INTERNATIONAL MARITIME ORGANIZATION

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MARINE ENVIRONMENT PROTECTION COMMITTEE
56th session
Agenda item 2

MEPC 56/2/3
6 April 2007
Original: ENGLISH

HARMFUL AQUATIC ORGANISMS IN BALLAST WATER

Basic Approval of Active Substances used by Resource Ballast Technologies System (Cavitation combined with Ozone and Sodium Hypochlorite treatment)

Submitted by South Africa

SUMMARY

Executive summary: This document contains non-confidential information for Basic Approval of Active Substances used by Resource Ballast Technologies System (Cavitation combined with Ozone and Sodium Hypochlorite treatment) in accordance with the Procedure for approval of Ballast Water Management Systems that make use of Active Substances (G9) adopted by resolution MEPC.126(53). The relevant documents and application dossier will be provided to the Marine Environment Division of the Organization for review by the GESAMP – Ballast Water Working Group.

Action to be taken: Paragraph 6

Related documents: BWM /CONF/36; MEPC 53/24/Add.1 and MEPC 56/1

Introduction

1 The International Convention for the Control and Management of Ships' Ballast Water and Sediments states in regulation D-3 that Ballast Water Management systems which make use of Active Substances to comply with the Convention shall be approved by the Organization, based on a procedure developed by the Organization.

2 The Procedure for approval of Ballast Water Management systems that make use of Active Substances (G9) was adopted by resolution MEPC.126(53). Procedure (G9) requests IMO to establish a Technical Group (the GESAMP – Ballast Water Working Group) to review proposals for Basic Approval submitted by Administrations, and report its findings to the Organization. Procedure (G9) details in Section 8 the methodology to be followed by an Administration seeking Basic Approval of Active Substances.

3 MEPC 55 agreed to invite Members to submit application dossiers for Basic Approval of Active Substances. Documents for MEPC 56 containing more than 6 pages of text should be submitted by Friday, 6 April 2007 (MEPC 56/1).

4 South Africa hereby submits to the Organization, in the annex of this document, the non-confidential information related to the Resource Ballast Technologies System (cavitation combined with Ozone and Sodium Hypochlorite treatment). The introduction part of the annex describes the system's ability to meet the performance criteria detailed in the Guidelines for approval of Ballast Water Management systems (G8) as per resolution MEPC.125(53), while remaining safe to ship, crew and environment.

5 The complete proposal for Basic Approval including application dossier and relevant documents will be submitted to the Marine Environment Division of the Organization to be reviewed by the GESAMP-BWWG in accordance with Procedure (G9).

Action requested of the Committee

6 The Committee is invited to consider the above proposal for Basic Approval and decide as appropriate.

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ANNEX

INFORMATION FOR BASIC APPROVAL OF ACTIVE SUBSTANCES USED BY RESOURCE BALLAST TECHNOLOGIES (CAVITATION COMBINED WITH OZONE AND SODIUM HYPOCHLORITE TREATMENT)

1 INTRODUCTION

1.1 Background

Resource Ballast Technologies (Pty) Ltd. has over the past 5 years developed a solution to treat ballast water that meets the IMO standards. With the initial guidelines in mind and the subsequent IMO Guidelines (G8) and Procedure (G9), the solution was designed to achieve the desired results.

Particular attention was taken to the environmental requirements using cavitation as a primary treatment with small amounts of Active Substances to assist the cavitation process. The Active Substances (Ozone and Sodium Hypochlorite) are used as an assistant for the cavitation. Test results taken from water treated directly after the Resource Ballast Treatment solution as shows chlorine levels (free and total) – (Appendix 1) < 0.4mg/L and Boromoform at 19.6µg/L (Appendix 2). As a guideline, both these levels are within the recommended standards for drinking water (Appendix 3).

A full-scale land-based test facility has been built at Cape Town Harbour (for Type Approval), capable of testing the Resource Ballast Technologies (RBT) Systems for flow rates of 200m^3/hr to 1500m^3/hr. Through traditional testing methods, and with the use of a FlowCAM, tests conducted to date show that the RBT system consistently meets Guidelines (G8) requirement for Type Approval.

The Type Approval testing process is currently under way, with assistance from the University of Stellenbosch, The Aquaculture Institute of South Africa (AISA) and The Marine and Coastal Management Department of the Ministry of Environmental Affairs and Tourism.

The process is being co-ordinated under the supervision of the Department of Transport and the South African Maritime Safety Authority.

1.2 System configuration

1.2.1 Resource Ballast Technologies BWT Reactor

The RBT Reactor is an efficient, cost effective system designed to meet the requirements of IMO resolution MEPC 53/24. Two models of the reactor are currently available to suit installation in vessels with 6-inch and 10-inch ballast water pipe systems respectively.

The Reactor combines the use of mechanical cavitation, disinfection and physical separation methods to provide a system that is equally efficient in both salt-water and freshwater environments. In addition, this combination of methods means that it can handle diverse water conditions such as high turbidity and polluted water, including that containing high levels of organic and mineral matter.

The disinfectants employed are produced within the system and thus, no additional storage facilities are required.

A basic block diagram of a typical Ballast Water Management System (BWMS) is shown below in Figure 1 and a dual-reactor system for installation in a vessel with port and starboard ballast water pumps in Figure 2. A further option is to replace the bypass valves shown in the illustrations with a separate Reactor thus ensuring that ballast water treatment operations can continue even in the event of main reactor failure. If fitted, this Reactor would be connected to the same Electrical Control Panel (ECP) as the main Reactor.

The disinfection and mechanical processes are carried out within the Reactor while the physical separation is done by the electric filter located after the ballast pump. In the block diagram the Reactor is shown before the ballast pump but can be located after the ballast pump if necessary. If required, the ECP can be installed remote from the Reactor.

The BWMS is an automatic system with the amount of ballast water that it can treat being dependent on model of reactor installed. For example, a 6-inch Reactor can treat up to 450 m^3 of ballast water per hour whereas a 10-inch Reactor can treat up to 1400 m^3 per hour. Its automatic operation and remote monitor units allow the ship's crew to continue with their normal tasks while ballast water operations are in progress. In addition, the relatively small size makes the system suitable for retrofit operations.

The RBT Reactor operates on normal shipboard supply, i.e. 400 VAC, 60 Hz. Note that a basic 320 m^3 system requires a 7 kW supply.

A combination of units can be installed into a manifold/pod, to upscale the output capacity of the Reactor to the Treatment Rate Capacity (TRC) to suit the capacity of the ballast pumps installed on board the particular vessel.

System controls and indicators are located on the RMU, two of which are supplied. Real-time data, salinity and pH sensor readings are recorded on a data logging PC connected to the ECP.

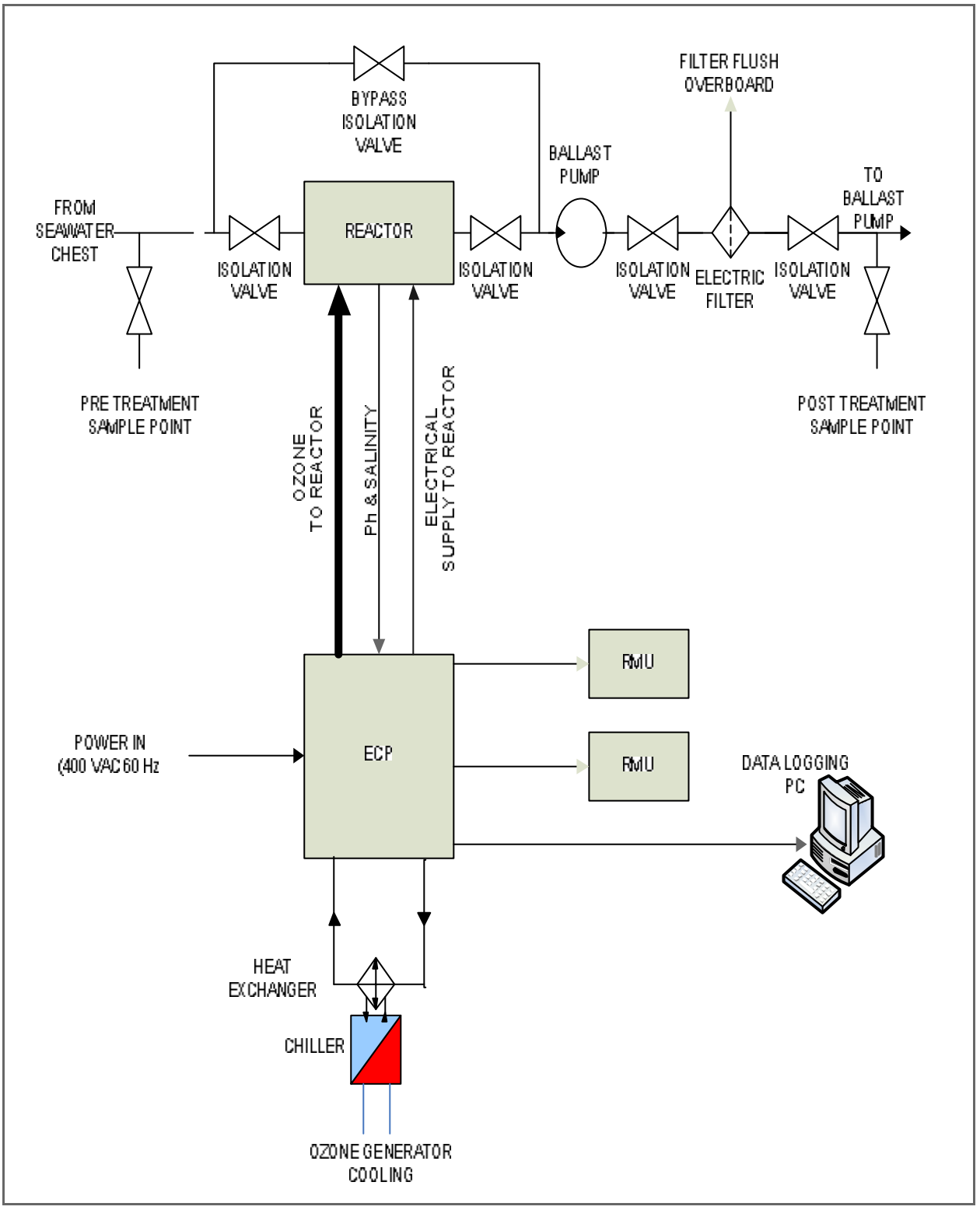

Figure 1: RBT Reactor System Block Diagram

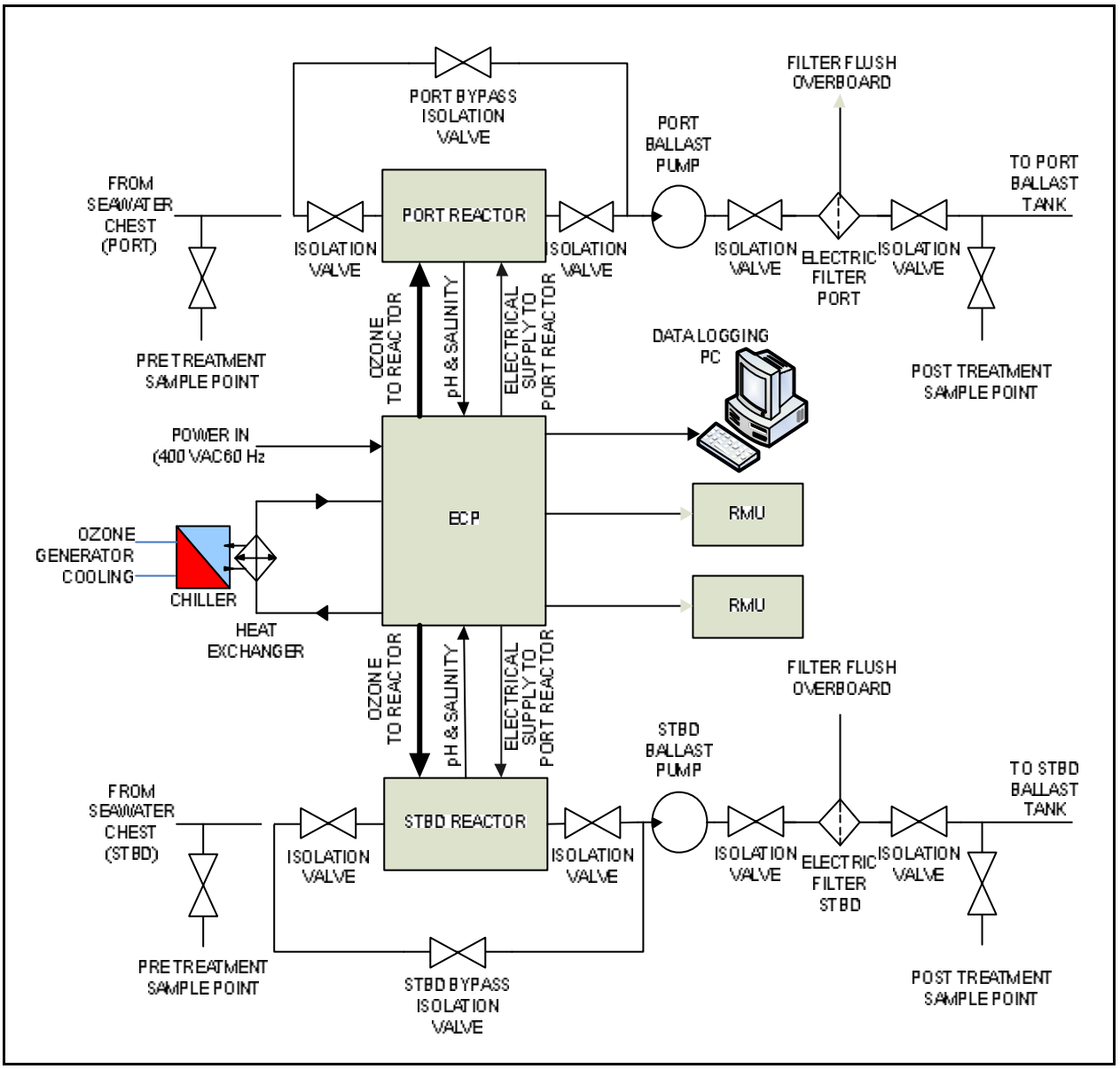

Figure 2: RBT Dual-Reactor System Block Diagram

1.3 Process Description

The BWMS incorporates a number of water treatment technologies. The methods used are as follows:

- Cavitation
- Electro-chemical (Chlorine)
- Ozone
- Filtration

It should be noted that no additional chemicals to those listed above are employed to allow the reactor to operate effectively. As such the safety of the crew and vessel are not jeopardized. Chlorine levels and Ozone levels are kept within the limits as required by manufacturers and suppliers of ballast tank coatings and should not pose any stress on the integrity of the ballast pipe work/system or ballast tank.

