Proposed Environmental Quality Standards for Chlorine Dioxide in Water

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R&D Technical Report P80

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This report reviews the available data on the use, fate/behaviour and aquatic toxicity of chlorine dioxide, which will assist Agency and SNIFFER staff in assessing the effect of this substance on water quality.

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FOREWORD

This report, which proposes Environmental Quality Standards (EQSs) for the protection of fresh and saltwater life and reference levels for water abstracted for potable supply for chlorine dioxide (and substances resulting from the use of chlorine dioxide), is one of a series of eleven produced under the Environment Agency (formerly the National Rivers Authority) and SNIFFER (Scotland and Northern Ireland Forum for Environmental Research) co-funded Phase IV EQS contract.

The other reports proposed EQSs for aluminium, cyanide, dioxins, fluoride, naphthalene, nonyl phenol, octyl phenol, mevinphos, monochlorophenols/2,4-dichlorophenol and sheep dip chemicals.

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EXECUTIVE SUMMARY

This report, prepared for the Environment Agency and the Scotland and Northern Ireland Forum for Environmental Research (SNIFFER), reviews and critically assesses information available on the uses, releases to the environment, environmental fate and toxicity of chlorine dioxide. This information is used, where possible, to propose Environmental Quality Standards (EQSs) for the protection of fresh and saltwater life and reference levels for abstraction to potable supply.

Chlorine dioxide is an orange gas which forms a yellow or greenish-yellow colour in water. It is a very reactive oxidising free radical, even in aqueous solutions. It has a wide scope of potential uses as a disinfectant and bleaching agent, predominately with aqueous media (e.g. drinking water treatment, process water treatment, industrial and domestic wastewater treatment). Stabilised solutions of chlorine dioxide are available for concentrations of up to 1000 mg l^{-1}, but most applications of chlorine dioxide involve the manufacture of chlorine dioxide *in-situ* using specially designed generators. Some chlorine dioxide may be present in gaseous industrial emissions but the principal route of entry of chlorine dioxide to the environment is likely to be via aqueous effluents, either to sewer or directly to the aquatic environment. However, because chlorine dioxide is rapidly converted to varying proportions of chlorite (ClO_2^-), chlorate (ClO_3^-) and chloride (Cl^-) ions in aqueous media, it is unlikely to be present in significant quantities by the time effluents are discharged to the aquatic environment. EQSs to protect aquatic life from exposure to chlorine dioxide are therefore considered inappropriate.

The relative amounts of chlorite, chlorate and chloride released to the aquatic environment as a result of using chlorine dioxide will depend upon a number of highly variable factors, such as the manufacturing process used (e.g. the precursor chemicals used), the manufacturing conditions (e.g. temperature), exposure to sunlight (chlorine dioxide and chlorite are susceptible to photolysis) and the demand of the receiving medium for oxidants (i.e. the amount of oxidisable matter present).

Although the limited data available on the toxicity of chlorite ion to aquatic life suggest that it is of high acute toxicity to fresh and saltwater crustaceans, with effect concentrations reported from 0.02 mg l^{-1} for the water flea (*Daphnia magna*) and 0.49 mg l^{-1} for the mysid shrimp (*Mysidopsis bahia*), there are insufficient data with which to derive EQSs to protect either fresh or saltwater life from exposure to chlorite. In particular, more toxicity data are required for aquatic invertebrates and algae.

The majority of toxicity data available for the chlorate ion suggest that it is generally of low acute and chronic toxicity to those fresh and saltwater organisms tested. However, there is evidence to suggest that chlorate is of high toxicity to both fresh and saltwater algae when nitrate is the only source of nitrogen available. This is believed to be due to the reduction of chlorate to chlorite by the nitrate-reductase enzyme system in algae. There is also some suggestion in the literature that the chlorate-to-nitrate ratio is important in determining the enzymatic reduction, and thus toxicity, of chlorate to algae, and the limited information available suggests that this ratio can vary considerably between species.

Table S1 Proposed Environmental Quality Standards for chlorine dioxide

Use	AA	MAC	Notes
Protection of freshwater life	-	-	1
Protection of saltwater life	-	-	1
Abstraction to potable supply	-	-	1

Notes to Table S1:

AA: Annual average.
MAC: Maximum allowable concentration.

[1] Because chlorine dioxide is rapidly reduced to chlorite (ClO_2^-), and chloride (Cl^-) (and possibly chlorate, ClO_3^-) before being released to the aquatic environment, EQSs for chlorine dioxide are considered inappropriate. Insufficient toxicity data are available for the derivation of EQSs to protect fresh and saltwater life and reference levels (abstraction to potable supply) for either chlorite and chlorate (refer to text for details).

In most freshwaters the amounts of nitrate present naturally should ensure that chlorate does not out-compete it for active sites on the reductase enzyme. However, research carried out in the Baltic Sea suggests that low levels of chlorate (>15 μg l^{-1}) may cause adverse effects on the growth of brown algae (e.g. *Fucus vesiculosus*, *Fucus serratus*) after long-term exposure in nitrogen-scarce intertidal zones. Basing EQSs for the protection of aquatic life on the lowest toxicity data available for chlorate (i.e. algal studies with nitrate as the sole source of nitrogen) may produce over-protective EQSs for aquatic environments with low chlorate-nitrate ratios. In order for reliable EQSs to be derived a more comprehensive dataset is required, showing clearly the effects of different nitrate concentrations on the toxicity of chlorate to different algal species. Until such data become available no EQSs for the protection of aquatic life from exposure to chlorate are proposed. Site-specific assessments (e.g. direct toxicity assessment) may provide a more practical solution to the regulation of chlorate-containing effluents which are derived from chlorine dioxide applications.

Based on WHO recommendations, because chlorine dioxide is rapidly reduced during drinking water treatment, a reference level (abstraction to potable supply) for chlorine dioxide is not considered appropriate. WHO reported a provisional guideline of 0.2 mg l^{-1} for chlorite in drinking water. However, a recent 90-day rat study has indicated some uncertainty about the study on which the WHO guideline was based, and, given this uncertainty, no reference level for chlorite is recommended. Insufficient toxicity data are available for the recommendation of a reference level for chlorate.

KEY WORDS

Environmental Quality Standards, EQS, chlorine dioxide, chlorite, chlorate, chloride, aquatic toxicity, bioaccumulation, freshwater, saltwater, mammalian toxicity, abstraction for potable supply.

1. INTRODUCTION

Chlorine dioxide is a strong oxidant and has a wide scope of potential uses as a disinfectant and bleaching agent (e.g. drinking water treatment, industrial wastewater and process water treatment).

This report reviews and critically assesses the information available on the inputs and concentrations of chlorine dioxide in the environment (Section 2), the analytical methods currently available (Section 3), the fate and behaviour of chlorine dioxide in the environment (Section 4 and Appendix A) and the aquatic and mammalian toxicity of chlorine dioxide and its principal by-products chlorate and chlorite (Section 5 and Appendices B to D). This information is used to propose Environmental Quality Standards (EQSs) for the protection of fresh and saltwater life and reference levels for the abstraction of water to potable supply.

2. CHLORINE DIOXIDE IN THE ENVIRONMENT

2.1 Physico-chemical properties of chlorine dioxide

A summary of the physico-chemical properties of chlorine dioxide is provided in Table 2.1. Chlorine dioxide is an orange gas which forms a yellow or greenish-yellow colour in water. It is a very reactive oxidising free radical, even while in dilute aqueous solutions and is readily soluble in water, with the upper boundaries of solubility proportional to temperature and partial pressure. The concentrated gas is explosively unstable at levels exceeding 10% (V/V%) in air and highly concentrated aqueous solutions (>10% W/V) are also unstable if compressed, subject to shock or ignition. The ratio of chlorine dioxide in water compared to chlorine dioxide in the gaseous phase decreases with increasing temperature (see solubility in Table 2.1 for details).

Table 2.1 Physico-chemical properties of chlorine dioxide

IUPAC CHEMICAL NAME	Chlorine dioxide
SYNONYMS/TRADE NAMES	Chlorine oxide Chlorine (IV) oxide Chlorine peroxide Chloroperoxyl Anthium dioxide Doxcide 50
CAS NUMBER	10049-04-4
MOLECULAR FORMULA	ClO+
MOLECULAR STRUCTURE	^{o}O-Cl=O
MOLECULAR MASS	67.46
APPEARANCE	Pure ClO_2 is an orange gas or liquid and forms a yellow or greenish-yellow solution in water (1, 2)
MELTING POINT	-59 ^{o}C (1)
BOILING POINT	11 ^{o}C (at 101.3 kPa) (3) 9.9 ^{o}C (at 97.2 kPa) (explodes) (4)
CRITICAL TEMPERATURE	153 ^{o}C (1)
DENSITY	gas: 2400 mg dm^{-3} (normal pressure and temp.) (1) liquid: 164 g cm^{3} (1)

EXPLOSIVE LIMITS	>10% v/v in air >10 % w/v (concentrated aqueous solutions)
VAPOUR PRESSURE (VP)	Up to 40 ^{o}C: log VP = (1.31 x 1375)/T (VP in kPa and T in ^{o}K) (1) Volatilises easily at temperatures >10-15 ^{o}C (3) 760 torr (at 20 ^{o}C) (4)
VAPOUR DENSITY	2.3 (4)
SOLUBILITY IN WATER	Soluble in water with slight hydrolysis to chlorous (chlorite ion) and chloric acid (i.e. chlorate ion) (4) S = 70±7 at 0 ^{o}C (see Note 1) (1) S = 45 at 15 ^{o}C (1) S = 26.5±0.8 at 35 ^{o}C (1) 3 g l^{-1} at 25 ^{o}C and 34.5 mm Hg partial pressure (2) 20 g l^{-1} at 0 ^{o}C and 75 mm Hg partial pressure (2)

Notes to Table 2.1:

[1] S-value is the solubility expressed as a ratio of the concentration in water divided by the concentration in the gas phase.

References:

1. CEN (1994)
2. Kaczur and Cawlfield (1993, cited by Gates and Harrington 1993)
3. Merck (1989)
4. Hazardtext (1997)

2.2 Manufacture

2.2.1 Manufacturing processes

Because of the explosive nature of chlorine dioxide, large scale chlorine dioxide manufacture is usually done *in-situ* with specially designed generators which mix precursor chemicals under vacuum at the site of use.

Chlorine dioxide is generated from sodium chlorite ($NaClO_2$) and either chlorine, hypochlorite or hydrochloric acid (chlorine gas is sometimes generated *in-situ* by combining sodium hypochlorite and hydrochloric acid to avoid chlorine storage).The reaction of $NaClO_2$ with chlorine gas is as follows:

$$2NaClO_2 + Cl_2\ (gas) \rightarrow 2ClO_2\ (gas) + 2NaCl. \qquad (2.1)$$

The above process has been adopted by Nalco (formerly Albright and Wilson) who are one of several suppliers of chlorine dioxide generators in the UK. The system used by Nalco generates Cl_2 gas from the reaction of sodium hypochlorite and hydrochloric acid. The

theoretical conversion of $NaClO_2$ to chlorine dioxide in the above reaction is 100% and Nalco have reported that the actual yields of chlorine dioxide from their generators are typically about 98% (Nalco, Personal Communication 1997). In addition to the desired formation of chlorine dioxide it has been suggested that chlorate ion (ClO_3^-) may be formed in this generation process as an undesired by-product in a competing reaction (Aieta and Berg 1986, AWWA 1994):

$$NaClO_2 + Cl_2 + OH^- \rightarrow NaClO_3 + HCl + Cl^-. \quad (2.2)$$

Information provided by the Rio Linda Chemical Company (patent holders of the above process in the United States and a subsidiary of Albright and Wilson Americas), however, indicates that after trials at three different generator concentrations of chlorine dioxide (820, 1308 and 1386 mg l^{-1}) chlorate was undetected in the generator product streams. However, chlorine (0, 21 and 23 mg l^{-1}, respectively) and chlorite (21, 61 and 17 mg l^{-1}, respectively) were detected in the product streams as well as chlorine dioxide. The percentage of unreacted chlorite was reported to range from 1.2-4.5%.

Some processes for the generation of chlorine dioxide require chlorine gas to be pre-dissolved in water. This can cause chlorine to hydrolyse and form a mixture of hypochlorous and hydrochloric acid as shown in reaction 2.3 below. When this mixture is reacted with $NaClO_2$ to form chlorine dioxide the yields are generally expected to be lower than if chlorine gas is reacted directly with $NaClO_2$ (Rio Linda 1997).

$$Cl_2\,(gas) + H_2O \leftrightarrow HOCl + H^+ + Cl^- \quad (2.3)$$

$$2NaClO_2 + HOCl \rightarrow 2ClO_2\,(gas) + NaCl + NaOH \quad (2.4)$$

The pre-dissolution of chlorine in water may also result in the further dissociation of hypochlorous acid to produce hypochlorite ion and other undesirable side reactions, such as the formation of sodium chlorate ($NaClO_3$) (see reactions 2.5-2.7).

$$HOCl \rightarrow H^+ + OCl^- \quad (2.5)$$

$$HOCl + NaClO_2 + OH^- \rightarrow NaClO_3 + Cl + H_2O \quad (2.6)$$

$$Cl_2 + H_2O + NaClO_2 \rightarrow NaClO_3 + 2Cl + 2H^+ \quad (2.7)$$

Another alternative process for generating chlorine dioxide involves the reaction of $NaClO_2$ with hydrochloric acid, as shown in reaction 2.8 (Aeita and Berg 1986). The theoretical conversion of $NaClO_2$ to chlorine dioxide in this process is 80%. It is likely that some chlorine will result from this process, as shown in reaction 2.9.

$$5NaClO_2 + 4HCl \rightarrow 4ClO_2 + 5NaCl + 2H_2O \quad (2.8)$$

$$4NaClO_2 + 4HCl \rightarrow 3ClO_2 + 0.5Cl_2 + 4NaCl + 2H_2O \quad (2.9)$$

Small volumes (<1000 mg l^{-1}) of stabilised chlorine dioxide solutions, which do not require generator equipment, are available for purchase but these are either solutions of sodium chlorite, which have to be acidified (e.g. with lactic acid) to produce chlorine dioxide (HSDB 1997), or chlorine dioxide in the presence of preservatives.

2.2.2 Manufactured quantities

As mentioned in Section 2.2.1, chlorine dioxide is mostly manufactured at sites of use and the doses applied vary depending upon the nature of its use (see Section 2.3) and the demand of the treatment medium (usually raw or wastewater) for chlorine dioxide. Because of this it is very difficult to estimate the amount of chlorine dioxide being manufactured in the UK at the present time. However, information provided by companies involved in the supply of sodium chlorite (the principal precursor of chlorine dioxide) in the UK suggest that approximately 1500 tonnes of sodium chlorite are sold per year for the manufacture of chlorine dioxide. Since the conversion of sodium chlorite to chlorine dioxide is quite high (certainly greater than 50% but generally 80-100%) the amount of chlorine dioxide manufactured in the UK is likely to be within the range 750-1500 tonnes per year. This range may increase in the future if the use of chlorine dioxide continues to gain popularity as an alternative disinfectant to chlorine in drinking water and wastewater treatment (see Section 2.3).

Companies which supply *in-situ* chlorine dioxide generators in the UK include Nalco (who have recently acquired this business from Albright and Wilson), Fospur Ltd and USF Wallace and Tiernan. None of these companies manufacture the precursor chemicals themselves. Nalco buys the appropriate precursor chemicals (including sodium chlorite) and estimates that 3-800 tonnes of sodium chlorite are supplied annually with their chlorine dioxide generators. Since the conversion of sodium chlorite to chlorine dioxide in their production system is reported to be about 98%, almost all of this will be produced as chlorine dioxide. Fospur Ltd are reported to have about 100 generators in operation at the moment and USF Wallace and Tiernan less than 10, but information on the quantities of sodium chlorite used by these generators was not available at the time of writing.

2.3 Uses

Due to its strong oxidising properties, existing over a wide pH range, chlorine dioxide has potential use as a disinfectant and a bleaching agent in a variety of industries. The general uses and industries to which chlorine dioxide may be applied are summarised in Table 2.2.

In the UK, suppliers of sodium chlorite (the main precursor of chlorine dioxide) and chlorine dioxide generator equipment indicate that chlorine dioxide currently has small applications in the treatment of drinking water and in the paper, textile and food industries, although the extent of these applications has not been quantified. The applications of chlorine dioxide in these UK industries are discussed in more detail in the following sections.

2.3.1 Drinking water treatment

Although chlorine dioxide was first used in drinking water treatment in the United Stated as long ago as 1944 (Aeita and Berg 1986) its use in the UK has been reasonably limited to date, partly because of the hazardous nature of chlorine dioxide and partly because of the popularity of other cost-effective disinfectants and oxidising agents (notably chlorine). The majority of processes available for manufacturing chlorine dioxide are now generally considered to sufficiently minimize the risk of accidental exposure of chlorine dioxide to

operators and the environment, but nevertheless, only a small number of treatment plants in the UK are believed to be using chlorine dioxide at the present time.

Many studies in the literature have reported that the use of chlorine dioxide results in the formation of fewer organic disinfection by-products than chlorine, and in particular, no trihalomethanes (THMs). However, if chlorine is used in conjunction with chlorine dioxide, or a small amount of chlorine is produced during the chlorine dioxide manufacturing process (see Section 2.2.1), some THMs may result (e.g. Rio Linda 1997, AWWA 1994, Lykins *et al* 1990, Myers *et al* 1990, Masschelein 1989, Lykins and Griese 1986, Rav-Ach 1984, Lin 1984, Stevens 1982). It has also been reported that unlike chlorine, chlorine dioxide does not react with phenolic compounds to form chlorophenols, which are often the cause of taste and odour complaints in drinking water (e.g. AWWA 1994).

2.3.2 Paper and pulp

In the paper and pulp industry chlorine dioxide has the potential as a bleaching agent as well as a biocide. However, since the UK imports most of its paper pulp from abroad, which is usually bleached prior to import, the UK industry does not generally employ chemical bleaching processes (The Paper Federation, Personal Communication 1997). Although specific details were not available it is believed that chlorine dioxide is currently being applied as a biocide in some UK paper making processes, probably to treat supply waters.

2.3.3 Textiles

Suppliers of chlorine dioxide generators have indicated its potential for use in the decolourisation of effluents from the textiles industry (e.g. Rio Linda 1997, Albright and Wilson 1997). However, this is not thought to be a significant application of chlorine dioxide in the UK at the present time. This is because most UK textile companies are small-medium enterprises and do not have the space or resources for *in-situ* treatment processes such as chlorine dioxide (British Textile Confederation, Personal Communication 1997). Nevertheless, there are some indications from the suppliers of chlorine dioxide generators that a few textile companies in the UK are currently using chlorine dioxide, possibly to treat process waters. Specific details of these uses, however, were not available.

2.3.4 Food

A report prepared by the Camden and Chlorley Wood Research Association (unpublished at the time of writing) for the food industry identified chlorine dioxide as one potential alternative to chlorine in the disinfection of process waters. There are already some reported applications of chlorine dioxide in the UK food industry (possibly for flour bleaching) but information on the extent of its use was not available at the time of writing.

2.3.5 Cooling towers

Chlorine dioxide has the potential for use as a biocide to prevent the build up of algae, fungi and bacteria in cooling towers. The particular advantage of chlorine dioxide is that it can be

used over a wide pH range. However, this is not considered to be a major application of chlorine dioxide in the UK at the present time. It has been reported that none of the power stations in the UK are currently using chlorine dioxide, mainly because of safety concerns over the *in-situ* generation of chlorine dioxide (Nuclear Electric, Personal Communication 1997).

Table 2.2 Summary of potential uses

Industry	Potential uses
Drinking water treatment	Disinfection. Reduction of taste and odour problems. Reduction of THM and AOX. Removal of iron and manganese. Coagulation aid.
Waste treatment (industrial and domestic)	Disinfection and sterilisation. Removal of colour (e.g. effluents from textile finishing processes). Oxidation of phenols, sulphides, mercaptans, cyanides and other organics. Odour removal in wet scrubbers, liquid process streams and contaminant ponds.
Paper and pulp	Bleaching of pulp. Slimicide applications for supply water, wastewater and whitewater.
Textiles	Removal of colour from effluents (e.g. dyes). Sterilisation of supply waters and recirculated water. Cleaning and tanning of leather.
Food (e.g. food processing plants, dairies and poultry houses)	Sterilisation of supply waters and air. Bleaching flour and oils.
Others	Biocidal activity in cooling towers.

References:

Rio Linda Product Brochure (1997)
Albright and Wilson Product Brochure (1997)
Interox (1997)
US EPA (1994) - Federal Register Fri 29 July - Part II - Nat. Prim. DW Regs; Disin. and disin. byprods.; proposed rule - EPA 811-z-94-004

2.4 Entry into the environment

Chlorine dioxide is not known to occur naturally so the presence of any in the environment is assumed to be anthropogenic in origin. Low concentrations (<1000 mg l^{-1}) of stabilised

aqueous chlorine dioxide are available for small, and potentially diffuse releases, but the vast majority of chlorine dioxide released in the UK arises from point sources, specifically *in-situ* generators at sites of use (see Section 2.3 for details of uses).

Chlorine dioxide generators are generally automated units which pump the different precursor chemicals into a mixing chamber under vacuum and release chlorine dioxide, either as a liquid or as a gas, at regulated doses. The design of these generators should prevent large quantities of chlorine dioxide being released to the environment through leakages and spillages so the majority of chlorine dioxide will enter the environment via process discharges.

Some chlorine dioxide may be present in gaseous industrial emissions (aqueous chlorine dioxide easily volatilises at temperatures in excess of 10-15 ^{o}C) but since the water phase to gaseous phase ratio still favours the water phase at high temperatures (e.g. 45 at 15 ^{o}C, 26.5 at 35 ^{o}C), the principal route of entry is likely to be in aqueous effluents, either to sewer or directly to the aquatic environment. However, because chlorine dioxide rapidly disproportionates in water (including process waters) and degrades in air, very little chlorine dioxide is expected to be found in the environment. In water, the reaction products chlorite, chlorate and chloride (depending on pH, photolysis and the systems used to manufacture chlorine dioxide) are more likely to be released than chlorine dioxide itself (see Section 4 and Appendix A for details). The possible routes of entry of chlorine dioxide and its reduction products to the environment are summarised in Figure 2.1.

It has been suggested that some chlorine dioxide may reform in drinking water distribution systems if excess chlorite ions (ClO_2^-) and free chlorine are both present after disinfection with chlorine dioxide (e.g. AWWA 1994), but this is not expected to be a major factor in surface waters since chlorite will have a greater tendency to oxidise and photodegrade in surface waters. The oxidation and photodegradation products of chlorite will be chlorate (ClO_3^-) and chloride (Cl^-) in varying proportions (see Section 4 and Appendix A).

2.5 Recorded levels in the aquatic environment

Neither chlorine dioxide nor its reduction products, chlorite and chlorate (see Section 4 and Appendix A), are routinely monitored in the UK aquatic environment. Therefore there are no data available on the concentrations present in the UK environment. However, considering the manufacturing methods used, its applications, and the fact that it is unstable in water, chlorine dioxide will generally not be present in the environment in detectable concentrations. However, chlorite and chlorate, and ultimately, increased levels of chloride, may result from the use of chlorine dioxide. The chloride ion has been considered in a previous WRc EQS report (Gardiner and Smith 1992).

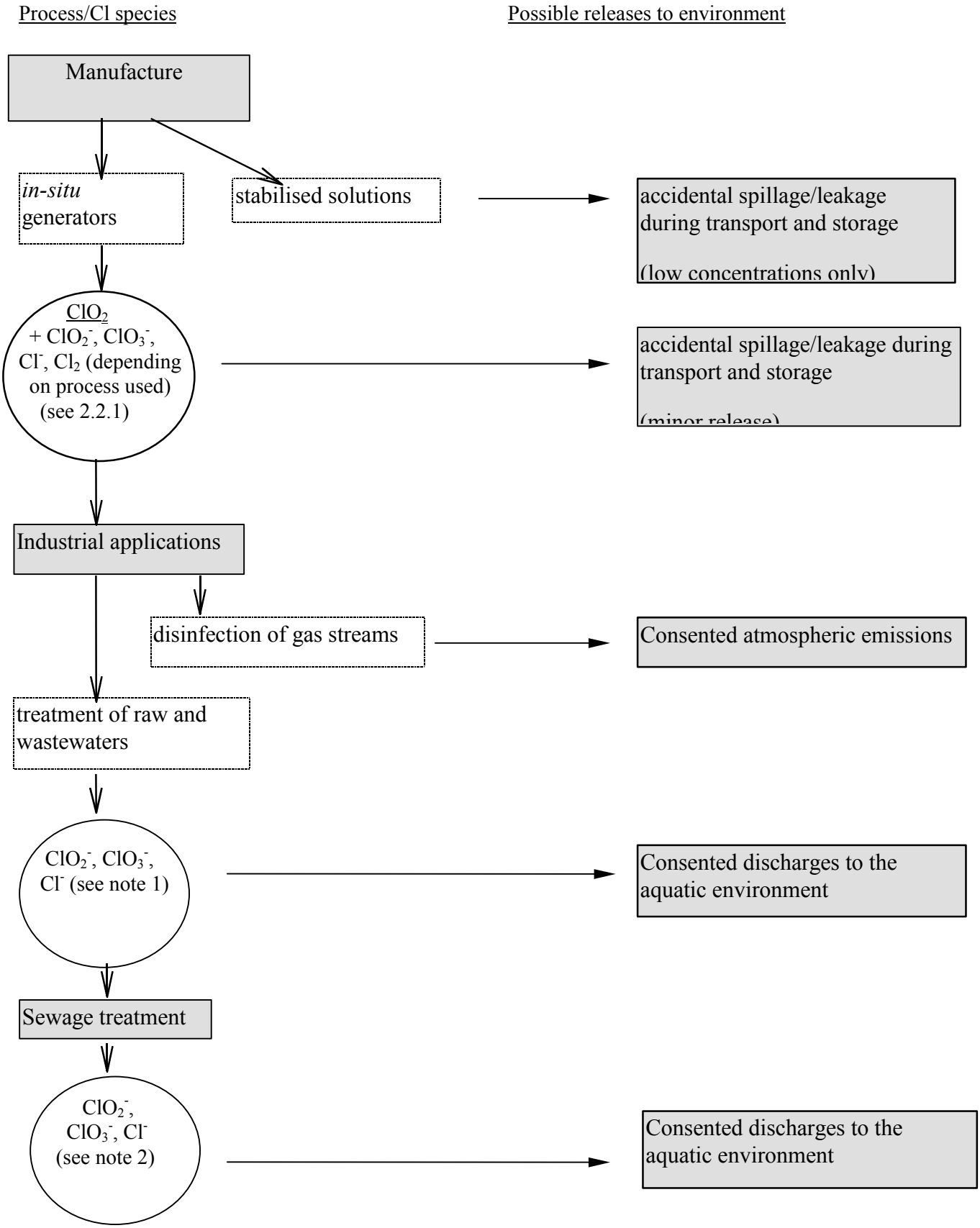

Figure 2.1 Possible environmental releases of chlorine dioxide and its reduction products

Notes to Figure 2.1:

ClO_2 Chlorine dioxide
ClO_2^- Chlorite ion
ClO_3^- Chlorate ion
Cl^- Chloride ion
Cl_2 Chlorine

[1] The relative proportions of chlorite, chlorate and chloride in raw waters and industrial wastewaters are dependent on:
(i) the manufacturing process (e.g. predissolved Cl_2 can lead to the reaction of HOCl and OCl^- with chlorite to form chlorate) (see Section 2.2.1 for details);
(ii) the manufacturing conditions (e.g. high temperatures increase the volatility of chlorine dioxide from water);
(iii) exposure to sunlight (chlorine dioxide and chlorite are readily photodegraded in water to form chlorate and chloride);
(iv) the demand of the receiving raw and wastewaters for oxidants (i.e. the amount of oxidisable matter present).

