bromamines, it is likely that the total bromine residual will decay fairly rapidly to bromide. Some formation of organic bromamines and brominated organics, which may well persist may occur (but this is outside the scope of this study).

It is therefore proposed that a safety factor of approximately 5 be applied to the lowest acute data for TRB a 96 hour LC50 of 0.065 mg l^{-1} TRB for the silverside, *Menidia beryllina*, (Great Lakes Chemicals (Europe) Ltd.). A higher safety factor than that used in the derivation of the chlorine EQS for the protection of saltwater organisms has been applied as the available dataset is more limited than for chlorine (there is no information on the toxicity of bromine to larval stages the most sensitive stage for chlorine) and there are no data for long term exposure.

In addition, because the use of bromine in cooling water disinfection often includes the use of chlorine in some form, either to activate the bromine or as part of the biocide substance added, it is possible that chlorine induced oxidants may form part of the oxidants present in the environment. It is therefore recommended that the standard be expressed as total residual oxidants, (as measured by the DPD or equivalent method).

Therefore, a tentative EQS of 10 µg l^{-1} TRO (as measured by the DPD or equivalent method) to be expressed as a maximum allowable concentration (MAC) is proposed for the protection of saltwater organisms.

Since annual average standards are designed to permit the survival of healthy aquatic communities during long term exposure, it is felt that until data on the fate and behaviour and the exposure of marine organisms to bromamines are available an annual average concentration cannot be proposed.

Because of the potentially different range of oxidants that could be classed under the term TRO, the EQS have been expressed along with an analytical method, so as to partially define the range of oxidants. Working ranges for this method have been quoted in the range 10 µg l^{-1} to 5 mg l^{-1}. However, in natural waters there may be problems with the background absorbance of the sample. Therefore, the standards proposed may be just below the limit of detection and improvements to the analytical methods are therefore required for the monitoring of the proposed EQS.

3.4 Abstraction to potable supply

The EC Directive relating to the quality of water intended for human consumption (CEC 1980) does not lay down standards specifically for bromine.

In addition, no standards for bromine are laid down in the EC Surface Water Directive (CEC 1975) for the protection of water used for the abstraction of drinking water.

When considering the mammalian toxicity of bromine in water (see Appendix C) it is also necessary to consider the impact of brominated by-products that may be formed as the result of its use as a disinfectant. It is important to note that brominated species may already be present in the water prior to treatment with oxidants/disinfectants such as ozone or chlorine and that treatment may produce a number of further brominated by-products which may be more toxic than the original brominated species or may increase the concentration of a particular brominated compound already present. The latter case may be important with regard to concentrations which may have been acceptable prior to treatment but which exceed toxicity guideline values after treatment. Such a case may be envisaged with the brominated trihalomethanes (THMs) which each have a guideline value proposed by the World Health Organisation. In addition, there is a prescribed concentration values of 100 $\mu g\ l^{-1}$ for total THMs in drinking water in the UK, set out in the Water Supply (Water Quality) Regulations 1989; an increase in concentration of one or more of the THMs can raise the total concentration above that which is acceptable.

The toxicity associated with elemental bromine, which is a vapour under normal atmospheric conditions does not apply when it comes into contact with water since it hydrolyses to hypobromous acid which further dissociates to give hypobromite ions and hydrogen ions, the degree of dissociation being highly pH dependent. Photolysis of free bromine will yield bromide and bromate. Hence, the review in Appendix C deals with the mammalian toxicity associated with bromate, bromide, and the major organobromine compounds that are produced from the reactions of bromine with natural organic matter. Relevant standards for these substances are outlined below.

3.4.1 Bromate

Using the linearised multistage model which is conservative in its estimate, the concentration of bromate in drinking water that would produce an excess risk of cancer of 1×10^{-5} (i.e. one extra case of cancer in a population of 100 000) is 3 $\mu g\ l^{-1}$. However, due to limitations in the available analytical and treatment methods, WHO have proposed a provisional guideline value of 25 $\mu g\ l^{-1}$ which equates to an excess risk of cancer of 7×10^{-5} (WHO 1993). The difference between the associated risks of cancer between the 3 $\mu g\ l^{-1}$ health based value and the 25 $\mu g\ l^{-1}$ provisional guideline value based upon the practical quantitation limit, is not considered to be toxicologically significant (Fawell and O'Neill 1993). The European Commission are currently revising the Drinking Water Directive and are proposing a limit of 10 $\mu g\ l^{-1}$ for bromate.

3.4.2 Bromide

Based on this, FAO/WHO provide an estimate of the Acceptable Daily Intake for Man (ADI) of 0-1 mg l^{-1} body weight bromide (FAO/WHO 1988).

Using the ADI estimated by the FAO/WHO assuming a 60 kg adult drinking 2 litres of water per day and allowing 10% of the ADI to water, a Suggested No Effect Level of 3 mg l^{-1} may be calculated, a value that is very comparable with that calculated by NAS (NAS 1980).

3.4.3 By-products

Relevant standards for the major by-products for which there was sufficient data with which the WHO were able to establish guideline values are described below.

Bromoform

The International Agency for Research on Cancer (IARC), have classified bromoform in their Group 3 category for agents that are not classifiable as to their carcinogenicity to humans due to inadequate evidence of carcinogenicity to humans and inadequate or limited evidence for carcinogenicity in experimental animals (IARC 1991). A tolerable daily intake (TDI) of 17.9 µg l^{-1} body weight has been derived for bromoform based upon a NOAEL of 25 mg kg^{-1} body weight/day observed in a 90-day study in rats. Using an allocation of 20% of the total intake to water and assuming a 60 kg adult drinks 2 litres of water per day, a drinking water guideline value of 100 µg l^{-1} has been proposed by WHO (WHO 1993).

Bromodichlorormethane (BDCM)

IARC have classified bromodichloromethane in Group 2B, a category for agents that are possibly carcinogenic to humans, determined by inadequate evidence of carcinogenicity in humans but sufficient evidence of carcinogenicity in experimental animals (IARC 1991). Using the linearised multistage model for extrapolation from the risk of cancer at the doses given to laboratory animals to the levels to which humans are exposed to in the environment a guideline value of 60 µg l^{-1} has been proposed for BDCM by the World Health Organisation. This is associated with an excess risk of cancer (upper 95% confidence interval) of 1 x 10^{-5} (WHO 1993). In their current revision of the Drinking Water Directive, the European Commission are proposing a limit of 15 µg l^{-1} BDCM (ex-works) although this can be raised to 25 µg l^{-1} if chloroform levels are ≤30 µg l^{-1}.

Dibromochloromethane

A TDI of 21.4 µg l^{-1} body weight has been calculated based upon a 90-day rat study in which a NOAEL of 30 mg kg^{-1} body weight/day was observed. Allocating 20% of the total intake to water and assuming that a 60 kg adult drinks 2 litres of water per day, a guideline value of 100 µg l^{-1} has been proposed by WHO.

Dibromoacetonitrile (DBAN)

The NOAEL observed for a 90-day study with rats was determined to be 23 mg kg^{-1} body weight day^{-1} (Hayes *et al.* 1986). Based on this NOAEL, an allocation of 20% of the total intake of DBAN to water, and assuming that a 60 kg adult drinks 2 litres of water per day, a provisional guideline value of 100 $\mu g\, l^{-1}$ has been proposed by WHO. The guideline is provisional because of the limitations of the database.

4. CONCLUSIONS

1. Many of the reactions of bromine in water are analogous to those of chlorine. In aqueous solution bromine hydrolyses to hypobromous acid (HOBr) and further dissociates to give hypobromite ions and hydrogen ions. Bromine will rapidly react with ammonia and nitrogen-containing organic compounds to form bromamines and organic material in water to form by-products broadly analogous to those formed by chlorine.

2. The degree of dissociation of bromine is highly pH dependent. The major bromine species present over the typical range of pH found in natural waters will be hypobromous acid.

3. For freshwaters, a tentative EQS of 5 $\mu g\ l^{-1}$ TRO (as measured by the DPD or equivalent method) expressed as a maximum allowable concentration (MAC) is proposed. This is derived by applying a safety factor of approximately 5 to the lowest acute data for hypobromous acid, a 96 hour LC50 of 0.032 $mg\ l^{-1}$ TRB reported for the amphipod, *Hyalella azteca.*

4. In addition, a tentative EQS of 2 $\mu g\ l^{-1}$ TRO (as measured by the DPD or equivalent method) to be expressed as an annual average is also proposed for freshwaters.

5. For marine waters, a tentative EQS of 10 $\mu g\ l^{-1}$ TRO (as measured by the DPD or equivalent method) expressed as a maximum allowable concentration (MAC) is proposed. This is derived by applying a safety factor of 5 to the lowest acute data for TRB (as opposed to bromamines), a 96 hour LC50 of 0.065 $mg\ l^{-1}$ TRB for silverside, *Mendidia beryllium.*

6. Based on the currently available analytical techniques, the EQSs proposed are below the available detection limits.

7. No environmental monitoring data for discharges of bromine into the aquatic environment and effects on the aquatic biota have been reported.

8. Limited data are available on the toxicity of bromamines, long-term exposure effects, and effects on different lifestages for both fresh and marine waters.

9. For freshwaters there are no available data on the toxicity of bromine to insects, the species found to be the most sensitive to chlorine.

10. The toxicity of bromine to freshwater organisms is in a range similar to that found for the majority of chlorine toxicity data. Therefore, based on the available data, it is unlikely that discharges of chlorine into waters where there are high concentrations of bromide will result in an overall increase in toxicity due to the formation of bromine species.