1.3.1 Cavitation

Cavitation can be defined as the rapid creation and collapse of bubbles in liquid. Within the RBT Reactor there are several cavitation chambers which mechanically produce the bubbles as the ballast water flows through the reactor. When the bubbles reach a critical mass, they implode producing a shock wave which kills the organisms within the ballast water. Note that both the electro-chemical and Ozone processes assist in increasing the cavitation effect (see Electro-chemical and Ozone sections for details).

1.3.2 Electro-Chemical

Mounted within the RBT chambers are a number of electrodes. The purpose of these electrodes is as follows:

The electrolytic production of sodium hyper chlorite (chlorine) compound (at levels of less than 1ppm) which occurs when direct current is applied to the electrodes. This assists with the sterilization process and helps to maintain a sterile environment within the ballast tanks of the vessel. The amount of Sodium Hyper chlorite produced by the electrodes has a half-life of 12 hours and does not result in any additional hazardous or harmful by-products.

To assist the cavitation effect that is achieved by means of the addition of gaseous bubbles to the ballast water flow which are produced when direct current is applied to the electrodes.

The electrical charge induced into the water while passing through the Reactor has a detrimental effect on certain organisms.

1.3.3 Ozone

The ECP contains two Ozone generators. The Ozone gas produced is infused into the ballast water by means of a Venturi injector that only becomes operational once the RBT Reactor is powered. The maximum level of Ozone is 0.001mg/lt.

Ozone has a very short life span in seawater; however the primary effects that the infusion of the Ozone gas causes are as follows:

- provide an effective method of killing Aquatic Nuisance Species (ANS).

- provide an effective method of treating ANS in both salt-water and freshwater. For example, Should a vessel take on ballast water in freshwater ports the electrolytic production of chlorine (as described in 1.2.2.2) will either not occur or be greatly reduced, and therefore the Ozone gas produced by the Reactor system compensates for this.

- assist the cavitation effect by increasing the levels of dissolved gases within the ballast water being treated.

1.3.4 Filtration

In the BWMS filtration is carried out before the ballast water enters the ballast tanks. The filter is located after the ballast pump and incorporates both coarse and fine filter screens. The standard fine screen is a 40-micron screen.

Cleaning of both the coarse and fine screens is carried out automatically with the "cake" from the screens being fed directly overboard.

1.4 Environmental

The BWMS generates a number of oxidising biocides to destroy cells in the ballast water. The oxidising agents and their quantities that the system produces are a maximum of:

- Ozone (0.001mg/l)
- Sodium Hypochlorite (<1ppm)

No additional conditioning of the ballast water is required to remove these agents from the ballast water. This is due to the fact that such small amounts are employed coupled with the fact that Sodium Hyperchlorite has a half life of less than 10 hours within seawater and Ozone has a half-life of 5.3 seconds in seawater.

The process also produces other by-products such as Trihalomethanes and Bromoform in limited quantities, which are well within the limits allowed for international standards for potable water.

Waste streams are limited to that from the cleaning of the electric filter screen. It is recommended that this stream is discharged directly back into the port or location from where the ballast water is being taken on board, thereby ensuring that any filtered organisms and contamination will remain in the water of the area of origin, and not be transported to other destinations. Note that all discharges cease when ballast operations cease as the filter and RBT reactor are electronically linked directly to the ballast pump.

2 IDENTIFICATION OF THE SUBSTANCE OR PREPARATION

2.1 Preparations

Active Substances to be used for treatment of ballast water using the RBT Reactor include Ozone which is produced by a generator provided on board and other disinfectants such as the hydroxyl radical which are introduced directly into the media using an electrochemical system. The electrochemical system also allows for direct oxidation of micro-organisms and other contaminants on the electrode surface. Neither of these processes involves the use of commercial preparations using additives, etc., and as such are not described in this application form.

2.2 Active Substances

In this application form, Ozone and the hydroxyl radical OH, are defined as Active Substances. Ozone exists in oxygen (raw material) at a ratio of 6 to 12%. Therefore, when it is used for treating the ballast water, it is in a state of Ozone gas. Hydroxyl radicals are produced at the electrodes of the electrolytic cell. They are highly reactive and decay within a few nanoseconds. Relevant chemical identifiers and components of Ozone gas and the hydroxyl radical are listed in Table 1.

Table 1. Components and relevant details (chemical identifiers) for Ozone gas and the hydroxyl radical

Name of substance	IUPAC International Chemical Identifier	CAS number	Molar mass	Empirical formula	Structural formula	Ratio	Stabilizers/ additives
Ozone	InChI=1/O3/c1-3-2	10028-15-6 6	48.00	O_3	O=O=O	~ 12v/v%	None
Oxygen	InChI=1/O2/c1-2	7782-44-7 80	32.00	O_2	O=O	~ 88v/v%	None
Nitrogen	InChI=1/N2/c1-2	7727-37-9 2	28.01	N_2	N≡N	~ 3v/v%	None
Argon	InChI=1/Ar	7440-37-1 4	39.95	Ar	Ar	~ 5v/v%	None
Hydroxyl radical	InChI=1/HO/h1H	3352-57-6	17.01	O*H	HO	-	None

Note that basic approval has been issued for use of all of these Active Substances by the GESAMP-Ballast Water Working Group (GESAMP-BWWG) (MEPC 55/2, 2006). Full details on the nature, toxicity and risks posed by these Active Substances is nonetheless provided in this application for completeness.

2.3 Relevant Chemicals

Ozone is a very unstable molecule and, after injection into raw water (e.g., natural seawater or freshwater), it decomposes very rapidly. The chemistry of the breakdown process in seawater, however, differs from that in freshwater because of the presence of bromide ion in seawater. Ballast water taken up by a vessel may include fresh and salt water and hence both are relevant here. In seawater, Ozone reacts with bromide ions to produce hypobromous hypobromous acid (HOBr) and and hypobromite (OBr–) which are in equilibrium with one another (von Gunten and Oliveras 1998, von Gunten 2003a, b). These compounds have disinfection properties. Bromoform (CHBr3), a disinfection byproduct, is also formed by a reaction with natural organic matter in the water. In freshwater Ozone breaks down more slowly (minutes rather than seconds) into oxygen and water. The rate of decomposition increases with the presence of organic impurities and increasing pH. As pH increases, Ozone turns into the very short-lived (microseconds) hydroxyl radicals (OH) (Rice and Wilkes, 1992).

The electrochemical system developed by the applicant produces a number of Active Substances and relevant chemicals at detectable concentrations including hydroxyl radicals (OH), free active chlorine (Cl_2) and hydrogen gas (H_2). Hydroxyl radicals (OH) are the primary Active Substances to be produced in freshwater, and is a very reactive oxidative species with a half-life in the order of nanoseconds. In seawater, small amounts of chlorine gas (Cl_2) are also likely to form on the anode surface of electrochemical system due to the presence of chloride ions. Reactions with the cathode on the electrochemical system will also produce other free active chlorine species – HOCl and/or OCl^- – depending on the pH. Chlorine gas (Cl_2) and the free active chlorine species (HOCl and OCl^-) will rapidly decay back into chloride ions. Trihalomethanes (THMs) (trifluoromethane CHF_3, chlorodifluoromethane $CHClF_2$, trichloromethane $CHCl_3$) may also be formed in the reactor due to the reaction of chlorine species with organic matter in the ballast water.

In this application, Ozone, bromate ions, bromoform, hydroxyl radicals, chlorine gas (Cl2), free free active chlorine species (HOCl and ClO-), and hydrogen gas generated during treatment of the ballast water are defined as relevant chemicals. Relevant chemical identifiers of these substances are provided in Table 2.

Table 2. Physical and chemical properties of the preparations and Active Substances used in the RBT system

Chemical name of substance	**Ozone**	**Bromate ion (in the form of Potassium bromate)**	**Bromoform**	**Hydroxyl radical**	**Chlorine gas**	**Sodium hypochlorite**	**Total residual oxidants**	**Hydrogen gas**	**Treated ballast water**
Liquid Concentration	1358g/L (at -112 °C)	-	2880g/L (at 20 °C)				-	-	-
CAS number	10028-15-6	7758-38-0	75-25-2	3352-57-6	7782-50-5	7681-52-9	-	01333-74-0	-
UN number and shipping name		UN1484 Potassium bromate	UN2515 Bromoform	-	UN1017 Chlorine	UN1971 Sodium hypochlorite	-	UN1049 Hydrogen	-
Active Substance (AS)/ stabilizer/inhibitor/ solvent	AS	AS	AS	AS	AS	AS	AS	AS	-
Molecular weight	48	167	252.73	17.01	70.9	74.4	-	2.015	-
Empirical formula	O_3	$KBrO_3$	$CHBr_3$	HO	Cl_2	NaClO	-	H_2	-
Structural formula	O=O-O			O*H	Cl-Cl	Na-OCl	-	H – H	-
Purity[1]						Usually 5-15% solution		>99%	-
Necessary stabilisers & additives	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	-
Melting point	-193°C	350°C	8.3 °C	-	N/A	-20 to -30 °C		-259 °C	-
Boiling point	-112°C	370°C	150 °C	-	N/A	96-120 °C	-	-253 °C	-
Flammability (flash point) °C	Non-combustible but promotes burning of other substances	N/A	>107 °C	N/A	Non-flammable	N/A	N/A	N/A	-
Density (20°C)		3.27 g/m^3				1.1 (relative density)		0.0899x10^{-3} g/cm^3	-
Vapour pressure (20°C)	>1atm		6.4hPa		5.85 x 103mmHg				-
Vapour Density (air=1)	1.6	5.8	8.7					0.0695	-
Water solubility/dissociation constant (pKa)	Definition for liquid; no relation with gas	8.62							-
Oxidation/reduction potential	2.07V		2.06V	2.8V	1.5V	1.7V			-
Corrosivity to the materials or equipment of normal ship construction	Yes	Yes	Yes		Yes	Yes			-
Autoignition temperature °C	Non-combustible but promotes burning of other substances				Forms explosive mixtures with flammable gases and mixtures			565.5	-

Chemical name of substance	Ozone	Bromate ion (in the form of Potassium bromate)	Bromoform	Hydroxyl radical	Chlorine gas	Sodium hypochlorite	Total residual oxidants	Hydrogen gas	Treated ballast water
Explosive properties	May cause fire or explosion when heated or coming into contact with a combustible material (alkene, ether)					Anhydrous sodium hypochlorite is explosive		Extremely especially at high temperatures	-
Oxidizing properties	Yes	Yes				Yes		Yes	-
Surface tension	3.84×10^{-2} N/mm (at -183°C)							-	-
Viscosity	1.5×10^{-3} Pa.s (at -183°C)	66.6-67.6						-	0.01Pa.s
Thermal stability and identity of relevant breakdown products	Oxygen								-
Reactivity towards container material	Yes								-
pH	-	-	-	-	-	-	-	-	5-8
Salinity	-	-	-	-	-	-	-	-	
TOC, DOC, % particulate matter	-	-	-	-	-	-	-	-	
Other known relevant physical or chemical hazards	Nothing noteworthy					Incompatibility with Nitrogen compounds, ammonium salts, acids, methanol. Copper, Nickel and cobalt speed up decomposition of NaOCl			Nothing noteworthy
Analytical methods at environmentally relevant concentrations									

2.4 Other chemicals

No other relevant chemicals are produced by the RBT reactor.

3 DATA ON EFFECTS ON AQUATIC PLANTS, INVERTEBRATES AND FISH, AND OTHER BIOTA, INCLUDING SENSITIVE AND REPRESENTATIVE ORGANISMS

3.1 Ozone (Active Substance)

Ozone is an unstable gas that cannot be stored. In pure (fresh) water Ozone has a half life of 10-30 minutes. In seawater the half life is reduced to 5.3 seconds. Ozone decomposition rate increases in accordance with the increase of temperature and pH. The rate increases fast in the neutral to alkaline range at any temperature. This tendency accelerates with the increase of temperature. The pH value of ballast water is generally between 5 and 8, in which range the decomposition of Ozone progresses quickly. Therefore it is highly unlikely that Ozone will have an effect on any ecosystem by the time the ballast water is discharged.

Note that Basic Approval has been recommended by the GESAMP-Ballast Water Working Group (GESAMP-BWWG) (MEPC 55/2, 2006) for use of Ozone at concentrations greater than those produced by the RBT reactor. Full details on the nature, toxicity and risks posed by this compound is nonetheless provided in this report for completeness.

3.1.1 Acute aquatic toxicity

The PAN pesticides database was used to obtain data on the acute toxicity of Ozone to aquatic organisms. Table 3 shows the effects of Ozone on representatives of three taxonomic groups (fish, molluscs and crustaceans). The acute toxicity of Ozone (LC_{50}) used as an Active Substance for this system is 8.1µg/L in freshwater and 50µg/L in seawater.

Table 3. Effects of Ozone on representatives of two taxonomic groups (fish and molluscs)

Test water substance	Organism	Scientfic/Common name	Stage	Endpoint	Effect	Effect Measurement	Duration Exp. Type	Concentration as Ozone (µg/L)	Reference
Ozone	Fish	*Lepomis macrochirus* Bluegill	3.82g	LC50	Mortality	Mortality	24 hrs	60.0	Paller and Heidinger (1979)
		Morone saxatilis Striped bass	Larvae, 20mm	LC50	Mortality	Mortality	6 hrs	150.0	Hall *et al.* (1981)
		Pimephales promelas Fathead minnow	Larvae	LC50	Mortality	Mortality	0.03 hrs	100.0	Asbury and Coler (1980)
	Molluscs	*Dreissena polymorpha* Zebra Mussel	Adult	LC50	Mortality	Mortality	5.78 hrs	520.0-540.0	Harrington *et al.* (1997)

There is limited data on the chronic aquatic toxicity of Ozone. The concentration of Ozone will break down to <11 µg/L (NOEC for *Daphnia magna*, Leynan *et al.* 1997) within 40 seconds in seawater and within 3.5 hours in freshwater, and to <0.14 µg/L (natural background levels) within 75 seconds in seawater and 6.5 hours in freshwater. Thus, it is supposed that Ozone has no chronic aquatic toxicity.

