FOREWORD INTRODUCTION

CALCIUM HYPOCHLORITE

CAS N°: 7778-54-3

SIDS Initial Assessment Report

For

SIAM 18

Paris, France, 20-23 April 2004

1.	**Chemical Name:**	Calcium hypochlorite
2.	**CAS Number:**	7778-54-3
3.	**Sponsor Country:**	Japan Mr. Motohiko Kato Director Second International Organisations Div. Ministry of Foreign Affairs 2-2-1 Kasumigaseki, Chiyoda-ku, Tokyo 100-8919
4.	**Shared Partnership with:**	Mr. Keigo Kato Nippon Soda Co., Ltd.
5.	**Roles/Responsibilities of the Partners:**	See below
•	Name of industry sponsor /consortium	Nippon Soda Co., Ltd., Tosoh Corporation, Nankai Chemical Industry Co., Ltd
•	Process used	The document was written by Mitsubishi Chemical Safety Institute LTD.
6.	**Sponsorship History**	
•	How was the chemical or category brought into the OECD HPV Chemicals Programme?	This substance is sponsored by Japan under the ICCA Initiative and is submitted for first discussion at SIAM 18.
7.	**Review Process Prior to the SIAM:**	Japanese government peer-reviewed the documents and audited selected studies.
8.	**Quality check process:**	Japanese government peer-review committee performed spot checks on randomly selected endpoints and compared original studies with data in the SIDS Dossier.
9.	**Date of Submission:**	
10.	**Date of last Update:**	23 January 2004
11.	**Comments:**	No

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	7778-54-3
Chemical Name	Calcium hypochlorite
Structural Formula	$Ca(OCl)_2$

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

Calcium hypochlorite is a white or grayish-white powder. This substance is dissociated into calcium ion (Ca^{++}) and hypochlorite ion (ClO^-) in water. Human health effect may be caused by contact with the solid powder, the aqueous solution, or accidentally generated chlorine gas. The calcium ion can generate a strong alkaline condition at the application site. Concerning hypochlorite ion toxicity, the exposure scenarios to calcium hypochlorite are common to sodium hypochlorite (liquid) or chlorine gas which is utilized as a source of hypochlorite ions, and they are thoroughly assessed in competent/pertinent international risk assessment programmes of organizations like WHO or the EU. Substantial parts of the description on hypochlorite-ion-related effects are common to those in the assessment documents for chlorine (CAS No 7782-50-5) which is also assessed in the OECD HPV Chemicals Programme.

Most of the data for toxicity of this substance by the oral route are from studies performed with sodium hypochlorite or chlorine gas. In biological systems, characterized by pH values in the range of 6-8, the most abundant active chemical species is HOCl, in equilibrium with ClO^-. Such available chlorine is readily absorbed via the oral route and distributed into plasma, bone marrow, testis, skin, kidney and lung. Only ca. 50% is excreted mainly with the urine followed by excretion with feces. HOCl is not enzymatically metabolized.

The acute oral LD_{50} of calcium hypochlorite was 790 mg/kg in male rats. Inhalation exposures to concentrations of greater than about 500 ppm (10 min or more) may be fatal for rats. Based on human experience and control studies in volunteers, it can be concluded that the acute NOAEL for humans was considered to be 0.5 ppm (1.5 mg/m^3). In a 13-week study, male and female F-344 rats (10/sex/group) received NaClO in drinking water at level of 0.025, 0.05, 0.1, 0.2, or 0.4 %. A weight gain was significantly decreased in male rats at 0.2 and 0.4 % and in females at 0.4 %. These effects were dose related and obviously correlated with reduced water consumption. No histopathological changes attributable to the treatment were found. But an increase of AAT in the blood gave evidence of the adverse effects on the liver. Based on significant body-weight reduction at the top dose, a subchronic NOAEL of 59.5 mg/kg bw/day as free available chlorine (FAC*) (at 0.1% NaClO level in the drinking water) can be calculated for male rats. For female rats a subchronic NOAEL of 215.7 mg/kg bw/day as FAC (at 0.2 % NaClO level in the drinking water) can be calculated. A NOAEL of 950 ppm available chlorine (59.5 mg/kg bw/day) can be derived from a 13-week rat study with sodium hypochlorite in drinking water.
In a life-time guideline NTP-study, 70 male and female F344 rats and B6C3F1 mice were administered chlorine via drinking water at dose levels of 0, 70, 140 and 275 mg (equivalent to FAC)/L in buffered water. These concentrations were equivalent to 0, 4.8, 7.5 and 13.9 mg/kg bw/day for male rats and 0, 3.8, 6.9 and 13.2 mg/kg bw/day for female rats. Mean body weights of male and female rats were similar among treated and control groups at both 14-week and 66-week interim evaluations. Those of male mice were significantly lower at week 66. Dose-related decrease in water consumption was observed throughout the study in both species and sexes. Food consumption was comparable among chlorine-treated and control groups. There were no clinical findings, alterations in haematological parameters and biologically significant differences in relative organ weights attributable to the treatment at 14/15-week and 66-week interim evaluations. Survival rate in chlorine-treated groups of rats and mice were similar to those of the controls after two groups. There was no evidence for non-neoplastic lesions to be associated with the consumption of chlorinated drinking water [NTP, 1992]. Based on these findings, a NOAEL (chronic) can be calculated to be approximately 14 mg available chlorine /kg bw/day for rats and 22.5 mg available chlorine /kg bw/day for mice.

Calcium hypochlorite is reported to be corrosive to the skin and has severe effects that can be expected from exposure to the eyes, which is ascribable to the alkalinity of calcium cation (pH=12.0 at 1 % FAC*). Moderate to

severe lesions in the respiratory tract were reported after exposure to chlorine that may emerge in case of accidental misuse of hypochlorite salts. Exposure to chlorine at 9 ppm (27 mg/m^3) for 6 h/day during 1, 3 and 5 days was reported to cause epithelial necrosis, cellular exfoliation, erosion, ulceration and squamous metaplasia in the nasal passage of rats and mice. For either of Ca or Na salt, reliable skin sensitization studies are not available and case reports are available but no reliable case report could be found showing a sensitization potential in humans.

There are data from *in vitro* studies to suggest that solutions of chlorine/hypochlorite have some mutagenic potential, but it can be concluded that they are not mutagenic *in vivo*.

No carcinogenicity was observed in mice or rats exposed by inhalation to chlorine and orally to sodium hypochlorite, except some equivocal results were reported for female rats by oral route. For human carcinogenicity, no causal relationship between hypochlorite exposure and tumor incidence was observed. The observation is applicable to calcium hypochlorite.

No reproductive toxic effects were shown up to 5 mg/kg (highest dose tested) of sodium salt (equivalent to 4.8 mg/kg of Calcium salt) in a one generation oral study in rats. No evidence of adverse developmental effects were reported in animals. Moreover, epidemiological studies in humans did not show any evidence of toxic effects on reproduction and development.

{*Hypochlorite ion is predominant at alkaline pH values, while Cl_2 is mainly present at pH below 4. Therefore the concentration of chlorine in an aqueous solution is generally expressed as free available chlorine (FAC) which is the sum of Cl_2 + HOCl + ClO^-, regardless whether these species stem from dissolved gaseous chlorine or from dissolved sodium/calcium hypochlorite.}

Environment

Calcium hypochlorite is a white or grayish-white powder with chlorine like odor at ambient temperatures and pressures. Density is 2.35 g/cm^3 and vapour pressure is not applicable. This substance is a strong oxidizer. It is highly soluble in water (214 g/L). The anion of this substance dissolved in water is brought to equilibrium between active chlorine species like chlorine (Cl_2), hypochloric acid (HOCl) or hypochlorite ClO^-. The relative amounts of the components are dependent on ionic strength and pH. At the pH in the natural environment (6-8), HOCl or ClO^- is dominating (HClO: pKa = 7.53). A diluted aqueous solution of HOCl will decompose very slowly in the dark, but more rapidly in the presence of light, particularly rapidly in full sun light, by producing hydrogen chloride and oxygen. Some chlorine and chloric acid ($HClO_3$) may also develop. The physico-chemical properties indicate that chlorine released into the environment as HClO or Cl_2 is distributed into water and air. Consequently, the effects that may manifest in the natural environment are considered common to those assessed for the other source of hypochlorite.

In the natural water, in the presence of organic or inorganic compounds, the free available chlorine immediately reacts forming various chlorinated and/or oxidized by-products e.g. chloramines or chloromethanes. They are mainly distributed to the hydrosphere, but are also able to transfer to some extent to the atmosphere depending on their intrinsic properties. A potential for bioaccumulation or bioconcentration of active chlorine species can be disregarded, because of their water solubility and their high reactivity.
Valid freshwater short-term toxicity data are available only for invertebrates: the LC50 for *Ceriodaphnia dubia* is 5 µg FAC/l (FAC=Free available chlorine). Adequate standard acute tests in fish are not available, but from many reliable studies performed under intermittent exposure conditions a 96h LC50 of 60 µg TRC/L and a 168h LC50 of 330 µg TRC/L can be derived (TRC = total residual chlorine = the sum of combined and free residual available chlorine). Due to the intermittent regime (three 45 minutes pulses per day) a 96h LC50 << 60 µg TRC/l can be expected for fish in a standard test. Most lowest result for algae is reported for *Thalassiosira pseudonana* with a IC_{50} of 75 µg/L (20°C).
Regarding long-term toxicity to freshwater organisms, the lowest NOEC was 5 µg/L (*Ictalurus punctatus*, 133d, growth). In microcosm and field studies the most sensitive parameter was the density of zooplankton with a NOEC of 1.5 µg TRC/L, and zooplankton is more sensitive to chlorine than algae.
For salt water, valid short-term toxicity data are available for mollusks and for fish (*Oncorhynchus kisutch* 96 h LC50 = 32 µg TRO/L) (TRO = Total Residual Oxidant) showing comparable sensitivity. For long term toxicity the molluscs are more sensitive than fish showing a 15d NOEC of 6.2 µg TRO/L. It is impossible to delineate representative toxicity indicator figures because of the unique feature of the chemical to be tested in standard methods. However, the accumulated scientific information covering a wide range of species, temperature, application regime or field studies can be used for the hazard assessment.

Exposure

Calcium hypochlorite is a basic chemical, and used as algicide, bactericide, deodorant, disinfectant, fungicide, oxidizing agent, bleaching agent and so on. Chlorine (gas) or sodium hypochlorite (liquid) is used in far higher amounts for the same purpose. The production volume of calcium hypochlorite was estimated to be 16,940 tonnes/year in Japan in 2001, and the total nameplate capacity worldwide including the PRC was approximately 230,000 t/year in 2002.

Exposure to this substance can occur through accidental events in industry (e.g. during filling operations of chlorine gas, using procedure as bleaching agents), during transport and storage, during professional water purification and disinfection measures for swimming-pools.
There is no available official recommendation and regulation for an occupational exposure limit. However, there are some recommendations and regulations for chlorine. This product is a solid and direct contact to the powder can be irritating or corrosive. The product is therefore usually pelletted with water to avoid dust generation and to control exposure during handling or transportation.

For consumers exposure to chlorine gas can occur through accidental events during the use of this chemical for disinfection of swimming-pools and the use of hypochlorite-containing cleaning products. For example, mixing of household cleaning agents, hypochlorite and acids eventually causes chlorine release and inhalation.

RECOMMENDATION

Human Health: The chemical is currently of low priority for further work.

Environment: The chemical is a candidate for further work

RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

Human Health:

The chemical possesses properties (corrosive effects and acute respiratory toxicity) indicating a hazard for human health. Although there are some open uses, consumer exposure is sufficiently regulated under the drinking and other water acts and occupational exposure is adequately controlled in the Sponsor country to ensure safe handling, and therefore this chemical is currently of low priority for further work. Countries may desire to investigate any exposure scenarios that were not presented by Sponsor countries.

Environment:

The substance has hazardous properties for the environments. As there are some open uses of the substance an exposure assessment and if necessary risk assessment should be performed for these uses. The formation of chlorinated by products should be taken into account. Work to that effect is being or has been performed for sodium hypochlorite in many countries and also within the framework of the EU Existing Substances Regulation. The action that may be taken should be common to that for sodium hypochlorite.

1 IDENTITY

1.1 Identification of the Substance

CAS Number: 7778-54-3

IUPAC Name: Calcium hypochlorite

Molecular Formula: CaCl2O2

Structural Formula: Ca(OCl)2

Molecular Weight: 142.98

Synonyms:
ACE-CHLON
BK Powder
Bleaching powder
Calcium hypochloride
Calcium hypochlorite, dry
Calcium oxychloride
Chloride of lime
Chlorinated lime
Chlorkalk
HI-CHLON
HTH
Hy-Chlor
Hypochlorous acid, calcium salt
J-CHLON
Lime chloride
Lo-Bax
Losantin
Mildew remover X-14
NEW STAR-CHLON
NICLON
Oxicloruro de calcio
Perchloron
Pittchlor
STAR-CHLON
TOYO-CHLON

1.2 Purity/Impurities/Additives

There are two production methods. The calcium method is to chlorinate slaked lime by chlorine directly, and the sodium method is to react sodium hypochlorite with the product of the calcium method to remove calcium chloride which is a by-product of the calcium method. The sodium method is predominant at present. Most of the production from the sodium method is marketed as hydrated salt to increase the safety.

Purity: The nominal purity in commercial products is usually 60% or 70%. But, generally, its actual purity is higher than nominal value by several percents.

Impurities: Impurity content varies widely by the manufacturers, grade and production grade. Typical impurities of the hydrated product from the sodium method are as follows:

NaCl	7 - 20%
$CaClO_3$	0 - 5%
$CaCl_2$	0 - 5%
$Ca(OH)_2$	0 - 5%
Water (hydrated)	6 - 15% (hydrated salt)

1.3 Physico-Chemical properties

Table 1 Summary of Physico-Chemical Properties

Property	Value	Reference
Physical state	Solid Pure product has not been reported.	Merck Index, 2001
Melting point	Decomposes at 175 °C	Kirk-Othmer , 1991-present
Boiling point	Not applicable	
Relative density	2.35 g/cm^3	Weast, 1983-1984
Vapour pressure	Not applicable	
Water solubility	approximately 214 g/L (20 °C)	Kirk-Othmer , 1987-1984
Partition coefficient n-octanol/water (log value)	Not applicable	
Henry's law constant	As HClO at pH=5.5; 20 °C H=0.4 x 10^{-4} (mg/L in air divided by mg/L in water)	Draft document of EU Risk Assessment Report as of May 2003
Appearance	White or grayish-white powder with chlorine-like odor	Merck Index, 2001

1.4 Species in aqueous solution as a function of pH

Calcium hypochlorite dissociates into calcium cation and hypochlorite in water. There are three species of hypochlorite in water: dissolved gaseous chlorine, hypochloric acid (HClO) and the hypochlorite anion (ClO-). The sum {$[Cl_2]$+[HClO]+$[ClO^-]$} may be called TRC (Total Residual Chlorine), available chlorine, active chlorine or active free chlorine. For example, at pH 7.5 (at 5 ppm where it may work as a water disinfectant), half of the chlorine is active as HClO and half is available as ClO^-. When this substance or sodium hypochlorite is dissolved in water, the same function as the case where chlorine is dissolved in water operates as shown below.

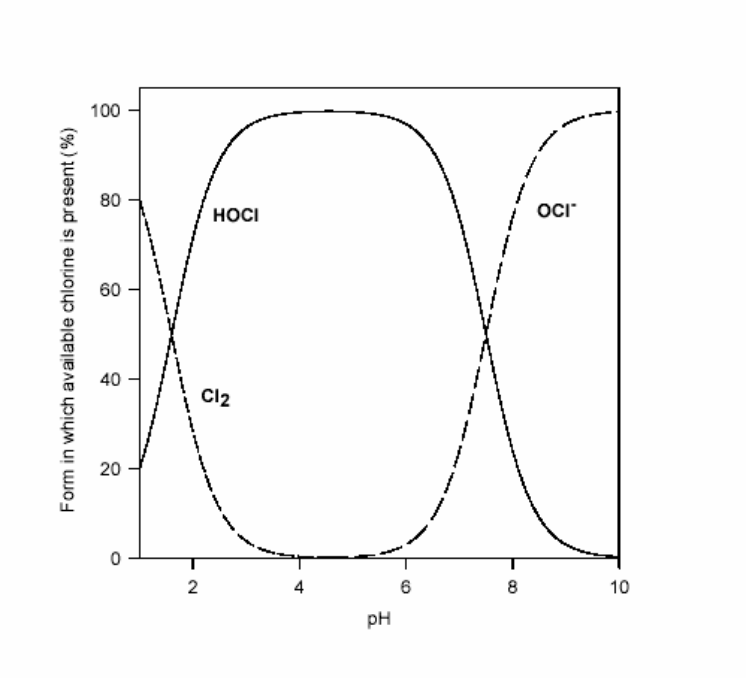

Figure 1 Calculated variation in composition of a chlorine solution with degree of acidity or alkalinity for 0.1 mol/L Cl_2 in water at standard temperature and pressure.

Decomposition

In a concentrated calcium hypochlorite solution at a pH value higher than 11, the content of available (or active) chlorine decreases because ClO^- tends to disproportionate to chloride (Cl^-) and chlorate (ClO_3^-):

$$3ClO^- \rightarrow ClO_3^- + 2Cl^-$$

The process is depending on the time, temperature and concentration of the calcium hypochlorite solution. At constant temperature the inverse of the active chlorine concentration is a linear function of the time. The speed of decomposition is doubled each 5 degree centigrade. That means, the higher the temperature the more available chlorine is lost. It is reported that a solution of NaClO (surrogate) dosed at 150 g/L available (or active) chlorine which is kept away from sunlight and at constant 15 °C, loses 1/6 of its concentration within less than 3 months. In diluted hypochlorite solutions the losses are minor. However, in sun-light decomposition is particularly rapid by the following reaction producing Cl^- and O_2. Calcium hypochlorite solutions are very sensitive to impurities, especially to metals (e.g. nickel and copper). Even minor amounts of these impurities can cause the decomposition of the hypochlorite solution with generation of oxygen:

$$2ClO^- \rightarrow 2Cl^- + O_2$$

In acid media under pH 4 hypochloric acid will be transformed to dissolved chlorine gas.

$$HClO + H^+ + Cl^- \rightarrow Cl_2 + H_2O$$

Between pH 4 and 11, there is mixing of ClO^- and HClO, the latter being much more active. Such a pH is obtained when diluted or all the calcium hydroxide has been carbonated. Degradation of HClO is more rapid than the degradation of ClO^-.

$$2HClO \rightarrow 2HCl + O_2$$

[see corresponding SIDS Documents for Chlorine, CAS No 7782-50-5]

2 GENERAL INFORMATION ON EXPOSURE

2.1 Production Volumes and Use Pattern

The production volume of calcium hypochlorite was estimated at 16,940 t/year in Japan in 2001, and the total name plate capacity worldwide including PRC is approximately 230,000 t/year in 2002 [Nippon Soda, unpublished report]. This substance is a basic chemical, and used as an algicide, bacteriocide, deodorant, disinfectant, fungicide, oxidizing agent, bleaching agent.

2.2 Environmental Exposure and Fate

2.2.1 Sources of Environmental Exposure

Exposure to residual hypochlorite by the oral route occurs mainly from its use as a drinking water disinfectant (mainly in the form of calcium hypochlorite, sodium hypochlorite or hypochlorous acid). Environmental releases from industrial sources are minimised by waste and emission control and management which include effluent treatment (settlement, pH adjustment, chlorine removal) and analytical control.

2.2.2 Photodegradation

The calcium hypochlorite solution is very sensitive to light [Kirk-Othmer, 1985]. Direct sunlight may cause rearrangement and decomposition resulting in the formation of chloride and oxygen. In natural water, the Cl_2 molecule as well as hypochlorite ions are not stable due to the presence of organic and inorganic matter. The half-life of hypochlorite is estimated to be less than 2 hours due to reduction and photolysis. In the atmosphere, chlorine mainly undergoes photolysis:

$$Cl_2 + h\nu \rightarrow 2Cl^{\circ}.$$

The half-lifetime for that process has been estimated to be in the order of 1–4 hours, depending on the time of the day.[see SIDS Documents for Chlorine, CAS No 7782-50-5]

2.2.3 Stability in Water

See Section 1.4 Species in aqueous solution as a function of pH

2.2.4 Transport between Environmental Compartments

The fugacity model is not applied to estimate the distribution of this substance in the environment because this substance decomposes rapidly in each compartment (air, water, soil and sediment). Therefore, this substance itself does not exist in nature.

2.2.5 Biodegradation

High water solubility and rapid reaction with organic matter leads to rapid disappearance of the hypochlorite moiety. Biodegradation of this substance cannot be measured A product of the reaction of calcium hypochlorite with organic matter is calcium chloride.

2.2.6 Bioaccumulation

The bioaccumulation potential of this substance can be disregarded, because of its water solubility and its high reactivity. Nevertheless, hypochlorite may be found in living organism. Hypochlorite is also produced naturally *in vivo* for cell defense process. The natural production of halo-oxo acids is widespread and related to haloperroxidases, which is well documented in the literature. A good overview of biohalogenation is given by Geigert et al. [Geigert et al., 1986] and more recently by Winterton [Winterton, 1997]. Hypochlorite is produced by chloroperoxidases, which are, among others, produced by mammals (in white blood cells), lichens and in many fungal species [Vollenbroek et al., 1995].

2.3 Human Exposure

Hypochlorous ions are physiologically present in the human body, being formed by white blood cells (neutrophils and monocytes) as a powerful antimicrobial agent during inflammation process. When the recognition of "non-self" proteins in an invading micro-organism triggers the immune response, the enzyme myeloperoxidase located in mammalian neutrophils catalyses hypochlorous acid formation trough the oxidation of chloride ion in combination with hydrogen peroxide. The endogenously formed hypochlorous acid plays a key role in the process of phagocytosis through which bacteria are killed. Due to its potent cytotoxic action, hypochlorite is also responsible for neutrophil-mediated tissue damage associated with the inflammatory response. Its high efficiency as antimicrobial agent is associated with the lack of a catalytically active detoxifying mechanism for HOCl in both bacteria and mammalian cells. Although it has been suggested that HOCl-induced cytotoxicity can be associated to the degradation of a number of functionally important molecules the primary mechanism of action is still not fully elucidated.

2.3.1 Occupational Exposure

A major production method is the conversion of the reaction product from the calcium method by addition of NaClO (referred as sodium method in section 1.2). Usually, the production of this substance is conducted in the same factory as sodium hypochlorite production and the process is very similar. Therefore, occupational exposures at production sites are similar to each other. It may occur by inhalation of gaseous chlorine. There is no available official recommendation and regulation for occupational exposure limit specific to calcium hypochlorite itself. However, there are some recommendations and regulations about chlorine to be applied to sodium hypochlorite production. The regulations are normally achieved. Namely, all TWA values measured for NaClO producers surveyed in an EU risk assessment program were below 0.5 ppm in ambient air. The same result should also apply to the calcium salt. During handling of the product, exposure through the dermal route by contact with the solid product of calcium salt is possible. There is no available monitoring data. All systems for production of this substance are semi-closed systems. The product is handled as pellets. Normally, workers wear protections for eye/face, skin, and respiratory system.

Exposure to hypochlorite ion or gaseous chlorine can occur through accidental events in various industries (e.g. during filling operations of chlorine gas, in the pulp and paper industry using chlorine, HCl or chlorine dioxide as bleaching agents), during transport and storage, or during professional water purification and disinfection measures for swimming-pools.

Exposure control

Although there is no occupational exposure limit for calcium hypochlorite most countries adopted the threshold for chlorine. Japan and most European countries have a limit for long term exposure (8 hour TWA) to chlorine of 0.5 ppm, some have a limit of 1 ppm.

2.3.2 Consumer Exposure

Exposure to calcium hypochlorite can occur through accidental events during the use of calcium hypochlorite for disinfection of swimming-pools and the use of hypochlorite-containing cleaning products, e.g. through mixing of household cleaning agents, such as hypochlorite and acids eventually associated with chlorine release and inhalation. No quantitative report is available at the European level to detail the frequency and importance of the swimming pool accidents. A report prepared by RPA for the European Commission [RPA, 1997] stated that despite the misuse of domestic hypochlorite bleaches resulting in fatal accidents in some rare instances, overall these products do not appear to present a significant risk to the consumer. In case of accidental misuse, moderate to severe lesions in the respiratory tract were reported after exposure to chlorine.

Exposure control

Exposure to hypochlorite can occur in general through disinfected drinking water. Controls are exist on drinking water content of active chlorine which is generally between 0.1 to 0.5 ppm. For example, the WHO recommendation regarding the maximum content of active chlorine in drinking water is 0.5 mg/L(see also chapter 2.3.1). The sponsor country Japan has set a lower limit to warrant tap water quality at 0.1 mg/L of FRC (free available chlorine after a reaction; see Glossary in the Annex) and a higher limit at 1.0 mg/L for amenity reasons. The lower limit (requirement) for swimming pools is 0.4 mg/L and the higher limit (recommendation) is 1.0 mg/L WHO has published a relevant report (Guidelines for Safe Recreational-water Environments. Volume 2: Swimming Pools, Spas and Similar Recreational-water Environments. Chapter 4. Chemical Hazards. Final Draft for Consultation August 2000). This covers calcium hypochlorite (as well as chlorine and sodium hypochlorite)

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

Calcium hypochlorite is a powder. Human health effect specific to the calcium salt may be caused by contact with the solid powder, the aqueous solution, or accidentally by generated chlorine gas. This substance is dissociated into calcium ion (Ca^{++}) and hypochlorite ion (ClO^{-}) in water. Calcium ion can cause a strong alkaline condition at the application site. Concerning hypochlorite ion toxicity, the exposure scenarios to calcium hypochlorite are common to sodium hypochlorite (liquid) or chlorine gas which is utilized as a source of hypochlorite ion, and they are thoroughly assessed in competent/pertinent international organization like WHO or in the EU risk assessment program. Therefore, a substantial portion of the description on hypochlorite-ion-related effects is taken over from those documents as well as the SIDS Documents for chlorine.

Most of the data for toxicity of this substance by the oral route came from studies performed with sodium hypochlorite or chlorine gas. In biological systems, characterized by pH values in the range of 6-8, the most abundant active chemical species is HClO, in equilibrium with ClO^{-}. Such available chlorine is readily absorbed via the oral route and distributed into plasma, bone marrow, testis, skin, kidney and lung. Only ca. 50% is excreted mainly with the urine followed by excretion with feces. HClO is not enzymatically metabolized.

3.1.1 Toxicokinetics, Metabolism and Distribution

The primary available chlorine species in aqueous solution are not different from those coming from hypochlorous acid at similar pH. In biological systems, characterised by pH values in the range of 6-8, the most abundant active chemical species is HClO, in equilibrium with ClO^-. The latter is predominant at alkaline pH values, while Cl_2 is mainly present at pH values below 4. Therefore, the studies performed with hypochlorite and its salts are used in this document. Limited data are available for the oral route only.

Studies in Animals

In vivo Studies

Abdel-Rahman and Suh [Abdel-Rahman and Suh, 1983] studied the toxicokinetics of hypochlorous acid (HClO) in Sprague-Dawley rats which were orally administered with different quantities of $H^{36}ClO$ solution. ^{36}Cl is readily absorbed and found into the bloodstream: a peak of radioactivity in rat plasma occurred 2 hours after $H^{36}ClO$ administration in fasted rats and 4 hr after administration in non-fasted rats. ^{36}Cl radioactivity was distributed throughout the major tissues at 96 hr after $H^{36}ClO$ administration. The higher levels were found in plasma, whole blood, bone marrow, testis, skin, kidney and lung. The lower levels were found in the liver, carcass and fat tissue. $H^{36}ClO$-derived radioactivity was not detected in expired air throughout the 96 hr-study. 36.43% ± 5.67 (mean ± S.E.) of the administered dose was excreted through the urinary route, while 14.8% ± 3.7 was recovered in the faeces, giving a poor total recovery of 51.23% ± 1.97.

It is well known from studies on HClO in inflammation processes that HClO is not enzymatically metabolised and its (bio)transformation readily occurs through direct reactions with organic compounds or with other chemicals present in the cellular environment, including hydrogen peroxide. The toxicokinetic study showed that chloride ion accounted for >80% ^{36}Cl radioactivity present in rat plasma.

When Sprague-Dawley rats were administered HClO at 0, 1, 10 or 100 mg/L daily in drinking water for one year, no significant chloroform concentrations were observed in rat blood at 4, 6, 9 and 12 months [Abdel-Rahman et al., 1984].

The formation of organochlorinated compounds in the stomach and blood was investigated in Sprague-Dawley rats. The rats were administered by gavage with 7 ml of a 8 mg/ml solution of sodium hypochlorite at pH 7.9 (about 140 mg/kg bw) and sacrificed after one hour: trichloroacetic acid, dichloroacetic acid and chloroform were detected only in the stomach of animals [Mink et al., 1983]. In the same laboratory, a multiple dose study was also carried out in rats administered for 8 days orally with 8 and 16 mg/kg bw/day NaClO, a much lower concentration with respect to the acute study by Mink et al. and more consistent with drinking water intake. No organo-chlorinated compounds were detected in the urine [Kopfler et al., 1985].

3.1.2 Acute Toxicity

Studies in Animals

Oral route

There are several studies available regarding the acute oral toxicity of sodium hypochlorite (not cited). However, with regard to the calcium salt, one report was considered to be reliable [Nippon Soda, 1985a].

Wister-derived albino rats were dosed by gavage (vehicle: water) at 890, 1000, 1120, and 1260 mg/kg bw (10 males/group). Eight/10, 5/10, 9/10, and 10/10 deaths occurred at 890, 1000, 1120, and 1260 mg/kg, respectively. Moderate depression of the central nervous system was found at 1 hour after administration. Most survivors showed a mild to moderate persistent anorexia. Most affected animals showed diarrhea for several days. The LD50 was calculated to be 790 mg/kg.

Because the content of the hypochlorite moiety in the molecule of the sodium salt is almost the same as for the calcium salt, (69.1% for sodium, 72% for calcium), data obtained using sodium hypochlorite may be, with minimal correction, used if necessary.

Inhalation

Calcium hypochlorite is incompatible with acidic conditions. As calcium hypochlorite can react with acids to release chlorine gas, toxicity data on the latter might be relevant for occupational exposure, accidents or misuse. Thus, the acute inhalation section of this document deals with chlorine gas. The acute inhalation toxicity of chlorine gas was investigated in many species including rodents, rabbits, guinea pigs, dogs, cats, and even pigs [Demnati et al., 1995; Demnati et al., 1998ab]. The key results generated on rodents can be summarised as follows: Exposures to concentrations of greater than about 500 ppm (10 min or more) may be fatal and is assumed to produce severe histological changes on the respiratory and oropharyngeal system which do not subside until 14 to 30 days in surviving animals [Zwart and Woutersen, 1988]. The following mortality data can be derived: Lethality thresholds for animals can be derived from dose response relationships. LC01 values calculated for 30-minute exposures are 112 ppm (336 mg/m^3) for mice and 420 ppm (1240 mg/m^3) for rats. The individual mortality data for all species tested for up to 60-minute exposures showed no lethality below 62 ppm (186 mg/m^3).

Studies in Humans

Inhalation

The inhalation toxicity of chlorine gas may be relevant for occupational exposure or accidental misuse because calcium hypochlorite is incompatible with acidic conditions (see section 1.4). Thus, the acute inhalation section of this document deals with chlorine gas (see also paragraph "Studies in humans, inhalation" in section 3.1.5.).

Chlorine has a characteristic pungent odor. Individual perception data range from about 0.6 mg/m^3 (0.2 ppm) up to 6.0 mg/m^3 (2.0 ppm). While 1 ppm (3 mg/m^3) already causes significant harassment through uncomfortable irritation, workers used to inhale low concentrations of chlorine get adapted to a certain extent and will tolerate otherwise irritating concentrations of chlorine [Wirth et al., 1994].

Pre-disposed/-sensitized people appear to have a lowered irritation threshold. Nasal airway resistance was increased in persons with seasonal allergic rhinitis exposed to 0.5 ppm (1.5 mg/m^3) chlorine for 15 minutes. These persons reported also nasal irritation and congestion. However, no evidence of any significant change in nasal airway resistance was observed in normal persons under the same conditions [Shusterman et al., 1998]. These findings in normal individuals were confirmed by the findings that exposure to up to 0.5 ppm (1.5 mg/m^3) for 6 hours on 3 consecutive days failed to induce any change in lung function parameters and nasal lavage measurements [Schins et al., 2000].

In Table 2 important results from controlled studies in human volunteers are summarised. These irritant or non-irritant chlorine levels seem to refer to short term exposure intervals (up to eight hours) and can be considered tolerable without inducing serious tissue damage, but can have some significant transient impact on the lung capacity and function. The critical concentration over 8

hours appears to be about 1 ppm, while 0.5 ppm (1.5 mg/m^3) does not cause significant changes in lung function parameters [Rotman et al., 1983].

Five case reports on human exposure to chlorine leading to acute effects are available. These data are mainly based on experiences from accident cases and comprise those chlorine levels over short intervals (up to one hour) which apparently are no longer tolerable and may produce serious to life-threatening lesions in the respiratory tract. Exposure concentration, exposure time and clinical symptoms are shown in Table 3.

Following exposures to low to moderate chlorine concentrations, symptoms started within 10 minutes of exposure, and dysfunctions disappeared within 1 to 2 months [Kaufman et al., 1971; Beach et al., 1969; Plysongsang et al., 1982].

In cases where no pulmonary oedema was evident, symptoms resolved within 1 week in subjects whose major complaint was cough. A slower resolution was noted in subjects whose initial complaint was dyspnea. In these subjects pulmonary function was still impaired 2 weeks after exposure [Hasan et al., 1983].

For example: In one well documented incident in Bombay, 88 workers in a chemical plant were exposed to about 66 ppm (198 mg/m^3) chlorine gas for about one hour. All of them suffered from dyspnea and coughing, as well as irritation of the throat and eyes, headache, giddiness, chest pain and abdominal discomfort. Radiological investigation of 28 of the 88 patients in the hospital revealed in some persons' hilar congestion and prominent bronchial vasculature markings. Respiratory incapacity was observed in 62 persons 48 hours after the exposure. A bronchoscopy after 5 days revealed tracheobronchial congestion in 56 persons and chronic bronchitis in 12 persons. In 28 persons scattered hemorrhagic spots were noted under the bronchial mucosa. Seven persons showed evidence of bronchial erosion and had persistent cough and respiratory distress. Cytopathological features were observed in bronchial brushings up to 25 days after exposure [Shroff et al., 1988].

Table 2 Effects of Acute Exposure to Chlorine Reported in Human Volunteers' Studies

Concentration		Exposure Time	Clinical symptoms on acute exposure	Number of subjects	Reference
ppm	mg/m^3				
0.06–0.2	0.18–0.6	n.r.	itching in the nose	3	Rupp et al., 1967
0.35–0.72	1.05–2.16	15 min	burning of conjunctivae	19	Rupp et al., 1967
0.1–0.5	0.3–1.5	n.r.	slight tickling in the nose and throat, cough, sensations in the ocular conjunctiva, sensation of choking	10–13	Beck, 1959
0.5	1.5	8 h	no impairment of pulmonary function, irritating effects	30	Anglen, 1981
0.5	1.5	8 h	no significant impairment of pulmonary function	n.r.	Rotman et al., 1983
0.5	1.5	2 h	borderline effects	8	Joosting and Verbeck, 1975
0.5	1.5	6 h on 3 consecutive days	no changes in lung function and nasal lavage	n.r.	Schins et al., 2000

Concentration		Exposure Time	Clinical symptoms on acute exposure	Number of subjects	Reference
ppm	mg/m^3				
1.0	3.0	30 min	tickling and stinging in the nose, scratchiness and dryness in the throat; in single case: dull sensation in the teeth and a slight metallic taste, headache and pressure, burning of ocular conjunctiva / outer skin, coughing, constriction of breathing	10	Beck, 1959
1.0	3.0	60 min	impairment of lung function: decrease in FEV1 (Forced Expiratory Volume)	n.r.	D'Alessandro et al., 1996
1.0–1.3	3.0–3.9	35 min	dyspnea and cough with violent headache	1	Rupp et al., 1967
1.0	3.0	4–8 h	sensory irritation and impairment of pulmonary function		Rotman et al., 1983
0.5–1.0	1.5–3.0	4 h	slight irritation, induced coughing reflex	30	Anglen, 1981
1.0	3.0	2 h	individual variation in sensibility with respect to eye irritation and coughing reflex.	8	Joosting and Verbeck, 1975
2.0	6.0	2 h	significant irritation throughout: cough, eye, nose, throat, but clearly tolerable without impairment of pulmonary function	8	Joosting and Verbeck, 1975, Anglen, 1981
2.0	6.0	2–4 h	pronounced signs of irritation, increased nasal mucus secretion	30	Anglen, 1981
2.0	6.0	15 min	no significant irritation and impairment of pulmonary function	30	Anglen, 1981
2.5–4.0	7.5–12.0	5–16 min	immediate burning of the eyes, itching in the pharynx, coughing, and nasal congestion	1	Matt, 1989

n.r.: not reported.
Note that the studies reporting this data are not robust.

Table 3 Case Reports on Effects of Acute Exposure to Chlorine

Concentration		Exposure Time	Clinical symptoms on acute exposure	Reference
(ppm)	mg/m^3			
15	45	< 30 min	significant ocular, nasal and pharyngeal irritation	Lheureux et al., 1993 [a]
20	60	about 30 min	dangerous	Wirth et al., 1994
30	90	< 30 min	cough, laryngospasm, chest pain, nausea, vomiting	Lheureux et al., 1993 [a]
40–60	120–180	< 30 min	tracheobronchitis, pneumonia, RADS ("Reactive Airways Dysfunction Syndrome")	Lheureux et al., 1993[a], Shroff et al., 1988
50	150	30–60 min	lethal	Wirth et al., 1994
430	1290	< 30 min	minimal lethal concentration reported	Lheureux et al., 1993 [a]

Concentration		Exposure Time	Clinical symptoms on acute exposure	Reference
(ppm)	mg/m^3			
690–1000	2070–3000	rapid	Lethal	Wirth et al., 1994, Lheureux et al., 1993 [a]

[a]Based on Hedges and Morrissey (1979) cited in [Lheureux et al., 1993].

Conclusion

The acute oral LD50 value obtained specifically from an experiment using calcium hypochlorite was 790 mg bw/kg in male rats. Chlorine gas inhalation data are equally relevant in safety assessment. Based on human experience and control studies in volunteers, it can be concluded that the acute NOAEL for humans is considered to be 0.5 ppm (1.5 mg/m^3) for 4 hours.

3.1.3 Irritation

Calcium hypochlorite is often quoted as "corrosive to the skin". The source of this statement, was, however, not retrievable and no additional information was available to substantiate it. Severe effects on the eyes can be expected due to the alkalinity of the calcium cation and non-reacted calcium hydroxide (pH=12.0 at 1 % FAC[1]).

Sensory irritation of chlorine in mice and rats was evaluated by quantifying the decrease in the respiratory rate following short-term exposure. In mice, no sensory irritation response appeared at 0.7 ppm (2.1 mg/m^3) [Barrow et al., 1977]. The concentration that resulted in a 50 % decrease in respiration rate (RD50) after 60 min exposure was 3.5 ppm (10.5 mg/m^3). For 10-min exposure, the RD50 for mice was 9.3 ppm (27.9 mg/m^3) [Gagnaire et al., 1994]. The histological changes caused by the irritation were investigated. In rats and mice, moderate to severe lesions in the respiratory tract after exposure to 9 ppm (27 mg/m^3) chlorine for 6 h/d for 1, 3 and 5 days were reported: mainly in the nasal passage, epithelial necrosis, cellular exfoliation, erosion, ulceration and squamous metaplasia. The changes were noted to associate with widespread loss of respiratory and olfactory cilia, were noted. This concentration corresponded to the RD50 [Jiang et al., 1983].

Generally, much of the toxicity seen in repeated inhalation and dermal exposure is irritant in nature. This may have prevented to elevate the dose level enough to show systemic effects.

3.1.4 Sensitisation

There are no experimental data. For either of Ca or Na salt, skin sensitization studies are not available and no reliable case report is found showing a sensitization potential in humans.

3.1.5 Repeated Dose Toxicity

Table 4 shows representative repeated dose studies. A long-term inhalation study has been conducted using chlorine gas in rats and mice. This study was a comprehensive study but lesions revealed were those upon local effects in the respiratory and oropharyngeal tract [Wolf et al., 1995].

[1] Hypochlorite ion is predominant at alkaline pH values, while Cl_2 is mainly present at pH below 4. Therefore the concentration of chlorine in an aqueous solution is generally expressed as free available chlorine (FAC) which is the sum of Cl_2+HClO+ ClO^-, regardless whether these species stem from dissolved gaseous chlorine or from dissolved hypochlorite salt.

In comprehensive and reliable testing programs, oral subchronic and lifetime studies have been performed on sodium hypochlorite in both rats and mice. In any studies, no systemic effect of chlorine was observed. No specific target organ could be identified, except that a decrease of body weight and body weight gain presumably due to low water intake were observed after oral administration at the highest dose [Furukawa et al., 1980; NTP, 1992].

Table 4 Effects of Repeated Exposure to Hypochlorite / Chlorine in Mammals

Dose level	Exposure Time	Effects of repeated exposure	Reference
0, 0.4, 1.0, 2.5 ppm 1 ppm = 3 mg/m^3	2 years inhalation study in rats 6 h/d, 5 d/week males, 6 h/d, 3 d/week females. In mice 6 h/d, 5 d/week males and females.	Lesions of the nasal passage in males and females; most severe in the anterior nasal cavity. All the group exposed to chlorine showed the change of some significance, NOEL s were not deduced in both species.	Wolf et al., 1995
0, 70, 140, 275 mg/L chlorine in drinking water. for male rat 0,4.8,7.5,13.9 mg/kg bw day for female rat 0,3.8, 6.9, 13.2mg/kg bw day for female mouse 0,8,14,24 mg/kg bw day for male mouse 0,7,14,24mg/kg bw day	life-time drinking water study in mice and rats	No clinical findings, alterations in haematologic parameters and biologically significant differences in relative organ weights at 14/15-week and 66-week interim evaluations. No microscopic abnormalities in a comprehensive range of tissues and organs.	NTP, 1992
0, 0.05, 0.1 % (males) 0.1, 0.2 % (females) NaClO 0, 13.5 27.7 mg/kg bw day(male) 0,34, 63 mg/kg bw day(female)	2 year drinking water study in rats	Dose-related decrease in body weight gain.	Hasegawa et al., 1986
0, 0.025, 0.05, 0.1, 0.2 to 0.4 % NaClO 0, 7, 14, 28, 55 to 118 mg/kg bw day	13 weeks drinking water, study in rats	Decrease in body weight gain was observed in both sexes. No histological changes attributable to the treatment.	Furukawa et al., 1980
Sprague-Dawley rats at 0, 25, 100, 175 and 250 mg chlorine/L correspond to 0, 3.5, 12.6, 19.5 and 24.9 mg chlorine/kg bw/day (male) and to 0, 2.1, 7.5, 12.8 and 16.7 mg chlorine/kg bw/day (females)	90-day study, chlorine in drinking water.	The highest dose of chlorine tested (250 mg/L in drinking water, 17–25 mg/kg bw/day) was concluded to be a NOAEL, because no toxic effects were observed in any dose group.	Daniel et al, 1990; Daniel et al., 1991
B6C3F1 mice at 0, 12.5, 25, 50, 100 and 200 mg chlorine/L correspond to 0, 2.7, 5.1,	90-day study, chlorine in drinking water.	In mouse non-specific effects (decreased body weight gain, reduced organ weight and lower levels of serum enzymes) were observed, which could be a consequence of the decreased water consumption,	Daniel et al, 1990; Daniel et al., 1991

Dose level	Exposure Time	Effects of repeated exposure	Reference
0, 0.4, 1.0, 2.5 ppm 1 ppm = 3 mg/m^3	2 years inhalation study in rats 6 h/d, 5 d/week males, 6 h/d, 3 d/week females. In mice 6 h/d, 5 d/week males and females.	Lesions of the nasal passage in males and females; most severe in the anterior nasal cavity. All the group exposed to chlorine showed the change of some significance, NOEL s were not deduced in both species.	Wolf et al., 1995
10.3, 19.8 and 34.3 mg chlorine/kg bw/day (males) and to 0, 2.8, 5.8, 11.7, 21.2 and 39.2 mg chlorine/kg bw/day (females)		associated with taste aversion and not chemically induced toxicity per se. According to the authors, 50 mg chlorine/L (10–12 mg/kg bw/day) is considered to be a NOAEL	

Studies in Animals

Inhalation

Groups of approx. 70 female and male F344/N rats and B6C3F1 mice each were exposed to chlorine gas at 0, 0.4, 1.0, 2.5 ppm (0, 1.2, 3.0, 7.5 mg/m^3) for 6 h/d, 5 d/week (mice and male rats) or 3 alternate days/week (female rats) for 2 years, with an interim necropsy of 10 rats/sex performed at 12 months. Exposure-dependent lesions were confined to the nasal passage in both sexes of both species. The respiratory and olfactory epithelial degeneration, septal fenestration, mucosal inflammation, respiratory epithelial hyperplasia, squamous metaplasia, goblet cell hypertrophy and hyperplasia, and secretory metaplasia of the transitional epithelium of the latera meatus were observed severely in the anterior nasal cavity. Statistically-significantly increase in incidence of nasal lesions was found. The severity of these lesions was dependent on the concentration of chlorine [Wolf et al., 1995]. The LOAEL for respiratory irritation was 0.4 ppm (1.2 mg/m^3). The NOAEL could not be established.

Dermal

A solution of chlorite, common to sodium hypochlorite solutions, was found to have an irritant effect on skin. An irritant effect on skin was found during 4 days of continuous topical application of a 0.1% solution of sodium hypochlorite in female SENCAR mice. These effects disappeared after cessation of application [Robinson et al., 1986].

Oral

In a 13-week study, 6 groups of male and female F-344 rats (10/sex/group) received NaClO in drinking water at levels of 0.025, 0.05, 0.1, 0.2, or 0.4 %. The weight gain was significantly decreased in male rats at 0.2 and 0.4 % and in females at 0.4 %. These effects were dose related and obviously correlated with reduced water consumption. No histopathological changes attributable to the treatment were found. The increase of AAT in the blood gave evidence of adverse effects on the liver. Although absolute weights of the lung, liver and spleen of males and the salivary gland, lung, heart and brain of females were significantly lower in the highest-dose group compared to the controls, relative weights were not changed. A maximum tolerated dose of sodium hypochlorite given in the drinking-water was estimated to be between 0.1 and 0.2 % for male rats and 0.2 and 0.4 % for female rats [Furukawa et al., 1980]. Based on significant body-weight reduction at the top dose, a subchronic NOAEL of 22.5 mg/kg bw per day as chlorine equivalent (at 0.1% NaClO in the drinking water) can be calculated for male rats. For female rats a subchronic NOAEL of 55.6 mg/kg bw per day as chlorine equivalent (at 0.2 % NaClO in the drinking water) can be calculated.

In a 90-day study, B6C3F1 mice and Sprague-Dawley rats (each consisting of 10 males and 10 females) were administered chlorine in drinking water. Doses administered to mouse were 0, 12.5, 25, 50, 100 and 200 mg chlorine/L which corresponded to 2.7, 5.1, 10.3, 19.8 and 34.3 mg chlorine/kg bw/day for males and to 2.8, 5.8, 11.7, 21.2 and 39.2 mg chlorine/kg bw/day for females. Rats received chlorine at 25, 100, 175 and 250 mg chlorine/L drinking water which corresponded to 3.5, 12.6, 19.5 and 24.9 mg chlorine/kg bw/day for males and to 2.1, 7.5, 12.8 and 16.7 mg chlorine/kg bw/day for females. In the rat study the highest dose of chlorine tested (250 mg /L in drinking water, 17–25 mg/kg bw/day) was concluded to be a NOAEL, because no toxic effects were observed in any dose group. In mice non-specific effects (decreased body weight gain, reduced organ weight and lower levels of serum enzymes) were observed, which could be a consequence of the decreased water consumption, associated with taste aversion and not chemically induced toxicity per se. The NOAEL is considered to be the highest dose employed, although the authors claimed 50 mg chlorine/L (10–12 mg/kg bw/day) to be a NOAEL [Daniel et al, 1990; Daniel et al., 1991].

In a life-time guideline NTP-study, 70 male and female F344 rats and B6C3F1 mice were administered chlorine (NaClO) via drinking water at dose levels of 0, 70, 140 and 275 mg/L buffered water. These concentrations were equivalent to 0, 4.8, 7.5 and 13.9 mg/kg bw/day for male rats and 0, 3.8, 6.9 and 13.2 mg/kg bw/day for female rats. Mean body weights of male and female rats were similar among treated and control groups at both 14-week and 66-week interim evaluations. Those of male mice were significantly lower at week 66. Dose-related decrease in water consumption was observed throughout the study in both species and sexes. Food consumption was comparable among chlorine-treated and control groups. There were no clinical findings, alterations in haematological parameters and biologically significant differences in relative organ weights attributable to the treatment at 14/15-week and 66-week interim evaluations. Survival rate in chlorine-treated groups of rats and mice were similar to those of the controls. There was no evidence for non-neoplastic lesions to be associated with the consumption of chlorinated drinking water [NTP, 1992]. Based on these findings, a NOAEL (chronic) can be calculated to be approximately 13 mg Cl_2/kg bw per day for rats and 22.5 mg Cl_2/kg bw per day for mice.

A second, less well documented long-term study using 50 male and 50 female F344 rats receiving 0.05 and 0.1 % (males) and 0.1 and 0.2 % (females) NaClO in drinking water for 104 weeks is in full agreement with the observations from the NTP study: Dose-related decrease in body weight gain was noted in both sexes. Drinking water intake and food consumption were comparable among treated and control groups. Haematology and serum biochemical analysis did not show significant treatment-related changes for any parameter in either sex. Histopathological results are described later with tumor evaluation. There were no specific increases in non–neoplastic lesions [Hasegawa et al., 1986]. The chronic NOAEL can be assumed to be of the order of that found in the NTP study or somewhat higher.

In a few non-standard studies, effects on the immune system of rats and mice in relation to the administration of chlorinated drinking water are reported. These effects (reductions in spleen weight and delayed-type hypersensitivity reactions reduction in macrophage function, increase in prostaglandin E2) were noted at low doses (15 to 30 ppm) after a short exposure time (4 to 9 weeks) [Exon et al., 1987], but an influence on delayed-type hypersensitivity was not consistent with a subchronic study in mice focussed on the same aspect [Hermann et al., 1982].

Studies in Humans

Inhalation

Exposure to sub-lethal doses of chlorine gas leads to obstructive disturbances of pulmonary ventilation which are usually reversible. Weill et al. [Weill et al., 1969] and Jones et al. [Jones et al.,

1986] did not find abnormalities up to 6 years after accidental chlorine exposures that could not be attributed to other underlying lung diseases or smoking. Also Leroyer et al. [Leroyer et al., 1998] in their 4-year follow-up of 13 workers with accidental chlorine exposure showed complete recovery in three months for the individual who had decreased forced expiratory volume (FEV1) and two individuals with decreased PC20 [Leroyer et al., 1998]. Hasan et al. [Hasan et al., 1983] found improvement in respiratory symptoms forced vital capacity (FVC) and FEV1 within 5 months. In this study, however, the bronchial hyperresponsiveness was not assessed [Hasan et al., 1983].

A syndrome, defined by Brooks et al. [Brooks et al., 1985] as "Reactive Airways Dysfunction Symptom" (RADS) has also been related to acute chlorine exposure, which is a sudden onset type of asthmatic illness following acute inhalation of high-dose irritant gases. In rare cases, persons may acquire a chronic bronchial hyperreactivity after acute or repeated contact with irritant gases like chlorine or sulfur dioxide. This depends on the individual disposition rather than on an allergic mechanisms.

Chronic occupational exposure to 0.01–1.4 ppm (0.03–4.2 mg/m^3) of chlorine gas is reported to have produced no lesions in the exposed workers [Patil et al., 1970]. .

Conclusion

The LOAEL for respiratory irritation was 0.4 ppm (1.2 mg/m^3) in animal studies. A NOAEL could not be established.

A NOAEL (chronic, oral) can be calculated to be approximately 13 mg Cl_2/kg bw/day for rats and 22.5 mg Cl_2/kg bw/day (corresponding to chlorine gas generated from 26 and 44.3 mg of $Ca(ClO)_2$) for mice. Immunotoxcity results are equivocal in light of the present state of the art of the evaluation science.

3.1.6 Mutagenicity

Solutions of chlorine and hypochlorite have been studied in a fairly extensive range of mutagenicity assays, both *in vitro* and *in vivo*. There are deficiencies in the conduct and/or reporting of most of the studies which in particular appear to be hampered by the fact that hypochlorite is highly cytotoxic. No mammalian cell gene mutation studies were identified. Results of several studies on the genotoxicity of chlorine are summarized in Table 5.

Table 5 Mutagenicity Tests with Hypochlorite Solutions

Type	Result	Notes	Reference
Ames / *Salmonella typhimurium*	Positive TA100 (+S9) Negative TA98	limited data presented TS : calcium hypochlorite	Kawachi et al., 1980
Ames / *Salmonella typhimurium*	Negative TA97 TA102 (±S9)	limited data presented	Fujita and Sasaki, 1987
Ames / *Salmonella typhimurium*	Negative TA100, TA1537, TA1538, TA98, WP2*uvr*A		Nippon Soda, 1985b
Ames / *Salmonella typhimurium*	Negative TA 100 TA 98 TA 102	not standard assay	Le Curieux et al., 1993
Ames / *Salmonella typhimurium*	Not applicable	supplemental information	Tsuda et al., 1983

Type	Result	Notes	Reference
Rec Assay / B. Subt.	Negative (±S9)	supplemental information	Kawachi et al., 1980
SOS chromotest	Negative	supplemental information	Klimm et al., 1989
CA / CHL cells	Positive (-S9)	limited data presented TS; calcium and sodium salt	Ishidate et al., 1984
CA / CHL Cells	Positive (+S9)	limited data presented	Matsuoka et al., 1979
CA / HEF Cells	Negative	limited data presented	Sasaki et al., 1980
SCE / HEF Cells	Positive	limited data presented	Sasaki et al., 1980
MN / Mouse BM	Negative	data well presented	Hayashi et al., 1988
MN / Mouse BM	Negative	data on bone marrow toxicity is missing	Meier et al., 1985
CA / Mouse BM	Negative	data on bone marrow toxicity is missing	Meier et al., 1985
Sperm Abn./ Mouse	Positive	supplemental information	Meier et al., 1985
DNA adduct/ Rat kidney	Negative	supplemental information	Kasai et al., 1987

In vitro Studies

The majority of the *in vitro* assays have shown positive or ambiguous responses.

Chromosomal aberrations were analysed in Chinese hamster cells treated for 24 or 48 hours with three different doses of calcium hypochlorite, in the absence of metabolic activation. A positive increase in chromosomal aberrations was observed only in a culture treated with 0.5 µg/ml (6.7 µmol/L = approx. 3.5 µmol/L active chlorine) for 48 hours. All the other reported experimental results were negative [Kawachi et al., 1980; Ishidate et al., 1981; Ishidate et al., 1984].

Chinese hamster cells were treated for three hours with 0.5 µg/mL (6.7 µmol/L = approx. 3.5 µmol/L active chlorine) of the agent in the presence of a metabolic activation system with S9 mix from the livers of PCB-treated Wistar rats. A slight increase of chromosomal aberration was observed [Matsuoka et al., 1979].

In human cells, a non-standard embryo fibroblast line (HE2144) was used for the analysis of chromosomal aberrations and SCE. In these cells no increase of chromosomal aberrations was reported at both 0.0744 µg/mL (10^{-6} mol/L) and 0.1488 µg/mL (2 x·10^{-6} mol/L). No other information was provided. In the same cell line the agent was tested for the induction of SCE after 40–48 hours treatment. A doubling and a 50% increase of the background level of SCE was produced at the highest (0.1488 µg/mL) and the lowest tested doses (0.0744 µg/mL), respectively [Sasaki et al., 1980].

The data of these studies suggest that chlorine/hypochlorite solutions may be mutagenic in these tests. However, the relevance of the available data set is limited due to the chemical property of the test substance that rapidly deteriorates the test systems.

In vivo Studies

In a series of assays, sodium hypochlorite has been tested for its ability to induce chromosomal aberrations and micronuclei in bone marrow of CD-1 mice [Meier et al., 1985]. In these assays, chlorine at pH 8.5, where hypochlorite predominates, was administered orally at dose levels equivalent to 1.6, 4 and 8 mg/kg/day for 5 days (1 mL of a solution of 200, 100 or 40 mg/L per

animal). In a mouse micronucleus assay, a small but statistically significant increase in the percentage of micronucleated polychromatic erythrocytes was observed in the combined male and female data, but not separately. The results were in the range of other control groups in the same study. The statistical significance of the increase is considered to be due to the low value recorded in the concurrent vehicle control rather than to any clastogenic effects of sodium hypochlorite. In the same study in CD1 mice, no statistically or biologically significant increase in the frequency of either structural or numerical chromosomal aberrations was observed.

In another well conducted mouse micronucleous assay, no statistically or biologically significant increase in micronucleated polychromatic erythrocytes was observed in the bone marrow following a single intraperitoneal injection at dose levels from 312.5 to 2500 mg/kg of sodium hypochlorite. An additional study involving the use of 4 repeated doses of 300 mg/kg, 24 hours apart, with a single sampling time at 24 hours following the final dose, was also clearly negative [Hayashi et al., 1988].