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APPENDIX A FRESHWATER TOXICITY AND BIOACCUMULATION

A1. FRESHWATER TOXICITY

Few available data on the acute and chronic toxicity of hypobromous acid or bromine releasing biocides to freshwater organisms are available in the open literature. A number of studies which compare the biocidal efficiency of hypobromous acid or bromine releasing biocides in cooling water systems with hypochlorous acid, i.e. investigating the amount of biofouling occurring, have been reported. However, the data are not relevant to EQS derivation and so are not outlined below. In addition, as mentioned in Section 2.4 those studies which investigated the toxicity of municipal effluents disinfected by bromine or bromine releasing biocides are also not reported here due to the difficulties in assessing the toxicity of the numerous by-products likely to be present.

The way in which bromine is used in cooling water disinfection often includes the use of chlorine in some form, either to activate the bromine or as part of the biocide substance added. While the reactions with chlorine and bromine in freshwaters are such that they ultimately lead to the removal of chlorine, it is possible that chlorine induced oxidants may form a part of the oxidants present in these tests.

A1.1 Algae

Limited toxicity data of relevance to EQS derivation are available for freshwater algae. A static 72 hour LC50 of 2.0 mg BCDMH l^{-1} for the green algae (*Scenedesmus subspicatus*) has been reported (Great Lakes Chemicals (Europe) Ltd.). No further data are available.

A number of authors have investigated the biofouling efficiencies of bromine and bromine releasing biocides, including the efficiency of removing algal biomass. However, these studies are not presented here since they were conducted using the high doses of biocide usually applied in power stations and have only reported the presence/absence of biofouling organisms rather than specific effect values.

A1.2 Invertebrates

For *Daphnia magna,* 24 hour LC50s have been reported in the range 1.05 - 1.5 mg l^{-1} TRB. For 48 hour exposure the sensitivity of *Daphnia magna* was at least a factor of 10 higher, with reported LC50s in the range <0.038 - 0.71 mg l^{-1} TRB. As may be expected from the relatively short persistence of TRB, *Daphnia magna* are far more sensitive to TRB under flow-through than static conditions. When exposed to intermittent concentrations (40 minutes every 8 hours for the given time period) a slight decrease in sensitivity was reported for *Daphnia magna* with a 48 hour intermittent LC50 (ILC50) of 0.061 mg l^{-1} TRB compared to an estimated 48 hour LC50 of <0.038 mg l^{-1} TRB when exposed continuously under flow-through conditions (Great Lakes Chemicals (Europe) Ltd). A 'less than value' has been reported for the 48 hour continuous exposure LC50 for *Daphnia magna* since survival was less than 50% at the level of oxidant quantitation (six survivors reported at the lowest test concentration, initially two replicates of ten organisms tested).

For the amphipod, *Hyalella azteca* sensitivity decreased by a factor of 10 when exposed to intermittent conditions for 96 hours. A 96 hour LC50 <0.032 mg l^{-1} TRB was reported under continuous flow-through exposure conditions compared to a 96 hour ILC50 (intermittent LC50) of 0.33 mg l^{-1} TRB (for exposure for 40 minutes every 8 hours) (Great Lakes Chemicals (Europe) Ltd). A 'less than value' has been reported for the 96 hour continuous exposure LC50 since survival was less than 50% at the level of oxidant quantitation (only one survivor reported at the lowest test concentration, initially two replicates of ten organisms tested).

Belanger *et al.* (1991) investigated the biocidal potential of bromine for controlling juvenile and adult Asiatic clam (*Corbicula fluminea*) by exposure to a bromacide for 28 days. Juvenile and adult clams from two locations were used; one exposed to high temperatures (average temperature of 30 °C); the other exposed to normal river water temperature not influenced by thermal discharges (average temperature 17.7 - 25.4 °C). For juvenile clams from the area not influenced by discharges, the LT50 when exposed to 0.25 mg l^{-1} TRB was 20.9 days: 2.6 days when exposed to 0.50 mg l^{-1} TRB and 2.4 days when exposed to 0.75 mg l^{-1} TRB. Adult clams from the same area appeared generally less sensitive with LT50s of 15.4 days when exposed to 0.25 mg l^{-1} TRB: 10.7 days when exposed to 0.50 mg l^{-1} TRB and 6.9 days when exposed to 0.75 mg l^{-1} TRB. Adult clams from the area previously exposed to high temperatures showed the least sensitivity with 16.7% mortality occurring when exposed to 0.25 mg l^{-1} TRB for 28 days; LT50s of 16.2 days when exposed to 0.50 mg l^{-1} TRB and 10.3 days when exposed to 0.75 mg l^{-1} TRB.

Only one study is available on the toxicity of bromine to insects and this investigated both the acute toxicity of HOBr and HOCl to mayfly larvae of *Cloeon sp* (Bromine Biocides Group). The toxicity was determined over a period of 96 hours, using sealed, semi static system with renewal of the test media at 12 hour intervals. The analytical method determined the concentration of Br_2 and Cl_2 in the test media which was assumed to reflect the concentrations of reaction products HOBr and HOCl from the addition of bromine and sodium hypochlorite to water respectively.

Based on nominal concentrations the 24 to 96 hour LC50 of HOBr was calculated to be 0.46 mg l^{-1}. The lowest concentration of HOBr resulting in 100% mortality after 96 hour was 1.0 mg l^{-1} and the highest concentration resulting in no mortality was 0.32 mg l^{-1}. Based on immobilisation of Cloeon sp the highest concentration resulting in no effects was observed to be 0.32 mg l^{-1} HOBr.

For HOCl the 24, 48 and 72 and 96 hour LC50 were calculated to be 0.22. 0.16, 0.13 and 0.12 mg l^{-1} respectively. The lowest concentration of HOCl resulting in 100% mortality after 96 hours was 0.18 mg l^{-1} and the highest concentration resulting in no mortality was <0.1 mg l^{-1}. Based on immobilisation of *Cloeon sp*. The highest concentration resulting in no effects was observed to be <0.1 mg l^{-1} (the lowest concentration tested).

Nominal concentrations were used to determine the toxicity since mean measured loss over the 12 hour renewal period was significant for both Br_2 and Cl_2, although, in general, greater for Br_2.

For Br_2 the mean initial measured concentrations and mean losses over 12 hours were:

Nominal conc Br_2 ($mg\ l^{-1}$)	Mean initial conc ($mg\ l^{-1}$)	Mean % loss over 12 hours
0.1	0.07	0.6
0.32	0.24	91.3
1.0	1.03	83.3
3.2	3.31	48.3
10	11.5	17.2

while for Cl_2 the mean initial measured concentrations and mean losses over 12 hours were :

Nominal conc Cl_2 ($mg\ l^{-1}$)	Mean initial conc ($mg\ l^{-1}$)	Mean % loss over 12 hours
0.1	0.08	62.3
0.18	0.16	68.2
0.32	0.37	79.9
0.56	0.69	56.7
1.0	1.29	48.1

The results outlined above appear to indicate that HOCl is of higher toxicity to *Cloeon sp* than HOBr, however, the raw data indicate a significantly greater loss of Br_2 over the 12 hour period with actual exposure concentrations for Br_2 probably lower.

The only available data for bromamine toxicity are for *Daphnia magna* with a 48 hour LC50 of $<0.032\ mg\ l^{-1}$ TRB (assumed to be as bromamines) (Great Lakes Chemicals (Europe) Ltd). For this experiment $0.3\ mg\ l^{-1}$ ammonia-nitrogen was added. However, while measurements following the addition of this ammonia to the bromine treatment did indicate the presence of relatively large concentrations of free available oxidant (FAO), this was attributed to the amperometric titration methods used measuring bromamines as FAO under the conditions employed. The authors of this study therefore assumed that since ammonia was added in relative excess complete conversion of bromine oxidants into bromamines occurred and hence the observed toxicity reflected bromamine toxicity. It is difficult to assess whether the quantity added was in excess of the break-point concentration. A `less than value' has been reported since survival was less than 50% at the level of oxidant quantitation (eight survivors reported at the lowest test concentration, initially two replicates of ten organisms tested).

Conversion of bromine oxidants to bromamines appears to increase toxicity. This was attributed to the presence of the bromamines and not to un-ionised ammonia. Since ammonia was measured in excess, complete conversion of bromine oxidants into bromamines was assumed.

A1.3 Fish

Only 96 hour LC50 values have been reported the toxicity of bromine to freshwater fish these are presented in Table A1 and further outlined below. Only one study is available on the toxicity of bromamines to fish.

From the available data the most sensitive fish species is rainbow trout (*Oncorhynchus mykiss*) with a 96 hour LC50 of 0.068 mg l^{-1} TRB under flow-through conditions (Great Lakes Chemicals (Europe) Ltd.). Under static conditions an LC50 of 0.23 mg l^{-1} TRB was reported, a decrease in sensitivity by a factor of ten (all mortalities were reported in the first 24 hours, with the exception of one at 48 hours; LC50 calculation based on analytical results at the start of the experiment). When exposure was flow-through but intermittent (40 minutes exposure every eight hours for the given time period) sensitivity again decreased with a 96 hour ILC50 of 0.484 mg l^{-1} TRB reported (Great Lakes Chemicals (Europe) Ltd.). However, this values was still lower than LC50s reported for other species (see Table A1).

For golden shiner (*Notemigonus crysoleucas*) a 96 hour LC50 of 0.288 mg l^{-1} TRB has been reported under flow-through conditions. Under flow-through intermittent exposure (40 minutes exposure every eight hours for the given time period) sensitivity decreased with an ILC50 of 0.790 mg l^{-1} TRB reported (Great Lakes Chemicals (Europe) Ltd.).