3.1.2 Endocrine disruption

There have been no documented studies on the endocrine disruptive properties of Ozone. Due to its rapid dissolution (see paragraph 3.2), it is considered not to be an endocrine disruptor.

3.1.3 Sediment toxicity

The decomposition speed of Ozone is high enough so that there is no concern of toxicity to benthic organisms (see paragraph 3.2).

3.1.4 Bioavailability/biomagnification/bioconcentration

Ozone decomposes very rapidly in natural water (see paragraph 2.2.1.1). Therefore there is no risk that Ozone may become concentrated in organisms.

3.1.5 Food web/population effects

Ozone does not persist or concentrate in organisms therefore there is no concern of effect on any animal high up the food chain.

3.1.6 Data on mammalian toxicity

The toxicity of Ozone to mammals is based on its strong oxidizing property. Ozone causes irritation to the eyes and to the pharynx, trachea, bronchi and bronchiole when inhaled.

3.1.6.1 Acute toxicity

Acute toxicity data for Ozone is available from test conducted on mice (Cheminfo by Canadian Centre for Occupational Health and Safety). Data has been submitted for inhalation only and not for oral and dermal toxicity as Ozone is a gas and thus inhalation is the main exposure route of concern. The acute toxicity of Ozone (4 hours, LD_{50}) is 5.9-6.8ppm.

3.1.6.2 Effects on skin and eye

Ozone gives no effect to the skin but causes irritation to the eyes at levels of 2 ppm or more when exposed for several minutes.

3.1.6.3 Repeated-dose toxicity

See chronic toxicity.

3.1.6.4 Chronic toxicity

In a series of studies, mice and rats exposed to 0.01-0.02 mg/kg bw/day for 105 to 130 weeks displayed increased incidence of non-carcinogenic lesions of the nose and lungs (NTP, 1994). Several studies also examined the effects of long-term exposure to monkeys. Exposures ranging from 0.003-0.013 mg/kg bw/day for 3-18 months consistently showed significant structural alterations to lungs. Many of the changes seemed irreversible six months after exposure had stopped.

3.1.6.5 Developmental and reproductive toxicity

A study on offspring of rats continuously exposed to 0.020 or 0.03 mg/kg bw for four days during middle or late pregnancy produced reduced growth rates after birth and delayed behavioural development. Maternal toxicity was not reported.

Another study reported a decrease in fetal body weight gain in the offspring of mice exposed to 0.024 but not to 0.008 or 0.016mg/kg bw, on days 7 to 17 of pregnancy. Signs of maternal toxicity were observed (Tox. Applied Pharmac. 1979).

Embryo toxicity in the form of increased resorption was observed in rats exposed to 0.03 and 0.04 mg/kg bw, but not 0.013-0.025 mg/kg bw. Maternal toxicity was not reported (COSHH).

Continuous exposure of mice from six days prior to mating to postnatal day 22 resulted in reduced body weight and slight effects in neurobehaviour development of the offspring. Maternal toxicity was not evaluated. No effects on reproductive performance (proportion of successful pregnancies, litter size, offspring viability and sex ratio) were noted (Arch. Toxicology, 1995).

3.1.6.6 Carcinogenicity

From a study with rats and mice exposed to 0.0021, 0.01 and 0.02 mg/kg bw of Ozone for 105-130 weeks, there was no evidence of carcinogenicity in male and female rats, inconclusive evidence in male mice and some evidence in female mice. Ozone showed no tumour promoter properties in these studies (NTP, 1994).

3.1.6.7 Mutagenicity/genotoxicity

There are a number of studies which examine the potential for mutagenicity of Ozone in humans but due to some deficiencies not all of them seem to be conclusive.

However cytogenetic aberrations have been observed in the lymphocytes of hamsters exposed to 0.0048-0.086 mg/kg bw for 5 hours and in the macrophages of female rats exposed 0.0024-0.016 mg/kg bw for 6 hours. Several studies have also shown that Ozone is mutagenetic in bacteria and in human and mammalian cells in vitro following inhalation exposure (NTP, 1994; Mutation Research, 1992).

3.1.6.8 Toxicokinetics

Ozone is absorbed in both the upper and lower respiratory tract. It is a potent oxidant that reacts with protein and lipids, particularly within biological membranes. A small amount of Ozone is absorbed into the blood. The extreme reactivity of Ozone limits its ability to accumulate.

3.1.7 Data on environmental fate and effect under aerobic and anaerobic conditions

Ozone reacts with both organic and inorganic compounds when injected into ballast water. The Ozone molecule is an unstable substance and its decomposition in water is promoted by higher temperatures and higher pH. In seawater, Ozone depredates with a half life of between 5 and 30 seconds (Haag *et al.* 1983). It is finally decomposed to oxygen and water in the water.

Some of the main reactions when Ozone is injected into seawater are as follows:

1. $O_3 + Br \rightarrow O_2 + OBr$
2. $O_3 + OBr \rightarrow 2O_3 + Br$
3. $2O_3 + OBr \rightarrow 2O_2 + BrO_3^-$
4. $Br^- + O3 \leftrightarrow OBr$
5. $OBr^- + H^+ \leftrightarrow HOBr$
6. $HOBr + \text{Organic Matter} \rightarrow CHBr_3$
7. $HOBr + NH_3 \leftrightarrow NH_2Br$

Reactions 6 and 7 do not occur in water with a pH higher than 8 or in water with high contents of bromium compounds.

3.1.7.1 Modes of degradation (biotic; abiotic)

Ozone breaks down in an abiotic process into oxygen (O_2).

3.1.7.2 Bioaccumulation, octanol/water partition coefficient

Bioaccumulation is a general term for the accumulation of substances, such as pesticides or other organic chemicals in an organism or part of an organism. The accumulation process involves the biological sequestering of substances that enter the organism through respiration, food intake, epidermal (skin) contact with the substance, and/or other means. The sequestering results in the organism having a higher concentration of the substance than the concentration in the organism's surrounding environment.

The level at which a given substance is bioaccumulated depends on the rate of uptake, the mode of uptake (e.g. through the gills of a fish, ingested along with food, contact with epidermis), how quickly the substance is eliminated from the organism, transformation of the substance by metabolic processes, the lipid (fat) content of the organism, the hydrophobicity of the substance, environmental factors, and other biological and physical factors. As a general rule the more hydrophobic a substance is the more likely it is to bioaccumulate in organisms, such as fish. Another way of saying this is that bioaccumulation of a substance is correlated to the octanol-water partition coefficient (K_{OW}) of the substance. Increasing hydrophobicity (lipophilicity) leads to an increasing propensity to bioaccumulate.

Bioccumulation data for Ozone shows the octanol/water partition coefficient to be less than 1 for Ozone. This means that it has a low propensity to bioaccumulate.

3.1.7.3 Reaction with organic matter

Cyclo addition (Criegee mechanism)

Consequentially to its dipolar structure, an Ozone molecule can undergo a 1-3 dipolar cyclo addition with saturised compounds (double or triple bonds). This leads to the formation of a compound called ozonide (Figure 3).

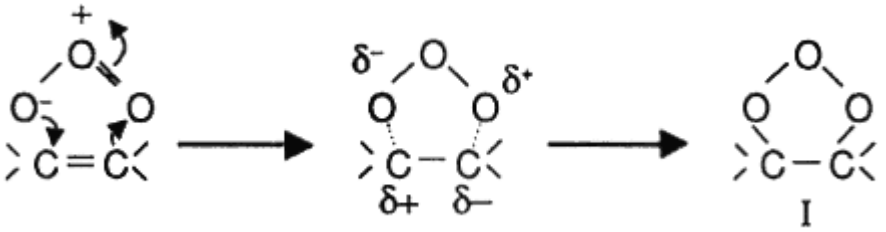

Figure 3. Dipolar cyclo addition (Lenntech database)

In a protonic solution, such as water, primary ozonide disintegrates into an aldehyde, a keton or a zwitter ion (Figure 4). The zwitter ion will eventually be disintegrated further into hydrogen peroxide and carboxyl compounds.

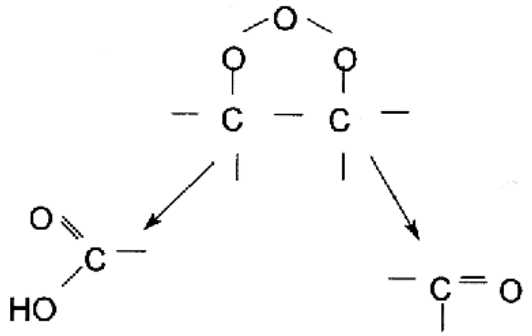

Figure 4. Disintegration of ozonide (Lenntech database)

Electrophilic reactions

Electrophilic reactions occur in molecular solutions that have a high electronic density and mainly in solutions that contain a high level of aromatic compounds. Aromatic compounds that are substituted by electron donors (such as OH and NH_2), have a high electronic density on the carbon compounds in ortho and para position. Consequentially, in these positions aromatic compounds react actively with Ozone. Figure 5 is an example of a reaction between Ozone and Phenol. Phenol groups react with Ozone relatively quickly.

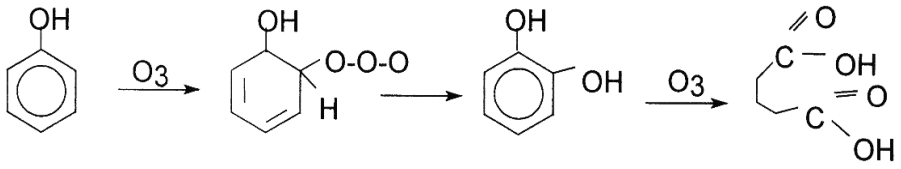

Figure 5. Reaction between phenol and Ozone (Lenntech database)

Nucleophilic reactions

Nucleophilic reactions mainly take place where there is a shortage of electrons and particularly at carbon compounds that contain electron-retreating groups, such as –COOH and $-NO_2$. For electron-retreating groups, the reaction speed is much lower.

From the above-mentioned data, it appears that direct oxidation of organic matter by Ozone is a selective reaction mechanism, during which Ozone reacts quickly with organic matter that contains double bonds, activated aromatic groups or amines. It is also stated that Ozone reacts quicker with ionized and dissociated organic compounds than with the neutral (non-dissociated) type.

For most inorganic compounds in drinking water, the reaction speed is relatively high. The main reaction mechanism for oxidation of inorganic compounds is determined by transfer of the extra oxygen atom of Ozone to the inorganic compounds. For inorganic compounds, reaction speed is also higher for ionized and dissociated compounds.

In summary, Ozone oxidizes organic compounds selectively and partly. A large number of inorganic compounds are oxidized fast and completely.

3.1.7.4 Potential physical effects on wildlife and benthic habitats

As the half life of Ozone is 5.8 seconds in seawater and 30 minutes in fresh water, it decomposes too quickly for the treated ballast water to have any potential effects on wildlife and benthic habitats.

3.1.7.5 Potential residues in seafood

As Ozone is not accumulated, there will be no risk of residue in seafood.

3.1.7.6 Any known interactive effects

Ozone produces oxygen when decomposed in water. As a result it increases the amount of oxygen dissolved in the water which helps aquatic organisms. In a closed system, such as a seawater aquaculture system, Ozone removes nitrogen in the form of ammonia that is discharged from fish.

3.2 Bromate Ion (Related Chemical)

Ozone reacts with bromide in seawater to form the bromate ion (BrO_3^-). The half life of the bromate ion is approximately 12 hours. Freshwater typically contains a very low concentration of the bromide ion and therefore the production of the bromate ion does not occur in freshwater.

Note that bromate ions are formed through reaction of Ozone with compounds in seawater and that Basic Approval has been recommended by the GESAMP-Ballast Water Working Group (GESAMP-BWWG) (MEPC 55/2, 2006) for use of Ozone at concentrations greater than those produced by the RBT reactor. Full details on the nature, toxicity and risks posed by this compound is nonetheless provided in this report for completeness.

3.2.1 Acute aquatic toxicity

The PAN pesticides database was used to obtain data on the acute toxicity of bromate to aquatic organisms. Effects of bromate on representatives of three taxonomic groups (fish, molluscs, and crustaceans) are summarized in Table 4.

Table 4. Effects of bromate on three taxonomic groups (fish, molluscs and crustaceans)

Test water substance	Organisms	Scientfic/ Common Name	Stage	Toxicity Endpoint	Effect	Effect Measurement	Duration Exp. Type	Toxic dose (µg/L)	Reference
Sewater Bromate	Fish	*Cymatogaster aggregater* Shiner Perch	2.5-6cm	LC100	Mortality	Mortality	72 hrs	880,000	Crecelius (1979)
		Oncorhynchus Keta Chum salmon	Juvenile 3cm	LC50	Mortality	Mortality	96 hrs	512,000	Crecelius (1979)
	Molluscs	*Crassostrea gigas* Pacific oyster	Larvae	EC50	Development	Developmental changes	48 hrs	30,000	Crecelius, 1979
		Macoma inquinata Bent-nosed clam	2-3cm	LC100	Mortality	Mortality	72 hrs	880,000	Crecelius (1979)
		Protothaca staminea Littleneck clam	2-5cm	LC100	Mortality	Mortality	72 hrs	880,000	Crecelius (1979)
		Crassostrea virginica American oyster	Fertilized eggs	NR	Mortality	Mortality	48 hrs	1,000	Stewart *et al.* (1979)
	Crustaceans	*Pandalus danae* Coon stripe shrimp	4-5cm	LC100	Mortality	Mortality	72 hrs	880 000	Crecelius (1979)
		Neomysis awatschnsis Opossum shrimp	Adult 1.5-3.0g	LC50	Mortality	Mortality	24 hrs	176 000	Crecelius (1979)
Freshwater Bromate	Not applicable	-	-	-	-	-	-	-	-

3.2.2 Endocrine disruption

No instances have been reported for the endocrine disruptive properties of bromate ions.

3.2.3 Sediment toxicity

Soil absorption factor of bromate ion is estimated as follows from the relation with the octanol/water partition coefficient, P_{ow}:

$$K_{oc} = 0.41 \times \log P_{ow} (-7.18)^{8} = 2.7 \times 10^{-8} \text{ L/kg}$$

This estimate of K_{oc}: 2.7×10^{-8} L/kg is below the value of anticipated toxicity to benthic organisms and there is therefore no risk of toxicity to benthic organisms.