A negative result in the induction of chromosomal aberrations in rat bone marrow has been reported by Kawachi et al. [Kawachi et al., 1980]. No other information was provided.

At the level of germ cells the induction of sperm head abnormalities has been evaluated in B6C3F1 mice treated for 5 days with 1.6 or 4 or 8 mg/kg [Meier et al., 1985]. Statistically significant increases in the frequency of sperm head abnormalities, at 3 weeks post-treatment, were observed at the two higher doses. No abnormalities were detected for sampling times of 1 and 5 weeks. The effect was reproduced in an independent repeated experiment and, in addition, an increase was observed at 1.6 mg/kg/day. The increases were, however, small (approximately 2 fold), and plateau'd between 4 and 8 mg/kg/day. The range of values observed in the vehicle historical control was wide, and the values observed in the animals treated with hypochlorite were only slightly outside this range.

In another assay, rats given 900 mg/kg orally showed no evidence of oxidative DNA damage, detected as 8-hydroxyguanosine, in the kidney [Kasai et al., 1987].

The overall data suggest that chlorine/hypochlorite solutions are not mutagenic *in vivo*.

Conclusion

There are data from *in vitro* studies to suggest that solutions of chlorine/hypochlorite have some mutagenic potential, but it can be concluded that they are not mutagenic *in vivo*.

3.1.7 Carcinogenicity

In vivo Studies in Animals

The potential carcinogenicity of chlorine has been examined in a comprehensive 2-years inhalation study in Fisher 344 rats and B6C3F1 mice [Wolf et al., 1995], in a long-term studies via chlorinated drinking water (by addition of NaClO) in Fisher 344 rats and/or B6C3F1 mice [Hasegawa et al., 1986; Kurokawa et al., 1986; NTP, 1992], furthermore, within a multigeneration study in BDII (cPah albino) rats administered highly chlorinated drinking water [Druckrey et al., 1968]. One promotor/initiator study was reported [Kurokawa et al., 1984].

Inhalation

In the above mentioned inhalation studies in rats and mice, the incidence of neoplasia was not increased by exposure, indicating that inhaled chlorine in rodents is an upper respiratory tract toxicant [Wolf et al., 1995]. No evidence of carcinogenic activity was seen in a high quality study

where rats and mice (about 70/sex/species/group) were exposed at up to 2.5 ppm chlorine gas for 6 h/day, on 5 days/wk (mice and male rats) or 3 alternate days/week (female rats) for 2 years. A comprehensive range of tissues and organs were examined microscopically.

Oral

In the two-years NTP study in F344 rats and B6C3F1 mice receiving 70, 140 or 275 mg/L in drinking water, there was no evidence of neoplastic effects in the animals, but a marginal, not clearly dose-related increase in the incidence of mononuclear cell leukaemia in female rats (control, 8/50; low-dose, 7/50; mid-dose 19/50; high-dose 16/50). The proportion of female rats that died of leukaemia before the end of the study and the mean time for observation of animals dying with leukaemia were similar among all dose groups and controls. Although the marginal increase in leukaemia incidence in the mid- and high-dose female rats suggested a possible association with the administration of chlorinated water, the incidence of leukaemia was not clearly dose related. There was no indication of reduced latency of leukaemia and the incidence of leukaemia in concurrent controls was less than the mean for historical controls; furthermore there was no supporting evidence of an effect in male rats and both sexes of mice.

In a study of Hasegawa, et al. [Hasegawa et al., 1986], 50 males and females F344 rats were supplied drinking water containing sodium hypochlorite at concentrations of 0, 0.05 or 0.1 % for males and 0, 0.1 or 0.2 % for females. After treatment for 104 weeks, all surviving animals were given untreated tap-water for a further 8 weeks, and then examined. The overall incidence of tumors in each group was 98-100 % in males and 70-80 % in females. There were no significant differences between the control and sodium hypochlorite-treated groups with respect to the total tumor incidences of the animals. It was concluded that the tumours observed in this study were unrelated to treatment of sodium hypochlorite at levels up to 0.1% in males and 0.2 % in females. Sodium hypochlorite had no carcinogenic effect in F344 rats.

In a study of Kurokawa, et al. [Kurokawa et al., 1986], 50 males and females F344 rats and B6C3F1 mice were supplied drinking water containing sodium hypochlorite at concentrations of 0, 300 or 600 ppm for rats and 0, 250 or 500 ppm for mice. After treatment for 85 weeks, no statistically significant differences were observed in the incidences of tumor formation in rats. In mice, the combined incidences of hyperplastic nodules and hepatocellular carcinomas of the liver in low-dose group, and adenomas and adenocarcinomas of the lung in a high-dose group, were marginally increased compared to controls. However, these incidences in treated males were within the range of values of historical control data.

Highly chlorinated water containing free chlorine at a level of 100 mg/L was given daily as drinking water over the whole lifespan (maximum of 2 years) to 236 BDII (cPah albino) rats in seven consecutive generations. There was no difference in survival or in tumor incidence in any generation group when compared to the untreated controls [Druckrey et al., 1968].

In conclusion, there was no evidence of carcinogenicity in mice and in male rats, but equivocal evidence in female rats. The overall genotoxicity data evaluated in this document suggest that aqueous solutions of chlorine/hypochlorite are not mutagenic *in vivo*. This is consistent with the absence of any definite carcinogenic effects in the oral carcinogenicity bioassays in rats or mice.

Promoting effect

Twenty female SENCAR mice with dimethyl-benzanthracene as initiator were used. Sodium hypochlorite was applied to the dorsal skin twice per week exposures to a 1-% solution for 51 weeks. No epidermal hyperplasia was observed, therefore sodium hypochlorite was inactive as a promoter [Kurokawa et al., 1984].

Studies in Humans

From the available literature, there is no evidence of a possible carcinogenic effect in human populations exposed to low levels of chlorine at the workplace for up to 20 years or even longer [WHO, 1982; Mvros et al., 1991].

Several epidemiology studies attempted to evaluate the carcinogenicity of chlorinated drinking water [McGeehin et al., 1993; Cantor et al., 1998; Hildesheim et al., 1997]. In the majority of these studies, weak associations between consumption of chlorinated surface water and increased relative risks for getting cancer of the gastro-intestinal tract (including stomach, colon, rectum, and bladder) have been calculated.

The IARC review in 1991 stressed the shortcomings of these studies and the difficulties in the interpretation of the data for an evaluation of the carcinogenicity of chlorinated drinking water. In the performed studies, there are several methodological inadequacies, many confounding variables, and no causal link between an apparent increased cancer risk with the expectation of some correlation between the higher risk for cancer of urinary bladder and the long-term consumption of chlorinated drinking water in some studies. Therefore, the IARC overall evaluation was that chlorinated drinking water and hypochlorite salts are not classifiable as to their carcinogenicity to humans and that there is inadequate evidence for the carcinogenicity of chlorinated drinking water and hypochlorite salts in humans [IARC, 1991]

Conclusion

There is inadequate evidence for the carcinogenicity of chlorinated drinking water and hypochlorite salts. The IARC has evaluated the carcinogenicity of hypochlorite salts and concluded that there were no data available from studies in human on their carcinogenicity and inadequate evidence for their carcinogenicity in experimental animals. Hypochlorite salts were assigned to group 3: the compounds are not classifiable as to their carcinogenicity to humans (IARC 1991).

The NTP (1992) studies, which appeared after the IARC overall evaluation, in rats and mice provided no evidence of neoplastic effects apart from a marginal, not dose-related effect on mononuclear cell leukaemia in female rats. Overall, it should be regarded to have strengthened the evidence (for positivity) is more inadequate. This conclusion is consistent with WHO's overview EHC 216 (2000) that reads "evidence from (these) animal and human studies suggests that chlorine, hypochlorite solutions, chloramine and chlorine dioxide themselves probably do not contribute to the development of cancer or any toxic effects".

3.1.8 Toxicity for Reproduction

The potential reproductive and developmental effects of chlorine have been examined in three studies, in Long-Evans rats after oral administration of chlorine by gavage [Carlton et al., 1986], in BDII rats given chlorinated drinking water in a "multigeneration study" [Druckrey et al., 1968], and in Sprague-Dawley rats given chlorinated drinking water prior to and throughout gestation [Suh et al., 1983]. An additional *in vivo* germ-cell study in male B6C3F1 mice can be considered to be not relevant [Meier et al., 1985].

Studies in Animals

Effects on Fertility

Potential reproductive effects were assessed in Long-Evans rats [Carlton et al., 1986]. The protocol was in good compliance with current standards. Males (12 per group) were administered at 0, 1, 2, and 5 mg/kg bw/day of aqueous chlorine, (as HClO, pH 8.5) corresponding to about 0.7, 1.4 and 3.5

mg/kg bw/day available chlorine. Doses chosen were the highest practicable considering solution stability and potential gastric irritation. Administration was performed by gavage from 56 day prior to mating and throughout the 10-day mating period. Female rats (24/group) received the same dose of aqueous chlorine by gavage for 14 d prior and throughout the mating, gestation and lactation period (21 days after parturition).

No clinical signs of toxicity, haematological changes or body weight depression were observed in the chlorine-treated male and female parent rats. No alterations in sperm count, sperm motility or sperm morphology were seen, and there were no histopathological lesions in the reproductive tract of parent male and female rats at any dose level.

The length of gestation was not influenced by chlorine exposure. There were no dose-related effects on fertility, fetal viability, litter size, fetal body weight, or day of eye opening. No alterations in estrous cyclicity or day of vaginal opening were observed among F1 females.

The study of Druckrey et al. deserves attention because of the unusual treatment regimen over several generations and the use of a high dose of available chlorine: A group of 60 male and female BDII rats (sex ratio not specified) was given tap water containing 100 mg/L available chlorine prepared with chlorine gas.

The animals were mated and the treatment was continued for the life-span through the six following generations from 1955 to 1964, with the exception of F3 and F4 animals which were treated during the weaning period only. All together, 236 animals in five generations were exposed. Two control groups were used (sex and age not specified), one starting in 1955 (n = 20) and the other in 1962 (n = 36). The highly chlorinated water was well tolerated. There was no evidence of toxic effects on fertility, growth, survival, blood parameter or on histology of the main organs [Druckrey et al., 1968].

Developmental Toxicity

From the results of the study of Druckrey et al. it can be concluded that solutions of chorine do not have effects on fetal development [Druckrey et al., 1968].

In a study of Suh et al. [Suh et al., 1983], female SD rats (6 per group) were administered chlorine in drinking water for 2.5 months prior to conception and throughout gestation. Chlorine was administered as hypochlorous acid (HClO) at concentrations of about 0.7, 6.6 and 66 mg/L (available chlorine). Doses can be estimated to be about 0.05, 0.5 and 5 mg/kg bw/day available chlorine. There were no statistical differences between the control and chlorine-treated groups for fetal viability and weight as well as the number of resorptions. There was no statistical difference between the control and chlorine-treated groups in the incidences of skeletal defects and soft tissue anomalies. Fetal weights were slightly decreased in the high dose group. From the limited data the authors of this study concluded that chlorine is not teratogenic but may be slightly embryotoxic when administered at high doses in drinking water to pregnant rats.

The study of Suh et al. is considered as invalid as no justification for dose selection and no historical control data was provided. Only 6 animals/dose level were regarded and maternal effects are not mentioned. And, while not stated, Suh et al. must have used the fetus and not the litter for their statistical analysis.

Studies in Humans

Developmental Toxicity

Limited epidemiological data, essentially on chlorinated drinking water is available. Two case-control studies did not identify any concern relative to pregnancy outcomes (including miscarriage)

[Aschengrau et al., 1993; Savitz et al., 1995]. A cross-sectional study reported a possible increased risk of shorter body length and shorter cranial circumference in new-borns from mothers drinking chlorinated tap water. There are evident deficiencies in methodology (e.g. lack of water consumption data) and obvious bias in pregnancy outcomes [Kanitz et al., 1996]. A possible causal relationship between risk of spontaneous abortion and chlorinated tap water drinking has been reported in a review of a series of retrospective studies (but inconclusive because of obvious bias) [Swan et al., 1992]. In a recent prospective study [Swan et al., 1998], it was reported that an increased risk of abortion was associated only with high consumption of cold tap water in the same area where the causal relationship has been recorded in previous retrospective study. But such relation was not found in two others areas studied. This causal relationship appears to be inconsistent with the author's causality hypothesis involving chlorinated drinking water by-products and especially trihalomethanes.

Conclusion

WHO (from EHC 216) has concluded as follows. "The existing epidemiological data are insufficient to allow the importance of the observed association of chlorinated drinking-water or THMs (trihalomethanes) and adverse pregnancy outcomes to be assessed. Although several studies have suggested that increased risks of neural tube defect and miscarriage may be associated with THMs or selected THM species, additional studies are needed to determine whether the observed associations are spurious." However, such advanced studies are beyond the current initial assessment.

3.2 Initial Assessment for Human Health

This substance is a white or grayish-white powder. This substance is dissociated into calcium ion (Ca^{++}) and hypochlorite ion (ClO^-) in water. Human health effect may be caused by contact with solid powder, aqueous solution, or accidentally generated chlorine gas. The Calcium ion can cause a strong alkaline condition at the application site. Concerning hypochlorite ion toxicity, the exposure scenarios to calcium hypochlorite are common to sodium hypochlorite (liquid) or chlorine gas which is utilized as a source of hypochlorite ion, and they are thoroughly assessed in competent/pertinent international organization like WHO or the EU. Therefore, substantial parts of the description on hypochlorite-ion-related effects are common to those in the assessment documents for chlorine (CAS No 7782-50-5) which is also assessed in the OECD HPV Chemicals Programme.

Most of the data for toxicity of this substance by the oral route are from studies performed with sodium hypochlorite or chlorine gas. In biological systems, characterized by pH values in the range of 6-8, the most abundant active chemical species is HClO, in equilibrium with ClO^-. Such available chlorine is readily absorbed via the oral route and distributed into plasma, bone marrow, testis, skin, kidney and lung. Only ca. 50% is excreted mainly with the urine followed by excretion with feces. HClO is not enzymatically metabolized.

The acute oral LD50 for Calcium hypochlorite was 790 mg/kg in male rats. Calcium hypochlorite is reported to be corrosive to the skin and has severe effects that can be expected from exposure to the eyes, which is ascribable to the alkalinity of the calcium cation (pH=12.0 at 1 % FAC[2]). Moderate to severe lesions in the respiratory tract were reported after exposure to chlorine that may

[2] Hypochlorite ion is predominant at alkaline pH values, while Cl_2 is mainly present at pH below 4. Therefore the concentration of chlorine in an aqueous solution is generally expressed as free available chlorine (FAC) which is the sum of Cl_2+HClO+ClO^-, regardless whether these species stem from dissolved gaseous chlorine or from dissolved sodium/calcium hypochlorite.

emerge in case of accidental misuse of hypochlorite salts. Exposure to chlorine at 9 ppm (27 mg/m^3) for 6 h/day during 1, 3 and 5 days was reported to cause epithelial necrosis, cellular exfoliation, erosion, ulceration and squamous metaplasia in the nasal passage of rats and mice. For either of Ca or Na salt, skin sensitization studies are not available and no case report is found showing a sensitization potential in humans.

A NOAEL of 950 ppm available chlorine (59.5 mg/kg bw per day) can be derived from a 13-week rat study with sodium hypochlorite in drinking water. A NOAEL of 14 mg Cl_2/kg bw per day for rats and a NOAEL of 22.5 mg Cl_2/kg bw per day for mice can be derived from a two year study with sodium hypochlorite in drinking water.

For genotoxicity the majority of *in vitro* assays for sodium salt have shown positive or ambiguous responses, suggesting that sodium hypochlorite may be mutagenic *in vitro*, however cytogenetic effects were not seen *in vivo*.

No carcinogenicity was observed in mice or rats exposed by inhalation to chlorine and orally to sodium hypochlorite, except some equivocal results were reported for female rats by oral route. For human carcinogenicity, no causal relationship between hypochlorite exposure and tumor incidence was observed. The observation is applicable to the calcium hypochlorite.

No reproductive toxic effects were shown up to 5 mg/kg bw (highest dose tested) of sodium salt (equivalent to 4.8 mg/kg bw of Calcium salt) in a one generation oral study in rats. No evidence of adverse developmental effects were reported in animals. Moreover, epidemiological studies in humans did not show any evidence of toxic effects on reproduction and development.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

The aquatic effect of calcium hypochlorite in the environment, where pH value is supposed to range from 6 to 8, is common to other hypochlorite-generating chemicals like chlorine gas or sodium hypochlorite. The biologically significant chemical species are hypochloric acid or hypochlorite ion. Thus, the data used for assessment are common to those used for the evaluation of other source material. In this chapter, the unit of concentration is expressed in TRC (Total Residual Chlorine[3] = Sum of weight of chlorine (Cl2) equivalent to the sum of the species of active chlorine), unless noted otherwise. Active chlorine is consumed rather rapidly in the aquatic test system for the effects by oxidizing reactions with organic material. The description is made consistent with the description in the SIAR for Chlorine (CAS No 7782-50-5).

Acute Toxicity Test Results

The acute toxicity of chlorine/hypochlorite in fish has been widely studied: Table 6 summarises data on acute toxicity of chlorine to fish. Out of more than 30 studies including some 17 freshwater species, three studies, fully valid with respect to test design and conditions, were chosen for the robust summaries [Thatcher, 1978; Heath, 1978; Wilde et al., 1983ab], all of which were published in peer reviewed papers. The lowest LC50 values were found in the studies of Thatcher [Thatcher, 1978] and of Heath [Heath, 1978]. It should be noted that the data by Heath were obtained from a pulse application of the test compound. The lowest 96h LC50 value was determined by Thatcher [Thatcher, 1978] for juvenile *Oncorhynchus kisutch* (Coho salmon, LC50 = 0.032 mg TRO/L)

[3] In other words, the molecular weights for HClO, ClO and Cl_2 are assumed to be 71.0, the molecular weight of Cl_2.

(TRO = Total Residual Oxidant) and juvenile *Oncorhynchus gorbuscha* (pink salmon, LC50 = 0.023–0.052 mg TRO/L). Both tests were performed in seawater that was chlorinated by a commercial product containing sodium hypochlorite. Ten fish were tested per concentration level. Since this study was conducted to assess the environmental impact of chlorinated effluents from operating power plants, fish were acclimated at one temperature and exposed to test material in water 5°C higher to include thermal stress (acclimatisation temperature 10°C; exposure temperature 14.8°C). Based on the above studies, the 96h LC50 for fish is in the range of 26–60 µg TRC/L. In the study of Heath [Heath, 1978], the lowest 96h LC50 in freshwater was determined for *Ictalurus punctatus* (channel catfish, LC50 = 0.06 mg free chlorine/L) and for *Salmo gairdneri* (rainbow trout, LC50 = 0.06 mg free chlorine/L). Note that the chlorine concentration refers to free chlorine. Tests were performed in tap water dechlorinated by activated charcoal. Ten fish were tested per concentration level.

The LC50 of the standard invertebrate test with *Daphnia magna* (48 hours) was 0.116 mg/L at 20°C (equivalent to OECD 202 test) [Cairns et al., 1978]. A comparison of LC50 values determined at different temperatures showed an increasing toxicity, i.e. decreasing LC50 values with increasing temperature. The lowest LC50 was 0.076 mg/L at 25°C (highest temperature investigated) [Cairns et al., 1978]. Table 7 summarises acute toxicity of chlorine to invertebrates. Thatcher [Thatcher, 1978] has investigated acute toxicity to seven invertebrates of which two shrimps (*Pandalus goniurus* and *Crangon niricauda*) were found to be most sensitive to chlorine. The 96h LC50 of *Pandalus goniurus* was 0.09 mg TRO/L (95% ci: 0.063–0.119 mg TRO/L) which is in the range of the LC50 of the most sensitive fish species. The study of Thatcher included thermal stress of ca. 5°C [Thatcher, 1978]. Lowest effect concentrations were described by Taylor for *Ceriodaphnia dubia* in a 24 hour test flow through test at 25°C (LC0 is equal or greater than 0.0015; LC50 is equal or greater than 0.005; LC100 is equal or greater than 0.008 mg FRC/L) [Taylor, 1993]. The LC50 determined under static conditions was 0.048 mg FRC/L which is in the range of the values reported in in Cairns et al. [Cairns et al., 1978] at the same temperature. The results of Taylor's study give a good impression of the *in situ* toxicity of chlorine. However, due to the high reactivity the total amount of chlorine applied in a flow through test is much higher than reflected by the concentration. Therefore the data were judged as valid with restriction.

Data on the acute toxicity of hypochlorite to aquatic plants is summarised in Table 8. Only results from static systems are available. Acute toxicity to algae has been investigated by Gentile [Gentile et al., 1976] in a 24 hours test with post exposure monitoring. As the active chemical species TRC diminished rather rapidly in the test system, the 24-hour data may be adopted as an alternative to the standard 72-hour data. 10 Species have been exposed to chlorine solutions at two different temperatures. The diatom *Thalassiosira pseudonana* was found the most sensitive algae with a reduction of the growth to 50% of the control at 0.075 mg Cl_2/L [Gentile et al., 1976]. The concentration of chlorine was determined only in the sample with the highest chlorine concentration. The median of the growth rate LC50 values was lower for species cultured at 10°C than for the ones cultured at 20°C indicating increased chronic toxicity of chlorine to algae for increased temperatures.

Table 6 Summary of the Acute Toxicity of Hypochlorite to Fish

Species	Duration	Type of study	Endpoint	Concentration (mg/L)	Reference
Freshwater					
Salmo gaidneri	96 h	Flow through; with analysis	LC50	0.08 (5°C) 0.06 (12°C) 0.09 (17°C) Free Chlorine	Heath, 1978
Ictalurus punctatus	96 h	Flow through; with analysis	LC50	0.08 (5°C); 0.06 (24°C) Free Chlorine	Heath, 1978
Notemigorus crysoleucas	96 h	Flow through; with analysis	LC50	0.27 (5°C); 0.19 (24°C) Free Chlorine	Heath, 1978
Lepomis macrochirus (yearling)	96 h	Flow through; with analysis	LC50	0.45 (6 °C) 0.44 (15°C) 0.39 (25°C) 0.455 (32°C) Free Chlorine	Heath, 1978
Salvelinus fontinalis	96 h	Flow through; with analysis	LC50	0.102 (10°C); 0.15 (15°C)	Thatcher et al., 1976
Lepomis macrochirus (yearling)	96 h	Flow through; with analysis	LC50	0.88 (21.1°C)	Wilde et al., 1983a
Lepomis macrochirus (yearling)	96 h	Flow through; with analysis	LC50	0.44 (27.7°C)	Wilde et al., 1983b
Pimephales promelas (juvenile)	96 h	Flow through; with analysis	LC50	0.18 (21.1°C)	Wilde et al., 1983a
Pimephales promelas (juvenile)	96 h	Flow through; with analysis	LC50	0.08 (21.7°C)	Wilde et al., 1983b
Pimephales promelas (yearling)	96 h	Flow through; with analysis	LC50	0.58 (21.1°C)	Wilde et al., 1983a
Pimephales promelas (yearling)	96 h	Flow through; with analysis	LC50	0.35 (21.7°C)	Wilde et al., 1983b
Saltwater					
Oncorhynchus kisutch (fresh water, marine)	96 h	Flow through; with analysis	LC50	0.032 (14.8°C)	Thatcher, 1978
Oncorhynchus gorbuscha (fresh water, marine)	96 h	Flow through; with analysis	LC50	0.023-0.052 (14.8°C)	Thatcher, 1978
Leiostomus xanthurus	96h	Flow through with analysis	LC50	0.090 (14.2-16.0°C)	Bellanca and Baily, 1977
Clupea harengus (estuary, marine)	96 h	Flow through; with analysis	LC50	0.065 (14.8°C)	Thatcher, 1978
Cymatogaster aggregata	96 h	Flow through;	LC50	0.071	Thatcher, 1978

Species	Duration	Type of study	Endpoint	Concentration (mg/L)	Reference
(estuary)		with analysis		(14.8°C)	
Gasterosteus aculeatus (estuary, marine)	96 h	Flow through; with analysis	LC50	0.167 (14.8°C)	Thatcher, 1978
Oncorhynchus tschawytscha (fresh water, marine)	96 h	Flow through; with analysis	LC50	0.032 (14.8°C)	Thatcher, 1978
Parophrys vetulus (marine)	96 h	Flow through; with analysis	LC50	0.038–0.065 (14.8°C)	Thatcher, 1978
Ammodytes hexapterus	96 h	Flow through; with analysis	LC50	0.073 (14.8°C)	Thatcher, 1978

Table 7 Summary of the Acute Toxicity of Hypochlorite to Invertebrates

Species	Duration	Type of study	Endpoint	Concentration (mg/L)	Reference
Freshwater					
Daphnia magna	48 h	Static; with analysis	LC50	0.116 (5°C) 0.076 (25°C)	Cairns et al., 1978
Ceriodaphnia dubia	24 h	Flow through; with analysis	LC50; LC0	0.005-0.006 0.0015-0.002	Taylor, 1993
Saltwater					
Pandalus goniurus	96 h	Flow through; with analysis	LC50	0.09 (15°C)	Thatcher, 1978

Table 8 Summary of the Acute Toxicity of Hypochlorite to Aquatic Plants

Species	Duration	Type of study	Endpoint	Concentration (mg/L)	Reference
Thalassiosira pseudonana	24 h	Static; with analysis	IC50 of growth rate	0.075 (20°C)	Gentile et al., 1976
Skeletonema costatum	24 h	Static; with analysis	IC 50	0.095 (20°C)	Gentile et al., 1976
Rhodomonas baltica	24 h	Static; with analysis	IC 50	0.110 (20°C)	Gentile et al., 1976
Dunaliella tertiolecta	24 h	Static; with analysis	IC 50	0.110 (20°C)	Gentile et al., 1976
Monochrysis lutheri	24 h	Static; with analysis	IC 50	0.200 (20°C)	Gentile et al., 1976
Chaetoceros decpiens	24 h	Static; with analysis	IC 50	0.140 (10°C)	Gentile et al., 1976
Thalassiosira nordensholdii	24 h	Static; with analysis	IC 50	0.195 (10°C)	Gentile et al., 1976
Thalassiosira rotula	24 h	Static; with analysis	IC 50	0.330 (10°C)	Gentile et al., 1976
Asterionella japonica	24 h	Static; with analysis	IC 50	0.250 (10°C)	Gentile et al., 1976
Chaettoceros didymum	24 h	Static; with analysis	IC 50	0.125 (10°C)	Gentile et al., 1976
Detonula confervacea	24 h	Static; with analysis	IC 50	0.200 (10°C)	Gentile et al., 1976

Chronic Toxicity Test Results

Table 9 summarises prolonged toxicity to fish. In a long-term study (133 days) reported by Hermanutz et al., no relationship between treatment concentration and the growth and survival of bluegill (*Lepomis macrochirus*), white sucker (*Catostomas commersoni*), and rainbow trout (*Salmo gairdneri*) was observed [Hermanutz et al., 1990]. There was, however, a consistent pattern of reduced growth of channel catfish (*Ictalurus punctatus*) with increasing TRC concentrations. The mean final weights of catfish at the highest TRC exposure were 64% (of control). The addition of ammonia (3 mg/L nitrogen) changed the effects of chlorine. Bluegills were still unaffected; growth and survival of channel catfish were reduced at all concentrations of chlorine: no survival was observed at mean TRC levels > 24 µg/L.

In a 147-day study with fathead minnows (*Pimephales promelas*) Arthur and Eaton investigated the effect of chlorinated water on the reproduction of fish [Arthur and Eaton, 1971]. Ten fish were tested per concentration level. During spawning, eggs were attached to the undersides of tunnel-like substrates and were immediately fertilised. Approximately one month after spawning started, the number of sexually mature males, as judged by secondary sexual characteristics and aggressive behaviour, was reduced to a total of two per test chamber to reduce competition for available spawning sites. The test was terminated after reproduction had slowed to less than one spawning a day among all the tanks for a week. As spawning rates at the various concentrations did not change near the end of the test, the spawning results presumable would not have been altered if the test had been allowed to continue.

For long term toxicity, the lowest NOEC is 5 µg/L which was determined in a non-standard study with juvenile *Ictalurus punctatus*. However, a number of studies have been performed under intermittent exposures due to fact that the chlorine/hypochlorite-concentration decreases rapidly over time. Nevertheless, these data are retrieved as useful supportive information because they mimic the short-term exposures expected in some scenarios and give an idea of the potential of sodium hypochlorite to produce acute effects in fish.

Liden et al. [Liden et al., 1980] have investigated chronic toxicity of chlorinated water to *Brevoortia tyrannus* in a 19 day study. Up to the maximal concentration of 0.062 mg/L no differences ($P>0.05$) in survival between chlorine and reference treatments was found.

Chronic toxicity to invertebrates has been investigated by Klerks and Fraleigh in a continuous flow system for 56 days at 11°C and for 28 days at 8°C (Table 10). There was 55% mortality at the nominal hypochlorite application of 0.5 mg Cl_2/l (i.e. 0.08 mg FRC/L) after 56 days. The 30 day LC50 value of 0.285 mg FRC/L was calculated by probit analysis [Klerks and Fraleigh, 1993].

Liden et al. have investigated chronic toxicity in a flow-through study with *Crassostrea virginica* and *Rangia cuneata*. The survival of juvenile oysters and clams was not affected after 15 days exposures at levels up to 62 µg/L. However a sublethal effect was observed (shell deposition) in oysters going from 3.5 mm in the control, which was dechlorinated (30 minutes after chlorination), to 2.3 mm when oysters were exposed to 62 µg/L. No sublethal effect was observed in clams [Liden et al., 1980].

The long-term toxicity of hypochlorite to the freshwater biota has also been studied in microcosm (laboratory) for 28 days and in mesocosm (outdoor) for 24 days [Pratt et al., 1988]. However the specific species present are not documented. Results of the micro- and mesocosm studies are summarised in Table 11.

Table 9 Summary of the Prolonged Toxicity of Hypochlorite to Fish

Species	Duration	Type of study	Endpoint	Concentration (mg/L)	Reference
Freshwater					
Lepomis macrochirus (juvenile)	133 d	Flow through with analysis	NOEC (growth, survival)	0.183	Hermanutz et al., 1990
Salmo gairdneri (juvenile)	133 d	Flow through with analysis	NOEC (growth, survival)	0.207	Hermanutz et al., 1990
Ictalurus punctatus (juvenile)	133 d	Flow through with analysis	NOEC (growth)	0.005	Hermanutz et al., 1990
Pimephales promelas	147 d	Flow through with analysis	NOEC (growth, survival)	0.016	Arthur and Eaton, 1971
Saltwater					
Brevoortia tyrannus (juvenile, estuarine)	19 d	Flow through with analysis	NOEC (survival)	0.062	Liden et al., 1980
Leistomus (juvenile, estuarine)	20 d	Flow through with analysis	NOEC (survival)	0.062	Liden et al., 1980

Table 10 Summary of the Chronic Toxicity of Hypochlorite to Invertebrates

Species	Duration	Type of study	Endpoint	Concentration (mg/L)	Reference
Freshwater					
Dreissena polymorpha (zebra mussel)	28 d, 56 d	Flow through with analysis	LC50 (56d) LC50 (28d)	0.5 (nominal) = 0.08 (measured) 0.285 (free Cl, calc.)	Klerks and Fraleigh, 1993
Saltwater					
Crassostrea virginica (oyster)	15 d	Flow through with analysis	NOEC (survival)	0.062	Liden et al., 1980
Rangia cuneata (calm)	15 d	Flow through with analysis	NOEC (survival) NOEC (shell deposition)	0.062 0.062	Liden et al., 1980

Toxicity to Microorganisms

In the 28-d laboratory microcosm colonised species of microscopic organisms from low trophic levels (bacteria, phytoplankton, zooplankton, protozoa) were exposed to six nominal test concentrations covering the range of 0–308 µg/L. Taxonomic parameters were measured, whilst on day 28 the non-taxonomic responses including total protein, extracellular alkaline phosphatase activity, chlorophyll a, potassium and ATP were also determined. Taxonomic and non-taxonomic data were analyzed to define LOEC and NOEC values. The most sensitive parameters were the number of species, the chlorophyll content and the alkaline phosphatase with a NOEC of 2.1 µg TRC/L.

The Sediment-Water Mesocosm was carried out in lake water with five nominal concentrations over the range of 10-1000 µg/L at 22.5°C. On days 3, 10, 17 and 24 the colonisation with protozoa was examined. Additional parameters determined at the end of the study were total protein, extracellular alkaline phosphatase activity, and chlorophyll a. Taxonomic data and non-taxonomic data were analysed to define LOEC and NOEC values.

The most sensitive parameter was the density of the zooplankton with a NOEC of 1.5 µg TRC/L. The NOEC of the other parameters investigated were 79 µg TRC/L and above [Pratt et al., 1988].

A general conclusion from this study is that zooplankton is more sensitive to chlorine than algae.

Table 11 Summary of the Chronic Toxicity of Hypochlorite In Micro- and Mesocosms

Species	Duration	Type of study	Endpoint	Concentration (mg/L)	Reference
Mixed culture (plankton/protozoa)	7 d	Flow through with analysis	IC20 (NOEC)	0.0027	Cairns et al., 1990
Laboratory Microcosm	28 d	Flow through with analysis	NOEC (Species + chlorophyll)	0.0021	Pratt et al., 1988
Outdoor Mesocosm	24 d	Flow through with analysis	NOEC (zooplankton); NOEC (algal genera)	0.0015 0.0079	Pratt et al., 1988

4.2 Terrestrial Effects

Due to the high reactivity of hypochlorite and its solution, the lifetime in soil is very short.

Bisessar and Mcllveen have investigated the effect of 1.5 to 150 mg/L hypochlorite in the water which was used for watering plants (*Poa pratensis*). Plant heights, fresh and dry weights were generally, and in some cases significantly higher in the treated soil compared to untreated control [Bisessar and Mcllveen, 1992]. Data on wild terrestrial vertebrates are not available, it is possible to use laboratory species as surrogates that appear in mammalian toxicity section.

4.3 Other Environmental Effects

4.4 There are no further data on environmental effects. Initial Assessment for the Environment

This substance is a white or grayish-white powder with chlorine like odor at ambient temperatures and pressures. Density is 2.35 g/cm^3 and vapour pressure is not applicable. This substance is a strong oxidizer and a chlorinating agent. It is highly soluble in water (214 g/L). The anion of this substance dissolved in water is brought to equilibrium between active chlorine species like chlorine (Cl_2), hypochloric acid (HClO) or hypochlorite ClO^-. The relative amounts of the components are dependent on ionic strength and pH. At the pH in the natural environment (6-8), HClO or ClO- is dominating. Diluted aqueous solution of HClO will decompose, very slowly in the dark, but more rapidly in the presence of light, particularly rapidly in full sun light, by producing hydrogen chloride and oxygen. Some chlorine and chloric acid ($HClO_3$) may also develop. The physico-chemical properties indicate that chlorine released into the environment as HClO or Cl_2 is distributed into water and air. Consequently, the effects that may manifest in the natural environment are considered common to those assessed for other sources of hypochlorite.

In natural water, in the presence of organic or inorganic compounds, the free available chlorine immediately reacts forming various chlorinated and/or oxidized by-products e.g. chloramines or chloromethanes. They are mainly distributed to the hydrosphere, but are also able to transfer to some extent to the atmosphere depending on their intrinsic properties. A potential for

bioaccumulation or bioconcentration of active chlorine species can be disregarded, because of their water solubility and their high reactivity.

It is impossible to delineate representative toxicity indicator figure because of unique feature of the chemical to be tested in standard methods. However, accumulated scientific information covering a wide range of species, temperature, application regime or field studies may be useful in for this hazard assessment. Valid freshwater short-term toxicity data are available only for invertebrates: the LC50 for *Ceriodaphnia dubia* is 5 µg FAC/L (FAC=Free available chlorine). Adequate standard acute tests in fish are not available, but from many reliable studies performed under intermittent exposure conditions a 96h LC50 of 60 µg TRC/L and a 168h LC50 of 330 µg TRC/L can be derived (TRC = total residual chlorine = the sum of combined and free residual available chlorine). The revealed toxicity seems dependent on the regime of feeding chemicals rather than duration. Due to the intermittent regime (three 45 minutes pulses per day) a 96h LC50 << 60µg TRC/l can be expected for fish in a standard test.

For freshwater long-term toxicity, no valid NOEC values from standard long-term tests are available, but data can be derived from some microcosm and field studies. The most sensitive parameter was the density of the zooplankton with a NOEC of 1.5 µg TRC/L, and zooplankton is more sensitive to chlorine than algae.

For salt water short-term toxicity valid data are available for molluscs and for fish (*Oncorhynchus kisutch* 96 h LC50 = 32 µg TRO/L) (TRO = Total Residual Oxidant) showing comparable sensitivity. For long term toxicity the molluscs are more sensitive than fish showing a 15d NOEC = 6.2 µg TRO/L.

5 RECOMMENDATIONS

Human Health: The chemical is currently of low priority for further work. The chemical possesses properties (corrosive effects and acute respiratory toxicity) indicating a hazard for human health. Although there are some open uses, consumer exposure is sufficiently regulated under the drinking and other water acts and occupational exposure is adequately controlled in the Sponsor country to ensure safe handling, and therefore this chemical is currently of low priority for further work. Countries may desire to investigate any exposure scenarios that were not presented by Sponsor countries.

Environment: The chemical is a candidate for further work. The substance has hazardous properties for the environments. As there are some open uses of the substance an exposure assessment and if necessary risk assessment should be performed for these uses. The formation of chlorinated by products should be taken into account. Work to that effect is being or has been performed for sodium hypochlorite in many countries and also within the framework of the EU Existing Substances Regulation. The action that may be taken should be common to that for sodium hypochlorite.

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ANNEX

GLOSSARY OF TERMS

FAC	Free Available Chlorine
FO	Free Oxidant
FRC	FAC measured after a reaction
TAC	Total Available Chlorine
TCF	Total Chlorine Free
CPO	Chlorine Produced Oxidant
TRC	Total Residual Chlorine
TRO	Total Residual Oxidant

	weight (g) equivalent to 1.000g TAC	**weight (g) equivalent to 1.000g CPO**	**Remarks**
Cl_2	1.000	1.000	
NaOCl	1.050	1.050	
$Ca(OCl)_2$	1.008	1.008	
HClO	0.740	0.740	
ClO^-	0.726	0.726	
NCl_3		0.566	
$HClO_3$		0.397	
available chlorine	1.000		
FAC	1.000		Free Available Chlorine
F O	1.000		Free Oxidant
FRC	1.000		FAC measured after a reaction
TRC	1.000		TAC measured after a reaction
TCF	1.000		Total Chlorine Free
TRO	1.000		C P O measured after a reaction

I U C L I D

D a t a Set

Existing Chemical	ID: 7778-54-3
CAS No.	7778-54-3
EINECS Name	calcium hypochlorite
EC No.	231-908-7
TSCA Name	Hypochlorous acid, calcium salt
Molecular Formula	Ca.2ClHO

Producer Related Part	
Company:	Masanobu Katoh
Creation date:	26-OCT-2005
Substance Related Part	
Company:	Masanobu Katoh
Creation date:	26-OCT-2005
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1.0.1 Applicant and Company Information

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30-OCT-2005

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30-OCT-2005

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30-OCT-2005

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30-OCT-2005

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30-OCT-2005

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30-OCT-2005

Name: NEUBER GES.M.B.H.
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Phone: 0222/599950
Telefax: 0222/5970200

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Name: OLIN CORPORATION
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Name: PQS BRENNTAG
Street: Crta. Madrid/C'adiz Km.554,4
Town: 41700 Dos Hermanas (Sevilla)
Country: Spain
Phone: 954919400
Telefax: 954919443

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Name: Solvay S.A.
Street: Rue du Prince Albert 33

Town: 1050 Bruxelles
Country: Belgium

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1.0.2 Location of Production Site, Importer or Formulator

1.0.3 Identity of Recipients

Name of recip.: Mr. Motohiko Kato, Ministry of Foreign Affairs, Economic Affairs Bureau, Second International Organizations Div.
Street: 2-2-1 Kasumigaseki, Chiyoda-ku
Town: 100-8919 Tokyo
Country: Japan
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1.0.4 Details on Category/Template

1.1.0 Substance Identification

1.1.1 General Substance Information

Substance type: inorganic
Physical status: solid
Purity: ca. 60 - 70 % w/w

Remark: Nominal purity in commercial products is 60% or 70% usually. But, generally, its actual purity is higher than nominal value by several %.
30-OCT-2005 (171)

Remark: CAS NUMBER: 7778-54-3
NAME (OECD NAME): calcium hypochlorite
NAME (IUPAC): calcium hypochlorite
MOLECULAR FORMULA and WEIGHT: Ca(OCl)2, 142.98
STRUCTURAL FORMULA: Cl-O-Ca-O-Cl
APPEARANCE: white or grayish-white powder with chlorine-like odor
30-OCT-2005 (32) (51)

1.1.2 Spectra

1.2 Synonyms and Tradenames

ACE-CHLON

13-JAN-2004 (171)

BK powder

16-SEP-2003 (52)

Bleaching powder

16-SEP-2003 (155)

Calcium hypochloride

16-SEP-2003 (52)

Calcium hypochlorite, dry

16-SEP-2003 (52)

Calcium oxychloride

30-OCT-2005 (52)

Chloride of lime

16-SEP-2003 (52)

Chlorinated lime

16-SEP-2003 (52)

Chlorkalk

13-JAN-2004 (164)

HI-CHLON

13-JAN-2004 (171)

HTH

16-SEP-2003 (52)

Hy-Chlor

30-OCT-2005 (52)

Hypochlorous acid, calcium salts

16-SEP-2003 (52)

J-CHLON

13-JAN-2004 (171)

Lime chloride

16-SEP-2003 (52)

Lo-Bax

16-SEP-2003 (52)

Losantin

16-SEP-2003 (52)

Mildew remover X-14

17-SEP-2003 (52)

NEW STAR-CHLON

13-JAN-2004 (171)

NICLON

30-OCT-2005 (171)

Oxicloruro de calcio

30-OCT-2005 (183)

Perchloron

16-SEP-2003 (52)
Pittchlor

16-SEP-2003 (52)

STAR-CHLON

30-OCT-2005 (171)

TOYOCHLON

30-OCT-2005 (171)

1.3 Impurities

EINECS-Name: OTHER: SEE REMARK

Remark: Impurity content is changed widely by the manufacturers, grade and production method.
Typical value of hydrated product in sodium method(See below) is as follows.
NaCl 7 - 20%
CaClO3 0 - 5%
CaCl2 0 - 5%
Ca(OH)2 0 - 5%
Water(hydrated) 6 - 15%(hydrated salt)

30-OCT-2005 (171)

1.4 Additives

1.5 Total Quantity

Quantity: 100000 - 500000

Remark: The total name plate capacity worldwide including PRC is approximately 230,000 t/year in 2002.

30-OCT-2005 (171)

Quantity: tonnes produced in 2001

Remark: 16,940 tonnes produced in Japan (2001)
30-OCT-2005 (227)

1.6.1 Labelling

Labelling: as in Directive 67/548/EEC
Symbols: (O) oxidizing
(C) corrosive
(N) dangerous for the environment
(E) For substances ascribed Nota E the risk phrases R20, R22 to R28 and all combinations of these risk phrases shall be preceded by the word 'also'. E.g. R23 'also' toxic by inhalation
Specific limits: yes
R-Phrases: (8) Contact with combustible material may cause fire
(22) Harmful if swallowed
(31) Contact with acids liberates toxic gas
(34) Causes burns
(50) Very toxic to aquatic organisms
S-Phrases: (1/2) Keep locked up and out of reach of children
(26) In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
(36/37/39) Wear suitable protective clothing, gloves and eye/face protection

(45) In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)
(61) Avoid release to the environment. Refer to special instructions/Safety data sets

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1.6.2 Classification

Classified: as in Directive 67/548/EEC
Class of danger: corrosive
R-Phrases: (22) Harmful if swallowed

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Classified: as in Directive 67/548/EEC
Class of danger: corrosive
R-Phrases: (34) Causes burns

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Classified: as in Directive 67/548/EEC
Class of danger: dangerous for the environment
R-Phrases: (50) Very toxic to aquatic organisms

30-OCT-2005

Classified: as in Directive 67/548/EEC
Class of danger: oxidizing
R-Phrases: (8) Contact with combustible material may cause fire

30-OCT-2005

Classified: as in Directive 67/548/EEC
R-Phrases: (31) Contact with acids liberates toxic gas

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1.6.3 Packaging

1.7 Use Pattern

Type: type
Category: Non dispersive use

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Type: type
Category: Use in closed system

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Type: type
Category: Wide dispersive use

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Type: industrial
Category: Basic industry: basic chemicals

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Type: industrial
Category: Paper, pulp and board industry

30-OCT-2005

Type: industrial
Category: Personal and domestic use

30-OCT-2005

Type: industrial
Category: Public domain

30-OCT-2005

Type: industrial
Category: Textile processing industry

30-OCT-2005

Type: industrial
Category: other: industrial, municipal and swimming-pool water treatments (sterilization and/or deodorization)

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Type: industrial

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Type: industrial
Category: other

30-OCT-2005

Type: use
Category: Bleaching agents

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Type: use
Category: Cleaning/washing agents and disinfectants

30-OCT-2005

Type: use
Category: Heat transferring agents

30-OCT-2005

Type: use
Category: Non agricultural pesticides

30-OCT-2005

Type: use
Category: other: water sterilization and deodorization

30-OCT-2005

Type: use

30-OCT-2005

1.7.1 Detailed Use Pattern

1.7.2 Methods of Manufacture

1.8 Regulatory Measures

1.8.1 Occupational Exposure Limit Values

Type of limit: TLV (US)

Remark: The TLV for calcium hypochlorite is not established. Based on guidelines for similar compounds and the extreme irritation seen in animal studies, the OLIN internal standard is established at 1 mg/m3 STEL.
30-OCT-2005

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1.8.2 Acceptable Residues Levels

1.8.3 Water Pollution

1.8.4 Major Accident Hazards

1.8.5 Air Pollution

1.8.6 Listings e.g. Chemical Inventories

1.9.1 Degradation/Transformation Products

1.9.2 Components

1.10 Source of Exposure

Method:	Chlorine levels in air were measured near swimming pools used by swimmers of the national team of Spain and the autonomous team of Catalonia. The chlorine level in air was measured near the water (<10 cm) on four sides of the pool at the same times during the day for a total of 5 days. The days were not consecutive.
Remark:	Human: exposure of the consumer/bystander
Result:	The concentration of chlorine in the air near the water surface was related to the number of swimmers present in the pool. The average concentration was 0.15 mg/m3 when < 5 swimmers were present and 0.42 mg/m3 when > 6 swimmers were present. In general, the concentration of chlorine also gradually increased from the first to the last measurements of the day.
Source:	MITSUBISHI CHEMICAL SAFETY INSTITUTE LTD. Tokyo

17-SEP-2003 (68)

1.11 Additional Remarks

Memo:	Fire Hazards of Calcium Hypochlorite
Remark:	"Calcium hypochlorite, 70% available chlorine " is a commercial chemical which is transported in large quantities generally packed in steel drums. It is classified by the I.M.C.O. code as an Oxidizing Agent. During the past five years there have been about a dozen serious accidents in ships involving this material. The losses caused have been very large, amounting to many millions of pounds sterling.A number of the P. and I. Clubs in London set up a sub-committee to investigate the hazards associated with the substances. The investigations include a research program carried out at the Royal Armament Research and development Establishment, Woolwich, England.It has been found that ignition may occur in some circumstances spontaneously, by mechanical stressing or by admixture with some combustible substances. The commercial material is not a definite

chemical compound but a mixture, the nature and properties of which may vary according to the nature of the raw materials and method of manufacture.Accidental ignition may be followed by explosive effects and the five evolves large quantities of oxygen, which procedure an almost uncontrollable burning of any combustible material nearby. It is apparent that the hazards are far more serious than ordinarily associated with other oxidizing agents.

13-JAN-2004 (55)

Memo: Process of manufacture

Remark: This substance is a basic chemical, and used as algicide, bacteriocide, deodorant, disinfectant, fungicide, oxidizing agent, bleaching agent and so on.
This product is manufactured by two kinds of production method. The form in the market is granule or tablet usually.

-Process of manufacture ,P (Calcium method): Slaked lime is chlorinated by chlorine directly. Usually calcium chloride of by-product is removed to increase the purity of calcium hypochlorite, but not perfectly removed.

-Process of manufacture 2 (Sodium method):As calcium chloride gives adverse effect to this product's stability. in this method it is substituded to sodium chloride by the reaction sodium hypochlorite and calcium chloride. Usually sodium chloride also is removed.

This is the major method in manufacture of this product at present.
The most products in this method is sold as hydrated salt to increase the safety.

13-JAN-2004 (112)

Memo: Speculation

Remark: Gaseous chlorine dissolved in water is in a fast equilibrium with hypochlorous acid which again is in a fast equilibrium with hypochlorite ions. (See chapter 2.6.1 and 3.1.2) If ammonia is present chloramines are formed rapidly. Chloramines still have oxidizing properties.

13-JAN-2004

Memo: Terms and definitions

Remark: The concentration of chlorine in an aqueous solution is generally expressed by the following terms, which are also used throughout the dossier.

Free (available) Residual Chlorine, FRC.
That portion of the total available residual chlorine composed of dissolved chlorine gas (Cl2), hypochlorous acid (HOCl), and/or hypochlorite ion (OCl-) in water. FRC does not include chlorine that has combined with ammonia, nitrogen, or other compounds.

Combined (available) Residual Chlorine.
The concentration of residual chlorine which is combined with ammonia (NH3) and/or organic nitrogen in water as a chloramine (or other chloro derivative) yet is still

available to oxidize organic matter and utilize its bactericidal properties.

Total Residual Chlorine (TRC).
The amount of available chlorine (remaining after a given contact time). TRC is the sum of the combined available residual chlorine and the free available residual chlorine.

Total Residual Oxidant (TRO)
The total amount of dissolved compounds with oxidizing properties.
In synthetic media, TRO and TRC, are almost identical.

17-SEP-2003

Remark: Storage Conditions: Keep tightly sealed. Store in a cool, dry, well ventilated area. Do not store at temperatures above
52 deg. C. Do not store or transport with acids, other oxidizers, organic materials, or corrosive liquids.
Transportation Information: This material is regulated as a DOT HAZARDOUS MATERIAL. It is subject to DOT Haz. Mat. Regulations via the following modes: rail, motor, water, air; in bulk, and non-bulk quantities. Applicable packaging sections in 49 CFR are 173.153 and 173.217.
Waste Disposal: If this product becomes a waste, it meets the criteria of a hazardous waste as defined under 40 CFR 261 and would have the following EPA hazardous waste number: D001.

13-JAN-2004 (73)

Remark: Pure product has not been prepared.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

30-OCT-2005 (32)

1.12 Last Literature Search

Type of Search: Internal and External

Remark: ACGIH
AQUIRE (CIS, STN)
BEILSTEIN (STN)
BIOSIS (STN, Dialog)
CHEMCATS (STN)
CHRIS (CIS, CHEM-BANK)
CSCHEM (STN)
ChemFinder
ECDIN
GMELIN (STN)
HODOC(STN)
HSDB (CIS, STN, DataStar, CHEM-BANK)
IARC
IRIS (CIS, CHEM-BANK)
IUCLIDMSDS-CCOHS (STN, Dialog)
MEDLINE (STN, Dialog, Datastar)
MSDS-OHS (STN)
NCI
NIOSHOHMTADS (CIS, CHEM-BANK)
NIOSHTIC(STN, Dialog)

PROMT(STN, Dialog)
REGISTRY (STN, Dialog)
RTECS(STN, CIS, Dialog, CHEM-BANK)
SPECINFO (STN)
SRC PhysPro Database(SRC: Syracuse Research Corporation)
TOXCENTER (STN)
TOXFILE (Dialog, Datastar)
TSCATS (CIS)
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1.13 Reviews

2.1 Melting Point

Decomposition: yes at 175 degree C

Remark: Decomposes rapidly and exothermically giving off oxygen and chlorine monoxide gases when heated above 175 degree C. Reacts vigorously or explosively with oxidizable materials.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
13-JAN-2004 (126)

Decomposition: yes at 177 degree C

Remark: Decomposes with release of heat and oxygen
Reliability: (4) not assignable
13-JAN-2004 (51)

Value: 100 degree C

Reliability: (4) not assignable
13-JAN-2004 (52)

Value: 100 degree C

Reliability: (4) not assignable
13-JAN-2004 (6)

Decomposition: yes at 100 degree C

Reliability: (4) not assignable
13-JAN-2004 (241)

Decomposition: yes at ca. 130 degree C

Remark: Melting point: Not applicable
Reliability: (4) not assignable
13-JAN-2004 (163)

2.2 Boiling Point

Value:

Remark: Boiling point: Not applicable
17-SEP-2003 (163)

2.3 Density

Type: density
Value: 2.35 g/cm³

Reliability: (2) valid with restrictions

Flag: Critical study for SIDS endpoint
13-JAN-2004 (51) (52) (241)

Remark: The bulk density for loose granules is 0.8 g/cc and the bulk density for tablets is 1.9g/cc.
Reliability: (4) not assignable
13-JAN-2004

2.3.1 Granulometry

2.4 Vapour Pressure

Remark: Not applicable
13-JAN-2004

2.5 Partition Coefficient

Remark: Not applicable
21-JAN-2004

log Pow: -2.46

Method: other (calculated)

Remark: calculated using: KOWWIN version 1.66 - 2000 U.S. Environmental Protection Agency
Reliability: (4) not assignable
21-JAN-2004

2.6.1 Solubility in different media

Value: ca. 18 vol% at 25 degree C
pH value: ca. 10.5 - 11.5
Conc.: 1 vol% at 25 degree C

Reliability: (4) not assignable
13-JAN-2004 (177)

Remark: Decomposes
17-SEP-2003 (52)

Remark: Souble with release chlorine gas
17-SEP-2003 (51)

Value: ca. 200 g/l at 20 degree C

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

13-JAN-2004 (163)

Solubility in: Water
Value: 21.4 vol% at 25 degree C

Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
26-OCT-2005 (125)

2.6.2 Surface Tension

2.7 Flash Point

Remark: This material is non-flammable.
13-JAN-2004 (177)

2.8 Auto Flammability

Value:

Remark: This material is non-flammable but it will decompose exothermally above 177 degrees celcius.
13-JAN-2004 (177)

2.9 Flammability

Remark: This materai is non-flammable.
13-JAN-2004 (176)

2.10 Explosive Properties

Remark: This materail is not expolosive.
13-JAN-2004 (177)

2.11 Oxidizing Properties

Remark: OLIN calcium hypochlorite products meet the specification of ASTM method E-487-74 as set forth in 49CFR SEC. 173.21, Title 49-Code of Federal Regulations, US Department of Transportation.
13-JAN-2004 (177)

2.12 Dissociation Constant

2.13 Viscosity

2.14 Additional Remarks

Memo: Henry's law constant:

Remark: As HClO at pH=5.5 ;20 □‹ CH=0.4x 10-4 (mg/l in air divided by mg/l in water)
13-JAN-2004 (77)

Memo: handling

Remark: Calcium hypochlorite marketed was pelletized as containing 13.1 % of water.
13-JAN-2004 (171)

3.1.1 Photodegradation

Remark: The calcium hypochlorite solution is very sensitive to light. Direct sunlight may cause rearrangement and decomposition resulting in the formation of chloride and oxygen.
30-OCT-2005 (127)

Remark: Not applicable
30-OCT-2005

3.1.2 Stability in Water

Test substance: other TS

Remark: The pH of calcium hypochlorite solution

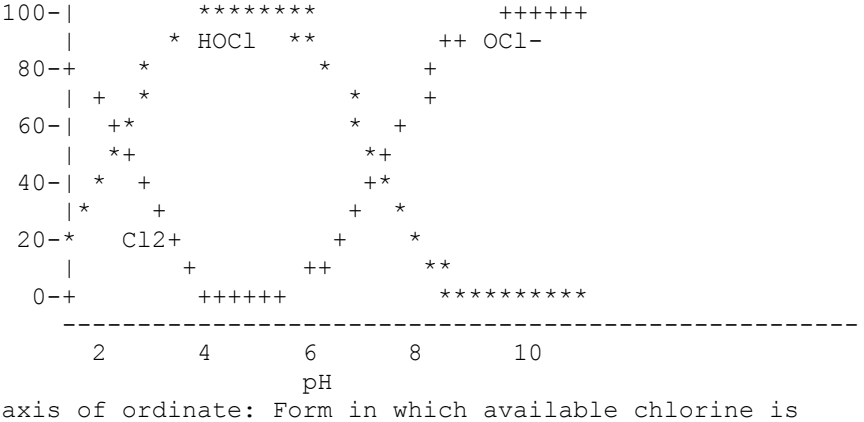
10 g/L; pH = 12.0
5.0 g/L; pH = 11.7
1.0 g/L; pH = 10.6
0.5 g/L; pH = 9.8
0.1 g/L; pH = 8.9
0.05 g/L; pH = 8.6
0.01 g/L; pH = 7.9
0.005 g/L; pH = 7.5
0.001 g/L; pH = 7.3

Test substance: Lot No.: NBB-25 (Nippon Soda Co., Ltd.)