Under static conditions the 96 hour LC50 for bluegills (*Lepomis macrochirus*) was estimated to be 0.52 mg l^{-1} TRB (since all mortalities were observed within the first 24 hours of the exposure period the LC50 calculation was based on the analytical results from the start of the experiment). The estimated no observed effects concentration was 0.30 mg l^{-1} (Great Lakes Chemicals (Europe) Ltd.).

Wilde *et al.* (1983) investigated the acute toxicity of 1-bromo-3-chloro-5,5- dimethylhydantoin BCDMH to juvenile (six week old) and yearling fathead minnows (*Pimephales promelas*) and young-of-the-year bluegills (*Lepomis macrochirus*) in a flow-through freshwater system used for cooling a nuclear reactor, with an intermittent exposure period of approximately two hours per day. DPD differential measurements showed >95% of the oxidant residual resulting from BCDMH treatment was bromine. Therefore the residuals from this treatment were considered to be total and free residual bromine. The 96 hour LC50s were reported as 0.81, 1.17 and 1.43 mg l^{-1} TRB for juvenile fathead minnows, adult fathead minnows and bluegills respectively. FRB contributed 50.4% to TRB during all exposure periods.

Static 96 hour LC50 for rainbow trout (*Oncorhynchus mykiss*) and fathead minnow (*Pimephales promelas*) exposed to BCDMH have been reported as 0.87 and 2.25 mg l^{-1} respectively. The corresponding LC50 data for 5,5-dimethylhydantoin, the dehalogenated by-product of BCDMH were 12 700 and 14 200 mg l^{-1} respectively (Great Lakes Chemicals (Europe) Ltd).

The acute toxicity of bromamines to rainbow trout (Oncorhynchus *mykiss*) was determined in a 96 hour semi-static test with renewal of the test media at 12 hour intervals (Bromine Biocides Group). Bromamines were formed from the addition of a HOBr solution to an ammonia/ammonium solution to produce nominal concentrations in the range 1.0 to 0.1 mg l^{-1}.

Table A1 Toxicity of bromine to freshwater life

Species	Life stage	Test type	Analysis	Temp. (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure time	Concn (mg l^{-1})	Effect	Comments	Ref
ALGAE											
Scenedesmus subspicatus (Green algae)	-	S	-	-	-	-	72 hr	2.0	LC50	Solution of BCDMH tested	1
MOLLUSCS											
Corbicula fluminea (Asiatic clam)	juvenile	F	Y	30	-	-	20.9 d	0.25	LT50	As TRB. Organisms not previously exposed to thermal or industrial discharges	2
Corbicula fluminea (Asiatic clam)	juvenile	F	Y	30	-	-	2.6 d	0.50	LT50	As TRB. Organisms not previously exposed to thermal or industrial discharges	2
Corbicula fluminea (Asiatic clam)	juvenile	F	Y	30	-	-	2.4 d	0.75	LT50	As TRB. Organisms not previously exposed to thermal or industrial discharges	2
Corbicula fluminea (Asiatic clam)	adult	F	Y	30	-	-	15.4 d	0.25	LT50	As TRB. Organisms not previously exposed to thermal or industrial discharges	2

Species	Life stage	Test type	Analysis	Temp. (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure time	Concn (mg l^{-1})	Effect	Comments	Ref
Corbicula fluminea (Asiatic clam)	adult	F	Y	30	-	-	10.7 d	0.50	LT50	As TRB. Organisms not previously exposed to thermal or industrial discharges	2
Corbicula fluminea (Asiatic clam)	adult	F	Y	30	-	-	6.9 d	0.75	LT50	As TRB. Organisms not previously exposed to thermal or industrial discharges	2
Corbicula fluminea (Asiatic clam)	adult	F	Y	30	-	-	28 d	0.25	LC16.7	As TRB. Organisms previously exposed to a thermal discharge of approx. 30 °C	2
Corbicula fluminea (Asiatic clam)	adult	F	Y	30	-	-	16.2 d	0.50	LT50	As TRB. Organisms previously exposed to a thermal discharge of approx. 30 °C	2
Corbicula fluminea (Asiatic clam)	adult	F	Y	30	-	-	10.3 d	0.75	LT50	As TRB. Organisms previously exposed to a thermal discharge of approx. 30 °C	2
ARTHROPODS - CRUSTACEANS											
Daphnia magna (Water flea)	-	S	-	-	-	-	48 hr	0.47	LC50	Solution of BCDMH tested	1

Species	Life stage	Test type	Analysis	Temp. (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure time	Concn (mg l^{-1})	Effect	Comments	Ref
Daphnia magna (Water flea)	-	S	Y	21-22	186	7.6-8.0	24 hr	1.05	LC50	As TRB (substance tested BrCl). All organisms killed outright, no immobilisation or other effects occurred.	1,5
Daphnia magna (Water flea)	-	S	Y	-	-	-	48 hr	0.71	LC50	As TRB (substance tested BrCl). Since all mortalities occurred in the first 24 hours the 24 hour LC50 of 1.05 mg l^{-1} TRB is a more appropriate expression of toxicity.	1,5
Daphnia magna (Water flea)	-	F	Y	25 ± 2	145	7	48 hr	<0.038	LC50	As TRB.	1
Daphnia magna (Water flea)	-	F	Y	25 ± 2	179	8	48 hr	0.061	ILC50	As TRB. Intermittent exposure 40 mins every eight hours	1
Daphnia magna (Water flea)	-	F	Y	25 ± 2	142	7	48 hr	<0.032[a]	LC50	As TRB. Continuous exposure with 300 µg l^{-1} ammonia	1
Daphnia magna (Water flea)	-	-	-	-	-	-	24 hr	1.5	LC50	As TRB.	4
Hyalella azteca (Amphipod)	-	F	Y	25 ± 2	138	7-8	96 hr	<0.032	LC50	As TRB.	1

Species	Life stage	Test type	Analysis	Temp. (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure time	Concn (mg l^{-1})	Effect	Comments	Ref
Hyalella azteca (Amphipod)	-	F	Y	25 ± 2	138	7-8	96 hr	0.333	ILC50	As TRB. Intermittent exposure 40 mins every eight hours	1
ARTHROPODS - INSECTS											
Cloeon sp (Mayfly)	larvae	SS	N	14.7-15.8	60.2	6.2 - 7.2	96 hr	0.46	LC50	As HOBr. Nominal concentration due to significant loss from test solution over the 12 hour renewal period	6
Cloeon sp (Mayfly)	larvae	SS	N	14.7-15.8	60.2	6.2 - 7.2	96 hr	1.0	LC100	As HOBr. Nominal concentration due to significant loss from test solution over the 12 hour renewal period	6
Cloeon sp (Mayfly)	larvae	SS	N	14.7-15.8	60.2	6.2 - 7.2	96 hr	0.32	HNOEC	As HOBr. Nominal concentration due to significant loss from test solution over the 12 hour renewal period	6
FISH **non-salmonid**											
Pimephales promelas (Fathead minnow)	-	S	-	-	-	-	96 hr	2.25	LC50	Solution of BCDMH tested	1

Species	Life stage	Test type	Analysis	Temp. (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure time	Concn (mg l^{-1})	Effect	Comments	Ref
Pimephales promelas (Fathead minnow)	(juvenile)	F	Y	19 - 23	-	6.7 - 7.1	96 hr	0.81	ILC50	As TRB. Intermittent exposure of approx two hours per day	3
Pimephales promelas (Fathead minnow)	(yearling)	F	Y	19 - 23	-	6.7 - 7.1	96 hr	1.17	ILC50	As TRB. Intermittent exposure of approx two hours per day	3
Lepomis macrochirus (Bluegill)	-	S	Y	21 - 22	-	7.0 - 7.7	96 hr	0.52	LC50 (estimated)	As TRB (substance tested BrCl). Since all mortalities were observed in the first 24 hours, the LC50 was based on 0 hour analysis.	1,5
Lepomis macrochirus (Bluegill)	-	S	Y	21 - 22	-	7.0 - 7.7	96 hr	0.30	NOEC (estimated)	As TRB (substance tested BrCl)	1,5
Lepomis macrochirus (Bluegill)	young-of-the-year	F	Y	19 - 23	-	6.7 - 7.1	96 hr	1.43	ILC50	As TRB. Intermittent exposure of approx two hours per day	3
Notemigonus crysoleucas (Golden shiner)	-	F	Y	25 ± 2	184	8	96 hr	0.288	LC50	As TRB	1
Notemigonus crysoleucas (Golden shiner)	-	F	Y	25 ± 2	145	7	96 hr	0.790	ILC50	As TRB. Intermittent exposure 40 mins every eight hours	1

Species	Life stage	Test type	Analysis	Temp. (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure time	Concn (mg l^{-1})	Effect	Comments	Ref
Salmonid											
Oncorhynchus mykiss (Rainbow trout)	-	S	-	-	-	-	96 hr	0.87	LC50	Solution of BCDMH tested	1
Oncorhynchus mykiss (Rainbow trout)	-	S	Y	13 - 14	-	7.3 - 8.0	96 hr	0.23	LC50	As TRB (substance tested BrCl).	1,5
Oncorhynchus mykiss (Rainbow trout)	-	S	Y	13 - 14	-	7.3 - 8.0	96 hr	0.1	NOEC	As TRB (substance tested BrCl).	5
Oncorhynchus mykiss (Rainbow trout)	-	F	Y	15 ± 1	160	7	96 hr	0.068	LC50	As TRB	1
Oncorhynchus mykiss (Rainbow trout)	-	F	Y	15 ± 1	160	7	96 hr	0.484	ILC50	As TRB. Intermittent exposure 40 mins every eight hours	1
Oncorhynchus mykiss (Rainbow trout)	-	SS	N	13.4-14.5	47.5 - 60.4	6.5 - 7.6	24-48 hr	0.54	LC50	Bromamines. Due to high loss over 12 hour renewal period, concentrations are based on nominal.	6
Oncorhynchus mykiss (Rainbow trout)	-	SS	N	13.4-14.5	47.5 - 60.4	6.5 - 7.6	72 hr	0.49	LC50	Bromamines. Due to high loss over 12 hour renewal period, concentrations are based on nominal.	6

