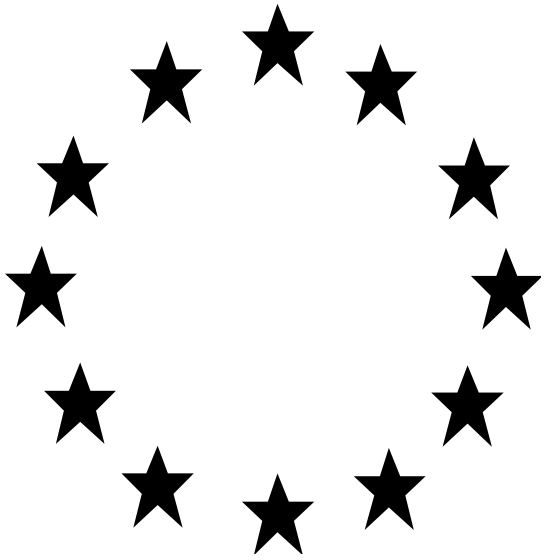

Draft Assessment Report (DAR)

- public version -

Initial risk assessment provided by the rapporteur Member State the Netherlands for the existing active substance

SODIUM HYPOCHLORITE

of the fourth stage of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC

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B.6 TOXICOLOGY AND METABOLISM

The data/studies/evaluations with regard toxicology and metabolism presented below are taken from the RAR (Risk Assessment Report) for sodium hypochlorite (November 2007), which was written by Italy under the Existing Substances Regulation. For this DAR, the references referred to in the RAR have not been individually evaluated by the RMS, neither were the references submitted by the notifier.

Sodium hypochlorite (NaClO) is produced as an aqueous solution containing 10 – 12% w/w available chlorine. In diluted form it is also known as bleach or soda bleach. Household bleaches usually contain about 5% sodium hypochlorite (about pH 11, irritant), and more concentrated bleaches contain 10-15% sodium hypochlorite (about pH 13, corrosive).

Sodium hypochlorite has a long history of use in the home for both bleaching of textiles and cleaning and disinfection of household surfaces. It is increasingly used in a very wide range of formulations for household, institutional or industrial applications. Sodium hypochlorite is used:

- for household and laundry cleaning, sanitation, deodorizing and disinfection
- for municipal water, sewage and swimming pool disinfection
- for medical environment disinfection
- for disinfection purposes in food industry and food manipulation
- for textile industry and pulp and paper bleaching
- for chemical synthesis
- as a multisite fungicide in agriculture and horticulture
- as an oxidant in a very wide range of activities

A sodium hypochlorite solution contains three chemical species, in equilibrium with each other: chlorine (Cl_2), hypochlorous acid (HOCl), and hypochlorite ion (ClO^-). Their concentration depends on the pH of the solution (see B.2.1.18). As a consequence, different concentration units are found in literature to measure the species present in a hypochlorite solution. The pH of commercial solutions is above 11 and the only species effectively present is ClO^-. The studies on the effects of a sodium hypochlorite solution found in literature use different units to define the concentrations tested:

- Available chlorine: it measures the concentration of the three species (in practice, only HOCl and OCl^- can be present, because chlorine is formed only at very low pH values)
- Active chlorine: it measures the concentration of Cl_2 and HOCl (in practice, only HOCl)
- HOCl or OCl^- (mostly used in cases in which, because of the pH value of the tested solution, one of the two species was predominant).
- Sodium hypochlorite (NaClO): the “theoretical” concentration of sodium hypochlorite in the solution, calculated on the basis of the available chlorine concentration.

In this DAR, the units used in the original studies will be reported in the description of the studies. For the purpose of Risk Characterisation, available chlorine unit will be used, since it covers all the different pH situations of the hypochlorite solution. If the unit used in the original studies is different, the use of available chlorine unit will be evaluated on a case by case basis.

Validity of available data as well as their relevance for the risk assessment have been assessed as described below:

Category 1 – Reliable without restriction

For study reports or literature data

- which were carried out or generated according to generally valid and/or internationally accepted testing guidelines (preferably according to GLP) or
- in which the test parameters documented are based on a specific (national) testing guideline (preferably according to GLP) or
- in which all parameters are described closely related/comparable to a guideline method.

General interpretation/conclusion on a TOX chapter (NOAEL, etc.) may be based on such data, if relevant for the risk assessment.