3.2.4 Bioavailability/biomagnification/bioconcentration

The octanol/water partition coefficient log value of bromate ion is -7.18 and is therefore considered not to pose a risk of concentration in organisms.

3.2.5 Food web/population effects

The bromate ion does not accumulate and there will therefore be no affect on organisms high in the food chain.

3.2.6 Data on mammalian toxicity

The toxicity of the bromate ion to mammals is based on its strong oxidizing properties. Bromate ions may effect the skin and eyes by exposure and cause coughing, pharynx pain, nausea, vomiting, abdominal pain, diarrhea or central nervous system disturbance if taken orally.

3.2.6.1 Acute toxicity

Experiments on rats have shown a single dose of Potassium bromate of concentration LD_{50} 321 mg/kg given orally is lethal (Sinochem chemical data sheet).

3.2.6.2 Effects on skin and eye

Bromate ions will cause skin and eye inflammation and pain.

3.2.6.3 Repeated-dose toxicity

The exposure of rats to bromate for 15 weeks demonstrated a No Observed Adverse Effect Level (NOAEL) of 30 mg/kg bw/day.

3.2.6.4 Chronic toxicity

The half life period of the bromate ion in seawater is approximately 12 hours longer than Ozone. Although the acute toxicity is relatively weak (approximately 1/1000 of that of Ozone) it is expected that the chronic effects are more likely due to the fact that it takes longer to decompose.

The carcinogenicity and chronic toxicity of potassium bromate ($KBrO_3$) was studied in male B6C3F1 mice and F344/N rats to confirm and extend the results of previous work. Mice were treated with 0, 0.08, 0.4, or 0.8 g/L $KBrO_3$ in the drinking water for up to 100 wk, and rats were provided with 0, 0.02, 0.1, 0.2, or 0.4 g/L $KBrO_3$. Animals were euthanatized, necropsied, and subjected to a complete macroscopic examination. Selected tissues and gross lesions were processed by routine methods for light microscopic examination. The present study showed that $KBrO_3$ is carcinogenic in the rat kidney, thyroid, and mesothelium and is a renal carcinogen in the male mouse, $KBrO_3$ was carcinogenic in rodents at water concentrations as low as 0.02 g/L (De Angelo, 1998).

3.2.6.5 Developmental and reproductive toxicity

Limited data are available on the reproductive or developmental effects of bromate by the oral route. Only one screening-level study (Wolf and Kaiser, 1996 in EPA/635/R-01/002, 2001) evaluates reproductive effects in rats. Although this study reports that pups were evaluated on postnatal day 5, no information on developmental endpoints is provided. No reliable multigenerational studies are available. Another study showed several multigenerational studies in which rats or mice were fed bread made from flour treated with potassium bromate (Kurokawa *et al*., 1986 in EPA/635/R-01/002, 2001), however, because most potassium bromate added to flour is converted to bromide during the bread baking process it is unlikely that the animals in these multigenerational studies were actually exposed to bromate. No data are available evaluating reproductive or developmental effects by the inhalation route (EPA/635/R-01/002, 2001).

In a study conducted for NTP, the potential reproductive and developmental toxicity of sodium bromate in Sprague-Dawley rats was evaluated following oral administration in the drinking water at concentrations of 0.25 ppm (2.6 mg/kg-day), 80 ppm (9.0 mg/kg-day), or 250 ppm (25.6 mg/kg-day) over a 35-day period. (Equivalent bromate ion doses are 2.2, 7.7, and 22 mg BrO_3 -/kg-day). (Wolf and Kaiser 1996 in EPA/635/R-01/002, 2001). Two groups of female rats were treated. Group A females (10/group) were dosed from study day 1 to 34 to test for effects during conception and early gestation. Group B females (13/group) were dosed from gestation day 6 to postnatal day 1 to test for effects during late gestation and birth. Male rats (10/group) were cohabited with Group B females for 5 days before dosing (study days 1-5) and were then dosed from study day 6 to day 34/35. Endpoints evaluated in males included clinical pathology, organ weight, sperm analysis, and histopathology. Endpoints evaluated in females included maternal body weight, number and weight of pups, and number of uterine implantations. Females in Group B were allowed to litter, and the pups were observed through postnatal day 5. However, there is no indication of the developmental endpoints that were evaluated in these pups or if any effects were observed. Treated males in the 250-ppm dose group demonstrated a statistically significant decrease (18%) in epididymal sperm density. All other endpoints evaluated were comparable between controls and treated groups. Female reproductive function was not adversely affected. There were no treatment-related gross or microscopic changes in the kidney, liver, spleen, testis, or epididymis. These results indicated that sodium bromate treatment did not produce any adverse signs of general toxicity in any of the dose levels tested; a MTD was not reached. On the basis of changes in sperm density, this study identifies a NOAEL of 80 ppm (7.7 mg BrO_3 -/kg-day) and a LOAEL of 250 ppm (22 mg BrO3 -/kg-day). (Taken directly from EPA/635/R-01/002, 2001).

3.2.6.6 Mutagenicity/genotoxicity

The carcinogenicity of KBrO to newborn rats and mice has been investigated. Male and female newborn F344 rats and ICR mice (24 hours old) were given single subcutaneous injections of 9.6, 19, 38, 77, or 154 mg BrO_3 -/kg. Another group of newborn rats and mice received four weekly injections of 9.6, 19, 38, 77, or 154 mg BrO_3 -/kg until weaning. Control animals received injections of olive oil. Rats were killed at 82 weeks, mice were killed at 78 weeks, and organs were examined histologically. No significant differences in the incidence of tumors in male or female rats or mice were observed. Under the conditions of the study, potassium bromate had no potential for carcinogenicity in newborn male or female rats or mice (Matsushima *et al*., 1986 in EPA/635/R-01/002, 2001).

Another study tested the tumor initiation potential of bromate in a 104-week study in which male F344/NCr rats (29 or 39 per group) were given an intragastric dose of 300 mg $KBrO_3$/kg (231 mg BrO_3 -/kg), the maximum tolerated single dose. The rats were administered bromate alone, bromate followed by 4,000 ppm sodium barbital in the animal diet as a promoter, or sodium barbital in the diet alone. Sodium barbital was added to the diet starting 2 weeks after the animals were dosed with potassium bromate. Rats were examined at 30, 52, and 104 weeks for nephropathy. At 30 weeks, renal damage (dysplastic tubular foci) was evident in the rats exposed to potassium bromate followed by sodium barbital and in rats exposed to sodium barbital, but not in those exposed to potassium bromate alone. The results indicated that a single oral dose of 300 mg $KBrO_3$/kg (231 mg BrO_3 -/kg) administered to rats does not initiate renal tumors within a 104-week observation period (Kurata *et al.* 1992 in EPA/635/R-01/002, 2001).

Groups of 8, 14, 20 and 26 male F344 rats were supplied with water containing 500 mg BrO_3 -/L for up to 104 weeks to assess the time-course of renal cell tumor induction. The average daily consumption of potassium bromate was 41.9 mg/kg (32.3 mg BrO_3 -/kg). At 104 weeks, the surviving animals were killed and examined histopathologically for dysplastic foci, renal adenomas and adenocarcinomas, thyroid follicular cell tumors, and peritoneal mesotheliomas. All were significantly increased with continuous treatment. Dysplastic foci and renal adenomas were first observed following 26 weeks of continuous treatment. Renal dysplastic foci and adenomas were each significantly increased over controls by 52 weeks of treatment (mean number of renal cell tumors/rat was 0.81 vs. 0 in the controls). Continuous potassium bromate administration over 104 weeks resulted in renal adenocarcinomas in 3/20 (15%) and renal adenomas in 6/20 (30%) rats. At 104 weeks the mean number of renal cell tumors/rat was 1.25 compared with 0 in the controls. The combined incidence of follicular adenomas and adenocarcinomas of the thyroid was increased significantly (7/20 [35%]; $p < 0.01$) in rats receiving treatment for 104 weeks. The authors concluded that the minimum induction time for renal adenoma development was 26 weeks (Kurokawa *et al.*1987 in EPA/635/R-01/002, 2001).

The genotoxic potential of potassium bromate was examined in a variety of tests with V79 Chinese hamster cells, including cytotoxicity, micronucleus, chromosome aberration, HPRT gene mutation, and comet assays. In addition, analysis was conducted on the HPRT mutations and for 8-oxodeoxyguanosine. Bromate was cytotoxic, increased the frequency of cells with micronuclei, increased the number of chromosome aberrations, and increased DNA migration in the alkaline comet assay. The majority of chromosome aberrations observed were chromatid breaks and chromatid exchanges. High-pressure liquid chromatography analysis revealed significantly increased levels of 8-oxodeoxyguanosine after potassium bromate treatment. Furthermore, potassium bromate clearly induced gene mutations at the HPRT locus. Molecular analysis of potassium bromate-induced mutations indicated a high proportion of deletion mutations. Three out of four point mutations were G-to-T transversions, which typically arise after replication of 8-oxoguanine. These results are consistent with oxidative damage induced by bromine radicals (Speit *et al.*1999 in EPA/635/R-01/002, 2001).

3.2.6.7 Toxicokinetics

Bromate may be converted into hypobromic acid by hypochloric acid in the stomach. Liver and kidney tissues may degrade bromate to bromide by a process involving glutathione, although only a small amount appears to be reduced in this way. Bromate is excreted mainly by urine as bromate and bromide and some may also be eliminated by faeces. Bromate concentrations in urine peak at about 1 hour, and bromate is not detectable in plasma after 2 hours.

In anaerobic conditions, the bromate ion is decomposed by anaerobic bacteria such as methanogen.

3.2.7 Data on environmental fate and effects under aerobic and anaerobic conditions

3.2.7.1 Modes of degradation (biotic; abiotic)

Bromine is reportedly removed from seawater by several mechanisms (Perrins *et al.* 2006). HOBr/OBr_ reacts with natural organic matter to form bromoform ($CHBr_3$) by the haloform reaction. In the presence of ammonia, $HOBr/OBr^-$ will react rapidly to form monobromamine which can subsequently disproportionate to $NHBr_2$ and NH_3 or with excess $HOBr/OBr^-$ it can react further to form N_2 and bromide. The rate of these reactions varies light and salinity concentration.

3.2.7.2 Bioaccumulation, octanol/water partition coefficient

Small amounts of bromine (1–2 ppm) were detected in the adipose tissue of mice, but not of rats, fed bread treated with potassium bromate in a lifetime study (Kurokawa *et al.*, 1990 in EPA/635/R-01/002, 2001).

3.2.7.3 Reaction with organic matter

Bromate does not volatilize and is only slightly adsorbed onto soil or sediment. Because it is a strong oxidant, its most likely fate is reaction with organic matter, leading to the formation of bromide ion (WHO, 2005). Potassium bromide is also reportedly harmful if ingested in large amounts and it can cause skin and eye irritations.

3.2.7.4 Potential physical effects on wildlife and benthic habitats

The half life of the bromate ion in seawater is fairly short (~12 hours) and its estimated absorption coefficient is very low (2.7kg x 10-8 L/kg), there is no potential of effects on benthic organisms. The bromate ion is not accumulated and therefore will not affect high-order organisms in a food chain.

3.2.7.5 Potential residues in seafood

The bromate ion is not accumulated and therefore there will be no potential residues in seafood.

3.2.7.6 Any known interactive effects

Under normal environmental conditions, it is difficult for the bromate ion to be reduced. However, it may be reduced to the bromide ion where ferrous ions, activiated carbon or other carbonaceous substances exist.

3.3 Bromoform (Tribromethane) – (Related Chemical)

Note that bromoform is produced through the reaction of Ozone with bromide ions in seawater and that Basic Approval has been recommended by the GESAMP-Ballast Water Working Group (GESAMP-BWWG) (MEPC 55/2, 2006) for use of the Ozone at concentrations greater than those produced by the RBT reactor. Full details on the nature, toxicity and risks posed by this

compound is nonetheless provided in this report for completeness.

3.3.1 Acute aquatic toxicity

The PAN pesticides database was used to obtain data on the acute toxicity of bromoform to aquatic organisms. Table 5 shows the effects of Bromoform on representatives of four taxonomic groups (fish, molluscs, crustaceans and phytoplankton).

Table 5. Effects of bromoform on representatives of four taxonomic groups (fish, molluscs, crustaceans and phytoplankton)

Test water substance	Organisms	Scientfic Name Common Name	Stage	Endpoint	Effect	Effect Measurement	Duration Exp. Type	Minimum Toxic dose (µg/L)	Reference
Bromoform	Fish	*Brevoortia tyrannus* Atlantic menhaden	NR	LC50	Mortality	Mortality	96 hrs	9,000	Anderson (1979)
		Cyprinodon variegates Sheepshead minnow	8-15mm, 14-28 days	LC50	Mortality	Mortality	48 hrs	16,000	Heitmuller *et al.* (1981)
		Lepomis macrochirus Blue Gill	Juvenile, 0.32-1.2g	LC50	Mortality	Mortality	24 hrs	33,000	Buccafusco *et al.* (1981)
	Molluscs	*Crassostrea virginica* American oyster	75g	NR	Mortality	Mortality	96 hrs	40,000	Gibson *et al.* (1981)
		Mercenaria mercenaria Hard clam	141g	NR	Mortality	Mortality	96 hrs	140,000	Gibson *et al.* (1981)
	Crustaceans	*Penaeus aztecus* Brown shrimp	3.3g	LC50	Mort ality	Mortality	96 hrs	20,000	Gibson *et al.* 1981
	Phtyoplankton	*Selenastrum capricornutum* Green algae	NR	EC50	Biochemistry	Chlorophyll	24 hrs	18,700	US EPA, 1978
		Selenastrum capricornutum Green algae	NR	EC50	Population	Population changes, general	96 hrs	28,100	US EPA, 1978

NR=Not recorded

3.3.2 Endocrine disruption

No information is available on disruption of endocrine functions by bromoform.

3.3.3 Sediment toxicity

Bromoform and dibromochloromethane have a minor tendency to be adsorbed by soils and sediments. Calculated and measured values of K_oc (the organic carbon/water partition coefficient, an index of the relative mobility of a material in water-soil systems) for bromoform range from 62 to 126 (Hassett *et al.* 1983; Hutzler *et al.* 1986; Mabey *et al.* 1982 in U.S. Agency

for Toxic Substances and Disease Registry, 2005). These relatively low values imply that bromoform will exhibit only a minor affinity for soil materials and will tend to be highly mobile (Roy and Griffin 1985 in U.S. Agency for Toxic Substances and Disease Registry, 2005). This low tendency for adsorption to soil has been confirmed in laboratory studies (Curtis *et al*. 1986 in U.S. Agency for Toxic Substances and Disease Registry, 2005) and in field studies by (Roberts *et al*.1986 in U.S. Agency for Toxic Substances and Disease Registry, 2005).