Analytical data
-available chlorine; 73.26 %
-NaCl; 7.42 %
-CaCl2; 1.38 %
-Ca(Cl3)2; 0.68 %
-as Ca(OH)2; 4.16 %
-water; 13.1 %

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
30-OCT-2005 (171)
Type: abiotic

Remark: Pattern diagrams



axis of ordinate: Form in which available chlorine is

present (%)
axis of abscissas : pH
Species in aqueous solution as a function of pH
There are three species of chlorine in water: gaseous chlorine, HOCl gas and ClO-. For example, at pH 7.5 half of the chlorine is active as HOCl and half is available as ClO-. The pH of commercial solutions is above 11 and the only species is ClO-.
The reaction of chlorine with water and the speciation of the "degradation products" were investigated and published in several papers.

The data provided refers to literature. The experiments were not explicitly performed according to a guideline procedure and no information on GLP can be provided.

There is no need to perform an additional guideline study as the solution of chlorine in water is consistently characterized by various sources (3 cited).

Result: Gaseous chlorine which is dissolved in water is in a fast equilibrium with hypochlorous acid which again is in a fast equilibrium with hypochlorite ions. (See attached document.) The total amount of chlorine dissolved in water depends on ionic strength and pH. Below 10 degree C chlorine forms hydrates, which can be separated as greenish-yellow crystals.

In pure water the equilibrium products are stable. In the presence of organic or inorganic contaminants the free available chlorine reacts with the contaminants forming various chlorinated by-products which may be toxic. UV components of sunlight induces the formation of atomic chlorine which forms hydrochloric acid in water.

Flag: Critical study for SIDS endpoint

30-OCT-2005 (33) (91) (199)

3.1.3 Stability in Soil

Type: other: none available

30-OCT-2005

Remark: The high water solubility indicates high soil mobility, although chlorine as vapour or aqueous solution is normally irreversibly combined with soil organic compounds within the first few millimetres or centimetres of the soil surface.

30-OCT-2005 (160)

3.2.1 Monitoring Data (Environment)

Type of measurement: other: no data available

30-OCT-2005

3.2.2 Field Studies

3.3.1 Transport between Environmental Compartments

Type: other: no data available

30-OCT-2005

3.3.2 Distribution

Media: water - air

30-OCT-2005

Remark: Not applicable
30-OCT-2005

3.4 Mode of Degradation in Actual Use

3.5 Biodegradation

Remark: Not applicable
30-OCT-2005

3.6 BOD5, COD or BOD5/COD Ratio

Method: other: no data available
Year:
Method:
30-OCT-2005

3.7 Bioaccumulation

Remark: A potential of this substance can be disregarded, because of their water solubility and their high reactivity. Nevertheless, hypochlorite may be found in living organism. Hypochlorite is also produced naturally in vivo for cell defense process. The natural production of halo-oxo acids is widespread and related to haloperroxidases, which is well documented in the literature. A good overview of biohalogenation is given by Geigert et al. [Geigert et al., 1986] and more recently by Winterton [Winterton, 1997]. Hypochlorite is produced by chloroperoxidases, which are, among others, produced by mammals (in white blood cells), lichens and in many fungal species [Vollenbroek et al., 1995].
Reliability: (2) valid with restrictions
30-OCT-2005 (88) (237) (247)

Species: other: no data available yet

30-OCT-2005

3.8 Additional Remarks

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Remark: Calcium hypochlorite which is dissolved in water is in a fast equilibrium with hypochlorous acid which again is in a fast equilibrium with hypochlorite ions. In general the concentration of chlorine in a test system is achieved by dissolving hypochlorous acid or sodium hypochlorite and not by application of gaseous chlorine.

Because of this equilibrium concentrations, in general, are provided as free available chlorine or as total residual chlorine (TRC). (See chapter 1.11 for definition.)
The studies were performed by independent laboratories and published in peer reviewed papers. Many studies were performed in the 70ties, when effects of the biocidal application of chlorine were carefully reinvestigated. Therefore the studies are not performed according to recent guidelines and no GLP information is provided.

With regard to the extensive number of tests that were already performed with chlorine no further studies according to recent guidelines were conducted to avoid further animal testing.
Flag: Critical study for SIDS endpoint
30-OCT-2005

Type: flow through
Species: Ictalurus punctatus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no data
LC50: = -
LC50 (5 degree C) :
= .06 -
LC50 (24 degree C) :
= .08 -

Method: other
Year: 1978
GLP: no data
Test substance: other TS: calcium hypochlorite

Method: Fingerling size fish (10 per concentration group) from a private hatchery, Windsor, Virginia were used. Water for the holding and bioassay tanks was dechlorinated by passing through a column of activated carbon. Some water quality characteristics were: average hardness 45 mg/L, conductivity 150 mMHOS, dissolved oxygen near saturation, copper 0.05 mg/L or less and zinc less than 0.02 mg/L. Test fish were acclimated to the experimental temperatures at least 2 weeks before being bioassayed. Four concentrations in duplicate aquaria were tested. In order to supply intermittent chlorination to the test aquaria, metering pumps were used to draw from a concentrated calcium hypochlorite solution which was injected into the water inflow of each aquarium. The metering pumps were controlled by a timer that turned them on for 45 minutes three times each 24 hours. Dead fish were removed and weighed approximately hourly for the first 12 hours and subsequently, every 4 hours except between the hours of 23:00 and 08:00. Chlorine measurements were made

twice daily and both free and combined chlorine were determined each time. All concentrations were measured at the peak of the chlorine pulse.

Result: Concentration refer to free chlorine, not to total residual chlorine

Same conditions:

Duration	5 degree C Result (mg/l)	24 degree C Result (mg/l)
48 h	0.20	ND
72 h	0.120	0.093
96 h	0.082	0.064
120 h	0.050	0.051

Test condition: Life stage: juvenile
Dechlorinated tap water Temperature: 5/24 degree C, pH=7.35
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
30-OCT-2005 (102)

Type: flow through
Species: Lepomis microlophus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
See Result : -

Method: other
Year: 1978
GLP: no data
Test substance: other TS: sodium hypochlorite

Result: Concentrations refer to free chlorine, not to total residual chlorine.

LC50:(96 hours)
LC50 (6 degree C); 0.45 mg/L
LC50 (15 degree C); 0.44 mg/L
LC50 (25 degree C); 0.39 mg/L
LC50 (32 degree C); 0.455 mg/L

Duration	Result (mg/l)
48 h	: 0.54 (24 degree C), 0.47 (32 degree C)
72 h	:0.53 (24 degree C), 0.41 (25 degree C), 0.47(32 degree C)
168 h	: 0.33 (6 degree C), 0.41 (25 degree C), 0.37 (32 degree)

Test condition: Life stage: juvenile
Dechlorinated tap water
Temperature: 6, 15, 25, and 32 degree C, pH=7.35
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
30-OCT-2005 (101)

Type: flow through
Species: Salmo gairdneri (Fish, estuary, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes

LC50 (5 degree C) :
= .08 -
LC50 (12 degree C) :
= .06 -
LC50 (17 degree C) :
= .09 -

Method: other
Year: 1978
GLP: no data
Test substance: other TS: calcium hypochlorite

Method: Fingerling size fish from a private hatchery, Windsor, Virginia were used. Water for the holding and bioassay tanks was dechlorinated by passing through a column of activated charcoal. Some water quality characteristics were: average hardness 45 mg/L, conductivity 150 mMHOS, dissolved oxygen near saturation, copper 0.05 mg/L or less and zinc less than 0.02 mg/L. Test fish were acclimated to the experimental temperatures at least 2 weeks before being bioassayed. Four concentrations in duplicate aquaria were tested. In order to supply intermittent chlorination to the test aquaria, metering pumps were used to draw from a concentrated calcium hypochlorite solution which was injected into the water inflow of each aquarium. The metering pumps were controlled by a timer that turned them on for 45 minutes three times each 24 hours. Dead fish were removed and weighed approximately hourly for the first 12 hours and subsequently, every 4 hours except between the hours of 23:00 and 08:00. Chlorine measurements were made twice daily and both free and combined chlorine were determined each time. All concentrations were measured at the peak of the chlorine pulse.
Remark: Analytical monitoring: total residual chlorine (TRC)
Result: Concentrations refer to free chlorine not to total residual chlorine (TRC)
Same conditions:at 5, 12, 17 degree C
- 24 h; LC 50; 0.294, 0.258, 0.263 mg/L
- 48 h; LC 50; 0.162, 0.090, 0.124 mg/L
- 72 h; LC 50; 0.103, 0.069, 0.074 mg/L
- 96 h; LC 50; 0.082, 0.062, 0.095 mg/L
- 120 h; LC 50; 0.074, 0.052, 0.089 mg/L
Test condition: Life stage: juvenile
Dechlorinated tap water
Temperature: 5, 12, and 17 degree C, pH=7.35,
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
30-OCT-2005 (101)

Type: flow through
Species: other: Notemigonus crysoleucas
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50: = -
LC50 (5 degree C) :
= .27 -
LC50 (24 degree C) :
= .19 -

Method: other
Year: 1978

GLP: no data
Test substance: other TS: calcium hypochlorite

Method: Fingerling size fish from a private hatchery, Windsor, Virginia were used. Water for the holding and bioassay tanks was dechlorinated by passage through a column of activated charcoal. Some water quality characteristics were: average hardness 45 mg/L, conductivity 150 mMHOS, dissolved oxygen near saturation, copper 0.05 mg/L or less and zinc less than 0.02 mg/L. Test fish were acclimated to the experimental temperatures at least 2 weeks before being bioassayed. Four concentrations in duplicate aquaria were tested. In order to supply intermittent chlorination to the test aquaria, metering pumps were used to draw from a concentrated calcium hypochlorite solution which was injected into the water inflow of each aquarium. The metering pumps were controlled by a timer that turned them on for 45 minutes three times each 24 hours. Dead fish were removed and weighed approximately hourly for the first 12 hours and subsequently, every 4 hours except between the hours of 2300 and 0800. Chlorine measurements were made twice daily and both free and combined chlorine were determined each time. All concentrations were measured at the peak of the chlorine pulse.
Result: Concentrations refer to free chlorine, not to total residual chlorine.
Same conditions:

Duration	Result (mg/l) 5 degree C	24 degree C
30 h	0.84	0.26
48 h	0.55	0.22
72 h	0.39	0.21
96 h	0.27	0.19
120 h	0.18	0.18
144 h	0.18	0.18

Test condition: Life stage: juvenile
Dechlorinated tap water
Temperature: 5 and 24 degree C, pH=7.35
Reliability: (2) valid with restrictions

Flag: Critical study for SIDS endpoint
30-OCT-2005 (101)

Type: flow through
Species: Lepomis microlophus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50: = .88 -

Method: other: American Public Health Association (APHA) (1980). Standard methods for the examination of water and wastewater. 15th Ed. Washington, D.C.
Year: 1982
GLP: no data
Test substance: other TS: 5% solution of sodium hypochlorite reagent grade.

Method: Groups of 10 bluegills were placed in 15 L test chambers. Studies were conducted in duplicate. Holding chambers had

screens at each end to allow circulation through the chamber and prevent predation.

A Hydrolab 8000 system was used to make daily measurements of dissolved oxygen, temperature, conductivity and pH in the test chambers receiving 100, 35%, 2% and 0% biocide solutions. Alkalinity was measured by APHA standard methods (APHA 1980).

Two 80 L stock solutions were prepared daily. Stock biocide solutions were added to their respective dilutor systems for 1 hour/day, 0, 24, 48 and 72 hour after testing began. Water samples (ca. 20 ml) were collected from each of the test chambers and tanks containing stock solutions at 10minute intervals during the periods (ca. 2 hr/day) those biocide residuals were measurable in the test chambers. Levels of total and free residual chlorine were determined by DPD spectrophotometric method (APHA 1980).

Biocide dosages were calculated as follows:
96-hr peak = single highest biocide residual detected during the four days of testing.
96-hr mean maximum = average maximum biocide residual detected during the four days of testing.
96-hr intermittent exposure mean = mean biocide residual level during the four ~2-hr exposure periods.
96-hr accumulative exposure = total 96-hr biocide exposure in mg/L residual x minutes of exposure (area under a time-concentration curve).

Result: For the 96-hr peak, mean maximum and intermittent exposure mean, highest results were obtained with the 96-hr peak and the lowest results obtained with 96-hr intermittent exposure mean.
Chlorine is expressed as total residual chlorine (TRC).
- 96-hr peak (mg/L): 2.48 (2.20-2.64)
- 96-hr intermittent exposure mean (mg/L): 0.88 (0.82-0.98)
- For the 96-hr accumulative (mg/L x min): 421 (387-465)

Test condition: Life stage: young of the year
Water quality measurements were as follows:

Parameter	Mean + SE	Range
Temp (degree C)	21.1 + 0.1	19.9 - 22.9
pH	7.0 + 0.1	6.7 - 7.1
DO (mg/L)	7.8 + 0.1	6.5 - 9.1
Cond (mmhos/cm)	66.6 + 0.1	63 - 71
Alkalinity (mg/L)	15.3 + 0.1	14 - 16

Test substance: 5% solution of sodium hypochlorite reagent grade.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
30-OCT-2005 (246)

Type: flow through
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50: = .58 -

Method: other: American Public Health Association (APHA) (1980).

Standard methods for the examination of water and wastewater. 15th Ed. Washington, D.C.

Test substance: other TS

Method: Groups of 10 juvenile and yearling minnows were placed in 15 L test chambers. Studies were conducted in duplicate. Juvenile fathead minnows were placed in small glass holding chambers which were suspended in the test chambers. Holding chambers had screens at each end to allow circulation through the chamber and prevent predation.

A Hydrolab 8000 system was used to make daily measurements of dissolved oxygen, temperature, conductivity and pH in the test chambers receiving 100, 35%, 2% and 0% biocide solutions. Alkalinity was measured by APHA standard methods (APHA 1980).

Two 80 L stock solutions were prepared daily. Stock biocide solutions were added to their respective dilutor systems for 1 hour/day, 0, 24, 48 and 72 hour after testing began. Water samples (ca. 20 ml) were collected from each of the test chambers and tanks containing stock solutions at 10minute intervals during the periods (ca. 2 hr/day) those biocide residuals were measurable in the test chambers. Levels of total and free residual chlorine were determined by DPD spectrophotometric method (APHA 1980).

Biocide dosages were calculated as follows:
96-hr peak = single highest biocide residual detected during the four days of testing.
96-hr mean maximum = average maximum biocide residual detected during the four days of testing.
96-hr intermittent exposure mean = mean biocide residual level during the four ~2-hr exposure periods.
96-hr accumulative exposure = total 96-hr biocide exposure in mg/L residual x minutes of exposure (area under a time-concentration curve).
Groups of 10 juvenile and yearling minnows were placed in 15 L test chambers. Studies were conducted in duplicate. Juvenile fathead minnows were placed in small glass holding chambers which were suspended in the test chambers. Holding chambers had screens at each end to allow circulation through the chamber and prevent predation.

Result: For the 96-hr peak, mean maximum and intermittent exposure mean, highest results were obtained with the 96-hr peak and the lowest results obtained with 96-hr intermittent exposure mean.

Chlorine is expressed as total residual chlorine (TRC).

96-hr peak (mg/L)
Juvenile fatheads 0.44 (0.22-0.62)

Yearling fatheads 1.56 (1.34-1.79)

96-hr intermittent exposure mean (mg/L)
Juvenile fatheads 0.18 (0.11-0.24)
Yearling fatheads 0.58 (0.51-0.65)

For the 96-hr accumulative (mg/L x min)
Juvenile fatheads 85 (48-113)

Yearling fatheads 274 (240-308)
Test condition: Life stage: juvenile (six-week old) and adult (1 year) Water quality measurements were as follows:

Parameter	Mean + SE	Range
Temp degree	21.1 + 0.1	19.9-22.9
pH	7.0 + 0.1	6.7-7.1
DO (mg/L)	7.8 + 0.1	6.5-9.1
Cond (mmhos/cm)	66.6 + 0.1	63-71
Alkalinity (mg/L)	15.3 + 0.1	14-16

Test substance: sodium hypochlorite, reagent grade, 5% solution
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
30-OCT-2005 (246)

Type: flow through
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50: = .18 -

Method: other: see TC
Year: 1982
GLP: no data
Test substance: other TS

Method: Fish were acclimated to pond water for 10 days prior to the test. Ten fish were placed in each chamber and there were two chambers for each concentration. A proportional, flow-through dilutor system was used. Each system delivered approximately 0, 2, 20, 35, 60, 75 and 100% of the stock solution to duplicate test chambers using pond water as the dilutent. Stock biocide solutions were added to the dilutor system for 1 hour/day at 0, 24, 48 and 72 hours after testing began. Water samples were collected from each test chamber at 10 minute intervals during the periods (approximately 2 hours/day) that biocide residuals were measurable in the test chambers. The 96-hour intermittent exposure mean corresponds to the mean biocide residual level during the four ~2-hour exposure periods.
Result: The 96-hour intermittent exposure LC50 is 0.18 mg/L (95% confidence interval 0.11-0.24 mg/L). Concentration refers to total residual chlorine (TRC).
The concentrations are calculated intermittent exposure mean during the 4 exposure periods of 2 hours.
Test condition: Life stage: juvenile (6 weeks)
Temperature: 21.1 +/-0.1 degree C, pH: 7.0+/-0.1, DO: 7.8+/-0.1 mg/L,
Cond: 66.6+/- 0.1 umhos/cm, Alkalinity: 15.3+/-0.1.
Test substance: 5% solution of sodium hypochlorite reagent grade
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
30-OCT-2005 (246)

Type: flow through
Species: Lepomis macrochirus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no data
LC50: = .44 -

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Method:           other: according to EPA guidelines
  Year:           1978
   GLP:           no data

Result:           Concentration expressed as total residual chlorine (TRC).
                  LC50 (96 hours) is 0.44 mg/L (95% cofidential interval
                  0.28-1.00 mg/L)
                  Temperature: 27.7 degree C
                  pH: 7.0
                  DO: 6.8 mg/L
Test condition:   Age = 1 year; Temperature = 27.7 degree C; pH = 7.0;
                  dissolved O2 = 6.8 mg/l
Reliability:      (2)  valid with restrictions
Flag:             Critical study for SIDS endpoint
30-OCT-2005                                                          (245)

Type:             flow through
Species:          Pimephales promelas  (Fish, fresh water)
Exposure period:  96 hour(s)
Unit:             mg/l                   Analytical monitoring: no data
LC50:             = .08 -

Method:           other: according to EPA guidelines
  Year:           1978
   GLP:           no data
Test substance:   other TS: TRC

Result:           Concentration expressed as total residual chlorine (TRC).
                  LC50 (96 hours) is 0.08 mg/L (95% cofidential interval
                  0.06-0.11 mg/L)
                  Temperature: 27.7 degree C
                  pH: 7.0
                  DO: 6.8 mg/L
Test condition:   Age = juvenile fish (4-week-old); Temperature = 28 degree C;
                  pH = 7.0; dissolved oxygen = 6.8 mg O2/l.
Test substance:   Chlorine is expressed as total residual chlorine (TRC).
Reliability:      (2)  valid with restrictions
Flag:             Critical study for SIDS endpoint
30-OCT-2005                                                          (245)

Type:             flow through
Species:          Pimephales promelas  (Fish, fresh water)
Exposure period:  96 hour(s)
Unit:             mg/l                   Analytical monitoring: no data
LC50:             = .35 -

Method:           other: according to EPA guidelines
  Year:           1978
   GLP:           no data
Test substance:   other TS

Result:           Concentration expressed as total residual chlorine (TRC).
                  LC50 (96 hours) is 0.35 mg/L (95% cofidential interval
                  0.20-1.08 mg/L)
                  Temperature: 27.7 degree C
                  pH: 7.0
                  DO: 6.8 mg/L
Test condition:   Age = adult fish (ca. 1-year-old); Temperature = 28 degree
                  C; pH = 7.0; dissolved oxygen = 6.8 mg O2/l
Test substance:   Chlorine is expressed as total residual chlorine (TRC).
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Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
30-OCT-2005 (245)

Type: other
Species: Leiostomus xanthurus (Fish, estuary, marine)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50: = .09 -

Remark: Bellanca and Bailey (1977) evaluated the short-term toxicity of chlorine to the estuarine fish, which consisted principally of free chlorine. This data is rated 1.
Result: Leiostomus xanthurus (ocean spot) was exposed ot hypochlorite in a flow through laboratory experiment, using a continuous flow serial diluter fed with river water. The authors calculated a 96h-TLm (equivalent to an LC50)= 0.090 mg/l of TRC.
Temperature: 14.2 - 16.0 degree C, pH: 7.5, Oxygen: 6.9 - 7.4 mg/L
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
30-OCT-2005 (20)

Type: flow through
Species: Clupea harengus (Fish, estuary, marine)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50: = .065 -

Method: other: American Public Health Association, 1971
Year: 1978
GLP: no data
Test substance: other TS: Clorox (trademark registered)

Method: Fish were collected in Sequim Bay, Washington and acclimated for only 2 or 3 days prior to testing due to difficulties in maintaining them for several weeks under laboratory conditions. Ten fish were tested per concentration level. Test chambers were 45 L aquaria. Flow rates were approximately 0.5 l/min, giving a calculated 99% replacement time of 7 hours. This rate of exchange maintained dissolved oxygen concentrations above 7 mg/L. The pH values were 8(+/-0.2) and salinity was 28 (+/-1). Since this study was conducted to assess the environmental impact of chlorinated effluents from operating power plants, fish were acclimated at one temperature and exposed to test material in water 5 degree C higher to include thermal stress.
Result: Results expressed as Total Residual Oxidant (TRO)
The 96 hour LC50 is 0.065 mg/L (95% confidence interval 0.033-0.097 mg/L).
Test condition: Life stage: juvenile
Temperature: 14.8 degree C, acclimatization at 10 degree C, pH=8, Oxygen > 7 mg/l, salinity 28 (seawater).
Test substance: Commercial product of Clorox, Oakland CA:
- 5.25 % sodium hypochlorite
- 4.12 % sodium chloride
- 0.20 % sodium carbonate
- 0.01 % sodium hydroxide
in water

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Reliability:      (2)  valid with restrictions
Flag:             Critical study for SIDS endpoint
30-OCT-2005                                                    (7) (226)


Type:             flow through
Species:          Cymatogaster aggregata  (Fish, estuary)
Exposure period:  96 hour(s)
Unit:             mg/l                   Analytical monitoring: yes
LC50:             = .071 -

Method:           other: American Public Health Association, 1971
  Year:           1978
   GLP:           no data
Test substance:   other TS: Clorox (trademark registered)

Method:           Fish were acclimated for at least two weeks prior to
                  testing.  Ten fish were tested per concentration level. Test
                  chambers were 45 L aquaria. Flow rates were approximately
                  0.5 l/min, giving a calculated 99% replacement time of 7
                  hours. This rate of exchange maintained dissolved oxygen
                  concentrations above 7 mg/L. The pH values were 8(+/- 0.2)
                  and salinity was 28 (+/-1). Since this study was conducted
                  to assess the environmental impact of chlorinated effluents
                  from operating power plants, fish were acclimated at one
                  temperature and exposed to test material in water of 5
                  degree C higher to impose thermal stress, additonally.
Result:           Results expressed as Total Residual Oxidant (TRO)
                  The 96 hour LC50 is 0.071 mg/L (95% confidence interval
                  0.045-0.098 mg/L).
Test condition:   Life stage: juvenile
                  Temperature: 14.8 degree C, acclimatization at 10 degree C,
                  pH=8, Oxygen > 7 mg/l, salinity 28 (seawater).
Test substance:   Commercial product of Clorox, Oakland CA:
                  - 5.25 % sodium hypochlorite
                  - 4.12 % sodium chloride
                  - 0.20 % sodium carbonate
                  - 0.01 % sodium hydroxide
                  in water
Reliability:      (2)  valid with restrictions
Flag:             Critical study for SIDS endpoint

Type:             flow through
Species:          Gasterosteus aculeatus  (Fish, estuary, marine)
Exposure period:  96 hour(s)
Unit:             mg/l                   Analytical monitoring: yes
LC50:             = .167 -

Method:           other: American Public Health Association, 1971
  Year:           1978
   GLP:           no data
Test substance:   other TS: Clorox (trademark registered)

Result:           Results expressed as Total Residual Oxidant (TRO)
                  The 96 hour LC50 is 0.167 mg/L (95% confidence interval
                  0.141-0.193 mg/L).
Test condition:   Temperature: 14.8 degree C, acclimatization at 10 degree C,
                  pH=8, Oxygen > 7 mg/l, salinity 28 (seawater).
Test substance:   Commercial product of Clorox, Oakland CA:
                  - 5.25 % sodium hypochlorite
                  - 4.12 % sodium chloride
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- 0.20 % sodium carbonate
- 0.01 % sodium hydroxide
in water
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
30-OCT-2005 (7) (226)

Type: flow through
Species: Oncorhynchus gorbuscha (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes

Method: other: American Public Health Association, 1971
Year: 1978
GLP: no data
Test substance: other TS: Clorox (trademark registered)

Method: Ten fish were tested per concentration level. Test chambers were 45 L aquaria. Flow rates were approximately 0.5 l/min, giving a calculated 99% replacement time of 7 hours. This rate of exchange maintained dissolved oxygen concentrations above 7 mg/L. The pH values were 8(+/- 0.2) and salinity was 28 (+/-1). Since this study was conducted to assess the environmental impact of chlorinated effluents from operating power plants, fish were acclimated at one temperature and exposed to test material in water of 5 degree C higher to impose thermal stress, additonally.
Result: Results expressed as Total Residual Oxidant (TRO). There were no deaths at 0.023 mg/L and all died at 0.052 mg/L. Thus the 96 hr LC50 is expected to be between 0.023 and 0.052 mg/L.
Test condition: Life stage: juvenile
Temperature: 14.8 degree C, acclimatization at 10 degree C, pH=8, Oxygen > 7 mg/l, salinity 28 (seawater).
Test substance: Commercial product of Clorox, Oakland CA:
- 5.25 % sodium hypochlorite
- 4.12 % sodium chloride
- 0.20 % sodium carbonate
- 0.01 % sodium hydroxide
in water
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
30-OCT-2005 (7) (226)

Type: flow through
Species: Oncorhynchus kisutch (Fish, fresh water, marine)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50: = .032 -

Method: other: American Public Health Association, 1971
Year: 1978
GLP: no data
Test substance: other TS: Clorox (trademark registered)

Method: Fish were acclimated for at least two weeks prior to testing. Ten fish were tested per concentration level. Test chambers were 45 L aquaria. Flow rates were approximately 0.5 l/min, giving a calculated 99% replacement time of 7 hours. This rate of exchange maintained dissolved oxygen concentrations above 7 mg/L. The pH values were 8(+/- 0.2)

and salinity was 28 (+/-1). Since this study was conducted to assess the environmental impact of chlorinated effluents from operating power plants, fish were acclimated at one temperature and exposed to test material in water of 5 degree C higher to impose thermal stress, additonally.
Result: Results expressed in Total Residual Oxidant (TRO). The 96 hour LC50 is 0.032 mg/L (95% confidence interval 0.026-0.038 mg/L).
Test condition: Life stage: juvenile
Temperature: 14.8 degree C, acclimatization at 10 degree C, pH=8, Oxygen > 7 mg/l, salinity 28 (seawater).
Test substance: Commercial product of Clorox, Oakland CA:
- 5.25 % sodium hypochlorite
- 4.12 % sodium chloride
- 0.20 % sodium carbonate
- 0.01 % sodium hydroxide
in water
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
30-OCT-2005 (7) (226)

Type: flow through
Species: Oncorhynchus tschawytscha (Fish, fresh water, marine)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50: = .032 -

Method: other: American Public Health Association, 1971
Year: 1978
GLP: no data
Test substance: other TS: Clorox (trademark registered)

Result: Results expressed in Total Residual Oxidant (TRO)
Test condition: Life stage: juvenile
Temperature: 14.8 degree C, acclimatization at 10 degree C, pH=8, Oxygen > 7 mg/l, salinity 28 (seawater).
Test substance: Commercial product of Clorox, Oakland CA:
- 5.25 % sodium hypochlorite
- 4.12 % sodium chloride
- 0.20 % sodium carbonate
- 0.01 % sodium hydroxide
in wa
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
30-OCT-2005 (7) (226)

Type: flow through
Species: Parophrys vetulus (Fish, marine)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50: = .038 - .065

Method: other: American Public Health Association, 1971
Year: 1978
GLP: no data
Test substance: other TS: Clorox (trademark registered)

Result: Results expressed in Total Residual Oxidant (TRO)
Test condition: Life stage: juvenile
Temperature: 14.8 degree C, acclimatization at 10 degree C, pH=8,

```
                  seawater
Test substance:   Commercial product of Clorox, Oakland CA:
                  - 5.25 % sodium hypochlorite
                  - 4.12 % sodium chloride
                  - 0.20 % sodium carbonate
                  - 0.01 % sodium hydroxide
                  in water
Reliability:      (2)  valid with restrictions
Flag:             Critical study for SIDS endpoint
30-OCT-2005                                                          (226)

Type:             flow through
Species:          other: Ammodytes hexapterus
Exposure period:  96 hour(s)
Unit:             mg/l                    Analytical monitoring: yes
LC50:             = .073 -

Method:           other: American Public Health Association, 1971
  Year:           1978
   GLP:           no data
Test substance:   other TS: Clorox (trademark registered)

Result:           Results expressed in Total Residual Oxidant (TRO)
                  The 96 hour LC50 is 0.073 mg/L (95% confidence interval
                  0.062-0.102 mg/L).
Test condition:   Life stage: juvenile
                  Temperature: 14.8 degree C, acclimatization at 10 degree C,
                  pH=8, Oxygen > 7 mg/l, salinity 28 (seawater).
Test substance:   Commercial product of Clorox, Oakland CA:
                  - 5.25 % sodium hypochlorite
                  - 4.12 % sodium chloride
                  - 0.20 % sodium carbonate
                  - 0.01 % sodium hydroxide
                  in water
Reliability:      (2)  valid with restrictions
Flag:             Critical study for SIDS endpoint
30-OCT-2005                                                    (7) (226)

Type:             flow through
Species:          Perca flavescens  (Fish, fresh water)
Exposure period:  30 minute(s)
Unit:             mg/l                    Analytical monitoring: yes
LC0:              = .48 - 5.1
LC50:             = .7 - 8
LC100:            = .95 - 15

Method:           other: see TC
  Year:           1975
   GLP:           no data
Test substance:   other TS: sodium hypochlorite

Remark:           Analytical monitoring: total residual chlorine (TRC)
Result:           LC0, LC50 and LC100 decreased with increasing temperature.
                  Concentration ranged from the highest temperature (the
                  lowest value) to the lowest temperature (the highest value).
                  More detailed results in the reference.
                  Result refers to the average of the initial and final TRC
                  concentration.
Test condition:   Life stage: young of the year
                  Acclimatization: min. 2 weeks
                  Temperature: 10, 15, 20, 25 and 30 degree C, pH=8.2-8.8,
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                freshwater
                Mortality assessed after 24-72 hours in recovery tanks.
Reliability:    (4)  not assignable
30-OCT-2005                                                          (29)

Type:           flow through
Species:        Salmo gairdneri  (Fish, estuary, fresh water)
Exposure period: 30 minute(s)
Unit:           mg/l                  Analytical monitoring: yes
LC0:            = .3 - .65
LC50:           = .43 - .99
LC100:          = .56 - 1.6

Method:         other: see TC
  Year:         1977
   GLP:         no data
Test substance: other TS: sodium hypochlorite

Remark:         Analytical monitoring: total residual chlorine (TRC)
Result:         LC0, LC50 and LC100 decreased with increasing temperature.
                Concentration ranged from the highest temperature (the
                lowest value) to the lowest temperature (the highest value)
                more detailed results in the reference.
                Result refers to the average of the initial and final TRC
                concentration.
Test condition: Life stage: juvenile
                Acclimatization: min. 2 weeks
                Temperature: 10, 15 or 20 degree C, pH=7.81-8.33, freshwater
                Mortality assessed after 24-72 hours in recovery tanks
Reliability:    (4)  not assignable
30-OCT-2005                                                          (29)

Type:           static
Species:        Alosa pseudobarengus  (Fish, fresh water)
Exposure period: 30 minute(s)
Unit:           mg/l                  Analytical monitoring: yes
LC0:            = .8 - 1.1
LC50:           = .3 - 2.15
LC100:          = .63 - 4.6

Method:         other
  Year:         1978

   GLP:         no data
Test substance: other TS: sodium hypochlorite

Remark:         Analytical monitoring: total residual chlorine (TRC)
Result:         LC0, LC50 and LC100 decreased with increasing temperature.
                Concentration ranges from the highest temperature (the
                lowest value) to the lowest temperature (the highest value).
                More detailed results in the reference.
                Result refers to the average of the initial and final TRC
                concentration.
Test condition: Life stage: juvenile
                Acclimatization: min. 2 weeks
                Temperature: 10, 15, 20 or (30) degree C, pH=8.23-8.53,
                freshwater
                Mortality assessed after 48 hours in recovery tanks.
Reliability:    (4)  not assignable
30-OCT-2005                                                          (205)
```

Type: static
Species: Oncorhynchus kisutch (Fish, fresh water, marine)
Exposure period: 30 minute(s)
Unit: mg/l Analytical monitoring: yes
LC0: = .21 - .91
LC50: = .29 - 1.38
LC100: = .54 - 1.7

Method: other
Year: 1976
GLP: no data
Test substance: other TS: sodium hypochlorite

Remark: Analytical monitoring: total residual chlorine (TRC)
Result: LC0, LC50 and LC100 decreased with increasing temperature. Concentration ranges from the highest temperature (the lowest value) to the lowest temperature (the highest value). More detailed results in the reference.
Result refers to the average of the initial and final TRC concentration.
Test condition: Life stage: juvenile
Acclimatization: min. 2 weeks
Temperature: 10, 15 or 20 degree C, pH=8.16-8.33, freshwater
Mortality assessed after 48 hours in recovery tanks.
Reliability: (4) not assignable
30-OCT-2005 (205)

Type: static
Species: Osmerus mordax (Fish, fresh water)
Exposure period: 30 minute(s)
Unit: mg/l Analytical monitoring: yes
LC50: = 1.27 -
LC10 : = .72 -
LC90 : = 2 -

Method: other
Year: 1978
GLP: no data
Test substance: other TS: sodium hypochlorite

Test condition: Life stage: adult
Temperature: 10 degree C, pH=8.46
30 min exposure and 48 hours observation
Reliability: (4) not assignable
30-OCT-2005 (205)

Type: static
Species: other: Notropis hudsonius
Exposure period: 30 minute(s)
Unit: mg/l Analytical monitoring: yes
LC0: = .38 -
LC50: = .53 - 2.41
LC100: = .83 -

Method: other
Year: 1978
GLP: no data
Test substance: other TS: sodium hypochlorite

Remark: Analytical monitoring: total residual chlorine (TRC)
Result: LC50 decreased with increasing temperature. Concentration

ranges from highest temperature (lowest value) to lowest temperature (highest value). LC0 and LC100 were determined at 20 degree C only. More detailed results in the reference.
Result refers to the average of the initial and final TRC concentration.
Test condition: Life stage: juvenile
Acclimatization: min. 2 weeks
Temperature: 10, 15 or 20 degree C, pH=8.24-8.41, freshwater
Mortality assessed after 48 hours in recovery tanks.
Reliability: (4) not assignable
30-OCT-2005 (205)

Type: flow through
Species: Perca flavescens (Fish, fresh water)
Exposure period: 5 minute(s)
Unit: mg/l Analytical monitoring: yes
LC0: <= 17 -
LC50: = 9 - 22.6
LC100: <= 37 -

Method: other: see TC
Year: 1974
GLP: no data
Test substance: other TS: sodium hypochlorite

Remark: Analytical monitoring: total residual chlorine (TRC)
Result: LC0 and LC100 were only determined at 10 degree C. LC50 wasdetermined at 10 degree C (higher value) and at 20 degree C (lower value).
More detailed results in the reference.
Result refers to the average of the initial and final TRC concentration.
Test condition: Life stage: young of the year
Acclimatization: at least 2 weeks
Temperature: 10, 20 degree C, pH=8.2-8.5, freshwater
Mortality assessed after 24-72 hours in recovery tanks.
Reliability: (4) not assignable
30-OCT-2005 (29)

Type: flow through
Species: Salmo gairdneri (Fish, estuary, fresh water)
Exposure period: 5 minute(s)
Unit: mg/l Analytical monitoring: yes
LC0: = 1 - 1.7
LC50: = .82 - 2.87
LC100: = 1.5 - 2.5

Method: other: see TC
Year: 1975
GLP: no data
Test substance: other TS: sodium hypochlorite

Remark: Analytical monitoring: total residual chlorine (TRC)
Result: LC0, LC50 and LC100 decreased with increasing temperature. Concentration ranges from highest temperature (lowest value) to lowest temperature (highest value). More detailed results in the reference.
Result refers to the average of the initial and final TRC concentration.
Test condition: Life stage: juvenile

Acclimatization: min. 2 weeks
Temperature: 10, 15 or 20 degree C, pH=7.81-8.33, freshwater
Mortality assessed after 24-72 hours in recovery tanks
Reliability: (4) not assignable
30-OCT-2005 (29)

Type: flow through
Species: Fundulus heteroclitus (Fish, estuary, marine)
Exposure period: 30 minute(s)
Unit: mg/l Analytical monitoring: yes
LC100: = .65 -

Method: other
Year: 1977
GLP: no data

Test condition: Life stage: juvenile
Temperature : 24 degree C, pH=8, 30 min exposure and 48 h observation
Reliability: (4) not assignable
30-OCT-2005 (42)

Type: flow through
Species: Gambusia affinis (Fish, fresh water)
Exposure period: 1 hour(s)
Unit: µg/l Analytical monitoring: no data
LC50: = 840 -

Method: other: Fish toxicity test
Year: 1981
GLP: no data
Test substance: no data

Test condition: Fish were 2 to 3.5 mm long. The water had a temperature of 21 degree C and a pH of 8.2
Reliability: (3) invalid
30-OCT-2005 (151)

Type: flow through
Species: Ictalurus punctatus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: µg/l Analytical monitoring: no data
LC50: = 70 -

Method: other: not specified
Year: 1979
GLP: no

Remark: Channel catfish is a freshwater species. Gill sodium uptake was drastically impaired.
Result: Concentration expressed as total residual chlorine (TRC).
Reliability: (3) invalid
30-OCT-2005 (75)

Type: flow through
Species: Menidia menidia (Fish, estuary, marine)
Exposure period: 96 hour(s)
Unit: µg/l Analytical monitoring: yes
LC50: = 37 -

Method: other: Acute fish toxicity

```
  Year:           1975
   GLP:           no data
Test substance:   other TS: calcium hypochlorite

Remark:           Species were selected from river estuaries.
Test condition:   Fish were tested under flow-through condition; temperature
                  ranged from 17 to 28 degree C and salinity from 18.2 to
                  20.4; dissolved O2 was always near saturation.
Test substance:   Stock solution was prepared by dissolving calcium
                  hypochlorite in deionized water buffered to ca. pH=8.
                  Chlorine is expressed as total residual chlorine (TRC).
Reliability:      (3)  invalid
30-OCT-2005                                                          (187)

Type:             flow through
Species:          Morone saxatilis  (Fish, estuary, marine)
Exposure period:  48 hour(s)
Unit:             mg/l                  Analytical monitoring: yes
LC50:             = .04 -

Test substance:   other TS: sodium hypochlorite

Result:           Same conditions:
                  Larvae (2d old) : LC50 = 0.04 mg/l
                  Larvae (12d old) : LC50 = 0.07 mg/l
                  Larvae (34d old) : 25 min : Avoidance test = 0.29-0.79 mg/l
                  Eggs (8/9h old) : 100% no hatch = 0.21 mg/l
Test condition:   Life stage: juvenile (30 d old)
                  Temperature: 18 degree C, pH=6.8
Reliability:      (4)  not assignable
30-OCT-2005                                                          (157)

Type:             flow through
Species:          Oncorhynchus gorbuscha  (Fish, fresh water)
Exposure period:  8 minute(s)
Unit:             mg/l                  Analytical monitoring: yes
LC50:             = .5 -

Test substance:   other TS: sodium hypochlorite

Result:           Results expressed as Total Residual Oxidant (TRO)
Test condition:   Life stage: juvenile
                  Temperature: 13.6 degree C, pH=7.53
Reliability:      (3)  invalid
30-OCT-2005                                                          (216)

Type:             flow through
Species:          Oncorhynchus kisutch  (Fish, fresh water, marine)
Exposure period:  60 minute(s)
Unit:             mg/l                  Analytical monitoring: yes
LC50:             = .13 -

Test substance:   other TS: sodium hypochlorite

Result:           Results expressed in Total Residual Oxidant (TRO)
                  Same conditions except temperature : 13 degree C LC50 =
                  0.208 mg/l
Test condition:   Life stage: adult
                  Temperature: 20 degree C, pH=7.9
Reliability:      (4)  not assignable
30-OCT-2005                                                          (215)
```

```
Type:              flow through
Species:           Oncorhynchus mykiss  (Fish, fresh water)
Exposure period:   96 hour(s)

Unit:              µg/l                    Analytical monitoring: no data
LC50:              = 14 -

Method:            other: Fish toxicity test
   GLP:            no data
Test substance:    no data

Test condition:    Fish length was 10-13 cm.
                   Water quality:
                   temperature = 2 - 6.5 degree C
                   hardness = 180 mg CaCO3/l
                   alkalinity = 155 mg CaCO3/l
                   pH = 8.2
Reliability:       (3)  invalid
30-OCT-2005                                                          (16)

Type:              flow through
Species:           Oncorhynchus tschawytscha  (Fish, fresh water, marine)
Exposure period:   60 minute(s)
Unit:              mg/l                    Analytical monitoring: yes
LC50:              = .25 -

Test substance:    other TS: sodium hypochlorite

Test condition:    Juvenile, temperature: 11.7 degree C, pH=7.53
Reliability:       (3)  invalid
30-OCT-2005                                                         (216)

Type:              flow through
Species:           Pseudopleuronectes americanus  (Fish, estuary, marine)
Exposure period:   30 minute(s)
Unit:              mg/l                    Analytical monitoring: yes
LC100:             = .55 -

Test substance:    other TS:sodium hypochlorite

Test condition:    Life stage: juvenile
                   Temperature: 24 degree C, pH=8
                   30 min exposure and 48 hours observation
Reliability:       (4)  not assignable
30-OCT-2005                                                          (42)
Type:              flow through
Species:           Salvelinus alpinus  (Fish, marine)
Exposure period:   6 day(s)
Unit:              µg/l                    Analytical monitoring: no data
Effect concentration :
                   > 19 -

Method:            other: Fish Toxicity Test
   GLP:            no data
Test substance:    other TS: TRC

Remark:            Measured endpoint was behaviour and recovery (activity,
                   thigmotaxis).
Test condition:    Age/Life stage of arctic char = 10-17 cm, 30-50 g, Water
                   temperature = 10 - 12.5 degree C
```

Test substance: Chlorine is expressed as total residual chlorine (TRC).
Reliability: (3) invalid
30-OCT-2005 (116)

Type: flow through
Species: Salvelinus fontinalis (Fish, estuary, fresh water)
Exposure period: 96 hour(s)
Unit: µg/l Analytical monitoring: no data
LC50: = 102 - 179

Method: other: Fish toxicity test
Year: 1976
GLP: no
Test substance: no data

Remark: When fish (10 - 15 cm long) were tested in water of 10 degree C and 20 degree C, the LC50 values were 102 to 179 microg/l, respectively. When fish with a length of 7.5 - 10 cm were tested at 15 degree C, the LC50 value was 153 microg/l.
Test condition: Water quality:
- temperature = 10, 15, or 20 degree C
- alkalinity = 45 to 80 mg CaCO3/l
- pH = 7.8 - 8.2
- dissolved O2 = > 8 mg/l
Reliability: (3) invalid
30-OCT-2005 (225)

Type: flow through
Species: other: Cymatogaster aggregata
Exposure period: 60 minute(s)
Unit: mg/l Analytical monitoring: yes
LC50: = .308 -

Test substance: other TS: sodium hypochlorite

Result: Same conditions except temperature: 20 degree C
LC50 = 0.230 mg/l
Test condition: Life stage: juvenile
Temperature: 13 degree C, pH=8
Reliability: (4) not assignable
30-OCT-2005

Type: flow through
Species: other: Gobiosoma bosci and Syngnathus fuscus
Exposure period: 96 hour(s)
Unit: µg/l Analytical monitoring: no data
LC50: = 80 - 270

Method: other: Acute fish toxicity
Year: 1975
GLP: no
Test substance: no data

Remark: Gobiosoma bosci were more susceptible to chlorine (LC50 = 80 microg/l) than Syngnathus fuscus (LC50 = 270 microg/l). Species were selected from river estuaries.
Test condition: Fish were tested under flow-through condition; temperature ranged from 17 to 28 degree C and salinity from 18.2 to 20.4 per mill; dissolved O2 was always near saturation.
Reliability: (4) not assignable

30-OCT-2005 (187)

Type: flow through
Species: other: Rhinichthys atratulus
Exposure period: 11 hour(s)
Unit: mg/l Analytical monitoring: no data
LC50: = .15 -

Method: other: Acute fish toxicity
Year: 1976
GLP: no
Test substance: other TS: free chlorine

Remark: The LC50's for 2.5 and 0.5 hour exposure was 0.740 and 6.6 mg/l.
Test condition: Fish had a length of about 4 cm.
Water quality:
- temperature = 20.9 - 21.9 degree C
- hardness = 71.3 - 103.8 mg CaCO3/l
- pH = 7.08 - 7.64
- dissolved O2 = 7.7 - 7.9 mg/l
Reliability: (4) not assignable
30-OCT-2005 (229)

Type: flow through
Species: other: Stenotomus versicolor
Exposure period: 30 minute(s)
Unit: mg/l Analytical monitoring: yes
LC100: = .65 -

Test substance: other TS: sodium hypochlorite

Test condition: Life stage: juvenile
Temperature: 24 degree C, pH=8
30 min exposure and 48 hours observation
Reliability: (4) not assignable
30-OCT-2005 (42)

Type: semistatic
Species: Salmo gairdneri (Fish, estuary, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50: = .35 -

GLP: yes

Remark: Analytical monitoring: total residual chlorine (TRC)
Result: Same conditions:
24 h; LC 50; 0.43 mg/l
Test condition: Life stage: juvenile
Temperature: 15 degree C, pH=7, freshwater
Reliability: (4) not assignable
30-OCT-2005 (214)

Type: static
Species: Alburnus alburnus (Fish, estuary)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC50: = 32 - 37

```
Test substance:   other TS

Test condition:   Life stage: adult
                  Temperature: 10 degree C, pH=7.8, brackish water (salinity
                  7/1000)
Test substance:   sodium hypochlorite, technical grade, 8-12% active chlorine
Reliability:      (3)  invalid
30-OCT-2005                                                            (142)

Type:             static
Species:          Brachydanio rerio  (Fish, fresh water)
Exposure period:  96 hour(s)
Unit:             mg/l                   Analytical monitoring: no
LC100:            = 8.7 -

Test substance:   other TS: sodium hypochlorite

Test condition:   Life stage: juvenile
                  Temperature: 23 degree C, pH=7.6
Reliability:      (3)  invalid
30-OCT-2005                                                            (109)

Type:             static
Species:          Carassius auratus  (Fish, fresh water)
Exposure period:  24 hour(s)
Unit:             mg/l                   Analytical monitoring: yes
LC50:             = .27 -

Test substance:   other TS: sodium hypochlorite

Test condition:   Life stage: adult
                  Temperature: 17/22.5 degree C, pH=7.4-8.7
                  exposure: During 24 hour every 4 hour exposure of 15 min
Reliability:      (4)  not assignable
30-OCT-2005                                                             (65)

Type:             static
Species:          Cynoscion nebulosus  (Fish, marine)
Exposure period:  48 hour(s)
Unit:             mg/l                   Analytical monitoring: yes
LC50:             = .17 - .28

Test substance:   other TS

Test condition:   Life stage: larvae (1h)
                  Temperature: 25 degree C, pH=7.8
Reliability:      (4)  not assignable
30-OCT-2005                                                            (114)

Type:             static
Species:          Lepomis cyanellus  (Fish, fresh water)
Exposure period:  96 hour(s)
Unit:             µg/l                   Analytical monitoring: no data
LC50:             = 820 -

Method:           other: Fish toxicity test
  Year:           1976
   GLP:           no
Test substance:   no data

Test condition:   Fish were weighing 1 to 1.5 g.
```

```
                  Water quality:
                  - temperature = 12 degree C
                  - Alkalinity = 30 to 35 mg CaCO3/l
                  - hardness = 40 to 48 mg CaCO3/l
                  - pH = 8.5
                  - dissolved O2 = >60% saturation
Reliability:      (3)  invalid
30-OCT-2005                                                          (146)

Type:             static
Species:          Pimephales promelas  (Fish, fresh water)
Exposure period:  96 hour(s)
Unit:             mg/l                   Analytical monitoring: yes
LC50:             = 4.8 - 8

Test substance:   other TS: sodium hypochlorite

Remark:           Analytical monitoring: amperometric titration
Test condition:   Life stage: adult
                  Temperature: 22 degree C, pH=7.2-7.9, freshwater
Reliability:      (4)  not assignable
30-OCT-2005                                                           (57)

Type:             static
Species:          Pimephales promelas  (Fish, fresh water)
Exposure period:  96 hour(s)
Unit:             mg/l                   Analytical monitoring: no
LC50:             = 10 -


Test condition:   Life stage: juvenile
                  Temperature: 20 degree C, pH=6.5-8.5, Lake Ontario water
Test substance:   Cl2 5.25% solution
Reliability:      (3)  invalid
30-OCT-2005                                                           (78)

Type:             static
Species:          Salmo trutta  (Fish, fresh water, marine)
Exposure period:  48 hour(s)
Unit:             mg/l                   Analytical monitoring: no data
LC50:             = 5 -

Method:           other: Fish toxicity test
  Year:           1974
   GLP:           no
Test substance:   no data

Test condition:   Water quality:
                  - temperature = 10 degree C
                  - alkalinity = 165 to 200 mg CaCO3/l
                  - hardness = 210 to 290 mg CaCO3/l
                  - pH = 7.6 to 8
                  - dissolved O2 = >50% saturation
Reliability:      (3)  invalid
30-OCT-2005                                                          (253)

Type:             static
Species:          other: Barbus sarana
Exposure period:  96 hour(s)
Unit:             µg/l                   Analytical monitoring: no data
LC50:             = 580 -
```

```
Method:           other: Fish toxicity test
   GLP:           no data
Test substance:   as prescribed by 1.1 - 1.4

Remark:           Freshwater fish
Test substance:   Chlorine is expressed as total residual chlorine (TRC).
Reliability:      (3)  invalid
30-OCT-2005                                                    (165) (166)

Type:             static
Species:          other: Channa punctatus (snake-head catfish)
Exposure period:  1 hour(s)
Unit:             µg/l                  Analytical monitoring: no data
Effect concentration :
                  = 1250 -

Method:           other: Fish toxicity test
   GLP:           no data
Test substance:   no data

Remark:           Measured endpoint was lethality.
Test condition:   Age/Life stage = 18.5 mm, 0.6 g
Reliability:      (3)  invalid
30-OCT-2005                                                          (185)

Remark:           Additional toxicity studies of aquatic organisms are cited
                  in the document of US EPA (Ambient Water Quality, 1984).

                  In general, freshwater fish, saltwater fish and
                  invertebrates had similar ranges of sensitivity to "free"
                  chlorine (=refers to strongly oxidative forms also known as
                  TRC or CPO). The
                  reported values ranged from 28-710 microg/l for 33
                  freshwater species and 26-1400 microg/l for 28 saltwater
                  species. Toxicity is dependent upon factors such as
                  temperature, form of TRC and light. Sensitivity generally
                  rises with temperature.
Reliability:      (3)  invalid
30-OCT-2005                                                           (76)

Type:             other
Species:          Carassius auratus  (Fish, fresh water)
Exposure period:  24 hour(s)
Unit:             µg/l                  Analytical monitoring: no data
Median toxic level :
                  = 170 -

Method:           other: not specified
  Year:           1977
   GLP:           no
Test substance:   other TS: chlorine

Test condition:   Freshwater species; intermittent chlorination (Cl2
                  addition); water temperature 17 to 25.50C
Reliability:      (3)  invalid
30-OCT-2005                                                           (75)

Type:             other
Species:          Leiostomus xanthurus  (Fish, estuary, marine)
Exposure period:  24 hour(s)
```

Unit: µg/l Analytical monitoring: no data
Median toxic level :
= 140 -

Method: other: not specified
Year: 1980
GLP: no data
Test substance: no data

Test condition: Saltwater species ocean spot; York River (VA) water; no additional information
Reliability: (3) invalid
30-OCT-2005 (75)

Type: other
Species: Lepomis cyanellus (Fish, fresh water)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no data
LC0: = 2 -
LC28% : = 3 -

Method: other: not specified
GLP: no
Test substance: other TS: free chlorine

Remark: No mortality was found with 48-hour exposure to 2 mg/l of chlorine.
Several other freshwater species were listed in McKee and Wolf (1963):

Fish	Exposure time	Effect	Concentration (microg/l)
Carp	12-16 days	25% killed	150 - 200
Trout	1 hour	killed	1000
	2 hours	killed	300
Small trout	47 minutes	killed	800
Rainbow trout	168 hours	half killed	80
Young salmon	28 days	critical level	50
Golden shiners	4 hours	killed	800
Channel catfish	5 hours	killed	250

Reliability: (3) invalid
30-OCT-2005 (153)

Type: other
Species: Notropis atherinoides
Exposure period: 30 minute(s)
Unit: µg/l Analytical monitoring: no data
LC50: = 230 - 280

Method: other: not specified
Year: 1979
GLP: no
Test substance: other TS: TRC

Remark: Yearling Emerald shiners were slightly more susceptible (LC50=230 5g/l) than adults (LC50=280 5g/l).
Test condition: Freshwater species; Lake Superior water; water temperature

25 degree C
Test substance: Chlorine is expressed as total residual chlorine (TRC).
Reliability: (3) invalid
30-OCT-2005 (75)

Type: other
Species: Oncorhynchus kisutch (Fish, fresh water, marine)
Exposure period: 60 minute(s)
Unit: µg/l Analytical monitoring: no data
LC50: = 208 -

Method: other: not specified
Year: 1980
GLP: no data
Test substance: no data

Test condition: Saltwater species (coho salmon); water temperature: 13 degree C; no additional information
Reliability: (3) invalid
30-OCT-2005 (75)

Type: other
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: µg/l Analytical monitoring: no data
LC50: = 100 -

Method: other: not specified
Year: 1973
GLP: no
Test substance: no data

Remark: - The 1-hour LC50 was 880 5g/l of chlorine for yellow perch and 740 microg/l of chlorine for largemouth bass.
- The safe concentration for fathead minnow was 16.5 microg/l.
- The 15-hour median mortality for smallmouth bass was 500 microg/l.
- A concentration of 1000 microg/l for 30-60 min was lethal to the white sucker.
Test condition: Additionally tested freshwater species were yellow perch (perca flavescens), largemouth bass (micropterus salmoides), smallmouth bass (micropterus dolomieui) and white sucker (catostomus commersoni).
Reliability: (3) invalid

Type: other
Species: Salmo gairdneri (Fish, estuary, fresh water)
Exposure period: 96 hour(s)
Unit: µg/l Analytical monitoring: no data
LC50: = 140 - 290

Method: other: not specified
Year: 1973
GLP: no

Remark: Results refer to total residual chlorine (TRC)
The 168-hr LC50 was 80 microg/l.
Reliability: (3) invalid
30-OCT-2005 (31)

Type: other
Species: Salvelinus fontinalis (Fish, estuary, fresh water)
Unit: µg/l Analytical monitoring: no data
Other : = 500 -

Method: other: not specified
Year: 1972
GLP: no
Test substance: other TS: TRC

Remark: Mean survival time for brook trout (freshwater species) was 48, 18, or 9 hours at 40, 80 and 350 microg/l of TRC. At 5 microg/l activity was depressed.
Median mortality was found at 500 microg/l of TRC for 90 min. At a concentration of 10 microg/l of TRC for 96 hours a 67% lethality was found.
Reliability: (3) invalid
30-OCT-2005 (31)

Type: other
Species: other: Oncorhynchus gorbuscha, kisuthch, tschawytscha
Exposure period: 48 hour(s)
Unit: µg/l Analytical monitoring: no data
LC0: = 50 -
LC100: = 80 - 200

Method: other: not specified
Year: 1973
GLP: no
Test substance: other TS: TRC

Remark: LC100 for pink salmon and coho salmon was 80-100 and 130-200 microg/l of TRC, respectively, within 48 hours. The maximum concentration which was not lethal for both salmons was 50 microg/l .
At a concentration of 250 microg/l of TRC the first Chinook salmon died after 2.2 hours.
Test condition: Freshwater species Coho salmon (Oncorhynchus kisutch), Pink salmon (Oncorhynchus gorbuscha) and Chinook salmon (Oncorhynchus tschawytscha) were tested.