Category 2 – Reliable with restrictions

For study reports or literature data

- in which the test parameters documented do not totally comply with the specific testing guideline, but are sufficient to accept the data or
- in which investigations are described which cannot be subsumed under a testing guideline, but which are nevertheless well documented and scientifically acceptable.

General interpretation/conclusion (NOAEL, etc.) may be based on such data if relevant for the RA in consideration, but with a lower weight compared to category 1.

Category 3 – Not reliable

For study reports or literature data

- in which there are interferences between the measuring system and the test system or
- in which organisms/test system were used which are not relevant in relation to the exposure, (e.g. justification by expert judgement) or
- which were carried out or generated according to a method which is not acceptable, the documentation of which is not sufficient for an assessment and which are not convincing for an expert judgement.

These studies may be helpful for RA but not sufficient in itself to conclusion (so giving qualitative rather than quantitative information).

Category 4 – Not assignable

- Which are only listed in short abstracts or secondary literature (books, reviews, etc.)

These studies are not useful for RA because it is impossible to judge their quality.

Relevance of data for the current use has also to be considered. Data classified in validity category 1 or 2 may be judged as not relevant for the Risk Assessment considered, e.g. the route of exposure in the animal study is not relevant for human exposure.

Results obtained by Biotest Laboratories have been reported as supportive information but they are not used for Risk Characterisation.

B.6.1 ABSORPTION, DISTRIBUTION, EXCRETION AND METABOLISM (TOXICOKINETICS) (ANNEX IIA 5.1)

Sodium hypochlorite dissolved in water exists as a mixture of different chlorine species, whose relative amounts depend mainly on the pH. In the biological systems, characterised by pH values in the range 6-8, the most abundant active chemical species are HOCl and ClO^-, in equilibrium. The latter is predominant at alkaline pH values, while Cl_2 is mainly present at pH below 4. Sodium hypochlorite readily interacts with organic molecules and cellular components, leading to the formation of chlorinated organic compounds possessing their own inherent toxicity (BIBRA, 1990).

Very few data have been produced on ADME for HOCl and are limited to the oral route. No information is available on dermal exposure or inhalation.

Endogenous occurrence

Hypochlorous acid is physiologically present in the human body, being formed by white blood cells (neutrophils and monocytes) as a powerful antimicrobial agent during inflammation processes. When the recognition of “non-self” proteins in an invading microorganism triggers the immune response, the enzyme myeloperoxidase located in mammalian neutrophils catalyses hypochlorous acid formation through the oxidation of chloride ion in combination with hydrogen peroxide (Weiss, 1989; Babior, 1984; IARC, 1991). 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, hypochlorous acid 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 (Weiss, 1989; Bernofsky, 1991) the primary mechanism of action is still not fully elucidated.

Besides being an oxidant itself, HOCl can react with H_2O_2 and superoxide anion to generate other highly reactive oxidizing molecules (singlet oxygen and hydroxyl radical), which very likely contribute to the onset of toxicity. In addition, hypochlorite can react with a number of cellular components, such as

amino-acids, thiolic compounds, nucleotides and lipoproteins, forming organochloride species (Weiss, 1989; Bernofsky, 1991; Fleming, 1991), some of which endowed with their own toxicity. As well as many N-chloramines, derived from reaction with both nucleotides and amino acids, chlorohydrins of unsatured fatty acids (Winterbourn, 1992) and chlorinated sterols (Hazen *et al.*, 1996 a ,b) have been identified as by-products of *in vitro* reactions of the myeloperoxidase/peroxidase/ chloride system.

Based on a mean HOCl production rate of 3.15 x 10^{-8} µM/cell-h for the myeloperoxidase-catalysed reaction and assuming that about 0.1% of total neutrophils are triggered at any one time, Haas (1994) estimated a production ratio of 16 µM/day HOCl from the human immune system. Considering a possible 1-5% yield, the total amount of hypochlorite corresponds to a total generation of organochlorine compounds in the human body in the range of 5.7 to 28 µg/day (equal to 0.16 - 0.8 µM/day).