Species	Life stage	Test type	Analysis	Temp. (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure time	Concn (mg l^{-1})	Effect	Comments	Ref
Oncorhynchus mykiss (Rainbow trout)	-	SS	N	13.4-14.5	47.5 - 60.4	6.5 - 7.6	96 hr	0.40	LC50	Bromamines. Due to high loss over 12 hour renewal period, concentrations are based on nominal.	6
Oncorhynchus mykiss (Rainbow trout)	-	SS	N	13.4-14.5	47.5 - 60.4	6.5 - 7.6	96 hr	1.0	LC100	Bromamines. Due to high loss over 12 hour renewal period, concentrations are based on nominal.	6
Oncorhynchus mykiss (Rainbow trout)	-	SS	N	13.4-14.5	47.5 - 60.4	6.5 - 7.6	96 hr	0.32	LC0	Bromamines. Due to high loss over 12 hour renewal period, concentrations are based on nominal.	6

Notes:

a : as bromamines, since ammonia was added in excess, complete conversion of bromine oxidants to bromamines was assumed. Toxicity was attributed to amines and not to the formation of unionised ammonia.
NOEC - no observed effect concentration
ILC50 - Intermittent LC50

References:

1. Great Lakes Chemicals (Europe) Ltd
2. Belanger *et al.* (1991)
3. Wilde *et al.* (1983)
4. Le Blanc (1980)
5. Data supplied by Bromine and Chemicals Ltd, Dead Sea Bromine Group and Rohm and Haas
6. Bromine Biocides Group

Mean losses over 12 hours were in the range 81-94%, the authors suggested that this corresponded to, and was consistent with, the known persistence of bromamines in water. Since there were concentrations less than the limit of detection at the completion of the exposure periods for some concentrations the results of the toxicity test were expressed in relation to nominal concentrations of bromamines in the test media.

The 24 and 48 hours LC50 was 0.54 mg l^{-1}, the 72 hour LC50 was 0.49 mg l^{-1} while the 96 hour LC50 was 0.40 mg l^{-1}. The highest test concentration causing no mortality after 96 hours was 0.32 mg l^{-1} and the lowest test concentration causing 100% mortality was 1.0 mg l^{-1}. The significant loss over the 12 hour period indicate that actual exposure concentrations may be lower than nominal.

A2. BIOACCUMULATION

There are no available data on the bioaccumulation of bromine or bromamines in freshwater organisms. Since chlorine and chloramines do not appear to have any potential for bioaccumulation or bioconcentration (CCREM 1987). It is reasonable to assume that this is probably the same for bromine and bromamines, although additional data are needed to confirm this. However, the reaction of residual oxidants with organic substances may yield brominated organic compounds which may well bioaccumulate.

A3 COMPARISON OF TOXICITY OF BROMINE AND CHLORINE TO FRESHWATER ORGANISMS

As previously mentioned EQSs for chlorine have been proposed in NRA R&D Note 332. Far more data are available on the toxicity of chlorine and chloramines to freshwater organisms than for bromine and bromamines to freshwater organisms.

For chlorine, sub-lethal effects in fish have been reported as low as 0.001 mg l^{-1} TAC (avoidance by rainbow trout, Sprague and Drury 1969). The lowest reported acute toxicity of chlorine to freshwater are: 100% mortality of brook trout in 45 hours at 0.010 mg l^{-1} TAC (Pike 1971 cited in Alabaster and Lloyd 1992) and a 48 hour LC50 of 0.0093 mg l^{-1} TAC reported for mayfly, *Isonychia* sp., Gregg 1974 cited in Mattice and Zittel 1976). This compares to a 96 hour LC50 of 0.068 mg l^{-1} TRB for rainbow trout and a 96 hour LC50 for the amphipod *Hyalella azteca* of <0.032 mg l^{-1} TRB (Great Lakes Chemicals (Europe) Ltd.). While there are no available data on sub-lethal effects of bromine, the majority of acute mortality data for chlorine is in the same range as that reported for bromine.

Due to differences in test designs and procedure, comparison of the toxicity of chlorine and bromine from different tests is difficult. However, studies in which the toxicity of chlorine and bromine are compared under the same test procedures are available and are outlined below.

Wilde *et al.* (1983) and a study from Great Lakes Chemicals (Europe) Ltd have directly compared the toxicity of bromine and chlorine to freshwater organisms. Both studies indicate that while there are some differences in toxicity values, they are in the same order of magnitude. The results are presented in Table A2.

Table A2 Comparison of the toxicity of bromine and chlorine to freshwater organisms

Species	Life stage	Test type	Analysis	Temp. (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure time	Concn (mg l^{-1})	Effect	Comments	Ref
Daphnia magna (Water flea)	<24 h	F	Y	-	144.5	7.31-7.71	48 hr	0.032	LC50	as chlorine l^{-1}	1
Daphnia magna (Water flea)	<24 h	F	Y	-	145	7.28-7.77	48 hr	<0.03	LC50	as bromine l^{-1}	1
Daphnia magna (Water flea)	<24 h	F	Y	-	161	8.21-8.34	48 hr	0.055	ILC50	as chlorine l^{-1}	1
Daphnia magna (Water flea)	<24 h	F	Y	-	178.7	8.21-8.34	48 hr	0.061	ILC50	as bromine l^{-1}	1
Daphnia magna (Water flea)	<24 h	F	Y	-	142	7.35-7.90	48 hr	<0.018	LC50	as chlorine l^{-1} (in the form of chloramines as 300 µg l^{-1} ammonia was added)	1
Daphnia magna (Water flea)	<24 h	F	Y	-	142	7.33-7.89	48 hr	<0.032	LC50	as bromine l^{-1} (in the form of bromamines as 300 µg l^{-1} ammonia was added)	1
Hyalella azteca (Amphipod)	juvenile	F	Y	-	138	7.84-8.00	96 hr	0.078	LC50	as chlorine l^{-1}	1
Hyalella azteca (Amphipod)	juvenile	F	Y	-	138	7.81-8.00	96 hr	<0.032	LC50	as bromine l^{-1}	1
Hyalella azteca (Amphipod)	juvenile	F	Y	-	138	7.84-8.00	96 hr	0.301	ILC50	as chlorine l^{-1}	1

Species	Life stage	Test type	Analysis	Temp. (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure time	Concn (mg l^{-1})	Effect	Comments	Ref
Hyalella azteca (Amphipod)	juvenile	F	Y	-	138	7.81-8.01	96 hr	0.333	ILC50	as bromine l^{-1}	1
Notemigonus (Golden shiner)	young	F	Y	-	184.3	8.16-8.36	96 hr	0.340	LC50	as chlorine l^{-1}	1
Notemigonus (Golden shiner)	young	F	Y	-	184.3	8.11-8.33	96 hr	0.288	LC50	as bromine l^{-1}	1
Notemigonus (Golden shiner)	young	F	Y	-	145.5	7.41-7.68	96 hr	0.572	ILC50	as chlorine l^{-1}	1
Notemigonus (Golden shiner)	young	F	Y	-	145.5	7.41-7.68	96 hr	0.790	ILC50	as bromine l^{-1}	1
Pimephales promelas (Fathead minnow)	(juvenile)	F	Y	19 - 23	-	6.7 - 7.1	96 hr	0.81	ILC50	As TRB. Intermittent exposure of approx two hours per day	2
Pimephales promelas (Fathead minnow)	(juvenile)	F	Y	19 - 23	-	6.7 - 7.1	96 hr	0.39	ILC50	As TRC. Intermittent exposure of approx two hours per day	2
Pimephales promelas (Fathead minnow)	(yearling)	F	Y	19 - 23	-	6.7 - 7.1	96 hr	1.17	ILC50	As TRB. Intermittent exposure of approx two hours per day	2

Species	Life stage	Test type	Analysis	Temp. (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure time	Concn (mg l^{-1})	Effect	Comments	Ref
Pimephales promelas (Fathead minnow)	(yearling)	F	Y	19 - 23	-	6.7 - 7.1	96 hr	1.37	ILC50	As TRC. Intermittent exposure of approx two hours per day	2
Lepomis macrochirus (Bluegill)	young-of-the-year	F	Y	19 - 23	-	6.7 - 7.1	96 hr	1.43	ILC50	As TRB. Intermittent exposure of approx 2 hours per day	2
Lepomis macrochirus (Bluegill)	young-of-the-year	F	Y	19 - 23	-	6.7 - 7.1	96 hr	2.13	ILC50	As TRC. Intermittent exposure of approx 2 hours per day	2
Oncorhynchus mykiss (Rainbow trout)	15 days old	F	Y	-	160	7.20-7.64	96 hr	0.059	LC50	as chlorine l^{-1}	1
Oncorhynchus mykiss (Rainbow trout)	15 days old	F	Y	-	160	7.24-7.41	96 hr	0.068	LC50	as bromine l^{-1}	1
Oncorhynchus mykiss (Rainbow trout)	15 days old	F	Y	-	160	7.20-7.64	96 hr	0.373	ILC50	as chlorine l^{-1}	1
Oncorhynchus mykiss (Rainbow trout)	15 days old	F	Y	-	160	7.24-7.41	96 hr	0.484	ILC50	as bromine l^{-1}	1

Notes:
ILC50 - Intermittent LC50
F - Flow through

References:
1. Great Lakes Chemicals (Europe) Ltd
2. Wilde *et al.* 1983

Wilde *et al.* (1983) compared the relative toxicity (96 hour LC50s) of chlorine and BCDMH to fathead minnows (*Pimephales promelas*) and bluegills (*Lepomis macrochirus*). Juvenile fathead minnows were slightly more tolerant of TRB than TRC. TRC and TRB 96 hour LC50 values for adult fathead minnows were not significantly different while bluegills were slightly more tolerant of TRC than TRB. On the basis of this study Wilde *et al.* (1983) concluded that chlorine and BCDMH additions resulted in similar overall toxicity to fish.