3.3.4 Bioavailability/biomagnification/bioconcentration

Bioaccumulation potential of the trihalomethanes appears to be low, compared to many chlorinated organic compounds. 28 day uptake and depuration tests were conducted on five commercially and recreationally important species. These included three species of Penaeid shrimp and one species of fish (Gibson *et al* 1979 in UK Marine Special Areas of Conservation Database). The authors found that both uptake and depuration were rapid, with an equilibrium reached after 24 hours. Bioconcentration factors were relatively low (between <1 and 10 times the water concentration). Experiments using the marine oyster *Crassostrea virginica* have shown that exposure to 90 µg l^1 bromoform for 24 hours resulted in a bioconcentration factor of 7.6. Uptake and depuration of bromoform were both rapid, both being essentially complete in 24-48 hours. However, it was concluded that the rate of uptake and depuration was dependent on the individual, the species and the concentration of bromoform in the water.

3.3.5 Food web/population effects

There is inconclusive evidence as to whether bromoform is concentrated up the food chain but it appears unlikely that bromoform persists in organisms.

3.3.6 Data on mammalian toxicity

Bromoform has been considered to be a moderately toxic chemical that can be absorbed through the lungs, gastrointestinal tract, and skin. The oral LD_{50} value for tribromomethane is 1,400 and 1,550 mg/kg in male and female ICR Swiss mice (Bowman *et al*., 1978 in Melnick, 1989) and 1,388 and 1,147 mg/kg in male and female Sprague Dawley rats (Chu *et al*., 1980 in Melnick, 1989). Tribromomethane was slightly less acutely toxic than chloroform, bromodichloromethane, or chlorodibromomethane in rats and mice. In male rats, the intraperitoneal LD_{50} value for tribromomethane is 414 yl/kg (1,196 mg/kg) (Agarwal and Mehendale, 1983 in Melnick, 1989). Exposure to bromoform vapors may cause irritation to the respiratory tract, lacrimation, and liver damage (von Oettingen, 1955 in Melnick, 1989).

The liver and kidney have been identified as target organs of trihalomethane toxicity in single exposure and short-term studies. Liver changes, described as variation in size of hepatocytes and vesiculation of biliary epithelial nuclei, were observed in female Sprague Dawley rats that survived a single oral dose of tribromomethane (1,071 mg/kg or higher) (Chu *et al*., 1982 in Melnick, 1989). Kidney changes were observed in Sprague Dawley rats that survived single oral doses of chloroform, bromodichloromethane, or chlorodibromomethane; however, these changes were not observed in rats exposed to tribromomethane. No pathologic changes were observed in Sprague Dawley rats exposed to tribromomethane at 5,50, or 500 ppm (equivalent to 0.13, 1.5, and 14 mg per rat per day, respectively) in drinking water for 28 days (Melnick, 1989).

3.3.6.1 Acute toxicity

Oral LD_{50} values for bromoform are 1388 and 1147 mg/kg for male and female rats, respectively (Chu *et al*., 1980 in Faust, 1995) and 1400 and 1550 mg/kg for male and female mice, respectively (Bowman *et al*. 1978 in Faust, 1995). The principal cause of death in laboratory animals following acute oral exposure is CNS depression. Clinical signs recorded in rats included piloerection, sedation, flaccid muscle tone, ataxia, prostration, and hypothermia. Enlargement of the livers and kidneys was also observed (Chu *et al*. 1980). At doses of 1000 mg/kg, ataxia, sedation, and anesthesia occurred in mice within 60 minutes, with sedation persisting for about 4 hours (Bowman *et al*., 1978 in Faust, 1995). In rats, a single gavage dose of 1000 mg/kg produced reductions in liver cytochrome P-450 content and aminolevulinic acid-dehydratase activity and increases in porphyrin content, suggesting disturbances in hepatic heme metabolism (Moody and Smuckler, 1986 in Faust, 1995).

3.3.6.2 Effects on skin and eye

Direct contact with bromoform in liquid form causes redness and pain and may also be absorbed.

3.3.6.3 Repeated-dose toxicity

In a gavage study, F344/N rats and B6C3F1 mice were treated 5 times weekly for 13 weeks with bromoform at doses of 0, 12, 25, 50, 100, or 200 mg/kg (rats) or 0, 25, 50, 100, 200, or 400 mg/kg (mice) (NTP, 1989 in Faust, 1995). All male rats treated with 100 or 200 mg/kg and all female rats treated with 200 mg/kg were lethargic. Dose-related increases of cytoplasmic vacuolization of hepatocytes were observed in males of both species, but not in females.

Bromoform was administered to male and female mice by gavage at daily doses of 50, 125, or 250 mg/kg/day for 14 days. Several indices of humoral and cellular immunity were depressed in male but not in female mice exposed to the highest dose. In addition, liver weights and serum glutamate oxaloacetate transaminase (SGOT) levels were increased and serum glucose and blood urea nitrogen (BUN) levels were decreased in the high-dose animals (Munson *et al*. 1982 in Faust, 1995). In a similar study,72, 145, or 289 mg/kg/day of bromoform was fed by gavage to CD-1 mice for 14 days. BUN levels and serum creatinine values were not altered, but serum glutamate-pyruvate transaminase (SGPT) levels were elevated in high-dose animals. Histological changes were seen in the kidneys and liver in the midand high-dose groups and included hyperplasia of tubular epithelial cells, and hypertrophy and degenerative changes of the glomeruli in the kidney, and centrilobular cytoplasmic pallor and slight focal inflammation of the liver (Condie *et al*. 1983 in Fasut, 1995).

3.3.6.4 Chronic toxicity

Sprague Dawley rats were exposed to tribromomethane at 5,50, 500, **or** 2,500 ppm in drinking water for 90 days (Chu *et al*., 1982 in Melnick, 1989). Liver lesions were observed and the severity was increased in male rats dosed with 2,500 ppm tribromomethane and in female rats dosed with 500 or 2,500 ppm tribromomethane.

Tribromomethane was fed by gavage to CD-1 mice for 14 days at doses of 0, 72, 145, or 289 mg/kg. Blood urea nitrogen (BUN) and serum creatinine levels were not altered; however, active uptake of p-aminohippurate into renal cortical slices was reduced, and serum

glutamate-pyruvate transaminase (SGPT) levels were elevated in the high dose animals. Similar effects were observed in animals administered chloroform, bromodichloromethane, or chlorodibromomethane. Histopathologic changes in the kidney (hyperplasia of tubular epithelial cells and hypertrophy and degenerative changes in the glomerular mesangium) and liver (centrilobular cytoplasmic pallor and slight focal inflammation) were observed in the mid dose and high dose groups (Condie *et al*. 1983 in Melnick , 1989).

3.3.6.5 Developmental and reproductive toxicity

Teratology studies of organic concentrates were prepared from the drinking water of five U.S. cities. In addition, because low molecular weight organohalides may be lost during the concentration procedure, a synthetic mixture was prepared based on the EPA monitoring survey of the concentrations of these compounds in 110 U.S. cities. The latter mixture contained 68.9% chloroform, 16.4% bromodichloromethane, 10.0% chlorodibromomethane, and 3.6% tribromomethane. Each of these preparations, dissolved in dimethyl sulfoxide, was administered by gavage to groups of pregnant CD-1 mice on gestation days 7-14 at dose levels equivalent to 300, 1,000, and 3,000 times the anticipated human exposure. The mice that were administered the synthetic mixture at 3,000 times the human exposure level received 10.3 mg organohalide/kg per day (the tribromomethane dose level was 0.37 mg/kg per day). No effects on fetal weight, mortality, or the occurrence of skeletal or visceral anomalies were observed in any of the exposed groups (Kavlock *et al*. 1979 in Melnick, 1989).

Chloroform was administered to pregnant Sprague Dawley rats by gavage from day 6 to day 15 of gestation at doses of 100, 200, or 400 mg/kg and tribromomethane, bromodichloromethane, and chlorodibromomethane at doses of 50,100, or 200 mg/kg. Corn oil was used as the vehicle for all studies. Only tribromomethane did not have an effect on maternal body weight gain. No histopathologic changes were observed for any of the four trihalomethanes in any of the dams or fetuses, nor were there any changes in the number of resorption sites, number of fetuses per dam, average fetal weight, or occurrence of visceral anomalies (Ruddick *et al*. 1983 in Melnick, 1989).

3.3.6.6 Mutagenicity/genotoxicity

Human data are considered inadequate in providing evidence of cancer by exposure to bromoform, while animal data indicate that long-term oral exposure can cause liver and intestinal tumors. Bromoform has been classified as a Group B2, probable human carcinogen (US EPA, 2000).

3.3.6.7 Toxicokinetics

Bromoform and dibromochloromethane can be absorbed by inhalation ingestion, and dermal routes of exposure. In humans and laboratory animals, bromoform is generally absorbed quickly (Backer *et al*. 2000 in U.S. Agency for Toxic Substances and Disease Registry, 2005). Although bromoform and dibromochloromethane are lipophilic, they do not appear to accumulate in adipose tissue (Stanley 1986 in U.S. Agency for Toxic Substances and Disease Registry, 2005). Bromoform and dibromochloromethane are thought to be metabolized by at least two route-independent pathways: oxidation by cytochrome P-450 mixed function oxidase system (Ahmed *et al*. 1977; Anders *et al*. 1978 in U.S. Agency for Toxic Substances and Disease Registry, 2005) and conjugation via glutathione S-transferase (DeMarini *et al*. 1997; Pegram *et al*. 1977 in U.S. Agency for Toxic Substances and Disease Registry, 2005). Bromoform is primarily excreted via exhalation as the parent compound or carbon dioxide (Mink *et al*. 1986 in

U.S. Agency for Toxic Substances and Disease Registry, 2005).

3.3.7 Data on environmental fate and effect under aerobic and anaerobic conditions

3.3.7.1 Modes of degradation (biotic; abiotic)

Bromoform tends to volatilize from water when exposed to the air, where it is relatively stable with a half-life of 1-2 months (ATSDR, 1990). Most measurements of the concentration of bromoform in air indicate that levels are quite low (<10 ppt).

3.3.7.2 Bioaccumulation, octanol/water partition coefficient

Bromoform may be slightly bioconcentrated by aquatic organisms. The octanol/water partition coefficient (K_{ow}) (an index of the partitioning of a compound between octanol and water) is approximately 2.38 for bromoform (Mabey *et al*. 1982 in U.S. Agency for Toxic Substances and Disease Registry, 2005). The magnitudes of these values suggest that the chemicals will tend to partition to fat tissues of aquatic organisms (U.S. Agency for Toxic Substances and Disease Registry, 2005).

3.3.7.3 Reaction with organic matter

Trihalomethanes such as bromoform are formed as a byproduct when chlorine or bromine are used to treat ballast water. They result from the reaction of chlorine and/or bromine with organic matter in the water being treated. However, once the bromoform is formed it does not appear to have any further reactions with organic matter.

3.3.7.4 Potential physical effects on wildlife and benthic habitats

No information is available on the effects of bromoform on wildlife or benthic habitats.

3.3.7.5 Potential residues in seafood

No information is available on bromoform residues in seafood.

3.4 Free active chlorine (Active Substance)

The following compounds are all treated under this heading:

- Chlorine gas (Cl_2)
- HOCl and ClO^- (Sodium hypochlorite)
- Total Residual Oxidants (TRO)

Note that Basic Approval has been recommended by the GESAMP-Ballast Water Working Group (GESAMP-BWWG) (MEPC 55/2, 2006) for use of the chlorine compounds produced through electrochemical reaction for treatment of ballast water at concentrations greater than those produced by the RBT reactor. Full details on the nature, toxicity and risks posed by this compound is nonetheless provided in this report for completeness.

3.4.1 Acute aquatic toxicity

The US EPA database was used to obtain data on the acute toxicity of chlorine to aquatic organisms. Effects of chlorine on representatives of four taxonomic groups (fish, molluscs, crustaceans and phytoplankton) is reported in Table 6.

Table 6. Effects of chlorine on representatives of four taxonomic groups (fish, molluscs, crustaceans and phytoplankton)

Test water substance	Organisms	Scientfic Name Common Name	Stage	Toxicity Endpoint	Effect	Effect Measurement	Duration Exp.	Concentration (mg/L)	Reference
Chlorine gas (Cl_2)	Fish	*Salvelinus fontinalis* Brook trout	11-15cm	LC100	Mortality	Mortality	9 hrs	350.0	Dandy, (1972)
		Morone saxatilis Striped bass	Fry, 1g	LC50	Mortality	Mortality	6 hrs	610.0	Bills *et al.* (1992)
	Molluscs	*Crassostrea gigas* Pacific oyster	Veliger	LC50	Mortality	Mortality	1 hr	556.0	Chien and Chow, 1989
		Mercenaria mercenaria Hard Clam	Larvae	LC50	Mortality	Mortality	48 hrs	5.0	Bellanca and Bailey, 1977
	Crustaceans	*Asellus aquaticus* Aquatic sowbug	Mature, non-reproducing	LC50	Mortality	Mortality	24 hrs	754.0	Kaniewska-Prus, 1982
		Callinectus sapidus Blue crab	Adult	LC50	Mortality	Mortality	48 hrs	750.0	Vreenegoor *et al.* 1977
	Algae	*Chlorophyceae* Green Algae	8 species	NR	Population	Abundance	24 hrs Static system	100.0	Toetz *et al.* 1977
Sodium hypochlorite	Fish	*Clupea pallasii* Pacific herring	Juvenile	LC50	Mortality	Mortality	96 hrs	65.0	Thatcher, 1978
	Molluscs	*Lepomis macrochirus* Bluegill	Juvenile	LC50	Mortality	Mortality	96 hrs	580.0	Office of pesticide programs, 2000
		Dreissena polymorpha Zebra mussel	Adult	LT95	Mortality	Mortality	17.7 hrs	90.0	Harrington *et al.* 1997
		Crassostrea virginica American oyster	Larvae (7 days)	LC50	Mortality	Mortality	0.5 hrs	120.0	Capuzzo, 1979
		Clibanarius humilis Hermit crab	NR	LC50	Mortality	Mortality	96 hrs	2,500.0	Best, 1991
		Hemigrapsus nudus Shore crab	Adult	LC50	Mortality	Mortality	96 hrs	1,418.0	Thatcher, 1978

Test water substance	Organisms	Scientfic Name Common Name	Stage	Toxicity Endpoint	Effect	Effect Measurement	Duration Exp.	Concentration (mg/L)	Reference
	Crustaceans	*Chlorella sorokiniana* Green algae	225 cells/ml	NR	Mortality	Mortality	20 hrs	200.0	Kott and Edlis, 1969
		Plankton	*Chaetoceros, Gracilis, Dunaliella*	LC50	Mortality	Mortality	96 hrs	90.0	Best, 1991

3.4.2 Endocrine disruption

No information is available on endocrine disruption by chlorine

3.4.3 Sediment toxicity

Chlorine may react with soil and sediment components to form chlorides, which, depending on their water solubility, are easily washed out. (Seiler *et al*. 1988 from US EPA database).