B.6.1.1 Toxicokinetic studies

Absorption, distribution and excretion

Abdel-Rahman *et al.* (1983) studied the toxicokinetics of hypochlorous acid (HOCl). Three groups of 4 Sprague-Dawley rats were orally administered with different quantities of HO^{36}Cl solution (range of specific radioactivity 1340-2190 dpm/µg ^{36}Cl): the first group of 4 nonfasted rats received 3 ml of 250 mg/L HO^{36}Cl aqueous solution (0.75 mg per animal; ca. 3.0 mg/kg bw, calculated assuming a body weight of 0.25 kg); the second group of 4 fasted rats received 200 mg/L HO^{36}Cl aqueous solution (0.60 mg per animal; ca. 2.4 mg/kg bw, calculated assuming a body weight of 0.25 kg). Blood samples were taken from animals of these two groups at different times (0- 96hr) and tissue specimen were prepared at sacrifice for ^{36}Cl content assessment. The third group of fasted rats receiving 200 mg/L HO^{36}Cl 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 ^{36}Cl radioactivity measurement.

^{36}Cl is readily absorbed and found into the bloodstream: a peak of radioactivity in rat plasma occurred 2 hours after HO^{36}Cl administration in group I (fasted rats) (7.9 µg/ml) and 4 hr after administration in group II (non-fasted rats) (10.7 µg/ml). The half-life of ^{36}Cl 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 (Abdel-Rahman *et al.*, 1983).

Indirect indication of rapid absorption through the GI 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 (Abdel-Rahman and Suh, 1984).

^{36}Cl radioactivity was distributed throughout the major tissues, 96 hr after HO^{36}Cl administration. The higher levels were found in plasma (1.92 µg/g), whole blood (1.59 µg/g), bone marrow (1.55 µg/g), testis (1.26 µg/g), skin (1.20 µg/g), kidney (1.13 µg/g) and lung (1.04 µg/g). The lowest levels were found in the liver (0.51 µg/g), carcass (0.40 µg/g), and fat tissue (0.09µg/g) (Abdel-Rahman *et al.*, 1983).

The distribution of ^{36}Cl in plasma and whole blood studied 24 hr after treatment showed that plasma ^{36}Cl content was 4-fold higher than radioactivity measured in packed cells. In plasma about 20% of total ^{36}Cl was bound to protein, while in red cells a high percentage of ^{36}Cl was loosely bound to the erythrocyte membrane or exchangeable with chloride in saline. The subcellular distribution of ^{36}Cl 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) (Abdel-Rahman *et al.*, 1983).

$HO^{36}Cl$-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 (Abdel-Rahman *et al.*, 1983).

Metabolism

As previously indicated, HOCl 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.

Results from the toxicokinetic study carried out by Abdel-Rahman *et al.* (1983), showed that the 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 any time (4, 6, 9, 12 months) during the treatment (Abdel-Rahman and Suh, 1984).

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: the results were expressed as detectable or not-detectable for the very low levels of reaction products (detection limit range: 0.06-1.3 µg/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 (Mink 1983).

In the same laboratory, a multiple dose study was also carried out dosing rats 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* (1983)

and more consistent with drinking water intake. Following the final dose rats were placed in metabolism cages, and urine was collected in water-cooled vials. No organo-chlorinated compounds were detected in urine extract by means of GC/MS analyses (Kopfler *et al*, 1985). The presence of Cl^- was not assessed.

Human data

No specific studies on humans have been conducted so far. Nevertheless, it is possible to obtain some information from some reported cases of accidental ingestion. Reported effects included some systemic symptoms, such as laboured breathing, decreased blood pressure, increased sodium levels in the blood and acidosis, probably due to the formation of hypochlorous acid and Cl_2 gas at the low pH typical of the gastric environment (Done, 1961; Ward and Routledge, 1988). The systemic effects could suggest the absorption and distribution of NaOCl, although it is not possible to exclude they are secondary to its local irritating and/or corrosive action producing tissue damage.

Intoxications caused by the direct inhalation of hypochlorite vapours have never been reported; it is generally due to misuse of bleaching solutions, when mixed with ammonia or acids, responsible for dramatic pH changes.

B.6.1.2 Summary and conclusions

Animal data suggest that after exposure via oral route, HOCl is absorbed and excreted mainly through urine as chloride (36.43% of the administered dose after 96h); a lesser extent of $HO^{36}Cl$-derived radioactivity not necessarily associated with absorption was detectable in the faeces 96h after exposure (14.8%). Plasma levels peaked after 4 hours. Elimination half-life was 88.5 hours.

Although this will be an underestimation, oral absorption is at least 40% (rounded value) based on urinary excretion only, as the RAR-summary did not quantify the amount recovered from tissues, organs and residual carcass.

Once in the body, HOCL is not enzymatically metabolised and it reacts directly with organic molecules to form some organochlorinated compounds, characterised by their own toxicity.