Reliability: (3) invalid
30-OCT-2005 (31)

Type: flow through
Species: Ictalurus punctatus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: µg/l Analytical monitoring: yes
LC0: 82 -

Reliability: (4) not assignable
30-OCT-2005

Type: static
Species: Lepomis macrochirus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
NOEC: < .032 -
LC50: = .049 - .16

Method: other: EPA-660/3-75-009
Year: 1975
GLP: no data

Reliability: (4) not assignable
30-OCT-2005 (3)

Type: static
Species: Salmo gairdneri (Fish, estuary, fresh water)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: yes
NOEC: ca. .1 -
LC50: ca. .15 - .21

Method: other

Reliability: (4) not assignable
30-OCT-2005 (4)

Type: static
Species: Salmo gairdneri (Fish, estuary, fresh water)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: yes
NOEC: < .01 -
LC50: ca. .15 - .21

Method: other

Reliability: (4) not assignable
30-OCT-2005 (204)

4.2 Acute Toxicity to Aquatic Invertebrates

Remark: Calcium hypochlorite which is dissolved in water is in a fast equilibrium with hypochlorous acid which again is in a fast equilibrium with hypochlorite ions. In general the concentration of chlorine in a test system is achieved by dissolving hypochlorous acid or sodium hypochlorite and not by application of gaseous chlorine.

Because of this equilibrium concentrations, in general, are provided as free available chlorine or as total residual chlorine (TRC). (See chapter 1.11 for definition.)
The studies were performed by independent laboratories and published in peer reviewed papers. Many studies were performed in the 70ties, when effects of the biocidal application of chlorine were carefully reinvestigated. Therefore the studies are not performed according to recent guidelines and no GLP information is provided.

With regard to the extensive number of tests that were already performed with chlorine no further studies according to recent guidelines were conducted to avoid further animal testing.
Flag: Critical study for SIDS endpoint
30-OCT-2005

Type: flow through
Species: other: Pandalus goniurus
Exposure period: 96 hour(s)

Unit: mg/l Analytical monitoring: yes
EC50: = -
LC50 : = .09 -

Method: other: American Public Health Association, 1971
GLP: no data
Test substance: other TS: Clorox (trademark registered)

Result: Results in mg/l of total residual oxidant (TRO)
LC50 = 0.09 mg/L (0.063 - 0.119 mg/L)
Test condition: Life stage: adult
Temperature: 15 degree C, acclimatization at 10 degree C, pH=8.0, seawater
Test substance: Commercial product of Clorox, Oakland CA:
- 5.25 % sodium hypochlorite
- 4.12 % sodium chloride
- 0.20 % sodium carbonate
- 0.01 % sodium hydroxide
in water
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
30-OCT-2005 (7) (226)

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no data
EC50: = .116 -

Method: other: Invertebrate toxicity test
Year: 1978
GLP: no
Test substance: other TS: NaOCl or Ca(OCl)2

Method: Procedure follows Standard Methods (APHA, 1974) with modifications for static tests (Cairns and Messenger, 1974; Buikema et al., 1974a, 1974b; and Newman, 1975). Ten Daphnia were placed in beakers with 300 ml of water. Three replicates of each concentration and controls were run. Beakers were covered to retard evaporation. All studies were conducted in
environmental growth chambers maintained at the appropriate temperature (+/-1C); photoperiod was approximately 12L:12D, and light intensity was approximately 60 foot candles. All animals were preacclimated to test conditions for 2-4 days. The tests were conducted without aeration or renewal of test material. Temperatures were 5, 10, 15, 20 and 25 degree C. Note temperature differences with those listed in results. The numbers of live organisms were recorded at 24 and 48 hours and were based on visible external or internal motion. LC50 values were obtained by probit analyses.
Result: The 48-hour LC50 decreased as the water temperature increased.
The LC50 at 20 degree C is typically used for OECD 202 tests.

LC50 concentrations [mg/L] at 24 hours and 48 hours exposure and different temperatures.

	Temperature [degree C]				
	5	10	15	20	25
24 hrs	0.16	0.15	0.145	0.14	0.076
48 hrs	0.15	0.13	0.12	0.116	0.085

Test condition: Static test condition at 25 degree C water temperature.
Water quality:
- Alkalinity = 42+5 mg CaCO3/l
- Hardness = 45+5 mg CaCO3/l
- pH = 7.5+0.05

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
30-OCT-2005 (35)

Type: flow through
Species: other: Ceriodaphnia dubia
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: yes
LC0 : = .0015 - .002
LC50 : = .004 - .006
LC100 : = .008 - .01

Method: other: not specified
Year: 1991
GLP: no data
Test substance: other TS: sodium hypochlorite

Remark: The study gives a good idea of the evolution of the active chlorine with different backgrounds. It gives information about the toxicity of the by products of hypochlorite in natural surroundings, monochloramine and dichloramine, with a very low toxicity by comparison (see RS).
Result: Results in mg/l of hypochlorite ion. The toxicity was slightly pH dependent. Lower LC values provided were found at pH=7, the higher values were found at pH=8.

Presented LC data were for the test at pH=7 and with food.

Toxicity of the by products of hypochlorite in natural surroundings, monochloramine and dichloramine:
0.016 mg/l for monochloramine and 0.027 for dichloramine
0.016 mg/l for monochloramine and 0.027 for dichloramine
Test condition: Evaluation made in free flow and static condition, with or without feeding in freshwater. Temperature : 25 degree C, pH=7 and pH=8.

The applied chlorine reacts very rapidly (< 1 min) with the food provided. The standard toxicity test procedure therefore rather determines the toxicity of chlorinated food than of free chlorine.
Therefore also tests without feeding were performed. (Decay of free chlorine in the static system was for 7 hours.)
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
30-OCT-2005 (224)

Type: flow through
Species: Palaemonetes pugio (Crustacea)
Exposure period: 96 hour(s)

```
Unit:              mg/l                    Analytical monitoring: yes
EC50:              = .22 -

Method:            other: Acute toxicity test
  Year:            1975
   GLP:            no
Test substance:    other TS: TRC

Remark:            Species were selected from river estuaries.
Test condition:    Test was performed under flow-through condition; temperature
                   ranged from 17 to 28 degree C and salinity from 18.2 to 20.4
                   per mill; dissolved O2 was always near saturation.
Test substance:    Stock solution was prepared by dissolving calcium
                   hypochlorite in deionized water buffered to ca. pH8.
                   Chlorine is expressed as total residual chlorine (TRC).
Reliability:       (2)  valid with restrictions
Flag:              Critical study for SIDS endpoint
30-OCT-2005                                                              (187)

Type:              flow through
Species:           other: shore crab
Exposure period:   96 hour(s)
Unit:              mg/l                    Analytical monitoring: yes
EC50:              = 1.24 - 1.53

Method:            other: American Public Health Association, 1971
   GLP:            no data

Remark:            species involved: Hemigrapsus nudus and Hemigrapsus
                   oregonensis
Result:            Results in mg/l of total residual oxidant (TRO)
Test condition:    Life stage: juvenile and adult
                   Temperature: 15 degree C, acclimatization at 10 degree C,
                   pH=8.0, seawater
Test substance:    Commercial product of Clorox, Oakland CA:
                   - 5.25 % sodium hypochlorite
                   - 4.12 % sodium chloride
                   - 0.20 % sodium carbonate
                   - 0.01 % sodium hydroxide
                   in water
Reliability:       (4)  not assignable
30-OCT-2005                                                        (7) (226)

Type:              static
Species:           Daphnia pulex  (Crustacea)
Exposure period:   48 hour(s)
Unit:              mg/l                    Analytical monitoring: no data
EC50:              = .04 -

Method:            other: Invertebrate toxicity test
  Year:            1978
   GLP:            no
Test substance:    other TS: NaOCl or Ca(OCl)2

Method:            Procedure follows Standard Methods (APHA, 1974) with
                   modifications for static tests (Cairns and Messenger, 1974;
                   Buikema et al., 1974a, 1974b; and Newman, 1975). Ten Daphnia
                   were placed in beakers with 300 ml of water. Three
                   replicates of each concentration and controls were run.
                   Beakers were covered to retard evaporation. All studies were
                   conducted in environmental growth chambers maintained at the
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appropriate temperature (+/-1C); photoperiod was approximately 12L:12D, and light intensity was approximately 60 foot candles. All animals were preacclimated to test conditions for 2-4 days. The tests were conducted without aeration or renewal of test material. Temperatures were 5, 10, 15, 20 and 25 degree C. Note temperature differences with those listed in results. The numbers of live organisms were recorded at 24 and 48 hours and were based on visible external or internal motion. LC50 values were obtained by probit analyses.

Result: LC50 concentrations [mg/L] at 24 hours and 48 hours exposure and different temperatures.

Temperature [degree C]	5	10	15	20	25
24 hrs	0.14	0.13	0.1	0.095	0.05
48 hrs	0.11	0.091	0.075	0.04	0.03

Test condition: Static test condition at 20 degree C water temperature.
Water quality:
- Alkalinity = 42 mg CaCO3/l
- Hardness = 45 mg CaCO3/l
- pH = 7.5

Reliability: (4) not assignable
30-OCT-2005 (35)

Type: semistatic
Species: other aquatic mollusc
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no
EC50: = 1.8 -

Method: other
Year: 1976
GLP: no data
Test substance: other TS: sodium hypochlorite

Remark: Species: Physa integra (freshwater pouch snail)
Result: Results expressed as Cl2
Same conditions 24 h, LC50=2.0 mg/l
Test condition: Semistatic; 1 renewal
temperature: 23.5 plus or minus 2.5 degree C, pH: 7 to 8.6, lake water:
hardness: 137 to 171 mg/l CaCO3
dissolved O2: 5 to 9 mg/l
Reliability: (4) not assignable
30-OCT-2005 (36)

Type: semistatic
Species: other aquatic mollusc
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no
EC50: = 6.2 -

Method: other
Year: 1976
GLP: no data
Test substance: other TS: sodium hypochlorite

Remark: Species: Goniobasis livescens (river snail); adult.
Result: Results expressed as Cl2
same conditions: 24 h LC50=10.4 mg/l
Test condition: Semistatic; 1 renewal
temperature: 23.5 plus or minus 2.5 degree C, pH: 7 to 8.6,
lake water:
hardness: 137 to 171 mg/l CaCO3
dissolved O2: 5 to 9 mg/l
Reliability: (4) not assignable
30-OCT-2005 (36)

Type: flow through
Species: Ceriodaphnia sp. (Crustacea)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
EC50: = .118 - .151

Method: other: American Public Health Association, 1971
Year: 1978
GLP: no data
Test substance: other TS: Clorox (trademark registered)

Method: Shrimp were acclimated for at least two weeks prior to testing. Ten shrimp were tested per concentration level. Test chambers were 45 L aquaria. Flow rates were approximately 0.5
l/min, giving a calculated 99% replacement time of 7 hours. This rate of exchange maintained dissolved oxygen concentrations above 7 mg/L. The pH values were 8(+/- 0.2) and salinity was 28% (+/-1%). Since this study was conducted to assess the environmental impact of chlorinated effluents from operating power plants, shrimp were acclimated at one temperature and exposed to test material in water of 5 degree C higher to impose thermal stress, additonally.
Remark: Species: Crangon nigricauda
Test condition: Life stage: adult
Temperature: 15 degree C, acclimatization at 10 degree C,
pH=8, seawater
Test substance: Commercial product of Clorox, Oakland CA:
- 5.25 % sodium hypochlorite
- 4.12 % sodium chloride
- 0.20 % sodium carbonate
- 0.01 % sodium hydroxide
in water
Reliability: (4) not assignable
30-OCT-2005 (226)

Type: semistatic
Species: other aquatic mollusc
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no
EC50: = 13.6 -

Method: other
Year: 1976
GLP: no data
Test substance: other TS: sodium hypochlorite

Remark: Lymnaea emarginata angulata (freshwater pond snail); adult.
Result: Results expressed as Cl2
same conditions: 24 h LC50=21.8 mg/l

Test condition: Semistatic; 1 renewal

temperature: 23.5 degree C, pH: 7 to 8.6,
lake water:
hardness: 137 to 171 mg/l CaCO3
dissolved O2: 5 to 9 mg/l
Reliability: (4) not assignable
30-OCT-2005 (36)

Type: flow through
Species: other: Anonyx sp
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
EC50: = .118 - .173

Method: other: American Public Health Association, 1971
Year: 1978
GLP: no data
Test substance: other TS: sodium hypochlorite

Result: Results in mg/l of total residual oxidant (TRO)
Test condition: Life stage: adult
Temperature: 15 degree C, acclimatization at 10 degree C,
pH=8, seawater
Test substance: Commercial product of Clorox, Oakland CA:
- 5.25 % sodium hypochlorite
- 4.12 % sodium choride
- 0.20 % sodium carbonate
- 0.01 % sodium hydroxide
in water
Reliability: (4) not assignable
30-OCT-2005 (7) (226)

Type: static
Species: other: Neomysis sp.
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
EC50: = .15 - .175

Method: other: American Public Health Association, 1971
Year: 1978
GLP: no data
Test substance: other TS: Clorox (trademark registered)

Result: Results in mg/l of total residual oxidant (TRO)
Test condition: Life stage: adult
Temperature: 15 degree C, acclimatization at 10 degree C,
pH=8.0, seawater
Test substance: Commercial product of Clorox, Oakland CA:
- 5.25 % sodium hypochlorite

- 4.12 % sodium chloride
- 0.20 % sodium carbonate
- 0.01 % sodium hydroxide
in water
Reliability: (4) not assignable
30-OCT-2005 (7) (226)

Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)

Unit: µg/l Analytical monitoring: no data
EC50: = 17 -

Method: other: National Environmental Research Center (1974): Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians, U.S. Environmental Protection Agency, Corvallis, OR 63 pp.
Year: 1981
GLP: no data
Test substance: no data

Method: The method was outlined by the National Environmental Research Center of the U.S. Environmental Protection Agency. The effluent used in these tests was from the wastewater treatment plant at Grandville, Michigan. The wastewater was primarily of domestic origin and, after treatment by the activated sludge process with chemical removal of phosphate, resulted in a reasonably good quality effluent (some typical characteristics were: total suspended solids, 19 mg/L; turbidity, 23 J.T.U.; COD, 38 mg/L; total phosphate 0.6 mg/L; and pH 7.2).

The plant chlorinating system was utilized to provide a chlorinated effluent stream. The water used for diluting the effluent stream delivered to the fish tanks was well water from which excess iron was removed by passing through an iron removal filter. This water had the following characteristics: hardness 464.0 mg/L, calcium 160.0 mg/L, chloride 8.0 mg/L and pH 7.6.

Daphnia magna less than 1 day old and 3 days old were used.
Result: The freshwater macroinvertebrate, Daphnia magna, was unable to tolerate 100% non-disinfected effluent.
Total residual chlorine concentrations of 0.220 mg/L and 0.070 mg/L were lethal to three-day-old D. magna in 5.5 and 10.5 hours, respectively. In a 48-hour acute test with D. magna less than one day old, an LC50 of 0.017 mg/L total residual chlorine was observed. Thus, extremely low levels of chlorinated effluent may affect adversely.
Reliability: (4) not assignable
30-OCT-2005 (75) (239)

Type: flow through
Species: other: Pandalus danae
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
EC50: = .159 - .199

Method: other: American Public Health Association, 1971
Year: 1978
GLP: no data
Test substance: other TS: Clorox (trademark registered)

Test condition: Life stage: juvenile and adult
Temperature: 15 degree C, acclimatization at 10 degree C,
pH=8.0,
seawater
Test substance: Commercial product of Clorox, Oakland CA:
- 5.25 % sodium hypochlorite

```
                - 4.12 % sodium chloride
                - 0.20 % sodium carbonate
                - 0.01 % sodium hydroxide
                in water
Reliability:    (4)  not assignable
30-OCT-2005                                                    (7) (226)

Type:           static
Species:        Daphnia magna  (Crustacea)
Exposure period: 24 hour(s)
Unit:           mg/l                  Analytical monitoring:
CE(1) :         = .06 - .1

Test substance: other TS: hypochlorite solution

Test condition: juvenile; static with the T90301 norm Temperature: 20 degree
                C
Test substance: Hypochlorite solution 12.7% active chlorine w/w
Reliability:    (3)  invalid
30-OCT-2005                                                        (228)

Type:           static
Species:        Daphnia magna  (Crustacea)
Exposure period: 48 hour(s)
Unit:           mg/l                  Analytical monitoring: no
EC50:           = .02 -

Test substance: other TS: sodium hypochlorite

Remark:         This data was already in the ECB IUCLID file. The study is
                not available to the notifier.
Test condition: Static Juvenile (< 24 h).
                Temperature: 17.5-19 degree C, pH=8.4
Reliability:    (4)  not assignable
30-OCT-2005                                                        (232)

Type:           flow through
Species:        other: Pontogeneiy sp.
Exposure period: 96 hour(s)
Unit:           mg/l                  Analytical monitoring: yes
EC50:           = .583 - .864

Method:         other: American Public Health Association, 1971
   GLP:         no data

Result:         Results in mg/l of total residual oxidant (TRO)
Test condition: Life stage: juvenile
                Temperature: 15 degree C, acclimatization at 10 degree C,
                pH=8.0, seawater
Test substance: Commercial product of Clorox, Oakland CA:
                - 5.25 % sodium hypochlorite
                - 4.12 % sodium chloride
                - 0.20 % sodium carbonate
                - 0.01 % sodium hydroxide
                in water
Reliability:    (4)  not assignable
30-OCT-2005                                                    (7) (226)

Type:           static
Species:        Daphnia magna  (Crustacea)
Exposure period: 48 hour(s)
```

Unit: mg/l Analytical monitoring: no
EC50: = 1.7 -

Test substance: other TS: sodium hypochlorite

Test condition: Life stage: juvenile Temperature: 20 degree C, pH=7.8
Reliability: (3) invalid
30-OCT-2005 (109)

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
EC50: = 2.1 -

GLP: no data

Test condition: 1st and 2nd larvae;
Temperature : 20 degree C, pH: 6.5 to 8.5, freshwater.
Test substance: 5.25% solution
Reliability: (3) invalid
30-OCT-2005 (78)

Species: Daphnia pulex (Crustacea)
Exposure period: 96 hour(s)
Unit: µg/l Analytical monitoring: no data
EC50: = 490 -

Method: other: not specified
GLP: no data
Test substance: no data

Remark: no additional information given
Reliability: (3) invalid
30-OCT-2005 (75)

Type: static
Species: Gammarus fasciatus (Crustacea)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
EC50: = 4 -

GLP: no data

Test condition: juvenile;
Temperature : 20 degree C, pH: 6.5 to 8.5, freshwater.
Test substance: 5.25% solution
Reliability: (3) invalid
30-OCT-2005 (78)
Type: static
Species: Nitocra spinipes (Crustacea)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
EC50: = 40 -

Method: other: GESAMP Reports and Studies No. 17 (IMO, London) 1982
Test substance: other TS: sodium hypochlorite

Test condition: Life stage: adult
Temperature: 10 degree C, pH=7.8, brackish water (salinity

```
                  7/1000)
Test substance:   sodium hypochlorite technical grade in a solution containing
                  8-12% active chlorine.
Reliability:      (3)  invalid
30-OCT-2005                                                          (21) (142)

Type:             static
Species:          Palaemonetes pugio  (Crustacea)
Exposure period:  96 hour(s)
Unit:             mg/l                    Analytical monitoring:
EC50:             = 5.9 -

Test substance:   other TS: sodium hypochlorite

Remark:           Analytical monitoring: amperometric titration
Test condition:   Life stage: adult
                  Temperature: 22 degree C, pH=8.3-8.7, synthetic seawater
                  salinity 25 plus or minus 1 g/l
Test substance:   aqueous solution of sodium hypochlorite 4-6%
Reliability:      (4)  not assignable
30-OCT-2005                                                              (57)

Species:          other aquatic arthropod
Unit:             mg/l                    Analytical monitoring: no data
:                 = 4.5 - 10.5

Method:           other: Aquatic arthropod toxicity test
  Year:           1958
   GLP:           no
Test substance:   other TS

Reliability:      (3)  invalid
30-OCT-2005                                                             (254)

Type:             static
Species:          other aquatic arthropod: Hydropsyche pellucidulla (caddisfly,
                  trichoptera)
Exposure period:  72 hour(s)
Unit:             µg/l                    Analytical monitoring: no data
EC50:             = 1.73 -

Method:           other: Invertebrate toxicity test
  Year:           1991
   GLP:           no data
Test substance:   other TS: TRC

Test condition:   Endpoint was development of larvae in a static test.
                  Water:
                  temperature = 15.5 degree C
                  hardness = 25.1 - 25.6 mg CaCO3/l
                  alkalinity = 36.8 - 37 mg CaCO3/l
                  dissolved O2 = 9 - 9.2 mg/l
                  pH = 7.5
Test substance:   Chlorine is expressed as total residual chlorine (TRC).
Reliability:      (3)  invalid
30-OCT-2005                                                              (38)

Type:             static
Species:          other aquatic mollusc
Exposure period:  96 hour(s)
Unit:             mg/l                    Analytical monitoring: no
```

EC50: = 59 -

GLP: no data
Test substance: other TS

Remark: Species: Helisoma trivolvis (Ramshorm snail)
Test condition: Life stage: juvenile Temperature: 20 degree C, pH=6.5-8.5, Lake Ontario water
Test substance: 5.25% solution
Reliability: (3) invalid
30-OCT-2005 (78)
Species: other aquatic mollusc: Corbicula fluminea
Exposure period: 28 day(s)
Unit: Analytical monitoring: yes

Year: 1991
GLP: no data
Test substance: other TS: TRC

Result: After a period of 28 days, 3.3 and 39 % mortalities were recorded for each life stage, respectively. At 23 degree C the same toxicant level resulted in lethal time LT50 of 14.4 and 23.3 days for juveniles and adults, respectively. All juveniles died by day 17, and 66.7 % of adults died by day 28.
Test condition: Corbicula adults and juveniles were exposed to 0.29 mg TRC/l(total residual chlorine) at 7 degree C.
Test substance: Chlorine is expressed as total residual chlorine (TRC).
Reliability: (3) invalid
30-OCT-2005 (19)

Species: other aquatic mollusc: Elimia clavaeformis (club elimia, gastropoda)
Exposure period: 24 hour(s)
Unit: µg/l Analytical monitoring: no data
Effect concentration :
> 400 -

Method: other: not specified
Year: 1990
GLP: no data
Test substance: no data

Test condition: Flow-through test; water temperature = 24 degree C; measured endpoint was immobilization.
Reliability: (3) invalid
30-OCT-2005 (34)

Species: other aquatic mollusc: Mercenaria mercenaria
Exposure period: 48 hour(s)
Unit: µg/l Analytical monitoring: yes
EC50: = 6 -

Method: other: Acute toxicity test
Year: 1975
GLP: no
Test substance: other TS: TRC

Remark: Species were selected from river estuaries.
Test condition: A constant addition test system was used; temperature ranged from 17 to 28 degree C and salinity from 18.2 to 20.4 per

mill; dissolved O2 was always near saturation.
Test substance: Stock solution was prepared by dissolving calcium hypochlorite in deionized water buffered to ca. pH8. Chlorine is expressed as total residual chlorine (TRC).
Reliability: (3) invalid
31-OCT-2005 (187)

Type: static
Species: other aquatic mollusc: Nitocris sp.
Exposure period: 48 hour(s)
Unit: µg/l Analytical monitoring: no data
LC50 : = 5300 -

Method: other: Invertebrate toxicity test
Year: 1978
GLP: no
Test substance: other TS: NaOCl or Ca(OCl)2

Result: The 24-hour LC50 was 8300 microg/l.
Test condition: Static test condition at 25 degree C water temperature. Water quality:
- Alkalinity = 42 mg CaCO3/l
- Hardness = 45 mg CaCO3/l
- pH = 7.5
Reliability: (3) invalid
31-OCT-2005 (35)

Species: other aquatic crustacea: Crassostrea virginica
Exposure period: 48 hour(s)
Unit: µg/l Analytical monitoring: yes
EC50: < 5 -

Method: other: Acute toxicity test
Year: 1975
GLP: no
Test substance: other TS: TRC

Remark: Species were selected from river estuaries.
Test condition: A constant addition test system was used; larvae were aerated; temperature ranged from 17 to 28 degree C and salinity from 18.2 to 20.4 per mill; dissolved O2 was always near saturation.
Test substance: Stock solution was prepared by dissolving calcium hypochlorite in deionized water buffered to ca. pH=8. Chlorine is expressed as total residual chlorine (TRC).
Reliability: (3) invalid
31-OCT-2005 (187)

Type: static
Species: other aquatic crustacea: Crassostrea virginica
Exposure period: 48 hour(s)
Unit: µg/l Analytical monitoring: yes
EC50: = 110 -

Method: other: Acute toxicity test
Year: 1975
GLP: no
Test substance: other TS: TRC

Remark: Species were selected from river estuaries.

Test condition: Test was performed under static condition with larvae; intermittent chlorine addition; temperature ranged from 17 to 28 degree C and salinity from 18.2 to 20.4 per mill; dissolved O2 was always near saturation.
Test substance: Stock solution was prepared by dissolving calcium hypochlorite in deionized water buffered to ca. pH8. Chlorine is expressed as total residual chlorine (TRC).
Reliability: (3) invalid
31-OCT-2005 (187)

Type: flow through
Species: other aquatic crustacea: Crassostrea virginica
Exposure period: 48 hour(s)
Unit: µg/l Analytical monitoring: yes
EC50: ca. 23 -

Method: other: Acute toxicity test
Year: 1975
GLP: no
Test substance: other TS: TRC

Remark: The EC50-value was extrapolated. Lowest tested concentration was 40 microg/l. Species were selected from river estuaries.
Test condition: Test was performed under flow through condition with juveniles; lowest tested concentration was 0.04 mg/l; temperature ranged from 17 to 28 degree C and salinity from 18.2 to 20.4 per mill; dissolved O2 was always near saturation.
Test substance: Stock solution was prepared by dissolving calcium hypochlorite in deionized water buffered to ca. pH8. Chlorine is expressed as total residual chlorine (TRC).
Reliability: (3) invalid
31-OCT-2005 (187)

Species: other aquatic crustacea: Grass shrimp
Exposure period: 96 hour(s)
Unit: µg/l Analytical monitoring: no data
Median toxic level :
= 220 -

Method: other: not specified
Year: 1977
GLP: no
Test substance: no data

Test condition: Saltwater species; York river (VA) water; no additional information.
Reliability: (3) invalid
31-OCT-2005 (75)

Type: flow through
Species: other aquatic crustacea: Orconectes rusticus
Unit: µg/l Analytical monitoring: no data
Lethal concentration :
= 50 -

Method: other: Acute toxicity test
Year: 1975
GLP: no
Test substance: other TS: TRC

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Test condition:   Test was performed under flow through conditions and at
                  water temperature of 8 degree C and 25 degree C.
Test substance:   Chlorine is expressed as total residual chlorine (TRC).
Reliability:      (3)  invalid
31-OCT-2005                                                          (148)

Species:          other: Acartia tonsa
Exposure period:  48 hour(s)
Unit:             µg/l                  Analytical monitoring: yes
EC50:             = 50 -

Method:           other: Acute toxicity test
  Year:           1975
   GLP:           no
Test substance:   other TS: TRC

Remark:           Species were selected from river estuaries.
Test condition:   A constant addition test system was used; temperature ranged
                  from 17 to 28 degree C and salinity from 18.2 to 20.4 per
                  mill; dissolved O2 was always near saturation.
Test substance:   Stock solution was prepared by dissolving calcium
                  hypochlorite in deionized water buffered to ca. pH=8.
                  Chlorine is expressed as total residual chlorine (TRC).
Reliability:      (3)  invalid
31-OCT-2005                                                          (187)

Type:             flow through
Species:          other: Acartia tonsa (crustacea copepod)
Exposure period:  30 minute(s)
Unit:             mg/l                  Analytical monitoring:
EC50:             = .82 - .86
EC100:            = 3.5 - 3.6

Test substance:   other TS: sodium hypochlorite

Test condition:   Life stage: no data
                  Temperature: 10/15/20/25/28 degree C, pH=8
                  30 min exposure and 48 hours observation
Reliability:      (4)  not assignable
31-OCT-2005                                                           (40)

Type:             static
Species:          other: Asellus intermedius
Exposure period:  96 hour(s)
Unit:             mg/l                  Analytical monitoring: no
EC50:             = 32 -

   GLP:           no data
Test substance:   other TS: sodium hypochlorite

Test condition:   Life stage: juvenile Temperature: 20 degree C, pH=6.5-8.5,
                  Lake Ontario water
Test substance:   5.25% solution
Reliability:      (3)  invalid
31-OCT-2005                                                           (78)

Type:             static
Species:          other: Branchionus calyciflorus (rotifer)
Exposure period:  24 hour(s)
Unit:             mg/l                  Analytical monitoring:
```

```
EC50:             = .37 -

Test substance:   other TS: sodium hypochlorite

Test condition:   Life stage: juvenile Temperature: 25 degree C, pH=7.9
Reliability:      (3)  invalid
31-OCT-2005                                                          (213)

Type:             flow through
Species:          other: Branchionus plicatilis (rotifer)
Exposure period:  30 minute(s)
Unit:             mg/l                    Analytical monitoring:
EC50:             = .01 - .18
EC100:            = .46 - 1.76


Test condition:   Temperature: 20/25/27.5 degree C, pH=8,
                  30 min exposure and 48 hours observation
Reliability:      (4)  not assignable

31-OCT-2005                                                           (41)

Type:             flow through
Species:          other: Crassostrea virginica (mollusc bivalve)
Exposure period:  30 minute(s)
Unit:             mg/l                    Analytical monitoring: yes
EC50:             = .08 - .12
EC100:            = .86 - 1.4

Test substance:   other TS: sodium hypochlorite

Test condition:   Life stage: larvae (7d)
                  Temperature: 20/25 degree C, pH=8,
                  30 min exposure and 48 hours observation
Reliability:      (4)  not assignable
31-OCT-2005                                                           (40)

Type:             flow through
Species:          other: Dreissena polymorpha
Exposure period:  18 hour(s)
Unit:             mg/l                    Analytical monitoring: yes
EC100:            = 1 -

Test substance:   other TS: sodium hypochlorite

Test condition:   Life stage: larvae (veligers)
                  Temperature: 20 degree C, pH=8.3
Reliability:      (3)  invalid
31-OCT-2005                                                           (23)

Type:             static
Species:          other: Dugesia tigrina
Exposure period:  96 hour(s)
Unit:             mg/l                    Analytical monitoring: no
EC50:             = 32 -

   GLP:           no data
Test substance:   other TS: sodium hypochlorite

Test condition:   Life stage: juvenile Temperature: 20 degree C, pH=6.5-8.5,
                  Lake Ontario water
```

```
Test substance:   5.25% solution
Reliability:      (3)  invalid
31-OCT-2005                                                          (78)

Type:             flow through
Species:          other: Homarus americanus (crustacea decapod)
Exposure period:  60 minute(s)
Unit:             mg/l                  Analytical monitoring: yes
EC50:             = .41 - 2.89

Test substance:   other TS: sodium hypochlorite

Test condition:   Life stage: larvae (stage I)
                  Temperature: 20/25/30 degree C, pH=8,
                  60 min exposure and 48 hours observation
Reliability:      (4)  not assignable
31-OCT-2005                                                          (43)

Species:          other: Keratella cochlearis (rotifer)
Exposure period:  4 hour(s)
Unit:             µg/l                  Analytical monitoring: no data
LC50 :            = 19 -

Method:           other: not specified
  Year:           1981
   GLP:           no data
Test substance:   no data

Remark:           no additional information given
Reliability:      (3)  invalid
31-OCT-2005                                                          (75)

Species:          other: Larval clam
Exposure period:  100 hour(s)
Unit:             µg/l                  Analytical monitoring: no data
LC100 :           = 500 -

Method:           other: not specified
  Year:           1981
   GLP:           no data
Test substance:   no data

Remark:           no additional information given
Reliability:      (3)  invalid
31-OCT-2005                                                          (75)

Type:             static
Species:          other: Lumbriculus variegatus
Exposure period:  96 hour(s)
Unit:             mg/l                  Analytical monitoring: no
EC50:             = 3.2 -

   GLP:           no data
Test substance:   other TS: sodium hypochlorite

Test condition:   Life stage: juvenile Temperature: 20 degree C, pH=6.5-8.5,
                  Lake Ontario water
Test substance:   5.25% solution
Reliability:      (3)  invalid
31-OCT-2005                                                          (78)
```

Remark: Additional toxicity studies of aquatic organisms are cited in the document of US EPA (Ambient Water Quality, 1984). In general, freshwater fish, saltwater fish and invertebrates had similar ranges of sensitivity to "free" chlorine (=refers to strongly oxidative forms also known as TRC or CPO). The reported values ranged from 28-710 5g/l for33 freshwater species and 26-1400 5g/l for 28 saltwater species. Toxicity is dependent upon factors such as temperature, form of TRC and light. Sensitivity generally rises with temperature.
Source: MITSUBISHI CHEMICAL SAFETY INSTITUTE LTD. Tokyo
Reliability: (4) not assignable
08-JAN-2004 (76)

4.3 Toxicity to Aquatic Plants e.g. Algae

Remark: Calcium hypochlorite which is dissolved in water is in a fast equilibrium with hypochlorous acid which again is in a fast equilibrium with hypochlorite ions. In general the concentration of chlorine in a test system is achieved by dissolving hypochlorous acid or sodium hypochlorite and not by application of gaseous chlorine.

Because of this equilibrium concentrations, in general, are provided as free available chlorine or as total residual chlorine (TRC). (See chapter 1.11 for definition.)
The studies were performed by independent laboratories and published in peer reviewed papers. Many studies were performed in the 70ties, when effects of the biocidal application of chlorine were carefully reinvestigated. Therefore the studies are not performed according to recent guidelines and no GLP information is provided.

With regard to the extensive number of tests that were already performed with chlorine no further studies according to recent guidelines were conducted to avoid further animal testing.
Flag: Critical study for SIDS endpoint
31-OCT-2005

Species: other algae: Thalassiosira pseudonana (diatom)
Endpoint: growth rate
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no
EC50: = .075 -

Test substance: other TS: sodium hypochlorite

Method: Algae were exposed to chlorine for 24 hours and chlorine action was stopped either by the addition of sodium thiosulfate or transfer of the test species to clean seawater. Post exposure response of growth, photosynthesis and mortality were monitored for 48 to 96 hours. Thiosulfate was shown to have no effect on the organisms at the levels used.

Rates of photosynthesis were determined immediately after dosing ceased by labelling an aliquot of the culture with

Na214CO3 (0.1 microCi/2.4 microMc/ml). Triplicate light and duplicate dark exposures were incubated for four hours, filtered at less than 5 psi, and filters were exposed to HCl fumes for 60 seconds, and the assimilated radioactivity was counted by liquid scintillation spectrometry.

In order to determine if significant amounts of chlorine were lost during the 24-hour period, seawater dosed at 5 ppm was monitored every four hours. After 24 hours this value decreased to 4.3 ppm. Thus approximately 14% was lost over a24 hour period.

Remark: The intention of the study was to investigate the influence of the discharge of cooling water that contains chlorine for antifouling purposes on non-target organisms. As part of these study 11 species of marine phytoplanktion were investigated (see additional entries below). Four species were chosen for an more extensive investigation (see additional entries below). The diatom Thalassiosira pseudonana was found to be the most susceptible and chosen for the most extensive investigation.

The report has been reviewed by the Environmental Research Laboratory, U.S. EPA and approved for publication.

Result: Concentration given produced a 50% reduction in the growth rate during a 24 hours exposure period. Results expressed as Cl2.

Table 1: Post exposure growth rates (24-hours) after exposure to chlorine for 10 seconds to 20 minutes.

Exposure	Chlorine concentration [mg/L]					
[s]	1.0	0.5	0.4	0.3	0.2	0.15
control	1.92	1.82	2.44	2.96	2.70	2.42
10	0.06	1.55	2.30			
15		0.90	2.30	2.95		
20	0.03	0.55	2.20			
		- 80/255 -				
30	0.03	0.01	2.20	2.75	2.60	
60	N.G.	N.G.	1.26	2.83	2.50	
150			N.G.	0.96	2.70	
300				N.G.	2.60	2.60
600					2.10	2.30
1200					1.10	2.10

N.G.: No growth rate measurable

Table 2: Immediate effects on photosynthesis [% of control] due to exposure to chlorine for 10 seconds to 20 minutes.

Exposure	Chlorine concentration [mg/L]					
[s]	1.0	0.5	0.4	0.3	0.2	0.15
control	100	100	100	100	100	100
10	16	68	79			
15		21	67	100		
20	14	17	65			
30	13	14	58	100	99	
60	7	6	30	88	90	
150			24	25	68	
300				0	70	100

	600 64 62 1200 33 48 _____ 24-hours IC50 values [mg/l] for other species measured at 20 degree C: Skeletonema costatum 0.095 Rhodomonas baltica 0.110 Dunaliella tertiolecta 0.110 Monochrysis lutheri 0.200 24-hours IC50 values [mg/l] for other species measured at 10 degree C: Chaetoceros decpiens 0.140 Thalassiosira nordensholdii 0.195 Thalassiosira rotula 0.330 Asterionella japonica 0.250 Chaettoceros didymum 0.125 Detonula confervacea 0.200
Test condition:	Synthetic sea water, 2500 lux illumination. Temperature 20 degree C. Growth rates were determined daily be cell counts using an electronic particle counter
Reliability:	(2) valid with restrictions
Flag:	Critical study for SIDS endpoint
31-OCT-2005	(90)
Species:	Dunaliella tertiolecta (Algae)
Endpoint:	growth rate
Exposure period:	24 hour(s)
Unit:	mg/l Analytical monitoring: no
EC50:	= .11 -
Test substance:	other TS: sodium hypochlorite
Method:	Algae were exposed to chlorine for 24 hours and chlorine action was stopped either by the addition of sodium thiosulfate or transfer of the test species to clean seawater. Post exposure response of growth, photosynthesis and mortality were monitored for 48 to 96 hours. Thiosulfate was shown to have no effect on the organisms at the levels used. Rates of photosynthesis were determined immediately after dosing ceased by labelling an aliquot of the culture with Na214CO3 (0.1 microCi/2.4 microMc/ml). Triplicate light and duplicate dark exposures were incubated for four hours, filtered at less than 5 psi, and filters were exposed to HCl fumes for 60 seconds, and the assimilated radioactivity was counted by liquid scintillation spectrometry. In order to determine if significant amounts of chlorine were lost during the 24-hour period, seawater dosed at 5 ppm was monitored every four hours. After 24 hours this value decreased to 4.3 ppm. Thus approximately 14% was lost within a 24-hour period.
Remark:	The report has been reviewed by the Environmental Research Laboratory, U.S. EPA and approved for publication.
Result:	Concentration given produced a 50% reduction in the growth rate during a 24 hours exposure period. Results expressed as Cl2.
Test condition:	Synthetic sea water, 2500 lux illumination. Temperature 20 degree C. Growth rates were determined daily from cell

counts using an electronic particle counter.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (90)

Species: Skeletonema costatum (Algae)
Endpoint: growth rate
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no
EC50: = .095 -

Method: other
Year: 1976
Test substance: other TS: sodium hypochlorite

Method: Algae were exposed to chlorine for 24 hours and chlorine action was stopped either by the addition of sodium thiosulfate or transfer of the test species to clean seawater. Post exposure response of growth, photosynthesis and mortality were monitored for 48 to 96 hours. Thiosulfate was shown to have no effect on the organisms at the levels used.
Rates of photosynthesis were determined immediately after dosing ceased by labelling an aliquot of the culture with Na214CO3 (0.1 microCi/2.4 microMc/ml). Triplicate light and duplicate dark exposures were incubated for four hours, filtered at <-5 psi, and filters were exposed to HCl fumes for 60 seconds, and the assimilated radioactivity was counted by liquid scintillation spectrometry.
In order to determine if significant amounts of chlorine were lost during the 24-hour period, seawater dosed at 5 ppm was monitored every four hours. After 24 hours this value decreased to 4.3 ppm. Thus approximately 14% was lost over a 24 hour period.
Remark: The report has been reviewed by the Environmental Research Laboratory, U.S. EPA and approved for publication.
Result: Concentration given produced a 50% reduction in the growth rate during a 24 hours exposure period. Results expressed as Cl2
Test condition: Synthetic sea water, 2500 lux illumination. Temperature 20 degree C. Growth rates were determined daily be cell counts using an electronic particle counter.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (90)

Species: other algae: Monochrysis lutheri (sea water algae)
Endpoint: growth rate
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no
EC50: = .2 -

Remark: The report has been reviewed by the Environmental Research Laboratory, U.S. EPA and approved for publication.
Result: Concentration given produced a 50% reduction in the growth rate during a 24 hours exposure period. Results expressed as Cl2
Test condition: Synthetic sea water, 2500 lux illumination. Temperature 20 degree C. Growth rates were determined daily be cell counts using an electronic particle counter.

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (90)

Species: other algae: Thalassiosira rotula (diatom)
Endpoint: growth rate
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring:
EC50: = .33 -

Remark: The report has been reviewed by the Environmental Research Laboratory, U.S. EPA and approved for publication.
Result: Results expressed as Cl2
Test condition: Synthetic sea water, 2500 lux illumination. Temperature 20 degree C. Growth rates were determined daily be cell counts using an electronic particle counter.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (90)

Species: Dunaliella sp. (Algae)
Endpoint: growth rate
Exposure period: 72 hour(s)
Unit: mg/l Analytical monitoring: yes
EC50: = .4 -
EC100 : = .6 -

Test substance: other TS: sodium hypochlorite

Result: Results expressed as Cl2.
Toxicity increases with decreasing cellular concentration.
Test condition: Temperature: 20 degree C
Reliability: (3) invalid
31-OCT-2005 (235)

Species: Phaeodactylum tricornutum (Algae)
Endpoint: biomass
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: yes
EC20 : = .6 -
EC100 : = .8 -

Result: Results expressed as Cl2
Test condition: Temperature: 20 degree C
Reliability: (3) invalid
31-OCT-2005 (235)

Species: other algae: Pavlova lutheri
Unit: mg/l Analytical monitoring: yes
EC50: = 3.5 - 4

GLP: no data
Test substance: other TS: sodium hypochlorite

Test condition: Temperature: 20 degree C, seawater
Reliability: (3) invalid
31-OCT-2005 (235) (236)

Species: Chlorella sp. (Algae)

Endpoint: other: mortality
Exposure period: 20 hour(s)
Unit: mg/l Analytical monitoring:
EC40 : = .6 -

Remark: Species: Chlorella sorokiniana
Algae number: same conditions : EC27 = 0.2 mg/l.
Test condition: Temperature: 30 degree C, pH=7
Reliability: (3) invalid
31-OCT-2005 (133)

Species: other algae
Endpoint: other: chlorophyll A production

Test substance: other TS: sodium hypochlorite

Result: At 0.1 mg/l (as Cl2), slight change in chlorophyll A for phytoplankton At 1 mg/l (as Cl2), decrease chlorophyll A, increase phaeophytin A
Reliability: (3) invalid
31-OCT-2005 (28)

Species: other algae: Scenedesmus acuminatus (green algae)
Endpoint: other: sinking rates
Exposure period: 30 minute(s)
Unit: µg/l Analytical monitoring: no data
Effect concentration :
= 7500 -

Method: other: not specified
GLP: no data
Test substance: no data

Test condition: Static test; water temperature = 20 degree C
Reliability: (3) invalid
31-OCT-2005 (181)

Species: other algae: marine phytoplankton
Endpoint: biomass
Exposure period: 23 day(s)
Unit: mg/l Analytical monitoring: yes
EC70 : = .25 -

Test substance: other TS: sodium hypochlorite

Result: Results expressed as Cl2
Test condition: temperature: 23.6 to 24.4 degree C
pH: 7.7 to 7.9
Reliability: (3) invalid
31-OCT-2005 (194)

Species: other algae: plankton
Endpoint: other: photosynthesis
Exposure period: 3 hour(s)
Unit: µg/l Analytical monitoring: no data
EC50: = 90 -

Method: other: Algae toxicity test
Year: 1978
GLP: no

```
Test substance:   no data

Test condition:   Test was performed under static condition at a water
                  temperature of 21 degree C.
Reliability:      (3)  invalid
31-OCT-2005                                                          (61)


Species:          other algae: plankton
Endpoint:         other: physiological effects (not specified)
Exposure period:  14 day(s)
Unit:             µg/l                   Analytical monitoring: no data

Effect concentration :
                  = 1000 -

Method:           other: Algae toxicity test
  Year:           1979
   GLP:           no
Test substance:   no data

Test condition:   Test was performed under flow through condition.
Reliability:      (3)  invalid
31-OCT-2005                                                         (161)

Species:          other aquatic plant: Lemna minor
Endpoint:         biomass
Unit:             µg/l                   Analytical monitoring: no data
EC10:             = 930 -

Method:           other: Aquatic plant toxicity test
  Year:           1986
   GLP:           no data
Test substance:   other TS: chlorine

Test condition:   Test was performed under static conditions and at a water
                  temperature of 27 degree C (pH = 7.5).
Reliability:      (3)  invalid
31-OCT-2005                                                         (238)

Species:          other aquatic plant: Macrocystis pyrifera
Endpoint:         other: photosynthesis
Exposure period:  2 day(s)
Unit:             µg/l                   Analytical monitoring: no data
Effect concentration :
                  >= 5000 -

Method:           other: see reference
  Year:           1963
   GLP:           no

Test substance:   other TS: free chlorine

Remark:           Exposure of giant kelp to 1 mg/l of chlorine for 5 days did
                  not affect photosynthetic capacity. Exposure to 5 to 10 mg/l
                  of chlorine led to a 10-15% reduction in photosynthesis
                  after 2 days and to a 50-70% reduction after 5 to 7 days.
Reliability:      (3)  invalid
31-OCT-2005                                                         (153)

Species:          other aquatic plant: Myriophyllum spicatus
```

Endpoint: other: growth and length of sprouts
Exposure period: 96 hour(s)
Unit: µg/l Analytical monitoring: no data
Effect concentration :
= 50 - 100

Method: other: Aquatic plant toxicity test
Year: 1984
GLP: no data
Test substance: other TS: chlorine

Remark: Chlorine affected growth and decreased length of sprouts (16% reduction) at a concentration of 50 5g/l and reduced chlorophyll content at a concentration of 100 5g/l.
Test condition: Test was performed under flow through conditions. Plants were 5 cm lo
Reliability: (3) invalid
31-OCT-2005 (240)

Species: other aquatic plant: Phaeodactylum tricornutum, Pavlova lutheri
Endpoint: other: growth, LD50
Unit: µg/l Analytical monitoring: no data
Toxic concentration :
>= 600 -

Method: other: not specified
Year: 1979
GLP: no
Test substance: no data

Remark: Phaeodactylum: reduced or ceased growth at 600 5g/l Pavlova: LD50 = 4000 5g/l
no additional information
Reliability: (3) invalid
31-OCT-2005 (75)
Species: other algae

Method: Chlorine toxicity to an alge community was investigated in an laboratory microcosm for 28 days. The microcosm was an artificial flow-through system that comprised colonized species of microscopic organisms from low trophic levels (bacteria, phytoplankton, zooplankton, and protozoa). The substrates were placed in a headbox, illuminated with 5000 lux for 12 h in every 24 h. The feed water was mixed with stock solutions of sodium hypochlorite to produce triplicates of the six nominal test concentrations covering the range 0-300ug/l. The flow rate of toxicant and diluent was maintained at about 12 turnover volumes per day, and the mean temperature was 13.5 degree C (range 9.6-17.0 degree C). The TRC was determined by titration three times weekly for each test chamber, and indicated that the nominal concentrations were quite well maintained and that virtually all the chlorine was present in its free form. The island substrates were examined on days 3, 7, 14, 21 and 28. On each sampling day, taxonomic parameters were measured, whilst on day 28 the non-taxonomic responses including total protein, extracellular alkaline phophatase activity, chlorophyll a, potassium and ATP were also determined. The non-taxonomic data were analyzed using the one-way ANOVA and Duncan's multiple range test, to define LOEC and NOEC

values.
Reliability: (4) not assignable
31-OCT-2005

4.4 Toxicity to Microorganisms e.g. Bacteria

Remark: The toxicity to microorganisms in other words the disinfection capacity of chlorine is very wide against bacteria, fungi, viruses and algae from concentration as low as 0.1 mg/l of active chlorine.

Calcium hypochlorite which is dissolved in water is in a fast equilibrium with hypochlorous acid which again is in a fast equilibrium with hypochlorite ions. In general the concentration of chlorine in a test system is achieved by dissolving hypochlorous acid or sodium hypochlorite and not by application of gaseous chlorine.

Because of this equilibrium concentrations, in general, those are provided as free available chlorine or as total residual chlorine (TRC). (See chapter 1.11 for definition.)

Bacterial toxicity of chlorine is mostly due to undissociated hypochlorous acid (HOCl). At a lower pH, the proportion of HOCl will be higher and the toxicity will increase. The
toxicity is also highly dependent on temperature, dissolved oxygen, and synergism or antagonism of other dissolved materials.

A large number of studies were performed to investigate the disinfection capacity of chlorine. Parts of these studies were provided in the HEDSET submitted by the manufacturers under the EU Existing Chemicals Regulation 793/93. The notifier has revised several of them However, some entries provided with the HEDSET have not been revised but were left in the IUCLID file all the same to avoid loss of information.

The studies are not performed according to recent guidelines and no GLP information is provided. With regard to the numerous tests that were already performed with chlorine no further studies according to recent guidelines were conducted. The data presented is extensive and accurate and reflects the effects of chlorine on microorganisms.
Flag: Critical study for SIDS endpoint
31-OCT-2005

Type: other: laboratory microcosms and field enclosures
Unit: Analytical monitoring: yes
See Result : -

Method: other
Year: 1988
GLP: no
Test substance: other TS

Method: Laboratory microcosm and field enclosures were used to evaluate effect of chlorine on microbial community structure

and function. Microcosms were exposed to chlorine (as sodium hypochlorite) at concentrations up to 308 micro-gram/L total residual chlorine (TRC) for 28 days.
Test systems were sampled weekly to evaluate protozoan species accrual, biomass distribution, microbial enzyme activity, and macronutrient retention.

Result: Protozoan species numbers were depressed at all sampling times at TRC concentrations greater than 25 micro-gram/L.
Algal biomass (chlorophyll a) was adversely affected at 2 micro-gram/L, alkaline phosphotase activity was inhibited at greater than 6 micro-gram/L. Other biomass measures and macronutrient retention were affected at 25 to 308 micro-gram/L.
Oxygen production was depressed at greater than 25 micro-gram/L.
Field (enclosures) sediment-water mesocosms) were dosed daily with chlorine, resulting in average chlorine doses up to 261 micro-gram/L.

Protozoan species numbers were depressed at chlorine dosed greater than 79 micor-gram/L, and zooplankton density was affected at 24 micro-gram/L.
Algal biomass and total biomass were adversely affected the highest chlorine level, 261 micro-gram/L. Nontaxonomic measures were typically less sensitive than community structure responses to chronic stress.
Estimated effect levels for both experiments overlapped; however the response of specific variables (i.e., stimulation, inhibition, no effect) chlorine differed between the two tests.
These results support the importance of experimental design and dosage regime in chronic toxicity testing.

Laboratory microcosm
Results of nonlinear regression of species number in time based on the MacArthur-Wilson equilibrium model in the laboratory microcosm tes.

Treatment	Seq
Control	40.7
2.1 ug/L	39.1
6.1	31.0*
25	30.4*
100	25.7*
308	18.7*

NOEC = 2.1 micro-gram/L

Outdoor mesocosms
Number of algal genera and zooplankton density in outdoor mesocosms on 24 day

Treatment	Algal genera	Zooplankton (/mL)
Control	44.0	10.2
0.4 ug/L	42.7	11.4
1.5	41.3	7.0
24	40.3	5.4*
79	39.0	4.3*
261	30.0*	1.7*

NOEC = 79 micro-gram/L(Algal genera)
NOEC = 1.5 micro-gram/L(Zooplankton)
Test substance: chlorine as hypochlorite ion
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (184)

Type: aquatic
Species: other bacteria: aerobic and anaerobic microorganisms, various Gram+ and Gram- bacteria
Unit: mg/l Analytical monitoring:
LC100 : .055 - 50

Test condition: Exposure period 2 seconds to 2 hours
Reliability: (3) invalid
31-OCT-2005 (72)

Type: aquatic
Species: other protozoa
Exposure period: 2 hour(s)
Unit: µg/l Analytical monitoring: no data
LC53 : = 1450 -

Method: other: Microorganism toxicity test
GLP: no
Test substance: other TS: chlorine

Remark: 53% of protozoa were killed with 3 Cl2 additions and 94% were killed with 7 Cl2 additions.
Test condition: Chlorine was added to Douglas Lake water 3 or 7 times (intermittent chlorination of water).
Reliability: (3) invalid
31-OCT-2005 (75)

Type: aquatic
Species: other protozoa
Exposure period: 7 day(s)
Unit: mg/l Analytical monitoring: yes
EC50: = .0316 -

Test substance: other TS: sodium chloride

Remark: more detailed results given in chapter 4.7
Reliability: (3) invalid
31-OCT-2005 (37)

Remark: species: Vibrio cholorea, Salmonella typhimurium, Staphylococcus aureus
Result: CL100 = 0.036 % active chlorine solution
Reliability: (3) invalid
31-OCT-2005 (192)

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

Remark: Calcium hypochlorite which is dissolved in water is in a fast equilibrium with hypochlorous acid which again is in a fast equilibrium with hypochlorite ions. In general the concentration of chlorine in a test system is achieved by dissolving hypochlorous acid or sodium hypochlorite and not by application of gaseous chlorine.

Because of this equilibrium concentrations, in general, are provided as free available chlorine or as total residual chlorine (TRC). (See chapter 1.11 for definition.)
The studies were performed by independent laboratories and published in peer reviewed papers. Many studies were performed in the 70ties, when effects of the biocidal application of chlorine were carefully reinvestigated. Therefore the studies are not performed according to recent guidelines and no GLP information is provided.

With regard to the extensive number of tests that were already performed with chlorine no further studies according to recent guidelines were conducted to avoid further animal testing.

Flag: Critical study for SIDS endpoint
31-OCT-2005
Species: Pimephales promelas (Fish, fresh water)
Endpoint: other: growth and survival
Exposure period: 147 day(s)
Unit: µg/l Analytical monitoring: no data
NOEC: = 16 -

Method: other
Year: 1971
GLP: no
Test substance: no data

Method: Juvenile fathead minnows used to start the chronic study were 3 months old and ranged from 33 to 50 mm long; mean wet weight was 0.46 g. The test was conducted from Jan 14, 1970 to June 12, 1970. Ten individuals were assigned randomly to each 5-gallon test chamber. The temperature was maintained at 23 +/- 1 degree C and the photoperiod at 16 hours of light each day. Fish were fed daily with Oregon Moist pellets. Since food remaining in the fish tanks temporarily lowered the chloramine concentration by as much as 50%, excess food was removed by siphoning 2 hour after the fish were fed. The regular diet was supplemented 1-2 times/week with live Daphnia. Procedures employed during spawning were similar to those described by Mount (1968). The spawning sites provided to the fish were 6-inch-long, semicircular sections of concrete-asbestos water pipe (irrigation tiles); one tile was placed in each test tank with the concave surface downward. Eggs were attached to the undersides of these tunnel-like substrates during spawning and were immediately fertilized. Between 10 and 12AM each day, 25 or 50 unbroken eggs were placed in gently moving egg-incubation cups and the remainder discarded after counting, or (weekends) the eggs on the tiles were counted and discarded. Approximately one month after spawning started, the number of sexually mature males, as judged by secondary sexual characteristics and aggressive behavior, was reduced to a total of two per test chamber to reduce competition for

available spawning sites. No additional males were removed for the duration of the test. During incubation, live eggs were counted and dead ones were removed daily until hatching, on about the 4th day. Larvae were retained in the egg cups until the 7th day, when they were either counted and discarded or transferred to the set of 2-gal larval tanks for observation for 30 more days. Larvae were fed finely ground Oregon Moist "starter mash". The test was terminated after reproduction had slowed to less than one spawning a day among all the tanks for a week. As spawning rates at the various concentrations did not change near the end of the test, the spawning results presumable would not have been altered if the test had been allowed to continue.

Remark: Growth and survival not affected by continuous exposure of adults to 43 microg/l residual chlorine.

Result: All 20 fathead minnows in the tanks containing 154 microg/L total chloramines were killed within 72 hours. In the next highest concentration (85 microg/L), the first died after 7 days. No significant differences (P=0.05) in growth or survival of adult fathead minnows were found at concentrations <43 microg/L total chloramine. Survival and growth of adult fathead minnows in duplicate tanks after 21 weeks exposure:

Mean ug/L	chloramine	# surviving (males/females)	Mean adult Weight (g) Males	Females
0	A	2/5	3.33	1.36
	B	2/5	3.05	2.07
6.6	A	5/4	2.27	1.05
	B	4/5	2.74	1.20
16	A	3/7	3.08	1.36
	B	7/2	2.58	1.23
43	A	5/5	2.14	0.95
	B	3/5	2.18	1.33
85	A	3/0	2.42	--
	B	4/3	2.27	1.20
154	A	0	--	--
	B	0	--	--

A/B denote two tanks set up in parallel.

Spawning was practically eliminated at a concentration of 85 mg/L total chloramine. The number of spawning/female was significantly reduced (P=0.05) at 43 microg/L, and fewer eggs per female were produced at this concentration than in the lower test concentrations and control. Among groups of eggs incubated, no toxicant-induced differences were observed in the percentage of larvae surviving after 7 days. Spawning activity of fathead minnow adults and survival of larvae after 7 days

Mean chloramine (ug/L)		Total Spawning	Spawning Female	/Eggs Spawning	/ Eggs/ Female	Mean % Survival
0	A	25	5.0	77	383	75
	B	35	7.0	140	982	88
6.6	A	32	6.4	77	613	77
	B	24	4.8	86	411	80

16	A	37	5.3	109	578	69
	B	8	4.0	129	517	37
43	A	13	2.6	94	245	24
	B	16	3.2	67	214	79
85	A	0	0	0	0	96
	B	1	0.3	18	6	78

A/B denote two tanks set up in parallel.