A comparison of the toxicity (under continuous and intermittent exposure) of HOBr and HOCl has been carried out with four freshwater organisms (*Daphnia magna, Hyalella azteca, Notemigonus crysoleucas* and *Oncorhynchus mykiss*). An additional study also compared the toxicity of chloramines and bromamines to *Daphnia magna*, Table A2 (Great Lakes Chemicals (Europe) Ltd.).

In general, the chlorine-based oxidants were slightly more toxic than the bromine-based oxidants. However, the difference tends to be small (approximately a factor of 2).

Conversion of chlorine oxidants into chloramines and bromine oxidants into bromamines appears to increase acute toxicity although this effect was slightly less pronounced in the case of bromamines. The increase in toxicity can be attributed to amines and not to the formation of un-ionised ammonia (Great Lakes Chemicals (Europe) Ltd).

APPENDIX B SALTWATER TOXICITY AND BIOACCUMULATION

B1 SALTWATER TOXICITY

The aim of the following section is to present information on the toxicity of bromine to saltwater organisms.

The way in which bromine is used in cooling water disinfection often includes the use of chlorine in some form, either to activate the bromine or as part of the biocide substance added, e.g. bromine chloride. Although the chemical reactions of chlorine in saltwater ultimately lead to the removal of detectable free and combined chlorine it is feasible that some chlorine induced oxidants may be present in the test solutions, thus potentially resulting in a mixture of bromine and chlorine oxidants.

B1.1 Algae

There are no available data on the toxicity of bromine to saltwater algae.

B1.2 Invertebrates

Only acute toxicity data are available for saltwater invertebrates. These are presented in Table B1 and the more relevant data are further outlined below.

Flow-through 96 hour LC50s for mysid shrimp (*Mysidopsis bahia*) have been reported in the range 0.092 - 0.17 mg l^{-1} TRB. Sensitivity of mysid shrimp decreased when exposure was intermittent (40 minutes exposure every eight hours for the given time period) with a 96 hour ILC50 of 0.367 mg l^{-1} TRB reported (Great Lakes Chemicals (Europe) Ltd).

For eastern oysters (*Crassostrea gigas*), a 96 hour flow-through EC50 (shell deposition) was calculated by linear regression to be 0.54 mg l^{-1} TRB (Great Lakes Chemicals (Europe) Ltd). Following 96 hours exposure shell deposition was reduced by 87% amongst oysters exposed to 0.88 mg l^{-1}. No statistically significant reduction in shell deposition was observed in oysters exposed to 0.31 mg l^{-1} and less.

American oysters (*Crassostrea virginica*) were reported to be slightly more sensitive than Eastern oysters when exposed to BrCl with an EC50 (shell growth) of 0.16 mg l^{-1} BrCl (Roberts and Gleeson 1978). Forty-eight hour EC50s (failing to reach the straight hinge stage) for larvae were in the range 0.1 - 0.21 mg l^{-1} BrCl.

The toxicity of 1-bromo-3-chloro-5,5-dimethylhydantoin (BCDMH) to grass shrimp (*Palaemonetes pugio*) and the American oyster (*Crassostrea virginica*) has been investigated. Reported static 96 hour LC50s were 13.0 and >640 mg l^{-1} respectively, while the corresponding LC50 data for 5,5-dimethylhydantoin, the dehalogenated by-product of BCDMH were 1300 and 13 300 mg l^{-1} respectively (Great Lakes Chemicals (Europe) Ltd). No further data are available.

Table B1 Toxicity of bromine to saltwater life

Species	Life stage	Test type	Analysis	Temp. (°C)	Salinity (ppt)	pH	Exposure time	Concn (mg l^{-1})	Effect	Comments	Ref
MOLLUSCS											
Crassostrea virginica (American oyster)	-	S	-	-	-	-	96 hr	>640	LC50	Solution of BCDMH tested	1
Crassostrea virginica (American oyster)	larvae	F	Y	19-28	20	-	48 hr	0.1	EC50	BrCl added; preliminary test effect failing to reach the straight hinge stage	4
Crassostrea virginica (American oyster)	larvae	F	Y	19-28	20	-	48 hr	0.21	EC50	BrCl added; preliminary test effect failing to reach the straight hinge stage	4
Crassostrea virginica (American oyster)	juvenile	F	Y	19-28	20	-	96 hr	0.16	EC50	BrCl added; shell growth	4
Crassostrea gigas (Eastern Oyster)	-	F	Y	19-20	32	7.8-8.1	96 hr	0.54	EC50 (shell deposition) (calculated by linear regression)	As TRB. (Hypobromous acid expressed as bromine) Added as BrCl	1,5
Crassostrea gigas (Eastern Oyster)	-	F	Y	19-20	32	7.8-8.1	96 hr	0.88	EC87 (shell deposition)	As TRB. (Hypobromous acid expressed as bromine) Added as BrCl	1,5

Species	Life stage	Test type	Analysis	Temp. (°C)	Salinity (ppt)	pH	Exposure time	Concn (mg l^{-1})	Effect	Comments	Ref
Crassostrea gigas (Eastern Oyster)	-	F	Y	19-20	32	7.8-8.1	96 hr	0.31	No statistically significant reduction in shell deposition	As TRB. (Hypobromous acid expressed as bromine) Added as BrCl	1,5
Crassostrea gigas (Eastern Oyster)	-	-					96 hr	0.47	LC50	As bromine l^{-1}	6
ARTHROPODS - CRUSTACEANS											
Palaeomonetes sp. (Grass shrimp)	-	S	-	-	-	-	96 hr	13.0	LC50	Solution of BCDMH tested	1
Palaeomonetes sp. (Grass shrimp)	-	F	Y	19 ± 1	7	-	24 hr	1.1	LC50	As total residual bromine chloride	2
Palaeomonetes sp. (Grass shrimp)	-	F	Y	19 ± 1	7	-	48 hr	0.8	LC50	As total residual bromine chloride	2
Palaeomonetes sp. (Grass shrimp)	-	F	Y	19 ± 1	7	-	96 hr	0.6	LC50	As total residual bromine chloride	2
Palaeomonetes sp. (Grass shrimp)	-	F	Y	-	18-20	-	48 hr	0.8	LC50	As total residual bromine chloride	3
Palaeomonetes sp. (Grass shrimp)	-	F	Y	-	18-20	-	96 hr	0.7	LC50	As total residual bromine chloride	3
Palaeomonetes sp. (Grass shrimp)	-	F	Y	19 ± 1	7	-	48 hr	0.4	EC50	As total residual bromine chloride: Change in colour	2

Species	Life stage	Test type	Analysis	Temp. (°C)	Salinity (ppt)	pH	Exposure time	Concn (mg l^{-1})	Effect	Comments	Ref
Palaeomonetes sp. (Grass shrimp)	-	F	Y	19 ± 1	7	-	96 hr	0.5	EC50	As total residual bromine chloride: Change in colour	2
Palaeomonetes sp. (Grass shrimp)	-	F	Y	19-28	20	-	24 hr	>1.13	LC50	As BrCl	4
Palaeomonetes sp. (Grass shrimp)	-	F	Y	19-28	20	-	48 hr	0.82	LC50	As BrCl	4
Palaeomonetes sp. (Grass shrimp)	-	F	Y	19-28	20	-	96 hr	0.70	LC50	As BrCl	4
Mysidopsis bahia (Mysid shrimp)	-	F	Y	23-26	31	7.7-8.0	24 hr	0.26	LC50(estimated by non-linear interpolation)	As TRB. (Hypobromous acid expressed as bromine)	5
Mysidopsis bahia (Mysid shrimp)	-	F	Y	23-26	31	7.7-8.0	48 hr	0.18	LC50(calculated by probit analysis)	As TRB. (Hypobromous acid expressed as bromine)	5
Mysidopsis bahia (Mysid shrimp)	-	F	Y	23-26	31	7.7-8.0	96 hr	0.17	LC50(calculated by probit analysis)	As TRB. (Hypobromous acid expressed as bromine)	1,5
Mysidopsis bahia (Mysid shrimp)	-	F	Y	25 ± 2	20.7	8	96 hr	0.092	LC50	As TRB.	1
Mysidopsis bahia (Mysid shrimp)	-	F	Y	25 ± 2	20.5	8	96 hr	0.367	ILC50	As TRB Intermittent exposure 40 mins every eight hours	1