Human data are very scant and indirect. Absorption is suggested by some transient and not severe systemic symptoms following ingestion, although the possibility they are secondary to a local effect could not be ruled out with certainty.

B.6.2 ACUTE TOXICITY INCLUDING IRRITANCY AND SKIN SENSITISATION (ANNEX IIA 5.2)

B.6.2.1.1 Acute toxicity – animal data

The effect values reported below refer to sodium hypochlorite expressed as available chlorine and are reported in Table 6.2.4.1.

Oral route

Several acute toxicity studies, the majority in rats, have been reported and are summarised in Table 6.2.4.1. The LD_0 value (the lowest dose to produce lethality) for a 3.6 % solution (as available chlorine) was reported to be greater than 10.5 g/kg bw (corresponding to 0.378 g/kg bw as available chlorine, for pure 100% sodium hypochlorite). No deaths and no alteration of the gastric mucosae of the exposed animals were reported (CERB 1985). Similarly, the LD_0 of 3.6% solution of sodium hypochlorite was reported to be > 11.8 g/kg bw (>0.425 g/kg bw as available chlorine, for pure 100% sodium hypochlorite) (AISE, 1997). The LD_{50} of a solution of 5.25 % sodium hypochlorite was reported to be approximately 13.0 g/kg bw, corresponding to 0.682 g/kg bw as available chlorine (for pure 100% sodium hypochlorite) (Chlorine Institute 1982).

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 bw. Gastric lesions were found in all animals exposed and sacrificed after 14 days of observation (CERB, 1985).

An oral LD_{50} of 8.8 g/kg bw 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 occurred in most cases within 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 LD_{50} 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 626 mg/kg bw of pure 100% sodium hypochlorite expressed as available chlorine) (Kaestner, 1981 in BIBRA, 1990).

Osterberg (1977) reported an LD_{50} > 5.0 g/kg bw for commercial bleach containing 4.74% of available chlorine, corresponding to a value > 0.237 g/kg bw available chlorine, for pure 100% sodium hypochlorite.

Using an unspecified commercial solution of sodium hypochlorite an LD_{50} value of 8.91 g/kg bw (6.83-11.68 g/kg bw) was reported for the the Male Albino rat. Signs of intoxication reported were hypoactivity, muscular weakness, hemorragic rhinitis, emaciation and death. No significant findings were observed following examination of both survivors and decedents (Industrial Bio-Test Laboratories Inc., 1970).

In the mouse the LD_{50} was reported as 5.8 ml/kg bw and 6.8 ml/kg bw for females and males respectively for a commercial solution of sodium hypochlorite of 10% as available chlorine diluted 50% v/v with water, leading to 0.36 and 0.42 g/kg bw available chlorine respectively, for pure 100% sodium

hypochlorite. Signs of toxicity consisted of depression of spontaneous activity and irritation of the gastrointestinal tract (Momma, 1986).

An LD_{50} of 0.88 g/kg bw sodium hypochlorite solution in the mouse is also reported in the literature (Klimm, 1989). The concentration of sodium hypochlorite was not reported and the methodology used was not fully explained. Therefore the value was not considered to be relevant for the risk assessment.

Dermal route

An LD_0 value > 10.0 g/kg bw in rabbits was reported for a sodium hypochlorite solution of unspecified concentration. The animals showed no signs of intoxication, however moderate to severe erythema was observed at the site of the application. No adverse effects were found at necropsy at the end of the observation period (Industrial Bio-Test Laboratories Inc., 1970).

Acute dermal toxicity is reported to be > 2.0 g/kg bw for a 5.25% available chlorine solution, corresponding to a value greater than 0.105 g/kg bw available chlorine, for pure 100% sodium hypochlorite (Chlorox unpublished data, in AISE, 1997).

Inhalation route

The LC_0 value by inhalation in rat was found to be greater than 10.5 mg/L for 1 hour exposure, using an unspecified commercial solution. The test was carried out at room temperature with a total air flow of 10 litres per minute. No death occurred and there was no sign of inactivity or lacrimation and no significant gross pathological changes reported (Industrial Bio-Test Laboratories Inc., 1970). This study is considered of limited interest since inhalation exposure of sodium hypochlorite is only possible if aerosols are formed.

Other routes

The only information available is a very old study (Taylor *et al* 1918), where sodium hypochlorite was administrated subcutaneously and intraperitoneally in mice and guinea pigs. The results demonstrated the low toxicity of sodium hypochlorite. However, these routes of administration are not relevant for the direct human exposure and therefore the studies are not considered for the risk assessment.