Growth and survival of fathead minnow larvae for the next 30 days were reduced only at 108 microg/L. Survival and growth of fathead minnow larvae for 30 days

Mean chloramine (ug/L)		Initial # Larvae	%	Final Mean Survival Wt (g)
0	A	43, 49	72, 71	0.038 0.041
	B	43	93	0.047
3.8	A	44	98	0.074
	B	49	100	0.069
17	A	34	76	0.044
	B	--	--	--
40	A	37	68	0.032
	B	25	80	0.036
108	A	24	38	0.014
	B	--	--	--

A/B denote two tanks set up in parallel.

Test condition: Routine water-chemistry determinations were conducted once a week during the long-term studies. Dissolved oxygen ranged between 5.2-10.4; pH ranged from 7.2-8.6; total hardness ranged between 44-48; alkalinity ranged between 42-48 and acidity ranged from 0.7-4.4 mg/L.

Conclusion: NOEL was 16 mg/L. The effect of the lowest toxic concentrations was a reduction in the number of offspring produced.

Reliability: (2) valid with restrictions

Flag: Critical study for SIDS endpoint

31-OCT-2005 (9)

Species: other
Endpoint: weight of young fish
Exposure period: 133 day(s)
Unit: µg/l Analytical monitoring: yes

Method: other: See ME
Year: 1985
Test substance: other TS: Commercial hypochlorite solution.

Method: Bluegill, Channel catfish, White suckers and Rainbow trout (all juvenile) were exposed to chlorine in the presence and absence of ammonia for 134 days or 49 days (rainbow trout). The source of chlorine was 10 % sodium hypochlorite solution. Fish were exposed in test streams of 518 m length supplied with Mississippi River water. Two streams were used as control, the other four streams were dosed with measured (nominal): 6.0 (10), 3.9 (10), 52.5 (50) and 182.9, (250) microg/l and 0 or 3 mg/l ammonia.

(Note that there is confusion with regard to the

concentrations applied as one Table mentions the same values but units of mg/l). Addition of ammonia decreased the pH due to nitrification
processes. This pH effect was compensated by adding hydroxide).

Chlorine concentration was determined twice a day at 2 streams and in a 2 weeks interval in each stream.
Characteristics of the Mississipe River water:
Alkalinity: 116-160 mg/l as CACO3
Hardness: 132-178 mg/l as CACO3
pH: 7.4-8.5
dissolved Oxygen: 5.8-9.2 mg/l

Result: No relationship between treatment concentration and the growth and survival of bluegills (Lepomis macrochirus), white suckers (Catostomas commersoni), and rainbow trouts (Salmo gairdneri) was observed.
There was, however, a consistent pattern of reduced growth of channel catfish (Ictalurus punctatus) with increasing TRC concentrations. The mean final weights of catfish at the highest TRC exposure were 64% (of control).
The addition of ammonia (3 mg/l nitrogen) changed the effects of chlorine. Bluegills were still unaffected; growth and survival of channel catfish were reduced at all concentrations of chlorine: no survivals at mean TRC levels >= 24 microg/l.

Control/Low-dose/Medium-Dose/High-Dose
---
measured concentration (ug/L):0/5.0/52.5/182.9
Bluegills: NOEC =182.9
-Survival(%):64.2/48.4/70.0/50.0
-Mean weight(g):40.2/36.8/42.9/38.5
Channel catfish NOEC =52.5
-Survival(%):79.5/92.0/90.0/88.0
-Mean weight(g):24.5/23.6/17.8/15.7
White suckers: NOEC was unknown.
-Survival(%):89.0/80.0/86.0/88.0
-Mean weight(g):39.0/25.1/29.9/32.7
---
measured concentration (ug/L):0/5.4/54.9/206.5
Rainbow trout: NOEC = 206.5
-Survival(%):85.3/87.5/82.5/77.5
-Mean weight(g):33.2/36.8/40.5/31.6

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (105)

Species: Brevoortia tyrannus (Fish, estuary, marine)
Exposure period: 19 day(s)
Unit: mg/l Analytical monitoring: yes
NOEC: = .062 -

Test substance: other TS: sodium hypochlorite

Method: The fish were acclimated for 30 days in separate 1000L tanks supplied continuously with dechlorinated (sodium thiosulfate added to dechlorinate) discharge water at flow rates of approximately 0.19L/sec. Basic water quality of the

acclimation water, monitored daily, was as follows: temperature 30.9 + 1.31 degree C; pH 7.0 + 0.62; dissolved oxygen 3.8 + 1.23 mg/L; ammonia - N 0.3+0.28 mg/L and salinity 2.1+0.71%. Maintenance diets of trout chow were apportioned to the fish on a twice-daily schedule, during both acclimation and test periods.
During the test period, fish were placed in troughs constructed of 1.9 cm thick marine plywood covered with polyester-resin-impregnated fiberglass. The troughs were partitioned with four 0.64 cm thick methyl methacrylate polymer dividers which were spaced equidistantly across the troughs. Each was secured to opposite ends of the trough to form a 12 M long serpentine channel. Cooling water inflow rates were maintained at 0.38 L/sec resulting in hydraulic residence periods of approximately 60 minutes in all troughs, simulating cooling water retention times observed in the plant's discharge canal. A second trough, supplied with dechlorinated cooling water, served as the reference exposure trough. In all studies, test organisms were retained in the troughs at positions where the cooling waters had "aged" approximately 5, 30 and 60 minutes.
Fish were placed in submerged nylon cages (0.19M x 0.33M x 0.74M) located in the troughs at positions of 5, 30 and 60 minutes of halogen decay. Approximately 30 Brevoortia tyrannus (menhaden) were randomly distributed to segregated paired cages. Observations of fish mortality were made at hours 1, 2, 4, 16 and 24 hours during the first 24 hours and twice daily thereafter during the 19-day exposure period. Total length and wet-weight data of dead menhaden.

Result: Mean Survival of Brevoortia tyrannus in 19 day study:

Conc., mg/L	Reference (Control)	Chlorine
0.014	93.1	96.7
0.032	100	100
0.062	100	96.5

There were no differences P>0.05 in survival between chlorine and reference treatments.
There were no significant differences between the chlorinated and reference stations: Temperature 31.2 + 1.23 degree C; pH 7.1 + 0.49; Dissolved oxygen 4.1 + 0.91; ammonia - N 0.2 + 0.36; salinity 2.0 + 0.63. Total residual oxidant concentrations were measured twice daily and varied during these studies because of plant equipment malfunctions. Two temporary plant shutdowns for equipment repair occurred, during which periods the oxidant levels of <0,003 mg/L were recorded. However the downtime interruptions totaled less than 8 hours.

Reference water is 30 minutes "Old " aged to less than 0.002 mg/L (measured).

Test condition: Flow-through. Juvenile.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (141)

Species: Leiostomus xanthurus (Fish, estuary, marine)
Exposure period: 20 day(s)
Unit: mg/l Analytical monitoring: yes

NOEC: = .062 -

Test substance: other TS: sodium hypochlorite

Method: The fish were acclimated for 30 days in separate 1000L tanks supplied continuously with dechlorinated (sodium thiosulfate added to dechlorinate) discharge water at flow rates of approximately 0.19L/sec. Basic water quality of the acclimation water, monitored daily, was as follows: temperature 30.9 + 1.31C; pH 7.0 + 0.62; dissolved oxygen 3.8 + 1.23 mg/L; ammonia - N 0.3+0.28 mg/L and salinity 2.1+0.71%. Maintenance diets of trout chow were apportioned to the fish on a twice-daily schedule, during both acclimation and test periods.

During the test period, fish were placed in troughs constructed of 1.9 cm thick marine plywood covered with polyester-resin-impregnated fiberglass. The troughs were partitioned with four 0.64 cm thick methyl methacrylate polymer dividers which were spaced equidistantly across the troughs. Each was secured to opposite ends of the trough to form a 12 M long serpentine channel. Cooling water inflow rates were maintained at 0.38 L/sec resulting in hydraulic residence periods of approximately 60 minutes in all troughs, simulating cooling water retention times observed in the plant's discharge canal. A second trough, supplied with dechlorinated cooling water, served as the reference exposure trough. In all studies, test organisms were retained in the troughs at positions where the cooling waters had "aged" approximately 5, 30 and 60 minutes.

Fish were placed in submerged nylon cages (0.19M x 0.33M x 0.74M) located in the troughs at positions of 5, 30 and 60 minutes of halogen decay. Approximately 20 Leiostomus xanthurus (spot) were randomly distributed to segregated paired cages. Observations of fish mortality were made at hours 1, 2, 4, 16 and 24 hours during the first 24 hours and twice daily thereafter during the 19-day exposure period.

Remark: Although the authors report a statistically significant difference between chorine and reference exposed fish, the greatest difference was in fish exposed to the lowest concentration. There was very little difference noted in the fish exposed to 0.062 mg/L and respective controls.

Result: Mean Survival of Leiostomus xanthurus in 19 day study:

Conc., mg/L	Reference (Control)	Chlorine
0.014	87.0	73.9
0.032	--	76.0
0.062	82.3	78.3

Survival of chlorine-treated spot was significantly more ($P<0.05$) than that of the reference fish.
Temperature 31.2 + 1.23C; pH 7.1 + 0.49; Dissolved oxygen 4.1 + 0.91; ammonia - N 0.2 + 0.36; salinity 2.0 + 0.63%.
Total residual oxidant concentrations were measured twice daily and varied during these studies because of plant equipment malfunctions. Two temporary plant shutdowns for equipment repair occurred, during which periods the oxidant levels of <0,003 mg/L were recorded. However the downtime interruptions totaled less than 8 hours.

Reference water is 30 minutes "Old " aged to less than 0.002 mg/L (measured).
Test condition: Flow through, juvenile
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (141)

4.5.2 Chronic Toxicity to Aquatic Invertebrates

Remark: Calcium hypochlorite which is dissolved in water is in a fast equilibrium with hypochlorous acid which again is in a fast equilibrium with hypochlorite ions. In general the concentration of chlorine in a test system is achieved by dissolving hypochlorous acid or sodium hypochlorite and not by application of gaseous chlorine.

Because of this equilibrium concentrations, in general, are provided as free available chlorine or as total residual chlorine (TRC). (See chapter 1.11 for definition.)
The studies were performed by independent laboratories and published in peer reviewed papers. Many studies were performed in the 70ties, when effects of the biocidal application of chlorine were carefully reinvestigated. Therefore the studies are not performed according to recent guidelines and no GLP information is provided.

With regard to the extensive number of tests that were already performed with chlorine no further studies according to recent guidelines were conducted to avoid further animal testing.
Flag: Critical study for SIDS endpoint
31-OCT-2005

Species: other: Crassostrea virginica (bivalves, estuary, marine)
Endpoint: mortality
Exposure period: 15 day(s)
Unit: mg/l Analytical monitoring: yes
NOEC (survival) : .062 -

GLP: no data
Test substance: other TS: sodium hypochlorite

Method: The bivalves were acclimated for 30 days in separate 200L tanks supplied continuously with dechlorinated (sodium thiosulfate added to dechlorinate) discharge water at flow rates of approximately 0.19L/sec.

During the test period, bivalves were placed in troughs constructed of 1.9 cm thick marine plywood covered with polyester-resin-impregnated fiberglass. The troughs were partitioned with four 0.64 cm thick methyl methacrylate polymer dividers which were spaced equidistantly across the troughs. Each was secured to opposite ends of the trough to form a 12 M long serpentine channel. Cooling water inflow rates were maintained at 0.38 L/sec resulting in hydraulic residence periods of approximately 60 minutes in all troughs, simulating cooling water retention times observed in the plant's discharge canal. A second trough, supplied with dechlorinated cooling water, served as the reference

exposure trough. In all studies, test organisms were retained in the troughs at positions where the cooling waters had "aged" approximately 5, 30 and 60 minutes. Bivalves were placed in submerged nylon socks located in the troughs at positions of 5, 30 and 60 minutes of halogen decay. Groups of 20 Crassostrea virginica (oysters) were randomly distributed to segregated paired cages. Observations of bivalve mortality were made three times each week following the 15-day exposure. New shell deposition was measured after the end of the experiment.

Total residual oxidant concentrations were measured twice daily and varied during these studies because of plant equipment malfunctions. Two temporary plant shutdowns for equipment repair occurred, during which periods the oxidant levels of <0,003 mg/L were recorded. However the downtime interruptions totaled less than 8 hours.

Result: Mean Survival of Crassostrea virginica in 15 day study

Conc., mg/L	Control	Chlorine
0.014	--	100.0
0.032	100.0	92.0
0.062	--	100.0

Survival of chlorine-treated oysters was comparable (P<0.05) to the reference oysters. All mortalities were attributed to apparent suffocation of individuals that had escaped from the retaining "socks" and had fallen into the anaerobic layer present in the trough bottoms.

NOEC (survival) = 0.062 mg/L

Mean shell deposition in Crassostrea virginica in 15 day study

Conc., mg/L	Control	Chlorine
0.014	--	3.0 + 0.21*
0.032	3.5 + 0.17	2.7 + 0.21*
0.062	--	2.3 + 0.15*

*:significantly (p<0.05)

Restricted shell deposition, indicative of sublethal stress, was evident among juvenile oysters. Significantly shell deposition (P<0.05) was generated among reference oysters compared to all chlorine exposed oysters.

NOEC (shell depositon) was not calculated.

Reference water is 30 minutes "Old " aged to less than 0.002 mg/L (measured).

Test condition: Test condition: Flow-through, Juvenile. Basic water quality of the acclimation water was monitored daily:
temperature 30.9 + 1.31C;
pH 7.0 + 0.62;
dissolved oxygen 3.8 + 1.23 mg/L;
ammonia - N 0.3 + 0.28 mg/L;
salinity 2.1 + 0.71%.

There were no significant differences between the chlorinated and reference stations at test conditions: Temperature 31.2 + 1.23C; pH 7.1 + 0.49; Dissolved oxygen 4.1 + 0.91; ammonia - N 0.2 + 0.36; salinity 2.0 + 0.63.

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (141)
Species: other: Rangia cuneata (bivalves, estuary, marine)
Endpoint: mortality
Exposure period: 15 day(s)
Unit: mg/l Analytical monitoring: yes
NOEC: = -
NOEC (survival and shell deposition) :
= .062 -

GLP: no data
Test substance: other TS: sodium hypochlorite

Method: The bivalves were acclimated for 30 days in separate 200L tanks supplied continuously with dechlorinated (sodium thiosulfate added to dechlorinate) discharge water at flow rates of approximately 0.19L/sec.

During the test period, bivalves were placed in troughs constructed of 1.9 cm thick marine plywood covered with polyester-resin-impregnated fiberglass. The troughs were partitioned with four 0.64 cm thick methyl methacrylate polymer dividers which were spaced equidistantly across the troughs. Each was secured to opposite ends of the trough to form a 12 M long serpentine channel. Cooling water inflow rates were maintained at 0.38 L/sec resulting in hydraulic residence periods of approximately 60 minutes in all troughs, simulating cooling water retention times observed in the plant's discharge canal. A second trough, supplied with dechlorinated cooling water, served as the reference exposure trough. In all studies, test organisms were retained in the troughs at positions where the cooling waters had "aged" approximately 5, 30 and 60 minutes.

Bivalves were placed in submerged nylon socks located in the troughs at positions of 5, 30 and 60 minutes of halogen decay. Groups of 25 Rangia cuneata (clams) were randomly distributed to segregated paired cages. Observations of bivalve mortality were made three times each week furring the 15-day exposure. New shell deposition was measured after the end of the experiment.

Result: Mean Survival of Rangia cuneata in 15 day study:

Conc., mg/L	Control	Chlorine
0.014	--	100.0
0.032	100.0	80.0
0.062	--	100.0

Survival of chlorine-treated clams was comparable ($P<0.05$) to the reference clams. All mortalities were attributed to apparent suffocation of individuals that had escaped from the retaining "socks" and had fallen into the anaerobic layer present in the trough bottoms.

NOEC (survival) = 0.062 mg/L
Growth was comparable between the control and treated clams. Data reported as >3.0 for each exposure group.

NOEC (shell deposition) = 0.062 mg/L

Reference water is 30 minutes "Old " aged to less than 0.002 mg/L (measured).

There were no significant differences between the chlorinated and reference stations: Temperature 31.2 + 1.23C; pH 7.1 + 0.49; Dissolved oxygen 4.1 + 0.91; ammonia - N 0.2 + 0.36; salinity 2.0 + 0.63%. Total residual oxidant concentrations were measured twice daily and varied during these studies because of plant equipment malfunctions. Two temporary plant shutdowns for equipment repair occurred, during which periods the oxidant levels of <0,003 mg/L were recorded. However the downtime interruptions totaled less than 8 hours.

Test condition: Flow-through. Juvenile

Basic water quality of the acclimation water were monitored daily:
temperature 30.9 + 1.31C;
pH 7.0 + 0.62;
dissolved oxygen 3.8 + 1.23 mg/L;
ammonia - N 0.3 + 0.28 mg/L;
salinity 2.1 + 0.71%.

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (141)

Species: other: Dreissena polymorpha (Zebra mussel)
Endpoint: mortality
Exposure period: 56 day(s)
Unit: mg/l Analytical monitoring:
LC50 : = .5 -

GLP: no
Test substance: other TS

Method: Hypochlorite was compared for their effectiveness against adult zebra mussels (Dreissena polymorph). The effect at applied concentrations of 0.5 - 2.5 mg/L were contrasted in continueous and intermittent 28-day static renewal tests. In addition, 0.5-10 mg free Chlorine was applied continuously for 28 or 56 days in flow-through systems.

Result: Chlorine was less toxic at lower temperature, and that less chlorine was needed for a given kill when lower concentrations were applied.

nominal concentration: 0.5 mg/L
measured chlorine concentration: 0.08 +/- 0.009 mg/L

LC50 (28 days) was calculated : 0.285 mg/L

LC50 (56 days) : niminal 0.5 mg/L, measured 0.08 mg/L

Test substance: Free chlorine
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

31-OCT-2005 (128)

Species: other: Dreissena polymorpha (Zebra mussel)
Endpoint: mortality
Exposure period: 12 day(s)
Unit: mg/l Analytical monitoring: yes
EC50: = 1 -

Remark: Test duration: 295 hours
Criteria for mortality: valve gape with no response to probing of exposed mantle tissues.
Result: Same conditions: 460 h : LC10 = < 1 mg/l
Test condition: Life stage: juvenile
static, Temperature: 22 degree C, pH=7.8
Reliability: (4) not assignable
31-OCT-2005 (147)

Species: other aquatic mollusc
Endpoint: mortality
Exposure period: 60 day(s)
Unit: mg/l Analytical monitoring:
LC46 : = .11 -

Test substance: other TS: sodium hypochlorite

Remark: Species: Crassostrea virginica (American oyster). Data confounded due to poor survival in controls. Only 58 percent of the controls survived 60 days which was attributed to the effects of the parasitic fungus, Dermocystidium marinum.
Result: Results as Cl2
LC98, 60 days = 0.66 mg/l
LC58, 60 days = 0.211 mg/l
Other data concerning Histology, residues, reproduction, respiratory rate .
Test condition: Life stage: adult
static, Temperature: 24-31 degree C, pH=7.4-8.3
Reliability: (3) invalid
31-OCT-2005 (202)

Species: other aquatic crustacea: Pandalus danae (marine species, coon stripe shrimp)
Endpoint: other: growth, mortality
Unit: Analytical monitoring: yes

Result: At 0.05mg/l Cl2, growth unaffected
At 0.08mg/l Cl2, slight decrease in growth
All died at 0.18 mg/l Cl2
Reliability: (3) invalid
31-OCT-2005 (92)

Species: other: Dreissena polymorpha (Zebra mussel)
Endpoint: mortality
Exposure period: 7 day(s)
Unit: mg/l Analytical monitoring: yes
EC50: = 2.5 -

Remark: Test duration: 178 hours
Criteria for mortality: valve gape with no response to

probing of exposed mantle tissues.
Test condition: Life stage: juvenile
static, Temperature: 22 degree C, pH=7.8
Reliability: (4) not assignable
31-OCT-2005 (147)

Species: other: Dreissena polymorpha (Zebra mussel)
Exposure period: 7 day(s)
Unit: mg/l Analytical monitoring:
EC50: = 50 -

GLP: no data

Remark: Test duration: 157 hours
Criteria for mortality: valve gape with no response to
probing of exposed mantle tissues.
Result: Same conditions: 264 h: LC100 = 5 mg/l
Test condition: Life stage: juvenile
static, Temperature: 22 degree C, pH=7.8
Reliability: (4) not assignable
31-OCT-2005 (147)

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Sediment Dwelling Organisms

4.6.2 Toxicity to Terrestrial Plants

Species: other terrestrial plant: Poa pratensis
Endpoint: growth

Method: other: not specified
Year: 1992
GLP: no data
Test substance: other TS: NaOCL tablets

Remark: Result:
Plants heights, fresh and dry weights were generally, and in some cases significantly higher in the treated soil compared to the untreated control.
Result: Plant heights, fresh and dry weights were generally, and in some cases significantly higher in the treated soil compared to untreated contr
Test condition: Plants at 8 weeks of age were subjected to 1.5 microg/ml or 150 microg/ml chlorine solutions. 7 pots (20 cm diameter) containing sandy loam soil were used for each treatment, each pot received 400 ml of solution four times at two weeks intervals.
Test substance: NaOCl tablets (0.5%) dissolved with tap water.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (24)

4.6.3 Toxicity to Soil Dwelling Organisms

Remark: Not applicable
31-OCT-2005

4.6.4 Toxicity to other Non-Mamm. Terrestrial Species

4.7 Biological Effects Monitoring

Method: Periphytic communities on artificial substrates were exposed to chlorine solutions for 7 days. Mean measured total residual chlorine (TRC) were 6.3 +/- 3.9 microg/l and 56.6 +/- 24.5 microg/l, the free residual chorine was 73.0 +/- 19.9 %.
Result: Exposure to the high chlorine treatment resulted in significant reduction in species richness of protozoan communities. 2.7 microg TRC/l reduced species richness by 20% (IC20).
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (37)

4.8 Biotransformation and Kinetics

4.9 Additional Remarks

Remark: Electrolytically generated chlorine (free chlorine level: 40-50 microg/ml) was injected into citrus microirrigation system. The minimum contact time of chlorine with water was found to be 11 min. The pH of the water was maintained below7. Result: The chlorine treatment eradicated Phytophthora nicotianae, P. citrophthora, Fusarium sp., algae and protozoan populations present in the water; bacterial population levels were drastically reduced.
10-SEP-2003 (96)

Remark: Juvenile fishes were exposed to 4, 6, 52.5, and 183 microg TRC/l (total residual chlorine) for 134 days or 49 days (rainbow trout). The source of chlorine was 10 % sodium hypochlorite solution.
No relationship between treatment concentration and the growth and survival of bluegills (Lepomis macrochirus), white suckers (Catostomas commersoni), and rainbow trouts (Salmo gairdneri) was observed.
There was, however, a consistent pattern of reduced growth of channel catfish (Ictalurus punctatus) with increasing TRC concentrations. The mean final weights of catfish at the highest TRC exposure were 64% (of control).
The addition of ammonia (3 mg/l nitrogen) changed the effects of chlorine. Bluegills were still unaffected; growth and survival of channel catfish were reduced at all concentrations of chlorine: no survivals at mean TRC levels >/= 24 microg/l, growth at mean TRC concentration of > 1 5g/l was 34% of control.
10-SEP-2003 (105)

Remark: Chlorine reacts under physiological conditions (37 degree C, pH=7.4) with water in the tissues to give nascent oxygen, hydrogen chloride and hypochlorous acid. The following reactions can take place:

1) Cl2 + H2O -------> HCl + HClO
2) 2 Cl2 + H2O -------> 4 HCl + O2
3) 3 Cl3 + 3 H2O -------> 5 HCl + HClO3
The hypochlorous acid in equation (1) can dissociate:
4) 2 HClO -------> 2 HCl + O2
5) HCl = H2O -------> H3O+ + Cl-

31-OCT-2005 (46)

5.0 Toxicokinetics, Metabolism and Distribution

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Remark: Calcium hypochlorite is a white or grayish-white powder which when dissolved in water dissociate into calcium ion (Ca++) and hypochlorite ion (ClO-). Human health effect may be caused by either of contacting with solid powder, aqueous solution or accidentally generated chlorine gas. Calcium ion can generate strongly alkaline condition at the application site. As for hypochlorite ion toxicity concerns, the exposure scenarios are common to sodium hypochlorite (liquid) or chlorine gas which is utilized further more in amount as source of hypochlorite ion and thoroughly assessed in component/pertinent international organization like WHO or EU risk assessment program. Therefore, substantial portion of description on hypochlorite-ion-related effects are made as much as common to those and SIAP for chlorine in this HPV program.
Most of the data for this substance's toxicity by oral route came from studies performed with sodium hypochlorite or chlorine gas. In biological systems, characterized by pH values in the range 6-8, the most abundant active chemical species is HOCl, in equilibrium with ClO-. Available chlorine is readily absorbed via oral route and distributed into plasma, bone marrow, testis, skin, kidney and lung. Only ca. 50% is excreted mainly with the urine followed by excretion with feces. HOCl is not enzymatically metabolized.
Flag: Critical study for SIDS endpoint
31-OCT-2005
Type: LD50
Species: rat
Strain: Wistar
Sex: male
No. of Animals: 40
Vehicle: water
Value: = 790 mg/kg bw

GLP: no data
Test substance: other TS: 70% calcium hypochlorite

Result:

Dose Level	Dead	Average of body weight(g)
890(mg/kg)	8/10	147
1000(mg/kg)	5/10	140
1120(mg/kg)	9/10	147
1260(mg/kg)	10/10	148

General observations
-At 2 highest dose levels, 7/19 deaths occurred in 1.5 - 5 hours, 4/19 deaths occurred in 24 - 48 hours.
-At 2 lower dose level, 9/13 deaths occurred in 2 - 5 days.

-Most survivors showed a mild to moderate persistent anorexia.
-Moderate central depression within 1 hour.
-Most affected animals showed diarrhea for several days.
-Dilatation of pupil
-No effects on corneal, lid or pain reflexes.
-No salivation or lachrymation.

LD50 = 790 (654-955) mg/

Test condition: Conditions:
-Fasten 24 hours before dosing
-Concentration of test substance; 100mg/mL
-Dosing volume; 1.11 - 2.16 mL
-Statistical analysis; Litchfield-Wecoxon method

Conclusion: Acute oral LD50 in fasten male rats, in the toxicity test was 79-0 mg/kg.

Reliability: (2) valid with restrictions

Flag: Critical study for SIDS endpoint

31-OCT-2005 (170)

Type: LD50
Species: rat
Strain: Wistar
Sex: male
No. of Animals: 30
Vehicle: water
Value: = 1260 mg/kg bw

GLP: no data

Test substance: other TS: 70% calcium hypochlorite

Result:

Dose Level	Dead	Average of body weight(g)*
800(mg/kg)	0/5	179.8
960(mg/kg)	0/5	169.4
1150(mg/kg)	1/5	138.0
1380(mg/kg)	4/5	144
1660(mg/kg)	5/5	---
2000(mg/kg)	5/5	---

*: at the end of test period (9 days after dosing), only survivors

-Mortality
2000 mg/kg; All animals were died on the dosing day.
1660 mg/kg; Four animals were died on the dosing day, one was died on the 3rd day after dosing.
1380 mg/kg; Four animals were died within 6 days after dosing.
1150 mg/kg; One animals was died in 4th day after dosing.
The other: No deaths

-Clinical observations
At higher dose level, hyposthenia and rough breathing were observed.
At greater than 1150 mg/kg, bleeding from nose and mouth were observed on the 1st day, piloerection and sparse fur were observed on the 5th day after dosing

LD50 = 1260 mg/kg (940 - 1680 mg/kg, 95% confidence limi
Test condition: -Animals; 6 weeks old
-Breeding; room temperature 22 plus or minus 1 degree C, humidity 55 plus or minus 5 %, acclimatization 1 week
-Body weight; 148 - 183 g
Reliability: (4) not assignable
31-OCT-2005 (168)

Type: LD50
Species: rat
Strain: Wistar
Sex: male/female
No. of Animals: 20
Value: = 3380 mg/kg bw

GLP: no data
Test substance: other TS

Result: Slight to marked depression was observed among the group which received 4.2, 3.5, 2.8, and 2.4 g/kg of Mildew-Rid.

All survivors appeared normal during 48 hours of dosage and throughout the fourteen-day post dosage observation period.

The clinical LD50 for Midew-Rid and its 95 to 100 confidence interval are estimated at 3.38 (2.83 - 3.90) g/kg body weight.
Test condition: Wister-derived albino rats, 120 - 160 g body weight, were maintained under standard laboratory conditions for a minimum of seven days, after which they were separated into groups of five and fasted for a period of 18 hours prior to administration of the test material.

The rats were dosed individually by gavage at 4.2, 3.5, 2.8, and 2.4 g/kg, after which they were returned to individual quarters where food and water available ad libitum.

The animals were observed for signs of pharmacologic activity and drug toxicity at 1, 3, 6, and 24 hours post-dosage. Observations were made daily until a total of fourteen days.

Computation of Median-Effective Dose (LD50) was performed by the moving average Method of Weil.
Test substance: The test material, supplied by Olin Research Center, was identified as Mildew-Rid, Sample #A-10199.
Reliability: (4) not assignable
31-OCT-2005 (167)

Remark: Ingestion is unlikely, since chlorine is a gas above -34.1 degree C at normal atmospheric pressure. An oral toxicity test of chlorine can therefore not be performed.
Dissolved in water chlorine is in a fast equilibrium with hypochlorous acid which again is in a fast equilibrium with hypochlorite ions. The acute toxicity of a solution of hypochlorous acid is found to be moderate (5800-6800 mg/kg bw in mouse, Momma et al. (1986)). There was one exceptional low LC50 value for oral toxicity in mouse (880 mg/kg bw) described by Klimm, W. et al., 1989.
Reliability: (4) not assignable

31-OCT-2005 (129) (159)

Type: LD50
Species: rat
Value: = 850 mg/kg bw

Year: 1975
GLP: no

Reliability: (4) not assignable
31-OCT-2005 (139)

Type: LDLo
Species: rat
Value: > 10500 mg/kg bw

Remark: The LD0 value for a 3.6 % solution (as available chlorine) was reported to be greater than 10.5 g/kg (the gastric mucosae of the exposed animals were reported.
Reliability: (4) not assignable
31-OCT-2005 (47)

Type: LDLo
Species: rat
Value: > 5800 mg/kg bw

Remark: A solution of sodium hypochlorite at a concentration of 12.5% (available chlorine) caused no mortality up to the level of 5.8 g/kg. Gastric lesions were found in all animals exposed and sacrificed after 14 days of observation.
Reliability: (4) not assignable
31-OCT-2005 (48)

Type: LD50
Species: rat
Strain: Wistar
Sex: male
No. of Animals: 10
Value: = 8830 mg/kg bw

Remark: An oral LD50 of 8.8 g/kg in rats was quoted for a 12.5% bleach solution (based on available chlorine). Five groups of 10 male Wistar rats each were given 20 ml/kg bw of a dilution of chlorine bleach containing 12.5% available chlorine. During the observation period of 14 days, the following symptoms of toxicity were recorded: ungroomed fur, light to moderate sedation, diarrhea, ataxia, and increased breathing of differing severity. The deaths observed in most cases within the 24 hours after application. Pathology upon dissection showed strong gas accumulation in the stomach and intestines, swelling of the liver, bleeding gastritis and enteritis. There were no symptoms noted in the animals that survived. The LD50 was determined to be 8.83 (8.2 - 9.51) g/kg bw, and the NOAEL was found to be 5.01 g/kg bw, all based on the 12.5% available chlorine solution (or 640 mg/kg bw as NaClO).
Reliability: (4) not assignable
31-OCT-2005 (118)

5.1.2 Acute Inhalation Toxicity

Remark: A large number of studies on acute inhalation toxicity of chlorine has been performed.

The studies provided in this chapter were performed by independent laboratories and published in peer reviewed papers. However, the studies are not performed according to recent guidelines and no GLP information is provided.

With regard to the large number of tests that were already performed with chlorine, no further studies according to recent guidelines were conducted to avoid further animal testing.

31-OCT-2005

Type: LC50
Species: rat
Strain: Wistar
Sex: male/female
No. of Animals: 10
Vehicle: other: whole body exposure
Exposure time: 60 minute(s)
Value: = 1.202 - 1.423 mg/l

Method: other: see reference
Year: 1987
GLP: yes
Test substance: other TS: purity >99.9% Cl2

Method: Rats were exposed to concentrations of chlorine varying from 1654 to 16801 mg/m3 for 5 minutes, from 1680 to 6519mg/m3 for 10 minutes, from 1586 to 1870 for 30 minutes or from 935 to 1725 mg/m3 for 60 minutes.
Satellite groups of 3 males and 3 females were exposed to chlorine and sacrificed two days after termination of the exposure for interim histopathological examination; clinical observations during the exposure included restlessness, dyspnea, wet nares, bubble formation and nasal discharge; and 14 days postexposure observation.

Result:

Exposure time (min)	LC01 (mg/l)
5	7.2 (7260 mg/m3)
10	3.0 (2986 mg/m3)
30	1.2 (1248 mg/m3)
60	0.8 (834 mg/m3)

Exposure time (min)	LC50 (mg/l)
5	15.9 (15949 mg/m3)
10	5.6 (5642 mg/m3)
30	2.0 (2033 mg/m3)
60	1.3 (1321 mg/m3)

Time-concentration-mortality relationship:
P=-16.67+1.33*ln(C)-4.31*ln(T)+1.01*lN(C)*ln(T)

P: Probit response
C: exposure concentration
T: exposure time

Most mortalities occurred within the first week of observation. Gross pathology showed that relative lung weights were generally increased, and the increases showed a positive correlation with concentration and duration of exposure. No dose-related effects for kidneys and liver were seen. Histopathologic examination of the lungs generally showed focal aggregates of polymorpho- or mononuclear inflammatory cells, increased septal cellularity, and squamous metaplasia of the bronchial epithelium.

Test condition: Doses: 935 to 1725 mg/m3
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
31-OCT-2005 (256) (257)

Type: LC50
Species: mouse
Strain: Swiss
Sex: male/female
No. of Animals: 10
Vehicle: other: whole body exposure
Exposure time: 30 minute(s)
Value: = 1.198 - 1.671 mg/l

Method: other: see reference
Year: 1987
GLP: yes
Test substance: other TS: purity >99.9 Cl2

Method: Mice were exposed to concentrations of chlorine varying from
* 1680 to 4798 mg/m3 for 10 minutes
or
* 1328 to 1870 mg/m3 for 30 minutes.

Satellite groups of 3 males and 3 females were exposed to chlorine and sacrificed two days after termination of the exposure for interim histopathological examination; clinical observations during exposure included restlessness, dyspnea, wet nares, bubble formation and nasal discharge; and 14 days postexposure observation.

Result: LC50 (exp. time: 10 min): 3.1 mg/l (3064 mg/m3)
LC50 (exp. time: 30 min): 1.5 mg/l (1462 mg/m3)

Most mortalities occurred within the second week of observation. Gross pathology showed that relative lung weights were generally increased, and the increases showed a positive correlation with concentration and duration of exposure. No dose-related effects for kidneys and liver were seen. Histopathologic examination of the lungs generally showed focal aggregates of polymorpho- or mononuclear inflammatory cells, increased septal cellularity, and squamous metaplasia of the bronchial epithelium.

Test condition: Doses: 1328 to 1870 mg/m3
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
31-OCT-2005 (256) (257)

Type: other: histopathologic changes after brief exposure to high

concentrations
Species: rat
Strain: Sprague-Dawley
Sex: male
No. of Animals: 74
Vehicle: other: Nose-only exposure
Exposure time: 10 minute(s)

Method: other
Year: 1995
GLP: no data
Test substance: other TS: purity >99.5% Cl2

Method: Rats were randomly divided into groups of four and were exposed in the flow-past chamber. Two series of exposures were made. The first consisted of exposing rats to concentrations of 50, 100, 200, 500 or 1500 ppm chlorine for periods of time ranging from 2 to 10 minutes. Pathological evaluation was conducted 72 hours after exposure. The second series consisted of exposing rats to 1500 ppm for 10 minutes with evaluation of histological changes at 1, 3, 6, 12, 24 and 72 hours. Control rats were placed in the chamber for similar periods of time as the exposed rats but breathed only room air.
Result: Lungs from the control group and from rats exposed to 50 or 100 ppm for 2 minutes were normal within 72 hours after exposure. In the lungs of rats exposed to 200 and 500 ppmfor 2 to 5 minutes, there was only slight perivascular edema present. Seventy-two hours after exposure to 1500 ppm for 2 minutes, there was again little difference from controls, with only mild perivascular edema and occasional small clusters of polymorphonuclear leukocytes in the mucosa of large airways. After 10 minutes exposure to 1500 ppm chlorine, significant epithelial and airspace abnormalities appeared. One hour after exposure, patchy but extensive separation of the epithelium from the airway wall occurred. Six, 12 or 24 hours after exposure, the changes were similar to those at 1 hour except that airspace edema was generally focal and mild. In addition, at each treatment there was a patchy infiltrate of polymorphonuclear leukocytes in the airway wall. This was thr most remarkable in the animals sacrificed at 12 hours and was relatively mild at the other two treatment. Seventy two hours after the exposure, the lungs of animals lacked the acute changes and instead showed a stratified epithelium. Goblet cell metaplasia was extensive in one animal and focal in another. Inflammatory cells were generally absent; however, focal small aggregates of eosinophils were present in the airway wall in some animals.
Test condition: Doses: 50, 100, 200, 500 or 1500 ppm
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (64)

Type: other: pulmonary function
Species: rat
Strain: Sprague-Dawley
Sex: no data
Exposure time: 5 minute(s)

Year: 1998

Test substance: no data

Method: Sprague-Dawley rats were exposed to 1500 ppm chlorine for 5 minutes. Lung resistance (RL), responsiveness to inhalaed methacholine (MCh), the airway epithelium and bronchoalveolar lavage (BAL) were assessed for a 3 month period after exposure.
Result: Lung resistance increased significantly up to 3 days after exposure, reaching a maximal change of 110 (+16%) from baseline. There was a significant decrease in the concentration of MCh required to increase RL by 0.20 cm H2O/ml/sec from days 1-7 after the exposure. In some rats, MCh hyperresponsiveness and RL changes persisted after the exposure for as long as 1 and 3 months, respectively. Histological evaluation with morphometric evaluation revealed epithelial flattening, necrosis, increase in smooth muscle mass and evidence of epithelial regeneration. BAL showed an increased number of neutrophils. The timing of maximal abnormality in the appearance of the epithelium (days 1-3) corresponded to that of the maximal functional changes. In summary, acute high chlorine exposure results in functional and pathological abnormalities that resolve in the majority of animals after a variable period; however, these changes can persist in some animals. Functional abnormalities in the initial stages may be related to airway epithelial damage.
Test condition: Doses: 1500 ppm
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (62)

Type: other: pulmonary function
Species: rat
Strain: Sprague-Dawley
Sex: no data
Exposure time: 5 minute(s)

Method: other
Year: 1998
GLP: no data
Test substance: no data

Method: Male Sprague-Dawley rats were exposed to 1500 ppm chlorine for 5 minutes and treated daily with either dexamethasone (DEX, 300 ug/kg/day) or saline intraperitoneally for 7 days. Lung resistance (RL), airway responsiveness to inhaled methacholine (MCh), airway wall morphometric measurements and bronchoalveolar lavage (BAL) cells were assessed over a 2-week period after exposure.
Result: DEX administration significantly attenuated both chlorine-induced increased RL and chlorine-induced increased responsiveness to methacholine compared with saline: -2.7 + 6.8% vs 102.3 + 36.6% change from baseline RL and 2.5 + 0.6 mg/ml vs 1.2 + 0.7 mg/ml in the MCh concentration required to double the RL from baseline. There was a tendency, albeit nonsignificant, for improvement in some indices of epithelial injury. DEX significantly attenuated the postexposure neutrophilic cellular response in BAL 1 day after the exposure (15.8 + 4.9% neutrophils in the DEX group vs 49.8 + 2.7% neutrophils in the saline group). In summary, the results show that DEX administration helps maintain

pulmonary function, reduces BAL inflammatory cell number and tends to improve some morphometric airway wall structure parameters in rats exposed to chlorine.
Test condition: Doses: 1500 ppm + DEX, 300 mg/kg/day
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (63)

Type: other: Respiratory rate (RD50)
Species: mouse
Exposure time: 10 minute(s)
Value: = .027 mg/l

Method: other
GLP: no data
Test substance: no data

Method: Irritating properties of chlorine were investigated. Male Swiss-Webster mice were exposed to chlorine in concentrations from 0.002 to 0.111 mg/l for 10 min. The response, measured as decrease in respiratory frequency, was dose-related with a plateau being apparent within 5 to 7 minutes of exposure.
Remark: RD50: Concentration that caused a 50% decrease in respiratory rate.
Test condition: Doses: 0.002 to 0.111 mg/l
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (12)

Type: other: acute none lethal toxicity
Species: mouse
Strain: other: OF1
Sex: male
Exposure time: 60 minute(s)

Method: other
Year: 1994
GLP: no data
Test substance: no data

Method: Groups of 8 male previously unexposed mice were exposed for 60 minutes to 1.7, 2.0, 2.3, 4.1, 4.4, 5.0, 6.3 or 8.8 ppm chlorine in body plethysmographs, while the head was enclosed in the inhalation chamber. Three additional groups of eight mice were exposed to 2.2, 4.6 or 6.6 ppm chlorine for 120 minutes under the same circumstances.
Result: The onset of the maximal response was gradual and occurred after 45 minutes. To ascertain that the response did not decrease any lower, three additional groups of mice were exposed to 3 concentrations of chlorine for 120 minutes. The response did not decrease any lower beyond 60 minutes of exposure. The response was characteristic of a sensory irritant, even at the highest concentration. After the 60-minute exposure, recovery was rapid and complete except for the two highest concentrations (6.3 and 8.8 ppm). The group of mice exposed to 8.8 ppm was tested 24 hours after the end of exposure. At this time the recovery was complete: 265 + 28 vs 282 + 36 breaths/minute before exposure. The RD50 was calculated to be 3.5 ppm.
Test condition: Doses: 1.7, 2.0, 2.3, 4.1, 4.4, 5.0, 6.3 and 8.8 ppm

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (86)

Species: rat
Strain: Wistar
Sex: male/female
No. of Animals: 30
Exposure time: hour(s)

GLP: no data
Test substance: other TS: 70% calcium hypochlorite

Result: The average chamber concentration of Mildew-Rid in air provided by the inhalation schedule was: 176.4 mg/L for the one hour exposure, and 158.3 mg/L for the three hour exposure. The dosages available to each test animal were approximately 27.46 and 73.94 mg/kg body weight, respectively.

During the exposure all animals showed evidence of ocular irritation and slightly reduced activity. These signs were no longer observed on the following day.

All animals survived the exposure and appeared normal for the 14-day post-exposure period.

Body weight increases were within normal ranges.

Five rats from each group sacrificed on the exposure day and the remaining rats autopsied on the 14th day, showed evidence of normal tissues and organs.
Test substance: The test material, supplied by Olin Research Center, was identified as Mildew-Rid, Sample #A-10199.
Test condition: Groups of fifteen Wistar-derived albino rats, 180 - 220 g in body weight, were housed in three-cubic-foot chambers for the one and three hour dynamic exposure periods. Preconditioned supportive air was supplied at 6.0 liters per minutes. an unsuccessful attempt was made to present the test material as a respirable aerosol via an Ohio Nebulizer following dilution with water to provide a working solution containing 3.5 % available chlorine.

Clogging of the jet aperture precluded the use of a working solution containing more than 1.8% available chlorine.

The material was expressed into the chamber at a level which would not cause excessive residue on the animals' fur.

Five animals were killed by cervical dislocation at the end of each exposure, and organs and tissues were examined with with emphasis on lungs and upper airways. The remaining animals were returned to individual quarters and observed for fourteen days, after which they were killed abnormal was fixed in 10 % buffered formalin for possible histopathological examination.
Reliability: (4) not assignable
31-OCT-2005 (167)

Type: LC100
Species: mouse

Strain: no data
Sex: male/female
Exposure time: 180 minute(s)
Value: = .064 mg/l

Method: other
Year: 1967
GLP: no
Test substance: no data

Result: 8 out of 10 mice died within 4 days. Pathological examination of these mice revealed pulmonary oedema and necrosis and inflammation of the respiratory epithelium.
Test condition: Doses: 0.029 mg/l
10 animals were exposed to 0.029 mg/l for 6 hours.
Reliability: (3) invalid
31-OCT-2005 (198)

Type: LC50
Species: mouse
Exposure time: 10 minute(s)
Value: = 1.82 mg/l

Method: other
GLP: no data
Test substance: no data

Reliability: (3) invalid
31-OCT-2005 (89)

Type: LC50
Species: mouse
Exposure time: 30 minute(s)
Value: = .37 mg/l

Method: other
Year: 1967
GLP: no
Test substance: no data

Method: Observation period: 4 days
Symptoms of toxicity: pulmonary edema, necrosis and inflammation of the respiratory epithelium
Reliability: (3) invalid
31-OCT-2005 (198)

Type: other: effects on pulmonary function
Species: rabbit
Exposure time: 30 minute(s)

Method: other
Year: 1975
GLP: no
Test substance: no data

Method: Study on lung function after exposure to 0.145, 0.29, 0.58 mg/l (50, 100, or 200 ppm); respiratory volumes, flow rates, pressure measurements, and pulmonary compliance were used for evaluating lung function, prior to exposure, and 30 min.,3, 14, and 60 days after exposure.

Result: Respiratory flow rates decreased initially in the two highest dose groups but returned to normal within 60 days after exposure; a decrease in pulmonary compliance was noted initially in all groups and returned to normal in the lowest dose group during the post-exposure phase.
Pathology: 0.29 and 0.58 mg/l: initial heamorrhage and oedema, followed by chronic inflammation during the post-exposure period.
Test condition: Doses: 0.145, 0.29, 0.58 mg/l
Reliability: (3) invalid
31-OCT-2005 (13)

Type: other: Lethal time (LT50)
Species: dog
Exposure time: 28 minute(s)
Value: = 2.9 mg/l

Method: other
GLP: no data
Test substance: no data

Remark: LT50 = time point when 50% of animals died;
symptoms of toxicity = pulmonary edema, hemorrhage
Reliability: (3) invalid
31-OCT-2005 (242)

Type: other: Respiratory rate
Species: rat
Exposure time: 10 minute(s)
Value: = .07 mg/l

Method: other: see reference
Year: 1982
GLP: no data
Test substance: no data

Result: RD50=0.07 mg/l, i.e. concentration that caused a 50% decrease in respiratory rate.
Reliability: (3) invalid
31-OCT-2005 (11)

Type: other: Respiratory rate
Species: rat
Exposure time: 10 minute(s)
Value: = .03 mg/l

Method: other
GLP: no data
Test substance: no data

Result: RD50=0.03 mg/l, i.e. concentration that caused a 50% decrease in respiratory rate.
Reliability: (3) invalid
31-OCT-2005 (50)

Type: LC50
Species: rat
Strain: Sprague-Dawley
Sex: male
Exposure time: 60 minute(s)
Value: ca. .86 mg/l

Method: other: see reference
GLP: no
Test substance: no data

Method: Inhalation LD50-value was calculated by the method of Thompson (1947) and Weil (1952) or, where enough information was available, by the probit method.
Result: The 1-hr LD50 value was 0.86 mg/l or 293 ppm (260-327).
Reliability: (4) not assignable
31-OCT-2005 (234)

Method: LT50 = time point when 50% of animals died was investigated. Groups of 4 male and female mice were exposed to 63, 250 or 1000 ppm for 16 hours or until death. Animals that survived and controls were observed for 5 months and then necropsied. Animals that died during the exposure were necropsied.
Result: The LT50 was >960, 440 and 28 minutes in mice exposed to 63, 250 and 1000 ppm, respectively. Animals exposed to 250 and 1000 ppm exhibited lacrimation initially followed by dyspnea,
prostration and convulsions. All of the mice exposed to 250 ppm were dead within 500 minutes and at 1000 ppm within 50 minutes.
Reliability: (3) invalid
31-OCT-2005

Type: LC50
Species: mouse
Exposure time: 10 minute(s)
Value: = .88 mg/l

Method: other
GLP: no data
Test substance: no data

Reliability: (3) invalid
31-OCT-2005 (5)

Type: LC50
Species: mouse
Exposure time: 10 minute(s)
Value: = 1.8 mg/l

Method: other
GLP: no data
Test substance: no data

Reliability: (3) invalid
31-OCT-2005 (143)

Type: LC50
Species: mouse
Exposure time: 160 minute(s)

Method: other: see reference
Year: 1978
GLP: no
Test substance: no data

Method: Mice were exposed to chlorine in concentrations of 0.84 and 0.49 mg/l. The exposure time was between 5 and 30 min for the high concentration and between 15 and 160 min for the lower concentration.
Result: Median lethal dose values in mice were calculated to be 0.84 mg/l for an 11 min exposure and to 0.49 mg/l for a 55 min for exposure.
Test condition: Doses: 0.84 and 0.49
Reliability: (3) invalid
31-OCT-2005 (26)

Type: LC50
Species: mouse
Exposure time: 30 minute(s)
Value: = 1.46 mg/l

Method: other
GLP: no data
Test substance: no data

Reliability: (3) invalid
31-OCT-2005 (249)

Type: LC50
Species: mouse
Exposure time: 60 minute(s)
Value: = .4 mg/l

Method: other: see reference
Year: 1977
GLP: no data
Test substance: no data

Method: Inhalation LD50-value were calculated by the method of Thompson (1947) and Weil (1952) or, where enough information was available, by the probit method.
Result: The 1-hr LD50 value was 0.40 mg/l or 137 ppm (119-159).
Reliability: (3) invalid
31-OCT-2005 (234)

Type: LC50
Species: mouse
Exposure time: 10 minute(s)
Value: = 1.73 mg/l

Method: other
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: 20 mice were exposed to various concentrations over a concentration range of 0.73-3.30 mg/L. Animals were observed for 10 days. Symptoms of toxicity: lethality
Reliability: (3) invalid
31-OCT-2005 (210)

Type: LC50
Species: mouse
Exposure time: 30 minute(s)
Value: = 2.05 mg/l

Method: other
GLP: no data
Test substance: no data

Reliability: (3) invalid
31-OCT-2005 (249)

Type: LC50
Species: mouse
Exposure time: 30 minute(s)
Value: = 1.89 mg/l

Method: other
GLP: no data
Test substance: no data

Method: Observation period: 3 days
Symptoms of toxicity: general excitement (restlessness, barking, urination, defecation); irritation of the eyes, sneezing, copious salivation, retching, vomiting; pulmonary oedema;
Pathology: necrosis of the epithelium lining the respiratory tract, destruction of the epithelium of the trachea and bronchi.
Reliability: (3) invalid
31-OCT-2005 (231)

Type: other: Lethal time (LT50)
Species: mouse
Exposure time: 53 minute(s)
Value: = 2.9 mg/l

Method: other
GLP: no
Test substance: no data

Remark: LT50 = time point at which 50% of the animals died; Symptoms of toxicity = pulmonary edema, hemorrhage
Reliability: (3) invalid
31-OCT-2005 (242)

Type: other: respiratory irritation
Species: mouse
Exposure time: hour(s)
Value: = ppm

Method: other: Test for assessment of sensory irritation
Year: 1990
GLP: yes
Test substance: no data

Result: The RD50 for an atmosphere of chlorine has been estimated as 5.7 ppm and an atmosphere of sodium hypochlorite (based on free chlorine) as 4.11 ppm.
The similarity of the results showed that the irritation of sodium hypochlorite is associated with the chlorine content of the material.

This test measures only respiratory (sensory) irritation and does not take into account other forms of toxicity.
Reliability: (3) invalid

31-OCT-2005 (107)

Type: other
Species: guinea pig
Exposure time: 30 minute(s)
Value: = .59 mg/l

Method: other: not specified
Year: 1970
GLP: no data
Test substance: no data

Result: Severe injury to mucous membranes of upper respiratory tract, irregular dilation and contraction of bronchi. Patches of acute emphysema and atelectasis, and inflammation.
Animals surviving 15 to 193 days after gassing showed emphysema, exudate in bronchioles consisting of fibroblasts, blood vessels with mononuclear cells.
Test condition: Doses: 0.59 mg/l
Guinea pigs were gassed for 15 to 30 minutes with 200 ppm (0.59 mg/l) of chlorine.
Reliability: (3) invalid
31-OCT-2005 (75)

Type: other

Method: other
GLP: no
Test substance: no data

Remark: Species: cat, rabbit, guinea-pig
Result:

Exposure (Dose + duration)	Effects
0.87 mg/l, 60 min.	asphyxiation
0.087 mg/l, several hours	pulmonary inflammation, hemorrhage
0.029 mg/l	inflammation of the respiratory mucosa
0.0087 mg/l	distinct irritation

no further information available
Reliability: (3) invalid
31-OCT-2005 (83)

Remark: Acute dermal toxification is unlikely with gaseous chlorine. Contact with liquid chlorine will cause bullous burn and frostbite. Yet, concomitant inhalation of chlorine gas will be main cause of hazard.
Flag: Critical study for SIDS endpoint
31-OCT-2005

Type: LD50
Species: rabbit
Value: > 2000 mg/kg bw

Year: 1975

Reliability: (4) not assignable
31-OCT-2005

5.1.4 Acute Toxicity, other Routes

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

Remark: Gaseous chlorine (and liquid chlorine) are already classified as irritating to the skin in the EU. Contact with liquid chlorine will cause burn skin and frostbite.
The following entries give results of a solution of sodium hypochlorite. If gaseous chlorine is dissolved in water it is in a fast equilibrium with hypochlorous acid which again is in a fast equilibrium with hypochlorite ions.
However dissolving chlorine in unbuffered water lowers the pH. Therefore a solution of chlorine in water is not directly comparable with the following entries.

Because of the above equilibria, in general, concentrations are provided as free available chlorine or as total residual chlorine (TRC). (See chapter 1.11 for definition.)
Flag: Critical study for SIDS endpoint
31-OCT-2005

Species: rabbit

Method: OECD Guide-line 404 "Acute Dermal Irritation/Corrosion"
Year: 1985
GLP: no data
Test substance: other TS: sodium hypochlorite

Result: Test compound: sodium hypochlorite in aqueous solution (2, 20, 35 or 50 %); test was done with rabbits, skin irritation index max. 8, erythema and edema were scored; test result: with 2 % irritation index 1.2, with 20 % 5.3, with 35 % 5.2 and with 50 % 5.3.
Reliability: (4) not assignable
31-OCT-2005 (144)

Species: rabbit
Concentration: 4.7 %
Exposure: Semiocclusive
Exposure Time: 24 hour(s)
Result: not irritating

Method: other: Federal Hazardous Substance Act Regulation 1973

Result: Primary irritation index < 5, not irritating.
Test condition: 0.5 ml of a 4.74 % sodium hypochlorite solution, semioccl., exposure period 24 h, examination after 48 h.
Reliability: (3) invalid
31-OCT-2005 (178)

Species: rabbit
Concentration: 2.6 %
Exposure Time: 30 minute(s)

Method: other
Year: 1951
GLP: no data
Test substance: other TS: bleach solution containing 5.25 of sodium hypochlorite

Method: The commercial solution was mixed 2:1 with water or various body fluids and than applied on the skin.
Result: Severe injury after 15 or 30 min. of exposure.
Reliability: (3) invalid
31-OCT-2005 (217)

Species: rabbit
Concentration: 12.5 %
Exposure Time: 24 hour(s)
Result: irritating

Method: other
Test substance: other TS: Concentrated Solution 12.5% of sodium hypochlorite w/w

Remark: EC classificat.: irritating
Reliability: (3) invalid
31-OCT-2005 (71) (206)

Species: other: rabbit, guinea pig
Concentration: 5.3 %
Exposure Time: 4 hour(s)
Result: slightly irritating

Method: other
Year: 1975
GLP: no data
Test substance: other TS: 5.25% solution of sodium hypochlorite

Method: Intact or abraded skin, 4 h covered patch test.
Reliability: (3) invalid
31-OCT-2005 (172)

Species: rat
Concentration: 2.6 %
Exposure Time: 30 minute(s)
Result: irritating

Method: other
Year: 1951
GLP: no data
Test substance: other TS: bleach solution containing 5.25% sodium hypochlorite

Method: The commercial solution was mixed 2:1 with water or variuos body fluids and than applied on the skin.
Result: Severe injury with the mixture with water.
Reliability: (3) invalid
31-OCT-2005 (217)

Species: rabbit

Test substance: other TS

Result: At 24 hours, Mildew-Rid caused moderate to severe erythema in four of the twelve intact sites, slight erythema in four

sites, and no erythema in four. Four of the twelve abraded sites showed moderate to severe erythema, four showed well-defined erythema, and the remaining four showed very slight erythema.

At 48 hours, ten of the twelve intact sites showed very slight erythema, and two showed no erythema. Three of the twelve abraded sites showed well-defined erythema, and eight showed very slight erythema, and one showed no erythema.