3.4.4 Bioavailability/biomagnification/bioconcentration

There is no potential for the bioaccumulation or bioconcentration of chlorine (HSDB, 1994).

3.4.5 Food web/population effects

As there is no potential for bioconcentration of chlorine, animals high in the food chain will not be affected.

3.4.6 Data on mammalian toxicity

Sodium hypochlorite solution causes moderate mucosal irritation, the extent of which depends on the volume ingested, the viscosity and concentration of the preparation and the duration of the contact. When swallowed it may cause gastrointestinal irritation, with nausea, vomiting and diarrhea. Haematemesis may occur with concentrated solutions.

3.4.6.1 Acute toxicity

In humans, oral doses of 2.5 mg chlorine/day, administered to 10 men for 12 weeks, had no adverse effects (U.S. EPA 1990). No adverse effects were noted in persons ingesting water containing 50-90 ppm of chlorine (~1.4 to 2.6 mg Cl/kg/day) for a short period of time (U.S. EPA 1989). Drinking water concentrations of >90 ppm chlorine caused irritation of membranes of throat and mouth (U.S. EPA 1989). Concentrations of chlorine in the drinking water of greater than 25 ppm make the drinking water unpalatable (U.S. EPA, 1989).

Chlorine is considered a primary irritant to the mucous membranes of the eyes, nose, and throat and to the linings of the entire respiratory tract (Stokinger 1982). The extent of acute injury to humans depends on the concentration and duration of exposure as well as the water content of the tissue involved and the presence of underlying cardiopulmonary disease (HSDB 1994). The estimated clinical effects of varying concentrations of chlorine are as follows: mild mucous membrane irritation at 1-3 ppm; moderate irritation of the upper respiratory tract at 5-15 ppm; immediate chest pain, vomiting, dyspnea, and cough at 30 ppm; toxic pneumonitis and

pulmonary edema at 40-60 ppm; death at 430 ppm for 30 minutes or 1000 ppm for a few minutes (HSDB 1994). Seventy-six individuals, exposed during a football game when approximately 1,100 pounds of chlorine gas were released from a plant, suffered no serious or prolonged incapacitation due to the release (HSDB 1994). If one survives acute exposure to chlorine, recovery is usually complete and rapid (U.S.EPA 1989).

In animals - LC50 values for rats and mice are estimated to be 293 ppm for 1 h and 137 ppm for 1 h, respectively (HSDB 1994). LCLo values for other species range from 330 ppm for 7 h (guinea pigs) to 660 ppm for 4 h (cats and rabbits) (U.S. EPA 1989). Mice and rats exposed to chlorine at the RD_{50} concentration (9-11 ppm, 6 h/day for 1, 3, or 5 days) developed degeneration of olfactory sensory cells in the olfactory mucosa, loss of cilia of the respiratory epithelium, and cellular exfoliation, primarily of the naso- and maxilloturbinates (HSDB 1994). Signs and symptoms of the acute oral toxicity of chlorine in rats include decreased blood glutathione (30 minutes after 0.2 mg chlorine/kg administered as HOCl); decreased hypothalamic norepinephrine levels and increases in normetanephrine levels (3 and 24 hours after intubation of 250 mg/kg free chlorine as HOCl); morphological and biochemical liver changes (within 2 days of dosing with 142.9 mg/kg free chlorine as NaOCl); kidney enlargement (200 mg/kg/day available chlorine for 14 days) (U.S. EPA 1989); and dose-related increase in liver and kidney weights (210 mg dietary chlorine/kg body weight/day for 28 days) (U.S. EPA 1987). C57Bl/6N mice given chlorine (25-30 ppm) in the drinking water for one to three weeks had significant decreases in the number of peritoneal exudate macrophages, and decreased in vitro cytotoxicity against mouse melanoma (B16) and fibrosarcoma (UV-112) target cells (U.S. EPA 1989).

3.4.6.2 Effects on skin and eye

Free active chlorine can cause mild skin irritation to corrosive injury depending on the duration of the contact, concentration and pH of the solution. An 8% sodium hypochlorite solution has been shown to not cause skin irritation. Mild eye irritation to corrosive injury can also be caused depending on the concentration and pH of the solution and the duration of contact.

3.4.6.3 Repeated-dose toxicity

See chronic toxicity.

3.4.6.4 Chronic toxicity

The major target organs for the subchronic/chronic toxicity of chlorine in humans are the respiratory tract and the blood. The major target organs for the subchronic/chronic toxicity of chlorine in animals are the immune system, the blood, the cardiovascular system and the respiratory tract. The US EPA has derived an oral RfD (reference dose) of 0.1 mg/kg/day for chlorine, based on a no-observed-adverse-effect level of 14.4 mg/kg/day in a chronic drinking water study in rats.

Fifty-five workers exposed to about 1.0 ppm of chlorine, 8 h/day for at least 90 days exhibited asthma, chronic bronchitis, tuberculosis, and emphysema (U.S. EPA 1987). The concentration of 1 ppm is roughly equivalent to 0.415 mg/kg/day. In one case study, exposure to 0.015 mg/L of chlorine, 8 h/day for 6 years resulted in dyspnea, marked emphysema of both lower lung lobes, and reduced respiratory mobility (U.S. EPA 1987). Fifteen male workers exhibited decreased residual levels of maximal mid-expiratory flow rates and FEV/FVC after exposure to 0.18 ppm chlorine, 8 h/day, 5 days/week for 8.9 years (U.S. EPA 1990). Workers

in 25 chlor-alkali plants (332 males), exposed to chlorine concentrations of 0.006 to 1.42 ppm (8-h TWAs) for about 10.9 years showed no dose-related correlation between chlorine exposure and the prevalence of colds, dyspnea, palpitation, chest pain, or permanent lung damage (544 exposed workers had chest x-rays) (Stokinger 1982 in US EPA, 1994).

Animals - Groups of 70 F344/N male and female rats were given drinking water containing up to 275 ppm of chlorine (14.4 mg/kg/day) for 2 years. No hematologic abnormalities were detected at interim sacrifices (14 and 66 weeks) and no non neoplastic abnormalities were observed in extensive gross and microscopic examinations at the end of the study (NTP 1992). The EPA used the no-observed-adverse-effect level, 14.4 mg/kg/day, to calculate a chronic oral RfD of 0.1 mg/kg/day for chlorine (U.S. EPA 1994).

Sprague-Dawley rats, given 5, 15, or 30 ppm chlorine in drinking water from weaning to 12 weeks of age exhibited significantly reduced spleen weights, suppressed delayed-type hypersensitivity reactions, and decreased oxidative metabolism by macrophages at the highest dose; no effects were noted for the other doses or on other parameters of immunotoxicity (NTP 1992). F344 rats given doses of chlorine up to 60 mg/kg/day for 92 days exhibited no histopathology in major organs (U.S. EPA 1989). Other species, exposed subchronically to chlorine exhibited the following: mice had no adverse effects on weight gain, food or water consumption, histological parameters (12.5 mg/kg/day for 50 days or 25 mg/kg/day for 33 days) (U.S. EPA 1989); New Zealand rabbits had increased hydroxyproline levels in heart tissue (1.6 mg chlorine/kg/day for 3 months; no effect at 0.1 mg/kg/day) (U.S. EPA 1989); Carneau pigeons exhibited cardiovascular effects (10 mg chlorine/kg/day in drinking water for 9 months) (U.S. EPA, 1989).

In various studies, Sprague Dawley and F344 rats treated with chlorine orally for 12-24 months at doses ranging from 0.14 to 22 mg/kg/day exhibited one or more of the following symptoms: significant decreases in red blood cell count and hematocrit (reversed at 6 months), increased osmotic fragility, increased mean corpuscular hemoglobin, significant decreases in body and liver weights, decreased brain and heart weights ($p<0.05$), and decreased salivary gland and kidney weights (U.S. EPA 1989), decreased spleen weight, and decreased thyroid weight (U.S. EPA, 1987).

Rhesus monkeys (4/sex/dose) exposed to ó2.3 ppm of chlorine 6 h/day, 5 days/week for 1 year experienced ocular irritation, conjunctival irritation, hyperplasia of the nasal mucosa, and, in some females, epithelial hyperplasia of the trachea with minimal evidence of early nonkeratinizing squamous metaplasia (U.S. EPA, 1990).

3.4.6.5 Developmental and reproductive toxicity

No conclusion on the developmental/reproductive toxicity of chlorine can be made based on the limited information available from human and animal studies.

Humans – No information was found in the secondary sources searched to indicate that chlorine is a developmental/reproductive toxicant in humans.

Animals – Female Sprague-Dawley rats were given chlorine concentrations of 0, 1.0, 10, or 100 mg/L in the drinking water (as HOCL) for 2.5 months prior to conception and throughout gestation, were killed on gestation day 20, and the fetuses were examined (NTP 1992 in U.S. EPA, 1994). Resorption was not increased at any concentration; however, fetuses in

the 100 mg/L group had soft tissue defects, including improper orientation of the heart and adrenal agenesis, and a slightly increased incidence of skeletal variants, such as incompletely ossified or missing sternebrae or rudimentary ribs. Statistical analyses were not available. In other studies, chlorine administered to pregnant mice was negative for reproductive and teratogenic effects when given in drinking water as 10-13 ppm of sodium hypochlorite and hydrochloric acid (to maintain water pH of 2.5) or when given in heavily chlorinated municipal drinking water (U.S. EPA, 1989).

Sperm head abnormalities were observed in B6C3F1 mice given 1.6 and 4.0 mg/kg/day of chlorine as OCl-, but not at 8.0 mg/kg or when given as HOCl (U.S. EPA 1994). BDII rats (236 total males and females) given 10 mg/kg/day of free residual chlorine in the drinking water throughout 7 generations had no adverse effects on weight gain, food consumption, water consumption, fertility, lifespan, growth pattern, hematology, histology (liver, spleen, kidney or other organs) (U.S. EPA, 1989).

3.4.6.6 Mutagenicity/genotoxicity

Chlorine was mutagenic in Salmonella typhimurium strains TA1530 and TA100, without metabolic activation; produced chromosome aberrations in human lymphocytes and other mammalian cells (ò20 ppm); interacted with DNA in E. coli polA test (as sodium hypochlorite); and was negative for the induction of erythrocyte micronuclei or chromosome aberrations of bone marrow cells of Swiss CD-1 mice (up to 8 mg/kg/day of NaOCl) for up to 5 days (U.S. EPA, 1989).

3.4.6.7 Toxicokinetics

No additional information is available on toxicokinetics for chlorine.

3.4.7 Data on environmental fate and effect under aerobic and anaerobic conditions

3.4.7.1 Modes of degradation (biotic; abiotic)

Chlorine hydrolyzes very rapidly in water (rate constants range from 1.5 x 10-4 at 0øC to 4.0 x 10-4 at 25øC; half-life in natural waters, 0.005 s) (U.S. EPA 1989). In fresh and wastewaters at pH >6, complete hydrolysis occurs with the formation of hypochlorous acid (HOCl) and chloride ion (Cl-). The hypochlorous acid ionizes to hydrogen ion (H+) and hypochlorite ion (OCl-). At pH values >5, OCl- predominates; at pH values <5, HOCl predominates (U.S. EPA 1989). Free chlorine (Cl2, HOCl, and OCl-) reacts rapidly with inorganics such as bromide and more slowly with organic material present in natural waters. These reactions yield chloride, oxidized organics, chloroganics (including trihalomethanes), oxygen, nitrogen, chlorate, bromate and bromoorganics.

3.4.7.2 Bioaccumulation, octanol/water partition coefficient

There is no potential for the bioaccumulation or bioconcentration of chlorine (HSDB 1994).

3.4.7.3 Reaction with organic matter

Chlorine reacts slowly with organic material present in natural waters. These reactions yield chloride, oxidized organics, chloroganics (including trihalomethanes), oxygen, nitrogen, chlorate, bromate and bromoorganics.

3.4.7.4 Potential physical effects on wildlife and benthic habitats

Chlorine has been shown to have high acute toxicity to many organisms (U.S. EPA, 1994). Low level chlorination (0.05 to 0.15 mg/L) results in significant shifts in the species composition of marine phytoplankton communities (HSDB 1994 in U.S. EPA, 1994). Chlorine is phytotoxic but is also essential to plant growth; crops need around 5 pounds or more of chlorine per acre. Acute toxicity to plants is characterized by defoliation with no leaf symptoms and, in higher plant forms, by spotting of the leaves (at 1.5 mg/m^3) and marginal and interveinal injury (at 150-300 mg/m^3) (Seiler *et al*. 1988 in U.S. EPA, 1994). No data were found regarding the toxicity of chlorine to terrestrial animals; however, the data from experimental studies indicate that injury to animals would occur only in the presence of high concentrations of chlorine, either in drinking water or the ambient atmosphere (U.S. EPA, 1994).

As chlorine and chlorine compounds do not persist in sediments it is not expected to affect benthic habitats unless directly applied in high concentrations.
Potential residues in seafood

As there is no potential for the bioaccumulation or bioconcentration of chlorine there is no risk of potential residues in seafood.

3.5 Hydrogen (Related Chemical)

Note that hydrogen gas is produced in the electrochemical cell of the RBT reactor as a byproduct of hydroxyl radicals. Basic Approval has been recommended by the GESAMP-Ballast Water Working Group (GESAMP-BWWG) (MEPC 55/2, 2006) for use of similar electrochemical systems designed for the treatment of ballast water that also produce hydrogen gas. Full details on the nature, toxicity and risks posed by this compound is nonetheless provided in this report for completeness.