[2] The relative proportions of chlorite, chlorate and chloride in sewage treatment effluents will depend upon those factors described in Note 1 above and on the type of treatments processes applied. All of these are very soluble in water and are unlikely to precipitate out during settlement. Chlorite may be oxidised and/or photodegraded to chlorate, but may also be toxic to micro-organisms. Chlorate is reportedly biodegraded in aerated ponds and under anaerobic conditions.

Information supplied by Rio Linda (1997) in a product brochure shows that the amounts of chlorine and chlorite present in generator product streams varied from 21.3 to 23 mg l^{-1} and 16.8 to 61.7 mg l^{-1}, respectively, when 820.5 to 1386.1 mg l^{-1} of chlorine dioxide was generated. No chlorate was detected in the product stream in the same tests (see Table 2.3). Although these data indicate the types and proportions of chlorine species likely to be present in the Rio Linda generator product stream (see Section 2.2 for manufacturing details), if this product stream is mixed with water for treatment (e.g. raw water or wastewater) the chlorine dioxide is likely to be consumed rapidly, forming more chlorite and possibly some chloride, before it is released to the drinking water distribution system, sewers or the aquatic environment. Some of the chlorine present in the product stream is likely to hydrolyse in water to form hypochlorous acid (HOCl), which can react with the chlorite (ClO_2^-) present to form chlorate (see Section 4 and Appendix A). The other *in-situ* manufacturing systems, which utilise chlorine pre-dissolved in water, have lower yields of chlorine dioxide and are likely to produce higher concentrations of chlorate because of the reactions between the hydrolysis products of chlorine (e.g. hypochlorous acid) and chlorite ions (see Section 2.2.1).

Table 2.3 Chlorine speciation of Rio Linda generator product streams

Chlorine dioxide (mg l^{-1})	Chlorine (mg l^{-1})	Chlorite (mg l^{-1})	Chlorate (mg l^{-1})	Calculated yield (%)	Unreacted chlorine (%)	Excess chlorine (%)
820.5	nd	21.2	nd	97.5	2.5	0
1308.5	21.3	61.7	nd	95.5	4.5	3.0
1386.1	23.0	16.8	nd	98.8	1.2	3.2

The concentrations of the different chlorine species discharged to the sewers or the environment via *in-situ* generators will vary depending on the chemical reactions used to produce chlorine dioxide (see Section 2.2.1), conditions during the manufacturing process (e.g. pH, temperature), the presence of free chlorine residuals, the presence of chlorate (ClO_3^-) in the feedstock, the demand for chlorine dioxide (i.e. the presence of oxidisable matter in the treatment medium) and exposure to sunlight (see Section 4 and Appendix A for details).

3. ANALYSIS

3.1 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 for 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 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, if an EQS of 1 $\mu g\ l^{-1}$ is proposed:

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

3.2 Analytical techniques

A method for the determination of chlorine dioxide and chlorite in potable waters has been produced by the Standing Committee of Analysts (SCA 1980) in the Blue Book "Chemical Disinfecting Agents in Water and Effluents, and Chlorine Demand 1980" as follows:

A sample of 100 ml is added to 2 ml of glycine solution. This is mixed with a solution of diethyl-p-phenylenediamine sulphate, disodium hydrogen phosphate and potassium dihydrogen phosphate buffer and disodium EDTA. This solution is then titrated with a ferrous ammonium sulphate solution until the colour is discharged. No accuracy and precision data are given but the limit of detection (LOD) is 0.03 $mg\ l^{-1}$ for chlorine dioxide. The LOD for chlorite is not stated.

A method for the determination of chlorite and chlorate in drinking water, rain water, groundwater and surface water has also been produced by the SCA in the Blue Book "The Determination of Anions and Cations, Transition Metals, Other Complex Ions and Organic Acids and Bases in Water by Chromatography 1990". The sample is injected directly into an ion chromatograph with a conductivity detector. The working range for chlorite was 0.05 to 20 mg l^{-1} and for chlorate it was 0.1 to 50 mg l^{-1}.

The American Water Works Association (1994) describes several methods for the determination of chlorine dioxide, chlorite and chlorate in water. These, as well as other methods are summarised in Table 3.1.

Table 3.1 Other analytical techniques for the determination of chlorine dioxide, chlorite and chlorate

Compounds Determined	Chlorine dioxide	Chlorine dioxide, chlorine, chlorite and chlorate	Chlorine dioxide, chlorine, chlorite and chlorate	Chlorine dioxide, chlorine, chlorite, chlorate and chloramine	Chlorine dioxide, chlorine, chlorite and chlorate	Chlorite, chlorate and other anions	Chlorine, chlorite and chlorate
Matrices	Potable waters	Drinking water	Drinking water	Drinking Water	Drinking Water	Drinking Water	Drinking Water
Concentration Range	Up to 0.4 mg l^{-1}	Up to about 1 g l^{-1}	0.3 - 10 mg l^{-1} (ClO_2) , 0.08 - 5 mg l^{-1} (Chlorate & Chlorite)	Not stated	Not stated	Not stated	Not Stated
Sample Size	50 ml	50 ml	Not stated	Not stated	Not stated	Not stated	Not stated
Extraction	None	None	None	None	None	None	None
Clean-up	Sodium cyclamate and thioacetamide suppress free chlorine interference	Chlorine species differentiated by pH adjustment and sample pre-treatment	Chlorine species differentiated by pH adjustment and sample pre-treatment	Chlorine species differentiated by pH adjustment and sample pre-treatment	Chlorine species differentiated by pH adjustment and sample pre-treatment	None	Chlorine species differentiated by pH adjustment and sample pre-treatment
Analysis	Absorbance measured at 570 nm using a UV-vis spectrophotometer and chlorophenol red	Amperometric or potentiometric determination of iodine formed by oxidation of iodide	Flow injection analysis with iodometric determination and spectrophotometric detection at 370 nm	DPD method , subsequent titrations with ferrous ammonium sulphate	Amperometric titration	Ion chromatography	Flow injection analysis with spectrophotometric detection

Limit of Detection	0.01 mg l^{-1}	0.05 mg l^{-1} (ClO_2), 0.02 mg l^{-1} (Chlorite), 0.25 mg l^{-1} (Chlorate)	0.3 mg l^{-1} (ClO_2), 0.08 mg l^{-1} (Chlorite), 0.08 mg l^{-1} (Chlorate)	0.01 mg l^{-1} (chlorite and chlorate)	0.02 mg l^{-1} (chlorite) 0.25 mg l^{-1} (chlorate)	0.01 mg l^{-1} (chlorite) 0.003 mg l^{-1} (chlorate)	0.04 mg l^{-1} (chlorite) 0.03 (chlorate)
Accuracy	Not stated	Mean recovery (n=3) = 96.4% at 0.55 mg l^{-1} (ClO_2), 100.0 % at 0.42 mg l^{-1} (Chlorite), 89.4 % at 0.66 mg l^{-1} (Chlorate)	Not stated	Not stated	Not stated	Not stated	Not stated
Precision	Relative Standard Deviation (RSD) = 2.8% at 0.3 mg l^{-1} (n=10)	RSD (n=3) = 0.6% at 0.55 mg l^{-1} (ClO_2), 0.5 % at 0.42 mg l^{-1} (Chlorite), 1.7 % at 0.66 mg l^{-1} (Chlorate)	Not stated	Not stated	Not stated	Not stated	Estimated uncertainties of analysis at 0.4 mg l^{-1} ± 0.01, 0.02 & 0.04 mg l^{-1} for ClO_2, chlorite and chlorate
Reference	Fletcher *et al.* (1985)	Aieta *et al.* (1984)	Gordon *et al.* (1989)	AWWA (1994)	AWWA (1994)	AWWA (1994)	AWWA (1994)

4. SUMMARY OF THE ENVIRONMENTAL FATE AND BEHAVIOUR OF CHLORINE DIOXIDE AND ITS REDUCTION PRODUCTS

Due to its manufacturing techniques and uses, chlorine dioxide is most likely to be released to the atmosphere and, in particular, the aquatic environment. Releases of chlorine dioxide to land are not expected to be common (see Section 2.4).

If used as a disinfectant of wastewaters or as an aqueous bleaching agent, chlorine dioxide will be rapidly reduced to chlorite, chlorate or chloride ions in varying proportions (see Section 4.1 below and Appendix A for more detail) and these chlorine species are more likely to be released to the aquatic environment than chlorine dioxide itself. Brief accounts of the stability of chlorite, chlorate and chloride in water have therefore been provided in Section 4.2.

4.1 Chlorine dioxide

4.1.1 Air

Although there is very little information available on the fate and behaviour of chlorine dioxide in the atmosphere, it is not generally expected to persist due to its susceptibility to photochemical degradation.

4.1.2 Soil

There does not appear to be any information specifically reported on the fate and behaviour of chlorine dioxide in soil. As explained in Section 2.4, the manufacture and usage of chlorine dioxide should generally prevent the release of this substance to soil. Chlorine dioxide is usually manufactured *in situ* and dosed immediately (without storage) into aqueous process streams, so if released to soil (e.g. via spillages or leakages), most chlorine dioxide is likely to be in the dissolved phase. Some aqueous chlorine dioxide will rapidly volatilise from the soil surface, with the amount volatilised depending on the temperature (it easily volatilises at temperatures greater than 10-15 ^{o}C). It is expected that chlorine dioxide will also be rapidly reduced by the oxidisable matter present in soil (e.g. humic and fulvic acids), and, based on the redox reactions of chlorine dioxide in water (see Section 4.3 and Appendix A), it will probably persist as chloride with possible traces of chlorite and chlorate.

4.1.3 Water

As previously suggested, the most likely route of chlorine dioxide to the aquatic environment is via sewage discharges (mainly originating from industrial trade wastes), although the fate and behaviour of chlorine dioxide in treated water (drinking water and wastewater) suggests that the majority of chlorine dioxide will be reduced to other chlorine species (chlorite, chlorate or chloride) before it is released to the aquatic environment (see Section 2.4).

The processes which influence the persistence of chlorine dioxide in water are entirely abiotic. Chlorine dioxide solutions volatilise easily at temperatures in excess of 10-15 ^{o}C although based on the reported water phase to gaseous phase ratios, the majority of chlorine dioxide will remain in solution even at relatively high temperatures (ratio is 45 at 15 ^{o}C and 26.5 at 35 ^{o}C). Chlorine dioxide is also susceptible to photodegradation, which reduces it to chlorate and chloride. The relative importance of these processes in treated waters will clearly depend upon their temperature and exposure to sunlight. They may also be dependent upon the redox potential of the water. Chlorine dioxide is a very strong oxidant and is likely to be almost completely reduced in water within a few hours to a few days. For example, concentrations of 1.5 and 3 mg l^{-1} almost completely disappeared after 11 and 15 hours in filtered water (see Section A1, Appendix A for details). The redox potential of the water, and thus the reduction of chlorine dioxide, will be determined by the amount of oxidisable matter present (e.g. metal ions, humic and fulvic acids).

The principal reduction product of chlorine dioxide during the treatment of raw waters for potable supply is widely reported to be the chlorite ion (ClO_2^-), with trace amounts of chlorate, chloride and chlorine possible (depending on the manufacturing conditions). Significant concentrations of the chlorate ion are reported to result from the use of chlorine dioxide in bleaching processes (e.g. pulp bleaching), both from the reduction of chlorine dioxide and the bleaching process itself. The stability of the chlorite and chlorate ions in water is considered in Sections 4.2 and 4.3, respectively.

Unlike chlorine, which is also used as an oxidising and bleaching agent, chlorine dioxide is not generally believed to be involved in substitution reactions. Chlorine dioxide, for example, does not react with ammonia to form chloramines as chlorine does, and oxidises (rather than substitutes) amines. However, it has been suggested that if added in excess, chlorine dioxide may result in the formation of monochloroacetic acid (and traces of di- and trichloroacetic acids) from humic substances.

In certain situations it is conceivable that small quantities of hypochlorous acid (HOCl) will be present in effluents from chlorine dioxide treatment processes. Hypochlorous acid may arise from the dissociation of chlorine if chlorine is used in conjunction with chlorine dioxide in the disinfection of water, or if chlorine residuals remain from the manufacture of chlorine dioxide (see Section 2.2.1). It has also been suggested that in neutral waters containing phenols, about 50% of chlorine dioxide may be converted to hypochlorous acid. In freshwaters, hypochlorous acid readily reacts with ammonia to form chloramines (high pH favours monochloramine while low pH favours dichloroamines) and in saltwater it may undergo reactions with other halide ions to form further halo-amines (e.g. bromamine). However, the overall significance and occurrence of chlorine dioxide derived hypochlorous acid in the aquatic environment is not known (the chemistry of hypochlorous acid is considered in more detail in the WRc EQS report for chlorine, Lewis *et al* 1994).

There is some evidence that chlorine dioxide can reform in the drinking water distribution system and persist for several days if chlorite ions (ClO_2^-) and free chlorine (e.g. hypochlorous acid) are present. This is unlikely to be significant in surface waters due to the higher demand of surface waters for oxidants (including chlorite) compared to treated drinking water and the volatile nature of chlorine dioxide, if reformed.

4.2 Stability of reduction products in water

4.2.1 Chlorite ion (ClO_2^-)

Information on the persistence of the chlorite ion in water is related almost entirely to the drinking water distribution system. Experiments carried out by the American Water Works Association showed that the chlorite ion was relatively stable in drinking water over a period of 18 days. After about 75 hours, an initial concentration of 1 mg ClO_2^- l^{-1} was reduced by approximately 12%, but in the proceeding 15 days it was reduced by only a further 2%.

Manufacturers have reported that sodium chlorite has strong oxidising properties and some of the reactions associated with chlorine dioxide (see Sections A1.1 to A1.5, Appendix A) appear to support this. Effective removal of chlorite from water has been achieved using PAC (powdered activated carbon) and ferrous iron, which also confirms its oxidising potential.

It has also been reported that chlorite can be photochemically degraded in water, ultimately to chlorate, chloride and oxygen, with potential intermediates being chlorine dioxide and chlorine.

The stability of chlorite in untreated raw waters and treated wastewaters will therefore largely depend upon the amount of oxidisable matter present (e.g. metal ions, humic acids) and exposure to sunlight. Because surface waters are likely to contain higher levels of oxidisable matter and be exposed more to sunlight, the reduction of chlorite will probably be higher and quicker than demonstrated in treated drinking water although actual reaction rates are not available.

4.2.2 Chlorate ion (ClO_3^-) in water

The chlorate ion (ClO_3^-) is expected to be relatively stable in natural waters compared to chlorine dioxide and the chlorite ion, although it too may be abiotically reduced (ultimately to the chloride ion) in waters containing high levels of oxidisable matter (e.g. metal ions, organic acids) or under acidic conditions. Tests carried out with treated drinking water showed that an initial concentration of 0.5 mg ClO_3^- l^{-1} remained stable for 18 days. The greater presence of oxidisable material in surface waters may reduce the level of stability somewhat, but not significantly.

The main processes for the removal of chlorate from water (including wastewaters) are likely to be biological. Chlorate can be taken up by algae, plants and some bacteria along with certain essential elements, particularly in nutrient-deficient conditions, and enzymatically reduced to chlorite. There is evidence that chlorate may be biodegraded in aerated ponds, depending on pH and temperature, and under anaerobic conditions chlorate may be completely degraded within a few hours.

4.2.3 Chloride ion (Cl^-)

The chloride ion (Cl^-) is generally very stable in water. Chloride ions in aqueous solution may form weak complexes with trace elements, although mercury and silver form stronger, more

covalent bonds. This complexation plays a greater role in saltwaters because of the high concentrations of chloride ion present, but in non-saline waters chloride will exist predominantly as the free hydrated ion. Chloride was considered in an earlier WRc EQS report for the National Rivers Authority (now the Environment Agency) (Gardiner and Smith 1992).

5. SUMMARY OF TOXICITY AND BIOACCUMULATION

Based on information on the likely fate and behaviour of chlorine dioxide in water (see Section 4 and Appendix A), chlorine dioxide is likely to be significantly reduced to the chlorate, chlorite or chloride ions in the aquatic environment. This section briefly considers the effects of chlorine dioxide, chlorate and chlorite on aquatic life (including bioaccumulation potential) and mammals. The information available on the toxicity of these substances to freshwater life, saltwater life and mammals are reviewed in more detail in Appendices B, C and D, respectively. The chloride ion has been considered in a previous EQS report (Gardiner and Smith 1992) (see Section 6).

5.1 Freshwater life

5.1.1 Chlorine dioxide

As chlorine dioxide is rapidly reduced in water, it is conceivable that the biological effects observed in laboratory tests are partly, if not predominantly, related to the presence of chlorine dioxide's reduction products (chlorite and chlorate). The same is likely to be true of chlorine dioxide released to the aquatic environment, although the rate of reduction will depend on the amount of oxidisable matter (e.g. metal ions, organics acids) present.

The overall dataset for laboratory-derived toxicity data is limited to five species of fish and one species of crustacean, mollusc and protozoan. These data are summarised in Table 5.1. The lack of data for lower organisms such as bacteria and algae is significant considering that chlorine dioxide is used as a biocide.

The only indication of the toxicity of chlorine dioxide to freshwater micro-organisms relates to a study with oocysts of the parasitic protozoan *Cryptosporidium parvum*. Oocysts of *C. parvum* are relatively resistant to chlorine, but one hour exposure to 1.3 mg l^{-1} of chlorine dioxide caused 90% inactivation (Korich *et al* 1990). This supports the suggestion that micro-organisms are particularly susceptible to chlorine dioxide, although this study is unlikely to be of ecological relevance.

Chlorine dioxide appears to be of high acute toxicity to freshwater mussels and fish when exposed continuously or intermittently. For example, continuous exposure of zebra mussels (*Dreissena polymorpha*) to 0.25 and 0.5 mg l^{-1} for four days in a flow-through test caused 40% and 60% mortality, respectively. In contrast, single 30-minute doses of 20-30 mg l^{-1} were required to achieve greater than 50% mortality of mussels (Matisoff *et al* 1996). Both fathead minnow (*Pimephales promelas*) and bluegill sunfish (*Lepomis macrochirus*) were sensitive to intermittent chlorine dioxide exposure. The 96-hour LC50s, expressed as the mean residual level during four approximate 2-hour exposure periods, were reported as 0.02 and 0.15 mg l^{-1}, respectively (Wilde *et al* 1983). In another study, 50% mortality of goldfish (*Carassius auratus*) was observed after 33.33 minutes exposure to 2 mg l^{-1} of chlorine dioxide (Parella *et al* 1986).

Table 5.1 Summary of the most pertinent freshwater toxicity data for chlorine dioxide

Species	Life stage	Test design	Temp(°C)	Hard.	pH	Exposure duration	Conc. mg ClO_2 l^{-1}	Effect	Ref
PROTOZOANS									
Cryptosporidium parvum (coccidian parasite)	oocyst	static, measured conc.	-	-	-	1 h	1.3	90% inactivation	1
MOLLUSCS									
Dreissena polymorpha (zebra mussels)	adult	flow-through, measured conc.	15-25	120-124	8.0-8.3	30 m	30	70% mortality (single dose)	2
						3-7 d	13	LC50 (single 30 m dose per day)	2
						28 d	5	25% mortality (single 30 m dose per day)	2
						4 d	0.35	LC50 (continuous exposure)	2
SALMONID FISH									
Salmo trutta (brown trout)	finger-lings	static, nominal conc.	10	165-200	7.6-8.0	48 h	200[1]	LC50	3

Species	Life stage	Test design	Temp(°C)	Hard.	pH	Exposure duration	Conc. mg ClO_2 l^{-1}	Effect	Ref
NON-SALMONID FISH									
Carassius auratus (goldfish)[7]	-	-	-	-	-	33.33 h	2	LT50	4
Pimephales promelas (fathead minnow)	juvenile	flow-through, measured conc.	24-30	-	6.7-7.5	96 h[2]	0.07	LC50	5
						96 h[3]	0.06	LC50	5
						96 h[4]	0.02	LC50	5
						96 h[5]	3.26	LC50	5
Lepomis macrochirus (bluegill sunfish)	young-of-the-year	flow-through, measured conc.	24-30	-	6.7-7.5	96 h[2]	0.56	LC50	5
						96 h[3]	0.42	LC50	5
						96 h[4]	0.15	LC50	5
						96 h[5]	80.5	LC50	5

[1] Applied as Doxcide 50 (2% chlorine dioxide)
[2] 96-hour peak, i.e. the single highest biocide residue level detected during the tests (refer to Section 5.?).
[3] 96-hour mean maximum, i.e. the average maximum biocide residual detected during the 96 hours of testing (refer to Section B1.1.5).
[4] 96-hour mean, i.e. the mean biocide residual level during four approximate 2-hour exposure periods (refer to Section B1.1.5).
[5] 96-hour accumulative exposure, i.e. the total 96-hour biocide exposure in mg l^{-1} residual x minimum of exposure (area under a time-concentration curve) (refer to Section B1.1.5).

References:

1. Korich *et al* (1990)
2. Matisoff *et al* (1996)
3. Woodiwiss and Fretwell (1974)
4. Parella *et al* (1986)
5. Wilde *et al* (1983)

The only study to examine the effects of long-term intermittent exposure to chlorine dioxide showed that the majority of zebra mussels (*Dreissena polymorpha*) exposed to 1 and 5 mg l^{-1} for 30 minutes every day for 28 days survived (10% and 25% mortality, respectively) (Matisoff *et al* 1996).

A number of studies have investigated the biological effects of wastewaters derived from processes which utilise chlorine dioxide (e.g. bleaching of pulp), and the majority of these effluents appear to be of lower acute toxicity to freshwater species (e.g. *Daphnia magna*, *Oncorhynchus mykiss*) than those derived from chlorine disinfection/bleaching processes. This may be partly due to the reduction in harmful organochlorine by-products formed with chlorine dioxide. These studies are discussed in more detail in Section B1.2, Appendix B.

5.1.2 Chlorate ion (ClO_3^-)

Chlorate salts are highly soluble (e.g. 957 g $NaClO_3$ l^{-1} at 20 ^oC) and relatively stable in freshwater (see Section 4.2 for details). Static tests based on nominal exposure concentrations are therefore adequate for assessing the toxicity of the chlorate ion to aquatic life.

Toxicity data are available for a wide range of freshwater taxa (see Table 5.2) and these data suggest that bacteria and algae are the most sensitive taxonomic groups to chlorate. The lowest concentration found to inhibit the growth of the bacteria *Desulfovibrio desulfuricans* and *Methanococcus vannielli*, and the unicellular green alga *Chlorella vulgaris* was 0.1 mg ClO_3^- l^{-1} (Cenci *et al* 1975). In addition, acute and chronic (7 days) toxicity thresholds of 3 and 0.24 mg ClO_3^- l^{-1} have been reported for the green alga *Scenedesmus* spp. (Bringmann and Kuhn 1959, 1980). However, very little background information was available for most of these tests (e.g. test design, test performance), and other, better reported studies with *Chlorella vulgaris* and *Scendesmus* spp. indicated a significant variation in inter-species sensitivity. For example, the NOECs (no-observed effect concentrations) determined for the growth of *S. quadricauda* and *S. subspicatum* in methods based on EEC/OECD test guidelines were >784 and 3137 mg ClO_3^- l^{-1} (Hutchinson 1994a), respectively, and after 48 hours exposure to 334 mg ClO_3^- l^{-1} only 5% inhibition of growth was observed with *C. vulgaris* (Solomonsson and Vennesland 1972).

The discrepancies between the different studies with algae may partly be due to differences in the culture media used, in particular the source and amount of nitrogen applied. A number of studies with algae and terrestrial plants have indicated that under nitrogen starvation conditions, chlorate is readily taken up and competes with nitrate for active sites on the nitrate-reductase enzyme. In these situations, chlorate may be reduced by the enzyme to the more toxic chlorite, and the degree of reduction (and thus toxicity) is dependent upon the ratio of chlorate to nitrate (see Sections B2.3 and C2.2 in Appendices B and C, respectively, for more details). This theory is supported by several of the tests carried out on freshwater algae. For example, no detectable inhibitory effects were found for *C. vulgaris* exposed to 334 and 3340 mg ClO_3^- l^{-1} in the presence of ammonium (NH_4^+) for four days or more, and only 5% growth inhibition was observed after 48 hours exposure to 334 mg ClO_3^- l^{-1} and 280 mg N l^{-1} (as nitrate). When the concentration of nitrate was reduced by a factor of 10 (28 mg N l^{-1}), however, 334 mg ClO_3^- l^{-1} caused about 61% growth inhibition (Solomonsson and Vennesland 1972).