At 72 hours, ten of twelve intact sites showed very slight erythema, and two of twelve showed no erythema. One of twelve abraded sites showed well-defined erythema, and ten of twelve showed very slight erythema, and one of twelve showed no erythema.

No edema was observed at 24, 48, or 72 hours on intact or abraded sites.

Test condition: A group of six albino New Zealand rabbits in a weight range of 1.8 - 2.4 kg, was used in this study. The test method is essentially that of Draize et al.

Briefly paraphrased, it consists of application of the test material (0.5mL) to clipped areas of intact and abraded skin to the extent of approximately 10 percent of the total body surface of the animal. The abrasions are longitudinal epidermal incisions sufficiently deep as to destroy the integrity of the derma. Following applications of the test material, the entire trunk of the animal is wrapped in an imperious sheeting. The animal is then immobilized. The sites are individually examined and scored separately for erythema and edema at 24 and 72 hours. The mean scores for 24- and 72-hour gradings are averaged to determine final irritation indices.

Scoring criteria for skin reactions are presented below.

Erythema and Eschar Formation

1: very slight erythema (barely perceptible)
2: well-defined erythema
3: moderate to severe erythema
4: severe erythema (beet redness) to slight eschar formation (injuries in depth)
Total possible erythema score = 4

1: very slight edema (barely perceptible)
2: well-defined edema (edges of area well-defined by definite raising)
3: moderate to severe edema (area raised approximately 1 mm)
4: severe edema (raised more than 1 mm and extending beyond area of exposure)
Total possible edema score = 4
Total possible primary irritation score = 4

Test substance: The test material, supplied by Olin Research Center, was identified as Mildew-Rid, Sample #A-10199.

Reliability: (4) not assignable
31-OCT-2005 (167)

Species: rabbit

Result: highly corrosive
EC classificat.: highly corrosive (causes severe burns)

Year: 1975
GLP: no

31-OCT-2005

5.2.2 Eye Irritation

Remark: Chlorine is already classified as irritating to eyes in the EU. Chlorine causes strong irritation of the eyes. Injury of cornea can result enduring impaired vision and blindness.

The following entries give results of a solution of sodium hypochlorite. If gaseous chlorine is dissolved in water it is in a fast equilibrium with hypochlorous acid which again is in a fast equilibrium with hypochlorite ions.
However dissolving chlorine in unbuffered water lowers the pH. Therefore a solution of chlorine in water is not directly comparable with the following entries.

Because of the above equilibria, in general, concentrations are provided as free available chlorine or as total residual chlorine (TRC). (See chapter 1.11 for definition.)
Flag: Critical study for SIDS endpoint
31-OCT-2005

Species: rabbit
Concentration: 5.5 %
Dose: .1 ml
Exposure Time: unspecified

Method: other: according to Draize.
Year: 1965
GLP: no data
Test substance: other TS: sodium hypochlorite solution 1-5.5%

Method: 0.1 milliliter instilled into the conjunctival sac of rabbits, non of the eyes was rinsed.
Remark: Vehicle: water
Result: The time for eyes to recover completely: with 5.5 % solution 7 to more than 35 days, with 1 % 14 days.
Reliability: (3) invalid
31-OCT-2005 (45)

Species: rabbit
Concentration: 5 %
Dose: .1 ml

Method: other: according to Draize
Year: 1985
GLP: no data
Test substance: other TS: solution of sodium hypochlorite

Method: Draize procedure with 0.01 or 0.1 ml/eye.
Result: Draize score = 11 (with 0.01 ml) or 40 (with 0.1 ml).
Reliability: (3) invalid
31-OCT-2005 (49) (67)

Species: rabbit
Concentration: 5 %
Dose: .1 ml
Result: irritating

Method: other: according to Draize
GLP: no data
Test substance: other TS: solution of sodium hypochlorite 5%

Method: Volumes of 0.01, 0.03 and 0.1 ml directly to the central corneal surface of rabbits, follow up 21 days, Draize scale.
Result: With 0.01 ml moderate irritation; with 0.03 ml substantial irritation and with 0.1 ml severe or corrosive reaction.
Reliability: (3) invalid
31-OCT-2005 (67) (97)

Species: rabbit
Concentration: 50 %

Method: other: according to Draize 1958
Year: 1986
GLP: no data
Test substance: other TS: sodium hypochlorite solution

Method: According to Graize et al.
Remark: Text of reference is in Japanese, the amount applied is not given in English.
Result: Without washing out the score 21 days after application was 48/110, with wash out after 30 sec. the score was 27/110 and with wash out after 4 sec. the 21-day score was 0/110.
Reliability: (3) invalid
31-OCT-2005 (67) (159)

Species: rabbit
Concentration: 4.7 %
Dose: .1 ml

Method: other
Year: 1977
GLP: no data
Test substance: other TS: liquid bleach containing sodium hypochlorite, pH=10.1

Method: 0.1 ml, examination after 24 h, day 2, 3 and 7.
Result: Severe reaction, not reversible within 7 days.
Reliability: (3) invalid
31-OCT-2005 (178)

Species: rabbit
Concentration: 5.3 %
Dose: .05 ml

Method: other
Year: 1985
GLP: no data
Test substance: other TS: sodium hypochlorite solution

Method: 0.05 ml/eye of sodium hypochlorite solution (5.25, 0,525, 0.052 and 0.005%), eyelid closed for 30 sec., scoring at 2, 6, 24, 48 and 72 h.
Result: With 0.52 and 5.25 % moderately severe conjunctival

palpebral edema and hyperemia, with 5.25 % slight corneal pitting.
Reliability: (3) invalid
31-OCT-2005 (179)

Species: rabbit
Concentration: .5 %
Result: irritating

Method: other
Year: 1983
GLP: no data
Test substance: other TS: sodium hypochlorite solution pH=9.0

Method: the cornea was washed for 3 to 5 minutes with the solution.
Result: After 6 h edema and redness of the eye, after 24 h severe edema, redness, discharge and corneal haziness; complete recovery within 14 days after an exposure of 3 minutes, no complete recovery after 5 min. exposure.

Reliability: (3) invalid
31-OCT-2005 (233)

Species: rabbit
Concentration: 5 %

Method: other

Method: Rabbit, in the eye, no data about volume.
Result: Edema and hemorrhage of conjunctiva and opacity of the cornea, reversible within 1 week. After rinsing the eyes 30 sec. only slight cornea opacity and edema of the conjunctiva, reversible within 1 day.
Reliability: (3) invalid
31-OCT-2005 (94)

Species: rabbit
Concentration: 5 %
Dose: .1 ml
Exposure Time: unspecified

Method: other: according to Draize
Year: 1962
GLP: no data
Test substance: other TS: sodium hypochlorite

Method: 0.1 milliliter instilled into the conjunctival sac of rabbits, eyes rinsed within 30 seconds.
Remark: Vehicle: water
Result: A 5% solution at pH 11.1-11.6 caused immediate pain, but if washed off with water within 30 seconds, left only slight, transient corneal epithelial haze and conjunctival edema, with return to normal within a day or less.
Reliability: (3) invalid
31-OCT-2005 (95)

Species: rabbit
Concentration: 15 %
Dose: 1 other: drop
Exposure Time: unspecified

Method: other: according to Draize
Year: 1962
GLP: no data
Test substance: other TS: sodium hypochlorite

Method: One drop instilled into the conjunctival sac of rabbits, eyes rinsed within 30 seconds.
Remark: Vehicle: water
Result: One drop of 15% solution at pH 11.2 caused immediate severe pain, and if the hypochlorite solution is not promptly washed away with water it causes hemorrhages from the conjunctiva and nose, plus rapid onset of ground-glass appearance of the corneal epithelium. This is followed by moderate bluish edema of the whole cornea, chemosis and discharge for several days. Such eyes have been observed to heal in 2-3 weeks with slight or no residual corneal damage, but there was neovascularization of the conjunctiva and distortion of the nictitating membrane by scarring.
Reliability: (3) invalid
31-OCT-2005 (95)

Species: monkey
Concentration: 5.5 %
Dose: .1 ml

Method: other
Year: 1965
GLP: no
Test substance: other TS: sodium hypochlorite solution

Method: 0.1 milliliter instilled onto the cornea with the lids held apart, non of the eyes was rinsed.
Result: The time for eyes to recover completely: with 5.5 % solution 2 days, with 1 % 1 day.
Reliability: (3) invalid
31-OCT-2005 (45)

Species: rabbit
No. of Animals: 6
Result: not irritating

Test substance: other TS

Result: Mildew-Rid was minimally irritating at 24 and 48 hours, and practically non-irritating at 72 jhours in the No Wash Group. The sample was minimally irritating at 24 hours, practically non-irritating at 48 hours, and non-irritating at 72 hours in the Four Second Wash Group.

Rabbit No.-Hour	Total Score
No Wash Group	
1-24	22
1-48	17
1-72	2
2-24	7
2-48	7
2-72	0
3-24	9
3-48	2
3-72	1

Average-24	13
Average-48	9
Average-72	1

Four Second Wash Group	
4-24	4
4-48	2
4-72	0
5-24	17
5-48	0
5-72	0
6-24	2
6-48	2
6-72	0
Average-24	8
Average-48	1
Average-72	0

Test condition: A group of six albino New Zealand rabbits in a weight range of 1.8 - 2.4 kg, was used in this study. The test method is essentially that of Draize et al.

Briefly paraphrased, it contains of applications of test material to the right eye of each animal with the left eye serving as control. Half of the animals receive no further treatment and the remaining animals receive an eye wash four seconds after instillation of test materials.

Eyes are scored at 24, 48 and 72 hours for evidence of injury or irritation of the cornea, iris, bulbar and palpebral conjunctiva, and graded according to a standard.

-Classification

Rating	Score Range
Non-irritating	0.0-0.5
Practically non-irritating	0.5-2.5
Minimally irritating	2.5-15.0
Mildly irritating	15.0-25.0
Moderately irritating	25.0-50.0
Severely irritating	50.0-80.0
Extremely irritating	80.0-110.0

Test substance: The test material, supplied by Olin Research Center, was identified as Mildew-Rid, Sample #A-10199.

Reliability: (4) not assignable

31-OCT-2005 (167)

Species: laboratory animal
Result: corrosive
EC classificat.: risk of serious damage to eyes

Year: 1975

31-OCT-2005

5.3 Sensitization

Remark: Chlorine gas is an irritating. A sensitization tests in animals is not appropriate for gaseous chlorine.

The following entries give results of a solution of sodium hypochlorite. If gaseous chlorine is dissolved in water it is in a fast equilibrium with hypochlorous acid which again is in a fast equilibrium with hypochlorite ions.

Because of the above equilibria, in general, concentrations are provided as free available chlorine or as total residual chlorine (TRC). (See chapter 1.11 for definition.)
Flag: Critical study for SIDS endpoint
31-OCT-2005

Type: Patch-Test
Species: human
Concentration 1st: Induction .5 other: ml
2nd: Challenge .5 other: ml
No. of Animals: 86
Vehicle: water
Result: not sensitizing

Method: other
Year: 1988
GLP: yes
Test substance: other TS: sodium hypochlorite solution 4 %

Method: Induction:
The material was applied in 0.5 ml aliquots to a 2 cm2 pad which was applied down the dorsal surface of the upper arm. Volunteers were instructed to keep the patches dry and clean and to remove and discard them after 24 hours. The patches were applied on Monday Wednesday and Friday of the first three weeks.

Challenge:
F14 days after the final insult patch challenges patches were applied to both arms of each subject. The results were graded after 48 and 96 hours.
Result: At challenge there was no evidence of skin sensitisation observed on the 86 subjects.
Reliability: (4) not assignable
31-OCT-2005 (212)

5.4 Repeated Dose Toxicity

Remark: The chronic toxicity of chlorine has been investigated very carefully. For both routes of administration, inhalation and drinking water, 90 days studies or even longer were performed.
Flag: Critical study for SIDS endpoint
31-OCT-2005

Species: rat Sex: male/female
Strain: Fischer 344
Route of administration: inhalation
Exposure period: 2 years
Frequency of treatment: 6 hours/day; 5 days/week (male); 3 days/week (female)

Post exposure period: no
Doses: 0. 0.4, 1.0 or 2.5 ppm
Control Group: yes
NOAEL: < .4
LOAEL: < .4

Method: other: essentially follows Combined Chronic Toxicity/Carcinogenicity Study Guideline
Year: 1993
GLP: yes
Test substance: other TS: chlorine 99.7% purity

Result: NOAEL and LOAEL were not provided in the reference but were deduced from the figures provided in the reference.

There was no difference in survival for male or female rats exposed to various concentrations of chlorine. Overall survival for female rats ranged from 80.2 - 85.2% and for males ranged from 72.9 - 80.4%. There were evidences of reduced body weight in male rats at all levels of chlorine exposure. Female rats exposed for 3 days/week exhibited significant effects at 1.0 and 2.5 ppm but not at 0.4 ppm. Hematology and clinical chemistry parameters were unaffected in rats exposed to chlorine for 12 or 24 months. Terminal body weight was decreased in male rats at the interim necropsy and in both sexes at the final necropsy. There were no biologically significant treatment-related changes in brain, liver or kidney weights in male or female rats but in male rats the liver weights were statistically significantly reduced. There were no treatment-related macroscopic findings in male rats at necropsy. High concentration exposed female rats had the macroscopic observation of cataract which was not seen in any other treatment group. However, following histopathologic examination, the eye was not considered a target organ.

Exposure-dependent lesions were confined to the nasal passage in all sex and species groups. Chlorine-induced lesions, which were most severe in the anterior nasal cavity, included respiratory and olfactory epithelial degeneration, septal fenestration, mucosal inflammation, respiratory epithelial hyperplasia, squamous metaplasia and goblet cell hypertrophy and hyperplasia, and secretory metaplasia of the transitional epithelium of the lateral meatus. Intracellular accumulation of eosinophilic proteinaceous material was also a prominent response involving the respiratory, transitional, and olfactory epithelia, and in some cases the squamous epithelium of the nasal vestibule. Many of these nasal lesions exhibited an increase in incidence and/or severity that was related to chlorine exposure concentration and were statistically-significantly increased at all chlorine concentrations studied.

Female rats were more sensitive to the effects of chlorine than male rats.

Selected Nasal Lesions in Rats
Respiratory Epithelial Eosinophilic Material (Level3):

Males	Females

Conc. [ppm]	affected [%]	Mean Severity	affected [%]	Mean Severity
0.0	3	1.4	70	1.2
0.4	33	1.5	85	1.2
1.0	51	1.6	84	1.3
2.5	73	1.6	93	2.0

Olfactory Epithelium Eosinophilic Material (Level 3):

Males			Females	
Conc. [ppm]	affected [%]	Mean Severity	affected [%]	Mean Severity
0.0	12	1.4	52	1.9
0.4	83	2.2	91	2.0
1.0	80	2.6	99	2.8
2.5	82	3.0	99	2.9

Test condition: Female and male F344 rats were exposed to chlorine gas for up to 2 years to determine chronic toxicity and carcinogenic properties. Groups of approximately 70 each of female and male rats were exposed to 0, 0.4 , 1.0, and 2.5 ppm chlorine gas for 6 h/day, 5 days/week (male rats), or 3 alternate days/week (female rats) for 2 years, with an interim necropsy of rats at 12 months (10 rats /sex/concentration group). A complete necropsy was performed on all animals. Histological examination was performed on all organs from high-concentration and control animals and selected target organs (nose and any gross lesions) from mid- and low-concentration groups.
Mice were also exposed to chlorine in the same chamber and are discussed separately.
Reliability: (1) valid without restriction

Flag: Critical study for SIDS endpoint
31-OCT-2005 (251)

Species: rat Sex:
Strain: Fischer 344
Route of administration: drinking water
Exposure period: 13 weeks
Doses: 0.025, 0.05, 0.1, 0.2 to 0.4 % NaClO
Control Group: yes, concurrent vehicle

Year: 1980
GLP: no
Test substance: other TS

Result: Six groups of male and female F-344 rats (10/group and sex) received NaClO in drinking water at levels from 0.025, 0.05, 0.1, 0.2 to 0.4 % in drinking water in a 13-week study. A dose-related decrease in body weight gain was observed in both sexes with a marked effect in high dose male and female groups, significant for 0.2 % and 0.4 % in males and 0.4 % in females, obviously correlated with reduced water consumption in affected groups. No histological changes attributable to the treatment were found. The increasing of GOT in the blood sera showed signs of slight liver effects

in the 0.2 and 0.4 % groups for both sexes. Absolute weights of the lung, liver and spleen of males and the salivary gland, lung, heart and brain of females were significantly lower in the highest-dose group than in the controls, relative weights were not changed. A maximum tolerated dose of sodium hypochlorite given in the drinking-water was estimated to be between 0.1 and 0.2 % for male and 0.2 and 0.4 % for female rats.
Test substance: Manufacturer: TSURUMI DODA, JAPAN (available chlorine 12%)
Conclusion: A maximum tolerated dose of sodium hypochlorite given in the drinking-water was estimated to be between 0.1 and 0.2 % for male and 0.2 and 0.4 % for female rats.
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
31-OCT-2005 (85)

Species: mouse Sex: male/female
Strain: B6C3F1
Route of administration: inhalation
Exposure period: 2 years
Frequency of treatment: 6 hours/day; 5 days/week
Post exposure period: no
Doses: 0, 0.4, 1.0 or 2.5 ppm
Control Group: yes
NOAEL: < .4 ppm
LOAEL: < .4 ppm

Method: other: essentially follows Combined Chronic Toxicity/Carcinogenicity Study Guideline
Year: 1993
GLP: yes
Test substance: other TS: chlorine 99.7% purity

Remark: An interim necropsy of rats was performed at 12 months (10 mice/sex/concentration group).
Result: NOAEL and LOAEL are not provided in the reference but were deduced from the figures provided in the reference.

There was no difference in survival for male or female mice exposed to various concentrations of chlorine. Overall survival for female mice ranged from 53.1 - 61.3% and for males ranged from 38.6 - 47.0% with the lowest survival noted in the controls. Male mice exposed to chlorine at 1.0 and 2.5 ppm exhibited significant body weight depression relative to controls. Female mice exhibited significant depression in body weight only at 2.5 ppm relative to controls. Hematology and clinical chemistry parameters were unaffected in mice exposed to chlorine for 24 months. Terminal body weights in female mice exposed to 1.0 or 2.5 ppm were decreased from control values. Terminal body weights in male mice were unaffected. There were no biologically significant treatment-related changes in gross observations at necropsy or in brain, liver or kidney weights but in female mice the liver weights were statistically significantly reduced.

Exposure-dependent lesions were confined to the nasal passage in all sex and species groups. Chlorine-induced lesions, which were most severe in the anterior nasal cavity, included respiratory and olfactory epithelial

degeneration, septal fenestration, mucosal inflammation, respiratory epithelial hyperplasia, squamous metaplasia and goblet cell hypertrophy and hyperplasia, and secretory metaplasia of the transitional epithelium of the lateral meatus. Intracellular accumulation of eosinophilic proteinaceous material was also a prominent response involving the respiratory, transitional, and olfactory epithelia, and in some cases the squamous epithelium of the nasal vestibule. Many of these nasal lesions exhibited an increase in incidence and/or severity that was related to chlorine exposure concentration and were statistically-significantly increased at all chlorine concentrations studied.

An increase in the incidence of ovarian abscesses and uterine inflammation was observed in female mice exposed to 2.5 ppm chlorine. A smaller proportion of female mice from the 0.4 and 1.0 ppm groups were also affected with this lesion. These lesions were attributed to the infectious condition described by Rao et al. (1987).

Male mice were more sensitive to the effects of chlorine than female mice.

Selected Nasal Lesions in Mice

Respiratory Epithelial Hyperplasia (Level 2)

Males			Females	
Conc. [ppm]	affected [%]	Mean Severity	affected [%]	Mean Severity
0.0	17	1.7	8	1.2
0.4	44	2.2	47	2.2
1.0	48	2.7	87	2.7
2.5	61	2.7	39	3.3

Olfactory Epithelium Atrophy (Level 3)

Males			Females	
Conc. [ppm]	affected [%]	Mean Severity	affected [%]	Mean Severity
0.0	13	1.5	3	2.0
0.4	12	1.5	20	2.2
1.0	28	2.7	21	2.3
2.5	42	2.2	39	2.8

Test condition: Female and male B6C3F1 mice were exposed to chlorine gas for up to 2 years to determine chronic toxicity and carcinogenic properties. Groups of approximately 70 each of female and male mice were exposed to 0, 0.4 , 1.0, and 2.5 ppm chlorine gas for 6 h/day, 5 days/week for 2 years. A complete necropsy was performed on all animals. Histological examination was
performed on all organs from high-concentration and control animals and selected target organs (nose, female reproductive tract and any gross lesions) from mid- and low-concentration

groups.
Rats were also exposed to chlorine in the same chamber and are discussed separately.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (53) (186) (251)

Species: rat Sex: male/female
Strain: Sprague-Dawley
Route of administration: drinking water

Exposure period: 90 days
Frequency of treatment: continuously in drinking water
Doses: 25, 100, 175, 250 mg/l drinking water, see also Results
Control Group: yes, concurrent vehicle
NOAEL: 16.7 mg/kg bw
LOAEL: > 16.7 mg/kg bw

Method: other: essentially follows OECD 409 Repeated Dose 90-Day Oral Toxicity Study.
Year: 1990
GLP: no data
Test substance: no data

Method: Amber-colored glass drinking-water bottles were used to reduce photolytic degradation. Double-balled stainless-steel sipper tubes were used to minimize drippage and to facilitate
accurate water consumption analysis. The bottles were filled to the top with fresh drinking solutions every other day. The concentration and purity of the disinfectant solutions were
determined before offering the test chemical to the animals and at the time of refilling bottles to determine the extent of degradation. The percentage of decomposition for chlorine during 72 hours in the water bottles was 3.6-17%.
Remark: The author did not consider the increased relative kidney weight effect to be evidence of an adverse effect.
Result: These dose-levels, 25, 100, 175 and 250 mg Cl/L corresponded to chlorine levels of 3.5, 12.6, 19.5 and 24.9 mg/kg/day and to 2.1, 7.5, 12.8 and 16.7 mg/kg/day for females and for males, respectively.

There were no deaths attributed to 90 days of dosing with any concentration of chlorine; however daily water consumption was decreased at all dose levels in males and 100, 175 and 250 mg/L in females. This was considered to be due to taste aversion. There were no clinical effects observed. There were no treatment-related effects on final body weights, weight gain and organ weights. The relative kidney weight for 250 mg/L females was significantly increased from control values.

Although there were several significant changes in hematology and clinical chemistry parameters, these were judged to be sporadic and not treatment-related. All gross and histopathologic observations during the 90-day study were considered to represent common, spontaneous lesions typical for Sprague-Dawley rats and were judged not related to exposure to chlorine.

LOAEL: greater than 250 mg/L the highest dose tested
NOAEL: 24.9 mg/kg/day for females

NOAEL: 16.7 mg/kg/day for males
Test substance: The chlorine solution was prepared by bubbling chlorine gas into double-distilled water to pH 9.4. The water for control animals was distilled water buffered with sodium bicarbonate to a pH of 8.0-8.5. The concentration of chlorine was determined by the N,N-diethylphenylenediamine method.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (59)

Species: mouse Sex: male/female
Strain: B6C3F1
Route of administration: drinking water

Exposure period: 90 days
Frequency of treatment: continuously in drinking water
Doses: 0, 12.5, 25, 50, 100 and 200 mg Chlorine/l
Control Group: yes, concurrent no treatment
NOAEL: ca. 10 mg/kg bw
LOAEL: 12.2 mg/kg bw

Method: other: essentially follows OECD 409 Repeated Dose 90-Day Oral Toxicity Study.
Year: 1991
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Stock solutions of chlorine were prepared by bubbling 99.5% chlorine gas into sodium hydroxide (243 g/4L) to a pH of 12.0, or approximately 50-55 g/L chlorine as determined by iodometric titration. The chlorine dosing solutions were prepared by diluting the stock chlorine solution with pH 9.4 carbonate buffer (1.38 g Na2CO3-10H20 and 1.5 g NaHCO3/L). The concentration of chlorine was determined by the N,N-diethylphenylenediamine method.

The concentrations equals to 2.7, 5.1, 10.3, 19.8 and 34.3 mg/kg/day for males and 2.8, 5.8, 11.7, 21.2 and 39.2 mg/kg/day for females, respectively.

The mice were assigned to 12 groups, each consisting of 10 males and 10 females.
Remark: Animals of the higher dose groups showed several changes, e.g., lower levels of serum enzymes and reduced organ weights, that were considered by the authors consistent with decreased water consumption, nutritional deficiencies and altered electrolyte balance rather than any specific indication of substance-induced toxicity. The authors also suggested that these effects could be a consequence of the decreased water consumption associated with taste aversion and not chemically induced toxicity per se.
Result: These dose-levels of 0, 12.5, 25, 50, 100 and 200 mg Chlorine/l corresponded to chlorine levels of 2.7, 5.1, 10.3, 19.8 and 34.3; and to 2.8, 5.8, 11.7, 21.2 and 39.2 mg/kg/day for males and females respectively.

Water consumption was significantly decreased in female mice

at 100 and 200 mg/L. At other concentrations, there was a slight concentration-related decreased water consumption in both males and females.
One female of the highest dose group died on day 82 of the experiment (necropsy: mild congestion of the lung and bronchus).

There were no clinical effects observed. There was a concentration-related decrease in weight gain for both sexes with a significant reduction in males at 100 and 200 mg/L (Table 1). Similarly, there was a significant reduction in body weight gain for males at 100 and 200 mg/L.

Tabulated body weight data for can be found in Table 1 of the attachement.

Exposure to chlorine in the drinking water produced only minor changes in hematology (Table 2). A slight increase in the RBC count was seen in males at 200 mg/L. In females, a slight decrease in RBC count was observed, reaching statistical significance at 25 and 200 mg/L. Except for slight decreases in the hematocrit for females at 100 mg/L and in MCV at 200 mg/L for males, there were no significant differences in the RBC parameters. In the females a dosage-related increase in WBCs was observed for the three highest concentrations. A slight (nonsignificant) increase was also observed in all treated male groups. There were no remarkable differences from the controls observed among the WBC counts.

Tabulated hematology measurements can be found in Table 2 of the attachment.

Alanine aminotransaminase (ALT) and alkaline phosphatase (AP) were consistently decreased in both males and females (Table 3). Alanine aminotransaminase was significantly lower for males at the two highest concentrations and for the highest dose female group. Alkaline phosphatase was significantly decreased at 25 mg/L for the males and at the four highest concentrations, 25, 50, 100 and 200 mg/L, for the females. Lactate dehydrogenase (LDH) was significantly increased in females at 25 mg/L and significantly decreased in females at 200 mg/L. The authors did not consider this to be treatment-related.

Tabulated clinical chemistry measurements can be found in Table 3 of the attachment.

Although numerous sporadic decreases in absolute and relative organ weights were observed, no consistent dosage-related increase in organ weights was seen in any of the treated groups for either sex (Table 4). Statistically significant decreases were seen in the weights of the adrenals, heart, liver, lung and spleen. In males, decreases were seen in both the absolute and relative weights of adrenal glands at two lower concentrations; in the liver at 25, 100 and 200 mg/L; in the lung at several intermediate concentrations; and in the spleen at the two highest concentrations. In females, a decrease in absolute and relative weights of the liver was observed at the highest concentration and of the heart at the two highest

concentrations.

Tabulated organ weight measurements can be found in Table 4 of the attachement.

All gross and histopathologic observations during the 90-day study were considered to represent agonal effects or to be incidental background findings and were judged not related to exposure to chlorine.

Overall, the correlation of the biochemical, hematology and organ weight data, in the absence of histopathology or of observable clinical signs of toxicity suggests that these drinking water exposures induced relatively mild, non-specific toxicity via an indirect mechanism, e.g. nutritional deficiencies, rather than by a direct toxicological effect on specific organs or tissues.
LOAEL: 19.8-12.2 mg/kg/day
NOAEL: 10-12 mg/kg/day

Table 1: Selected Body Weight Data for Mice (males)

Parameter	mg Chlorine/L in drinking water				
0.0	12.5	25.0	50.0	100.0	200.0
Initial BW [g]					
25.6+2.57	25.8+1.83	25.9+2.15	25.8+1.84	25.9+1.49	26.2+1.85
Final BW [g]					
33.0+2.42	32.7+2.13	32.1+2.15	32.2+2.32	31.5+1.49	32.1+2.73
Weight gain [g]					
7.3+1.64	6.9+1.06	6.2+1.21	6.5+1.20	5.6+1.00*	5.9+1.49*

* Statistically different from control, p<0.05.

Table 2: Selected Hematology Measurements for Mice

Parameter	mg Chlorine/L in drinking water				
0.0	12.5	25.0	50.0	100.0	200.0
Males					
RBC-106/ml					
7.45+0.3	7.52+0.25	7.27+0.33	7.41+0.37	7.59+0.35	7.63+
MCV-m3					
38.43+1.77	38.61+1.19	37.48+1.95	37.98+1.88	39.04+1.67	38.52+
WBC-103/ml					
2.39+0.52	2.50+0.75	2.61+0.63	2.76+0.47	2.44+0.66	2.52+
Females					
RBC-106/ml					
7.80+0.39	7.79+0.37	7.51+0.18*	7.66+0.14	7.56+0.16	7.46+
Hematocrit-%					
40.28+2.33	40.10+2.07	38.92+1.08	39.73+0.57	38.58+0.74*	39.02+1.
WBC-103/ml					
1.58+0.38	1.54+0.57	2.16+0.54	2.47+0.54*	2.80+0.65*	

3.04+1.*
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* Statistically different from control, p<0.05.

Table 3: Selected Clinical Chemistry Measurements for Mice

Parameter mg Chlorine/L in drinking water
0.0 12.5 25.0 50.0 100.0 200.0

Males
ALT
36.20+9.17 32.90+6.51 37.70+12.10 36.70+9.37 24.10+4.43*
22.30+10.95*
AP
48.60+3.95 48.30+4.79 44.00+2.71* 46.40+6.04 43.70+8.35
39.20+12.10
Females
ALT
38.70+20.90 33.10+14.00 32.13+12.52 24.00+3.12* 29.90+9.07
26.22+9.92*
AP
83.60+5.19 80.30+10.07 75.50+6.78* 73.25+9.57* 74.50+6.96*
68.33+5.00*
LDH
190.10+54.78 248.00+108.8 417.25+130.7* 187.38+76.35
181.20+48.88 105.78+18.14*
---
* Statistically different from control, p<0.05.

Table 4: Selected Organ Weight Measurements for Mice

Parameter mg Chlorine/L in drinking water
0.0 12.5 25.0 50.0 100.0 200.0

Males
Adrenals
0.017+0.004 0.013+0.007 0.012+0.003* 0.014+0.004
0.012+0.003* 0.014+0.004
Liver1.844+0.215 1.830+0.162 1.673+0.157* 1.733+0.105
1.627+0.117* 1.624+0.159*
Lung0.215+0.018 0.196+0.020* 0.194+0.020* 0.195+0.009*
0.202+0.022 0.203+0.017
Spleen0.098+0.022 0.090+0.016 0.084+0.010 0.086+0.016
0.079+0.010* 0.079+0.016*
Females
Liver
1.528+0.128 1.498+0.117 1.477+0.103 1.470+0.125 1.469+0.116
1.353+0.13
Heart
0.155+0.013 0.145+0.012 0.142+0.015* 0.143+0.011
0.139+0.016* 0.137+0.0
---
* Statistically different from control, p<0.05.

Test substance: Stock solutions of chlorine were prepared by bubbling 99.5% chlorine gas into sodium hydroxide (243 g/4L) to a pH of 12.0, or approximately 50-55 g/L chlorine as determined by iodometric titration.

Reliability: (2) valid with restrictions

Flag: Critical study for SIDS endpoint

31-OCT-2005 (60)

Species: rat Sex: male/female
Strain: Fischer 344
Route of administration: drinking water
Exposure period: 2 years
Frequency of treatment: daily ad libitum
Post exposure period: no
Doses: 3.8 to 13.9 mg/kg/day, details see Methods
Control Group: yes

Method: other: generally followed OECD 453 Combined Chronic Tox/Carcinogenicity Study.
GLP: yes
Test substance: other TS: sodium hypochlorite

Method: Doses: 70, 140 or 275 mg/l buffered water corresponded to 4.8, 7.5 and 13.9 mg/kg/day for male rats and 3.8, 6.9 and 13.2 mg/kg/day for female rats, respectively, based on weeks 53-101. Daily dose consumption was less for each group earlier in the study.
Result: No effect on survival; body weights of male and female rats were slightly lower than control values (97-98% of control values) during the last year of the study; dose-related decrease of water consumption in 140 and 275 mg/L of males and in all three doses of females.
Test substance: Chlorine dose formulations as sodium hypochlorite solutions were prepared by mixing the appropriate volume of stock solution with sodium chloride and bicarbonate-carbonate buffer solution, then diluting with deionized charcoal-filtered drinking water.
Stability studies indicated that the buffered hypochlorite stock solution was approximately 96% of its original concentration after 7 days at 5 □‹C. Chlorinated water formulations at levels of 70 to 275 ppm retained 95% of their original concentrations after storage for 1 day and 90% after 2 days. Thus the buffered hypochlorite stock solution used in these studies was stored at 5 □‹C for no longer than 7 days, and the dose solutions were stored at room temperature for no longer than 48 hours.
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
31-OCT-2005 (70) (175)

Species: mouse Sex: male/female
Strain: B6C3F1
Route of administration: drinking water
Exposure period: 2 years
Frequency of treatment: daily ad libitum
Post exposure period: no
Doses: 3.6 - 22.5 mg/kg/day details see Methods

Method: other: generally followed OECD 453 Combined Chronic Tox/Carcinogenicity Study.
Year: 1992
GLP: yes
Test substance: other TS: sodium hypochlorite

Method: Doses: 70, 140 or 275 mg/l buffered water corresponded to 7.2,14.0 and 22.5 mg/kg/day for male mice and 6.3, 12.1 and 19.8 mg/kg/day for female mice, respectively, based on weeks 53-101. Daily dose consumption was initially greater for

each group earlier in the study.
Result: No effect on survival; mean body weight of male mice ingesting 70, 140 or 275 mg/L were 97, 96 and 94%, respectively, of control value for the last year of the study; mean body weight of female mice ingesting 70, 140 or 275 mg/L were 96, 95 and 94%, respectively, of control values for the last year of the study; mean body weights during the first year were slightly decreased at 275 mg/L but were not affected at 70 and 140 mg/L; dose-related decrease of water consumption.
Test substance: Chlorine dose formulations as sodium hypochlorite solutions were prepared by mixing the appropriate volume of stock solution with sodium chloride and bicarbonate-carbonate buffer solution, then diluting with deionized charcoal-filtered drinking water.
Stability studies indicated that the buffered hypochlorite stock solution was approximately 96% of its original concentration after 7 days at 5C. Chlorinated water formulations at levels of 70 to 275 ppm retained 95% of their original concentrations after storage for 1 day and 90% after 2 days. Thus the buffered hypochlorite stock solution used in these studies was stored at 5C for no longer than 7 days, and the dose solutions were stored at room temperature for no longer than 48 hours.
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
31-OCT-2005 (70) (175)

Species: human Sex: male
Route of administration: other: inhalation study in human volunteers
Exposure period: 6 hours/day
Frequency of treatment: 3 consecutive days
Doses: 0, 0.1, 0.3, 0.5 ppm
Control Group: yes, concurrent vehicle
LOAEL: > .5 ppm

Method: other: see RM and ref.
Year: 1999
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Testing was conducted in 8 subjects using a repeated measures design. Subjects complying with all study selection criteria were exposed on three consecutive days, 6 hours/day, to four conditions: 0, 0.1, 0.3 or 0.5 ppm chlorine. The exposure periods were spaced eleven days apart. Subjects were exposed in two groups of four, based on the availability of subjects and by ballot. Exposure to the test substance and the effect measurements were conducted in a double-blind fashion, i.e., neither the subject nor the co-investigators were aware of exposure conditions. Nasal lavages and lung function were performed before and after each exposure and 1-day and 4-days after the third exposure.
Remark: Abstract:
The objectives of this study were. 1) to determine if chlorine exposure at low levels induces nasal effects in humans as it does in rodents; and 2) to establish a possible occurrence of respiratory effects in human volunteers exposed to chlorine vapour at concentrations of 0, 0.1, 0.3 and 0.5 ppm. The study was conducted in a double-blind fashion in 8 male volunteers using a repeated measures

design, with randomly selected exposure sequences. Subjects were exposed for 6 h/day. on 3 consecutive days to each of the 4 exposure conditions. In nasal lavage, interleukin-8 (IL-8), albumin, total cell number and percentages of neutrophils, lymphocytes, monocytes, eosinophils , and epithelial cells were determined. The lung function parameters that were analysed included forced Vital capacity (VFC), forced expiratory volume in first second (FEV1, FEV1/FVC ratio, and maximal mid expiratory flow (MMEF). Data analysis was limited to 7 subjects since one volunteer

decided to stop participating for reasons not related to the study. Nasal lavage measurements did not support an inflammatory responses or irritant effects on the nasal epithelium. For FVC FEV1 and FEV1/FVC , no significant differences were found. MMEF was significantly different between the 0 and 0.5 ppm exposure, but this was attributed to an unexplained shift in baseline values during control (0 ppm) exposure.
The present data does not support an inflammatory effect in the nose nor shows changes in respiratory function at repeated exposure up to 0.5 ppm. This discrepancy with previous data in rodents can be attributed at least in part to differences in respiratory tract airflow characteristics.

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (197)

Species:	rat	Sex: male/female
Strain:	Fischer 344	
Route of administration:	inhalation	
Exposure period:	6 weeks	
Frequency of treatment:	6 h/d, 5 d/w	
Post exposure period:	no	
Doses:	0.0029, 0.0087, 0.026 mg/l (1, 3, 9 ppm)	
Control Group:	yes	

Method: other: see reference
Year: 1978
GLP: no
Test substance: no data

Method: Groups of 10 rats/sex were exposed to 0, 1, 3 or 9 ppm for 6 hours/day, 5 days/week for 6 weeks. Chamber concentrations, temperature and relative humidity were measured four times/day/chamber. Total chamber airflow varied between 500 and 650 L/min in a 3.7m3 chamber. Animals were weighed three times/week beginning one week prior to exposure and continuing to the end of the study. Blood was obtained prior to the terminal sacrifice for routine hematological (hemoglobin concentration, hematocrit, erythrocyte count, and leukocyte count (total and differential) Urine was also obtained prior to the terminal sacrifice and included appearance, occult blood, specific gravity, protein, pH, ketones, glucose and bilirubin. All surviving rats were fasted overnight and sacrificed 1 (males) or 2 (females) days after the last day of exposure for gross pathological examination. Blood was collected at the terminal sacrifice for clinical chemistry determinations (blood urea nitrogen concentration and serum gamma glutamyl transpeptidase, alkaline phosphatase, glutamic pyruvic transaminase and

glutamic oxaloacetic transaminase activities). The weights of the following organs were recorded: brain, heart, kidneys, liver, lungs, spleen, thymus, testes or ovaries. Approximately 40 tissues were saved for histopathological evaluation.

Result: 0.0029 mg/l: slight irritation of nasal mucosa; slight decrease in body weight in females (Final body weight was 98% (males) and 92% (females) of control value); ; elevation in urine specific gravity of females (Specific gravity was 1.037 in controls and 1.050 in 1 ppm females); pathology: inflammatory reaction in respiratory tract.

0.0087 mg/l: eye and upper respiratory tract irritation; decreased body weight (Final body weight was 90% (males) and 85% (females) of control value); elevation in urine specific gravity (1.031 in controls and 1.048 in 3 ppm males; 1.037 in controls and 1.052 in 3 ppm females); pathology: inflammatory reaction in respiratory tract; minor hepatocellular cytoplasmic changes.

0.026 mg/l: eye and respiratory tract irritation; mortality in 3/10 females; decreased body weight (Final body weight was 56% (males) and 64% (females) of control value); elevation in segmented neutrophils and hematocrit (Segmented neutrophils in controls and 3 ppm males was 2.4 + 1.0 and 5.9 + 1.4 x103/mm3, respectively; hematocrit in control and 3 ppm males was 52.3 + 3.0 and 55.1 + 2.0%, respectively); elevation in urine gravity (1.031 in controls and 1.059 in 3 ppm males; 1.037 in controls and 1.066 in 3 ppm females); elevation in serum enzymes and urea nitrogen (Alkaline phosphatase in control and 9 ppm males was 97.0 + 10.0 and 138.0 + 19.0 mU/ml, respectively; SGGT in control and 9 ppm males was 4.0 + 1.0 and 9.0 + 2.0 mU/ml, respectively; BUN in control and 9 ppm males was 19.0 + 2.0 and 34.0 + 10.0 mg/100 ml, respectively); pathology: general toxicity indicated by decreased size of carcass, emaciation and decreased adipose reserves; inflammatory, necrotic, and hyperplastic reaction of respiratory tract; minor renal tubular and hepatocellular cytoplasmic changes.

Microscopic changes in the nasal turbinates and lungs of rats exposed to chlorine

Concentration, ppm							
0	1	3	9	0	1	3	9
Males				Females			
Nasal Turbinates							
Focal mucopurulent inflammation							
2	1	3	9	0	2	3	5
Multifocal mucopurulent inflammation							
0	0	0	1	0	0	0	3
Necrotic erosions							
0	0	0	3	0	0	0	2
Increased secretory material Lungs							
0	0	0	5	0	0	0	1
Increased secretory material in							
0	1	0	9	0	0	0	4
Bronchiolar lumen							

Epithelial hyperplasia of bronchioles
0 0 0 10 0 0 0 7
Peribronchiolar lymphoid activity +
0 1 2 0 1 0 0 7
++
4 0 1 2 7 3 2 4
+++
5 9 7 7 2 7 8 4
++++
1 0 0 1 0 0 0 0
Peribronchiolar inflammatory reaction
0 0 1 8 0 0 0 4
Inflammatory reaction around respiratory
Bronchioles and alveolar ducts +
0 3 0 6 0 0 0 3
++
0 1 0 2 0 0 0 3
+++
0 0 0 1 0 0 0 0
Increased numbers of alveolar macrophages within alveoli, primarily around alveolar duct
0 3 3 10 2 0 0 5

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (14) (15)

Species: other: rat and mouse Sex:
Strain: other: Fischer 344 and Swiss Webster
Route of administration: inhalation
Exposure period: 1, 3 or 5 days
Frequency of treatment: 6 hours/day
Post exposure period: no
Doses: 9 to 11 ppm
Control Group: yes

Method: other: see reference
Year: 1983
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Animals were exposed to chlorine at their respective RD50 concentration that is the concentration which reduces respiratory rate by 50% for 6 hours/day for 1, 3 or 5 days.
Result: Chlorine induced severe lesions in specific locations in both the olfactory and respiratory epithelia of the nasal passages with more widespread loss of respiratory and olfactory cilia.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (113)

Species: monkey Sex: male/female
Strain: other: Macaca mulatta
Route of administration: inhalation
Exposure period: 52 weeks
Frequency of treatment: 6 h/d, 5 d/w
Post exposure period: no
Doses: 0.1; 0.5; 2.5 ppm (ca. 0.00029, 0.00145, 0.00667 mg/l)
Control Group: yes
LOAEL: ca. .1 ppm

Method: other: see reference
Year: 1987
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Remark: 4 male and 4 female rhesus monkeys/group were used; pulmonary physiology, body weights, urinalysis, electrocardiographs, hematology, and clinical chemistry were evaluated monthly; blood gas evaluations were performed at three-month intervals and histopathologic, ophthalmologic, and neurologic parameters were evaluated after the one-year exposure period.
Result: The monkeys of the highest dose group exhibited signs of ocular irritation at the end of the daily exposures and a superficial conjunctival irritation was present at the end of the treatment period (no ocular irritation of the cornea); histopathological changes of the epithelium of the nasal passages and trachea (limited to focal); concentration-related epithelial hyperplasia with loss of cilia and decease in the number of goblet cells in affected areas; no exposure-related differences in the clinical chemistry, hematology, or urinalysis; no difference between exposed animals and control monkeys in lung function testing(diffusion capacity, distribution of ventilation). Tracheal lesions were confined to the 2.3 ppm group. The lesions observed at 2.3 ppm were not present in all animals.

At the lower chlorine concentrations, similar though less prominent respiratory epithelial lesions were observed. The latter changes were very minimal and were confined to the nasal passages of some treated monkeys and one male control animal. In the low concentration group, effects were only observed in females but not males.

The results of this study indicate that 2.5 ppm chlorine acts as an upper respiratory irritant in monkeys, while 0.5 and 0.1 ppm induce changes of questionable clinical significance. In addition, the monkey appears to be less sensitive than the rat to chlorine toxicity.
Reliability: (4) not assignable
31-OCT-2005 (131)

Species: mouse Sex: no data
Strain: no data
Route of administration: inhalation
Exposure period: 3 days
Frequency of treatment: 8 h/d
Doses: 0.0073, 0.0145 mg/l
Control Group: no data specified

Method: other
GLP: no data
Test substance: no data

Remark: no further information available
Result: Loss in body weight, microscopic examination of the lungs in the high dose group yielded findings similar to these following lethal or near lethal short- term exposures.
Reliability: (3) invalid
31-OCT-2005 (198)

```
Species:                 mouse                              Sex: no data
Route of administration: drinking water
Exposure period:         33 or 55 days
Frequency of treatment:  continuously in drinking water
Doses:                   0.1 (55 d), 0.2 g/l drinking water (33 d)
Control Group:           no data specified

Method:           other
   GLP:           no data
Test substance:   no data

Remark:           no further information available
Result:           no adverse effects were observed
Reliability:      (3)  invalid
31-OCT-2005                                                              (27)

Species:                 rat                                Sex: male/female
Strain:                  no data
Route of administration: oral feed
Exposure period:         30 days
Doses:                   0.071, 0.14, 0.21, 0.36%
NOAEL:                   ca. 160 mg/kg bw

  Year:           1972
   GLP:           no

31-OCT-2005

5.5 Genetic Toxicity 'in Vitro'

Type:                    Ames test
System of testing:       Salmonella typhimurium TA98, TA100,TA1535, TA1537,
                         TA1538, Escherichia coli WP2uvrA
Concentration:           -S9: 0.01- 50 ug/plate
                         +S9: 0.5-1000 ug/plate
Cytotoxic Concentration: See Result
Metabolic activation:    with and without
Result:                  negative

  Year:           1985
   GLP:           no
Test substance:   as prescribed by 1.1 - 1.4

Method:           S9: SD rat liver (phenobaribital and 5,6-benzoflabone)
                  Incubation: 37 degree C for 65 hours
                  Negative control: water
                  Positive control:
                  +S9 mix
                  -ENNG 5 ug/plate (TA1535)
                  -AF-2 0.01 (TA100), 0.02 (TA98), 0.01 ug/plate (WP2uvrA)
                  -9AA 10 ug/plate (TA1537)
                  -4NOPD 5 ug/plate (TA1538)

                  -S9 mix
                  -B(a)P 5ug/plate (TA100, TA1537, TA1538, TA98)
                  -2AA 2 (TA1535), 80 ug/plate (WP2uvrA)

                  Dose levels (ug/plate)
                  -S9: 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50
                  +S9: 0.5, 1, 5, 10, 50, 100, 500, 1000
```

Result: No increasing of revertant was observed in each strain at with and withot S9.

Dose observed cytotoxicity were shown below.
-S9
-TA1535, TA100, TA1537,TA1538; greater than 10 ug/plate
-TA98; greater than 5 ug/plate
-WP2uvrA; 50 ug/plate

+S9
-Each strain; greater than 500 ug/plate
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (169)

Type: other: modified Ames test
System of testing: Salmonella typhimurium TA98, TA100, TA102
Concentration: 0.1 - 100 ug/ml
Metabolic activation: without
Result: negative

Method: other: modified Ames test
Year: 1993
GLP: no data
Test substance: other TS: sodium hypochlorite solution

Method: A modification of the Ames test, the fluctuation test as described by Hubbard et al., (1984) was performed. Briefly, the test material is exposed to bacteria in a liquid medium in many replicate cultures (96-well microplate) instead of the agar plate used in the Ames assay. After the 3-day incubation period, bromothymol blue (600 ug/ml) was added.

Positive wells turn yellow whereas negative wells remain green.

Chemicals were tested twice using triplicate microplates for every concentration.
Result: Toxicity was noted at 50 mg/ml where a bacteriostatic effect was observed. No detectable mutagenic effect in the three strains was noted.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (137)

Type: Ames test
System of testing: Salmonella typhimurium TA 97 /TA 102(II)
Concentration: 0.01, 0.05, 0.1 and 0.5 mg/plate
Metabolic activation: with and without
Result: negative

Method: other: preincubation +/- S9 mix
Year: 1987
GLP: no data
Test substance: other TS: sodium hypochlorite solution

Remark: Methodology cannot be reviewed (in Japanese). Limited study conducted in only 2 tester strains. No rationale provided for selection of top concentration; may not be a defendable maximum tolerated concentration. Clear negative result based on limited data presented.

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (84)

Type: Ames test
System of testing: Salmonella typhimurium TA92, TA135, TA100, TA1537
Concentration: 6 concentrations up to 5 mg/plate
Metabolic activation: with and without
Result: positive

Method: other: Standard Ames test
GLP: no data
Test substance: other TS: Solution of sodium hypochlorite

Remark: There are two publications (Ishidate, 1981 and 1984) of the same group that probably refer to the same series of tests that was performed for the ministry of health and welfare of Japan. However, no evidence is given.
There is also a review on the ministry of health and welfare program (Kawachi, 1980).
Result: positive (232/plate) at 5 mg/plate in TA100 with S9. Similar result was found with calcium hypochlorite (491/plate at 5 mg/plate in TA100 with S-9 mix).
Source: MITSUBISHI CHEMICAL SAFETY INSTITUTE LTD. Tokyo
Test condition: Liver of rats those were pretreated with polychlorinated biphenyls. Incubation for 20 min before plating. Duplicate plates were used for each of six different concentrations. The number of revertant colonies was scored after incubation at 37C for 2 days.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
22-JAN-2004 (110) (111) (122)

Type: DNA damage and repair assay
System of testing: B. subtilis
Metabolic activation: with and without
Result: negative

GLP: no
Test substance: other TS: Solution of sodium hypochlorite

Remark: Review article; no methodology, concentrations or data provided. Cannot be evaluated. Of limited value.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (122)

Type: Cytogenetic assay
System of testing: human embryo fibroblast cells
Concentration: 0.1488 and 0.0744 mg/ml
Cytotoxic Concentration: no data
Metabolic activation: no data
Result: negative

Method: other
GLP: no data
Test substance: other TS: solution of sodium hypochlorite

Remark: Chromosome breakage was investigated.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

31-OCT-2005 (195)

Type: Sister chromatid exchange assay
System of testing: human embryo fibroblast cells (HE2144)
Concentration: 0.1488 and 0.0744 mg/ml
Metabolic activation: no data
Result: positive

Method: other
Year: 1980
GLP: no
Test substance: other TS: Solution of sodium hypochlorite

Remark: Only the results for the two highest tolerable concentrations were provided. Methods provided in Sasaki et al. (1980) is very brief only.
The experiments were performed within the scope of a cancer research project of the ministry of health Japan. Kawachi et al. (1980) provide a review on a series of endpoints investigated within this project.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (122) (195)

Type: other: in vitro chromosomal aberration assay
System of testing: Chinese Hamster (CHL)
Concentration: 0.5 mg/ml
Metabolic activation: with and without
Result: positive

Method: other
Year: 1979
GLP: no data
Test substance: other TS: Solution of sodium hypochlorite

Method: Only one concentration tested.
Incubation: 3 hours
Recovery: 24 hours
Rats for the S9 mix were pretreated to induce microsomal enzymes.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (149)

Remark: The potent mutagens 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2, 62450-07-1), 2-amino-6-methyldipyrido-[1,2-a:3', 2'-d]imidazole (Glu-P-1, 67730-11-4) and 2-amino-3-methylimidazo[4,5-f]quinoline (IQ, 76180-96-6), isolated from pyrolysates of tryptophan and glutamic acid and from broiled sardines, respectively, were effectively degraded by chlorinated tap water with a concomitant loss of mutagenicity toward Salmonella typhimurium TA98 and TA100. The half-life of 10 microM IQ in the presence of 1.5 ppm of residual chlorine was less than 10 sec; those of Glu-P-1 and Trp-P-2 were 0.5-1 and 2-3 min, respectively. This means that a glass of chlorinated tap water (150 ml) containing 1.5 ppm of residual chlorine can break down about 200 micrograms of these pyrolysate mutagens within a couple of minutes.

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (230)

Type: DNA damage and repair assay
System of testing: E. coli PQ37
Concentration: 0.192 mg/l
Metabolic activation: with and without
Result: ambiguous

Method: other: SOS-chromotest
Year: 1989
GLP: no
Test substance: other TS: solution of sodium hypochlorite

Remark: Test was performed in duplicate. Only one single concentration was tested. No details on methods are described. Publication is written in German.

Result: One of the duplicates was positive with S9 activation.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (130)

Type: Cytogenetic assay
System of testing: Chinese Hamster lung fibroblasts (CHL)
Concentration: 3 concentrations (only for 05. mg/ml details provided)
Metabolic activation: with and without
Result: ambiguous

Method: other
GLP: no
Test substance: other TS: solution of sodium hypochlorite

Method: Method is described in Ishidate et al., 1984.
Remark: There are two publications (Ishidate, 1981 and 1984) of the same group that very probably refer to the same series of tests that was performed for the ministry of health and welfare of Japan. However, no evidence is given.
There is also a review on the ministry of health and welfare program (Kawachi, 1980).
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (111) (122)

Type: other: in vitro chromosomal aberration assay
System of testing: Human embryo fibroblast cells (HE2144)
Concentration: 0.0744 and 0.1488 mg/l
Metabolic activation: without
Result: negative

Method: other
Year: 1980
GLP: no
Test substance: other TS: solution of sodium hypochlorite
Remark: Ishidate, M. et al., (1981) is a review article referencing the study detailed in Sasaki, M. et al. (1980).
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

31-OCT-2005 (110) (195)

Type: Ames test
System of testing: Salmonella typhimurium TA98, TA100, TA 1535, TA1537, TA1538 and Escherichia coli WP2uvrA
Concentration: 0.5, 1.0, 2.5, 5.0, 10 and 25 ppm
Metabolic activation: with and without
Result: negative

Method: other: according to Ames et al. (1975)
Year: 1975
GLP: no data
Test substance: other TS: Chlorine gas purity 99.9%

Method: Ames, B.N., McCann, J. and Yamasaki, E. (1975): Methods for detecting carcinogens and mutagens with the salmonella/mammalian microsome mutagenicity test. Mutat Res, 31, 347-364.
Result: Growth inhibition was observed at 25.0 ppm in all Salmonella strains. There was no evidence of toxicity in E coli at the same concentration.
Exposure to chlorine did not result in an increase in point mutations in the Ames test.
Test condition: Mutagenicity was tested according to the Salmonella/mammalian microsome test (Ames, 1975). A minimal glucose agar plate with 0.1 ml of one of the strains and 0.5 ml of S9 mix and was exposed to chlorine gas upside down without a lid in a glass chamber.
Reliability: (4) not assignable
31-OCT-2005 (207)

Type: Cytogenetic assay
System of testing: human lymphocyte cultures
Concentration: remark 0, 2.5, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 ppm
Metabolic activation: no data
Result: ambiguous

Method: other: Genetic toxicity test
Year: 1973
GLP: no
Test substance: other TS: sodium hypochlorite

Method: Human lymphocytes were grown for 24 hours before chlorine was added. At 72 hr of culture (48 hour exposure to chlorine) the cells were harvested and processed.
Result: Test was negative at chlorine concentrations < 20 ppm and positive at concentrations of 20 ppm and higher. At concentrations >40 ppm cell division was markedly inhibited.

Effects of 48-hour exposure to different concentrations of sodium hypochlorite on human lymphocytes

Conc. [ppm]	Total cells examined	Breaks per cell
0	611	0.0016
25	425	0.047
30	229	0.047
35	456	0.026

40	854	0.043
45	72	0
50	53	>0.184
55	16	>1.00

Data from 2.5-20 ppm not reported in paper.
Test substance: Sodium hypochlorite N.F. (Fisher Scientific Co.) with a minimum of 5% available chlorine.
Reliability: (4) not assignable
31-OCT-2005 (25) (156)

Type: Ames test
System of testing: Salmonella typhimurium TA1530, TA1535, TA1538
Concentration: 0.00014, 0.0014, 0.014 and 0.14 micro-moles/plate
Metabolic activation: without
Result: positive

Method: other: Standard Ames test (no details provided)
Year: 1975
GLP: no
Test substance: other TS: Solution of sodium hypochlorite

Remark: Increase of revertants with TA1530 and TA1535. Methodology details not provided. Limited study conducted in only 3 test strains in the absence of S9-mix only. Reported as positive in strains TA1530 and TA1535 but the data displayed an inverse dose response which may be due to toxicity.