B.6.2.1.2 Acute toxicity – human data

Some information is available from accidental exposure due to ingestion of commercial products or in patients undergoing hemodialysis where massive hemolysis, hyperkalemia, cyanosis and cardiopulmonary arrest were observed (Hoy, 1981, Dedhia, 1989).

Oral routes

The lethal dose of sodium hypochlorite in humans (adult) has been reported to be about 200 ml of a solution containing 3-6% of available chlorine (Bozza Marrubini *et al*., 1989, reported in Racioppi *et al*., 1994). But the survival of patients who swallowed up to 1 l of a 5.25 % NaClO solution (Strange *et al*., 1951) and about 500 ml of a 10% NaClO solution has also been reported (Ward and Routledge, 1988).

The distinctive smell/taste makes unintentional ingestion of large volumes of hypochlorite bleaches virtually impossible.

Suicides attempts by adults can lead to death after ingestion of at least 250-500 ml of fairly concentrated (12.5%) NaClO solutions (Racioppi *et al*., 1994).

Clorox (US commercial bleach preparation containing 5.25% sodium hypochlorite) is frequently listed among the corrosive substances commonly ingested. However, there have been no specific reports of tissue injury apart from one case of a 49 year old man who attempted suicide by drinking approximately 1000 ml of Clorox. He sustained a severe corrosive injury to the stomach with subsequent scarring that necessitated a total gastrectomy (Strange *et al*., 1951).

129 cases of sodium hypochlorite solution (5.25%) ingestion seen at the Children's Hospital of the District of Columbia have been reviewed and no severe complications have been encountered: 65 of these cases were examined by oesophagoscopy within 96 h after ingestion and only two cases exhibited any evidence of oesophageal injury (Pike *et al*., 1963).

Hook and Lowry (1974, cited in Racioppi *et al*., 1994) reported on 26 children admitted to the Children's Memorial Hospital of Chicago since 1969 with bleach ingestion as the reason for admission. Severe irritation of the oesophageal mucosa was observed in only one case, which evolved positively without symptoms of stricture. Only minor transient irritation effects were observed in some of the other 25 patients.

Yarington (1965) reported on injury from ingestion of Clorox (5.25% sodium hypochlorite) in his experience: in the 31 cases observed, 11 patients showed mouth burns, 17 oesophageal burns and 9 severe oesophageal burns.

Muehlendahl *et al*. (1978) investigated the consequences of household products ingested by children: 90% of the children involved were from 1 to 3 years old. On a total base of 1157 cases, only 23 involved NaClO solutions. One of these 23 cases showed signs of superficial burns in the oesophagus that had disappeared 2 weeks later when examined by oesophagoscopy.

These data are comparable to the previous observations made by French *et al*. (1970): on 160 patients admitted to the Tulane Services (New Orleans) because of the ingestion of household bleach, 5 showed oesophageal burns but only 2 developed oesophageal stricture.

The clinical effects of accidental chlorine bleach ingestion in 80 children, admitted to hospital in Turkey between 1976-1986, have been evaluated. It has been reported that bleaches manufactured in Turkey can cause significant oesophageal injury due to their high content of sodium hydroxide which increases the corrosive effect (Tanyel *et al*., 1988).

Metabolic consequences of bleach ingestion which are less investigated are hypernatraemia and hyperchloraemic acidosis: the first one due to the large sodium hydroxide load contained in household bleaches, the second one due to the reaction, in the stomach, with hydrochloric acid to form hypochlorous acid and chlorine (Ward and Routledge, 1988).

Table 6.2.1.2.1 Human acute cases

REFERENCE	PRODUCT	NOTE
ORAL		
Strange, 1951	1 liter of 5.25% sol.	Suicide attempt – total gastrectomy for severe corrosion
Pike, 1963	5.25% sol.	Children accidental ingestion – 129 cases with 2 cases of oesophageal injury
Hook and Lowry, 1974	nd	Children accidental ingestion – 26 cases: one irritation
Yarington, 1965	5.25% sol.	31 cases observed with different type of burns
Muehlendahl, 1978	nd	Children accidental ingestion – 23 cases: one with superficial oesophagus burn
French, 1970	nd	Accidental ingestion in 160 cases with 2 severe oesophageal effects
Tanyel, 1988	10-200 ml of 5-6% sol.	Children accidental ingestion – 80 cases: three with oesphageal effects
Ward, 1988	1 liter of 5.25% sol.	2 cases: hypermatraemia and hyperchloraemic acidosis due to ingestion
PARENTERAL		
Froner, 1987	0.3 ml of 5.25% sol.	Suicide attempt – no death
Becker, 1974	0.5 ml of 5.25% sol. injected in periapical tissue	Strong inflammation, hematoma – 1 month for normal status
Hermann, 1979	1.8 ml of 5.25% sol. injected in facial nerve	Pain, edema – two weeks to resolve
Hoy, 1981	30 ml of 5.25% sol. intravenous infusion	Cardio-respiratory arrest – resolved after recovery