Table 5.2 Summary of the most pertinent freshwater toxicity data for chlorate (ClO_3^-)

Species	Life stage	Test design	Temp. (°C)	Hard.	pH	Nitrogen mg N l^{-1}	Exposure duration	Conc mg ClO_3 l^{-1}	Effect/test substance	Ref
BACTERIA										
Desulfovibrio desulfuricans	-	-	-	-	-	-	-	0.1	LOEC (growth inhibition) - $NaClO_3$	1
Pseudomonas putida	-	-	-	-	-	NO_3 (173)	16 h	1.9	Toxicity threshold - $KClO_3$	2
Methanococcus vannielli	-	-	-	-	-	-	-	0.1	LOEC (growth inhibition) - $NaClO_3$	1
ALGAE										
Chlorella vulgaris (unicellular green)	-	-	-	-	-	-	-	0.1	LOEC (growth inhibition) - $NaClO_3$	1
Chlorella vulgaris (unicellular green)	-	-	-	-	-	NO_3 (28)	48 h	334	61% growth inhibition - $NaClO_3$	3
Chlorella vulgaris (unicellular green)	-	-	-	-	-	NO_3 (280)	48 h	334	5% growth inhibition - $NaClO_3$	3
Chlorella vulgaris (unicellular green)	-	-	-	-	-	NO_3 (280)	48 h	3340	79% growth inhibition - $NaClO_3$	3
Scenedesmus sp. (unicellular green)	-	static, nominal conc	24	-	-	NO_3 (79)	acute	3	Toxicity threshold - $NaClO_3$	4

Species	Life stage	Test design	Temp. (°C)	Hard.	pH	Nitrogen mg N l^{-1}	Exposure duration	Conc mg ClO_3 l^{-1}	Effect/test substance	Ref
Scendesmus quadricauda (unicellular green)	-	static, nominal conc	-	-	-	NH_4 (3.92)	96 h	≥784	NOEC - $NaClO_3$	5
Scendesmus quadricauda (unicellular green)	-	static, nominal conc	27	-	-	NO_3 (41)	7 d	0.24	Toxicity threshold - $KClO_3$	2
Scenedesmus subspicatum (unicellular green)	exp. growth phase	static, nominal conc	24	-	-	NH_4 (3.92)	72 h	3137	NOEC (growth rate, biomass) - $NaClO_3$	6
							72 h	>3137	LOEC (growth rate, biomass) - $NaClO_3$	6
Mixed diatom community	-	flow-through, measured conc	15-18.5	-	-	NO_3/NH_4 (0.01/ <0.005)	23 d	0.025-0.5	No-effect on growth rate and community structure	7
CRUSTACEANS										
Daphnia magna (water flea)	-	nominal conc	-	-	-	-	24 h	880	LC50 - $KClO_3$	8
Daphnia magna (water flea)	-	nominal conc	-	-	-	-	24 h	600	NOEC (survival) - $KClO_3$	8
Gammarus sp. (shrimp)	adult	field,[1] measured conc	7.8-12.6	-	6.4-7.2	-	48 h	44.7-1.49[1]	75% mortality - $NaClO_3$	9
							96 h	44.7-1.49[1]	90% mortality - $NaClO_3$	9
INSECTS										

Species	Life stage	Test design	Temp. (°C)	Hard.	pH	Nitrogen mg N l^{-1}	Exposure duration	Conc mg ClO_3 l^{-1}	Effect/test substance	Ref
Ephemera japonica (mayfly)	nymphs	field,[1] measured conc	7.8-12.6	-	6.4-7.2	-	48 h	44.7-1.49[1]	60% mortality - $NaClO_3$	9
							96 h	44.7-1.49[1]	70% mortality - $NaClO_3$	9
FISH (SALMONIDS)										
Oncorhynchus mykiss (rainbow trout)	-	static, nominal conc	15	-	6.3	-	96 h	1373	LC50 - $NaClO_3$	10
Oncorhynchus mykiss (rainbow trout)	8.8g	field,[2] nominal	-	-	-	-	7 w	47[2]	No effect on growth - $NaClO_3$	11
FISH (NON-SALMONIDS)										
Cyprinus carpio (carp)	juveniles	nominal conc	-	-	-	-	48 h	>1310	EC50 (behavioural/survival) - $NaClO_3$	12
Pimephales promelas (fathead minnow)	juveniles	nominal conc	-	-	-	-	96 h	≥784	NOEC (survival) - $NaClO_3$	13
Pimephales promelas (fathead minnow)	juveniles	static, nominal conc	16-23	140-200	7.2-7.6	-	96 h	10820	LC50 -$NaClO_3$	14
							96 h	10663	LC50 -$NaClO_3$	14
							96 h	10584	LC50 -$NaClO_3$	15

Species	Life stage	Test design	Temp. (°C)	Hard.	pH	Nitrogen mg N l^{-1}	Exposure duration	Conc mg ClO_3 l^{-1}	Effect/test substance	Ref
Leuciscus leuciscus (dace)	3.2 cm, 0.25g	static, nominal conc	20.7	-	7.4-7.9	-	96 h	7843	TLm - $NaClO_3$	15
							240 h (10 d)	4705	TLm - $NaClO_3$	15
Brachydanio rerio (zebrafish)	juveniles	static, nominal conc	-	-	-	-	96 h	≥784	NOEC (survival) - $NaClO_3$	16
Rasbora heteromorpha (harlequin fish)	1.3-3 cm	flow-through, measured conc	20	20	7.2	NO_3 (0.7)	24 h	6745	LC50 - $NaClO_3$	17

[1] $NaClO_3$ applied to a forest stream by helicopter. Invertebrates and fish in a nearby stream were caged (separately) prior to and during application. Concentrations of $NaClO_3$ in the water were measured from the time of application (57 mg $NaClO_3$ l^{-1} or 44.7 mg ClO_3^- l^{-1}) to 4 days after application (1.9 mg $NaClO_3$ l^{-1} or 1.49 mg ClO_3^- l^{-1}) (refer to Section 5.1.1 for details).

[2] Tests were conducted in artificial streams. An initial dose of 47 mg ClO_3^- l^{-1} was reduced to zero after 7 hours. Growth was measured by body length and weight. Refer to Section 5.1.1 for further details.

References:

1. Cenci *et al* (1975)
2. Bringmann and Kuhn (1980)
3. Solomonsson and Vennesland (1972)
4. Bringmann and Kuhn (1959)
5. Kroon (1993, cited by van Wijk and Hutchinson 1995)
6. Hutchinson (1994a)
7. Perrin and Bothwell (1992)
8. Bringmann and Kuhn (1977)
9. Matida *et al* (1975a)
10. Beech (1983, cited by Environment Canada 1985)
11. Matida *et al* (1975b)
12. Nishiuchi (1980)
13. Mark and Arends (1993)
14. Shifrer *et al* (1974)
15. Matida *et al* (1976)
16. Mark and Hantink-de Rooij (1991)
17. Alabaster (1969)

Only one study has examined the effects of long-term continuous exposure of freshwater algae to chlorate. No adverse effects were observed on the growth rate and community structure of mixed diatom populations exposed to 0.025-0.5 mg ClO_3^- l^{-1} for 23 days in flow-through apparatus with both nitrate and ammonium available as sources of nitrogen (Perrin and Bothwell 1992).

Chlorate appears to be of low acute toxicity to all of the freshwater macroinvertebrate and fish species tested in the laboratory, with the majority of effect concentrations reported to be greater than 880 mg ClO_3^- l^{-1} (see Table 5.2 and Appendix B for more details). However, a field trial in Japan with caged freshwater shrimps (*Gammarus* spp.) and mayfly nymphs (*Ephemera japonica*) showed relatively high mortality rates of these species when placed in a stream that had been contaminated with sodium chlorate after aerial application to a nearby forest (see Table 5.2). The initial concentration of 44.7 mg ClO_3^- l^{-1} detected in the contaminated stream was gradually reduced to 1.9 mg ClO_3^- l^{-1} after four days. Cherry salmon (*Oncorhynchus masou*) that had also been caged in the contaminated stream all survived the test period (Matida *et al* 1975a, 1975b, 1976).

The only study to have considered the effects of long-term exposure to fish reported that there had been no effects on the growth of rainbow trout (*Oncorhynchus mykiss*) when exposed to a nominal chlorate concentration of 47 mg ClO_3^- l^{-1} for seven weeks in artificial streams. However, the initial concentration was reduced to undetectable levels after only seven hours and the authors do not report any renewal of test solutions after this period. This study is not therefore a very reliable assessment of chronic chlorate toxicity (Matida *et al* 1975b).

These studies are reviewed in more detail in Section B2, Appendix B.

5.1.3 Chlorite ion (ClO_2^-)

Chlorite salts are readily soluble in water (e.g. 390 g $NaClO_2$ l^{-1} at 17 ^oC), and, although more stable than chlorine dioxide, the chlorite ion is also reported to be a strong oxidant. In treated drinking water, about 12% of the chlorite initially present was reduced in the first three days, but only a further 2% was reduced in the following 15 days. The rate of reduction is expected to be higher in most surface waters since they generally contain higher levels of oxidisable matter than treated drinking water. For this reason toxicity tests with chlorite should ideally include the renewal of test solutions and the determination of actual exposure concentrations. However, the majority of tests reported in the literature do not make clear whether these requirements have been accounted for.

Toxicity data (see Table 5.3) are available for the crustacean *Daphnia magna* and three species of freshwater fish. The EC50s reported for *D. magna* suggest that chlorite is of high acute toxicity to this crustacean, although there is some discrepancy between these EC50s. A 48-hour EC50 of 0.02 mg ClO_2^- l^{-1}, with a corresponding NOEC of <0.002 mg ClO_2^- l^{-1}, was determined using a standard method recommended by the ASTM (American Society for Materials and Testing) (Degusso Corporation 1984, cited by IUCLID 1996), but a second, unspecified test reported a 48-hour EC50 of 0.22 mg ClO_2^- l^{-1} (NOEC 0.075 mg l^{-1}) (Kirk-Othmer, cited by IUCLID 1996). However, all of these values were based on nominal concentrations of chlorite (applied as sodium chlorite) and specific details of each of these tests were unavailable for review.

Table 5.3 Summary of the most pertinent aquatic toxicity data for chlorite (ClO_2^-)

Species	Life stage	Test design	Temp (°C)	Hard./ Salinity	pH	Exposure duration	Conc.[1] (mg ClO_2 l^{-1})	Effect/test substance	Ref
FRESHWATER									
CRUSTACEANS									
Daphnia magna (water flea)	-	static, nominal conc.	-	-	-	48 h	<0.002	NOEC - $NaClO_2$ (80%)	1
						48 h	0.02	EC50 - $NaClO_2$ (80%)	1
Daphnia magna (water flea)	-	flow-through, nominal conc.	-	-	-	24 h	>0.75	EC50 - $NaClO_2$ (80%)	2
Daphnia magna (water flea)	-	no information	-	-	-	48 h	0.22	EC50 - $NaClO_2$	3
						48 h	0.075	NOEC - $NaClO_2$	3
SALMONID FISH									
Oncorhynchus mykiss (rainbow trout)	juvenile	static, nominal conc.	-	-	-	96 h	75	No mortality - $NaClO_2$ plus dechlorinated water	4
NON-SALMONID FISH									
Lepomis macrochirus (bluegill sunfish)	-	no information	-	-	-	48 h	156	LC50 - $NaClO_2$	3
						48 h	37.5	LC50 - $NaClO_2$	3

Species	Life stage	Test design	Temp (°C)	Hard./ Salinity	pH	Exposure duration	Conc.[1] (mg ClO_2 l^{-1})	Effect/test substance	Ref
Brachydanio rerio (zebra fish)	-	static, nominal conc.	-	-	-	96 h	75 to >375	LC50 - $NaClO_2$ (Hoechst products[2])	5
SALTWATER									
CRUSTACEANS									
Mysidopsis bahia (mysid shrimp)	-	static, nominal conc.	-	-	-	48 h	0.62	EC50 - $NaClO_2$ (80%)	2
						72 h	0.5	EC50 - $NaClO_2$ (80%)	2
						96 h	0.49	EC50 - $NaClO_2$ (80%)	2
						96 h	0.19	NOEC - $NaClO_2$ (80%)	2

[1] Concentrations have been converted to mg ClO_2 l^{-1} as 69% (based on molar proportions) of the concentrations reported for $NaClO_2$.
[2] A range of Hoechst sodium chlorite products were tested. Refer to Section 5.1.3 or Appendix B for details.

References:

1. Degussa Corporation (1984, cited by IUCLID 1996)
2. Environ Systems (1991, cited by IUCLID 1996)
3 Kirk-Othmer (cited by IUCLID 1996)
4. Cairns and Conn (1979)
5. Hoechst AG (1985a-e, cited by IUCLID 1996)

The limited data indicate that the chlorite ion is of moderate to low acute toxicity to freshwater fish, with effect concentrations from 37.5 mg ClO_2^- l^{-1}. The 48-hour EC50s reported for bluegill sunfish (*Lepomis macrochirus*) are 37.5 and 156 mg ClO_2^- l^{-1}, although no details of the type of tests carried out are provided. Tests based on OECD guidelines (Guideline Method 203), and carried out according to Good Laboratory Practices (GLP), were performed with zebra fish (*Brachydanio rerio*) for a range of sodium chlorite formulations (Hoechst 1985a-e). The 96-hour LC50s determined for these formulations ranged from nominal concentrations of 75 to >375 mg ClO_2^- l^{-1}. The OECD Method referred to indicates that semi-static or flow-through systems should be applied where appropriate, but no information was provided to indicate that either of these methods were adopted in this study.

No data were available on the effects of long-term exposure to chlorite.

5.2 Saltwater life

5.2.1 Chlorine dioxide

Laboratory-derived saltwater toxicity data are available for three species of fish, five species of invertebrates and two species of algae. The most pertinent data are summarised in Table 5.4.

Of the limited number of species tested, algae appear to be the most sensitive to acute chlorine dioxide exposure. The lowest concentration to significantly reduce the germination of kelp meiospores (*Macrocystis pyrifera*) after 48 hours exposure (i.e. the LOEC) was 25 mg l^{-1}, with a corresponding NOEC (no-observed effect concentration) of 2.5 mg l^{-1}. However, as these values were based on nominal concentrations of chlorine dioxide, without any renewal of the test media, they are likely to be underestimates of the true toxicity of chlorine dioxide to this species (Hose *et al* 1989).

Chlorine dioxide was of low acute toxicity to those saltwater invertebrates studied, with 48-hour LC50s of 500 mg l^{-1}, or greater, reported for the purple sea urchin (*Strongylocentrotus purpuratus*), the cockle (*Cerastoderma edule*), the Aesop shrimp (*Pandalus montagui*), the brown shrimp (*Crangon crangon*) and the shore crab (*Carcinus maenus*) (Portman and Wilson 1971, cited by CIS 1997). These values were determined using a semi-static test system with the biocide "Doxcide 50", which is only 2% chlorine dioxide. However, the source of this information does not make it clear whether these LC50s are for Doxcide 50 or for chlorine dioxide. If the former is true, the LC50s for chlorine dioxide will be 10 mg l^{-1} or greater (i.e. 2% of the LC50s for Doxcide 50), indicating moderate acute toxicity.

Insufficient data are available to properly assess the toxicity of chlorine dioxide to saltwater fish. An LT50 of 2.5 hours was reported for striped mullet (*Mugil cephalus*) exposed to 1 mg l^{-1} chlorine dioxide, indicating that chlorine dioxide is of high acute toxicity to this species (Parella *et al* 1986). However, few details of this study were available to review its suitability for use in the derivation of Environmental Quality Standards. The only other fish to have been tested is the kelp bass (*Paralabrax clathratus*). No significant effects were observed on the survival of eggs of this species when exposed to concentrations of up to 25 mg l^{-1} for 48 hours in static test vessels (Hose *et al* 1989).

Table 5.4 Summary of the most pertinent saltwater toxicity data for chlorine dioxide

Species	Life stage	Test design	Temp. (°C)	Salinity (‰)	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
ALGAE									
Macrocystis pyrifera (giant kelp)	meio-spores	static, nominal conc.	15	33	-	48 h	25	LOEC (reduction in germination)	1
						48 h	2.5	NOEC (reduction in germination)	1
						48 h	250	LOEC (germ tube length)	1
						48 h	25	NOEC (germ tube length)	1
Cladophora sp. (green filamentous)	-	static, measured conc.	30	-	7.0	24 h	52 (as Cl)	Cellular effects (change in organelle structure)	3
						24 h	2.6 (as Cl)	Cell morphology	3
ECHINODERMS									
Strongylocentrotus purpuratus (purple sea urchin)	embryo	static, nominal conc.	15	33	-	48 h	250	LOEC (% abnormalities)	1
						48 h	25	NOEC (% abnormalities)	1
MOLLUSCS									
Cerastoderma edule (cockle)	adult	semi-static, nominal conc.	15	-	-	48 h	>500[1]	LC50	4

Species	Life stage	Test design	Temp. (°C)	Salinity (‰)	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
CRUSTACEANS									
Pandalus montagui (Aesop shrimp)	adult	semi-static, nominal conc.	15	-	-	48 h	>500[1]	LC50	4
Crangon crangon (common shrimp)	adult	semi-static, nominal conc.	15	-	-	48 h	>500[1]	LC50	4
Carcinus maenas (shore or green crab)	adult	semi-static, nominal conc.	15	-	-	48 h	500[1]	LC50	4
FISH									
Paralabrax clathratus (kelp bass)	eggs	static, nominal conc.	20	33	-	48 h	<25	No significant effects on survival	1
Mugil cephalus (striped mullet)	-	no information	-	-	-	2.05 h	1	LT50	2

[1] Applied as doxcide (2% chlorine dioxide) but LC50s are assumed to be expressed as mg ClO_2 l^{-1}.

References:

1. Hose *et al* (1989)
2. Parrella *et al* (1986)
3. Betzer and Kott (1969
4. Portman and Wilson (1971, cited by CIS 1997))

A number of field and mesocosm-based studies in the Baltic identified brown algae (e.g. *Fucus* spp., *Cladophora* spp) as being highly sensitive to long-term exposure to effluents from pulp mills using chlorine dioxide in the bleaching process. As a result, a number of grazing invertebrates were also adversely affected, but these direct and indirect effects were related to the presence of chlorate in the effluents rather than chlorine dioxide itself (see Section 5.2.2 and Appendix B for more details).

5.2.2 Chlorate ion (ClO_3^-)

The toxicity data available for saltwater organisms are restricted mainly to bacteria and algae, and, although there are no laboratory-derived chronic toxicity data, several mesocosm studies have been carried out on the long-term effects of chlorate-containing pulp mill effluents to intertidal communities (see Table 5.5).

Insufficient reliable data are available to properly assess the effects of chlorate on saltwater bacteria. Growth inhibition of the luminescent bacterium *Photobacerium phosphoreum* (also known as *Vibrio fisheri*) has been reported at a concentration as low as 0.1 mg ClO_3^- l^{-1} (Cenci *et al* 1975), although bioluminescence (the principle of the Microtox test) was much less sensitive (15-minute EC50 34510 mg ClO_3^- l^{-1}) (Macauley, unpublished, cited by van Wijk and Hutchinson 1995). Neither of these studies were available for review.

Based on the toxicity data reported, chlorate appears to be of moderate to low acute and chronic toxicity to most saltwater algae. Tests with the unicellular alga *Phaedactylum tricornutum*, carried out according to standard ISO guidelines, recorded 72-hour LOECs of 100 and 200 mg ClO_3^- l^{-1} and NOECs of 50 and 100 mg ClO_3^- l^{-1} for biomass and growth rate, respectively (Hutchinson 1994b), and the 48-hour EC50 (growth inhibition) reported for the red macroalga *Gracilaria tenuistipitata* was 46 mg ClO_3^- l^{-1} (Haglund *et al* 1996).

A series of mesocosm studies in nitrogen-deficient saltwater identified a number of brown algae (*Fucus* spp., *Chorda filum*, *Ectocarpus siliculosus* and *Pilayella littoralis*) as being sensitive to long-term continuous exposure to chlorate-containing pulp mill effluents (derived from the bleaching of pulp with chlorine dioxide), while other macro- and microalgae (green algae, blue-green algae and red algae) were either unaffected or stimulated to grow more rapidly. The EC50s (frond growth) for the brown macroalgae *Fucus vesiculosus* and *Fucus serratus* after six months exposure were 0.08 and 0.13 mg ClO_3^- l^{-1}. Similar reductions in growth were also observed when chlorate was applied without effluent, confirming chlorate as the principal cause of the observed effects. Indirect effects on the macroinvertebrate communities were also observed, most notably on brown algal grazers such as *Ideothea* spp. On the basis of these studies, brown algae may require greater protection than other intertidal algal species.

These studies are reviewed in more detail in Section C2, Appendix C.

Table 5.5 Summary of the most pertinent saltwater toxicity data for chlorate (ClO_3^-)

Species	Life stage	Test design	Temp (°C)	Salinity (‰)	pH	Nitrogen (mg N l^{-1})	Exposure duration	Concn (mg l^{-1})	Effect/test substance	Ref
BACTERIA										
Photobactrium phosphoreum	-	no information	-	-	-	-	-	0.1	LOEC (growth inhibition) - $NaClO_3$	1
Photobactrium phosphoreum	-	no information	-	-	-	-	5 m	43137	EC50 (bioluminescence) - $NaClO_3$	2
							15 m	34510	EC50 (bioluminescence) - $NaClO_3$	2
ALGAE										
Phaeodactylum tricornutum (unicellular)	exp. growth phase	static, nominal conc.	20	-	-	NO_3 (8.25)	72 h	50	NOEC (biomass) - $NaClO_3$	3
								100	LOEC (biomass) - $NaClO_3$	3
								298	EC50 (biomass) - $NaClO_3$	3
Phaeodactylum tricornutum (unicellular)	exp. growth phase	static, nominal conc.	20	-	-	NO_3 (8.25)	72 h	100	NOEC (growth rate) - $NaClO_3$	3
								200	LOEC (growth rate) - $NaClO_3$	3

Species	Life stage	Test design	Temp (°C)	Salinity (‰)	pH	Nitrogen (mg N l^{-1})	Exposure duration	Concn (mg l^{-1})	Effect/test substance	Ref
								444	EC50 (growth rate) - $NaClO_3$	3
Gracilaria tenuistipitata (red macroalga)	-	static	-	25	8.0	NO_3/NH_4	48 h	46	EC50 (growth inhibition) - ClO_3^-	4
								10	NOEC (growth inhibition) - ClO_3^-	4
Fucus vesiculosus (bladderwrack)	adult	mesocosm, measured conc.	3-20	7	-	NO_3 (<0.039)	6 months	0.005	NOEC (frond growth) - ClO_3/Pulp mill effluent	5
								0.015	LOEC (frond growth) - ClO_3/Pulp mill effluent	6
								0.08	EC50 (frond growth) - ClO_3/Pulp mill effluent	6
Fucus serratus (brown macroalga)	adult	mesocosm, measured conc.	3-20	7	-	NO_3 (<0.039)	6 months	0.06	NOEC (frond growth) - ClO_3/Pulp mill effluent	6
								0.1	LOEC (frond growth) - ClO_3/Pulp mill effluent	6
								0.13	EC50 (frond growth) - ClO_3/Pulp mill effluent	6

Species	Life stage	Test design	Temp (°C)	Salinity (‰)	pH	Nitrogen (mg N l^{-1})	Exposure duration	Concn (mg l^{-1})	Effect/test substance	Ref
Mixed phytoplankton	-	static, measured conc.	-	-	-	NO_3 (<0.039)	8 h	0.05	NOEC (primary production	6
							8 h	50	LOEC (primary production)	6
PLANTS										
Zostera marina (Phanerogam)	-	mesocosm, measured conc.	3-20	7	-	NO_3 (<0.039)	6 months	>0.288	NOEC (growth)	6

References:

1. Cenci *et al* (1975)
2. Macauley (unpublished data, cited by Van Wijk and Hutchinson 1995)
3. Hutchinson (1994b)
4. Haglund *et al* (1996)
5. Rosemarin *et al* (1986)
6. Rosemarin *et* al (1994)

Key to Tables 5.1-5.5:

-	Information unavailable or not reported
Hard.	Hardness (mg $CaCO_3$ l^{-1})
Salinity	in ‰
w	Weeks
d	Days
h	Hours
LC50/TLm	Median lethal concentration
EC50	Median effect concentration
LOEC	Lowest observed effect concentration
NOEC	No-observed effect concentration

5.2.3 Chlorite ion (ClO_2^-)

Information on the effects of the chlorite ion on saltwater organisms is extremely sparse (see Table 5.3). The only laboratory-derived data available suggest that chlorite is of high acute toxicity to the mysid shrimp (*Mysidopsis bahia*), with 48-, 72- and 96-hour LC50s (based on nominal concentrations) of 0.62, 0.5 and 0.49 mg ClO_2^- l^{-1}, respectively (Environ Systems 1991, cited by IUCLID 1996).

A number of studies reported in the literature have suggested that the effects of the *chlorate* ion on algae and bacteria are, in fact, due to its reduction to chlorite by the nitrate-reductase enzyme system (see Section 5.2). Recent research has indicated that the chlorite ion is indeed "toxic" to bacteria, algae and fungi, although test data are not yet available for this work (Van Wijk, Personal Communication 1997). Mesocosm studies carried out with chlorate-containing pulp mill effluents showed that brown algae (e.g. *Fucus* spp., *Cladophora* spp., *Ectocarpus* sp.) were particularly sensitive to chlorate, and if the above theory on the mode of toxic action of chlorate is correct, one might also expect brown algae to be sensitive to direct chlorite exposure. However, the same unpublished study described above also indicated that chlorite was less toxic to the brown alga *Ectocarpus variabilis* than chlorate.

Further studies are required to properly assess the toxicity of chlorite to saltwater organisms, in particular, sensitive algae and bacteria.

5.3 Bioaccumulation (aquatic life)

Chlorine dioxide, chlorite and chlorate are all oxidants (in descending order of strength) and will therefore tend to be reduced by the oxidisable matter (e.g. metal ions, humic acids) present in water rather than be accumulated by aquatic organisms. There is evidence that chlorate may be taken up by plants and algae but in these cases it is rapidly reduced to chlorite by the nitrate-reductase enzyme system (see Section B2.3, Appendix B for more details).

5.4 Mammalian toxicity

No adverse health effects or abnormalities in the urine and blood of 10 healthy men were observed when exposed to chlorine dioxide, chlorite and chlorate concentrations in water of up to 24, 2.4 and 2.4 mg l^{-1}, respectively, for 16 days and to an equivalent dose of 36 μg kg^{-1} body weight in drinking water for 12 weeks. In an epidemiological study of a community where chlorine dioxide was used as a drinking water disinfectant for 12 weeks, no consistent changes were observed in any of the measured clinical parameters. Adult exposure to chlorine dioxide, chlorite and chlorate ranged from 0.25-1.1 mg l^{-1}, 3.2-7 mg l^{-1} and 0.3-1.1 mg l^{-1}, respectively.