3.5.1 Acute aquatic toxicity

There is no data on acute aquatic toxicity of hydrogen.

3.5.2 Endocrine disruption

None of the available data indicate toxicity for exposures of any duration.

3.5.3 Sediment toxicity

There is no data on sediemnt toxicity of hydrogen.

3.5.4 Bioavailability/biomagnification/bioconcentration

Hydrogen does not bioconcentrate in the tissues of organisms.

3.5.5 Food web/population effects

Hydrogen does not bioconcentrate or accumulate in the tissues of organisms therefore there is no risk to organisms up the food chain.

3.5.6 Data on mammalian toxicity

None of the available data indicate toxicity for exposures of any duration. Hydrogen is not listed by NTP, OSHA or IARC.

3.5.6.1 Acute toxicity

High concentrations of hydrogen in the air can cause a deficiency of oxygen with the risk of unconsciousness or death. Ingestion is not an observed route of exposure to gaseous hazardous materials. Exposure to moderate concentrations may cause dizziness, headache, nausea and unconsciousness.

3.5.6.2 Effects on skin and eye

No detrimental effects of skin or eye contact has been reported. Hydrogen only poses a risk of thermal burns if ignited.

3.5.6.3 Repeated-dose toxicity

None of the available data indicate toxicity for exposures of any duration.

3.5.6.4 Chronic toxicity

None of the available data indicate toxicity for exposures of any duration.

3.5.6.5 Developmental and reproductive toxicity

None of the available data indicate toxicity for exposures of any duration.

3.5.6.6 Mutagenicity/genotoxicity

None of the available data indicate toxicity for exposures of any duration.

3.5.6.7 Toxicokinetics

None of the available data indicate toxicity for exposures of any duration.

3.5.7 Data on environmental fate and effect under aerobic and anaerobic conditions

3.5.7.1 Modes of degradation (biotic; abiotic)

Hydrogen exists naturally in the atmosphere and will be dissipated rapidly in well ventilated areas.

3.5.7.2 Bioaccumulation, octanol/water partition coefficient

Hydrogen rapidly dissipates so there is no bioaccumulation.

3.5.7.3 Reaction with organic matter

Hydrogen is not known to react with organic matter.

3.5.7.4 Potential physical effects on wildlife and benthic habitats

No adverse ecological effects are expected. Hydrogen does not contain any Class I or Class II Ozone depleting chemicals (40 CFR Part 82). Hydrogen is not listed as a marine pollutant by DOT (49 CFR Part 171).

3.5.7.5 Potential residues in seafood

Hydrogen does not bioaccumulate so there is no risk of hydrogen residues in seafood.

4 PHYSICAL AND CHEMICAL PROPERTIES FOR THE ACTIVE SUBSTANCES AND PREPARATIONS AND TREATED BALLAST WATER

Data on the physical and chemical properties of the Active Substances, preparations and treated ballast water are provided in Table 2.

5 USE OF THE ACTIVE SUBSTANCE OR THE PREPARATION

5.1 Active Substance Preparation

The RBT reactor employs the use of two Active Substances in its working principals. These substances namely; Ozone and sodium hypochlorite, are generated on board the vessel from natural sources such as air from the atmosphere for Ozone production, and sodium chloride or other electrolyte substance found in the ballast water being pumped.

The main reason for the addition of these substances is to assist with the cavitation that forms the basis of the reactor; also these chemicals have the additional advantage of maintaining a sterile environment within the ballast tanks on board a ship.

Both Ozone and sodium Hypochlorite have been used to disinfect drinking water for many decades. The use of these chemical compounds for potable water treatment is well documented.

These chemical substances are added into the ballast water during ballasting operations and as such do not need to be handled by the crew at any stage.

Levels of these chemical are kept to a minimum i.e. 0.001mg/l for the O3 gas and <1.0ppm for the sodium Hypochlorite.

No additional preparation methods or handling of these chemicals is required. The RBT Reactor is supplied pre-calibrated for the specific ballast flow rate as determined by the individual vessel requirements.

5.2 The RBT "Coronatech" Ozone generator

Ozone gas that is produced and subsequently infused into the ballast water treatment system is generated by the corona method.

The corona discharge generator utilizes a high frequency transformer (typically 16-20KHz) This method is favoured because greater Ozone production can be achieved with less electrode surface area and less electrical consumption (0.75 amps).

The RBT Ozone discharge generator accelerates electrons so as to give them sufficient energy to split the oxygen-oxygen double bond upon impact with another oxygen molecule. The two oxygen atoms, which are produced by the collision, react with other diatomic oxygen molecules to form Ozone. Ozone is produced as a result of an electrical discharge.

Various factors that affect the production of Ozone gas via the generator have been taken into account i.e., temperature, humidity and oxygen supply. In order to maintain the efficient working temperature for the effective production of Ozone gas, a cooling system is integrated into the equipment. Filtered atmospheric air is cooled via a chiller; the chiller also acts to cool the corona discharge chamber. Ozone produced via the generator is approximately 2%.

5.2.1 Risks to ship safety and crew

The Ozone generator that forms part of the RBT ballast water treatment system is purpose built, baring in mind the safety of the ship its crew and the environment.

Specific consideration was given to the assurance that no Ozone leaks could or would take place during the normal operation of the O3 generator.

Safety features

Ozone is produced from the surrounding air, and is drawn into the ballast water by means of a venturi system. (Vacuum) As such the O3 generator will not produce any additional O3 gas in the event of a zero flow of air.

Two one-way / non-return, check valves are installed. One check valve on the Ozone delivery line and one check valve on the air intake of the Ozone generator. The purpose of these check valves is to ensure a one directional flow of air through the O3 generator and also that no Ozone gas can be dissipated into the surrounding air. In the most unlikely event that the check valves should fail, ballast water would be passed back into the corona tubes, thereby rendering the Ozone generator incapable of producing any O3 gas. An 5 milli-amp fuse protects the O3 generator.

Corrosion

Due to the fact that the RBT reactor makes use of only 0.0001 ppm of Ozone gas in its working principals no noticeable increase in corrosion of the ballast pipe work or tanks will occur. Surface coatings within the ballast tanks offer additional protection from any residual Ozone gas.

Venting

In the unlikely event of any Ozone gas build up within the ballast tank, any free Ozone gas that is not absorbed into the ballast water will be vented via the ballast tank venting system. At no time will the free Ozone reach any harmful or dangerous levels.

System failure

The Ozone generator has no moving parts and as such is maintenance free. In the event of Ozone system failure, warning signals are transmitted to the bridge and ballast control room.

5.3 Electro Chemical Production

Installed into the electrical control panel is an electrical rectifier that supplies a current to the electrodes that form part of the RBT reactor. Passing an electrical potential through a cathode and an anode produces Electro chemical production of chemical compounds such as Sodium Hypochlorite and gases. The dissolved salts and compounds within ballast water, namely Na Cl serve as an electrolyte in the process, produce compounds such as Hydrogen, Oxygen and Sodium Hypochlorite. The electrodes that form part of the RBT Reactor are selected from the group VIII metals on the periodic table – Ruthenium, Titanium.

Low concentrations of Sodium Hypochlorite are produced at the surface of the electrodes (< 1 ppm) Residual chlorine content assists in maintaining a sterile environment within the ballast tank. Other useful chemicals produced by the electrodes that are harmful or lethal to marine organisms include, Bromine, Hypobromous acid, Chlorine Dioxide, Chlorine, Hypobromite, Hydroxyl radical. These chemicals degrade over a period of time and would not be harmful to the environment when the ship de-ballasts.

Chlorine residuals are affected by temperature and pH of the water being treated, the effectiveness of chlorine decreases with an increase of pH above 8.0. For human consumption chlorine residuals of 1.2 to 1.5 ppm are acceptable. It is important to maintain chlorine (NaOCl) limits within the prescribed limits for human consumption, as no particular limits and standards are currently available for the treatment of seawater. The levels of NaOCl produced by the RBT reactor are less than 1 ppm.

Sodium Hypochlorite levels fall within the specifications of the paint manufacturers responsible for the manufacture and application of ballast tank paint protection systems

5.3.1 Risks to ship safety and crew

The rectifiers installed into the electrical control panel of the RBT reactor supplies a DC current directly to the electrodes of the reactor.

As with all electrical component that make up the treatment process of the equipment, ship and crew safety are taken into account. Where necessary the electrical equipment installed would be supplied with the necessary certification and build to ATEX Standards, should the equipment need to be installed into a hazardous area on board a vessel.

Safety features

Certain chemicals predominantly "sodium hypochlorite," are produced inside of the RBT reactor directly from the seawater, when the ballast water is being pumped into the vessel. The ship crew requires no additional handling of any chemicals.

Circuit breakers and fuses are installed into the ECP that would trip in the event of any direct short circuit of the rectifiers.

Corrosion

Chemicals produced by the electro chemical components of the RBT reactor can be potentially corrosive, however, levels of these chemicals and gases are low and will not increase the corrosion rate of the ballast piping and tanks.

Venting

Any un-dissolved gases such as chlorine gas and hydrogen will be vented through the ships ballast tank vents. Hydrogen gas levels would never reach dangerous levels (4%).

System failure

In the unlikely event of a rectifier or electrode failure, aural and visual alarm warnings are transmitted to the ballast control room as well as the bridge. Two rectifiers are installed to ensure that ballasting operations would not be affected in the event of a rectifier failure.

6 RISK ASSESSMENT

6.1 Risk to safety of ship

There is an increased risk of corrosion arising form the oxidizing properties of Ozone. However, because Ozone has such a short half-life (< 6 seconds in seawater), it will only exist for a very short time in the ballast tank.

Mitigation required will be to ensure that the machinery producing the Ozone and the piping leading into the ballast water is coated with a ceramic coating and/or constructed of anti-corrosive material such as stainless steel. Ozone is the most corrosive of the Active Substances applied directly to the ballast water and therefore any measures taken to prevent corrosion by Ozone will be sufficient to prevent corrosion by the other Active Substances. Hydrogen poses a flammability risk but quantities produced will be so small as to be of no concern. Hydroxyl radicals degradate within nanoseconds and are therefore of no concern with regards to corrosion.

There are no risks associated with the handling of active chemical as the Active Substances and the relevant chemicals are produced *in situ* and are therefore not handled.

Ozone Ship Risk Summary

Risk of substance: Medium
Certainty: High

Risk after mitigation: Very Low
Certainty: High

Bromate ion Ship Risk Summary

Risk of substance: Low
Certainty: High

Risk after mitigation: Very Low
Certainty: High

Bromoform Ship Risk Summary

Risk of substance: Low
Certainty: High

Risk after mitigation: Very Low
Certainty: High

Sodium hypochlorite (free active chlorine) Ship Risk Summary

Risk of substance: Medium
Certainty: High

Risk after mitigation: Very Low
Certainty: High

Hydrogen Ship Risk Summary

Risk of substance: Medium
Certainty: High

Risk after mitigation: None
Certainty: High

Hydroxyl radical Ship Risk Summary

Risk of substance: None
Certainty: High

Risk after mitigation: None
Certainty: High

6.2 Risks to human health

6.2.1 Ozone

Ozone is very toxic by inhalation route and there is also a potential for Ozone to cause mutagenicity after oral exposure. Therefore strict mitigation measures will have to be applied to avoid human exposure.

Mitigation: The Ozone generator is fabricated in such a way as to ensure that no Ozone gas can escape into the surrounding air. When the ballast pump is incited the Ozone generator is turned on automatically, and the Ozone gas that is generated is sucked directly into the ballast water being treated.

A non-return/one way valve, situated onto the corona reactor ensures that no Ozone gas escapes. Should there be no air flow through the corona reactor, no Ozone gas is produced thereby ensuring that the equipment is safe for the ship and its crew. Even in the event of accidental or purposely turning on of the Ozone generator it would be impossible for the Ozone generator to produce Ozone gas without an airflow through the corona reactor. The air flow required is created by a venturi that ensures that all of the Ozone gas is infused into the ballast water.

In addition, with regard to the health of the ship crew, placards relating to the general hazards of Ozone inhalation should be placed onboard to promote general awareness.

Ozone Human Health Risk Summary

Risk of substance: Low
Certainty: High

Risk after mitigation: Very Low
Certainty: High

6.2.2 Bromate ions

Several studies have indicated high potential for mutagenicity and carcinogenicity in humans. The current MCL (Maximum Contaminant Level) in the United States is 0.010mg/L and the MCL goal is zero because of the possibility that bromate may function as a genotoxic carcinogen. Therefore human exposure should be totally avoided.

Mitigation: Bromate ions will form in the ballast water which is pumped into the ship's ballast tanks mechanically and therefore there will be no human contact on board the ship with treated ballast water. Providing the treated water is released more than 12 hours (the half life of a bromate ion) after it was treated, bromate ions will pose no threat to human health outside of the ship either.

Bromate ion Human Health Risk Summary

Risk of substance: Low
Certainty: High

Risk after mitigation: Very Low
Certainty: High

6.2.3 Bromoform

Bromoform is a highly reactive compound capable of bioacumulating. Exposure to bromoform vapors may cause irritation to the respiratory tract, lacrimation, and liver damage. Bromoform has also been classified as a Group B2, probable human carcinogen (US EPA, 2000). Exposure should therefore be minimized.

Mitigation: According to tests conducted on water that was treated using Resource Ballast Technologies (Pty) Ltd., the concentration of all trihalomethanes (which was made up almost entirely of bromoform) was 19.6µg/L. The acceptable concentration level of total trihalomethanes in the United States for water used by humans is 80.0µg/L. Therefore the concentration levels of bromoform produced by this methodology are unlikely to pose a health risk to humans. However, further testing is recommended with seawater from a variety of sources.

Bromoform Human Health Risk Summary

Risk of substance: Low
Certainty: High

Risk after mitigation: Low
Certainty: High

6.2.4 Free active chlorine

Swallowing sodium hypochlorite can cause irritation, pain and inflammation of mucoses. In severe cases it can cause ulceration and perforation of the gastro intestinal tract. Vapours are not easily formed although under certain conditions, high concentration solutions in high pH mixed with acids, may produce chlorine gas which causes mild irritation of the nose and throat.

Mitigation: The sodium hypochlorite will be produced in situ and will be pumped directly into the ballast water. Therefore there will be no potential risk of the crew being exposed to chlorine in any form. Chlorine has a rapid half life (0.005) and does not bioaccumulate. Therefore it poses no risk to humans once after the treated ballast water has been released.