Species	Life stage	Test type	Analysis	Temp. (°C)	Salinity (ppt)	pH	Exposure time	Concn (mg l^{-1})	Effect	Comments	Ref
Mysidopsis bahia (Mysid shrimp)	-	F	Y	25 ± 2	21.0	8	96 hr	<0.050[a]	LC50	As TRB. Continuous exposure with 300 µg l^{-1} ammonia	1
Acartia tonsa (Copepod)	-	F	Y	19-28	20	-	48 hr	0.12	LC50	As BrCl; preliminary test	4
Acartia tonsa (Copepod)	-	F	Y	19-28	20	-	48 hr	0.11	LC50	As BrCl; definitive test	4
Callinectes-sapidus (Blue crab)	-	F	Y	19 ±1	7	-	48 hr	1.2	LC50	As total residual bromine chloride	2
Callinectes sapidus (Blue crab)	-	F	Y	19 ±1	7	-	96 hr	0.8	LC50	As total residual bromine chloride	2
Callinectes sapidus (Blue crab)	non-molt mature (7.6 - 10.2 cm wide)	F	Y	-	18-20	-	96 hr	1.0	LC50	As total residual bromine chloride	3
Callinectes sapidus (Blue crab)	non-molt juvenile (2.5 cm wide)	F	Y	-	18-20	-	96 hr	0.6	LC50	As total residual bromine chloride	3
FISH											
Cyprinodon sp. (Sheepshead minnow)	-	S	-	-	-	-	96 hr	20.0	LC50	Solution of BCDMH tested	1

Species	Life stage	Test type	Analysis	Temp. (°C)	Salinity (ppt)	pH	Exposure time	Concn (mg l^{-1})	Effect	Comments	Ref
Cyprinodon sp. (Sheepshead minnow)	-	F	Y	24-26	31	7.8-8.0	96 hr	0.19	LC50	As TRB. (Hypobromous acid as bromine expressed)	1,5
Cyprinodon sp. (Sheepshead minnow)	-	S	Y	24-26	31	7.8-8.0	96 hr	0.11	NOEC	As TRB. (Hypobromous acid as bromine expressed)	1,5
Menidia beryllina (Silversides)	-	F	Y	25 ± 2	20.7	8	96 hr	0.065	LC50	As TRB	1
Menidia beryllina (Silversides)	-	F	Y	25 ± 2	20.5	8	96 hr	0.344	ILC50	As TRB. Intermittent expressed 40 mins every eight hours	1
Menidia menidia (Atlantic silverside)	-	F	Y	19-28	20	-	24 hr	0.23	LC50	As BrCl	4
Menidia menidia (Atlantic silverside)	-	F	Y	19-28	20	-	48 hr	0.23	LC50	As BrCl	4
Menidia menidia (Atlantic silverside)	-	F	Y	19-28	20	-	96 hr	0.23	LC50	As BrCl	4
Brevoortia tyrannus (Atlantic menhaden)	-	F	Y	19-28	20	-	24 hr	0.31	LC50	As BrCl	4
Brevoortia tyrannus (Atlantic menhaden)	-	F	Y	19-28	20	-	48 hr	0.22	LC50	As BrCl	4

Species	Life stage	Test type	Analysis	Temp. (°C)	Salinity (ppt)	pH	Exposure time	Concn (mg l^{-1})	Effect	Comments	Ref
Brevoortia tyrannus (Atlantic menhaden)	-	F	Y	19-28	20	-	96 hr	0.21	LC50	As BrCl	4
Leiostomus xanthurus (Spot)	-	F	Y	19-28	20	-	24 hr	0.23	LC50	As BrCl	4
Leiostomus xanthurus (Spot)	-	F	Y	19-28	20	-	48 hr	0.22	LC50	As BrCl	4
Leiostomus xanthurus (Spot)	-	F	Y	19-28	20	-	96 hr	0.22	LC50	As BrCl	4

Notes:

a : as bromamines, since ammonia was added in excess, complete conversion of bromine oxidants to bromamines was assumed. Toxicity was attributed to amines and NOEC : no observed effect concentration

ILC50 : Intermittent LC50

References:

1. Great Lakes Chemicals (Europe) Ltd
2. Burton and Margrey (1978)
3. Roberts (1977) cited in Burton and Margrey (1978)
4. Roberts and Gleeson (1978)
5. Data supplied by Bromine and Chemicals Ltd, Dead Sea Bromine Group and Rohm and Haas
6. US EPA (1993)

The only available data on the toxicity of bromamines to saltwater invertebrates is for the mysid shrimp (*Mysidopsis bahia*), with a flow-through 96 hour LC50 of <0.05 mg l^{-1} TRB (as bromamines) reported (Great Lakes Chemicals (Europe) Ltd.).

Burton and Margrey (1978) investigated the acute toxicity of bromine chloride to two estuarine macroinvertebrates, the grass shrimp (*Palaeomonetes* sp.) and juvenile Blue crab (*Callinectes sapidus*). They concluded that while salinity appears to affect the toxicity of chlorine, it does not appear to affect the toxicity of bromine chloride, however, age and size of organisms tested may play a role in bromine chloride toxicity.

The 24, 48 and 96 hour LC50s for grass shrimp were 1.1, 0.8 and 0.6 mg l^{-1} total residual bromine chloride respectively. The 48 and 96 hour EC50s (change of colour) were 0.4 and 0.5 mg l^{-1} total residual bromine chloride.

Similar LC50 values (48 and 96 hour LC50s for grass shrimp of 0.8 and 0.7 mg l^{-1} total residual bromine chloride) were reported by Roberts 1977 (cited in Burton and Margrey 1978).

For blue crab, Burton and Margrey (1978) reported 48 and 96 hour LC50s of 1.2 mg l^{-1} and 0.8 mg l^{-1} total residual bromine chloride respectively. Roberts 1977 (cited in Burton and Margrey 1978) reported a 96 hour LC50 of approximately 1.0 mg l^{-1} (18 - 20 ‰) for non-molt mature blue crabs (7.6 - 10.2 cm wide) and a 96 hour LC50 of approximately 0.6 mg l^{-1} for non-molt juvenile blue crabs (2.5 cm wide).

B1.3 Fish

Only acute toxicity data are available for saltwater fish, these are presented in Table B1 and further outlined below.

In a 96 hour flow-through LC50 test with sheepshead minnow (*Cyprinodon variegatus*) using concentrations in the range 0.043 - 0.75 mg l^{-1} hypobromous acid expressed as bromine, no compound related deaths were observed at the three lowest levels (0.043, 0.082 and 0.11 mg l^{-1} hypobromous acid expressed as bromine) following 96 hours of exposure, while 100% mortality was observed at the highest levels (0.75 and 0.34 mg l^{-1} hypobromous acid expressed as bromine). The 96 hour LC50 was estimated (by nonlinear interpolation) to be 0.19 mg l^{-1} hypobromous acid expressed as bromine. The observed effect concentration was 0.11 mg l^{-1} (Great Lakes Chemicals (Europe) Ltd).

For the estuarine fish, Silverside, *Menidia beryllina*, a 96 hour continuous for LC50 of 0.065 mg l^{-1} TRB has been reported. Under intermittent exposure (40 minutes every eight hours for the given time period) the LC50 increased to 0.344 mg l^{-1} TRB (Great Lakes Chemicals (Europe) Ltd).

The toxicity of 1-bromo-3-chloro-5,5-dimethylhydantoin (BCDMH) to sheepshead minnow has been investigated. The static 96 hour LC50 was 20.0 mg l^{-1}, while the corresponding LC50 data for 5,5-dimethylhydantoin, the dehalogenated by product of BCDMH was 8100 mg l^{-1} (Great Lakes Chemicals (Europe) Ltd). No further data are available.

B2. BIOACCUMULATION

There are no available data on the bioaccumulation of bromine or bromamines in saltwater organisms. However, as proposed for freshwater organisms since chlorine and chloramines do not appear to have any potential for bioaccumulation or bioconcentration (CCREM 1987), it is reasonable to assume that this is probably the same for bromine and bromamines. However, additional data are needed to confirm this. In addition, the reaction of residual oxidants with organic substances may yield brominated organic compounds which may well bioaccumulate.

B3 COMPARISON OF TOXICITY OF BROMINE AND CHLORINE TO SALTWATER ORGANISMS

As previously mentioned, EQSs for chlorine have been proposed in a NRA R&D Note 332. Far more data are available on the toxicity of chlorine and chloramines to saltwater organisms than for bromine and bromamines and it is therefore difficult to compare the data in the two reports. However, some studies have compared the toxicity of bromine and chlorine, these are presented in Table B2 and further outlined below.

A comparison of the toxicity (under continuous and intermittent exposure) of HOBr and HOCl has been carried out with two saltwater organisms the silverside, *Menidia beryllina* and the mysid, *Mysidopsis bahia.* An additional study also compared the toxicity of chloramines and bromamines to *Mysidopsis bahia* (Great Lakes Chemicals (Europe) Ltd). When comparisons are made on a weight basis (i.e. $\mu g\ l^{-1}$) chlorine-induced oxidants appear slightly more toxic (by a factor of approximately two) than bromine-induced oxidants for all tests except the continuous 96 hour exposure for *Mendidia beryllina.*

Conversion of chlorine oxidants into chloramines and bromine oxidants into bromamines appears to increase toxicity although this effect was less pronounced in the case of bromamines. The increase in toxicity can be attributed to formation of the amines and not to the formation of un-ionised ammonia (Great Lakes Chemicals (Europe) Ltd).

A number of studies are reported below which compare the toxicity of bromine chloride with chlorine and report toxicity to be within the same order of magnitude.

Liden *et al.* (1980) used continuous flow bioassays to compare the effects of bromochlorinated and chlorinated condenser cooling effluent on several estuarine food-chain organisms. Two fish species, Atlantic menhaden (*Brevoortia tyrannus*) and spot (*Leiostomus xanthurus*), two bivalve species, American oyster (*Crassostrea virginica*) and brackish water clam (*Rangia cuneata*) have been investigated. Similar total survival of menhaden and spot as well as oysters and clams exposed to BrCl and Cl_2 treated effluents indicated that the toxicities of the residual oxidants were similar for both halogens.