Exposure to chlorine dioxide, chlorate and chlorite has been associated with adverse effects on red blood cells in laboratory animals. Haemolytic anaemia is the main symptom which is associated with oxidative damage to the red blood cells. Chlorite is the most potent of the three species and, at high doses, methaemoglobineama (a reduced capacity for red blood cells to carry oxygen) can be induced. A NOAEL of 1 mg kg^{-1} body weight has been identified for

chlorite based on decreased red blood cell glutathione levels in a 90 day drinking water study in rats. However, a more recent 90-day drinking water study with rats has suggested a higher NOAEL of 10 mg kg^{-1} body weight for chlorite based on effects on blood. In a 90 day rat study involving both sexes, a NOAEL (effects on red blood cells, organ weights and the thyroid gland) of 30.06 mg kg^{-1} body weight day^{-1} was established in males following exposure to sodium chlorate in their drinking water. Significant depression of thyroid hormones has also been observed in rats and monkeys exposed to chlorine dioxide. This may be because of the oxidising effect of chlorine dioxide on iodide in the gastrointestinal tract and food, thereby making iodide less bioavailable.

Mutagenicity studies with chlorite, chlorate and chlorine dioxide produced negative results, and long-term drinking water studies with chlorine dioxide and sodium chlorite have provided no evidence for carcinogenicity in laboratory animals. In addition, chlorite does not appear to be teratogenic. Although chlorine dioxide has been shown to impair neurobehavioural and neurological development in rats exposed perinatally, this is probably related to effects on thyroid function. A two-generation reproductive/neurodevelopmental study has recently been completed, although the full results are currently unpublished. Nonetheless, interim results which were presented at an ILSI meeting in 1995 indicated that no adverse neurological effects had been observed in F_1 animals.

The mammalian toxicity of chlorine dioxide, chlorate and chlorite is reviewed in more detail in Appendix D.

6. DERIVATION OF EQSs

6.1 Standards in other countries

No water quality standards for the protection of aquatic life appear to have been proposed for chlorine dioxide, or its by-products chlorate and chlorite, in the US, Canada or Europe. An EQS of 250 mg l^{-1}, expressed as an annual average concentration, has been adopted by the Environment Agency for the protection of freshwaters from a third possible reduction product of chlorine dioxide, the chloride ion (Cl^-) (Gardiner and Smith 1992).

The World Health Organisation (WHO) considered chlorite, chlorate as well as chlorine dioxide in its 1993 Drinking Water Guidelines (WHO, 1996). A provisional guideline of 0.2 mg l^{-1} was derived for chlorite, the provisional nature being due to the fact that the use of chlorine dioxide as a disinfectant may result in the chlorite value being exceeded. A guideline for chlorine dioxide was not considered because of its rapid breakdown and the chlorite provisional guideline was considered to be adequately protective for any potential toxicity. Due to the lack of suitable toxicity data, WHO declined to derive a guideline value for chlorate in drinking water.

6.2 EQSs for the protection of aquatic life

6.2.1 Chlorine dioxide

The limited aquatic toxicity data available for chlorine dioxide suggest that it may be of high to moderate acute toxicity to those freshwater and saltwater organisms tested (see Section 5), but due to the behaviour of chlorine dioxide in water, it is quite likely that the adverse effects observed in these tests are as much to do with the presence of its reduction products (most notably chlorite) than chlorine dioxide itself. However, none of these tests appear to have considered this possibility in detail (e.g. by measuring the conversion of chlorine dioxide to chlorite etc).

Chlorine dioxide is expected to be almost completely reduced to chlorite, chlorate and chloride (in varying proportions) during the treatment of raw or wastewaters and is therefore unlikely to be released to the aquatic environment in significant amounts. Any chorine dioxide that does enter the aquatic environment (e.g. accidental spillage, leakage) should also be rapidly reduced by the oxidisable matter present in the water. For this reason, it is not considered appropriate to derive EQSs for the protection of freshwater or saltwater life from exposure to chlorine dioxide.

6.2.2 Chlorate (ClO_3^-)

The majority of toxicity data available for chlorate indicate that it is generally of low acute and chronic toxicity to those freshwater and saltwater organisms tested (see Section 5 for details). However, there is evidence to suggest that chlorate is of high acute and chronic toxicity to algae (freshwater and saltwater) when nitrate is the only source of nitrogen available. It has

been suggested in the literature that nitrogen starvation enhances the uptake and reduction of nitrate (to nitrite) by algae (e.g. Rosemarin *et al* 1986) (see Section C2.2, Appendix C for further details). In such instances, chlorate, if present, also appears to be readily taken up by algae, and, competing with nitrate for active sites on the nitrate-reductase enzyme, may be reduced by the enzyme to the harmful chlorite ion (ClO_2^-). The chlorate-to-nitrate ratio is therefore likely to be important in determining the level of chlorate toxicity to aquatic life, although the limited data available in the literature suggests that this ratio can vary considerably between algal species.

In most freshwater systems the amounts of nitrate present naturally should ensure that chlorate does not out-compete nitrate for enzyme active sites and should therefore prevent significant conversion of chlorate to chlorite in most instances.

However, there is evidence from a series of research projects performed in the Baltic Sea area that the presence of chlorate (15 $\mu g\ l^{-1}$ and greater) in intertidal zones containing low levels of nitrate (typically <0.039 mg l^{-1}) can have adverse effects on brown saltwater algal communities. The disappearance of *Fucus vesiculosus* (bladderwrack) from a 12 km^2 stretch of the Baltic coast, for example, was attributed to the discharge of chlorate in pulp-mill effluents over several years, which was confirmed with several chronic exposure mesocosm studies (e.g. Rosemarin *et al* 1986, 1990, 1994). In these mesocosm tests, the growth of other species of intertidal algae (including various green and red algae) was either unaffected or stimulated. Although it is not known why brown algae are more sensitive to chlorate, this research suggests that brown saltwater algae require greater protection from chlorate in low nitrate saltwater environments.

As mentioned above, the few studies that have considered the effects of nitrate on the toxicity of chlorate to algae (freshwater and saltwater) seem to indicate that the ratio of chlorate to nitrate required to cause significant adverse effects varies considerably between species. Akzo Nobel in the Netherlands have recently carried out some tests on the toxicity of chlorate to various algae (mainly saltwater algae), but the results of these tests have not yet been published. However, a much more comprehensive dataset of toxicity data for algae is required, showing clearly the effects of different nitrate concentrations on the toxicity of chlorate to different algal species, and until such data become available no EQSs are proposed for the protection of aquatic life. It could be argued, in that case, that a more site-specific approach (e.g. direct toxicity assessment) to the regulation of effluents containing chlorate may be more practical.

6.2.3 Chlorite (ClO_2^-)

The aquatic toxicity data available for chlorite are limited to a few studies on freshwater invertebrates and fish and just one species of saltwater invertebrate (see Section 5 and Table 5.5). Tests carried out with the water flea (*Daphnia magna*) and the saltwater mysid shrimp (*Mysidopsis bahia*) indicate that chlorite is of high acute toxicity to these crustaceans. However, the 48-hour EC50s reported for *D. magna* differ by approximately an order of magnitude, and as there is very little background information available for either study, it is difficult to assess the suitability of these values for use in the derivation of EQSs.

Because of the lack of reliable toxicity data, in particular for lower organisms (e.g. algae), it is not possible to propose EQSs for the protection of freshwater or saltwater life from exposure to chlorite.

In order to propose tentative EQSs for the protection of aquatic life, additional data would be required for invertebrates and lower organisms (e.g. algae), and, in the case of saltwater life, fish. Because of the discrepancy in the acute toxicity values reported for *Daphnia magna*, confirmatory data on the sensitivity of this crustacean to chlorite are also needed prior to the derivation of EQSs. To allow for the possibility of other invertebrate species being more sensitive to chlorite than *D. magna*, toxicity data for other invertebrates would also be ideal. Some work has recently been carried out by Akzo Nobel in the Netherlands on the toxicity of chlorite to algae, bacteria and fungi, although the results of this work have not yet been published. The Akzo Nobel study is thought to have mainly considered marine species, so there may still be a need for toxicity data for freshwater algae before tentative EQSs can be proposed for freshwater life.

6.3 Reference levels for the protection of water abstracted for potable supply

Based on WHO recommendations, because it is rapidly reduced during drinking water treatment a reference level for chlorine dioxide is not considered appropriate. However, the WHO derived a tolerable daily intake (TDI) for chlorite of 10 $\mu g\ kg^{-1}$ body weight day^{-1}. This was determined from a NOAEL of 1 $mg\ kg^{-1}$ body weight, based on decreased glutathione levels in a 90-day rat study and incorporating an uncertainty factor of 100 (for inter- and intraspecies variation). The TDI derived in this manner was consistent with the NOAEL (36 $\mu g\ kg^{-1}$ body weight day^{-1}) in a 12 week clinical study involving a small number of human volunteers (see Section 5 and Appendix D). By allocating 80% of the TDI to drinking water a provisional guideline of 0.2 $mg\ l^{-1}$ was derived. However, a more recent 90-day rat study suggested a higher NOAEL of 10 $mg\ kg^{-1}$ body weight based on effects on the blood. This indicates some uncertainty about the study on which the WHO (1993) guideline was based, and, given this uncertainty, no reference level for chlorite has been recommended.

Insufficient toxicity data are available for the recommendation of a reference level for chlorate.

7. CONCLUSIONS

1. Chlorine dioxide is a very reactive oxidising free radical, even in aqueous solutions. It has a wide scope of potential uses as a disinfectant and bleaching agent, but predominantly with aqueous media (e.g. drinking water treatment, process water treatment, industrial and domestic wastewater treatment).

2. Approximately 1500 tonnes of sodium chlorite are sold annually in the UK for the manufacture of chlorine dioxide and from this figure it is estimated that between 750 and 1500 tonnes of chlorine dioxide are generated annually.

3. Chlorine dioxide is rapidly converted to varying proportions of chlorite (ClO_2^-), chlorate (ClO_3^-) and chloride (Cl^-) in water, and as a result, is unlikely to be present in significant concentrations when effluents are discharged to sewer or the aquatic environment. EQSs for the protection of aquatic life and reference levels for the abstraction of water to potable supply are not considered appropriate.

4. The relative amounts of chlorite, chlorate and chloride released to the aquatic environment following the application of chlorine dioxide will depend upon a number of variable factors, such as the manufacturing process used (e.g. the combination of precursor chemicals), the manufacturing conditions (e.g. temperature), exposure to sunlight (chlorine dioxide and chlorite are susceptible to photolysis) and the demand of the receiving medium for oxidants (i.e. the amount of oxidisable matter present).

5. The limited aquatic toxicity data available suggest that chlorite is of high acute toxicity to those fresh and saltwater crustaceans tested, with effect concentrations reported from 0.02 and 0.49 mg l^{-1} for the water flea (*Daphnia magna*) and mysid shrimp (*Mysidiopsis bahia*), respectively. However, insufficient data are available for the derivation of EQSs to protect fresh and saltwater.

6. The majority of aquatic toxicity data available for chlorate suggest that it is of low acute and chronic toxicity to those fresh and saltwater organisms tested. However, there is evidence to suggest that chlorate is of high toxicity to both fresh and saltwater algae when nitrate is available as the only source of nitrogen (e.g. acute effect concentrations of 100 μg l^{-1} reported for freshwater, and chronic effect concentrations reported from 15 μg l^{-1} in intertidal mesocosms). This is believed to be due to the reduction of chlorate to chlorite by the nitrate-reductase enzyme. However, not enough detailed information is available with which to propose reliable EQSs for the protection of fresh or saltwater life. However, site-specific assessments (e.g. direct toxicity assessment) may provide a more practical solution to the regulation of chlorate-containing effluents which are derived from chlorine dioxide applications.

7. Chloride has previously been considered by WRc and an EQS of 250 mg l^{-1}, expressed as an annual average concentration, has been adopted by the Environment Agency for the protection of freshwater life (Gardiner and Smith 1992).

8. WHO reported a provisional guideline of 0.2 mg l^{-1} for chlorite in drinking water. However, a recent 90-day rat study has indicated some uncertainty about the study on

which the WHO guideline was based, and, given this uncertainty, no reference level (abstraction to potable supply) for chlorite is recommended.

9. Insufficient toxicity data are available for the recommendation of a reference level (abstraction to potable supply) for chlorate.

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APPENDIX A FATE AND BEHAVIOUR IN THE ENVIRONMENT

Due to the hazardous properties of chlorine dioxide (e.g. strong oxidant, explosive), it is generally manufactured on the site of use by combining appropriate precursor chemicals (e.g. sodium chlorite, sodium hypochlorite, HCl) in specially designed units as needs dictate. Because of this ClO_2 is not transported or stored in significant quantities, and accidental spillage or leakage to surrounding land is unlikely. In the event of such incidents it is probable that spillages or leakages will be drained to sewers or volatilised to the atmosphere. The fate and behaviour of chlorine dioxide in air and land is summarised in Section 4, but since the majority of chlorine dioxide is likely to be released to the aquatic environment (e.g. via the sewerage system) this Appendix concentrates on the fate and behaviour of chlorine dioxide in water.

A1 FATE AND BEHAVIOUR IN WATER

The majority of information available on the fate and behaviour of chlorine dioxide in water relates specifically to drinking water and wastewater treatment processes. However, these studies provide an indication of the substances which may eventually be released to the aquatic environment as a result of its use, although this will also depend upon any additional treatment applied to wastewaters later on (e.g. on-site and municipal (sewage) treatment processes).

Chlorine dioxide is generally not expected to persist in water for very long (depending on the concentration and the demand of the water). For example, the half-lives of chlorine dioxide in filtered water at concentrations of 3 and 1.5 mg l^{-1} were about 1 and 3 hours, respectively. At these concentrations chlorine dioxide was found to almost completely disappear after about 15 and 11 hours, respectively (Howells and Pound 1982). The half-life of chlorine dioxide in nitrified-denitrified wastewater, however, was less than 15 minutes and the initial dose of 6 mg l^{-1} produced residuals of 1 mg l^{-1} after 15 minutes and 0.12 mg l^{-1} after 4 hours (Cairns and Conn 1979). These studies indicate that chlorine dioxide is less stable in water with greater amounts of oxidisable matter.

It has been suggested that chlorine dioxide may reform and persist for several days in the drinking water distribution system if chlorite and free chlorine are not removed after treatment with chlorine dioxide (AWWA 1994). However, this is unlikely to be of significance in natural waters because the greater presence of oxidisable matter, and thus the higher oxidant demand, will generally account for chlorite and any re-formed chlorine dioxide.

The persistence of chlorine dioxide in water is entirely influenced by abiotic processes, involving various disproportionation and redox reactions with the oxidisable components of water, volatilisation and photolysis (see Sections A1.1 to A1.5 for details). As many of these processes result in the reduction of chlorine dioxide to chlorite (ClO_2^-), chlorate (ClO_3^-) and chloride (Cl^-) ion, the stability of these ions in water has been considered briefly in Section A1.6.

A1.1 Disproportionation and redox reactions

Chlorine dioxide does not significantly hydrolyse in water and, without the presence of reducing substances, will exist as a dissolved gas in the pH range 2-10. In alkaline solutions (pH >10), however, chlorine dioxide will disproportionate to give a 1:1 molar ratio of chlorite (ClO_2^-) and chlorate (ClO_3^-):

$$2ClO_2 + 2OH^- = ClO_2^- + ClO_3 + H_2O \quad \text{(A1.1)}$$

The disproportionation of chlorine dioxide to chlorite and chlorate in alkali water is rapid. Half-lives are reported to be in the order of 20 minutes to 3 hours for 5-10 mg l^{-1} chlorine dioxide in water at a pH of 12 (Aieta *et al* 1984, cited by Aieta and Berg 1986). However, this is not a realistic temperature for the aquatic environment.

Chlorine dioxide is a strong oxidant and reacts with reducing substances in water, primarily by a one-electron oxidative pathway to form the chlorite ion (Aieta and Berg 1986, AWWA 1994):

$$ClO_2 + e^- = ClO_2^- \quad \text{(A1.2)}$$

As a result, the majority of chlorine dioxide in water will be rapidly converted to the chlorite ion. Chlorite ion is also an effective oxidising agent and will be consumed in oxidation-reduction reactions, although at a much slower rate than chlorine dioxide. The reduction of the chlorite ion results in the formation of chloride as follows:

$$ClO_2 + 4H + 4e^- = Cl^- + H2O \quad \text{(A1.3)}$$

During drinking water treatment approximately 50-70% of chlorine dioxide reacts and appears immediately as chlorite, with the remainder as chloride (Aieta *et al* 1984, Miltner 1976, cited by Aieta and Berg 1986). The residual chlorite continues to degrade in the water distribution system with oxidisable material in the finished water or in the distribution system. Under these conditions no chlorate is expected to be formed (Brauch *et al* 1981, cited by Aieta and Berg 1986).

Chlorate may, however, be formed if chlorine dioxide is generated from the reaction of sodium chlorite and chlorine predisolved in water (see Section 2.2) or if chlorine dioxide is used in conjunction with chlorine as a disinfectant (AWWA 1994). The use of chlorine may result in the formation of hypochlorous acid (HOCl) which reacts with chlorite (ClO_2^-) to produce chlorate (ClO_3^-).

$$HOCl + ClO_2^- \leftrightarrow ClO3^- + Cl^- + H^+ \quad \text{(A1.4)}$$

Aieta and Berg (1986) reported this reaction to be slow (15-20 days at concentrations of reactants commonly found in drinking water). However, Griesse *et al* (1993) showed that 20 minutes contact with hypochlorous acid caused an increase of 39% in the levels of chlorate detected in water pretreated with chlorine dioxide.

A1.2 Reactions with inorganic compounds

Several authors have previously reviewed the reactions of chlorine dioxide with inorganic compounds in industrial and wastewater treatment applications (e.g. Hoigne and Bader 1994, Noack and Iacoviello 1992, Aieta and Berg 1986). Because only low levels of chlorine dioxide are likely to be released directly to the aquatic environment these reactions are not likely to be of great significance to receiving waters, although the products of these reactions may ultimately find their way into the aquatic environment through discharges from treatment plants. This section summarises the main reactions described in the literature, principally Noack and Iacoviello (1992).

A1.2.1 Ammonia

Chlorine dioxide does not react with ammonia (Rav-Ach 1984, Stevens 1982), and, providing it is not used in conjunction with chlorine, should not directly result in the formation of harmful chloramines. Hoigne and Bader (1994) estimated the second order rate constant for the reaction of chlorine dioxide with ammonia as $<10^{-2}$ M^{-1} s^{-1} at pH 7-8.

A1.2.2 Cyanide ion

Cyanide ion (CN^-) is a common and highly toxic component of waste products in the metal plating, steel, gold and silver mining and chemical process industries. Chlorine dioxide reacts with free cyanide "instantaneously", yielding chlorite ion and cyanate ion as shown in reaction A1.5 below.

$$CN^- + 2ClO_2 + H_20 \rightarrow NCO^- + 2ClO_2^- + 2H^+ \quad \text{(A1.5)}$$

If copper or nickel are present (e.g. as cyanide complexes), a similar reaction takes place but following a slightly different stoichiometry (2 moles of CLO_2 are required to convert 5 moles of CN^- to 5 moles of NCO^- and 2 moles each of Cl^- and H^+). Zinc and cadmium complexes are reported to follow reaction A1.5, but the cyanide ligand in the ferro- and ferricyanide complexes is not oxidised by chlorine dioxide under normal reaction conditions, although the divalent iron in the ferrocyanide complex is readily oxidised to trivalent iron (e.g. the ferricyanide complex) (Noack and Iacoviello 1992).

A1.2.3 Sodium nitrite

Sodium nitrite may be present in effluents from the chemical process industry where it can be used in the manufacture of various organic chemicals, dyes and explosives (Noack and Iacoviello 1992). The reaction of nitrite (NO_2) with chlorine dioxide results in the formation of the nitrate ion (NO_3^-) and the chloride ion (Cl^-).

$$2ClO_2 + 5NO_2^- + H_2O \rightarrow 5NO_3^- + 2Cl^- + 2H^+ \quad \text{(A1.6)}$$

A1.2.4 Sulphur compounds

Sulphur suspended in water is reported to react slowly with chlorine dioxide to form hydrochloric acid (HCl) and sulphuric acid (H_2SO_4) as follows (Noack and Iacoveillo 1992):

$$5S + 6ClO_2 + 8H_20 \rightarrow 6H^+ + 6Cl^- + 5H_2SO_4 \quad \text{(A1.7)}$$

Noack and Iacoveillo (1992) suggested that the stoichiometry indicated in reaction A1.7 is only an approximation and indicated that the first step may, in fact, be the transfer of an oxygen atom from chlorine dioxide to sulphur to form sulphur oxide (A1.8).

$$ClO_2 + S \rightarrow ClO + SO \quad \text{(A1.8)}$$

Hydrogen sulphide (H_2S), hydrosulphide (HS^-) and sulphide ions (S^{2-}) are reported to be readily oxidised by chlorine dioxide (Noack and Iacoviello 1992). Possible products reported include elemental sulphur, sulphate ion (or sulphuric acid) and chloride ion (or hydrochloric acid), although Noack and Iacoviello (1992) state that there is no conclusive evidence of the stoichiometry of the reactions involved.

Chlorine dioxide reacts with S (IV) species (e.g. sulphur dioxide SO_2, bisulphite ion HSO_3^- and sulphurous acid HSO_3^-). Noack and Iacoviello (1992) cited studies which report that all of the sulphite (SO_3^{2-}) is oxidised to sulphate ion (SO_4^{2-}) and most of the chlorine dioxide is reduced to chloride ion (or HCl). However, some chlorate may also be formed. The overall reaction suggested by Noack and Iacoviello (1992) is given below (Reaction A1.9).

$$2ClO_2 + 2S(IV)^* + 2OH^- \rightarrow Cl^- + ClO_3^- + H_2O + 2SO_4^{2-} \quad \text{(A1.9)}$$

* denotes the sum of all species of tetravalent sulphur.

Sodium thiosulphate rapidly reacts with chlorine dioxide and chlorite as shown in reactions A1.10 and A1.11. Noack and Iacoviello (1992) suggest that the increase in acidity caused by these reactions may result in the re-formation of chlorine dioxide from any chlorites present unless the wastestream is first neutralised.

$$5Na_2S_2O_3 + 8ClO_2 + 9H_20 \rightarrow 10NaHSO_4 + 8HCl \quad \text{(A1.10)}$$

$$Na_2S_2O_3 + 2NaClO_2 + H_2O \rightarrow 2\ NaHSO_4 + 2NaCl \quad \text{(A1.11)}$$

A1.2.5 Ferrous and manganous ions

The oxidation of ferrous (Fe^{2+}) and manganous (Mn^{2+}) ions by chlorine dioxide has long been utilised in drinking water treatment (Noack and Iacoviello 1992). The main reaction products are the ferric ion (Fe^{3+}) and MnO_2, and as they are readily precipitated, they are more readily removed from water than their precursors during treatment (unless the metal ions are chelated). Whilst the ferrous ion reduces chlorine dioxide to the chloride ion (Cl^-), the manganous ion reduces it to the chlorite ion (ClO_2^-).

A1.3 Reactions with organic compounds

The reactions of chlorine dioxide with various organic compounds, principally in treatment waters, have previously been reviewed by several authors (e.g. Masschelein 1989, Rav-Ach *et al* 1989, Rav-Ach 1984, Stevens 1982). These reactions provide an indication of the types of organic compounds which, depending on the treatment processes employed (some by-

products may be removed during wastewater treatment), may be found in the aquatic environment as a result of the use of chlorine dioxide, or in the unlikely event of chlorine dioxide being directly released to the aquatic environment.

A1.3.1 Humic and fulvic substances

Chlorine dioxide reacts with humic and fulvic substances to form quinones and hydroquinones, aliphatic and aromatic carboxylic acids, aldehydes and glyoxal (including aryl and methyl derivatives). If chlorine dioxide is added in excess Masschelein (1989) suggested that monochloroacetic acid and traces of monochlorosuccinic acid, di- and trichloroacteic acids may result. Rav-Ach (1984) indicated that water solutions of humic materials consume ClO_2 rapidly in the first few hours and then the consumption decreases gradually over a period of a few days.

In comparison to chlorine (Cl_2), fewer chlorinated organic compounds are produced with humic materials (Rav-Ach 1984). This may be of significance to the aquatic environment as the use of chlorine dioxide in place of, or in conjunction with chlorine is reported to reduce the amounts of harmful di- and tri-chloroacetic acids (Rav-Ach 1984).

As part of a study on the photolysis of chlorine dioxide and chlorite in aqueous solutions (see Section A1.5 for details), Karpel vel Leitner *et al* (1992) discovered that chlorite (7×10^{-5} mol l^{-1}) in the presence of humic substances (10 mg l^{-1}) led to the production of organochlorine compounds. This was probably due to the reaction of chlorine, formed as an intermediate in the photolysis of chlorite. Although chlorine dioxide was not tested in the presence of humic substances, chlorine was also identified as a photolysis intermediate of chlorine dioxide. Based on this study, it is conceivable that if waters that are to be treated with chlorine dioxide (or treated waters containing chlorine dioxide-derived chlorite) are exposed to sunlight, some organochlorine compounds may result.

A1.3.2 Phenols and phenolic derivatives

Rav-Ach (1984) reported that chlorine dioxide reacts rapidly with phenols and that the reaction is first order with respect to each reactant. In addition, Rav-Ach also reported that 2 moles of ClO_2 are consumed by each mole of phenol in this reaction.

Masschelein (1979) showed a strong correlation between the consumption of chlorine dioxide by phenols and the formation of chlorite ions (ClO_2^-) and suggested that 90% of chlorine dioxide is reduced to chlorite in the presence. Wayon *et al* (1982, cited by Rav-Ach 1984), however, reported that at neutral pH only 50% is converted to chlorite, with the remaining 50% being converted to hypochlorous acid (HOCl).

Glabisz (1968, cited by Rav-Ach 1984) indicated that hydroquinone and monohydric phenols that are not *para*-substituted retain their ring structure after the reaction with chlorine dioxide and are oxidised mainly to quinones and chloroquinones. Phenols that are *para*-substituted, such as *p*-cresol, 3,4-xylenol, di- or tri-hydric phenols, resorcinol, pyrocatecol and pyrogallol, undergo ring cleavage with chlorine dioxide and are oxidised to organic acids (e.g. oxalic, maleic and fumaric) and carbon dioxide.

When small concentrations of chlorine dioxide were applied to excess concentrations of phenol Spanggord (1978, cited by Rav-Ach 1984) identified the following reaction products:

2-chlorophenol, 4-chlorophenol, 2,6-dichlorophenol, 2,4-dichlorophenol, 2,4,6 trichlorophenol, 2-chlorohydroquinone, resorcinol and fumaric acid. However, when chlorine dioxide was added in excess the chlorophenols were no longer detected. Stevens (1982) also exposed phenol to varying molar ratios of chlorine dioxide and similarly found that at low chlorine dioxide levels (4:5 ClO_2:phenol) chlorophenols were detected. Higher molar ratios (14:5 and 14:1) did not produce chlorphenols but favoured the formation of hydroquinones.