Free active chlorine human health risk summary

Risk of substance: Medium
Certainty: High

Risk after mitigation: None
Certainty: High

6.2.5 Hydrogen

Hydrogen is not toxic but if released in large enough quantities it may displace oxygen leading to unconsciousness or asphyxiation. It is also highly explosive in high concentrations.

Mitigation: The amount of hydrogen produced by the electro-chemical electrodes is miniscule and would not reach any dangerous levels, any excess that is not already absorbed/dissolved into the ballast water would be vented into the atmosphere via the vents in the ballast tanks.

Hydrogen human health risk summary

Risk of substance: Medium
Certainty: High

Risk after mitigation: Very Low
Certainty: High

6.2.6 Hydroxyl radicals

Hydroxyl radicals have an extremely raid half life (nanoseconds) and do not persist or bioaccumulate. They therefore do not pose a risk to human health.

7 RISKS TO THE AQUATIC ENVIRONMENT

7.1 Screening for persistence, bioaccumulation and toxicity

Active Substances and relevant chemicals produced or introduced by the RBT reactor for treatment of ballast water include the following:

- Ozone,
- bromate ions,
- bromoform,
- hydroxyl radicals,
- chlorine gas (Cl_2),
- free active chlorine species (HOCl and ClO^-), and
- hydrogen gas.

Of these, Ozone, hydroxyl radicals and chlorine compounds (chlorine gas and free active chlorine species) are extremely short lived and have very little or no potential for persistence or for bioaccumulation (see data presented in the preceding sections). Thus, although their toxicity to marine life may be high (which is the reason for their use in the RBT reactor) risks posed to the environment through the release of treated ballast water is considered negligible.

Bromate ions are only likely to be formed in seawater but have a longer half life than Ozone, hydroxyl radicals and the chlorine compounds mentioned above (approximately 12 hours). The octanol/water particial coefficient log value (P_{ow}) for bromate is -7.18 and such it is not considered to pose a risk of concentration in marine organisms or to animals higher up the food chain (PAN Pesticides Database). Acute toxicity of bromate is also low (approximately 1/1000 of that of Ozone) and concentrations in ballast water treated with the RBT reactor are very low relative to concentrations at which toxic effects have been demonstrated (see data presented in the preceding sections).

Bromoform has an octanol/water partition coefficient (P_{ow}) of approximately 2.38 which suggests that it will tend to partition to fat tissues of aquatic organisms, and hence may be slightly bioconcentrated by aquatic organisms. The concentration of bromoform in ballast water treated with the RBT reactor is very low (<20 µg/l) relative to concentrations at which toxic effects have been detected (9 000-140 000 µg/l, see preceding sections).

In conclusion, therefore it is clear that none of the Active Substances or relevant chemicals produced by the RBT reactor can be considered as PBT substances (Table 7).

Table 7. Risk characterization for Active Substances and relevant chemicals produced or introduced by the RBT reactor

Substance	Persistence (Half-life: > 60 days in marine water/> 40 days in freshwater, or > 180 days in marine sediment, or > 120 days in freshwater sediment	Bioaccumulation (Log Pow >3)	Toxicity (Chronic NOEC < 0.01 mg/l)
Ozone	No	No	Yes
Bromate ions	No	No	Yes
Bromoform,	Yes	No	Yes
Hydroxyl radicals	No	No	Yes
Chlorine gas (Cl_2)	No	No	Yes
Free active chlorine species (HOCl and ClO-)	No	No	Yes
Hydrogen gas	No	No	No

7.2 Evaluation of the treated ballast water

Toxicity test has not yet been undertaken on samples of treated ballast water due to time constraints but it is the intention of the proponents to undertake relevant toxicity tests as required by the GESAMP-Ballast Water Working Group (GESAMP-BWWG) (MEPC 55/2, 2006).

7.3 Aquatic toxicity of Active Substance & Relevant Chemical

7.3.1 Acute aquatic toxicity

An overview of acute toxicity data (lowest short-term LC_{50} test values) for up to three taxa (fish, crustacean, mollusc, algae) where available are presented in Table 8.

It can be concluded from these data that acute toxicity to Ozone can be seen at >500 µg/L, bromate ions at >176 000 µg/L, bromoform at >16 000 µg/L, chlorine gas at >600 µg/L, and sodium hypochlorite at around 100 µg/L. The concentration of bromoform and chlorine in ballast water treated with the RBT reactor are low (<20 µg/l, and 80-320 µg/l, resepctively) relative to levels at which acute toxicity can be expected.

Table 8. Overview of acute toxicity data (lowest short-term LC_{50} test values)

Test Substance	F C A	Species	Effect	Value (µg/L)
Ozone	**F**	Lepomis macrochirus	24h LC_{50}	60
	M	Dreissena polymorpha	5.8h LC_{50}	520
	A	-	-	-
Bromate Ion	**F**	Oncorhynchus keta	96h LC_{50}	512 000
	C	Neomysis awatschnsis	24h LC_{50}	176 000
	A	-	-	-
Bromoform	**F**	Cyprinodon variegates	48h LC_{50}	16 000
	C	Peneus aztecus	96h LC_{50}	20 000
	A	-	-	-
Chlorine gas (Cl_2)	**F**	Morone saxatilis	6h LC_{50}	610
	C	Asellus aquaticus	24h LC_{50}	754
	A	-	-	-
Sodium hypochlorite	**F**	Clupea pallasii	96h LC_{50}	65
	M	Crassotrea virginica	0.5 LC_{50}	120

F = Fish, C = Crustacea, A = Algae, M = Molluscs

7.3.2 Chronic aquatic toxicity

An overview of chronic toxicity data (lowest long-term LC_{50} test values) for up to three taxa (fish, crustacean, mollusc, algae) where available are presented in Table 9. It can be concluded from these data that chronic toxicity to Ozone and free active chlorine can both be expected at >5 µg/L. Considering the short half lives of these substances, they are not expected to be released into the environment above these levels.

Table 9. Overview of acute toxicity data (lowest long-term NOEC test values)

Test Substance	F C A	Species	Effect	Value (µg/L)
Ozone	**F**	*Oncorhynchus mykiss*	90d NOEC	5
	C	-	-	-
	A	-	-	-
Bromate Ion	**F**	-	-	-
	C	-	-	-
	A	*Skeletonema costatum*	7d NOEC	0.125 -
Free active chlorine	**F**	*Menidia penensulae* eggs	28d NOEC	40
	C	*Crassostrea virginica* & *Rangia cuneata*	15d NOEC	7
	A	Phytoplankton	21d effect	1

F = Fish, C = Crustacea, A = Algae, M = Molluscs

7.3.3 Derivation of the Predicted No Effect Concentration (PNEC)

According to the GESAMP-Ballast Water Working Group (GESAMP-BWWG) (MEPC 55/2, 2006), PNEC for Active Substances and relevant chemicals should be derived by dividing the lowest available effect concentration with an appropriate assessment factor, as provided in Table 10.

Table 10. Effect assessment for deriving PNECs for freshwater and saltwater (MEPC 55/2)

	Assessment Factor	
Data set	**PNEC long**	**PNEC short**
Freshwater assessment		
Lowest short-term L(E)C_{50} from fresh water species representing three trophic levels	1000	10-100
Lowest chronic NOEC from three freshwater or saltwater species representing three trophic levels	10	
Saltwater assessment		
Lowest short-term L(E)C_{50} from marine species representing three trophic levels	1000	10-100
Lowest chronic NOEC from three freshwater or saltwater species representing three trophic levels	100	
Lowest chronic NOEC from three saltwater species representing three trophic level	100	
Lowest chronic NOEC from three freshwater or saltwater species representing three trophic levels + at least two chronic NOECs from additional marine taxonomic groups	10	

Table 11. Predicted concentrations of Active Substance and relevant chemical in treated ballast water produced or introduced by the RBT reactor

Active Substance/ chemical	**PNEC**	**Concentration immediately after treatment (µg/L)**	**Concentration after 2 h (µg/L)**	**Concentration after 4h (µg/L)**	**Concentration after 8h (µg/L)**	**Concentration after 12h (µg/L)**
Ozone (freshwater)		1 000	nd	nd	nd	nd
Ozone (seawater)		1 000	62.5	3.9	0.015	5.9×10^{-5}
Bromoform (seawater)		19.6	19.6	19.6	19.6	19.6
Chlorine gas (freshwater)		0.32	nd	nd	nd	nd

nd = not detectable.

It is only relevant to evaluate PNEC values for Ozone in freshwater and bromoform in seawater for this study (the remaining Active Substances are either created in negligible concentrations in the other medium or decay to rapidly to be of consequence).

For Ozone there is only one chronic value available. An assessment factor of 100 is applied to this value resulting in a PNECchronic = 0.05 µg/L. There are 2 values available for acute data, the lowest of which is 60 µg/L. Apply a factor of 1000 to this yields a PNECacute = 0.06 µg/L. Of these two data the lowest value is taken as the resulting PNEC: PNEC = 0.05 µg/L as Ozone. In the case of Ozone introduced to freshwater a total time of at least 8 hours is required before the ballast water can safely be discharged.

For bromoform there is one chronic value available. An assessment factor of 100 is applied to this value resulting in a PNECchronic = 48 µg/L and 3 for the acute data, the lowest of which is 7.1 mg/L. Using an assessment factor of 1000 the PNECacute is established at 7.1 µg/L. Of these two data the lowest value is taken as the resulting PNEC: PNEC = 7.1 µg/L as bromoform. The half life of bromoform is in the order of 1-2 month and delaying release of ballast water is not considered feasible. Concentration in the treated ballast water (19.6 µg/L) are only marginally higher than the PNEC value (7.1 µg/L) and hence not considred to pose a major risk to the environment.

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* * *

APPENDIX 1

J. MULLER

LABORATORIES (PTY) LTD
Reg. No: 1980/004017/07
ANALYTICAL CHEMISTS

P.O. BOX 511
PAARDEN EILAND 7420
REP. OF SOUTH AFRICA
TELEPHONE: 27-021-5118301/2
FAX: 27-021-5103800
E-mail: jmlabs@iafrica.com

OFFICE & LABORATORIES AT:
36 MARINE DRIVE
PAARDEN EILAND 7405
REP. OF SOUTH AFRICA

Our Ref LN703955-9R-02

Date of Issue: 30 MARCH 2007

Certificate of Analysis

PAGE 1 OF 1

This is to certify that the samples listed below were analysed

SUBMITTED BY: RESOURCE BALLAST TECHNOLOGIES (PTY) LTD
P.O. BOX 431
SEA POINT
8060
ATTENTION: B JACOBS

SAMPLE TYPE: WATER
SAMPLE MARKS: 01. CONTROL
02. 200m³ p/H
03. 200m³ p/H + O_3
04. 320m³ p/H

DATE SAMPLE RECEIVED: 30 MARCH 2007
DATE ANALYSIS STARTED: 30 MARCH 2007
DATE ANALYSIS COMPLETED: 30 MARCH 2007

Analysis relates only to the sample/s tested:
Analysis gave:

		01	02	03	04
4500-Cl G	Free Chlorine (Cl_2) mg/L	<0,01	0,24	0,32	0,08
4500-Cl G	Total Chlorine (Cl_2) mg/L	<0,01	0,28	0,35	0,12

Values reported as less than (<) are lower than the limit of detection for the method employed.

ANALYST
C JOHNSON

LABORATORY MANAGER
C VENTER

J. MULLER LABORATORIES (PTY) LTD DIRECTOR: B.M. JOHNSON
CONDITIONS OF ISSUE SEE OVERLEAF

APPENDIX 2

APR.04'2007 13:36 0216816701 SABS #4060 P.001/003

TEST REPORT **SABS**

Your ref: Bank deposit
Our ref: 2819
Enquiries: JA Santer
Date: 2007-04-03
Report No: 2819/J1948
Page: 1 of 2

Resource Ballast Technologies (Pty) Ltd.
ATTENTION: Ian Vroom/Bernard Jacobs
PO Box 431
SEA POINT
8060

PRELIMINARY REPORT
WATER SAMPLES

1 **SCOPE OF ACCREDITATION**

The laboratory of the SABS Water Division, Western Cape Region is accredited according to ISO/IEC 17025 and registered with SANAS (South African National Accreditation System) as an accredited laboratory.

The scope of accreditation is specific and tests, which are marked 'NA' (Not Accredited) in this report, are currently not included in the SANAS Accreditation schedule for our laboratories.

2 **DESCRIPTION OF SAMPLES**

The following samples were submitted by Ian Vroom/Bernard Jacobs to our Cape Town laboratory on 08 March 2007.

SABS CODE	SAMPLE DESCRIPTION
CT0522	Control
CT0523	Ballast sea water

3 **ANALYTICAL DURATION**

The analysis commenced on 08 March 2007 and was completed on .. April 2007.

NOTE: Preservation techniques used, where required, were based on the recommendations supplied in ISO 5667/3 'Guidelines on the preservation and handling of samples' and Method 1060/C 'Sample preservation' from 'Standard Methods for the Examination of Water and Wastewater', APHA-AWWA-WPCF, 1995 19th Edition.

/4 RESULTS OF ANALYSIS

4 **RESULTS OF ANALYSIS**

DETERMINANDS	METHOD	LAB Ref.	RESULTS	
			CT0522	CT0523
pH at 25°C	SANS 5011	T0090	7,8	7,8
Turbidity in nephelometric units	SABS SM 197	T0090	2,4	2,0
Suspended solids at 105 °C in mg/L	SABS SM 1049	T0090	58	59
Sodium as Na in mg/L	SABS SM 1050	T0090	12 050	12100
Chloride as Cl in mg/L	SANS 374	T0090	18 590	18170
Dissolved Organic Carbon as DOC in mg/L	68	T0036		
Total Organic Carbon as DOC in mg/L	68	T0036		
Particulate Organic Carbon as POC in mg/L	68	T0036		
Total trihalomethanes in µg/L	67	T0036	<0,8	19,6
Chloroform in µg/L	67	T0036		<0,8
Bromodichloromethane in µg/L	67	T0036		<0,1
Dibromochloromethane in µg/L	67	T0036		<0,1
Bromoform in µg/L	67	T0036		19,6

J A SANTER
PRINCIPAL TEST OFFICER
WATER DIVISION
WESTERN CAPE
Santer/Water/J1948/mcromey-hawke