Roberts and Gleeson (1978) determined the acute toxicity of bromochlorinated estuarine seawater (ca 20 ‰) for several estuarine organisms. When the BrCl toxicity data were compared with Cl_2 toxicity data for the same species and LC50s are expressed as equivalents per litre, BrCl was found to be two to four times less toxic than Cl_2. The ranking of species in terms of sensitivity was found to be the same for both disinfectants.

Bradley (1977) reported a calculated 24 hour static LC50 for *Acartia tonsa* of 362 ± 26 $\mu g\ l^{-1}$ bromide chloride (applied in the form of sodium hypochlorite and sodium hypobromite). Toxicity was found to be similar to chlorine (applied as sodium hypochlorite) with a 24 hour static LC50 for *Acartia tonsa* of 403 ± 46 $\mu g\ l^{-1}$.

Table B2 Comparison of the toxicity of bromine and chlorine to saltwater organisms

Species	Life stage	Test type	Analysis	Temp. (°C)	Salinity (ppt)	pH	Exposure time	Concn (mg l^{-1})	Effect	Comments	Ref
Mysidopsis bahia (Mysid shrimp)	5 day	F	Y	-	20.7	8.36-8.52	96 hr	0.062	LC50	as chlorine l^{-1}	1
Mysidopsis bahia (Mysid shrimp)	5 day	F	Y	-	20.7	8.36-8.51	96 hr	0.092	LC50	as bromine l^{-1}	1
Mysidopsis bahia (Mysid shrimp)	5 day	F	Y	-	20.5	8.33-8.65	96 hr	0.210	ILC50	as chlorine l^{-1}	1
Mysidopsis bahia (Mysid shrimp)	5 day	F	Y	-	20.5	8.30-8.69	96 hr	0.367	ILC50	as bromine l^{-1}	1
Mysidopsis bahia (Mysid shrimp)	5 day	F	Y	-	21.0	8.00-8.11	96 hr	<0.021	LC50	as chlorine l^{-1} (in the form of chloramines as 300 µg l^{-1} ammonia was added)	1
Mysidopsis bahia (Mysid shrimp)	5 day	F	Y	-	21.0	8.02-8.16	96 hr	<0.050	LC50	as bromine l^{-1} (in the form of bromamines as 300 µg l^{-1} ammonia was added)	1
Menidia beryllina (Silversides)	8 days old	F	Y	-	20.7	8.36-8.52	96 hr	0.143	LC50	as chlorine l^{-1}	1
Menidia beryllina (Silversides)	8 days old	F	Y	-	20.7	8.36-8.51	96 hr	0.065	LC50	as bromine l^{-1}	1

Species	Life stage	Test type	Analysis	Temp. (°C)	Salinity (ppt)	pH	Exposure time	Concn (mg l^{-1})	Effect	Comments	Ref
Menidia beryllina (Silversides)	11 days old	F	Y	-	20.5	8.33-8.65	96 hr	0.193	ILC50	as chlorine l^{-1}	1
Menidia beryllina (Silversides)	11 days old	F	Y	-	20.5	8.30-8.69	96 hr	0.344	ILC50	as bromine l^{-1}	1

Notes:

ILC50 : Intermittent LC50

References:

1. Great Lakes Chemicals (Europe) Ltd

APPENDIX C MAMMALIAN TOXICOLOGY

C1. INTRODUCTION

The main uses of bromine in water processing are in cooling water disinfection and also in swimming pool disinfection. Disinfection may be achieved by the use of reactive chemical agents to destroy microbiological pathogens and thus there is the potential for such an agent (in this case, bromine), to react with other water constituents to produce potentially harmful compounds. Consequently, when considering the mammalian toxicity of bromine in water, it is also necessary to consider the impact of brominated by-products that may be formed as the result of its use as a disinfectant. It is important to note that brominated species may already be present in the water prior to treatment with oxidants/disinfectants such as ozone or chlorine and that treatment may produce a number of further brominated by-products which may be more toxic than the original brominated species or may increase the concentration of a particular brominated compound already present. The latter case may be important with regard to concentrations which may have been acceptable prior to treatment but which exceed toxicity guideline values after treatment. Such a case may be envisaged with the brominated trihalomethanes (THMs) which each have a guideline value proposed by the World Health Organisation. In addition, there is a prescribed concentration value of 100 $\mu g\,l^{-1}$ for total THMs in drinking water in the UK, set out in the Water Supply, (Water Quality) Regulations 1989; an increase in concentration of one or more of the THMs can raise the total concentration above that which is acceptable. The European Commission are currently revising their Drinking Water Directive and are proposing a parametric value of 15 $\mu g\,l^{-1}$ for bromodichloromethane (although this can be increased to 25 $\mu g\,l^{-1}$ provided chloroform levels are $\leq$30 $\mu g\,l^{-1}$).

Bromine is highly soluble in water, being up to three times more soluble than chlorine. However, the toxicity associated with elemental bromine, which is a vapour under normal atmospheric conditions does not apply when it comes into contact with water since it hydrolyses to hypobromous acid which further dissociates to give hypobromite ions and hydrogen ions, the degree of dissociation being highly pH dependent. Photolysis of free bromine will yield bromide and bromate. Hence, this review will deal with the mammalian toxicity associated with bromate, bromide, and the major organobromine compounds that are produced from the reactions of bromine with natural organic matter.

C2. BROMATE

Bromate is a powerful oxidising agent which has been shown to cause kidney and possibly other tumours in laboratory animals. *In situ* bromide is oxidised during the treatment of water with disinfectants (oxidants) such as ozone. The bromide appears to be oxidised to hypobromous acid, a process that parallels the oxidation of bromide during chlorination. However, hydroxyl radicals present during ozonation have the power to further oxidise hypobromite to bromate. This mechanism is supported by observations that bromate levels are higher in water treated by ozone/hydrogen peroxide where hydroxyl radicals are more abundant.

To date, data from only one *in vivo* study on the absorption, metabolism and excretion of potassium bromate are available. This study shows that bromate is absorbed, can be metabolised to bromide and can be, at least to some extent, excreted unchanged in the urine (Fujii *et al.* 1984). *In vitro* studies show strong metabolic activity by rat kidney and liver homogenates and showed substantial degradation of bromate by sulphydryl compounds such as cysteine and glutathione (Fujii *et al.* 1984).

C2.1 Toxicity to laboratory animals

Bromate appears to be of low acute toxicity to laboratory animals.

Sub-acute studies with rats indicate that potassium bromate causes effects on the renal tubular epithelium with an increase in eosinophilic droplets noted, relative to controls, which was reversible on cessation of treatment.(Onodera *et al.* 1985)

Carcinogenicity studies on rats showed a high incidence of renal cell tumours in treated male and female rats and in male rats there was an increase in the incidence of tumours of the body cavity lining (mesotheliomas) which were absent in both treated and untreated females (Kurokawa *et al.* 1986a, 1990). Long-term studies in Syrian Golden hamsters also demonstrated a small increase in kidney tumours in treated animals but the incidence was much lower than in the rat (Takamura *et al.* 1985). These results could not be confirmed in mice although there was some evidence of a small, but non-significant, increase in kidney tumours (Kurokawa *et al.* 1986a).

C2.2 Genotoxicity

Potassium bromate has been shown to be negative or only very weakly positive in its ability to cause mutations in bacterial test systems. It was positive in strains of *Salmonella typhimurium* (TA102 and TA104) that were sensitive to chemicals that generate oxygen radicals (Ishidate *et al.* 1982, Ishidate *et al.* 1984, Levin *et al.* 1982).

Potassium bromate has been demonstrated to cause significantly higher rates of chromosome aberrations in Chinese hamster lung cells, in a dose dependent manner, without metabolic activation (Ishidate *et al.* 1980, 1981, 1987) and also to induce chromatid breaks in cultured Chinese hamster DON-6 cells (Sasaki *et al.* 1980). i.e. the compound has clastogenic activity. *In vivo* administration of potassium bromate to rats shows significantly increased aberrant metaphase cells in bone marrow cells (Fujie *et al.* 1988) and induced micronuclei in the bone marrow cells of mice given high doses (Hayashi *et al.* 1988).

C2.3 Mechanisms of carcinogenicity

The evidence available suggests that potassium bromate can cause genotoxicity. For such carcinogens, there is theoretically no threshold to the risk of cancer and the risk merely reduces with reducing dose. Mathematical models are employed to calculate the risk and although they are simplistic and do not take into account important biological processes such as pharmacokinetics and DNA repair, they do provide a useful estimate of possible risks (Fawell 1992). Using the linearised multistage model which is conservative in its estimate, the concentration of bromate in drinking water that would produce an excess risk of cancer of 1×10^{-5} (i.e. one extra case of cancer in a population of 100 000) is 3 $\mu g\ l^{-1}$. However, due to

limitations in the available analytical and treatment methods, WHO have proposed a provisional guideline value of 25 $\mu g\ l^{-1}$ which equates to an excess risk of cancer of 7×10^{-5} (WHO 1993). The difference between the associated risks of cancer between the 3 $\mu g\ l^{-1}$ health based value and the 25 $\mu g\ l^{-1}$ provisional guideline value based upon the practical quantitation limit, is not considered to be toxicologically significant (Fawell and O'Neill 1993). The European Commission are proposing a parametric value of 10 $\mu g\ l^{-1}$ for bromate in drinking water. This value is also based on bromate's potential carcinogenicity.