A1.3.3 Amines and amino acids

Chlorine dioxide is reported to react with amines but the reaction rates depend greatly on the structure (Rav-Ach 1984). The reactivity of amines is of the order tertiary>secondary>primary, the reaction rate being dependent on the electron density of nitrogen (the greater the basicity of the amine the faster its reaction with chlorine dioxide).

Hull *et al* (1967, cited by Rav-Ach 1984) proposed a general mechanism for the reaction of tertiary and secondary amines possessing an *alpha*-hydrogen. This mechanism is as follows:

$$ClO_2 + (C_2H_5)_3N \leftrightarrow \textit{(slow)} \leftrightarrow ClO_2^- + (C_2H_5)_3N^+ \rightarrow (C_2H_5)_2N\text{-}CH\text{-}CH_3$$

$$\rightarrow \textit{(+}ClO_2\textit{)(fast)} \rightarrow ClO_2^- + (C_2H_5)_2N^+{=}CH\text{-}CH_3 \rightarrow (C_2H_5)_2NH + CH_3CHO + H^+.$$

Rosenblatt (1982) estimated that chlorine dioxide reacts ten times faster with triethylamine than phenol and nearly 10^4 times faster than permanganate. The overall reaction with triethylamine required 2 moles of chlorine dioxide per mole of amine.

Although some cyclic tertiary amines react with chlorine dioxide by similar mechanisms Rav-Ach (1984) suggested that some tertiary amines containing five or six membered rings are often oxidised to carbinolamines rather than undergoing a carbon-nitrogen cleavage. For example, *N*-butylisoindoline produces *N*-butyl-3-hydroyphthamidine (Rav-Ach 1984). Rav-Ach (1984) also proposed that some amines which cannot give up an *alpha*-hydrogen (e.g. quinuclidine) are oxidised to N-oxides. N-oxides can lead to the formation of nitrosamines, which have been found to be carcinogenic, but the author states that the amines which give rise to N-oxides are rarely found in either drinking water or wastewater effluents.

Amino acids which do not contain specific reactive groups (e.g. glycine, alanine, phenylalinine, serine and leucine) do not react with chlorine dioxide under water treatment conditions (Kennaugh 1957, cited by Rav-Ach 1984). Reactive amino acids, such as tryosine, histidine and cystine, have been found to react under mildly acidic conditions. For example, Rav-Ach (1984) suggests that at pH 3.5 cystine forms cystine bisulphoxide and cysteric acid.

A1.3.4 Other hydrocarbons

Most aliphatic and aromatic hydrocarbons (except phenols and some polycyclic aromatic hydrocarbons) do not react with chlorine dioxide under typical water treatment conditions (Rav-Ach 1984). Carboxylic acids and alcohols are generally unreactive with chlorine dioxide but under extreme conditions of pH (acidic) and temperature, alcohols may react with chlorine dioxide in excess to produce aldehydes and carboxylic acids.

Stevens (1982) identified many aldehydes in Ohio River water that had been treated with chlorine dioxide and indicated that the majority were not further oxidised to their respective

carboxylic acids. Carbonyl compounds, however, reacted quite quickly with chlorine dioxide, even under moderate conditions to produce carboxylic acids.

A1.4 Volatility

Aqueous chlorine dioxide is reported to volatilise easily at temperatures in excess of 10-15 ^{o}C (Merck 1989). According to the following formula (CEN 1994), the vapour pressure of chlorine dioxide increases with increasing temperature:

log VP = (1.31 x 1375)/T (where T is in ^{o}K).

Additional information on the solubility of chlorine dioxide in water (CEN 1994) also indicates a tendency for it to partition more to the gaseous phase as temperature increases:

S = 70±7 at 0 ^{o}C

S = 45 at 15 ^{o}C

S = 26.5±0.8 at 35 ^{o}C

Where S = ratio of chlorine dioxide in water to chlorine dioxide in the gaseous phase.

If chlorine dioxide is released directly to the aquatic environment, volatilisation is likely to have an important role in its removal. However, direct release of chlorine dioxide to the aquatic environment is expected to be rare and volatility from water is more likely to affect chlorine dioxide concentrations in process waters and effluents prior to release. There is no information available on the rate of volatilisation of aqueous chlorine dioxide.

A1.5 Photolysis

There is evidence which indicates that chlorine dioxide can be photolytically decomposed in water to chloride (Cl^-) and chlorate (ClO_3^-) ions (e.g. Griese *et al* 1993, Karpel vel Leitner *et al* 1992, Zika *et al* 1985, Zepp and Cline 1977). Griese *et al* (1993) reported chlorate concentrations of 0.36 to 0.97 mg l^{-1} in pilot studies at a water treatment plant in the United States of America where 3.56 to 3.99 mg l^{-1} chlorine dioxide had been applied. No chlorate was detected in the control tests (i.e. conducted in the dark). Karpel vel Leitner *et al* (1992) showed that 5 x 10^{-4} mol l^{-1} of chlorine dioxide was photodecomposed at neutral pH to chloride (0.4 mol/mol of ClO_2), chlorate (0.6 mol/mol of ClO_2) and oxygen as stable end-products, with chlorine and chlorite as intermediates. Moreover, experiments carried out at different pH values (4, 7.2 and 8) showed that the pH has no effect on the production of chloride and chlorate.

If photolysis is allowed to occur during drinking water or wastewater treatment processes, chlorate concentrations are therefore likely to be increased, and unless chlorates are removed from the treated water before release, this may result in an increase in the amounts of chlorate found in the drinking water distribution system or aquatic environment, respectively. In addition, Van vel Leitner *et al* (1992) found that chlorite was photochemically degraded to chlorate, chloride and oxygen in aqueous solution. If chlorite is present in the aquatic environment as a result of the use of chlorine dioxide, and depending on the extent of

exposure to sunlight, chloride and chlorate are likely to be the predominant by-products of chlorine dioxide.

A1.6 Reaction products

A.1.6.1 Chlorite

Information on the persistence of the chlorite ion in water is related almost entirely to the drinking water distribution system. Experiments carried out by the American Water Works Association (AWWA 1994) showed that the chlorite ion was relatively stable in drinking water over a period of 18 days. After about 75 hours, an initial concentration of 1 mg ClO_2^- l^{-1} was reduced by approximately 12%, but in the proceeding 15 days it was reduced by only a further 2%.

Manufacturers have reported that sodium chlorite has strong oxidising properties and some of the reactions described in Sections A1.1 to A1.5 appear to support this. It has also been reported that chlorite can be photochemically degraded in water, ultimately to chlorate, chloride and oxygen, with potential intermediates being chlorine dioxide and chlorine (Blatchley 1993). The stability of chlorite in untreated raw waters and treated wastewaters will therefore largely depend upon the amount of oxidisable matter present (e.g. metal ions, humic acids) and exposure to sunlight. Because surface waters are likely to contain higher levels of oxidisable matter and be exposed more to sunlight, the reduction of chlorite will probably occur to a greater extent and more quickly than demonstrated by AWWA (1994) in treated drinking water.

A1.6.2 Chlorate

The chlorate ion (ClO_3^-) is reported to be relatively stable in natural waters (IUCLID 1996). Chlorate may be abiotically reduced to the chloride ion in acidic solutions (IUCLID 1996), and in waters containing high levels of oxidisable matter, such as ferrous ion and organic acids. Tests carried out with treated drinking water showed that an initial concentration of 0.5 mg ClO_3^- l^{-1} remained stable for 18 days (AWWA 1994). The greater presence of oxidisable material in surface waters may reduce the level of stability somewhat, but probably not significantly.

The main processes for the removal of chlorate from water (including wastewaters) are likely to be biological. A number of studies have demonstrated the uptake and assimilation of chlorate by algae, plants and bacteria in association with nitrate, as well as the subsequent reduction of chlorate to chlorite by the nitrate-reductase enzyme system (see Sections B2.3 and C2.2). The IUCLID database (1996) indicates that chlorate can quickly be biodegraded in an aerated pond, depending on pH and temperature. Under anaerobic conditions chlorate may be completely degraded within a few hours (IUCLID 1996, Malmqvist *et al* 1992, 1991).

No information was available on the potential for chlorate to photodegrade.

A1.6.3 Chloride

The chloride ion (Cl^-) is generally very stable in water. Chloride ions in aqueous solution may form weak complexes with trace elements, although mercury and silver form stronger, more covalent bonds. This complexation plays a greater role in saltwaters because of the high

concentrations of chloride ion present. In non-saline waters, chloride will exist predominantly as the free hydrated ion. Chloride was considered in an earlier WRc EQS report for the National Rivers Authority (now the Environment Agency) (Gardiner and Smith 1992).

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APPENDIX B TOXICITY TO FRESHWATER LIFE

In this Appendix, information available on the freshwater toxicity of chlorine dioxide is reviewed (see Section B1 and Table B1.1). Because the fate and behaviour of chlorine dioxide in water (see Appendix A) indicates the possible release of the reduction products chlorite and chlorate to the aquatic environment following the use of chlorine dioxide, data for these compounds are also considered (see Sections B2 and B3, respectively). Another possible reduction product of chlorine dioxide is the chloride ion (Cl^-), which has been covered in a previous WRc EQS report (Gardiner and Smith 1992).

B1 CHLORINE DIOXIDE

Only a limited amount of data are available specifically on the toxicity of chlorine dioxide to freshwater organisms (see Section B1.1), although there is also some information on the environmental impact of wastewaters which have been treated with chlorine dioxide (see Section B1.2).

B1.1 Single-substance studies

Most of the limited toxicity data reported for chlorine dioxide are from a few single-species, acute laboratory tests, although one study examined the toxicity of fish in outdoor ponds. The reliability of most of these studies is questionable as few details of the test designs and performance (e.g. control mortality, dose-responses) have been provided.

The overall dataset comprises five species of fish (only one of which is a UK species) and one species of crustacean, mollusc and protozoan. The absence of data for lower organisms (e.g. bacteria and algae) may be significant, considering that one of chlorine dioxide's principal uses is as a microbiological biocide (e.g. drinking water, industrial process waters, industrial wastewaters). However, due to the chemistry of chlorine dioxide in water (see Appendix A), it could be argued that chlorine dioxide will not enter the aquatic environment in significant concentrations and therefore the lack of toxicity data for these species, and the paucity of toxicity data in general, is of less importance for chlorine dioxide than it is for the more persistent chlorite and chlorate ions.

B1.1.1 Protozoans

Korich *et al* (1990) studied the effects of various biocides, including chlorine dioxide, on *Cryptosporidium parvum* oocyst viability. *C. parvum* is an intestinal parasite which can infect humans via contaminated surface waters, where it persists as resistant oocysts. The authors found that exposure of oocysts to 1.3 mg l^{-1} of chlorine dioxide yielded 90% inactivation after one hour. Although this protozoan lives in surface waters for part of its life-cycle, the results of this study are of more significance to the treatment of surface waters intended for potable supply than the protection of the aquatic environment.

B1.1.2 Molluscs

Matisoff *et al* (1996) tested chlorine dioxide as a control agent for adult zebra mussels (*Dreissena polymorpha*) using single, intermittent and continuous exposures. Chlorine

dioxide gas, generated *in-situ*, was dosed at varying concentrations into test vessels which were set up with a flow-through of lake water. Exposure of mussels to a single 30-minute dose of 20 mg l^{-1} chlorine dioxide resulted in 50% mortality, while a dose of 30 mg l^{-1} caused 70% mortality. Intermittent exposure for 30 minutes each day showed that chlorine dioxide at concentrations of 10-20 mg l^{-1} caused significant mortality over three to seven consecutive days (LC50 13 mg l^{-1}), although concentrations of 1 to 5 mg l^{-1} applied daily over 28 consecutive days resulted in less than 30% mortality. In contrast, continuous exposure of mussels to chlorine dioxide concentrations of 0.25, 0.5, 1.0, 2.0 and 5.0 mg l^{-1} for four days induced 40, 60, 100, 100 and 100% mortality, respectively, with an LC50 of 0.35 mg l^{-1}.

B1.1.3 Crustaceans

In a study to investigate the removal of animals from drinking water distribution systems, Hansen *et al* (1990) reported that chlorine dioxide was lethal to the isopod crustacean *Asellus* sp. and suggested that the mechanism of toxicity was attributed to an effect on haemocyanin pigments which are responsible for oxygen transport. The authors also suggested that 40-80% of the chlorine dioxide applied was converted to the chlorite ion (ClO_2^-), which caused competitive inhibition of oxygen binding by haemocyanin. However, only the abstract of this study was available for review and the authors do not state in the abstract what concentrations of chlorine dioxide or chlorite were toxic to *Asellus*.

B1.1.4 Salmonid fish

In the only study to examine the toxicity of chlorine dioxide to salmonid fish, Woodiwiss and Fretwell (1974) reported a 48-hour LC50 of 10 000 mg l^{-1} for the brown trout (*Salmo trutta*) exposed to the commercial biocide Doxcide 50 (2% chlorine dioxide). The authors calculated the LC50 for chlorine dioxide to be approximately 200 mg l^{-1} (i.e. 2% of the LC50 for Doxcide 50). The authors used a static test system but only nominal concentrations of Doxcide 50 were applied, and since it is possible that some chlorine dioxide would have been consumed by the oxidisable material in the test water, the LC50s reported may have been overestimated slightly.

B1.1.5 Non-salmonid fish

Tooby *et al* (1975) reported 24-, 48- and 96-hour LC50s of 9600, 7400 and 6500 mg l^{-1}, respectively, for the harlequin fish (*Rasbora heteromorpha*) exposed to chlorine dioxide in a static test system. However, these results were reported as concentrations of the commercial biocide Doxcide 50, only 2% of which is chlorine dioxide. The LC50s for chlorine dioxide might therefore be estimated by calculating 2% of those reported for Doxcide 50, i.e. 192, 148 and 130 mg l^{-1}, respectively. During these tests the authors did not appear to measure the exposure concentrations of chlorine dioxide, so it is possible that the toxicity values reported are an underestimate of the actual toxicity.

The LT50 (the time at which 50% lethality occurs in the test populations) determined for the goldfish (*Carassius auratus*) exposed to 2 mg l^{-1} was 33.33 hours (Parrella *et al* 1986). Although this fish is thought to be a freshwater species, the authors indicate that it was tested in marine waters. Specific details of the test design and performance (e.g. control mortality, dose responses) were not provided to confirm this.

Table B1.1 Toxicity of chlorine dioxide to freshwater life

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
PROTOZOANS										
Cryptosporidium parvum (coccidian parasite)	oocyst	S	m	-	-	-	1 h	1.3	90% inactivation	1
MOLLUSCS										
Dreissena polymorpha (zebra mussels)	adult	F	m	15.4-25.4	120-124	8.0-8.3	30 m	30	70% mortality (single dose)	2
							3-7 d	13	LC50 (single 30 m dose per day)	2
							28 d	1	10% mortality (single 30 m dose per day)	2
							28 d	5	25% mortality (single 30 m dose per day)	2
							7 d	10	65% mortality (single 30 m dose per day)	2
							4 d	0.25	40% mortality (continuous exposure)	2
							4 d	0.5	60% mortality (continuous exposure)	2
							4 d	1-5	100% mortality (continuous exposure)	2

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
							4 d	0.35	LC50 (continuous exposure)	2
SALMONID FISH										
Salmo trutta (brown trout)	finger-lings	S	n	10	165-200	7.6-8.0	48 h	200[6]	LC50	3
NON-SALMONID FISH										
Rasbora heteromorpha (harlequin fish)	-	S	n	-	270	5.2	24 h	9600[1]	LC50	4
							48 h	7400[1]	LC50	4
							96 h	6500[1]	LC50	4
Carassius auratus (goldfish)[7]	-	-	-	-	-	-	33.33 h	2	LT50	5
Pimephales promelas (fathead minnow)	juvenile	F	m	24.5-30.4	-	6.7-7.5	96 h[2]	0.07	LC50	6
							96 h[3]	0.06	LC50	6
							96 h[4]	0.02	LC50	6
							96 h[5]	3.26	LC50	6
Pimephales promelas (fathead minnow)	adult	F	m	24.5-30.4	-	6.7-7.5	96 h[2]	0.63	LC50	6
							96 h[3]	0.46	LC50	6
							96 h[4]	0.17	LC50	6

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
							96 h[5]	93.47	LC50	6
Lepomis macrochirus (bluegill sunfish)	young-of-the-year	F	m	24.5-30.4	-	6.7-7.5	96 h[2]	0.56	LC50	6
							96 h[3]	0.42	LC50	6
							96 h[4]	0.15	LC50	6
							96 h[5]	80.5	LC50	6

Notes to Table B1.1:

[1] Concentrations assumed to be expressed as mg Doxcide 50 l^{-1} (2% chlorine dioxide). The approximate 24-, 48-, and 96-hour LC50s for the active ingredient (ClO_2) are estimated as 2% of the reported values (i.e. 192, 148 and 130 mg l^{-1}, respectively)

[2] 96-hour peak, i.e. the single highest biocide residue level detected during the tests (refer to Section B1.1.5).

[3] 96-hour mean maximum, i.e. the average maximum biocide residual detected during the 96 hours of testing (refer to Section B1.1.5).

[4] 96-hour mean, i.e. the mean biocide residual level during four approximate 2-hour exposure periods (refer to Section B1.1.5).

[5] 96-hour accumulative exposure, i.e. the total 96-hour biocide exposure in mg l^{-1} residual x minimum of exposure (area under a time-concentration curve) (refer to Section B1.1.5).

[6] Applied as Doxcide 50 (2% chlorine dioxide) (refer to Section B1.1.4)

[7] Although this is a freshwater species the authors indicate that it was tested in marine water. Specific details of the test design were not provided.

References:

1. Korich *et al* (1990)
2. Matisoff *et al* (1996)
3. Woodiwiss and Fretwell (1974)
4. Tooby *et al* (1975)
5. Parella *et al* (1986)
6. Wilde *et al* (1983)

Wilde *et al* (1983) studied the acute toxicity of chlorine dioxide to fathead minnows (*Pimephales promelas*) and bluegill sunfish (*Lepomis macrochirus*) using a flow-through system designed to simulate the dosing of biocides in a nuclear reactor cooling tower. Juvenile and adult fathead minnows and young-of-the-year bluegill sunfish were exposed to different biocide doses (100, 56, 32, 18, 10, 5.6 and 0% of the stock solution) in an outdoor pond for one hour every day for four days (96-hours). Due to the high initial demand of the test water, the exposure concentrations of chlorine dioxide were initially low in each instance. However, residual concentrations of chlorine dioxide were found to increase steadily over the one-hour dosing period as the demand was reduced, and after dosing had ceased, the levels of residual chlorine dioxide gradually reduced to zero again after about an hour. Fish were therefore exposed to chlorine dioxide for about two hours every day for four days. The four ways in which the chlorine dioxide doses were calculated by the authors are as follows:

1. 96-hour peak, i.e. the single highest chlorine dioxide residual level detected during the tests;

2. 96-hour mean maximum, i.e. the average maximum chlorine dioxide residue detected during the four dosing periods;

3. 96-hour mean, i.e. the mean chlorine dioxide residual level during the four two-hour exposure periods;

4. 96-hour accumulative exposure, i.e. the total 96-hour chlorine dioxide exposure in mg l^{-1} residual multiplied by the number of minutes of exposure (i.e. area under the time-concentration curve).

As a result, four different 96-hour LC50s were reported for each test, ranging from 0.02 to 3.26 mg l^{-1}, 0.17 to 93.47 mg l^{-1} and 0.15 to 80.5 mg l^{-1} for juvenile fathead minnows, adult fathead minnows and bluegill sunfish, respectively (see Table B1.1 for details). The 96-hour LC50s that are probably the most meaningful are those based on the 96-hour mean (i.e. the mean biocide residual level during the four two-hour exposure periods). The 96-hour mean LC50s were in fact the lowest LC50s reported (i.e. 0.02, 0.17 and 0.15 mg l^{-1}, respectively) and suggest that chlorine dioxide is of high acute toxicity to these fish, in particular juvenile fathead minnows, when dosed continuously for an hour every day.

B1.2 Studies with industrial effluents

Chlorine dioxide is used as a disinfectant or bleaching agent in industrial process waters and wastewaters (e.g. pulp mills) and a number of studies have therefore investigated the biological effects of effluents derived from these processes.

Haley *et al* (1995) examined the long-term effects (6-9 months) of effluents from bleached-kraft mills on experimental streams, before and after mill conversion to chlorine dioxide as a bleaching agent. Although fewer chlorinated organic constituents and adsorbable organic halides were detected in the effluent after conversion from chlorine to chlorine dioxide, no significant differences were noted on the effects of the effluent to the organisms present (periphyton, macroinvertebrates, rainbow trout). The authors suggested that the lack of response to the change in effluent composition was probably because the concentrations of constituent toxicants were below ecologically significant levels. The concentrations of

chlorinated phenols and AOX reported before conversion to chlorine dioxide were 39-43 and 22.5 μg l^{-1}, respectively and, after conversion, 4 and 19.8 μg l^{-1}, respectively.

In another study, Donnini (1983) tested the effects of replacing chlorine with chlorine dioxide in the bleaching of kraft softwood pulp on the water flea *Daphnia magna*. This study entailed static bioassays, performed in duplicate over 48 hours with serially diluted solutions of neutralised (pH 7.5) samples. The authors found that low levels of chlorine dioxide substitution (4-10%) caused a significant reduction in the toxicity of the effluent compared to pure chlorine bleaching. Higher substitutions (>30%) also provided a decrease in effluent toxicity but these were proportionally smaller than the lower levels of substitution. Intermediate levels of chlorine dioxide substitution (20 and 30%) produced effluents as toxic as those from pure chlorine treatments (LC50s 42.5 and 42.7% for 20 and 30% substitutions, respectively). The authors did not provide any possible explanations for these results, and as specific details of the effluent composition were not provided it is difficult to speculate.

Liebergott *et al* (1991) studied the change in toxicity of kraft pulp effluent to rainbow trout fingerlings (*Oncorhynchus mykiss*) when the bleaching agent was converted from chlorine (Cl_2) to chlorine dioxide (ClO_2). The acute LC50s determined for *O. mykiss* indicated a gradual decrease in toxicity with decreasing Cl_2:ClO_2 ratio. The 96-hour LC50s (expressed as percentage of effluent) reported for Cl_2:ClO_2 ratios of 90:10, 70:30, 50:50, 30:70 and 10:90 were 20, 27, 32, 70 and 100%, respectively. The authors also found that the chlorinated phenol and AOX content of the effluents decreased with increasing chlorine dioxide substitution.

Chlorine dioxide was used to disinfect municipal wastewater in Canada (Cairns and Conn 1979) and effluents which had been dosed with nominal concentrations of chlorine dioxide at or above 1 mg l^{-1} were found to be acutely toxic to rainbow trout (*O. mykiss*) after 96 hours exposure. However, in this study actual chlorine dioxide concentrations were not measured and the authors suggested that there had probably been a rapid loss of chlorine dioxide residual in the waste stream. Although the lethal concentration of chlorine dioxide could not be determined, an estimate of its relative toxicity with respect to chlorine was made by comparing the 96-hour LC50s associated with at least a 90% reduction in total coliforms. On this basis, effluent disinfected with chlorine dioxide was less toxic than effluent disinfected with chlorine and, at the same time, chlorine dioxide provided a consistently higher degree of disinfection than chlorine. For example, a 96-hour LC50 of 4.1% was reported for *O. mykiss* exposed to effluents from the chlorine disinfection process, where 99.98% total coliform reduction was achieved, while the 96-hour LC50 for chlorine dioxide was 31% with 99.93% total coliform reduction.

B2. CHLORATE (ClO_3^-)

The chlorate ion (ClO_3^-) is a potential by-product of chlorine dioxide in water and may therefore enter the aquatic environment via industrial discharges (either directly or via the sewerage system) where chlorine dioxide has been used. The following sections (also see Table B2.1) summarise the most pertinent information available on the toxicity of chlorate ion to freshwater life. The effects of effluents which contain chlorate (due to the use of chlorine dioxide, e.g. pulp mill effluents) on model outdoor ecosystems are discussed in Sections B1.2 (freshwater) and C1.2 (saltwater).