No meaningful positive data displayed.
Reliability: (4) not assignable
31-OCT-2005 (250)

Type: Ames test
System of testing: salmonella/mammalian-microsome mutagenicity assay
Concentration: 1.0 mg/plate
Metabolic activation: no data
Result: positive

Year: 1984

Remark: Results from these studies, namely weak mutagenicity and chromosomal aberrations, are not conclusive with respect to the potential for calcium hypochlorite to induce genetic damage. Both findings are from a single study published in 1984 and are without subsequent confirmation. Furthermore, the validity of using these in vitro test systems is questionable since calcium hypochlorite is functionally used to kill microorganisms, among which are those typically used as the testing species. The concentration which produces mutations in these assays was significantly greater than the concentrations used for disinfection. Based on the high cellular toxicity in these assays and the lack of mutagenicity observed in animals, the risk of genetic damage to humans is judged not significant. This chemical; therefore, is considered to be of questionable mutagenic potential.
Reliability: (4) not assignable
31-OCT-2005

Type: other
System of testing: Chinese hamster fibroblasts cells

Concentration: 0.06 mg/ml
Result: positive

Year: 1984

Reliability: (3) invalid
31-OCT-2005

5.6 Genetic Toxicity 'in Vivo'

Type: Micronucleus assay
Species: mouse Sex: male/female
Strain: B6C3F1
Route of admin.: gavage
Exposure period: 5 applications
Doses: 0, 1.6, 4.0 and 8 mg/kg/day administered by gavage for 5 successive days
Result: negative

Method: other: similar to OECD Guide-line 474
Year: 1985
GLP: no data
Test substance: other TS: Chlorine solved in water

Method: The test was performed very similar to the OECD guideline 474

Five males and five females were used for each treatment group. Three sub-chronic dose levels (five daily administrations ca. 24 hours apart) and positive and negative controls were applied. The positive control group was treated with 1 mg/kg triethylenemelamine (TEM) as a split dose (sub-chronic).
Animals were killed 6 hours after the last administration.

1000 polychromatic erythrocytes (PCEs) were scored for micronuclei for each animal. The percent micronucleated PCEs per animal was the endpoint used in the evaluation of the data.
Result: No significant increase of micronucleated (PCEs) at P=0.01 either for pooled or individual sex data. At P=0.05, pooled sex data showed a significant increase at pH=8.5 (OCl-) at the two highest dose levels. The increase was considered to be biologically not significant.

Activity of chemicals in the mouse micronucleus test

% Micronucleated cells Dose, mg/kg/day	0	1.6	4.0	8.0
OCl-	0.01+0.01	0.04+0.02	0.10+0.03	0.12+0.4
HOCl	0.10+0.02	0.05+0.02	0.06+0.02	0.08+0.03

All values were comparable to control values, $p < 0.01$.
Test substance: Solutions of hypochlorite were prepared by bubbling chorine gas into a solution of NaOH and adjusting the pH with 2.5 N HCl to either 8.5 (OCl- predominant) or 6.5 (HOCl predominant) in the following.

Concentrations of chlorine were determined by iodometric titration.
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
31-OCT-2005 (154)

Type: other: Bone marrow aberration
Species: mouse Sex: male/female
Strain: B6C3F1
Route of admin.: gavage
Exposure period: 5 applications
Doses: 0, 1.6, 4.0 and 8 mg/kg/day administered by gavage for 5 successive days.
Result: negative

Method: other: similar to Directive 2000/32/EC, B.11
Year: 1985
GLP: no data
Test substance: other TS: Chlorine solved in water

Method: The test was performed very similar to the Directive 2000/32/EC

Five males and five females were used for each treatment group. Three sub-chronic dose levels (five daily administrations ca. 24 hours apart) and 1 acute dose were applied. In addition positive and negative controls were performed. The positive control group was treated with 1 mg/kg triethylenemelamine (TEM) in a one time (acute) administration.

Animals of the sub-chronic group were killed 6 hours after

the last administration. Animals of the acute dose group were sacrificed 6, 24 and 48 hours after exposure.

A mitotic index was determined by scoring the number of cells in mitosis based on at least 500 cells. 50 metaphase spreads for each animal (where possible) were scored for structural and numerical aberrations.

4 endpoints were examined
(1) number of structural aberrations present per animal
(2) number of numerical aberrations present per animal
(3) Percentage of cells with at least one structural aberration present per animal and
(4) percentage of cells with two or more structural aberrations per animal

Data for male and female animals were analysed both separately and combined.
Result: No significant differences from control for any of the treatment groups for any of the endpoints investigated, were observed.

Activity of chemicals in the Mouse Bone Marrow Cytogenetics Assay

% Cells with chromosomal aberrations				
Dose, mg/kg/day	0	1.6	4.0	8.0

Sample	type	S	N	S	N	S	N	S	N
OCl-	Sub	0.8	1.8	0.0	2.6	0.0	2.8	2.0	1.0
	Ac	0.6	0.9	0.5	2.0	0.5	1.2	3.0	1.2
HOCl	Sub	1.5	2.0	3.0	1.2	0.8	1.9	2.0	1.1
	Ac	0.5	1.8	1.3	2.0	2.0	1.0	1.5	1.2

All values were comparable to control values, p < 0.01
S: Structural aberrations
N: Numerical aberrations
Sub: Subchronic dosing (5 daily administrations) with sacrifice 6 hours following the last dose.
Ac: Acute dosing with sacrifice 24 hour later.

Test substance: Solutions of hypochlorite were prepared by bubbling chorine gas into a solution of NaOH and adjusting the pH with 2.5 N HCl to either 8.5 (OCl- predominant) or 6.5 (HOCl predominant) in the following.
Concentrations of chlorine were determined by iodometric titration.
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
31-OCT-2005 (154)

Type: other: Sperm head abnormality test
Species: mouse Sex: male
Strain: B6C3F1
Route of admin.: gavage
Doses: animal 0, 1.6, 4.0 and 8 mg/kg/day administered by gavage for 5 successive days.
Result: ambiguous

Method: other: similar to OECD 483
GLP: no data
Test substance: other TS: Chlorine solved in water

Result: At pH 8.5 (where the hypochlorite ion, OCl-, predominates), at dose levels equivalent to approx. 4 and 8 mg/kg/day, an increase of sperm-head abnormalities was observed. No effect was found at any other pH. HOCl, the protonated form of chlorine in water failed to produce significant increases in sperm-head abnormalities.

Activity of Chemicals in the Mouse Sperm-Head Abnormality Assay

% Abnormal sperm-head Dose,mg/kg/day	0	1.6	4.0	8.0
OCl-	2.12+0.19	2.81+0.16	4.07+0.39**	3.68+0.47**
OCl-	0.91+0.09	1.41+0.15**	1.74+0.13**	1.37+0.10*
HOCl	2.73+0.31	2.07+0.19	1.36+0.11	1.43+0.08
HOCl	1.06+0.06	1.24+0.08	1.01+0.10	1.02+0.08

*Significantly elevated above control at p< 0.05.
**Significantly elevated above control at p< 0.01.

Test substance: Solutions of chlorine were prepared adjusting the pH with 2.5 N HCl to either 8.5 or 6.5.
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
31-OCT-2005 (154)

Type: Micronucleus assay
Species: mouse Sex: male
Strain: other: ddY
Route of admin.: i.p.
Exposure period: 1 and multiple applications
Doses: 0, 312.5, 625, 1250 and 2500 mg/kg for acute and 300 mg/kg for multiple applications
Result: negative

Method: other: similar to OECD Guide-line 474
Year: 1998
GLP: no data
Test substance: other TS: sodium hypochlorite dissolved in water

Method: The maximum dose levels were set at the supposed maximum tolerated dose referring to the LD50. Groups of 6 male mice were used. For the multiple application group, animals were administered 4 doses ip, 24 hours apart. Twenty four hours after the last dose, femoral marrow cells were collected.
Result: At 2500 mg/kg all animals died within 24 hours of ip administration. The number of micronucleated polychromatic erythrocytes were comparable between control and treated animals following single or multiple exposures.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (100)

Type: other: formation of DNA damaged 8-hydroxydeoxyguanosine
Species: rat Sex: male
Strain: Fischer 344
Route of admin.: gavage
Doses: 900 mg/kg
Result: negative

Method: other
GLP: no data
Test substance: other TS: sodium hypochlorite

Method: Male F-344 rats received single intragastric administrations of 900 mg/kg sodium hypochlorite. Animals were sacrificed and kidney and liver were removed, 0, 3, 6, 12, 24 and 48 hours after dosing. These tissues were homogenized for 10-20 seconds. DNA was isolated using Marmur's method, except that cells were lysed by 2% sodium dodecylsulfate at 37C for 30 minutes. DNA samples were heat denatured at 95C for 3 minutes and then ice cooled. DNA was digested to deoxynucleosides and then analyzed for 8-hydroxydeoxyguanosine (8-OH-dG) by HPLC.
Remark: While the methods section states that kidney and liver DNA were to be examined, the results only mentioned liver DNA from potassium bromate administered animals. Thus it is unclear whether liver DNA from NaClO dosed animals was examined.
Result: No significant increase of 8-OH-dG was observed in the kidney DNA of the NaClO dosed rats.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (120)

5.7 Carcinogenicity

Species: rat Sex: male/female
Strain: Fischer 344
Route of administration: drinking water
Exposure period: 2 years
Frequency of treatment: daily ad libitum
Post exposure period: no
Doses: 70, 140 or 275 mg/l buffered water
Result: ambiguous
Control Group: yes

Method: other: generally followed OECD 453 Combined Chronic Tox/Carcinogenicity Study.
GLP: yes
Test substance: other TS

Result: No neoplastic effect in the male rats (no evidence of carcinogenicity); increase of incidence of mononuclear cell leucemia in the mid-dose females only.
Incidence of Mononuclear Cell Leukemia in female rats:

Cl in drinking water [ppm]	Mononuclear Cell Leukemia
0	8/50
70	7/50
140	19/51*
275	16/50

* Statistically significantly different than control values.
Historical incidence for 2-year studies of all leukemias for untreated control groups in NTP studies is:25 -+ 6.1%, range 14-36% in dietary studies; 26 + 8.5%, range 16-33% in drinking water studies.

Test substance: Chlorine dose formulations as sodium hypochlorite solutions were prepared by mixing the appropriate volume of stock solution with sodium chloride and bicarbonate-carbonate buffer solution, then diluting with deionized charcoal-filtered drinking water.

Stability studies indicated that the buffered hypochlorite stock solution was approximately 96% of its original concentration after 7 days at 5C. Chlorinated water formulations at levels of 70 to 275 ppm retained 95% of their original concentrations after storage for 1 day and 90% after 2 days. Thus the buffered hypochlorite stock solution used in these studies was stored at 5C for no longer than 7 days, and the dose solutions were stored at room temperature for no longer than 48 hours.
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
31-OCT-2005 (175)

Species: mouse Sex: male/female
Strain: B6C3F1
Route of administration: drinking water
Exposure period: 2 years
Frequency of treatment: daily ad libitum
Post exposure period: no
Doses: 70, 140 or 275 mg/l buffered water
Result: negative

Control Group: yes

Method: other: : generally followed OECD 453 Combined Chronic Tox/Carcinogenicity Study.
Year: 1991
GLP: yes
Test substance: other TS

Result: No non-neoplastic effects; no neoplastic effect (no evidence of carcinogenicity).
Test substance: Chlorine dose formulations as sodium hypochlorite solutions were prepared by mixing the appropriate volume of stock solution with sodium chloride and bicarbonate-carbonate buffer solution, then diluting with deionized charcoal-filtered drinking water.
Stability studies indicated that the buffered hypochlorite stock solution was approximately 96% of its original concentration after 7 days at 5C. Chlorinated water formulations at levels of 70 to 275 ppm retained 95% of their original concentrations after storage for 1 day and 90% after 2 days. Thus the buffered hypochlorite stock solution used in these studies was stored at 5C for no longer than 7 days, and the dose solutions were stored at room temperature for no longer than 48 hours.
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
31-OCT-2005 (175)

Species: rat Sex: male/female
Strain: Fischer 344
Route of administration: inhalation
Exposure period: 2 years
Frequency of treatment: 6 hours/day; 5 days/week (male); 3 days/week (female)
Post exposure period: no
Doses: 0.4; 1.0; 2.5 ppm
Result: negative
Control Group: yes

Method: other: generally followed OECD 453 Combined Chronic Tox/Carcinogenicity Study.
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Remark: An interim necropsy of rats was performed at 12 months (10 rats/sex/concentration group).
Result: The incidence of neoplasia was not increased by exposure, indicating that inhaled chlorine is an upper respiratory tract toxicant but not a carcinogen.

Tumors Observed in Rats for All Organs:

Dose	Males	Females
All Organs Malignant Lymphoma		
0.0	0/69	0/69
0.4	2/70	0/69
1.0	0/70	0/69
2.5	0/69	0/70
All Organs Histiocytic Sarcoma		
0.0	2/69	0/69
0.4	0/70	0/69

1.0	0/70	0/69
2.5	1/69	0/70
All Organs Mononuclear Cell Leukemia		
0.0	9/69	27/69
0.4	9/70	12/69
1.0	9/70	13/69
2.5	10/69	32/70

Reliability: (1) valid without restriction

Flag: Critical study for SIDS endpoint
31-OCT-2005 (54) (251)

Species: mouse Sex: male/female
Strain: B6C3F1
Route of administration: inhalation
Exposure period: 2 years
Frequency of treatment: 6 hours/day; 5 days/week
Post exposure period: no
Doses: 0.4; 1.0; 2.5 ppm
Result: negative
Control Group: yes

Method: other: generally followed OECD 453 Combined ChronicTox/Carcinogenicity Study.
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Remark: An interim necropsy of rats was performed at 12 months (10 mice/sex/concentration group).
Result: The incidence of neoplasia was not increased by exposure, indicating that inhaled chlorine is an upper respiratory tract toxicant but not a carcinogen.

Tumors Observed in Mice for All Organs

Dose	Males	Females
All Organs Hemangiosarcoma		
0.0	0/64	0/66
0.4	2/66	0/67
1.0	0/69	2/69
2.5	4/67	0/61
All Organs Histiocytic Sarcoma		
0.0	3/64	2/66
0.4	1/66	4/67
1.0	0/69	2/59
2.5	1/67	1/61
All Organs Malignant Lymphoma: Histiocytic, Lymphocytic, Mixed		
0.0	0/64	11/66
0.4	2/66	6/67
1.0	2/69	5/59
2.5	1/67	5/61

Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
31-OCT-2005 (54) (252)

Species: rat Sex: male/female
Strain: other: BDII

Route of administration: drinking water
Exposure period: life-time in 7 generations
Frequency of treatment: daily
Post exposure period: no
Doses: 100 mg/l
Result: negative
Control Group: yes

Method: other: Toxicity test
Year: 1968
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Chlorine solution with a content of free chlorine of 100 mg/L were prepared by bubbling gaseous chlorine into untreated tab water (Freiburg, Germany, 1955-1964). The concentration of free chlorine was determined by titration with (Na2S2O3). Half live time of chlorine in the stock solution stored at 5□‹C was found to be 40 days. Nevertheless, stock solutions were prepared weekly.

The 100 mg/L chlorine solution was the only source of drinking water for the animals. To investigate the possible effects of using chlorinated drinking water for cooking, food pellets of the first generation were boiled in the stock solution. The subsequent generations pellets were fed untreated. All Animals were observed until natural death and were autopsied and underwent gross examinations. Organ weights were determined and selected organs and possible tumors underwent histologic examination.

The rats used in the experiments were BD II (cPah, albino) which are similar to Wistar rats.
Result: The highly chlorinated water (100 mg/L) was well tolerated. The study did not reveal any toxic effects on fertility, growth or blood picture, or on histology of liver, spleen, kidneys and other organs. The incidence of malignant tumors was the same in the experimental and control groups. No shortening of the lifespan of the rats treated with chlorine was observed.
Test substance: Free chlorine.
Reliability: (2) valid with restrictions

Flag: Critical study for SIDS endpoint
31-OCT-2005 (69)

Species: rat Sex: male/female
Strain: Fischer 344
Route of administration: drinking water
Exposure period: 104 weeks
Frequency of treatment: daily
Post exposure period: 8 weeks
Doses: 0.05 and 0.1% in males and 0.1 and 0.2% for females
Result: negative
Control Group: yes

Method: other: essentially the same as National Cancer Institute (1975). Bethesda, MD
Year: 1986
GLP: no data
Test substance: other TS: sodium hypochlorite

Method: Groups of 50 rats of each sex were supplied drinking water containing sodium hypochlorite at concentrations of 0, 0.05 or 0.1% for males and 0, 0.1 or 0.2% for females. During the experiment period, all animals were observed daily, and any clinical signs and mortality were recorded. Body weight was measured weekly during the first 6 weeks of the study and then every 4 week until the end of the experiment. Drinking water consumption was measured at regular intervals and the sodium hypochlorite intake was calculated. After treatment for 104 weeks, all surviving animals were given untreated tap-water for a further 8 weeks, and then killed under ether anaesthesia after a 24-hr fast. Blood samples were collected from the abdominal aorta of these rats for microscopic examination and serum biochemical studies. After macroscopic examination, organs including brain, pituitary gland, salivary glands, lungs, heart, liver, spleen, adrenal glands, kidneys, testes and ovaries, were weighed. All dissected organs and tissues were fixed in 10% buffered formalin and processed for histological examination. Moribund rats or animals dying spontaneously during the experiment were autopsied and underwent complete gross and microscopic examinations.

Result: The overall incidence of tumors in each group was 98-100% in males and 70-80% in females. There were no significant differences between control and experimental groups with respect to the total tumor incidences of the animals. The highest incidence of tumors is presented in Table 1. Most of the tumors found were of types that occur most commonly as spontaneous tumors in F344 rats. Therefore, it was concluded that the tumors observed in this study were unrelated to treatment and drinking sodium hypochlorite at levels up to 0.1% in males and 0.2% in females had no carcinogenic effect in F344 rats.

Incidence of neoplasia in Fischer 344 rats ingesting sodium hypochlorite in the drinking water for up to 104 weeks.

Site and tumor type					
Males			Females		
Effective # ats					
49	50	50	50	50	50
NaClO conc. (%)					
0	0.05	0.1	0	0.1	0.2
Hemopoietic systemMultiple organs - leukemia					
7	11	10	8	6	2
Endocrine systemPituitary adenoma (chromophobe)					
4	7	4	21	26	20
Thyroid gland C-cell adenoma					
5	7	4	3	5	3
Thyroid gland C-cell adenocarcinoma					
2	3	0	0	1	0
Adrenal gland Phaechromocytoma benign					
7	2	2	0	0	1
Adrenal gland Phaechromocytoma malignant					
0	0	1	0	2	0
Respiratory systemLung adenoma					
6	4	6	3	2	1
Reproductive systemTestis interstitial cell tumor					

```
                49    48    49          -     -     -
                Mammary gland fibroadenoma
                6     2     4           8     0*    1*
                ------------------------------------------------------------

                * Significantly different from control incidence, P<0.01.
Reliability:    (1)  valid without restriction
Flag:           Critical study for SIDS endpoint
31-OCT-2005                                                          (99)

Species:                mouse                           Sex: male/female
Strain:                 B6C3F1
Route of administration: drinking water
Exposure period:        85 weeks
Frequency of treatment: daily
Doses:                  500 and 250 ppm in males and females
Result:                 negative
Control Group:          yes

Method:         other: essentially the same as National Cancer Institute
                (1975). Bethesda, MD
  Year:         1986
   GLP:         no data
Test substance: other TS: sodium hypochlorite

Method:         Groups of 50 mice of each sex were supplied drinking water
                containing sodium hypochlorite at concentrations of 0, 250
                or 500 for males and females for 85 weeks.
Result:         Mice in tratment groups of both sexs were given NaClO2 at
                concentrations of 500or 250 ppm for 85 weeks, at which time
                all surviviors were sacrified. Dead or moribund malemice
                were found during the experimental earlier in control groups
                than in the treated groups because of severe fighting.
                Survival percentages at the end of study were 86%, 94%, and
                70% in male and 100%, 100%, and 94% in females,
                respectively, in high-dose, low-dose, and control groups.
                However, body weight increases were comparable among all
                groups of either sex.
                The incidences of liver tumors were higjer in treated males
                than in control males. Thses tumors were histologically
                diagnosed as hyperplastic nodules or hepatocellular
                carcinomas. The combined incidences of these tumors were
                significantly different in males of the low-dose group
                (p<0.05). The incidences of hyperplastic nodules of the
                liver in males were significantly higher in both high- and
                low-dose groups (p<0.05), although the incidences did not
                exhibit a dose-related effect. Also, the combined incidences
                of adenomas and adenocarcinomas and that of adenomas of the
                lung significantly higher in males of the high-dose group
                (p<0.05). Relatively higher tumor rates were observed for
                malignant lymphomas and/or leukemias and adenomas of
                Harderian gland in both sexes, and for tumors of the liver
                of malignant lymphomas and/or leukemias in the high-dose
                females group were samller by statistically significant
                margin. These incidences in treated males were within the
                range of values of historical control data.
Reliability:    (2)  valid with restrictions
Flag:           Critical study for SIDS endpoint
31-OCT-2005                                                         (136)

Species:                rat                             Sex:
```

Strain: Fischer 344
Route of administration: drinking water
Exposure period: 85 weeks
Frequency of treatment: daily
Doses: 600 and 300 ppm in males and females
Result: negative
Control Group: yes

Method: other: essentially the same as National Cancer Institute (1975). Bethesda, MD
Year: 1986
GLP: no data
Test substance: other TS: sodium hypochlorite

Method: Groups of 50 rats of each sex were supplied drinking water containing sodium hypochlorite at concentrations of 0, 300 or 600 for males and females for 85 weeks.
Result: This study was prematurely terminated at week 85 because of widespread Sendai virus infection in all groups, necessintating immediate sacrifice of all survivors. At necrpsy, pneumonias were found in all animals, and an abscess of the lung had developed in some case. Percentage of survivors at 85 week were 86%, 60%, and 68% in males and 100%, 88%, and 94% in females, respectively, in high-dose (600ppm), low-dose (300ppm), and control groups. Body weight increase was inhibited in a dose-dependent manner in both males and females. Drinking water intake in treated animals was slightly lower than that in control animals of both sexes. Daily consumption of sodium hypochlorite (mg/kg body weight/day) was 32.1 and 18.0 in males and 40.9 and 28.3 in females, respectivelyfor high- and low-dose group.
No statistically significant differences in the incidence of tumor-bearing animals were observed between treatment and control group of either sex. Incidences of tumors survival organs were appreciable, i.e., C-cell adenomas of the thyroid, pheochromocytomas of the adrenal, and interstitial cells tumors of the testis in males, and chromophobic adenomas of the pituitary and endometrial polys of the uterus in females. However, no statistically significant defferences in the rates of tumor development in any organs were observed between sodium hypochlorite-treated and control animals of either sex. serum biochemistry analysis revealed that levels of glutamic oxaloacetic transaminase in the liver were significantly decreased in the high-dose males. Hematalysis and urinalysis revealed no significant changes in blood or urine.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (136)

Species: rat Sex: male/female
Strain: Fischer 344
Route of administration: drinking water
Exposure period: 2 years
Frequency of treatment: continuously in drinking water
Doses: 0.05-0.3 mmol/kg/day
Result: ambiguous
Control Group: yes, concurrent vehicle

Method: other
GLP: no data

Test substance: no data

Result: Equivocal evidence for a carcinogenic response in female rats indicated by a marginal increase in mononuclear cell leukemia.
Reliability: (4) not assignable
31-OCT-2005 (70)

Species: mouse Sex: male/female
Route of administration: dermal
Exposure period: 18 months
Frequency of treatment: 3 times/week
Post exposure period: no data
Doses: 0.1 ml solution of 14.8%, noel=181 mg/kg/day

Year: 1974

Remark: IARC reviewed several hypochlorite salts and concluded that the hypochlorite salts are not classifiable as to their carcinogenicity to humans (Group 3).
31-OCT-2005

5.8.1 Toxicity to Fertility

Type: One generation study
Species: rat
Sex: male/female
Strain: Long-Evans
Route of administration: gavage
Exposure Period: ca. 66 days
Frequency of treatment: daily
Premating Exposure Period
male: 56 days prior to breeding
female: 14 days prior to breeding
Duration of test: ca. 66 days
Doses: 1.0, 2.0 and 5.0 mg/kg
Control Group: yes

Method: other: similar to OECD 415
Year: 1985
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Remark:
Organohalides formed through the reaction of chlorine and organic compounds in natural and waste waters pose potential health hazards. For this reason, alternative water disinfectants that do not form organohalides are being investigated with great interest. Limited data are available on the health effects, in particular reproductive toxicity effects, of these compounds. In our laboratory, we have examined the reproductive effects of chloramine and chlorine administered by gavage in Long-Evans rats. Animals were treated for a total of 66 to 76 days. Males were treated for 56 days and females for 14 days prior to breeding and throughout the 10-day breeding period. Females were treated throughout gestation and lactation. Following breeding, the males were necropsied and evaluated for sperm parameters and reproductive tract histopathology. Adult females and some

pups were necropsied at weaning on postnatal day 21. Other pups were treated postweaning until 28 or 40 days of age. These pups were evaluated for the day of vaginal patency and thyroid hormone levels. No differences were observed between control rats and those rats exposed to up to 5 mg/kg/day chlorine or 10 mg/kg/day chloramine when fertility, viability, litter size, day of eye opening, or day of vaginal patency were evaluated. No alterations in sperm count, sperm direct progressive movement (micron/sec), percent motility, or sperm morphology were observed among adult male rats. In addition, male and female reproductive organ weights were comparable to their respective control groups, and no significant histopathologic changes were observed among chlorine- or chloramine-treated male and female rat
Males were also dosed during 10 day breeding period. Females received chlorine throughout breeding, gestation, and lactation. Selected pups were dosed following weaning until day 40 or the day of vaginal opening.

In addition seminal fluid was obtained from the right cauda epididymis and sperm counts, sperm direct progressive movement (mm/sec), percent motility or sperm morphology were obtained.

Doses chosen were the highest practicable considering solution stability and potential gastric irritation.

Dosing period for male rats was 4 days shorter than 70 days recommended by OECD 415 guideline.

Result: No clinical signs of toxicity or body weight depression were observed. Fertility, fecundity, and litter weight were unaffected. The day of parturition was not influenced by chlorine exposure. No alterations in estrous cyclicity or day of vaginal opening were observed among F1 females. F0 males showed no adverse effects of chlorine exposure when sperm count, sperm morphology, motility, or velocity were evaluated. No histopathologic lesions of the reproductive tract were observed in males or females.

Test substance: Chlorine was given as aqueous solution.

Reliability: (1) valid without restriction

Flag: Critical study for SIDS endpoint

31-OCT-2005 (44) (50) (211)

Type: other: multigeneration study (7 consecutive generations)
Species: rat
Sex: no data
Strain: no data
Route of administration: drinking water
Exposure Period: lifetime
Frequency of treatment: continuously in drinking water
Duration of test: 7 consecutive generations

Doses: 100 mg/l drinking water
Control Group: yes

Method: other
Year: 1968
GLP: no
Test substance: no data

Method: Chlorine solution with a content of free chlorine of 100 mg/L were prepared by bubbling gaseous chlorine into untreated tab water (Freiburg, Germany, 1955-1964). The concentration of free chlorine was determined by titration with (Na2S2O3). Half live time of chlorine in the stock solution stored at 5 degree C was found to be 40 days. Nevertheless, stock solutions were prepared weekly.
The 100 mg/L chlorine solution was the only source of drinking water for the animals. To investigate the possible effects of using chlorinated drinking water for cooking, food pellets of the first generation were boiled in the stock solution. The subsequent generation pellets were fed untreated. All animals were observed until natural death, and were autopsied and underwent gross examinations. Organ weights were determined and selected organs and possible tumors underwent histologic examination.
The rats used in the experiments were BD II (cPah, albino) which are similar to Wistar rats.
Remark: No. of generation studies: 7
Result: The highly chlorinated water (100 mg/L) was well tolerated. The study did not reveal any toxic effects on fertility, growth or blood picture, or on histology of liver, spleen, kidneys and other organs. The incidence of malignant tumors was the same in the experimental and control groups. No shortening of the lifespan of the rats treated with chlorine was observed.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (69)

Species: human
Route of administration: drinking water

Test substance: other TS: sodium hypochlorite and chlorine dioxide

Method: A cross-sectional study in Genoa, Italy which treated drinking water with sodium hypochlorite, chlorine dioxide or both disinfectants and Chiavari, Italy which did not disinfect the drinking water. Some potential confounders, including maternal age, education level, smoking, alcohol consumption and sex of the child, were also collected. The authors state that they adjusted for these confounders.
Remark: They did not adjust for other confounders such as nutritional habits, amount of smoking and age distribution of the women.
Result: A higher frequency of small body length (<49.5 cm) and small cranial circumference (<35 cm) was observed in infants born to mothers who drank water treated with sodium hypochlorite or chlorine dioxide. Body length was not affected in children born to mothers who drank both sodium hypochlorite or chlorine dioxide treated water.
Reliability: (4) not assignable
31-OCT-2005 (119)

Type: other: there are no known or reported effects on reproductive function or fetal development.
Species: mouse
Sex: male
Route of administration: i.p.
Exposure Period: single dose
Frequency of treatment: once

Doses: 8.4, 16.8 mg/kg
NOAEL Parental: = 16.8 mg/kg bw

Year: 1972

Reliability: (4) not assignable
31-OCT-2005

5.8.2 Developmental Toxicity/Teratogenicity

Species: rat Sex: female
Strain: Sprague-Dawley
Route of administration: drinking water
Exposure period: ca. 3.5 months
Frequency of treatment: daily ad libitum
Duration of test: ca. 3.5 months (killed at gestational day 20)
Doses: 1, 10, or 100 mg/l of HClO
Control Group: yes
NOAEL Maternal Toxity: > 100 mg/l

Method: other
Year: 1982
GLP: no data
Test substance: other TS: HClO (see remark)

Method: Chlorine gas, ultra high purity was bubbled into double distilled water and titrated daily. Groups of 6 female rats were administered 0, 1, 10 or 100 mg/l of HOCl daily in the drinking water. After treatment for 2.5 months, the females were placed in the cages of untreated males in a ratio of 1 male:3 females. Thus, only two males were used per dose

level. Females with sperm-positive vaginal smears were allowed to drink their respective solutions throughout gestation. On day 20 of gestation the dams were sacrificed, the numbers of live and dead fetuses were noted as well as the number of resorptions. Individual fetal weights were recorded and a gross examination for external malformations was made. Half of the fetuses from each dam were examined for soft tissue anomalies while the other half were examined for skeletal anomalies. While the litter is the appropriate unit of analysis for developmental toxicity studies, this study used the fetus.
Remark: Due to the significant methodological deficiencies, such as small sample size in females and only two males used per dose level, it is impossible to identify whether a possible genetic effect is occurring. Based on the available literature, it is not possible to determine whether all of the effects observed were noted in one fetus, litter or present in all litters.
Result: No significant increase in resorptions and fetal viability and weight was observed. The fetuses from 10 and 100 mg/L HOCl group had a higher percentage of skeletal defects compared with the control; however, a chi-square analysis revealed no significant differences. The 100 mg/L group also had a greater incidence of soft-tissue defects compared with the control but there was no significant difference. The defects in the 100 mg/L group consisted of three cases of adrenal agenesis, one-right sided heart (dextrocardia), one case of improper orientation of the apex of the heart, and

one atrio-ventricular valve enlargement. The lower concentrations of 1 and 10 mg/L HOCl did not produce any soft-tissue defects. A slightly significant increase in skeletal variants and soft tissue defects was found at 100 mg/l, with no such difference at 10 and 1 mg/l. Fetal weights were slightly decreased at the high dose. The skeletal anomalies were common ones, such as incompletely ossified or missing sternebrae and rudimentary ribs. These results were interpreted by the author to mean that chlorine is slightly embryotoxic but not teratogenic.
Maternal toxicity was not evaluated.

Effect of chlorine in drinking water on the formation of skeletal and soft-tissue defects in rat fetuses:

Conc. mg/L	0	1	10	100
Defect found:				
Skeletal	34.5	23.8	59.1	57.7
Soft-tissue	7.1	0.0	0.0	19.2
Total	21.1	12.2	27.1	38.5*

Values represent percent of defects for all fetuses in each treatment group.
* Significantly different from control ($p<0.05$), chi-square analysis.

Effect of chlorine in drinking water on skeletal anomaly in rat fetuses

Conc. mg/L	0	1	10	100
Number of fetuses examined	58	41	52	52
Skeletal Anomaly found:				
Incomplete/bipartite sternebrae	7	4	3	16
Missing sternebrae	4	0	7	9
Rudimentary ribs	5	1	6	1
Extra ribs	0	0	1	0
Short ribs	0	1	0	0

Values represent absolute numbers of anomalies found in each treatment.

Conclusion: Limited data suggests that chlorine is not teratogenic but may be slightly embryotoxic when administered at high doses in drinking water to pregnant rats.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (221)

Species: rat Sex: female
Route of administration: gavage
Exposure period: day 6 through 16 of gestation
Frequency of treatment: 1/day
Duration of test: day 6 through day 16 of gestation
Doses: 12.5 and 25.0 mg/kg/day
NOAEL Maternal Toxity: > 25 mg/kg bw

Remark: All parameters examined after dosing did not differ from

those of the control females. These include maternal body weight, maternal mortality, implantation sites, resorption sites, viable fetuses, fetal external abnormalities, fetal skeletal development and fetal internal development.

Reliability: (4) not assignable
31-OCT-2005

5.8.3 Toxicity to Reproduction, Other Studies

5.9 Specific Investigations

5.10 Exposure Experience

Remark: Acute exposure to chlorine gas: Concentration-time to effects relation

1-Concentration (ppm)	2-Exposure Time	3-Clinical symptoms on acute exposure	4-Reference
1-15	2-< 30 min	3-significant ocular, nasal and pharyngeal irritation	4-Lheureux, P. et al. (1993) a
1-20	2-about 30 min	3-dangerous	4-Wirth, K.E. and Gloxhuber, (1994)
1-30	2-< 30 min	3-cough, laryngospasm, chest pain, nausea, vomiting	4-Lheureux, P. et al. (1993) a
1-40-60	2-< 30 min	3-tracheobronchitis, pneumonia, RADS ("Reactive Airways Dysfunction Syndrome")	4-Lheureux, P. et al. (1993) a, Shroff, C.P. et al. (1988)
1-50	2-30-60 min	3-lethal	4-Wirth, K.E. and Gloxhuber, C.(1994)
1-430	2-< 30 min	3-minimal lethal concentration reported	4-Lheureux, P. et al. (1993) a
1-690-1000	2-rapid	3-lethal	4-Wirth, K.E. and Gloxhuber, C.(1994),Lheureux, P. et al.(1993) a

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------------------------------------------------------------
Based on Hedges and Morrissey (1979) cited in Lheureux, P.
et al. (1993).
Acute exposure to chlorine gas: Concentration-time to
effects relation
------------------------------------------------------------
ORDER
1-Concentration (ppm)
2-Exposure Time
3-Clinical symptoms on acute exposure
4-Number of subjects
5-Reference
------------------------------------------------------------
1-0.06-0.2
2-n.r.
3-itching in the nose
4-3
5-Rupp, H. and Henschler, D.(1967)

1-0.35-0.72
2-15 min
3- burning of conjunctivae
4-19
5-Rupp, H. and Henschler, D.(1967)

1-0.1-0.5
2-n.r.
3-slight tickling in the nose and throat,cough, sensations

inthe ocular conjunctiva, sensation of choking
4-10-13
5-Beck, 1959

1-0.5
2-8 h
3-no impairment of pulmonary function,irritating effects

4-30
5-Anglen et al., 1980

1-0.5
2-8 h
3-no significant impairment of pulmonary function
4-n.r.
5-Rotman, H. et al. (1983)

1-0.5
2-2 h
3-borderline effects
4-8
5-Joosting and M. Verbeck.(1975)

1-0.5
2-6 h on 3 consecutive days
3-no changes in lung function and nasal lavage
4-n.r.
5-Schins, R. et al. (2000)