An important point to note is that the actual mechanism of carcinogenicity for bromate has not been determined and that the linearised multistage model is used for genotoxic carcinogens. Hence, if it is subsequently shown that the mechanism is not directly genotoxic and that consequently a threshold may be demonstrable, the linearised multistage model may not be the most appropriate model to use in order to determine the risk of cancer. Since it has been shown that tissue damage and subsequent regeneration is a mechanism of carcinogenesis, and that potassium bromate shows such effects, this may provide an additional mechanism for the carcinogenicity associated with potassium bromate (Melnick 1992). One way in which bromate could cause both genotoxicity and tissue damage resulting in tumour formation is by oxidative damage. This can occur by causing oxidation of lipids in cell membranes, producing active oxygen species which can in turn produce damage to other macromolecules such as DNA. Cells are well adapted to cope with oxidative damage since they possess "scavenger" molecules such as glutathione and other sulphydryl molecules together with a variety of other processes which are capable of reducing the active oxygen species to inert oxygen species. When these protective mechanisms are saturated, further production of the active oxygen species will lead to DNA damage via lipid peroxidation.

C3. BROMIDE

Pharmacokinetically, bromide behaves in a similar manner to chloride. It is readily absorbed by the gastrointestinal tract and is distributed throughout body fluids. The clearance of bromide in humans is approximately 0.9 litres day^{-1} which accounts for most of the excretion of this element. From this a theoretical biological half life of 12 days can be calculated (NAS 1980). This half life may be reduced to 3-4 days by administration of chloride due to competitive reabsorption in the renal tubule. There also appears to be a diurnal variation in the excretion of bromide and there is no excretion during sleep (NAS 1980). Specific bromides are well documented with regard to toxicological information because of their previous use in therapeutics. Bromide toxicity relates to the nervous system ranging from neuroses and psychoses through severe ataxia.

Bromide is of low, acute oral toxicity to laboratory animals (FAO/WHO 1988).

In a 90 day feeding study, male and female rats were given doses of 0, 75, 300, 1200, 4800, 19200 ppm in the diet of sodium bromide. Plasma bromide levels reached a plateau in each dose group within three weeks. In the highest male dose group and the three highest female dose groups, thyroid weights were significantly increased. Histopathological examination revealed hyperplasia of the thyroid in these groups. Also in the highest male groups there was a significant increase in adrenal weight and prostate weight relative to body weight. Histopathological examination of the testis revealed decreased spermatogenesis in the highest dose group (Van Logten 1974).

Dogs fed sodium bromide at 100-400 mg/kg/day for six weeks developed signs of gastrointestinal and nervous system toxicity when serum levels reached 1800-4000 mg l^{-1} (Rosenblum 1958).

Sodium and ammonium bromide were both found to be non-mutagenic in Ames tests using *Salmonella typhimurium* strains TA 98 and TA 100 (Voogd 1988).

One means of determining a suitable guideline value for chronic exposure from drinking water appears to be the concentrations of bromide found in the serum of normal individuals (1.5-50 mg l^{-1}). The mid-range figure, 26 mg l^{-1} corresponds to a daily intake of 26 mg l^{-1} x 0.06 x 15l = 23.4 mg or a daily dose of 0.334 mg l^{-1}. Assuming a 20% intake from water a Suggested No-Adverse-Response Level for chronic exposure may be calculated:

$$\frac{0.334 \text{ mg kg}^{-1} \times 70 \text{ kg} \times 0.20}{2 \text{ litres}} = 2.3 \text{ mg l}^{-1} \text{ (NAS 1980)}$$

An alternative approach used studies involving human volunteers, which did not show neurophysiological or endocrinological changes at doses of 0.4 and 9 mg bromide/kg body weight/day i.e. a NOAEL of 9 mg kg^{-1} was observed. Based on this, FAO/WHO provide an estimate of the Acceptable Daily Intake for Man (ADI) of 0-1 mg kg^{-1} body weight (FAO/WHO 1988).

Using the ADI estimated by the FAO/WHO assuming a 60 kg adult drinking 2 litres of water per day and allowing 10% of the ADI to water, a Suggested No Effect Level of 3 mg l^{-1} may be calculated, a value that is very comparable with that calculated by NAS (NAS 1980).

C4. BROMINATED BY-PRODUCTS

The reaction of bromine with natural organic substances leads to the production of brominated organic compounds. As with the formation of chlorinated by-products from the reaction of chlorine with natural organic substances, there are numerous possibilities of organo-brominated by-products formation and toxicological data for many is either scarce or non-existent making toxicological assessment of these difficult. Possible brominated by-products resulting from discharge to water are: Bromamines (mono-, di-, tri-, organo-); bromoform; bromoacetic acids; bromoacetonitriles (esp. dibromo-); bromoaldehydes; bromoketones; bromohydrins; 3-bromo 4-(dibromomethyl)-5 hydroxy 2(5H)- furanone (BMX); bromopicrin; cyanogen bromide; Dibromochloromethane; Dichlorobromomethane; Bromate. Bromination of natural organic matter is thought to occur primarily by HOBr whereas oxidation to bromate involves the conjugate base, hypobromite.

The major by-products for which there was sufficient data with which the WHO were able to establish guideline values are described below.

C4.1 Bromoform

Bromoform is readily absorbed from the gastrointestinal tract. Long term exposure to experimental animals led to a small increase in relatively rare tumours of the large intestine in rats of both sexes, but did not induce tumours in mice. There is also some evidence that bromoform is able to adversely affect foetal development at concentrations below those that

cause maternal toxicity. The International Agency for Research on Cancer (IARC), have classified bromoform in their Group 3 category for agents that are not classifiable as to their carcinogenicity to humans due to inadequate evidence of carcinogenicity to humans and inadequate or limited evidence for carcinogenicity in experimental animals (IARC 1991). A tolerable daily intake (TDI) of 17.9 $\mu g\ kg^{-1}$ body weight has been derived for bromoform based upon a NOAEL of 25 mg/kg body weight/day observed in a 90-day study in rats. Using an allocation of 20% of the total intake to water and assuming a 60 kg adult drinks 2 litres of water per day, a drinking water guideline value of 100 $\mu g\ l^{-1}$ has been proposed by WHO (WHO 1993).

C4.2 Bromodichloromethane (BDCM)

BDCM is readily absorbed from the gastrointestinal tract. Long term exposure to experimental animals led to liver and kidney damage. BDCM has been shown to induce kidney tumours (renal adenomas and adenocarcinomas) in both sexes of rats and in male mice, rare tumours of the intestine (adenomatous polyps and adenocarcinomas) in both sexes of rats and liver tumours (hepatocellular adenomas and adenocarcinomas) in female mice. The risks of cancer have been based on the increased incidence of renal tumours observed in male mice since these tumours yield the most protective value. The hepatic tumours observed in female mice were not considered due to the possible role of the corn oil vehicle in which BDCM was administered. Genotoxic assays with BDCM have yielded both positive and negative results (WHO 1993). IARC have classified bromodichloromethane in Group 2B, a category for agents that are possibly carcinogenic to humans, determined by inadequate evidence of carcinogenicity in humans but sufficient evidence of carcinogenicity in experimental animals (IARC 1991). Using the linearised multistage model for extrapolation from the risk of cancer at the doses given to laboratory animals to the levels to which humans are exposed to in the environment a guideline value of 60 $\mu g\ l^{-1}$ has been proposed for BDCM by the World Health Organisation. This is associated with an excess risk of cancer (upper 95% confidence interval) of 1×10^{-5} (WHO 1993). The European Commission are currently proposing a parametric value of 15 $\mu g\ l^{-1}$ for BDCM although this can be increased to 25 $\mu g\ l^{-1}$ provided chloroform levels are $\leq 30\ \mu g\ l^{-1}$.

C4.3 Dibromochloromethane (DBCM)

The following studies have been cited in WHO (1993).

DBCM is well absorbed from the gastrointestinal tract. Long term exposure to experimental animals led to kidney and liver damage. The available data on genotoxicity studies is inconclusive. IARC have classified DBCM in Group 3, a category in which the agent is not classifiable as to its carcinogenicity to humans due to inadequate evidence of carcinogenicity in humans and inadequate or limited evidence in experimental animals .

A TDI of 21.4 $\mu g\ kg^{-1}$ body weight has been calculated based upon a 90-day rat study in which a NOAEL of 30 mg/kg body weight/day was observed. Allocating 20% of the total intake to water and assuming that a 60 kg adult drinks two litres of water per day, a guideline value of 100 $\mu g\ l^{-1}$ has been proposed by WHO.

As well as there being individual guideline values proposed for each of the trihalomethanes (chloroform is included in these but is not discussed in this review) and because the four compounds usually occur together, the World Health Organisation have suggested a fractionation approach in order that a standard for total THM can be established to account for additive toxicity:

$$\frac{^{C}\text{bromoform}}{^{GV}\text{bromoform}} \pm \frac{^{C}\text{DBCM}}{^{GV}\text{DBCM}} \pm \frac{^{C}\text{BDCM}}{^{GV}\text{BDCM}} \pm \frac{^{C}\text{chloroform}}{^{GV}\text{chloroform}} \leq 1$$

where C = concentration and GV = guideline value

C4.4 Dibromoacetonitrile (DBAN)

The following studies are all cited in WHO 1993 and WRc 1992.

The major source of exposure to DBAN is drinking water, as a consequence of the reaction of chlorine with bromide and the subsequent reactions with amino acids, humic and fulvic acids or algae in water. DBAN shows some evidence of genotoxicity *in vitro* but this was not confirmed *in vivo* (Bull *et al.* 1985; Lin *et al.* 1986; Daniel *et al.* 1986).

The NOAEL observed for a 90-day study with rats was determined to be 23 mg/kg body weight/day (Hayes *et al.* 1986). Based on this NOAEL, an allocation of 20% of the total intake of DBAN to water, and assuming that a 60 kg adult drinks two litres of water per day, a provisional guideline value of 100 $\mu g\, l^{-1}$ has been proposed by WHO. The guideline is provisional because of the limitations of the database.

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