Table B2.1 Toxicity of chlorate (ClO_3^-) to freshwater life

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Nitrogen source (mg N l^{-1})	Exposure duration	Conc mg ClO_3 l^{-1}	Effect/test substance	Ref
BACTERIA											
Desulfovibrio desulfuricans	-	-	-	-	-	-	-	-	0.1	LOEC (growth inhibition) - $NaClO_3$	1
Escherichia coli	-	S	n	-	-	-	-	acute	4	Toxicity threshold - $NaClO_3$	2
Pseudomonas putida	-	-	-	-	-	-	NO_3 (173)	16 h	1.9	Toxicity threshold - $KClO_3$	3
Sphaerotilus natans	-	-	-	-	-	-	-	-	0.1	LOEC (iron metabolism inhibition) - $NaClO_3$	1
Leptothrix chloandii	-	-	-	-	-	-	-	-	0.1	LOEC (iron metabolism inhibition) - $NaClO_3$	1
Methanococcus vannielli	-	-	-	-	-	-	-	-	0.1	LOEC (growth inhibition) - $NaClO_3$	1
CYANOBACTERIA											
Microcystis aeruginosa	-	-	-	-	-	-	-	8 days	12	Toxicity threshold - $KClO_3$	8
Nostoc muscorum	-	-	-	-	-	-	-	-	0.1	LOEC (growth inhibition) - $NaClO_3$	1

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Nitrogen source (mg N l^{-1})	Exposure duration	Conc mg ClO_3 l^{-1}	Effect/test substance	Ref
ALGAE											
Chlorella vulgaris (unicellular green)	-	-	-	-	-	-	-	-	0.1	LOEC (growth inhibition) - $NaClO_3$	1
Chlorella vulgaris (unicellular green)	-	-	-	-	-	-	NO_3 (28)	48 h	334	61% growth inhibition - $NaClO_3$	5
Chlorella vulgaris (unicellular green)	-	-	-	-	-	-	NO_3 (280)	48 h	334	5% growth inhibition - $NaClO_3$	5
Chlorella vulgaris (unicellular green)	-	-	-	-	-	-	NO_3 (280)	48 h	3340	79% growth inhibition - $NaClO_3$	5
Scenedesmus sp. (unicellular green)	-	S	n	24	-	-	NO_3 (79)	acute	3	Toxicity threshold - $NaClO_3$	2
Scendesmus quadricauda (unicellular green)	-	-	-	-	-	-	NH_4 (3.92)	96 h	≥784	NOEC - $NaClO_3$	6
Scendesmus quadricauda (unicellular green)	-	S	n	27	-	-	NO_3 (41)	7 d	0.24	Toxicity threshold - $KClO_3$	3
Scenedesmus subspicatum (unicellular green)	exp. growth phase	S	n	24	-	-	NH_4 (3.92)	72 h	3137	NOEC (growth rate and biomass) - $NaClO_3$	7
								72 h	>3137	LOEC (growth rate and biomass) - $NaClO_3$	7

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Nitrogen source (mg N l^{-1})	Exposure duration	Conc mg ClO_3 l^{-1}	Effect/test substance	Ref
								72 h	1569	No effect on cell size and colour (visual observations only) - $NaClO_3$	7
								72 h	3137	LOEC (cell size and colour, visual observations only) - $NaClO_3$	7
Platymonas suecica	-	-	-	-	-	-	-	8 d	1350	EC50 (growth rate) - $NaClO_3$	9
Mixed diatom community	-	F	m	15-18.5	-	-	NO_3/ NH_4 (0.01/<0.005)	23 d	0.025-0.	No-effect on growth rate and community structure	30
PROTOZOANS											
Entosiphon sulcatum	-	-	-	-	-	-	NO_3 (27)	72 h	817	Toxicity threshold - $KClO_3$	3
PLATY-HELMINTHES											
Polycelius nigra (planarian/flatworm)	-	S	n	15-18	-	6.0	-	48 h	12549	LC50 - $NaClO_3$	12
CRUSTACEANS											

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Nitrogen source (mg N l^{-1})	Exposure duration	Conc mg ClO_3 l^{-1}	Effect/test substance	Ref
Daphnia magna (water flea)	-	-	n	-	-	-	-	-	3325	Toxicity threshold - $NaClO_3$	10
Daphnia magna (water flea)	-	-	n	-	-	-	-	24 h	880	LC50 - $KClO_3$	11
Daphnia magna (water flea)	-	-	n	-	-	-	-	24 h	600	NOEC (survival) - $KClO_3$	11
Daphnia magna (water flea)	juvenile	-	n	-	-	-	-	48 h	1098	LC50 - $NaClO_3$	12
Daphnia pulex (water flea)	juvenile	-	n	-	-	-	-	3 h	>31	NOEC (survival) - $NaClO_3$	13
Asellus hilgendorf (sowbug)	juvenile	S	n	19.2	-	-	-	48 h	7372	TLm - $NaClO_3$	14
								96 h	5019	TLm - $NaClO_3$	14
								24 h	3215[3]	TLm - grain formulation[2]	14
								48 h	2666[3]	TLm - grain formulation[2]	14
								96 h	2196[3]	TLm - grain formulation[2]	14
Gammarus sp. (shrimp)	adult	field[1]	m	7.8-12.6	-	6.4-7.2	-	6 h	44.7-1.49[1]	65% mortality	20

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Nitrogen source (mg N l^{-1})	Exposure duration	Conc mg ClO_3 l^{-1}	Effect/test substance	Ref
								24 h	44.7-1.49[1]	65% mortality	20
								48 h	44.7-1.49[1]	75% mortality	20
								72 h	44.7-1.49[1]	80% mortality	20
								96 h	44.7-1.49[1]	90% mortality	20
INSECTS											
Stenopysche griseipennis (caddisfly)	0.35g	S	n	17.2	-	8.5-9.0	-	24 h	2431[3]	LC50 grain formulation[2]	14
								48 h	2431[3]	LC50 grain formulation[2]	14
								96 h	2118[3]	LC50 grain formulation[2]	14
Cloeon dipterum (mayfly)	-	-	n	-	-	-	-	48h	>31	LC50 - $NaClO_3$	15
Ephemera japonica (mayfly)	nymphs	field[1]	m	7.8-12.6	-	6.4-7.2	-	6 h	44.7-1.49[1]	50% mortality	20
								24 h	44.7-1.49[1]	60% mortality	20
								48 h	44.7-1.49[1]	60% mortality	20
								72 h	44.7-1.49[1]	65% mortality	20
								96 h	44.7-1.49[1]	70% mortality	20
FISH (SALMONIDS)											

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Nitrogen source (mg N l^{-1})	Exposure duration	Conc mg ClO_3 l^{-1}	Effect/test substance	Ref
Oncorhynchus mykiss (rainbow trout)	-	-	n	-	-	-	-	24 h	3294	LC50 - $NaClO_3$	16
Oncorhynchus mykiss (rainbow trout)	-	S	n	-	-	-	-	48 h	2157	LC50 - $NaClO_3$	17
Oncorhynchus mykiss (rainbow trout)	-	-	n	-	-	-	-	48 h	930	LC50 - $NaClO_3$	18
Oncorhynchus mykiss (rainbow trout)	-	S	n	15	-	6.3	-	96 h	1373	LC50 - $NaClO_3$	19
Oncorhynchus mykiss (rainbow trout)	8.8g	field[4]	n	-	-	-	-	7 w	47	No effect on growth - $NaClO_3$	21
Oncorhynchus masou (cherry salmon)	finger-lings	SS	n	17.5	-	7.0-8.0	-	24 h	9412[3]	TLm - $NaClO_3$	14
								48 h	7843[3]	TLm - $NaClO_3$	14
								96 h	2510[3]	TLm - $NaClO_3$	14
Oncorhynchus masou (cherry salmon)	larvae	field[1]	m	4.1-12.8	-	6.4-7.2	-	48 h	44.7-1.49[1]	No mortality - $NaClO_3$	20

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Nitrogen source (mg N l^{-1})	Exposure duration	Conc mg ClO_3 l^{-1}	Effect/test substance	Ref
FISH (NON-SALMONIDS)											
Carassius auratus (goldfish)	6-9 cm, 3-5 g	S	n	18-23	"hard"	7.7	-	120 h	784	No apparent injuries	28
Cyprinus carpio (carp)	juveniles	-	n	-	-	-	-	48 h	>1310	EC50 (behavioural/survival) - $NaClO_3$	15
								96 h	5561	LC50 - $NaClO_3$	22
								96 h	5784	LC50 - $Mg(ClO_3)_2$	22
Tribolodon hakonensis (Japanese barbel)	4 cm, 0.5g	S	n	20.6	-	8.7-8.8	-	6 h	3843[3]	LC50 - grain formulation[2]	14
								12 h	3686[3]	LC50 - grain formulation[2]	14
								24 h	3294[3]	LC50 - grain formulation[2]	14
								48 h	2980[3]	LC50 - grain formulation[2]	14
								96 h	2980[3]	LC50 - grain formulation[2]	14
Perca fluvatilis (perch)	-	S	-	-	-	-	-	-	8628	Toxicity threshold - $NaClO_3$	29

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Nitrogen source (mg N l^{-1})	Exposure duration	Conc mg ClO_3 l^{-1}	Effect/test substance	Ref
Rutilus rutilus (roach)	juveniles	-	n	-	-	-	-	96 h	5561	LC50 - $NaClO_3$	22
								96 h	5784	LC50 - $Mg(ClO_3)_2$	2
Alburnus alburnus (bleak)	-	S	-	-	-	-	-	-	10192	Toxicity threshold - $NaClO_3$	29
Pimephales promelas (fathead minnow)	juveniles	-	n	-	-	-	-	96 h	≥784	NOEC (survival) - $NaClO_3$	23
	juveniles	S	n	16-23	140-210	7.2-7.6	-	1.1-1.3 h	18817	LT50 -$NaClO_3$	24
	juveniles	S	n	16-28	140-210	7.2-7.3	-	3.4-4.5 h	14113	LT50 -$NaClO_3$	24
	juveniles	S	n	15.8	140-210	7.2	-	96 h	10820	LC50 -$NaClO_3$	24
	juveniles	S	n	23	140-210	7.4	-	96 h	10663	LC50 -$NaClO_3$	24
	juveniles	S	n	28.7	140-210	7.5	-	96 h	10584	LC50 -$NaClO_3$	24
Phoximus phoximus (minnow)	juveniles	-	n	-	-	-	-	96 h	5561	LC50 - $NaClO_3$	22
								96 h	5784	LC50 - $Mg(ClO_3)_2$	22
Ictalurus punctatus (channel catfish)	-	-	n	-	-	-	-	24 h	2476	LC50 - $NaClO_3$	16

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Nitrogen source (mg N l^{-1})	Exposure duration	Conc mg ClO_3 l^{-1}	Effect/test substance	Ref
Leuciscus idus (golden orfe)	-	S	n	-	-	-	-	-	2880	LC50 - $KClO_3$	25 **
Leuciscus leuciscus (dace)	3.2 cm, 0.25g	S	n	20.7	-	7.4-7.9	-	24 h	9412[3]	E50 (behaviour/survival) - $NaClO_3$	14
								48 h	9098[3]	TLm - $NaClO_3$	14
								96 h	7843[3]	TLm - $NaClO_3$	14
								240 h (10 d)	4705[3]	TLm - $NaClO_3$	14
Brachydanio rerio (zebrafish)	juveniles	-	n	-	-	-	-	96 h	≥784	NOEC (survival) - $NaClO_3$	26
Rasbora heteromorpha (harlequin fish)	1.3-3 cm	F	n	20	20	7.2	NO_3 (0.7)	24 h	6745	LC50 - $NaClO_3$	27
Oryzias latipes (killifish)	-	-	n	-	-	-	-	24 h	>31	LC50 - $NaClO_3$	15
Opsariichtys uncirostris (toadfish)	juveniles	-	n	-	-	-	-	96 h	5561	LC50 - $NaClO_3$	22
								96 h	5784	LC50 - $Mg(ClO_3)_2$	22

Notes to Table B2.1

[1] $NaClO_3$ applied to a forest stream by helicopter. Invertebrates and fish in a nearby stream were caged (separately) prior to and during application. Concentrations of $NaClO_3$ in the water were measured from the time of application (57 mg $NaClO_3$ l^{-1} or 44.7 mg ClO_3^- l^{-1}) to 4 days after application (1.9 mg $NaClO_3$ l^{-1} or 1.49 mg ClO_3^- l^{-1}) (refer to Section B2.6 for details).

[2] The grain formulation (Kusa-tohru) was tested. This was reported to be 50% sodium chlorate and approximately 30% sodium bicarbonate and 20% "clayey" material.

[3] LC50s were reported by the authors as concentrations of the active ingredient (i.e. sodium chlorate) but have been converted here to mg chlorate l^{-1}.

[4] Tests were conducted in artificial streams. An initial dose of 47 mg ClO_3^- l^{-1} was reduced to zero after 7 hours. Growth was measured by body length and weight. Refer to Section B2.8.2 for further details.

-	Information unavailable or not reported
S	Static laboratory bioassay
SS	Semi-static (renewal) laboratory bioassay
F	Flow-through laboratory bioassay
field	Field bioassay
m	Measured concentration (i.e. analysed)
n	Nominal concentration (i.e. not analysed)
w	Weeks
d	Days
h	Hours
LC50	Median lethal concentration
EC50	Median effect concentration
LT50	Time taken to cause 50% mortality at a given concentration
LOEC	Lowest observed effect concentration
NOEC	No-observed effect concentration

References:

1. Cenci *et al* (1975)
2. Bringmann and Kuhn (1959)
3. Bringmann and Kuhn (1980)
4. Macauley (unpublished data, cited by van Wijk and Hutchinson 1995)
5. Solomonsson and Vennesland (1972)
6. Kroon (1993, cited by van Wijk and Hutchinson 1995)
7. Hutchinson (1994a)
8. Bringmann and Kuhn (1978)
9. Reynolds (1978)
10. McKee and Wolf (1963)
11. Bringmann and Kuhn (1977)
12. Jones (1941, cited by CIS 1997)
13. Nishiuchi (1982)
14. Matida *et al* (1976)
15. Nishiuchi (1980)
16. Pimental (1971, cited by Environment Canada 1985)
17. WQCDB (1971, cited by Environment Canada 1985)
18. Nishiuchi and Iwamura (1983)
19. Beech (1983, cited by Environment Canada 1985)
20. Matida *et al* (1975a)
21. Matida *et al* (1975b)
22. Agaev *et al* (1986)

23. Mark and Arends (1993)
24. Shifrer *et al* (1974)
25. Juhnke and Leudemann (1978)
26. Mark and Hantink-de Rooij (1991)
27. Alabaster (1969)
28. Ellis (1937)
29. Meinck (1956, cited by Shifrer *et al* 1974)
30. Perrin and Bothwell (1992)

A large toxicity dataset exists for chlorate, with data available for six species of bacteria, two species of cyanobacteria, four species of algae (and one mixed diatom community), one species of protozoan, one species of platyhelminthes, four species of crustaceans, three species of insects and 17 species of fish (see Table B2.1). The majority of studies reported in the literature relate to acute, single-species, static tests. However, there appears to be only very limited information on the effects of long-term exposure to chlorate.

Chlorate salts are very soluble in water (e.g. 957 g $NaClO_3$ l^{-1}) and information on the stability of the chlorate ion in water (see Section A1.6, Appendix A) indicate that it will not appreciably degrade during the course of most acute toxicity tests, although this will depend upon the level of oxidisable organic matter present since chlorate is an oxidant. The American Water Works Association (AWWA 1994) reported that 0.5 mg l^{-1} of chlorate in drinking water did not degrade after 20 days. The demand of surface waters for chlorate is likely to be higher than that of drinking water because surface waters generally contain higher levels of oxidisable organic matter. Chlorate is therefore likely to be less persistent in surface waters than drinking water. Nevertheless, the relative stability of chlorate suggests that static test systems based on nominal concentrations are suitable for determining the effects of acute exposure to chlorate, and although not ideal, may also be adequate for chronic exposures depending on the organic content of the test water used.

B2.1 Bacteria

Chlorate appears to be of high acute toxicity to most bacteria, with reported effect concentrations ranging from 0.1 to 4 mg ClO_3 l^{-1} (see Table B2.1). However, the majority of studies were poorly reported and few details of the test procedures used were provided. In addition, the ecological significance of some of the toxicity endpoints measured is not clear. For example, Cenci *et al* (1975) determined LOECs of 0.1 mg ClO_3^- l^{-1} for *Sphaerotilus natans* and *Leptothrix chloandii* using the inhibition of iron metabolism as an endpoint.

It has been suggested that the effects of chlorate on lower organisms are not directly related to chlorate itself but instead may be due to the enzymatic reduction of chlorate to chlorite, and possibly hypochlorite (Van Wijk and Hutchinson 1995). Van Wijk and Hutchinson (1995) cited a number of studies (although predominantly with algae) which indicate that the enzyme systems involved in the reduction of chlorate to chlorite are normally responsible for the reduction of nitrate, and that chlorate and nitrate compete for enzyme active sites. If this theory applies to bacteria it is likely that the source and amount of nitrogen available will influence the ability of bacteria to reduce chlorate and therefore influence the overall toxicity. However, a few studies also cited by Van Wijk and Hutchinson (1995) suggest that some bacteria may have separate chlorate-reducing enzyme systems (e.g. Oltmann *et al* 1976), in which case the effect of nitrate may be reduced. Further studies are required to confirm the likely mechanism of chlorate toxicity in bacteria.

B2.2 Cyanobacteria (blue-green algae)

The information available for cyanobacteria is limited to just two species and, because few details of the test procedures used were provided, the resulting data are difficult to interpret.

Cenci *et al* (1975) reported a LOEC (lowest observed effect concentration) of 0.1 mg ClO_3^- l^{-1} for *Nostoc muscorum*, based on the inhibition of growth, but they do not state the duration of

exposure or any other details of the test. Bringmann and Kuhn (1978) determined a toxicity threshold (cell multiplication inhibition) of 12 mg ClO_3^- l^{-1} for *Microcystis aeruginosa* after eight days exposure, which suggests that chlorate is of moderate chronic toxicity to this species.

The results of a number of studies on model outdoor ecosystems have indicated that the growth of some saltwater cyanobacteria may be stimulated by chlorate-containing pulp mill effluents over a period of months (e.g. Rosemarin 1990, 1994) (see Section C1.2, Appendix C). However, there does not appear to be any evidence of stimulated growth reported in the literature for freshwater cyanobacterium species.

B2.3 Algae

The data available for freshwater algae relate almost entirely to unicellular green algal species (i.e. *Chlorella* sp. and *Scenedesmus* spp.). Although some poorly reported studies suggest that chlorate may be toxic to green algae at concentrations as low as 0.1 and 3 mg ClO_3^- l^{-1}, other studies indicate that it is of low acute and chronic toxicity to the species tested, with LOECs and NOECs in excess of 300 mg ClO_3^- l^{-1}.

The lowest toxicity value reported is the LOEC of 0.1 mg ClO_3^- l^{-1} determined for *Chlorella vulgaris* by Cenci *et al* (1975) but, as the duration of this test was not reported it is not known whether this is an acute or chronic value. The next lowest acute toxicity value, therefore, is the acute toxicity threshold of 3 mg ClO_3^- l^{-1} recorded for *Scenedesmus* sp. (Bringmann and Kuhn 1959), where nitrate (NO_3) was used as a source of nitrogen. It was not clear from this paper (written in German) which toxicological end-point this LOEC was determined for but, based on other work published by Bringmann and Kuhn (e.g. Bringmann and Kuhn 1980), it is likely that cell multiplication inhibition was used as the end-point. As already mentioned, however, the values reported by Cenci *et al.* (1975) and Bringmann and Kuhn (1959), are not in agreement with those determined in other studies.

Solomonsson and Vennesland (1972) reported that exposure of *Chlorella vulgaris* to a nominal concentration of 334 mg ClO_3^- l^{-1} for 48 hours in a culture medium containing 280 mg NO_3^- l^{-1} caused only a 5% reduction in cell numbers compared to the control. Exposure to 3340 mg ClO_3^- l^{-1} in the same type of medium, however, caused a 79% reduction in cell numbers. The authors also obtained evidence which suggests that the toxicity of chlorate to *C. vulgaris* is dependent on the source and amount of nitrogen available to the growing cultures. For example, when cells were exposed to 334 mg ClO_3^- l^{-1} in media containing reduced nitrate (28 mg NO_3^- l^{-1}) a 61% reduction in cell numbers was recorded compared to control tests. The same concentrations of chlorate (334 and 3340 mg ClO_3^- l^{-1}) had no detectable inhibitory effects on cells grown for four days or more in media containing ammonium (NH_4^+) as a source of nitrogen.

Based on this information, and work previously carried out on terrestrial plants (e.g. Aberg 1947), the authors suggested that chlorate is not directly toxic to algae but, depending on the source of nitrogen available, may be enzymatically reduced to the more toxic chlorite ion (ClO_2^-). The enzyme system involved in the reduction of chlorate by algae (and some bacteria) has been identified as the nitrate-reductase system (Aberg 1947, Hofstra 1977, cited by Rosemarin *et al* 1986), and the popular theory is that chlorate, taken up along with nitrate, competes for nitrate-reductase active sites. The amount of chlorite produced from the

reduction of chlorate (and thus the level of toxicity induced) therefore depends on the ratio of chlorate to nitrate. Solomonsson and Vennesland (1972), for example, found that a ratio of approximately two was sufficient to significantly inhibit the cell multiplication of *C. vulgaris*. Those experiments carried out with ammonium (e.g. NH_4^+) as the only source of nitrogen suggest that ammonium represses the nitrate-reductase enzyme in algae, thus reducing the reduction of chlorate to chlorite. The condition of algae (i.e. nitrogen starvation, stage of growth and life cycle) may also determine how toxic a certain level of chlorate may be, and it therefore follows that chlorate toxicity should occur most often in nitrogen-limited systems (Rosemarin *et al* 1986).

If the above theory on the mode of toxic action of chlorate to algae is correct, proper comparisons of the different algal studies can only be made if the source and amount of nitrogen are known, and this could partly explain the differences between the effect concentrations reported by Solomonsson and Vennesland (1972) and Cenci *et al* (1975) for *C. vulgaris*. According to the findings of Solomonsson and Vennesland (1972), where a chlorate-to-nitrate ratio of two causes significant adverse effects on cell multiplication, the concentration of nitrate applied by Cenci *et al* (1975) would have to have been about half (i.e. 0.05 mg l^{-1}) of the chlorate applied. However, this information was not reported by Cenci *et al* (1975).

Hutchinson (1994a) and Kroon (1993, cited by Van Wijk and Hutchinson 1995) examined the effects of sodium chlorate on *Scenedesmus subspicaudum* and *Scendesmus quadricauda*, respectively, using tests based on the OECD Guideline Method 201. Hutchinson (1994a) found that there were no significant effects on the biomass and growth rate of *S. subspicaudum* after 72 hours exposure to nominal concentrations of up 3137 mg ClO_3^- l^{-1} (i.e. LOEC = >3137 mg ClO_3^- l^{-1} and NOEC = 3137 mg ClO_3^- l^{-1}) and Kroon (1993) reported that the 96-hour NOEC (inhibition of growth) for *S. quadricauda* was greater than the highest concentration tested (i.e. 784 mg ClO_3^- l^{-1}). In both tests the source of nitrogen used was ammonium. In contrast, tests carried out on *Scendesmus* sp. by Bringmann and Kuhn (1959 and 1980) suggest that chlorate may adversely affect growth following acute exposure (toxicity threshold 3 mg l^{-1}) and chronic exposure (7-day toxicity threshold 0.24 mg l^{-1}) with nitrate as the nitrogen source. Based on the findings of Solomonsson and Vennesland (1975) one might expect a higher level of chlorate toxicity with the presence of nitrate, but data produced by Bringmann and Kuhn suggest that a much lower chlorate-to-nitrate ratio will significantly reduce the growth of *Scenedesmus* sp. than for *Chlorella vulgaris* (see Table B2.1). However, few details of the test procedures and performance were available to review the earlier report published by Bringmann and Kuhn (1959) and there are some doubts about the relevance of their later study with *Scendesmus* sp. (Bringman and Kuhn 1980). In the latter study, cell multiplication was measured turbidimetrically and expressed by the extinction coefficient of light. The toxicity threshold was defined as being the concentration which caused a mean extinction value that was 3% or more below the mean extinction value for "non-toxic" dilutions, but the authors do not offer an explanation for the selection of 3% as an extinction threshold or its ecological relevance.

Mesocosm studies have identified certain "keystone" saltwater macroalgal species in the Baltic Sea (i.e. species that are key to the overall ecosystem structure) and have shown that these are more sensitive to chlorate than others (see Sections C1.2 and C2.2 for details). Using specially designed 'experimental trough apparatus' Perrin and Bothwell (1992) investigated whether chlorate had similar effects on freshwater diatom communities. Under low ambient

nitrate concentrations (approximately 0.01 mg l^{-1}), chlorate additions of up to 0.5 mg l^{-1} did not reduce the specific growth rates or change the taxonomic composition of the attached diatom community, which was dominated by *Fragilaria crotonensis*, *Synedra ulna*, *Fragilaria vaucheriae* and *Tabellaria fenestrata*. The authors concluded that chlorate discharged into river environments under these conditions will not cause changes to the dominant algal producers such as diatoms. However, data for more freshwater algae are ideally required to ensure that any potential freshwater keystone species have been considered.

B2.4 Protozoans

In the only study to have considered a freshwater protozoan, Bringmann and Kuhn (1980) determined a 72-hour toxicity threshold of 817 mg l^{-1} for *Entosiphon sulcatum*.

B2.5 Platyhelminthes

The flatworm (*Polycelius nigra*) was found to be tolerant to relatively high concentrations of chlorate (Jones 1941, cited by CIS 1997). In a static test, based on nominal concentrations, a 48-hour LC50 of 12 549 mg ClO_3^- l^{-1} was determined. However, this test was conducted more than 50 years ago, and although the original report was not available for review, it did not meet the quality criteria required by the US EPA AQUIRE database (CIS 1997).

B2.6 Crustaceans

Chlorate appears to be of low acute toxicity to the few crustaceans tested in the laboratory, with effect concentrations greater than 600 mg l^{-1} (see Table B2.1). However, there is some evidence from field trials that freshwater shrimps (*Gammarus* sp.) are significantly more sensitive. There are no data on the effects of long-term exposure to chlorate.

Bringmann and Kuhn (1977) determined a 24-hour LC50 of 880 ClO_3^- mg l^{-1}, with a corresponding NOEC of 600 mg ClO_3^- l^{-1} (based on nominal concentrations), for the water flea *Daphnia magna*.

Matida *et al* (1976) exposed *Asellus hilgendorffii* to varying nominal concentrations of reagent grade sodium chlorate and "Kusa-tohra" (a 50% grain formulation of sodium chlorate) in static laboratory tests. The 96-hour median lethal thresholds (TLm), which is considered equivalent to the LC50, were 5019 and 2196 mg ClO_3^- l^{-1}, respectively, indicating low acute toxicity to this species.

In an earlier study, Matida *et al* (1975a) examined the effects of sodium chlorate on caged *Gammarus* sp. (shrimps) following aerial application of sodium chlorate to a stream. Twenty gammarids were placed in single cages at each of three sites (a control stream, the contaminated stream and the confluence between the control and contaminated stream). When deploying gammarids in the field, however, it is preferable to isolate individuals since this genus can be cannibalistic. Analysis of contaminated stream water showed a peak chlorate concentration of 44.7 mg l^{-1} half-an-hour after application which was gradually reduced to about 1.9 mg ClO_3^- l^{-1} after about 96 hours. The results of this study, and those for the insect *Ephemera japonica*, are summarised in Table B2.2. It appears that exposure of *Gammarus* sp. to an initial chlorate concentration of 44.7 mg l^{-1} caused significant mortality compared to the control stream upstream of the contaminated area. Gammarids caged at the confluence of the

control and contaminated streams were exposed to concentrations of chlorate ranging from 29.8 to 0.9 mg l^{-1}, with 70% mortality after 96 hours.

Although several species of *Gammarus* may be found in UK waters, the tests carried out by Matida *et al* (1975a) were performed in Japan and therefore might be considered less relevant for assessing the effects of chlorate on UK species. In addition, it is conceivable that factors other than chlorate influenced the mortality of gammarids in this study (e.g. the presence of other toxicants, cannibalism), so the results cannot necessarily be attributed to the effects of chlorate alone. Data for known UK or European species of *Gammarus* are ideally required to confirm whether this genus is more sensitive to chlorate than other crustaceans and further field studies with crustaceans are needed to determine whether exposure to chlorate in the field is more significant than in the laboratory.

B2.7 Insects

The limited data available suggest that chlorate is of low acute toxicity to aquatic insects, although evidence from a single field study indicates that mayfly nymphs may be sensitive to chlorate. No chronic toxicity data are available.