1-1.0
2-30 min
3-tickling and stinging in the nose, scratchiness and
```

dryness in the throat; in single case: dull sensation in the
teeth and a slight metallic taste, headache and pressure, burning of ocular conjunctiva/outer skin, coughing, constriction of breathing
4-10
5-Beck, (1959)

1-1.0
2-60 min
3-impairment of lung function: decrease in FEV1 (Forced Expiratory Volume)
4-n.r.
5-D'Alessandro, A. et al. (1996)

1-1.0-1.3
2-35 min
3-dyspnea and cough with violent headache
4-1
5-Rupp, H. and Henschler, D.(1967)

1-1.0
2-4-8 h
3-sensory irritation and impairment of pulmonary function

5-Rotman, H. et al. (1983)

1-0.5-1.0
2-4 h
3-slight irritation, induced coughing reflex
4-30
5-Anglen et al., (1980)

1-1.0
2-2 h
3-individual variation in sensibility with respect to eye irritation and coughing reflex.
4-8
5-Joosting and M. Verbeck. (1975)

1-2.0
2-2 h
3-significant irritation throughout: cough, eye, nose, throat, but clearly tolerable without impairment of pulmonary function
4-8
5-Joosting and M. Verbeck.(1975), Anglen et al., 1980

1-2.0
2-2-4 h
3-pronounced signs of irritation, increased nasal mucus

secretion
4-30
5-Anglen et al., 1980

1-2.0
2-15 min
3-no significant irritation and impairment of pulmonary

function
4-30
5-Anglen et al., 1980

1-2.5-4.0
2-5-16 min
3-immediate burning of the eyes, itching in the pharynx, coughing, and nasal congestion
4-1
5-Matt, L. (1889)
---
n.r.: not reported.
Note that the studies reporting this data have not been made
robust.

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
30-OCT-2005 (8) (18) (58) (117) (140) (150) (189) (191) (197) (248)

Remark: Acute toxicity:
Chlorine is a highly reactive gas with an unpleasant odor; optical chronaxie, visual adaptometry and other behavioural tests have generally demonstrated effects only at or above the threshold for odor perception; the values for odor perception and irritation threshold level were similar, they
range from 0.0006-0.006 mg/l; at or above 0.003-0.006 mg/l the irritation becomes uncomfortable and above 0.012 mg/l intolerable. Below intolerable concentrations chlorine is a upper respiratory tract irritant. Inhalation of intolerable concentrations produces lung injury; the gas dissolves in the water saturated atmosphere of the airways releasing hydrochloric acid (HCl), leading to air way inflammation, injury and bronchospasm; chlorine also reachesthe alveoli, penetrates cell membranes and reacts with intracellular water to form HCl, HOCl and chlorine and oxygen free radicals, which causes protein coagulation and oxidation of intracellular components; these reactions develop slowly and
explain therefore the appearance or aggravation of symptoms many hours after exposure; dependent on duration and concentration of exposure the entire respiratory tract can be affected; pathological changes include bronchial epithelium sloughing, ulcerative tracheobronchitis, purulent
intraluminal exudate, and interstitial and alveolar pulmonary edema.
After inhalation of chlorine patients are suffering from lacrimation, conjunctival irritation, rhinorrhea, cough, headache, sore throat, chest burning, dyspnea, nausea, vomiting, and heightening of anxiety especially in those people prone to "neurosis"; severe exposure can lead to severe tracheobronchitis, pulmonary edema, and acute hypoxemic respiratory failure; short-term high level exposures can also aggravate pre-existing heart diseases, producing electrocardiographic changes and congestive heart failure; at sufficiently high doses (i.e. war-time conditions) the exposure to chlorine can cause shock, coma, respiratory arrest, and death; people exposed during physical exertion appear especially vulnerable; controlled human exposure data suggest some people to be more

responsive to the effects of chlorine gas; epidemiologic data also indicate that certain subpopulations (e.g. smokers) may have a greater risk of adverse effects due to chlorine inhalation.

Chronic toxicity:
The effects of long-term exposures to chlorine have been investigated mainly in workers exposed to time-weighted average levels of less than 0.0013 mg/l, but with a few exeptions exposed to average levels up to 0.0042 mg/l; minor
modifications of pulmonary function was found a substance-related effect.
other: human toxicity summary

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
16-SEP-2003 (103) (123) (145) (162) (190) (193) (201) (244)

Remark: Type of experience: other: Health records, other regarding accidents with sodium hypochlorite solutions
Type of experience: other: Health records, other regarding accidents with sodium hypochlorite solutions
Remark: Very few human eye injuries have been reported, presumable because most accidental splashes in the eye have been with the weaker 5% household solutions. A patient who accidentally splashed Clorox in her eyes washed her eyes with water several minutes later because of much burning discomfort, and when seen later the same day had only slight
superficial disturbance of the corneal epithelium which cleared completely in the next day or two without special treatment. Several other case reports provided similar results, essentially normal 48 hours after splashing the eye
with 5% solutions.
The more concentrated 15% solutions used in commercial laundries and in swimming pools as a disinfectant would naturally be expected to cause more serious injury from splash in the eye, and this is indicated in rabbit experiments. There seem to be no well-documented clinical reports, but there is one report of three eyes burned by strong sodium hypochlorite solutions, by Roth with the actual concentration not given, which had slow recovery; one
required a Denig graft.

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
22-JAN-2004 (94) (121) (134) (201)

Method: Baseline spirometry and methacholine challenge tests were performed in a cohort of 278 workers at risk of accidental chlorine inhalation as part of a prospective study. Workers in whom accidental inhalation led to intervention in a first
aid unit were reassessed 5 - 25 days after the accident and serially thereafter when there were notable changes.

Result: During a four-year follow up period, 13 workers were seen at
the first aid unit after a symptomatic accidental

inhalation. All 13 subjects reported an immediate onset of symptoms after accidental inhalation exposure. The main acute symptoms were throat irritation (n=7), cough (n=10), and shortness of breath (n=10). Mean duration of acute symptoms was brief (mean 14.4 hours, range 1-72 hours). Immediate treatment consisted of inhaled bronchodilators in 10 cases. No antiinflammatory drugs were given. Three of them experienced notable functional changes: one worker experienced a 10% fall in forced expiratory volume in one second (FEV1), and the other two had a notable fall in the concentration of methacholine that caused a 20% fall in FEV1
(PC20). Two workers were smokers and one had a personal history of atopy. Baseline assessment was within the normal range in these three workers. Recovery was complete three months after the accidental inhalation. Thus, transient but notable decreases in airway function or increases in bronchial responsiveness can occur after an accidental inhalation of high concentrations of chlorine in workers at risk.

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
22-JAN-2004 (138)

Method: A case-control study of bladder cancer and drinking water disinfection methods was conducted during 1990 -1991 in Colorado. A total of 327 histologically verified bladder cancer cases were frequency matched by age and sex to 261 other-cancer controls. Subjects were interviewed by telephone about residential and water source histories. This
information was linked to data from water utility and Colorado Department of Health records, including total trihalomethanes, nitrates and residual chlorine measurements, to create a drinking water exposure profile.

Remark: The authors demonstrated an increased OR with prolonged exposure to chlorinated water. However, this was not correlated with total trihalomethanes or residual chlorine. Thus it is unclear what the relationship between bladder cancer and chlorinated water really is.

Result: After adjusting for cigarette smoking, tap water and coffee consumption and medical history factors, the odds ratio for bladder cancer increased for longer duration of exposure to a level of 1.8 (95% confidence interval 1.1-2.9) for more than 30 years exposure to chlorinated surface water compared
with no exposure (Table 1). The increased bladder cancer risk was similar for males and females and for nonsmokers and smokers. Levels of total trihalomethanes, nitrates and residual chlorine were not associated with bladder cancer risk after controlling for years of chlorinated water exposure.

Table 1
Adjusted odds ratio (OR) and 95% confidence intervals (CI) for lifetime years of exposure to chlorinated water, Colorado, 1988-1989

No. of years	OR	95% CI
0	0.0	
1-10	0.7	0.4-1.3
11-20	1.4	0.8-2.5
21-30	1.5	0.8-2.9
>30	1.8	1.1-2.9

Odds ratios and 95% CI adjusted for coffee consumption, smoking, tap water intake, family history of bladder cancer, sex, and medical history of bladder infection or kidney stone.

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
23-JAN-2004 (152)

Method: A case-control study of bladder cancer in Iowa in 1986-1989 was conducted. Cases 40-85 years of age with histologically confirmed bladder cancer in the years 1986-1989, and

without previous diagnosis of a malignant neoplasm. Residential history, drinking water source, beverage intake and other factors with historical data from water utilities and measured contaminant levels to create indices of past exposure to chlorination byproducts. The study comprised 1123 cases and 1983 controls who had data relating to at least 70% of their lifetime drinking water source. The study was conducted in two phases. In the first phase (1986-1987), bladder cancer was one of six cancer sites and controls were frequency-matched by sex and 5-year age group, to all cases, resulting in a case:control matching ratio for the bladder cancer case series of approximately 2.3:1. In the second phase (1988-1989), we restricted the study to bladder cancer cases only and a control series, with frequency-matching of controls to cases at a ratio of 1:1. Cases of both in situ and invasive bladder cancer (transitional cell carcinoma and papillary transitional cell carcinoma) were included because they appear to share the same risk factors. Patients diagnosed in 1987 with in situ disease were included in the 1988-1989 study period, resulting in more cases in the second (N=894) than the first phase (N=558). In the spring and summer of 1987, surveys of all Iowa water utilities serving at least 1000 persons were conducted. Historical information from 280 utilities serving 345 Iowa communities with a total 1980 population of 1.94 million (state population = 2.92 million). Most of the state population not served by these community supplies used private wells. Samples of water were collected at each water utility for

analysis using EPA method 524.2 (trihalomethane measurement).

Remark: Although this study examined the relationship of chlorination byproducts, trihalomethanes, with bladder cancer, the chlorinated surface water was treated with chlorine.

Result: Positive findings of bladder cancer were restricted to men drinking chlorinated surface water and to ever-smokers. There was no association among men who never smoked (Table 1). Odds ratios increased both with smoking level and among the ever-smokers, with duration of chlorinated surface water use.

Table 1
Odds ratios (OR) and 95% Confidence Intervals among men for bladder cancer by cigarette smoking and Duration of residence served by chlorinated surface water sources

Years exposure Chlorinated surface water	Smoking Status Never Smoker	Past Smoker	Current Smoker
None	1.0	1.7 (1.3-2.3) [112,332]	3.5 (2.5-4.7) [236,387] [188,156]
1-19	1.0	1 (0.6-1.6) [27,75]	3.5 (1.4-2.8) (2.3-5.3) [92,131] [73,62]
20-39	0.8	2.0 (0.3-2.0) [6,22]	5.7 (1.2-3.5) (3.1-10.4) [30,42] [37,20]
>40	0.7	3. (0.3-1.9) [5,26]	5.8 (2.1-5.8) (3.0-11.3) [39,39] [29,16]

* Reference category
[ ] The numbers of cases and controls are shown in brackets.

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
22-JAN-2004 (39)

Result: There were trivial changes observed in the comparison of the sham exposure vs. 0.5-ppm exposure. Total lung capacity (TLC) was lower before 0.5 ppm exposure then before sham exposure, and the percent decrease in carbon dioxide pulmonary diffusing capacity was smaller 24 h after 0.5 ppm exposure than 24 h after sham exposure. Comparison of the sham vs. the 1 ppm exposure showed several differences in percent changes ($P<0.05$) e.g. forced vital capacity (FCV), forced expiratory volume at 1 s (FEV1), peak expiratory flow rate (PEFR), forced expiratory flow rate (PEFR), forced expiratory flow rate at 50 and 25% vital capacity (FEF50 and FEF25), and airway resistance (RAW).

Test condition: Eight healthy, unacclimated volunteers were exposed to chlorine gas in concentrations of 0.5, ppm or 1 ppm for 8 hours and several pulmonary function measurements were made.
Sham exposures were made as control.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
22-JAN-2004 (189)

Method: A case-control study of colon and rectal cancer in Iowa in 1986-1989 was conducted. Cases 40-85 years of age with histologically confirmed colon or rectal cancer in the years
1986-1989, and without previous diagnosis of a malignant neoplasm. Residential history, drinking water source, beverage intake and other factors with historical data from water utilities and measured contaminant levels to create indices of past exposure to chlorination byproducts. The study comprised 685 colon cancer and 655 rectal cancer cases
and 2434 controls who had data relating to at least 70% of their lifetime drinking water source. The study was conducted in two phases. In the first phase (1986-1987), colon and rectal cancer was one of six cancer sites and controls were frequency-matched by sex and 5-year age group,
to all cases, resulting in a case:control matching ratio for
the colon and rectal cancer case series of approximately 1:1.2. In the second phase (1988-1989), an additional 1175 controls were selected similar to those used for bladder cancer cases. In the spring and summer of 1987, surveys of all Iowa water utilities serving at least 1000 persons were conducted. Historical information from
280 utilities serving 345 Iowa communities with a total 1980
population of 1.94 million (state population = 2.92 million). Most of the state population not served by these community supplies used private wells. Samples of water were
collected at each water utility for analysis using EPA method 524.2 (trihalomethane measurement).

Result: For colon cancer and subsites, there was no important increase in risk associated with duration of chlorinated surface water, nor with trihalomethane estimates. For rectal
cancer, there was an association with duration of chlorinated surface water use, with adjusted odds ratios of 1.1, 1.6, 1.6 and 2.6 for 1-19, 20-39, 40-59 and >60 years exposure compared with no exposure. Low dietary fiber intake
or physical activity were found to have a larger relative risk estimate (Table 1).

Table 1
Odds ratios and 95% confidence intervals for risk of rectal cancer associated with years at chlorinated surface water sources and usual fiber intake, usual physical activity or average daily tapwater ingestion.

	Duration at chlorinated surface water (years)			
Dietary fiber intake				
Above median:	1.0*	1.28 (0.9-1.9)	1.2 (0.6-2.3)	0.89 (0.4-1.8)
Below median:	0.99 (0.7-1.3)	0.88 (0.6-1.3)	1.57 (0.9-2.7)	2.43 (1.5-4.0)
Usual physical activity (times/week)				
>1:	1.0*	0.96 (0.7-1.4)	0.89 (0.5-1.7)	1.16 (0.6-2.2)
<1, never:	1.1 (0.8-1.4)	1.25 (0.9-1.8)	2.28 (1.4-3.8)	2.22 (1.3-3.7)
Average daily tapwater (liters/day)				
<2.1:	1.0*	1.34 (0.9-1.9)	1.85 (1.1-3.1)	2 (1.2-3.3)
>2.1:	1.27 (0.9-1.6)	1.24 (0.9-1.8)	1.34 (0.8-2.3)	1.72 (1.0-3.0)

* Reference category

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
22-JAN-2004 (106)

Method: A case-control study was conducted from 14,130 obstetric patients at Boston Hospital for Women Division of Brigham and Women's Hospital, Boston, MA. Three case groups and one control group were selected from the women enrolled. The three case groups consisted of: 1) Congenital anomaly group 1314 cases, 2) Stillbirth group 121 cases and 3) Neonatal death group 76 cases. The control group consisted of 1490 cases. Medical records were reviewed. Those women for whom records were not located for review were excluded as were women with a diagnosis of diabetes, epilepsy, prenatal herpes, toxoplasmosis, rubella, a history of drug abuse, lived outside of Massachusetts or lived in a town with no public water supply. A subject also was excluded if she became pregnant more than once during the study period. The final study population consisted of 1039 congenital anomaly cases, 77 stillbirth cases, 55 neonatal death cases and 1177 controls. The women's address at the time of pregnancy outcome or, if available, during the first trimester was used to match each women to drinking water data collected routinely from her city or town. Information about drinking water quality was obtained from routine chemical and metal analyses of Massachusetts public water supplies. No information about tap water quality was obtained. Also gathered was information about drinking water source (surface, ground or mixed) and about surface water treatment (chlorination or chloramination). All Massachusetts surface water is treated by one of these methods; ground water, however is generally untreated. Routine water quality analyses are conducted periodically by the Massachusetts Department of Environmental Protection (DEP). The DEP analyzes tap water samples from preselected representative locations, usually a public building such as town hall, in every city and town that has a public drinking water system.

The routine chemical analyses, which have been conducted since the 1960s, include measurement of pH, alkalinity, hardness, sodium, potassium, iron, manganese, silica, sulfate, chloride, ammonia, nitrate, nitrite and copper. Available data on organic contaminants were too limited to permit meaningful analysis. Also, since 1977, under the provisions of the Safe Drinking Water Act, heavy metals-including arsenic, cadmium, chromium, lead, mercury, selenium, silver and fluoride- have been measured. Guidelines for analyses of the water quality parameters are supplied by the U.S. Environmental Protection Agency. The interval from the date of a matched chemical water sample to the date of conception ranged from 0 days to 4.1 years, and the median interval was 3.3 months.

Remark: The authors mention that risk estimates from this study are likely to be diluted because of nondifferenetial errors in the measurement, recordation and classificaton of exposure. The water composition in the public taps may not have reflected accurately the composition of the water in the homes of the women during pregnancy because of differences in pipes that supplied the water. Exposure misclassification may have also occurred because they were unable to obtain a first-trimester address for every subject. In addition, because they did not have any data on the amount of home tap water consumption, bottled water consumption, dietary sources of the trace elements, or air or occupational exposures, our exposure data may not have represented the levels to which the women and their developing embryos were exposed. The authors conclude that their study has many limitation and the few other published studies report inconsistent results.

Result: No material increases were observed in the frequency of any adverse pregnancy outcomes for women who used surface versus ground and mixed water. Use of chlorinated versus chloraminated surface water was associated with a 2.2 fold increased occurrence of stillbirths (95% CI, 1.3-3.9) and a 1.5 fold increased occurrence of major malformations (95% CI, 1.0-2.3). Both associations persisted after control for confounding (adjusted OR of 2.6 and 95% CI of 0.9-7.5 for stillbirths, adjusted OR of 1.5 and 95% CI of 0.7-2.1 for major malformations). The increased occurrence of major malformations among chlorinated surface water users consisted primarily of increases in the risk of respiratory (OR 3.2, 95% CI 1.1-9.5) and urinary tract defects (OR 4.1, 95% CI 1.2-14.1). Use of chlorinated surface water was not associated with increases in the occurrence of minor malformations, normal variants or neonatal deaths.

Reliability: (2) valid with restrictions

Flag: Critical study for SIDS endpoint

22-JAN-2004 (10)

Method: A population-based case-control study of miscarriage, preterm delivery and low birth weight was conducted in Alamance, Durham and Orange Counties in central North Carolina. Controls were selected in a one-to-one ratio to live birth cases from the deliveries immediately following a preterm or low birth weight of the same race and hospital but restricted to term, normal weight births. During telephone interviews, the source of drinking water (community water company, private well or bottled water)

and number of glasses of water consumed per day was ascertained.
A women's address was used to assign her to one of the five public water supplies serving residences in this region. The dates of pregnancy were used to assign the reported quarterly average trihalomethane (THM) value from the appropriate supplier as her THM score. For miscarriage cases and their controls, the fourth week of pregnancy was the time period used for making that assignment, and for preterm delivery cases, low birth weight cases and their controls, the 28th week of pregnancy was used to assign the nearest THM value. These periods reflect the most likely intervals in which any adverse effects would occur.

Remark: The high OR based on the continuous measure analysis of the

THM concentration was due to a much higher risk associated with the highest sextile of exposure with a low risk in the second to highest sextile (adjusted OR of 0.1, 95% CI =0.0 - 0.5). In the highest sextile, the THM concentration was >100 ppb, the US federal standard. There was no correlation with total dose of THM which makes this finding suspect.

Result: The Odds Ratio (OR) for THM concentration (ppb) and THM Dose (ppb x glasses water consumed/day) were not associated with miscarriage risk. However, using a continuous measure analysis the THM concentration predicted an OR of 1.7 per 50 ppb increment. Preterm delivery showed virtually no association with water source, THM concentration or THM dose. Analysis of low birth weight indicated no association with water source. In general, all three analyses, miscarriage, preterm deliver and low birth weight exhibited a decreased risk with increasing number of glasses water consumed/day.

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
22-JAN-2004 (196)

Method: 29 volunteers (age 20-33) were exposed to chlorine gas (0, 0.5, 1.0, 2.0 ppm) during 4 hours. Severity of irritation was subjectively measured by questionnaires from the subjects every 15-60 minutes and was categorized from barely perceptible to clearly objectionable.

Remark: Type of experience: Human

Result: 1.0 ppm induced a statistically significant decrease in mean FEV1 (-15.3%) following 8 hours exposure. A statistical significant increase in throat irritation in subjects exposed to 1.0 ppm began at 1 hour into exposure. 1 ppm was found to be the NOEL after 30 min exposure. Consistent throat irritation was not observed in subjects during a 4 hour exposure to 0.5 ppm. However, 0.5 ppm chlorine produced throat irritation and an urge to cough after a 4 hour exposure.

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
22-JAN-2004 (8)

Method: A cross-sectional epidemiology study on the association between somatic parameters at birth and drinking water disinfection with chlorine dioxide and/or sodium hypochlorite was conducted. Over a 2 year time period, the

births at two hospitals, one in Genoa (548 cases) and the other in Chiavari (128 cases) were examined. Data regarding both mother and child were obtained from hospital records. Different sections of Genoa are provided water treated with chlorine dioxide, sodium hypochlorite or both. The water in Chiavari is untreated.

Remark: The authors did not examine the amount of water consumed and several potential confounding factors, such as, nutritional habits, amount of smoking and age distribution. In addition, measurements for the control and treated groups were conducted at different hospitals which may have slightly different methods of conducting these routine measurements resulting in slight differences reported.

Result: The average birthweight of children was higher ($p<0.0001$) when mothers were older than 30 years of age and did not consume water disinfected with chlorine; the same was not true with young mothers. Body length and cranial circumference was significantly smaller only for the children of mothers older than 30 who consumed water disinfected either with chlorine dioxide (body length, $p=0.005$; cranial circumference, $p=0.022$) or sodium hypochlorite (body length, $p=0.003$; cranial circumference, $p=0.0001$). Average cranial circumference was also smaller when both disinfectants were used ($p=0.0003$).

Table 1
Mother's age and somatic parameters at birth according to drinking water disinfection treatment

	None	Chlorine dioxide	Sodium hypochlorite	Chlorine dioxide and sodium hypochlorite	Total
Mothers age (years)					
N:	128	277	108	163	676
Median :	30	30	29	30	30
Median absolute Deviation:	3	3	3	3	3
Length of pregnancy (weeks)					
N:	128	275	106	162	671
Median:	40	40	40	39	40
Median absolute Deviation:	1	1	1	1	1
Birthweight (g)					
N:	128	249	91	149	617
Mean:	3421.4	3185.2	3132.1	3176.7	3224.3
95% CI:	3340.9-3502	3133.0-3237.3	3055.5-3208.6	3111.7-3241.7	3190.5-3258.1
Body length (cm)					
N:	125	202	81	117	525
Mean:	49.85	49.18	49.19	49.92	49.4
95% CI:	49.5-50.2	48.9-49.4	48.9-49.6	49.1-49.7	49.2-49.6
Cranial circumference (cm)					
N:	125	200	82	117	524
Mean:	35.23	34.82	34.14	34.41	34.72
95% CI:	34.98-35.48	34.52-35.13	33.86-34.42	34.18-34.63	34.57-34.87

Reliability: (2) valid with restrictions

Flag: Critical study for SIDS endpoint
23-JAN-2004 (119)

Method: Approximately 2000 new cases were examined in the Occupational Health Clinic at the University of Cincinnati Medical Center in Cincinnati, Ohio between 1975 and 1982. About 25% of these cases were patients with apparent bronchial asthma, mostly suspected of being occupational or environmental in origin. Among the approximate 500 cases of asthma, there were 30 with suspected Reactive Airways Dysfunction Syndrome (RADS). Twenty of these cases did not have complete past histories or medical information or lacked some clinical criteria. Subsequently, ten individuals developing symptoms following a single exposure to irritating vapors, fumes or smoke were studied up to several years following acute exposure.
Remark: In this study, none of the ten cases were exposed to chlorine.
Result: In most instances, the high level exposure was the result of an accident occurring in the workplace or a situation where there was poor ventilation and limited air exchange in the area. In all cases symptoms developed within a few hours and often minutes after exposure. No documented preexisting respiratory illness was identified nor did subjects relate past respiratory complaints. In two subjects, atopy was documented, but in all others, no evidence of allergy was identified. In the majority of cases, there was persistence of respiratory symptoms and continuation of airways hyperreactivity for more than one year and often several years after the incident. The incriminated etiologic agent varied, but all shared a common characteristic of being irritant in nature. In two cases, bronchial biopsy specimens were available, and an airways inflammatory response was noted. This investigation suggests acute high level, uncontrolled irritant exposures may cause an asthma-like syndrome in some individuals which is different from typical ccupational asthma. It can lead to long-term sequelae and chronic airways disease. Nonimmunologic mechanisms seem operative in the pathogenesis of this syndrome. We have designated the illness as reactive airway dysfunction syndrome (RADS) because a consistent physiologic accompaniment was airways hyperreactivity.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
22-JAN-2004 (30)

Method: This is a review of 5 retrospective epidemiology studies which consisted of 2 cohort studies (Deane et al., (1992) and Wrensch et al., (1992)) and 3 case-control studies (Hertz-Picciotto et al., (1992), Windham et al., (1992) and Enster et al., (1992)). Two of the studies were designed initially to study spontaneous abortion in relation to exposure to contaminated water. After addressing the primary study hypothesis, each was analyzed with respect to water consumption.
Remark: Authors considered these 5 retrospective studies to have several methodological difficulties which confound the interpretation.

Result: The two retrospective cohort studies suggest an unusually low rate of spontaneous abortion (0-3%) among women who drank no tapwater or who filtered their tapwater. The studies by Dean and Wrensch, however, which found the strongest evidence of an association were conducted in connection with the much publicized episode of water contamination. Despite an increase in the use of bottled water during 1980-1985, shown by Wrensch, the spontaneous abortion rate did not appear to decline, although this study may not have had the power to detect such an decrease.
Associations between spontaneous abortion and ground and surface waters did not differ consistently, suggesting that the agent, if present would not be a chlorination by-product, nor would it be removed by chlorination. The authors conclude that the findings could be due to: 1) Chance (authors consider this unlikely), 2) Bias (authors consider biased reporting to be the most plausible since these were all retrospective studies) or 3) Causation (authors consider this to lack biological plausibility since a range of water systems were examined).
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
22-JAN-2004 (223)

Type of experience: other: Occupational Exposure

Remark: Patil et al. (1970) evaluated the exposure of 332 male diaphragm cell workers to 0.006-1.42 ppm chlorine gas (a range with a time-weighted average of 0.146 +0.287; most workers were exposed to less than 1 ppm). A control group consisting of 382 workers from 25 representative chlorine manufacturing plants was also studied. Both groups were comprised of men between the ages of 19-69 with a mean age of 31.2 +11.0 years. Physical examinations (blood and urine analysis, chest x-rays and electrocardiograms) were conducted, in most cases, within the first six months of the study year. At two month intervals, each plant was surveyed and chlorine levels were determined. Exposed employees were grouped according to job classification. Researchers found the average number of exposure years for the study group to be 10.9 + 2.8 years and concluded that the exposure level had no correlation to the number of years exposure. Ninety-eight of the 332 workers were found to have abnormal teeth and gums, but no dose-response relationship was concluded.
Similarly, no dose-response relationships were shown with the symptoms of sputum production, cough, dyspnea, history of frequent colds, palpitation, chest pain, vital capacity, maximum breathing capacity and forced expiratory volume. Any deterioration in pulmonary function was shown to be age related. Of the 332 exposed workers, 9.4% experienced abnormal EKGs. 8.5% of the control group showed the same abnormalities, but this difference was not significant. Above 0.5 ppm, an increase appeared in the incidence of fatigue. No neurological defects developed and there was no noted prolonged anoxia as a result of the chlorine exposure.
Also, no consistent gastrointestinal trouble or abnormal incidence of dermatitis was found. Exposed workers showed elevated white blood cell counts and decreased hematocrit

values compared to the control group.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
22-JAN-2004 (180)

Remark: Following the study by Swan et al described in previous entry, epidemiology study was conducted in three area. This study reported that an increased risk of abortion was associated with high consumption of cold tap water in the same area where the causal relationship has been recorded in previous retrospective study but not in two others areas.
The authors reported that this result was attributable to dissolved chlorine or trihalomethan potentially contained in cold water.

This causal relationship appears to be inconsistent with the causality hypothesis involving chlorinated drinking water by-products and especially trihalomethanes.
Reliability: (2) valid with restrictions
30-OCT-2005 (222)

Remark: Effects of the inhalation of chlorine on humans

Data gathered by the Environmental Protection Service of Canada
Conversion factor: 1 ppm = 2.95 mg/m3

Exposure Level (Duration)	Effects
Acute exposure	
1000 ppm	Fatal after a few breaths.
833 ppm (30-60 min)	Death.
40-60 ppm (30-60 min)	Pulmonary edema.
30 ppm	Choking, coughing, burning sensation
15 ppm (min)	Eye, nose and throat irritation
4 ppm (30-60 min)	No ill effects noted.
1.3 ppm (30 min)	Shortness of breath, headache
1 ppm	Minimum effect level; burning eyes, dry throat, coughing, difficulty in inhaling.
1 ppm (20 min)	Dull sensation in teeth, slight metallic taste, headache, burning of conjunctiva, skin, distinct taste, coughing, constriction of breathing.
<1 ppm	Acute obstructive ventilatory defects clearing within 24 to 48hrs.
0.5 ppm	One subject was accidentally exposed due to the use of a

poor fitting respirator. Several hours later, victim developed mediastinal emphysema.
0.5 ppm
Optical chronaxie increased.
0.45 ppm
Burning of conjunctival tissue.
0.2 ppm
Dry throat, slight cough, sensations in conjunctiva.
0.058 ppm
Tickling in the throat.
0.027 ppm
Tickling in the nose.

Chronic exposure:
<1 ppm (mean 10.9 yr)
Male workers were exposed. No dose response relationship correlation to occurrence of colds, dyspnea, chest pain, ventilatory capacity and volumes was found. A slight excess in abnormal ECGs among exposed workers was noted. Slight correlations of exposure to anxiety, dizziness, leukocytosis, and lowered hematocrit were present. No evidence of mutagenicity or carcinogenicity was noted.
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other: human toxicity summary
Results from several authors on exposure and inhalation of chlorine are given in the attached document.

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (75)

Method: Groups of five humans with and five without airway hyperresponsiveness (HR) were studied following exposure to 1.0 ppm chorine for 60 minutes. In addition, 5 persons, all with HR, were studied following exposure to 0.4 ppm chlorine for the same time period. Airflow and airway resistance were measured immediately before and immediately after exposure.
In addition, 24 hours before and 24 hours after exposure lung volumes, airflow, diffusing capacity, airway resistance and responsiveness to methacholine were measured. All volunteer subjects were between 18 and 30 years of age.
Subjects were considered HR if a nebulized methacholine dose <8 mg/ml induced a 20% or greater fall from baseline FEV1.
Three HR subjects were exposed to both 0.4 and 1.0 ppm chlorine. Exposures occurred greater than 2 months apart.

Remark: Type of experience: Human

Result: After 60 minutes exposure to 1 ppm chlorine, there was a statistically significant fall in FEV1 and FEF25-75 and a significant increase in Sraw among both normal and HR subjects (Table 1). There was also a fall in FVC that was statistically significant only when all subjects were analyzed together. Two subjects, both in the HR group, experienced respiratory symptoms following exposure. All remaining subjects were asymptomatic. Twenty four hours after exposure, there were no significant group changes for either normal or HR subjects.

There was no statistically significant response in airflow

or resistance after 0.4 ppm chlorine either immediately following exposure or after 24 hours. The subject with the most marked response following exposure to 1.0 ppm had virtually no response following exposure to 0.4 ppm.

Table 1

Pulmonary Function Change from Baseline	Normal Mean + SD	HR Mean + SD
FEV1		
Absolute change, mL	-150 + 64	-520 + 383
Relative change, %	-4 + 2	-16 + 13
FVC		
Absolute change, mL	-20 + 84	-420 + 460
Relative change, %	-0.4 + 1	-9 + 11
FEF25-75		
Absolute change, mL	-400 + 255	-540 + 378
Relative change, %	-11 + 8	-25 + 20
Sraw		
Absolute change, U	+2.1 + 1.6	+7.5 + 4.9
Relative change, %	+39 + 28	+108 + 93

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
23-JAN-2004 (58)

Method: Initially, eight male volunteers were exposed for 6 hours/day on 3 consecutive days to each of 4 exposure concentrations which were spaced 11 days apart. Males were exposed to 0, 0.1, 0.3 and 0.5 ppm. Four of the individuals were exposed to the four concentrations in the following sequence: 0.3, 0.1, 0 and 0.5 ppm. The remaining individuals were exposed in the following sequence: 0.3, 0.5, 0.0 and 0.1 ppm. Assignment to treatment sequences was random. The exposure to the test substance and the effect measurements were conducted in a double-blind fashion. One individual decided to stop participating for reasons not related to the study.
Measurements of lung function parameters (forced vital capacity, forced expiratory volume in first, second, maximal mid expiratory flow) and nasal lavage parameters (total cells, cell differentials, albumin, interleukin-8) were used to evaluate the potential respiratory effects of whole body exposure to chlorine vapor.
Remark: Type of experience: Human
Result: No significant differences were found between all 4 Exposure conditions with respect to forced vital capacity, forced expiratory volume in first, second and the ratio of these parameters. For the maximal mid expiratory flow, a statistically significant difference was observed between the control and 0.5 ppm exposures. However, this was attributed to an unexplained shift in baseline values in the control exposure week.
The possible inflammatory effects of exposure to chlorine were assessed by measurements in nasal lavage. The number of cells were counted and the proportion of neutrophils, lymphocytes, monocytes, eosinophils and epithelial cells was determined. In addition albumin, as an indicator for

epithelial permeability, and interleukin-8, as a sensitive biomarker of local inflammatory response were measured. Results of nasal lavage measurements, did not support evidence of an inflammatory response or irritant effects on the nasal epithelium.

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
16-SEP-2003 (74)

Method: Seven chemical workers who were accidentally exposed to chlorine gas in separate accidents were investigated. The natural history, chest radiographs and arterial blood of these workers were studied.

Result: Of the seven chemical workers, six had mild or moderate illnesses which lasted two to eight days. The usual symptoms were conjunctivitis, cough, breathlessness and chest pains.
With one exception, the onset of the symptoms followed within 10 minutes of exposure. In this one individual, symptoms appeared approximately 45 minutes after exposure. In anteroposterior radiographs, congestion, consolidation or basal nodules were observed. All of these acute changes cleared within a week.
The individual that did not have mild or moderate illness appeared to have been exposed to a higher concentration of chlorine gas. He quickly began to choke and developed dyspnoea, persistent cough and chest pain. Approximately 10 hours later, he was cyanostic, with rapid and shallow breathing, and coughing up pink, frothy sputum. On the second day the patient had a severe headache and pains in the limbs and chest which persisted for 2 days. He remained critically ill for 48 hours and then gradually improved. For 9 days the patient received continuous oxygen. On days 4 and 5 he was cyanosed when allowed to breathe air. The dyspnoea gradually decreased and by the tenth day there was none at rest. He was discharged from the hospital after 13 days.
Exercise dyspnoea persisted for 5 weeks. Two months after the exposure, there were no residual symptoms or signs and the chest radiograph and lung function tests were normal.

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
22-JAN-2004 (17)

Method: Four young adults accidentally exposed to chlorine gas were studied physiologically for one month.

Result: All patients were symptomatic with cough, tightness in the chest, and shortness of breath immediately after exposure. All had restrictive ventilatory defect with impaired diffusing capacity. There was evidence of some obstruction in small airways. There was inconsistent evidence of obstruction in large airways. All lung function impairment was temporary and cleared entirely within one month. There was no residual lung damage.

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
22-JAN-2004 (182)

Method: Eighteen young adults accidentally exposed to chlorine gas were studied for up to 5 months. Health histories were obtained shortly after exposure. Routine blood studies, liver function tests, chest roentgenograms, electrocardiograms were obtained. Arterial blood gas analysis was conducted as necessary during hospitalization. Pulmonary function tests were conducted within 18 hours of chlorine exposure and repeated at 1 and 2 weeks and, in 9 subjects, at 5 months after exposure. At 5 months, measurement of lung volume by helium dilution and single breath carbon monoxide diffusing capacity were also measured.

Result: Intense dyspnea and paroxysmal dry cough were the chief complaints in 6 and 12 subjects, respectively, upon admission to the hospital. Smoking history was more prevalent in the dyspnea group (6 of 6 subjects) than the cough group (3 of 12 subjects). A history of bronchial asthma or "wheezing" was present in 4 subjects, 3 of whom belonged to the dyspnea group. Subjects in the dyspnea and cough groups had a diminished FEV1.0, as well as low FEF25-75%, FEF50% and FEF25% on admission (Table 1). These latter abnormalities were still evident at 1 and 2 weeks after chlorine exposure in the dyspnea group. In contrast, the diminished flow rates in the cough group returned to normal by 7 days after chlorine exposure and remained so 1 week later.
In the nine subjects that were studied 5 months after exposure, no physiologic abnormalities were evident except for a slightly diminished FEF25-75% and mild hyperinflation in 2 subjects who have continued to smoke. In summary, subjects whose initial complaint was dyspnea had a slower resolution than those who complained of cough. Cigarette smoking and a history of asthma or wheezing was more prevalent in the group with slower resolution.

Table 1:

	Percent of predicted expiratory flow rates		
	Day 1	Day 7	Day 14
Cough (N = 12)			
FVC, L	89.5+12.8	87.9+14.0	90.7+12.1
FEV(1.0), L	72.5+14.6	88.0+14.8	93.6+11.4
FEV(1.0)/FVC,	65.7+10.4	81.8+ 3.2	84.8+ 4.2
FEF(25-75%), L/sec	64.5+16.9	91.4+16.6	97.0+17.4
FEF5(0%), L/sec	63.8+19.0	85.8+16.9	91.9+12.8
FEF(25%), L/sec	62.9+20.9	94.0+18.1	94.7+16.5
Dyspnea (N = 6)			
FVC, L	88.3+16.1	88.5+13.1	87.0+14.2
FEV(1.0), L	78.5+13.7	84.5+ 9.0	84.8+10.5
FEV(1.0)/FVC, %	75.5+ 8.2	75.6+ 7.3	77.9+ 7.1
FEF(25-75%), L/sec	65.5+12.8	68.2+11.3*	73.8+12.8*
FEF(50%), L/sec	56.3+18.6	65.0+15.7*	69.8+15.2*

* Significant difference between dyspnea and cough groups (P<0.05).

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
23-JAN-2004 (98)

Method: Twenty eight individuals exposed to approximately 66 ppm chlorine for one hour had bronchial brushings collected on day 5-, 15- and 25-post exposure. Histological examinations were performed on the brushings.
Result: Postexposure smears collected on day 5 showed basal-cell and goblet-cell hyperplasia, acute inflammation, and chromatolysis of columnar epithelial cells. Columnar epithelial cell syncytia were observed in 15 (53.57%) smears. Nine (32.14%) smears showed abundant nonpigmented alveolar macrophages. Seven (25%) smears from mucosal erosions showed proliferating fibroblasts and capillary fragments; on day 15 and day 25 repeat smears from these seven cases showed evidence of epithelial regeneration and repair by fibrosis.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
31-OCT-2005 (208)

Remark: Case Report:

Case #1;
A 39-year-old woman was using 65% calcium hypochlorite "shock treatment" to remove algae in her swimming pool. In order to make the granules more soluble she mixed them with water from the pool in a bucket. This is a recommended procedure on some labels. As soon as she added water it started bubbling. It was later said that there may have been residual amounts of "something like bleach" in the bucket.
She knocked the bucket into the swimming pooland when leaving the area inhaled the gas. She began coughing, and her eyes were watering profusely. Enroute to the hospital by ambulance she was given albuterol by inhalation, which caused her to go into severe bronchospasm, partial relieved by removal of the albuterol. In the emergency department she had severe respiratory distress presenting with rapid gasping, coughing, and pale skin. Attempts to obtain initial arterial blood gases (ABGs) were successful. She was immediately placed on 100% humidified oxygen by mask and an IV of D5W was started. One hour later the patient, still on O2 by mask, began expiratory wheezing, and she was transferred to intensive care. An initial chest X ray showed diffuse interstitial edema with a preponderance at both bases and hyperinflation of both lungs. Four hour after the exposure while on 100 plus O2, 50% humidity, she had audible wheezes and tightness in the chest on auscultation. She was given midazolam 2mg and morphine sulfate (MS) 5mg IV. In preparation for intubation, she was given 100mg lidocaine (for possible arrhythmias), 10 mg S, 10mg midazolam, 100mcg fentanyl, and 5mg pancuronium. After five attempts (made difficult by laryngospasm) she was successfully intubated.
Initial ventilation on FI02 0.45 was 12/min, volume 650mL, pressure 45mmHG, PEEP +5. ABGs at this time were pH 7.52, PaCO2 28, PaO2 250mmHg, and O2 saturation > 99%. The O2 was gradually reduced to 25% by 10 hours post exposure.
Additional medications included lorazepam 1mg every eight

hours as need; MS 2-4 mg every two hours as needed;

acetaminophen suppositories as needed for fever; ketamine 50mg, midazolam 5 mg, and/or haloperidol 40 mg every three to five hours as needed; dexamethasone 4mg every six hours; and IV fluids with 40mEq KCl at 75mL/h.Laryngoscopy 36 h inhalation showed no evidence of severe lung injuryand the primary problem was assessed as begin in the upper airway. At 60 h post exposure, the patient was extubated with lidocaine and haloperidol and placed on 30% O2 by aerosol mask. At 68 h, oxygen, IVs and steroids were discontinued, and the patient was transferred to a medical bed. Culture of blood and urine were negative but sputum culture yielded a heavy growth of Streptococcus pneumoniae. There was no clinical evidence of pneumonia, and antibiotics were not prescribed. At 88 h post the patient was discharged home with mild cough, wheezing.

Case #2;
A 34-year-old white male owned an above-ground swimming pool. Over the winter the cover blew off and on the first day of the new season the pool water was found to be full of debris and algae. After trying other chemicals, the owner then proceeded to use the chlorinating agent, TST, "Pool Shock". This mixture is 81% w/w TST with the reminder being inert filler.
A pail was filled with dry chemical and pool water was Added for dissolving prior to mixing into the pool. As the water was added to the dry chemical an explosion occurred. The explosion blasted a cloud of white smoke almost 30 feet in the air and the sound was heard for several blocks. The man was found covered in a white powder and running around frantically. Members of the household came to his assistance and turned the water house on him to wash off the powder; this accelerated chemical burns to his body. He was then rushed to the hospital.
Upon admission to the hospital at 1930, the only complaint from the patient was a burning of the eyes. No shortness of breath, rales or hypo-ventilation were present. The blood pressure was 90/95 mmHg and plus 64 bpm. The eyes were irrigated. The patient, being very agitated, was administrated diazepam at 1955. At 2000 his blood pressure was 90/38 with a pulse of 120, nail beds were cyanotic, and ABGs were PaO2 28mmHg, PaCO2 43 mmHg, and pH 7.24. The patient was intubated and given 100 % oxygen. A subsequent ABG at 2005 showed PaO2 30mmHg, PaCo2 34 mmHg and pH 7.14. The patient became cyanotic, had a cardiac arrest and resuscitation was unsuccessful.
At autopsy, the deceased was found to have chemical burns of the skin on his face and both arms and the eyes were opaque.
The lungs were chemically burnt and severely edematous, the right weighing 1400 g and the left 1300 g. Both were oozing fluid and rubbery in consistency. Other organs appeared grossly normal except for the liver which was congested. Death was attributed to severe pulmonary congestion, edema, and lung obstruction from sloughing of the bronchial tree due to chemical burns.
Case report: Explosion Risk from Swimming Pool.

Reliability: (4) not assignable
22-JAN-2004

Remark: The document contains additional references of acute and long-term human exposure to chlorine:

In a study of Shroff et al. [Diagn Cytopathol, 4, 28-32, 1988] bronchial brushings were used to evaluate the cytopathological changes in 28 out of a total of 88 patients who had been accidentally exposed to chlorine concentrations of 191 mg/m3 (66 ppm) for one hour following a gas leak in Bombay, India. Sixty-two of the 88 patients showed respiratory incapacitation, including both an obstructive and a restrictive pattern as well as a mixture.

In another study of accidental exposure to chlorine in a pulp mill, twenty construction workers were followed for up to 20 years with lung function testing [Schwartz et al. (1990) Chest, 97, 820-825]. Immediately following the exposure, all 20 workers experienced burning of the eyes, nose and throat and also developed a dry cough with chest tightness. Five individuals had transient infiltration on the X-ray. Although a high prevalence of airflow obstruction was observed throughout the period of observation, the authors ascribe this prevalence to smoking habits rather than the exposure to chlorine. The clinical complications following the exposure is unclear, and the small number of exposed and tested persons make conclusions difficult.

A sample of 147 men drawn form the workers in a pulp mill was compared with one of 124 men from a paper mill [Ferris et al. (1967) Brit J Industr Med, 24, 26-37]. The pulp mill workers were exposed to chlorine (average exposure 21 mg/m3 - traces), chlorine dioxide, and sulphur dioxide, while the paper mill workers mainly were exposed to SO2. A insignificant difference with respect to lung function parameters was observed between the chlorine-exposed workers and the control group. Ferris et al. [Brit J Industr Med, 36, 127-134] studied the mortality and morbidity experience of the same sub-groups in a ten-year follow-up investigation. No marked differences in mortality and morbidity were found.

An additional study of pulp mill workers revealed an airflow limitation that appeared to be associated with working in the production area of the plant, where the exposure was mainly to chlorine with a mean concentration (8-hr TWA) of 0.52 mg/m3 (0.18 ppm). [Enarson et al. (1984) Arch Environ Hlth, 39, 325-330]

Shi [Shi Z (1990) in: Occupational Epidemology, Sakurai et al. (eds.), Elsevier Science Publisher B.V., p. 173] reported on the effect of long-term exposure to chlorine in a Chinese diaphragm cell chlorine production plant with a workplace concentration of chlorine ranging from 2.6 - 11 mg/m3 (0.9 - 3.8 ppm; mean 4.82 mg/m3). The result of the lung function testing indicated that long-term exposure to low levels of chlorine produced abnormalities of the respiratory system, and in general the abnormalities were more frequent in workers with a long duration of exposure (>10 years). The result also showed that exposure to chlorine and cigarette smoking may have an additive effect which played an important role in the impairment of the

respiratory system.
Reliability: (4) not assignable
22-JAN-2004 (46)

Remark: In a Norwegian study on chlorinated drinking water no evidence for a causal relationship with colorectal cancer was observed.
Reliability: (4) not assignable
22-JAN-2004 (81)

Method: Three patients accidentally exposed acutely to sublethal doses of chlorine gas were followed. Bronchoalveolar lavage was performed 4 and 16 months after exposure.
Result: The patients complained of intermittent dyspnea in association with respiratory irritants and physical exertion for more than 2.5 years post-exposure. Four months after the accident, bronchoalveolar lavage showed an inflammatory cell reaction, whereas 16 months later the differential cytology proved nearly normal. Moderate to severe nonspecific bronchial hyperresponsiveness was assessed in intervals of 4, 20 and 30 months after the accident. All patients showed the typical features of the reactive airways dysfunction syndrome defined as an asthma-like occupational illness after acute exposure to highly concentrated respiratory irritants. We conclude that a single high exposure to chlorine gas may lead to acute respiratory injury and to long-term reactive airway dysfunction with typical symptoms of inflammatory changes of the airways and nonspecific bronchial hyperresponsiveness.
Reliability: (4) not assignable
22-JAN-2004 (200)
Remark: Intoxication of 76 children by chlorine gas is reported.
Intoxication of 76 children by chlorine gas is reported.

The contamination affected a small sector of the population of Zaragoza (Spain) in November 1981). It was caused by a leak of 300 liters of chlorine gas from the city reservoirs,when a canister, containing the gas under pressure, was being handled during the routine procedure of chlorination of drinking water. The gas spread rapidly throughout a sparsely populated residential area containing various primary and secondary schools.

Symptoms were irritative cough (90.7%), nasal-pharyngeal pruritus (65.5%), signs of irritation in the lower respiratory tracts such as chest pain, tachypnoea and dyspnoea (25; 19.7; and 14.4%, respectively). Eight children showed facial congestion, seven were affected by headache, four suffered from vomiting and nausea and two lost consciousness.

The longest period of hospitalization was 12 hours and, until the article was published, there has been no recurrence of symptoms.
Reliability: (4) not assignable
22-JAN-2004 (82)

Method: A case-report of anosmia (loss of sense of smell) is reported.
Result: A single case report of an individual losing the sense of smell following acute chlorine exposure is reported.
Reliability: (4) not assignable
22-JAN-2004 (22)

Remark: A retrospective study was performed to investigate the incidence of obstructive disease of the respiratory tract of 25 employees of chemical plants which were exposed to low concentrations of gaseous chlorine. The incidence was not statistically different from that of a control group.
Ref.: Strassburger KU (1981)

A retrospective study was performed to investigate the incidence of obstructive disease of the respiratory tract of employees of chemical plants which were exposed to low concentrations of gaseous chlorine. There was a trend for increased obstruction with increasing age. The tend was not statistically significant when compared to a control group.
Ref.: Strassburger KU and Thiess AM (1981), Strassburger DU (1984)
A prospective investigation of 23 workers, which were exposed to gaseous chlorine after an accident (30 ppm, 5-10 min), was performed. In all except 3 workers normal respiratory functions were found one year after the accident.
Ref.: Strassburger KU and Thiess AM (1981), Strassburger DU (1984)
Reliability: (4) not assignable
22-JAN-2004 (218) (219) (220)

Remark: Threshold Perception Properties:

Odour

Parameter	Media	Concentration
Threshold of Discomfort	In air	15.1 ppm (44.5 mg/m3)
Upper Recognition Threshold	In air	5.0 ppm (14.8 mg/m3)
Median Recognition Threshold	In air	3.0 ppm (8.9 mg/m3)
Odour Threshold	In air	0.3-0.4 ppm
Absolute Odour Threshold	In air	0.314 ppm (0.9 mg/m3)

Taste

Detection	In water	16.9 ppm (50 mg/m3)

Reliability: (4) not assignable
22-JAN-2004 (75)

Method: Eight subjects with Seasonal Allergic Rhinitis (SAR) and eight without were studied out of season. In a single-blind crossover study, subjects had their nasal airway resistance (NAR) measured in triplicate before, immediately after, and 15 minutes after a 15-minute exposure to either filtered air

or 0.5 ppm chlorine in filtered air, administered through a nasal mask in a climate-controlled chamber. Log-transformed NAR values were analyzed in a repeated-measures analysis of variance model, with confirmatory testing using paired t tests.

Result: The net (chlorine minus air day) percent change in NAR from baseline (before exposure) to immediately after exposure was +24% in the SAR group and +3% in the nonrhinitic group. The corresponding net changes from baseline to 15 minutes after exposure were +21% in the SAR group and -1% in the nonrhinitic group.

Conclusion: The observed augmented nasal congestive response of subjects with SAR versus nonrhinitic subjects to a controlled low-level chemical irritant provocation is consistent with epidemiologic surveys showing a higher prevalence of nasal symptoms among subjects with SAR than nonrhinitic subjects in environments involving irritant air pollutants.

Reliability: (4) not assignable

31-OCT-2005 (209)

Method: Age-adjusted mortality rates for cancer during 1982-1991 among 14 chlorinated municipalities (CHM) were compared to rates for 14 matched unchlorinated municipalities (NCHM). A CHM was defined as one in which more than 90% of the municipality population was served by the chlorinated water while the NCHM was defined as one in which less than 5% of the municipality population was served by chlorinated water.
The CHM and NCHM had similar urbanization levels and sociodemographic characteristics.

Result: The results of this study suggest a positive association between consumption of chlorinated drinking water and cancer of the rectum, lung, bladder and kidney.

Reliability: (4) not assignable

22-JAN-2004 (255)

Method: This population based case-control study in Ontario Canada examined the relationship between bladder cancer and exposure to chlorination by-products in public water supplies. Residence and water source histories and data from municipal water supplies were used to estimate individual exposure according to water source, chlorination status, and by-product levels (represented by trihalomethane (THM) concentration). Exposures were estimated for the 40-year period prior to the interview, using 696 cases diagnosed with bladder cancer between 1 September 1992 and 1 May 1994 and 1545 controls with at least 30 years of exposure information. Odds ratios (OR) adjusted for potential confounders were used to estimate relative risk.

Result: Exposure to chlorinated surface water for 35 or more years had an increased risk of bladder cancer compared with those exposed for less than 10 years (OR = 1.41, 95 % CI 1.10-1.81). Those exposed to an estimated THM level >50 ug/L for 35 or more years had OR of 1.63 (95% CI 1.08-2.46).
These results indicate that the risk of bladder cancer increases with both duration and concentration of exposure

to chlorination by-products, with population attributable risks of about 14 to 16 percent.

Reliability: (4) not assignable
22-JAN-2004 (124)

Method: Five healthy male and five healthy female nonsmokers were used if they met the following criteria: between 18 and 40 years old, with no history of hay fever, asthma, allergic rhinitis, chronic respiratory disease or cardiovascular disease, had not used medication within 1 week of the experiment, did not swim more than once a week in a chlorinated swimming pool and had an FEV1 to FVC ratio >75% of the predicted value. Using a mouthpiece or nasal cannula, the subject inhaled beginning at functional residual capacity while viewing a computer monitor on which the respiratory volume was displayed in real time. The subject controlled his or her breathing so that the respiratory volume signal followed a predrawn pattern corresponding to equal inspiratory and expiratory flows of 250 mls/sec and a tidal volume of 500 ml. This was followed by chlorine sessionss at 150 and 1000 mls/sec that were performed in an order that was randomized among subjects. At a predetermined time during inhalation, a 10 ml CL2 bolus was automatically injected into the inspired airflow. Penetration of the bolus into the respiratory system could be systematically varied from breath to breath by changing the bolus injection time relative to the time the subject switched from inhalation to exhalation. The earlier the injection time relative to the end of inhalation, the greater the penetration of the bolus distal to the airway opening.
Three experiments were conducted: oral breathing with a peak inhaled concentration of 3 ppm; nasal breathing with a peak inhaled concentration of 3 ppm and nasal breathing with a peak inhaled concentration of 0.5 ppm. The volume of each subjects airways was determined using a commercially available acoustic reflection apparatus (Eccovision Acoustic Rhinometry-Pharyngometry System, Hood Laboratories).

Result: From the bolus inhalation measurements, nearly all (>95%) of the chlorine inhaled at flow rates ranging from 150 to 1000 ml/sec is absorbed in the upper airways, whether the nose or the mouth is the site of air access.

Reliability: (4) not assignable
22-JAN-2004 (174)

Remark: Trained industrial hygienists found that 1.2 to 1.3 ppm (3.5 to 3.8 mg/m3) produced no effects (eye and respiratory irritation), 2.6 ppm (7.7 mg/m3) produced minimal respiratory irritation, 3.0 ppm (8.9 mg/m3) painful respiratory irritation, and 9.0 ppm (26.6 mg/m3) was intolerable. 7.7 ppm or 20 ppm of chlorine produced minimal and painful eye irritation, respectively.

Reliability: (4) not assignable
22-JAN-2004 (93)

Remark: Medical Conditions Aggrevated by Exposure: Asthma and respiratory and cardiovascular disease.

Reliability: (4) not assignable

22-JAN-2004

Remark: A study which monitored the respiratory effect/function of

persons acutely exposed to chlorine gas pursuant to a train derailment for 6 years after the incident suggests no persisting abnormal rate of decline in lung function as a result of the acute exposure. Initially 145 individuals, 10 years of age or older were considered part of the exposed group. The study group was reduced to 113 due to inability to locate or too far away to participate. Ultimately, sixty adults were followed in the study for the entire 6 years; changes in lung function correlated with smoking but not to distance from the exposure site or severity of injury suffered from exposure.

A study which monitored the respiratory effect/function of persons acutely exposed to chlorine gas pursuant to a train derailment for 6 years after the incident suggests no persisting abnormal rate of decline in lung function as a result of the acute exposure. Sixty adults were followed in the study; changes in lung function correlated with smoking but not to distance from the exposure site or severity of injury suffered from exposure.

Mean annual lung function changes during 6 years after exposure

	FVC	FEV1	FEF25-75
Total (n=60)	-0.027	-0.025	-0.030
Current Smoker (25)	-0.034	-0.034*	-0.045*
Ex or never smoked (35)	-0.018	-0.018	-0.020
By triage status			
Admitted (8)	-0.012	-0.013	-0.026
Abnormal (10)	-0.035	-0.032	-0.027
Normal (42)	-0.028	-0.025	-0.031
By distance from spill			
<1.05 miles (12)	-0.014	-0.015	-0.031
>1.05 miles (48)	-0.031	-0.027	-0.030

* 0.01<p<0.05 current vs ex or never smoked

Reliability: (4) not assignable
22-JAN-2004 (115)

Method: Twelve subjects were studied three and seven years following accidental exposure to chlorine gas. Eleven of the twelve had been hospitalized after exposure and represented the most severely effected individuals. The remaining person was the spouse of one of the hospitalized individuals and had had prominent symptoms after the accident following a similar exposure. Pulmonary edema, detected by physical examination and confirmed by chest films, was common in this group immediately following exposure. Follow up chest films showed normal lungs in all instances. Three and seven years following exposure, routine pulmonary function tests were conducted.

Result: All patients were free of respiratory symptoms at the time of the study except one, a 53-year-old man who had a

history of heavy cigarette smoking, symptoms of chronic bronchitis, and vascular changes on his chest film suggestive of ulmonary emphysema. In this subject, symptoms were appropriate for the mild degree of airway obstruction demonstrated, and he was able to carry out full-time employment. Two subjects were markedly obese at the time of the study, and one was in her last trimester of pregnancy. The clinical state of these patients affected pulmonary function results.

Reliability: (4) not assignable
22-JAN-2004 (243)

Method: A single case report following exposure to chlorine gas is reported.

Result: Six years after exposure to chlorine gas effects were still noted. The authors consider this to be a case of Reactive Airways Dysfunction Syndrome (RADS).

Reliability: (4) not assignable
22-JAN-2004 (66)

Method: A longitudinal study (1992-1994) was performed to determine the relation between accidental chlorine exposure and changes in lung function and airway responsivenesss in 239 workers in a metal production plant. These workers had participated in a cross-sectional survey in 1992. In both the initial and the follow-up survey, history of exposure to chlorine ("puffs"), accidental chlorine inhalation reported to the first-aid unit (gassing incidents), and of chronic symptoms were documented; spirometry and methacholine challenge tests were performed. At follow-up, 211 workers (88.2%) were seen.

Result: In workers with 20 pack-years or more of cigarette smoking, the fall in FEV1 was associated with having had a gassing incident during the follow-up period; the fall in FEV1/FVC (%) was predicted by the number of puffs causing mild symptoms between the two assessments. An increase in airway responsiveness (PC(20) decrease > 1.5-fold) was present in 19 workers; it was associated with accidents reported to the first-aid unit during the previous 2 year (OR 5.9, 95% CI:1.1 to 32.3). These findings suggest: (1) an effect on airway function related to the estimated number of puffs with mild symptoms and gassing incidents mostly among smokers; (2) a detectable increase in airway responsiveness associated with gassing incidents.

Reliability: (4) not assignable
31-OCT-2005 (87)

Method: Residents of Washington County as of 1975 with a first time diagnosis of pancreatic cancer from July 1975 through December 1989 were included in a case control study. A total of 101 cases and 206 controls were part of the final study sample. Parameters examined included smoking history, years of residence in present house, previous cancer history, source of drinking water, use of water softeners and other home water treatment.

Result: Chlorinated drinking water was used as a source of drinking water by 79% of cases and 63% of controls. As has been previously demonstrated, this study found a significant

odds ratio increase with advancing age and heavy cigarette smoking. The odds ratio for drinking chlorinated municipal water and developing pancreatic cancer was 2.2.

Table 1
Estimated risk of pancreatic by age, current smoking and chlorination of home drinking water in 1975, Washington County, Maryland, 1975-1989

Characteristic in 1975	Adjusted* Odds Ratio	95% Confidence Interval Adjusted*
Age (years)		
35-39	1.00	
40-49	1.44	0.39-5.30
50-59	4.79	1.39-16.50
60-69	8.71	2.44-31.01
>70	11.06	3.06-39.92
Current cigarette smoker		
Nonsmoker	1.00	
<15/day	0.42	0.13-1.32
15-24/day	1.24	0.55-2.78
>25/day	3.64	1.48-8.91
Drinking water (chlorinated)		
Nonmunicipal	1.00	
Municipal	2.18	1.20-3.95

*Adjusted odds ratios and 95% CI are adjusted for the effects of other characteristics in the table.

Reliability: (4) not assignable
23-JAN-2004 (108)

Method: Five healthy male and five healthy female nonsmokers were used if they met the following criteria: between 18 and 40 years old, with no history of hay fever, asthma, allergic rhinitis, chronic respiratory disease or cardiovascular disease, had not used medication within 1 week of the experiment, did not swim more than once a week in a chlorinated swimming pool and had an FEV1 to FVC ratio >75% of the predicted value. Using a mouthpiece or nasal cannula, the subject inhaled beginning at functional residual capacity while viewing a computer monitor on which the respiratory volume was displayed in real time. The subject controlled his or her breathing so that the respiratory volume signal followed a predrawn pattern corresponding to equal inspiratory and expiratory flows of 250 mls/sec and a tidal volume of 500 ml. At a predetermined time during inhalation, a 10 ml Cl2 bolus was automatically injected into the inspired airflow. Penetration of the bolus into the respiratory system could be systematically varied from breath to breath by changing the bolus injection time relative to the time the subject switched from inhalation to exhalation. The earlier the injection time relative to the end of inhalation, the greater the penetration of the bolus distal to the airway opening.
Three experiments were conducted: oral breathing with a peak inhaled concentration of 3 ppm; nasal breathing with a peak inhaled concentration of 3 ppm and nasal breathing

with a peak inhaled concentration of 0.5 ppm. The volume of each subjects airways was determined using a commercially available acoustic reflection apparatus (Eccovision Acoustic Rhinometry-Pharyngometry System, Hood Laboratories).

Result: From the bolus inhalation measurements, nearly all (>95%) of the chlorine inhaled during quiet breathing is absorbed in the upper airways, whether the nose or the mouth is the site of air access.

Reliability: (4) not assignable

22-JAN-2004 (173)

5.11 Additional Remarks

Type: Toxicokinetics

Method: Three groups of 4 Sprague-Dawley rats were orally administered with different quantities of HO36Cl solution (range of specific radioactivity 1340-2190 dpm/mg 36Cl): the first group of 4 non-fasted rats received 3 ml of 250 mg/l HO36Cl aqueous solution (0.75 mg per animal); the second group of 4 fasted rats received 200 mg/l HO36Cl aqueous solution (0.60 mg per animal). Blood samples were taken from animals of these two groups at different times (0-96hr) and tissue specimen were prepared at sacrifice for 36Cl content assessment. The third group of fasted rats receiving 200 mg/l HO36Cl aqueous solution (0.60 mg per animal) were housed in metabolic cages in order to collect urine, faeces and expired air at different times for 36Cl radioactivity measurement.

Remark: In Vitro/in vivo: In vivo
Type: Toxicokinetics
Species: rat
No. of animals, males: 12
Doses, males: See ME
Vehicle: water
Route of administration: oral unspecified

Result: 36Cl is readily absorbed and found into the bloodstream: a peak of radioactivity in rat plasma occurred 2 hours after HO36Cl administration in group I (fasted rats) (7.9 mg/ml) and 4 hr after administration in group II (non-fasted rats) (10.7 mg/ml). The half-life of 36Cl in group II resulted 2-fold higher (88.5 h) than the one measured in group I (44.1 h), very likely due to the different fasting conditions of animals

36Cl radioactivity was distributed throughout the major tissues, 96 hr after HO36Cl administration. The higher levels were found in plasma (1.92 mg/g), whole blood (1.59 mg/g), bone marrow (1.55 mg/g), testis (1.26 mg/g), skin (1.20 mg/g), kidney (1.13 mg/g) and lung (1.04 mg/g). The lowest levels were found in the liver (0.51 mg/g), carcass (0.40 mg/g), and fat tissue (0.09mg/g).

The distribution of 36Cl in plasma and whole blood studied 24 hr after treatment showed that plasma 36Cl content was 4-fold higher than radioactivity measured in packed cells. In plasma about 20% of total 36Cl was bound to protein, while in red cells a high percentage of was loosely bound to the erythrocyte membrane or exchangeable with chloride in saline. The subcellular distribution of 36Cl in the liver,

showed that the main fraction of the radioactivity recovered in hepatic homogenate was localised in the cytosol, and only 4% was bound to proteins (as measured in the TCA precipitate).
HO36Cl-derived radioactivity was not detected in expired air throughout the 96 hr study. During the same period, 36.43%+5.67 (mean+S.E.) of the administered dose was excreted through the urinary route, while 14.8%+3.7 was recovered in the faeces, giving a poor total recovery of 51.23%+1.97.

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

11-SEP-2003 (2)

Type: other: promotor effect

Method: Groups of 20 female mice were dosed twice/week with 0.2 ml of a 1% NaOCl dissolved in acetone. In the experiment for tumor-promoting activity, the mice received a single topical application of 20 nmol DMBA in 0.2 ml of acetone or acetone alone, followed 1 week later by application of NaOCl, 12-O-tetradecanoyl-phorbol-13-acetate (TPA) as positive control, or acetone alone as vehicle control for 51 weeks. To test the complete carcinogenic activity, NaOCl or acetone only were given topically for 51 weeks. The number and diameter of all skin tumors were recorded weekly.

Result: Sodium hypochlorite was inactive either as a promotor (Table 1) or a complete carcinogen (Table 2).

Table 1
Skin tumor promotion tests in female Sencar mice initiatedwith DMBA:

Chemical	# mice	Week 52 # mice with skin tumors	Maximum # skin tumors/mouse	squamouscell carcinoma(%)
Acetone	15	0	0	0
Sodium hypochlorite	20	3	0.2	1 (5)
TPA	20	20	40.1*	20(100)*

* Significantly different from vehicle control p<0.01.

Table 2
Complete skin carcinogenicity tests in female Sencar mice:

Chemical	# mice	Week 52 # mice with skin tumors	Maximum # skin tumors/mouse	squamous cell carcinoma(%)
Acetone	15	0	0	0
Sodium hypochlorite	20	0	0	0

No values were significantly different from vehicle control p<0.01.

Test condition: Endpoint: other: promoting or complete carcinogenic activity
Species: mouse

Strain: other: sencar
Sex: female
Route of administration: dermal
Vehicle: other: acetone
Frequency of treatment: twice per week
Doses: 0.2 ml of a 1% solution in acetone
Control Group: yes
Method: other
GLP: no data

Conclusion: Twenty female SENCAR mice with dimethyl-benzanthracene as initiator were used. Sodium hypochlorite was applied to the dorsal skin twice per week exposures to a 1-% solution for 51 weeks. No epidermal hyperplasia was observed, therefore sodium hypochlorite was inactive as a promoter.

Reliability: (2) valid with restrictions

Flag: Critical study for SIDS endpoint

23-JAN-2004 (135)

Type: Toxicokinetics

Method: Sprague-Dawley rats were administered HClO at 0, 1, 10 or 100 mg/l daily in drinking water for one year, no significant chloroform concentrations, measured by were observed in rat blood at any time (4, 6, 9, 12 months) during the treatment.

Remark: In Vitro/in vivo: In vivo
Type: Toxicokinetics
Species: rat
No. of animals, males: 16
Doses, males: 0, 1, 10, 100 mg/l
Vehicle: water
Route of administration: drinking water

Result: Indirect indication of rapid absorption through the g.i. tract was given by the occurrence of blood GSH depletion evidenced soon after (15-120 min) the acute treatment of Sprague Dawley male rats with 3 ml aqueous solution containing 10, 20, 40 mg/l HOCl by gavage.

Reliability: (2) valid with restrictions

Flag: Critical study for SIDS endpoint

21-JAN-2004 (1)

Type: Toxicokinetics

Method: The formation of organochlorinated compounds was tested in the stomach content and in the blood samples of four groups of three Sprague-Dawley rats each: fasted/non-fasted control group, fasted / non-fasted dosed group. The dosed groups were administered by gavage with 7 ml of a 8 mg/l solution of sodium hypochlorite at pH 7.9 (about 140 mg/kg bw) and sacrificed after one hour.

Remark: In Vitro/in vivo: In vivo
Type: Toxicokinetics
Species: rat
No. of animals, males: 12

Result: The results were expressed as detectable or not-detectable for the very low levels of reaction products (detection limit range: 0.06-1.3 mg/ml plasma). Qualitatively it resulted that acetic acid was found in all the blood and stomach content samples from all the 4 groups, including

controls.
Trichloroacetic acid, dichloroacetic acid and chloroform were detected only in the stomach content of dosed animals (fasted and not fasted), suggesting its formation independently from the presence of food content in the gut. On the contrary, dichloroacetonitrile detection was limited to gut samples from non-fasted rats. Some plasma samples of dosed animals resulted positive to the presence of trichloroacetic acid.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

21-JAN-2004 (158)

Type: other

Method: Groups of 12 animals were administered 0, 5, 15 or 30 ppm HOCl beginning at 3 weeks of age until termination at 12 weeks of age. Serum IgG antibody response to keyhole limpet hemocyanin (KLH) as measured by an enzyme-linked immunosorbent assay (ELISA); delayed-type hypersensitivity reaction (DTH) to bovine serum albumin (BSA) as measured by a footpad swelling technique; splenic NKC cytotoxicity to tumor cells; oxidative metabolism by macrophages as measured by a chemiluminescence (CL) method; phagocytic activity of macrophages as measured by their ability to ingest 51Cr-labeled opsonized sheep red blood cells (SRBC); PGE2 production by macrophages; and IL2 production by splenic lymphocytes. Antibody synthesis, DTH reactions, NKC cytotoxic responses and production of IL2 and prostaglandin were assessed in 1 group of animals. A separate group of rats were analyzed for oxidative metabolism and phagocytosis responses.
Remark: The author suggests that macrophage function is altered following in vivo exposure to relatively high levels of chlorine-based disinfectants and will ultimately shorten the life-span of animals. In the drinking water studies conducted in rats and mice, the life-span of animals treated to much higher concentrations of sodium hypochlorite was unaffected. Thus the relevance of this information is unknown.
Result: No significant effects on body weights, thymus weights, antibody response, NKC cytotoxicity, IL2 production or phagocytic activity. Rats treated with 30 ppm sodium hypochlorite had lower absolute or relative spleen weights compared to controls. The DTH reactions were also significantly less in this group of rats. The early macrophage CL response was significantly delayed compared to controls in groups of rats treated with 15 or 30 ppm sodium hypochlorite. Macrophage production of PGE2 of rats treated with 15 or 30 ppm sodium hypochlorite was significantly elevated.
Test condition: Species: rat
Strain: Sprague-Dawley Sex:
Route of administration: drinking water
Exposure Period: 63 day(s)
Frequency of treatment: daily ad libitum; weaning to 12 weeks of age
Doses: 5, 15 and 30 ppm HOCl
Control Group: yes
Reliability: (2) valid with restrictions

Flag: Critical study for SIDS endpoint

21-JAN-2004 (79)

Type: other

Remark: A multiple dose study was also carried out in rats given for 8 days orally with 8 and 16 mg/kg bw/day NaOCl, a much lower concentration with respect to the acute study by Mink et al. and more consistent with drinking water intake. No organo-chlorinated compounds were detected in the urine.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

21-JAN-2004 (132)

Type: other

Method: Groups of 30 male mice were administered deionized distilled water (0.1 ppm residual chlorine), tap water (0.9 ppm residual chlorine), hyperchlorinated water (15.0 ppm residual chlorine), hyperchlorinated water (30.0 ppm residual chlorine), chlorinated acidified water (15.0 ppm residual chlorine), hyperacidified water (0.1 ppm residual chlorine) and tetracycline water (0.1 ppm residual chlorine) in the drinking water for 120 days. All solutions, except for the tap water were prepared with deionized, distilled water three times/week. At the end of the 120 day study, delayed-type hypersensitivity using the foot pad test with sheep erythrocytes was measured in 15 mice/group. Serum antibody responses were also measured in 15 mice/group.
Result: There were no significant differences in the foot pad responses between the immunized mice drinking deionized distilled water and any of the other immunized groups. However, nonimmunized controls that received hyperchlorinated (30 ppm) water and subsequently challenged with phosphate buffered saline had a significantly greater increase in foot pad thickness at 24 hours than the nonimmunized controls receiving either tap water or deionized water. This difference in response disappeared by 48 hours. There were no significant differences in the change in foot pad thickness between any groups 48 hours following challenge with either sheep erythrocytes or phosphate buffered saline.
The increase in foot pad thickness in the nonimmunized controls receiving 30 ppm hyperchlorinated water was not reproducible in a repeat test.
No significant differences in antibody titers were seen between any of the treatment groups at either day 5 after intraperitoneal immunization or day 10 following foot pad immunization with sheep erythrocytes.
Mice receiving acidified water had significantly lower reticuloendothelial clearance rates when compared with the deionized distilled water controls. However, when adjusted for spleen and liver weights, significant differences were not observed between any of the treatment groups and controls.
Test condition: Species: mouse
Strain: CD-1
Sex: male
Route of administration: drinking water

Exposure Period: 120 day(s)
Frequency of treatment: daily ad libitum
Doses: 5, 15 and 30 ppm HOCl
Control Group: yes
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

13-JAN-2004 (104)

Type: other

Method: Groups of 5 female mice were treated with 1, 10, 100, 300 or 1000 ppm HOCl (pH 6.5) for four days. Negative controls received distilled water for the same duration and another group received 1000 ppm Na)Cl (pH 8.5) for four days. All whole-body (except head) exposures consisted of a 10-minute contact time per day of treatment.
12-O-Tetradecanoylphorbol-13-acetate (TPA) was applied topically at a dose of 1.0 ug and served as the positive control. In a separate study, a single treatment of HOCl or NaOCl was administered in the same manner and animals were held for up to 12 days before sacrifice to determine the time course associated with the hyperplastic response. Animals in each study were sacrificed 24 hours after final treatment, and a 1-cm2 section of dorsal skin was taken from each mouse for histopathologic evaluation.

Result: The epidermal layer in control animals measured 15.4 micro-m.When four daily treatments of 1, 10, 100, 300 and 1000 ppmHOCl were applied and animals were sacrificed on day 5,results for 1 and 10 ppm were not unlike those of controls(14.4 and 15.8 micro-m), but 100, 300 and 1000 ppm progressivelyincreased skin thickness to 21.9, 30.0 and 38.7 micro-m,respectively. In contrast when animals were exposed for 4days to NaOCl at a concentration of 1000 ppm and sacrificedon day 5, the epidermal thickness was only increased to 25.0micro-m. In the second study, the hyperplastic response measuredthroughout the 12 day recovery period was greatest in theHOCl group (30.8 micro-m, nearly equaling that of TPA (32.8micro-m).The response of HOCl, however, was delayed for 4 dayswhereas maximum response to TPA occurred on the second day.The hyperplasia resulting from HOCl exposure was sustainedconsiderably longer than associated with TPA treatment. Theepidermal thickness following HOCl exposure peakedsignificantly on day 8 at 30.8 micro-m after a sharp increase to26.8 mm on day 4 and was still at 26.1 micro-m on day 10 beforefalling to 19.1 micro-m on day 12. The increase with NaOCl wasless than with HOCl but was significant and was sustainedthroughout the 12-day period, with the highest valuesreached at day 10 (18.2 micro-m) and day 12 (19.8 micro-m),respectively.

Table 1
Skin hyperplasia producted by alternate drinking waterdisinfectants - dose response

Treatment	Dose ppm	Days treatment	Epidermal thickness, micro-m
Control (H2O)		4	15.4+1.5
Chlorine pH 6.5	1	4	14.4+1.7
	100	4	15.8+2.5

	300	4	30.0+13.0	
	1000	4	38.7+7.0	
*Chlorine pH 8.5	1000	4	25.0+6.2*	
TPA	1.0 micro-g	1	32.8+3.4	

* p<0.05. Statistical analyses were performed using Tukey'smultiple comparison test.

Test condition: Endpoint: other: epidermal thickness
Species: mouse
Strain: other: SENCAR
Route of administration: whole body except head
Exposure Period: 10 minute(s)
Frequency of treatment: 1 or 4 days
Doses: 1, 10, 100, 300 or 1000 mg/L for 4 days and 1000 mg/L for one day
Control Group: yes

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

23-JAN-2004 (188)

Method: Antimicrobial activity and tissue toxicity of two sodium hypochlorite solutions buffered to a physiologic pH were studied. Initially, a 0.5% NaOCl solution buffered with 3 g of NaH2PO4 per liter was examined. The solution had a pH of 7.49 and an osmolality of 352 mOsmol/liter. Because of the pH instability and basal cell toxicity, a 0.1% NaOCl solution buffered with NaH2PO4-Na2HPO4 was evaluated. This solution had an osmolality of 386 mOsmol/liter and a pH of 7.4 that was stable over 1 week. The 0.1 and 0.5% sodium hypochlorite solutions were applied to guinea pig skin for 2 weeks. The viability of basal cells was measured. In addition, the antibacterial activity was measured.

Result: When compared with unbuffered and NaHCO3-buffered 0.5% NaOCl solutions, the NaH2PO4-buffered solution was significantly more effective in killing Staphylococcus aureus in vitro. However, the pH of the NaH2PO4-buffered solution decreased over time with a concomitant decrease in antibacterial activity. At this concentration, a 15% decrease in basal cell viability was noted in guinea pigs in a two-week study. A freshly prepared 0.1% NaOCl solution decontaminated skin colonized with S. aureus, C. albicans, and P. aeruginosa within 10, 20, and 30 min, respectively. A 24-h-old solution did not completely decontaminate the colonized skin but significantly reduced the number of microorganisms on the skin surface (P less than 0.001). Application of this solution of guinea pig skin for 2 weeks produced no significant effect on basal cell viabilities.

08-JAN-2004 (56)

Type: other

Remark: In Vitro/in vivo: In vivo
Species: rat

Result: rat stomach fluid chlorinated with aqueous hypochlorite in vitro and in vivo: chloramines were formed.

Reliability: (3) invalid

22-JAN-2004 (203)

Type: other

Method:	Female mice 5-6 weeks of age received tap water (0.5 ppm HOCl) or hyperchlorinated drinking water (25-30 ppm HOCl) for up to 4 weeks. At weekly intervals groups of 5 mice each were injected ip with 2 ml of thioglycollate broth. Individual mouse peritoneal exudate cell (PEC) differential and total counts were conducted 5 days later.
Result:	The number of peritoneal exudate cells in the controls decreased after week 0. In the treated group, the number of PECs increased from 21 million to ~45 million at week 4.
Test condition:	Endpoint: other: RD50 Species: mouse Strain: other: C57BL/6N Sex: female Route of administration: drinking water Exposure Period: 28 day(s) Frequency of treatment: daily ad libitum Doses: 25 - 30 ppm HOCl Control Group: yes

22-JAN-2004 (80)

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