Matida *et al* (1976) studied the effects of a grain formulation (50% sodium chlorate) on caddisfly larvae in a static test. The authors determined 24, 48 and 96-hour LC50s of 2431, 2431 and 2118 mg $ClO_3^-l^{-1}$, respectively. However, the grain formulation also contained about 30% sodium bicarbonate and the high alkalinity recorded (pH 8.5-9.0), probably due to the presence of the bicarbonate, may have influenced the response of test animals to chlorate. The LC50s reported by Matida *et al* (1975a) are therefore likely to have been overestimated.

As with crustaceans, the results of the laboratory studies do not agree with the findings of a field study performed with caged mayfly nymphs (*Ephemera japonica*) in Japan (Matida *et al* 1975a). This study, which is summarised in more detail in Section B2.6, showed that after 96 hours exposure to an initial chlorate concentration of 44.7 mg l^{-1}, 70% of the caged nymphs died (see Table B2.2). In comparison, 35% of the nymphs caged in a control stream died after the same exposure period. However, for reasons similar to those described in Section B2.6, this study is not considered to be very reliable.

B2.8 Salmonid fish

The information on salmonid fish is restricted to just two species, the rainbow trout (*Oncorhynchus mykiss*) and cherry salmon (O*ncorhynchus. masou*), neither of which are particularly relevant to UK waters (rainbow trout are introduced to some lakes and rivers for sport fishing). Nevertheless, the data reported for both species suggest that chlorate is of low acute toxicity (effect concentrations greater than 930 mg ClO_3^- l^{-1}). One poor-quality study with *O. mykiss* reported no adverse effects on growth after seven weeks, although the initial dose of 47 mg ClO_3^- l^{-1} reduced to zero after seven hours. Better quality data are therefore required to assess the effects of long-term exposure to chlorate.

Table B2.2 The effects of aerial chlorate application on caged *Gammarus* sp. and *Ephemera japonica* (after Matida *et al* 1975a)

	Time (hours)					
	0.5	7	24	48	72	96
Contaminated stream:						
Measured Conc. (mg ClO_3^- l^{-1})	44.7	14.9	1.6	1.0	0.7	1.9
% Mortality of *Gammarus* sp.	NR	65	65	75	80	90
% Mortality of *E. japonica*	NR	50	60	60	65	70
Confluence of contaminated and control streams:						
Measured Conc. (mg ClO_3^- l^{-1})	29.8	7.8	2.4	0.9	0.4	0.9
% Mortality of *Gammarus* sp.	NR	35	40	45	55	70
% Mortality of *E. japonica*	NR	25	35	35	35	35
Control stream (upstream of release):						
Measured Conc. (mg ClO_3^- l^{-1})	ND	ND	ND	NR	NR	ND
% Mortality of *Gammarus* sp.	NR	10	22	25	30	30
% Mortality of *E. japonica*	NR	5	10	10	10	20

Notes

NR: not reported ND: not detected

B2.8.1 Acute toxicity

The lowest acute value reported was the 48-hour LC50 of 930 mg ClO_3^- l^{-1} determined by Nishiushi and Iwamura (1983, cited by Van Wijk and Hutchinson 1995) for juvenile *O. mykiss*, but as a translation of the original report, written in Japanese, was not available it was not possible to fully assess the reliability of this test. Acute toxicity values for *O. mykiss* have been cited by Environment Canada (1985) (i.e. 24-, 48- and 96-hour LC50s of 3294, 2157 and 1373 mg ClO_3^- l^{-1}, respectively), although all of these values were from different tests and few details of these tests were provided.

The 24-, 48- and 96-hour median lethal thresholds (equivalent to LC50) reported by Matida *et al* (1976) for cherry salmon fingerlings (*O. masou*) were 9412, 7843, 2510 mg ClO_3^- l^{-1}, respectively. A semi-static test system was employed by Matida *et al* (1976), with test solutions renewed approximately every 24 hours. In a field trial, Matida *et al* (1975a) applied sodium chlorate to a stream in a forested catchment by helicopter (peak concentration of

44.7 mg ClO_3^- l^{-1}) and found that all of the salmon (*O. masou*) that had been deployed in cages the day before survived. Further details of this field trial are provided in Section B2.6.

B2.92 Chronic toxicity

Matida *et al* (1975b) applied a single dose of 47 mg ClO_3^- l^{-1} to an artificial stream containing 30 rainbow trout (*Oncorhynchus mykiss*). The growth (measured by body length and weight) of test fish and control fish steadily increased over a period of seven weeks, but at no time was growth found to differ significantly from the control fish. In addition, the authors showed that the initial dose of chlorate reduced to zero after about seven hours, so for the majority of the seven-week test period fish were not exposed to any chlorate. This test cannot therefore be used to assess the effects of long-term chlorate exposure.

B2.9 Non-salmonid fish

With one possible exception, all the acute effect concentrations reported for non-salmonid fish (15 different species) are greater than 784 mg ClO_3^- l^{-1}, and the majority are greater than 2000 mg ClO_3^- l^{-1}. Chlorate is therefore considered to be of low acute toxicity to non-salmonid fish.

There are no chronic toxicity data available. However, a median lethal threshold (i.e. LC50) of 4705 mg ClO_3^- l^{-1} was reported after 10 days exposure of dace (*Leuciscus leuciscus*) to sodium chlorate, suggesting that chlorate is of low sub-chronic toxicity to this species.

B2.9.1 Acute toxicity

As mentioned above, the majority of acute effect concentrations reported for non-salmonid fish are greater than 784 mg ClO_3^- l^{-1}, and most of those are greater than 2000 mg ClO_3^- l^{-1}.

The one possible exception is a study performed by Nishiuchi (1980) with the Japanese killifish *Oryzias latipes*. In this case the LC50 was reported to be greater than the highest concentration tested, which was 31 mg ClO_3^- l^{-1}. Based on the evidence for other fish, the actual LC50 for *O. latipes* would be expected to be at least in the order of a thousand mg ClO_3^- l^{-1}, but without experimental confirmation the possibility of this species of fish being more sensitive to chlorate than others cannot be entirely discounted.

Shifrer *et al* (1974) found that temperature had no significant effect on juvenile fathead minnows (*Pimephales promelas*) exposed to sodium chlorate in a static test system for up to 96 hours. The 96-hour LC50s (based on nominal concentrations) reported for mean test temperatures of 15.8, 23 and 28.7 ^oC were 10 820, 10 663 and 10 584 mg ClO_3^- l^{-1}, respectively. As one would expect, higher concentrations of sodium chlorate were found to cause the same level of mortality (i.e. 50%) in much shorter times. For example, the LT50 for *P. promelas* exposed to 18 817 mg ClO_3^- l^{-1} ranged from 1.1 to 1.3 hours at temperatures from 16 to 23 ^oC. More recently, Mark and Arends (1993, cited by Van Wijk and Hutchinson 1995) reported that the LC50 for *P. promelas* in tests believed to have been carried out to EEC/OECD guidelines was greater than the highest concentration tested (i.e. 784 mg $ClO_3^-l^{-1}$), confirming the low acute toxicity of chlorate to non-salmonid fish.

Mark and Hantink-de Rooij (1991, cited by Van Wijk and Hutchinson 1995) are reported to have carried out tests on the zebrafish (*Brachydanio rerio*) following EEC/OECD test guidelines. No effects were observed at the highest concentrations tested (784 mg ClO_3^- l^{-1}), but a copy of the study report was not available for review.

B2.9.2 Sub-chronic toxicity

The longest test duration reported for non-salmonid fish exposed to chlorate in the laboratory was 10-days. The standard duration of chronic exposure studies recommended by the OECD for juvenile or adult fish is 14 days, so 10 days can only be considered as sub-chronic.

Matida *et al* (1976) exposed dace (*Leuciscus leuciscus*) to sodium chlorate in a static test system and reported a median lethal threshold (i.e. LC50), based on nominal concentrations, of 4705 mg ClO_3^- l^{-1} after 10 days exposure. It is not clear from the authors' description of the test procedure used whether or not the test solutions were renewed. In short-term tests with another fish (*Oncorhynchus masou*) they specify that the test solutions were renewed daily, but they do not provide any such information for the tests performed with dace. It is therefore conceivable that some of the sodium chlorate nominally applied degraded during the exposure period, in which case the toxicity value reported may be considered a slight underestimate. However, this is unlikely to affect the overall conclusion that chlorate is of low sub-chronic toxicity to *L. leuciscus*.

B3. CHLORITE (ClO_2^-)

Information on the toxicity of chlorite ion (ClO_2^-) to freshwater life is restricted to acute laboratory tests and three species of fish and one species of crustacean (see Table B3.1). The absence of toxicity data for lower organisms such as bacteria and algae may be significant to the overall assessment of chlorite since, like chlorine dioxide, it has strong oxidising properties.

All of the tests reported in the literature used the sodium chlorite salt. Sodium chlorite is highly soluble in water, increasing with increasing temperature (340 g l^{-1} at 5 ^oC, 390 g l^{-1} at 17 ^oC and 460 g l^{-1} at 30 ^oC) (Merck 1989). The molecular proportion of chlorite ion in sodium chlorite is about 75% (molecular weights of $NaClO_2$ and ClO_2^- are 90.5 and 67.5, respectively) and all the concentrations cited in this report have been converted to concentrations of the chlorite ion on this basis.

Chlorite is more stable in water than chlorine dioxide. Studies on the stability of chlorite in drinking water suggest that initial concentrations degrade rapidly (about 12%) within the first three days but then become relatively stable for up to at least 18 days (AWWA 1994). Because of the greater presence of oxidisable material in surface waters, chlorite is expected to be less stable in surface waters than drinking water, and depending on the type of water used, toxicity tests should ideally include the measurement of actual exposure concentrations to confirm nominal levels and/or renew test solutions. Although some of the toxicity tests reported for chlorite appear to have been based on standard guideline methods (e.g. OECD, ASTM), all are based on nominal concentrations.

The limited data suggest that chlorite is of high acute toxicity to the water flea *Daphnia magna*, with effect concentrations ranging from 0.018 to >0.69 mg ClO_2^- l^{-1}. The fish tested appear to be of lower sensitivity to chlorite, with acute LC50s reported from 34.5 mg ClO_2^- l^{-1}.

B3.1 Crustaceans

Toxicity data only appear to be available for the water flea *Daphnia magna*. All of these data are reported on the IUCLID database (1996), which provides only limited background information on the studies cited.

The acute EC50s reported for *D. magna* suggest that chlorite is of high acute toxicity to this species. However, there are significant differences between these values. A 48-hour EC50 of 0.018 mg ClO_2^- l^{-1} (with a corresponding NOEC of <0.002 mg ClO_2^- l^{-1}) was determined using the American Society for Testing and Materials (ASTM) standard method for static, acute toxicity tests (Degussa Corporation 1984), although the 48-hour EC50 calculated in a second, unspecified test was 0.2 mg ClO_2^- l^{-1} (corresponding NOEC 0.069 mg ClO_2^- l^{-1}) (Kirk-Othmer, cited by IUCLID 1996). A flow-through test with *D. magna* (apparently following US EPA FIFRA guidelines and Good Laboratory Practice) was also reported by the IUCLID database, with a 24-hour EC50 of >0.69 mg ClO_2^- l^{-1} (>1 mg $NaClO_2$ l^{-1}).

B3.2 Salmonid fish

Cairns and Conn (1979) exposed juvenile rainbow trout (*Oncorhynchus mykiss*) to nominal concentrations of 10 and 100 mg $NaClO_2$ l^{-1} (7.5 and 75 mg ClO_2^- l^{-1}, respectively) in non-disinfected wastewater and dechlorinated water, respectively, for 96-hours and found that all fish survived. In addition, more than 50% of fish survived when placed in the non-disinfected effluent without sodium chlorite, although the specific percentage survival was not stated. This study suggests that chlorite is of moderate acute toxicity to *O. mykiss*, but much more data are needed for confirmation.

B3.3 Non-salmonid fish

The limited data available indicate that chlorite is of moderate to low acute toxicity the bluegill sunfish (*Lepomis macrochirus*) and the zebra fish (*Brachydanio rerio*), with LC50s ranging from 37.5 to >375 mg ClO_2^- l^{-1}.

The two 48-hour LC50s reported for *L. macrochirus* are 37.5 and 156 mg ClO_2^- l^{-1}. Both values are referred to the same source (Kirk-Othmer, cited by IUCLID 1996), which provides no further information on the tests used to determine them.

Hoechst (1985a-e) carried out acute toxicity tests with the zebra fish (*Brachydanio rerio*) and a variety of sodium chlorite products (see Table B3.1) based on the OECD Guideline Method 203. The 96-hour LC50s reported range from 75 to >375 mg ClO_2^- l^{-1}, although these were based on nominal concentrations of chlorite. Specific information on the products studied (e.g. composition, purity) was not provided. However, since the LC50s were obtained from a datasheet for sodium chlorite it is assumed that they were expressed as concentrations

of the sodium chlorite rather than the product as a whole. These concentrations have been converted to concentrations of chlorite (ClO_2^-) in this report.

Table B3.1 Toxicity of chlorite (ClO_2^-) to freshwater life

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure duration	Conc.[1] (mg ClO_2 l^{-1})	Effect/test substance	Ref
CRUSTACEANS										
Daphnia magna (water flea)	-	S	n	-	-	-	24 h	0.0345	NOEC - $NaClO_2$ (80%)	1
							48 h	<0.002	NOEC - $NaClO_2$ (80%)	1
							48 h	0.018	EC50 - $NaClO_2$ (80%)	1
Daphnia magna (water flea)	-	-	n	-	-	-	24 h	>0.69	EC50 - $NaClO_2$ (80%)	2
							48 h	0.29	EC50 - $NaClO_2$	3
							48 h	0.069	NOEC - $NaClO_2$	3
SALMONID FISH										
Oncorhynchus mykiss (rainbow trout)	juvenile	S	n	-	-	-	96 h	6.9	No mortality - $NaClO_2$ plus wastewater effluent	9
							96 h	69	No mortality - $NaClO_2$ plus dechlorinated water	9

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure duration	Conc.[1] (mg ClO_2 l^{-1})	Effect/test substance	Ref
NON-SALMONID FISH										
Lepomis macrochirus (bluegill sunfish)	-	-	-	-	-	-	48 h	143.5	LC50 - $NaClO_2$	3
							48 h	34.5	LC50 - $NaClO_2$	3
Brachydanio rerio (zebra fish)	-	S	n	-	-	-	48 h	69-345	LC50 - $NaClO_2$ (Hoechst 40 N)	4
							96 h	69-345	LC50 - $NaClO_2$ (Hoechst 40 N)	4
							48 h	>345	LC50 - $NaClO_2$ (Hoechst 40 N)	5
							96 h	>345	LC50 - $NaClO_2$ (Hoechst 40 N)	5
							48 h	345	LC50 - $NaClO_2$ (Hoechst 80 K)	6
							96 h	345	LC50 - $NaClO_2$ (Hoechst 80 K)	6
							48	>345	LC50 - $NaClO_2$ (Hoechst 30 KS)	7
							96 h	>345	LC50 - $NaClO_2$ (Hoechst 30 KS)	7

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure duration	Conc.[1] (mg ClO_2 l^{-1})	Effect/test substance	Ref
							48 h	>345	LC50 - $NaClO_2$ (Hoechst 30 N)	8
							96 h	>345	LC50 LC50 - $NaClO_2$ (Hoechst 30 N)	8

Notes to Table B3.1:

[1] Concentrations have been converted to mg ClO_2 l^{-1} as 69% (based on molar proportions) of the concentrations reported for $NaClO_2$.

- Information unavailable or not reported
S Static laboratory bioassay
m Measured concentration (i.e. analysed)
n Nominal concentration (i.e. not analysed)
h Hours
LC50 Median lethal concentration
EC50 Median effect concentration
NOEC No-observed effect concentration

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1. Degussa Corporation (1984, cited by IUCLID 1996)
2. Environ Systems (1991, cited by IUCLID 1996)
3 Kirk-Othmer (cited by IUCLID 1996)
4. Hoechst AG (1985a, cited by IUCLID 1996)
5. Hoechst AG (1985b, cited by IUCLID 1996)
6. Hoechst AG (1985c, cited by IUCLID 1996)
7. Hoechst AG (1985d, cited by IUCLID 1996)
8. Hoechst AG (1985e, cited by IUCLID 1996)
9. Cairns and Conn (1979)

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APPENDIX C TOXICITY TO SALTWATER LIFE

Information available on the saltwater toxicity of chlorine dioxide is reviewed in this section (see Section C1 and Table C1.1). Because the fate and behaviour of chlorine dioxide in water (see Appendix A) indicates the possible release of the reaction products chlorite and chlorate to the aquatic environment following the use of chlorine dioxide, data for these compounds are also considered (see Sections C2 and C3, respectively). Another possible reaction product of chlorine dioxide is chloride ion (Cl^-) which has been covered in a previous WRc report (Gardiner and Smith 1992).

C1. CHLORINE DIOXIDE

Few studies appear to have specifically examined the effects of chlorine dioxide on saltwater organisms (see Section C1.1 and Table C1.1). However, some research has been carried out in recent years on the ecological effects of effluents from processes involving the use of chlorine dioxide, principally pulp bleaching processes. The results of these studies are summarised in Section C1.2.

C1.1 Single-substance studies

Data on the toxicity of chlorine dioxide to saltwater organisms are very limited and generally of poor quality. The available dataset comprises just two species of fish and one algal and one echinoderm species.

C1.1.1 Algae

In the only study to have considered saltwater algae, Hose *et al* (1989) exposed meiospores of the giant kelp (*Macrocystis pyrifera*) to nominal concentrations of a stabilised aqueous solution of 25% chlorine dioxide for 48 hours in a static test system. The lowest test concentration to cause a significant difference in meiospore germination compared to the control (i.e. the LOEC) was 25 mg l^{-1} and the highest no-observed effect concentration (i.e. the NOEC) was 2.5 mg l^{-1}. Germ tube length was found to be a less sensitive end-point as the LOEC and NOEC were 250 and 25 mg l^{-1}, respectively. The authors did not determine the actual exposure concentrations of chlorine dioxide, and since some chlorine dioxide is likely to have been consumed by the test media the reported toxicity values are probably underestimates of the true toxicity of chlorine dioxide.

The effects of chlorine dioxide on organelle structure and cell morphology of the green filamentous alga *Cladophora* sp. was studied by Betzer and Kott (1969). After 24 hours exposure, concentrations of 52 and 2.6 mg l^{-1} (expressed as Cl) were found to have significant effects on organelle structure and cellular morphology, respectively. However, the overall ecological significance of these endpoints is unknown.

Table C1.1 Toxicity of chlorine dioxide to saltwater life

Species	Life stage	Test type	Analysis	Temp (°C)	Salinity (‰)	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
ALGAE										
Macrocystis pyrifera (giant kelp)	meio-spores	S	n	15	33	-	48 h	25	LOEC (reduction in germination)	1
							48 h	2.5	NOEC (reduction in germination)	1
							48 h	250	LOEC (germ tube length)	1
							48 h	25	NOEC (germ tube length)	1
Cladophora sp. (green filamentous)	-	S	m	30	-	7.0	24 h	52 (as Cl)	Cellular effects (change in organelle structure)	3
							24 h	2.6 (as Cl)	Cell morphology	3
ECHINODERMS										
Strongylocentrotus purpuratus (purple sea urchin)	embryo	S	n	15	33	-	48 h	250	LOEC (% abnormalities)	1
							48 h	25	NOEC (% abnormalities)	1
MOLLUSCS										
Cerastoderma edule (cockle)	adult	SS	n	15	-	-	48 h	>500[1]	LC50	4

Species	Life stage	Test type	Analysis	Temp (°C)	Salinity (‰)	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
CRUSTACEANS										
Pandalus montagui (Aesop shrimp)	adult	SS	n	15	-	-	48 h	>500[1]	LC50	4
Crangon crangon (common shrimp)	adult	SS	n	15	-	-	48 h	>500[1]	LC50	4
Carcinus maenas (shore or green crab)	adult	SS	n	15	-	-	48 h	500[1]	LC50	4
FISH										
Paralabrax clathratus (kelp bass)	eggs	S closed	n	20	33	-	48 h	<25	No significant effects on survival	1
Mugil cephalus (striped mullet)	-	-	-	-	-	-	2.05 h	1	LT50	2

Notes to Table C1.1:

[1] Applied as doxcide (2% chlorine dioxide) but LC50s are assumed to be expressed as mg ClO_2 l^{-1}.

- Information unavailable or not reported
S Static laboratory bioassay
SS Semi-static (renewal) laboratory bioassay
m Measured concentration (i.e. analysed)
n Nominal concentration (i.e. not analysed)
h Hours
LC50 Median lethal concentration
LT50 Time taken to cause 50% mortality at a given concentration
LOEC Lowest observed effect concentration
NOEC No-observed effect concentration

References:

1. Hose *et al* (1989)
2. Parrella *et al* (1986)
3. Betzer and Kott (1969)
4. Portman and Wilson (1971, cited by CIS 1997)

C1.1.2 Echinoderms

Recently fertilised embryos of the purple sea urchin (*Strongylocentrotus purpuratus*) were exposed to varying concentrations of stabilised aqueous chlorine dioxide (25%) and after 48 hours were measured for abnormalities (i.e. pre-hatch malformations, retarded development, post-hatch malformations, skeletal malformations and gut malformations). The lowest observed effect concentration (LOEC) was 250 mg l^{-1}, and the highest no-observed effect concentration (NOEC) was 25 mg l^{-1} (Hose *et al* 1989). However, these values were based on nominal concentrations of chlorine dioxide. Because of the strong oxidising properties of chlorine dioxide it is likely that the actual exposure concentrations of chlorine dioxide were lower than the nominal concentrations applied. The toxicity values reported by Hose *et al.* (1989) may therefore be underestimates.

C1.1.3 Molluscs

The only data for saltwater molluscs are from a semi-static (renewal) study with adult cockles (*Cerastoderma edule*) (Portmann and Wilson 1971, cited by CIS 1997). In this study, the 48 hour LC50 was not found to be within the concentration range tested (maximum concentration tested 500 mg l^{-1}). Although actual exposure concentrations were not measured, test media were renewed at regular intervals in order to maintain the nominal concentrations applied. It is still likely, however, that some chlorine dioxide was lost during the test.

C1.1.4 Crustaceans

Data are available for three species of saltwater crustacean, although these data are all from the same study (Portman and Wilson 1971, cited by CIS 1997). The 48-hour LC50s reported for the Aesop shrimp (*Pandalus montagui*) and the brown shrimp (*Crangon crangon*) were greater than the highest concentration tested (500 mg l^{-1}), while for the shore crab (*Carcinus maenas*) an LC50 of 500 mg l^{-1} was recorded. These species were tested under semi-static (renewal) conditions in order to maintain the exposure concentrations of chlorine dioxide, but it is nevertheless likely that some chlorine dioxide was lost during the tests. The toxicity values reported may therefore be slight underestimates of the true toxicity.

C1.1.5 Fish

Hose *et al* (1989) exposed fertilised eggs of the kelp bass (*Paralabrax clathratus*) to five different concentrations of stabilised aqueous chlorine dioxide (up to 25 mg l^{-1}) in culture dishes for 48 hours (the eggs hatched 40 hours post-fertilisation). None of the concentrations tested were found to have significant effects on the survival of eggs compared to the control. For example, the percentage survival of eggs in the control test was 78%, and 82% when exposed to the highest test concentration of 25 mg l^{-1}.

Parrella *et al* (1986) reported that the time taken for 1 mg l^{-1} of chlorine dioxide to cause 50% mortality (i.e. LT50) of the striped mullet (*Mugil cephalus*) was 2.05 hours. In the same study the authors reported an LT50 of 33.33 hours for the goldfish (*Carassius auratus*) exposed to 2 mg l^{-1} of chlorine dioxide. Although *C. auratus* is a freshwater fish, the authors indicated that it had been tested in marine waters. Specific details of the test design, however, were not available to check this.

C1.2 Studies with industrial wastewaters

In recent years a number of studies have been carried out by researchers in Sweden and Finland on the exposure of pulp mill effluents to saltwater organisms (e.g. Rosemarin *et al* 1994, Tana *et al* 1994, Ladner *et al* 1994, Lehtinen *et al* 1990, Rosemarin *et al* 1990, 1986). Although adverse ecological effects have been observed due to the effects of chlorate, which can result from the use of chlorine dioxide, on certain "keystone" species (see Section C2.2), these studies generally indicate that pulp effluents derived from chlorine dioxide bleaching processes are less harmful than those from chlorine bleaching processes. In the UK, the majority of pulp is bleached before being imported, so pulp mill effluents of the nature described in this section are unlikely to be discharged to the saltwater environment. Nevertheless, these studies offer a useful indication of the possible effects of effluents derived from chlorine dioxide treatment processes, and in particular, chlorate-containing effluents.

Rosemarin *et al* (1990) investigated the effects of kraft pulp effluents treated with different bleaching methods (including chlorine dioxide and chlorine) on out-door mesocosms designed to simulate the littoral *Fucus vesiculosus* zone of the coastal Baltic sea. Two dilutions (2000 and 4000 times) were used, based on a normalised effluent volume of 50 m^3 $tonne^{-1}$ of pulp. All of the effluents tested caused severe community shifts, both in the algae and macroinvertebrate communities. The effects on the algae were attributed to the chlorate present in different effluents, which in turn was dependent on the amounts of chlorine dioxide used in the bleaching process (specific ClO_2 doses were not stated). The authors attributed the effects on macroinvertebrates to both direct toxic effects and indirect effects due to the chlorate-induced break-down of the *Fucus* algal community. However, the more chlorine was replaced by chlorine dioxide, the lower the direct toxic effects were on the fauna community, but high concentrations of chlorate in these effluents still caused indirect effects by eliminating the ecological niche of several species, notably crustaceans.

Lehtinen *et al* 1990 exposed immature rainbow trout (*O. mykiss*) to a total of six different bleached kraft mill effluents at two exposure concentrations (400 and 2000 times dilution) in a flow-through brackish water (8‰) system for seven weeks, during three consecutive years. The fish were exposed in outdoor tanks connected to the outgoing water from a parallel mesocosm study simulating the Baltic littoral zone described above (Rosemarin *et al* 1990). After exposure, haematological, osmoregulational and mixed function oxidase parameters were studied and the results showed that conventional bleaching, with and without treatment in aerated lagoons, had the strongest effects on the fish, while oxygen bleaching followed by 50% substitution of the active chlorine as chlorine dioxide had significantly reduced effects on the fish exposed.

Although a number of studies have indicated that using chlorine dioxide in place of, or in conjunction with chlorine to bleach pulp reduces the amounts of chlorinated organic compounds in pulp effluents (see Section B1.2, Appendix B2), Tana *et al* (1994) were unable to find any correlation between the concentration of adsorbable organic halides (AOX) and level of effect on algae, invertebrate or fish exposed to effluents in model ecosystems (as described by Rosemarin *et al* 1990, 1994). In addition, no correlation was observed between the concentration of conjugated chlorophenolics or resin acids in the bile of rainbow trout and physiological effects in fish.

Rosemarin *et al* (1994) examined further the effects of long-term exposure (six months) of Baltic Sea algae to pulp mill effluents containing chlorine dioxide derived chlorate (ClO_3^-) and to brackish water spiked with chlorate. Brown algae (*Phaeophyta*) were found to be highly sensitive to both chlorate and chlorate-containing effluents, with all brown algal species (e.g. *Fucus* spp., *Chorda filum*, *Pilayella littoralis* and *Ectocarpus siliculosus*) adversely affected down to μg ClO_3^- l^{-1} levels. The EC50s (inhibition of growth) reported for *Fucus vesiculosus* were about 80 μg ClO_3^- l^{-1}. Neither green algae (*Cladophora* sp., *Entermorpha ahlneriana*, *Spirogyra* sp., *Urospora* sp. and *Chaetomorpha* sp.), blue-green algae (*Rivularia* spp., *Lyngbya* spp. and *Anabeana* spp.), red algae (*Furcellaria lumbricalis*, *Phyllophora truncata*, *Ceramium tenuicorne* and *Polysiphonia* sp.), diatoms or the plant *Zostera* marina were adversely affected by any of the treatments applied. When exposed to effluents containing the highest chlorate concentrations some evidence of stimulation of green, blue-green and red alage was reported. This study is outlined in more detail in Section C2.2 as part of a general discussion on the toxicity of chlorate to saltwater algae.

C2 CHLORATE (ClO_3^-)

Laboratory-derived data on the toxicity of chlorate ion (ClO_3^-) to saltwater organisms are restricted to a few studies on bacteria and algae (see Table C2.1 and Sections C2.1 and C2.2). There appear to be no toxicity data for saltwater invertebrates or fish, and, although there are no laboratory-based chronic toxicity data, several mesocosm studies have been carried out on the long-term effects of chlorate-containing pulp mill effluents to intertidal algal communities. These are reviewed in a general discussion on the toxicity of chlorate to saltwater algae in Section C2.2.

Chlorate is relatively easy to test in standard ecotoxicology tests. Sodium chlorate, which has been used as the source of chlorate in most laboratory toxicity tests, has a solubility of 957 g l^{-1} in freshwater at 20 ^oC which increases with increasing temperature (Environment Canada 1985). This is not expected to be significantly different in saltwater. There appears to be no information on the stability of chlorate in saltwater, although based on the information available for freshwater (see Appendix A) it is unlikely to degrade significantly during the course of most acute toxicity studies. Acute tests based on static systems and nominal concentrations are therefore considered to be acceptable.

C2.1 Bacteria

The toxicity data available for marine bacteria are limited to two different studies on the same species. Cenci *et al.* (1975) reported that inhibited growth of the luminescent bacterium *Photobacterium phosphoreum* (now more commonly referred to as *Vibrio fisherii*) occurred at a concentration of 0.1 mg ClO_3^- l^{-1}. However, specific details of the test procedures employed (e.g. duration of exposure, the source and amount of nitrogen used) by Cenci *et al* (1975) were not reported. Van Wijk and Hutchinson (1995) cited data from a previously unpublished study (author name Macauley) which indicate that much higher chlorate concentrations are required to affect photoluminescence (the principle behind the Microtox test). The 5-minute and 15-minute EC50s reported by Van Wijk and Hutchinson (1995) were 43 137 and 34 510 mg ClO_3^- l^{-1}, respectively.

Table C2.1 Toxicity of chlorate (ClO_3^-) to saltwater life

Species	Life stage	Test type	Analysis	Temp (°C)	Salinity (‰)	pH	Nitrogen source (mg N l^{-1})	Exposure duration	Concn (mg l^{-1})	Effect/test substance	Ref
BACTERIA											
Photobactrium phosphoreum	-	-	-	-	-	-	-	-	0.1	LOEC (growth inhibition) - $NaClO_3$	1
								5 minutes	43137	EC50 (bioluminescence) - $NaClO_3$	2
								15 minutes	34510	EC50 (bioluminescence) - $NaClO_3$	2
ALGAE											
Phaeodactylum tricornutum (unicellular)	exp. growth phase	S	n	20	-	-	NO_3 (8.25)	72 h	50	102% of control biomass (NOEC) - $NaClO_3$	3
									100	83% of control biomass (LOEC)[1] - $NaClO_3$	3
									200	69% of control biomass[1] - $NaClO_3$	3
									400	35% of control biomass[1] - $NaClO_3$	3
									800	6% of control biomass[1] - $NaClO_3$	3

Species	Life stage	Test type	Analysis	Temp (°C)	Salinity (‰)	pH	Nitrogen source (mg N l^{-1})	Exposure duration	Concn (mg l^{-1})	Effect/test substance	Ref
									1600	0% of control biomass[1] - $NaClO_3$	3
									298	EC50 (biomass) - $NaClO_3$	3
Phaeodactylum tricornutum (unicellular)	exp. growth phase	S	n	20	-	-	NO_3 (8.25)	72 h	50	102% of control growth rate - $NaClO_3$	3
									100	96% of control growth rate (NOEC) - $NaClO_3$	3
									200	91% of control growth rate (LOEC)[1] - $NaClO_3$	3
									400	74% of control growth rate[1] - $NaClO_3$	3
									800	33% of control growth rate[1] - $NaClO_3$	3
									1600	2% of control growth rate[1] - $NaClO_3$	3
									444	EC50 (growth rate)	3
Gracilaria tenuistipitata (red macroalga)	-	S	-	-	25	8.0	$NO_3 + NH_4$	48 h	46	EC50 (growth inhibition) - ClO_3^-	4

Species	Life stage	Test type	Analysis	Temp (°C)	Salinity (‰)	pH	Nitrogen source (mg N l^{-1})	Exposure duration	Concn (mg l^{-1})	Effect/test substance	Ref
									10	NOEC (growth inhibition) - ClO_3^-	4
Fucus vesiculosus (bladderwrack)	apical fronds	S	n	-	-	-	NO_3 (<0.039)	70 hours	4	66% reduction in net photosynthesis rate - ClO_3/Effluent	5
									0.4	16% reduction in net photosynthesis rate - ClO_3/Effluent	5
									4	33% reduction in nitrogen uptake rate - ClO_3/Effluent	5
									0.4	no-effect on nitrogen uptake - ClO_3/Effluent	5
Fucus vesiculosus (bladderwrack)	adult	meso-cosm	m	3-20	7	-	NO_3 (<0.039)	6 months	0.005	NOEC (frond growth) - ClO_3/Effluent	5
									0.015	LOEC (frond growth) - ClO_3/Effluent	6
									0.08	EC50 (frond growth) - ClO_3/Effluent	6
									0.058	LOEC (nitrogen utilisation) - ClO_3/Effluent	6
Fucus serratus (brown macroalga)	adult	meso-cosm	m	3-20	7	-	NO_3 (<0.039)	6 months	0.06	NOEC (frond growth) - ClO_3/Effluent	6

Species	Life stage	Test type	Analysis	Temp (°C)	Salinity (‰)	pH	Nitrogen source (mg N l^{-1})	Exposure duration	Concn (mg l^{-1})	Effect/test substance	Ref
									0.1	LOEC (frond growth) - ClO_3/Effluent	6
									0.13	EC50 (frond growth) - ClO_3/Effluent	6
Mixed phytoplankton	-	S	m	-	-	-	NO_3 (<0.039)	8 h	0.05	NOEC (primary production	6
								8 h	50	LOEC (primary production)	6
PLANTS											
Zostera marina (Phanerogam)	-	meso-cosm	m	3-20	7	-	NO_3 (<0.039)	6 months	>0.288	NOEC (growth)	6

Notes to Table C2.1:

[1] Statistically significant (p=0.05)

-	Information unavailable or not reported
S	Static laboratory bioassay
mesocosm	Artificial ecosystem
m	Measured concentration (i.e. analysed)
n	Nominal concentration (i.e. not analysed)
h	Hours
LC50	Median lethal concentration
EC50	Median effect concentration
LOEC	Lowest observed effect concentration
NOEC	No-observed effect concentration

References:

1. Cenci *et al* (1975)

2. Macauley (unpublished data, cited by Van Wijk and Hutchinson 1995)
3. Hutchinson (1994b)
4. Haglund *et al* (1996)
5. Rosemarin *et al* (1986)
6. Rosemarin *et al* (1994)

From these two studies it is difficult to draw any firm conclusions about the likely toxicity of chlorate to bacteria, or indeed *P. phospheum*. Information obtained with freshwater microorganisms suggests that the source and amount of nitrogen made available can influence the toxicity of chlorate (see Sections B2.1 and B2.3, Appendix B). Neither of the studies with *P. phospheum* provided information on the nitrogen sources used, making them less easy to compare. The difference in toxicity values obtained from these tests may simply indicate that the inhibition of growth is a more sensitive toxicity end-point than bioluminescence. Further data are required to properly assess the sensitivity of chlorate to saltwater bacteria.

C2.2 Algae

Only three laboratory-based studies on the toxicity of chlorate to saltwater algae were available for review at the time of writing. These studies, along with several mesocosm and field experiments, suggest that chlorate is of relatively low acute and chronic toxicity to the majority of saltwater algae, including green algae (Chlorophyta), blue-green algae (Cyanophyta), red algae (Rhodophyta) and various diatoms. However, there is strong evidence to suggest that brown algae (Phaenophyta) are sensitive to chlorate at μg l^{-1} levels.

C2.2.1 Laboratory studies

Tests were carried out on the unicellular marine alga *Phaeodactylum tricornutum* by Hutchinson (1994b) according to ISO Standard 10253. Test solutions containing nominal chlorate concentrations of 50 to 3200 mg l^{-1} were inoculated with algae in the exponential growth phase and incubated at 20 ^{o}C for 72-hours in media containing 8.25 mg N l^{-1} as nitrate. Measurements of cell density were made after 24-, 48- and 72-hours and were expressed as areas under a growth curve (i.e. total biomass) and growth rates. The EC50s determined for biomass and growth rate were 298 mg ClO_3^- l^{-1} and 444 mg ClO_3^- l^{-1}, respectively, indicating that chlorate is of low acute toxicity to this species. The corresponding 72-hour NOECs (50 and 100 mg ClO_3^- l^{-1}) suggest that chlorate is also likely to be of low chronic toxicity to *P. tricornutum*.

The effect of chlorate on the growth of the red macroalga *Gracilaria tenuistipitata* was examined by Haglund *et al* (1996) in a static test system using seawater (25‰) with both nitrate and ammonium present as sources of nitrogen. The authors determined a 48-hour EC50 (growth inhibition) of 46 mg ClO_3^- l^{-1} and a corresponding NOEC of 10 mg ClO_3^- l^{-1}.

Rosemarin *et al* (1986) exposed excised apical fronds from the brown alga *Fucus vesiculosus* to chlorate and effluent containing chlorate in the laboratory. After 70 hours exposure to chlorate, the net photosynthesis rate was reduced by about 66% in 4 mg ClO_3^- l^{-1} and by about 16% in 0.4 mg ClO_3^- l^{-1}.

Recent laboratory tests carried out by Akzo Nobel with algae, bacteria and fungi found that chlorate is “very toxic” to the brown alga *Ectocarpus variabilis* and that all other species tested (including green and blue-green algae) were non-sensitive. In addition, when tested with nitrate as the nitrogen source, chlorate was found to be 10 times more toxic to *E. variabilis* than with ammonium. The source of nitrogen used did not appear to affect the sensitivity of other species to chlorate (Van Wijk, Personal Communication 1997). This study is currently being submitted for publication, but at the time of writing only a brief summary of the work carried out was available. Nevertheless, this information appears to support the

findings of various mesocosm studies (e.g. Rosemarin *et al* 1986, 1990, 1994) which also identified brown algae as being particularly sensitive to chlorate.

C2.2.2 Mesocosm and field studies

Research using mesocosms has largely been related to effluents derived from pulp mills in the Baltic which use chlorine dioxide as a bleaching agent. Much of this research has therefore already been discussed in Section C1.2 within the context of chlorine dioxide, but a few important studies require special mention here as they deal specifically with the effects of chlorate on intertidal ecosystems.

The perennial brown alga *Fucus vesiculosus* (bladderwrack) completely disappeared from an area of about 12 km^2 in the Baltic Sea due to the presence of chlorate (mean effluent concentration 0.053 mg ClO_3^- l^{-1}) in pulp mill effluents during the mid-eighties (Lindvall 1984). Transplant experiments in the receiving waters indicated that fresh *F. vesiculosus* were killed within three to five months as far as 1.5 km from the effluent source and reduced growth occurred at 4 km from the source (Rosemarin *et al* 1986). Removal of chlorate from these effluents subsequently led to the recolonisation of this area by *F. vesiculosus* (Notini 1991).

Rosemarin *et al* (1994) developed a system of outdoor, flow-through experimental ecosystems into which a variety of macro and microalgae were transplanted or colonised. In order to simulate the Baltic intertidal ecosystem a number of macroinvertebrate species normally associated with the test algae (e.g. *Ideothea* spp) were also introduced, as well as 100 stickleback larvae (*Gasterosteus aculeatus*) and five juvenile flounder (*Platichthys flesus*) per pool. After a two-month period to allow the ecosystems to adapt and stabilise to the test conditions, different combinations and dilutions of pulp effluent and chlorate were applied to each of six test pools, with one uncontaminated control pool. Three pools received the same amount of chlorate - one with chlorate alone, one with chlorate plus effluent and the third with effluent alone - in order to test whether there were any toxic effects associated with the effluents other than chlorate. Seawater and "test solutions" were renewed every 48 hours over a total period of six months, during which regular observations and qualitative measurements (e.g. presence or absence) of most algal species were made. The net growth of the brown alga *Fucus vesiculosus* (bladderwrack) was quantitatively measured by volume displacement and apical frond growth, and wet weight was used to assess the growth of another two brown algal species, *F. serratus* and *Chorda filum* (a perennial alga).

All of the brown algae tested (perennials and annuals) were found to be sensitive to the pulp effluents and to chlorate when applied alone. Since the effects on these algae were similar when exposed to the same chlorate concentration with and without effluent, the authors concluded that chlorate was the principal toxicant of the pulp mill effluents. The most sensitive species was *Fucus vesiculosus*. Significant effects on the volume of *F. vesiculosus* were found to occur from 0.015-0.02 mg ClO_3^- l^{-1} (the LOEC), with a corresponding NOEC of 0.005 mg ClO_3^- l^{-1} and an EC50 of 0.08 mg ClO_3^- l^{-1}. Effects on apical growth were detected at the lowest chlorate concentrations tested (0.001-0.02 mg ClO_3^- l^{-1}), although the EC50 for apical growth was also about 0.08 mg ClO_3^- l^{-1}. The EC50 (based on wet weight) for *Fucus serratus* was approximately 0.130 mg ClO_3^- l^{-1} and *Chorda filum* essentially disappeared from all treatments after three months (LOEC 0.02, NOEC <0.02 mg ClO_3^- l^{-1}). *Ectocarpus siliculosus* only survived in the control pool and the pool that received the lowest chlorate

concentration (LOEC 0.058 mg ClO_3^- l^{-1}, NOEC 0.02 mg ClO_3^- l^{-1}), while *Pilayella littoralis* only survived in the control pool (LOEC 0.02 mg ClO_3^- l^{-1}, NOEC <0.02 mg ClO_3^- l^{-1}).

The other algae monitored (green, blue-green, red and diatoms) were found to be largely unaffected by any of the treatments, and the growth of some (e.g. *Rivularia* sp. (blue-green alga), *Cladophora* spp. (green alga) and *Polysiphonia* sp. (red alga)) even appeared to be stimulated at the higher chlorate concentrations (i.e. >0.058 mg ClO_3^- l^{-1}).

Rosemarin *et al* (1994) demonstrated that the net productivity of the artificial ecosystems was negative (i.e. more CO_2 was evolved by respiration than was consumed in photosynthesis) during the summer and autumn treatments with pulp mill effluent. A negative hyperbolic dose-effect relationship resulted, with an EC50 of about 0.08 mg ClO_3^- l^{-1}. In August, water samples were collected from each pool and the rates of phytoplankton photosynthesis (using a C^{14} fixation technique) were determined. Low concentrations of chlorate (0.005-0.05 mg ClO_3^- l^-1) did not affect the photosynthesis of phytoplankton but exposure to 0.5 and 5 mg ClO_3^- l^{-1} had slight inhibitory effects (5-10%) and exposure to 50 mg ClO_3^- l^{-1} caused about 30% inhibition compared to the control.

In their 1994 report Rosemarin *et al* do not discuss the impact of chlorate on the whole intertidal ecosystem. However, in an earlier report of similar experiments (Rosemarin *et al* 1990), the authors indicated that due to the loss or reduction of brown algae, certain grazing macroinvertebrates were also indirectly affected.

As discussed previously in Section B2.3 (Appendix B), the source and amount of nitrogen available to algae may be important in determining the toxicity of chlorate. It is widely believed that chlorate only becomes toxic to algae if it is first reduced to chlorite, and there is evidence to suggest that this may be achieved in certain situations by the nitrate-reductase enzyme system. In algae it is well known that nitrogen starvation enhances both nitrate uptake and reduction (e.g. Hipkin *et al* 1980, cited by Rosemarin *et al* 1994). In such instances chlorate, if present, also appears to be readily taken up by algae and, competing with nitrate for active sites on the reductase enzyme, may be reduced to the harmful chlorite ion (ClO_2^-). The chlorate-to-nitrate ratio is therefore important in determining the level of chlorate toxicity. Rosemarin (1986), for example, demonstrated that in nitrogen-scarce conditions (<0.039 mg N l^{-1} as NO_3) 4 mg ClO_3^- l^{-1} of chlorate reduced the rate of nitrate uptake by *F. vesiculosus* (no-effect at 0.4 mg ClO_3^- l^{-1}, 33% reduction at 4 mg ClO_3^- l^{-1}). The mesocosm test system used by Rosemarin *et al* (1994) also incorporated low levels of nitrate (<0.039 mg N l^{-1}), but there is no explanation for why chlorate is more toxic to brown algae under these conditions than other algal species. Greater research is required on the characteristics of nitrogen reductase in brown algae and its affinity for chlorate.

C2.3 Plants

In the mesocosm study described above in Section B2.2.2, Rosemarin *et al* (1994) reported that growth of the Phanerogam *Zostera marina* was unaffected at concentrations above 0.288 mg ClO_3^- l^{-1} during six months continuous exposure to chlorate.

C3 CHLORITE (ClO_2-)

Information on the toxicity of chlorite to saltwater organisms is very sparse (see Table C3.1). Van Wijk (Personal Communication 1997) reported that a study on the toxicity of chlorate and chlorite to bacteria, fungia and algae has recently been carried out by Akzo Nobel in the Netherlands. Although a report of this study is currently being submitted for publication, details of the tests performed or the toxicity values generated have not been forthcoming. However, the author stated that chlorite was "toxic" to most of the species tested (concentrations not specified).

Based on mesocosm studies with chlorate-containing pulp mill effluents (see Section C2.2.2), there is evidence to suggest that brown algae are significantly more sensitive to chlorate than other algae. Van Wijk (Personal Communication 1997), however, reported that chlorite was less toxic to the brown alga *Ectocarpus variabilis*. Effect concentrations were not provided.

Chlorite appears to be of high acute toxicity to the mysid shrimp *Mysidopsis bahia*, with 48-, 72- and 96-hour LC50s of 0.62, 0.5 and 0.49 mg ClO_2^- l^{-1} (based on nominal concentrations), respectively (Environ Systems 1991, cited by IUCLID 1996). These values were determined using a method which was reported to follow US EPA test guidelines and Good Laboratory Practice (GLP) but as the original source of this information was not available this could not be confirmed.

There appear to be no toxicity data available for saltwater molluscs or fish and no chronic toxicity data.

Table C3.1 Toxicity of chlorite (ClO_2^-) to saltwater life

Species	Life stage	Test type	Analysis	Temp (°C)	Salinity (‰)	pH	Exposure duration	Conc.[1] (mg ClO_2 l^{-1})	Effect	Ref
CRUSTACEANS										
Mysidopsis bahia (mysid shrimp)	-	-	n	-	-	-	48 h	0.62	EC50 - $NaClO_2$ (80%)	1
							72 h	0.50	EC50 - $NaClO_2$ (80%)	1
							96 h	0.49	EC50 - $NaClO_2$ (80%)	1
							96 h	0.19	NOEC - $NaClO_2$ (80%)	1

Notes to Table C3.1:

[1] Concentrations have been converted to mg ClO_2 l^{-1} as 75% (molar proportions) of the concentrations given for sodium chlorite.

- Information unavailable or not reported
h Hours
LC50 Median lethal concentration
NOEC No-observed effect concentration

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1. Environ Systems (1991, cited by IUCLID 1996)

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APPENDIX D MAMMALIAN TOXICITY OF CHLORINE DIOXIDE, CHLORITE AND CHLORATE

During drinking water treatment, chlorine dioxide rapidly decomposes into chlorite (and to a lesser extent chlorate). This Appendix therefore considers the mammalian toxicity of chlorine dioxide and its breakdown products.

The World Health Organisation (WHO) considered chlorite, chlorate as well as chlorine dioxide in its 1993 Drinking Water Guidelines (WHO, 1996). A provisional guideline of 0.2 mg l^{-1} was derived for chlorite, the provisional nature being due to the fact that the use of chlorine dioxide as a disinfectant may result in the chlorite value being exceeded. A guideline for chlorine dioxide was not considered because of its rapid breakdown and the chlorite provisional guideline was considered to be adequately protective for any potential toxicity. Due to the lack of suitable toxicity data, WHO declined to derive a guideline value for chlorate in drinking water.

Clinical studies in humans have been carried out for chlorine dioxide, chlorite and chlorate. Firstly, ten normal healthy men were treated with successively increasing doses of chlorine dioxide, chlorite and chlorate or with untreated water. The concentrations administered were increased every third day up to 16 days. The concentration ranges for chlorine dioxide, chlorite and chlorate were 0.1-24 mg l^{-1}, 0.01-2.4 mg l^{-1} and 0.01-2.4 mg l^{-1}, respectively. Secondly, in a 12 week study, 500 ml of 5 mg l^{-1} (equivalent to a daily dose of 36 μg kg^{-1} body weight) of chlorine dioxide, chlorite or chlorate in drinking water were given to the same volunteers who were then observed for a further eight weeks. Urine and blood sample were collected at weekly intervals and subjected to complete routine analysis as well as special determinations for methaemoglobinaemia, glutathione, glucose 6-phosphatase dehydrogenase and thyroid function. In the third phase, three healthy men found to be deficient in the enzyme glucose 6-phosphatase dehydrogenase were studied, since such individuals are likely to be more susceptible to adverse effects on red blood cells. All three subjects ingested 500 ml of sodium chlorite solution at a concentration of 5 mg l^{-1}. In all three parts of the study, no apparent adverse health effects were noted, and abnormalities in the blood and urine were few and appeared to be randomly distributed and therefore the doses were considered to be NOAELs (WHO, 1996). However, this study is only short-term and effects on women and children were not included.

In an epidemiological study of a community where chlorine dioxide was used as a drinking water disinfectant for 12 weeks, no consistent changes were observed in any of the measured clinical parameters. Adult exposure to chlorine dioxide, chlorite and chlorate ranged from 0.25-1.1 mg l^{-1}, 3.2-7 mg l^{-1} and 0.3 - 1.1 mg l^{-1}, respectively. The conclusion of the study was that there was no association between the use of chlorine dioxide treated water and adverse health effects (WHO, 1996).

Exposure to chlorine dioxide, chlorate and chlorite are associated adverse effects on red blood cells in laboratory animals. Haemolytic anaemia is the main symptom which is associated with oxidative damage to the red blood cells. Chlorite is the most potent of the three species and, at high doses, methaemoglobineama (a reduced capacity for red blood cells to carry oxygen) can be induced. A NOAEL of 1 mg kg^{-1} body weight has been identified for chlorite based on decreased red blood cell glutathione levels in a 90 day drinking water study in rats. However,

a more recent 90-day drinking water study with rats has suggested a higher NOAEL of 10 mg kg^{-1} body weight for chlorite based on effects on blood (Harrington *et al* 1995a). In a 90 day rat study involving both sexes, a NOAEL of 30.06 mg kg^{-1} body weight day^{-1} was established in males following exposure to sodium chlorate in their drinking water. This was based on effect on red blood cells, organ weights and effects on the thyroid gland (McCauley *et al*, 1995). Significant depression of thyroid hormones has also been observed in rats and monkeys exposed to chlorine dioxide in a number of drinking water studies (WHO 1996). This may be because of the oxidising effect of chlorine dioxide on iodide in the gastrointestinal tract and food, thereby making iodide less bioavailable.

Mutagenicity studies have given negative results for chlorite, chlorate and chlorine dioxide (WHO, 1996). Long-term drinking water studies with chlorine dioxide or sodium chlorite have provided no evidence for carcinogenicity in laboratory animals (WHO 1996). In addition, chlorite does not appear to be teratogenic (WHO 1996; Harrington *et al* 1995b). Although chlorine dioxide has been shown to impair neurobehavioural and neurological development in rats exposed perinatally, this is probably related to effects on thyroid function (WHO 1996). A 2-generation reproductive/neurodevelopmental study has recently been completed, although the full results are currently unpublished. Nonetheless, interim results which were presented at an ILSI meeting (1995) indicated that no adverse neurological effects had been observed in F_1 animals.

In 1993, WHO derived a tolerable daily intake (TDI) for chlorite of 10 μg kg^{-1} body weight for chlorite. This was based on a NOAEL of 1 mg kg^{-1} body weight based on decreased glutathione levels in a 90 day rat study and incorporating an uncertainty factor of 100 (for inter- and intraspecies variation). The TDI derived in this manner was consistent with the NOAEL (36 μg kg^{-1} body weight day^{-1}) in a 12 week clinical study involving a small number of human volunteers. Allocating 80% of the TDI to water gave a provisional guideline of 0.2 mg l^{-1}. However, a more recent 90-day rat study suggested a higher NOAEL of 10 mg kg^{-1} body weight based on effects on the blood. This indicates some uncertainty about the study on which the WHO (1993) guideline was based.

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