nd: not determined

Parenteral exposure

Sodium hypochlorite is a standard disinfectant for various applications and an accidental exposure by parenteral route can happen.

Four cases have been reported:

- The first is referred to a suicide attempt by a 27-year-old man, who had ingested 60 ml and injected 0.3 ml of a 5.25% solution of sodium hypochlorite; he experienced no local pain during or following the injection, nor loss of consciousness; on physical examination he was lethargic but awake and he had normal vital signs (Froner *et al*.; 1987).
- The second involved an injection of 0.5 ml sodium hypochlorite solution (5.25%), commonly used as a disinfectant during root canal surgery, accidentally into periapical tissue, resulting in an acute reaction. The patient experienced extreme pain, inflammation of the mouth and face and hematoma formation. The patient's face returned to normal one month after the accident (Becker *et al*., 1974).
- The third involved injection of 1.8 ml of sodium hypochlorite solution (5.25%), which had been stored in an anesthetic bottle, into the mandibular branch of the facial nerve, resulting in pain, edema and trismus that resolved within two weeks (Hermann *et al*.; 1979).
- The final case involved intravenous infusion during hemodialysis of approximately 30 ml of sodium hypochlorite (5.25%), which had been inadvertently added to the dialysate, leading to a cardiorespiratory arrest but with eventual recovery (Hoy *et al*.; 1981).

B.6.2.2 Irritation/corrosivity

B.6.2.2.1 Studies in animals

Skin

A solution of sodium hypochlorite (4.74% available chlorine) in a mixture with other ingredients used as control bleach in a patch study was applied (0.5 ml) under a semiocclusive patch on the dorsal skin of the rabbits for a 24-hour period. The skin was examined for erythema and edema directly after patch removal and 48 hours later. The evaluation of the lesions was carried out according to the FHSA Regulation (1973). A primary irritation index (PII) of 5 or higher indicates a primary irritation response in accordance with FHSA Regulations. The compound was considered to be non irritant to the rabbit skin based on the PII reported as < 5. (Osterberg *et al*., 1977).

Sodium hypochlorite 5.25 % solution (pH 10.7, 0.5 ml) was applied on rabbit and guinea pig abraded and non-abraded skin in a 4-hour patch test as outlined in the revised FHSA procedure that had been proposed by FDA (Edwards, 1972). The skin was examined at 4, 24 and 48 hours after patch removal. Results showed the compound to be slightly irritant to both rabbits (PII = 1.2) and guinea pigs (PII = 0.8) (Nixon *et al*., 1975).

0.5 ml of sodium hypochlorite 12.5 % available chlorine was applied to the intact and abraded rabbit skin for 24 h. The skin was examined for effects up to 72 hours. The scoring of the irritation response was carried out according to the Draize classification. Initial solution (12.5 %) and dilution of ½ (6.25 %) were considered as severe irritant (with PII = 5.6 for both concentrations); a dilution of ¼ (3.12 %) was considered to be moderately irritant (PII = 4.0) and a dilution of 1/8 (1.56 %) as slightly irritant (PII = 1.9) (Duprat *et al*., 1974). It is pointed out that Duprat used a much longer exposure time (24 hours) compared to the standard classification patch test, which recommends patching for 4 hours. This can also explain the higher scores in comparison to the Nixon study, which did use the standard 4 hours exposure time.

0.5 ml of sodium hypochlorite 12.7 % active chlorine was applied to intact and abraded rabbit skin for 24 h. The skin was examined for effects up to 72 h. Scores from intact skin and abraded skin were added and a mean was calculated. Hypochlorite at 12.7 % was considered to be moderately irritant (PII = 4.04) (Colgate-Palmolive, unpublished data-1985).

In a dermal irritancy/corrosion test on 20 compounds in aqueous solution, sodium hypochlorite solution 8-12% available chlorine applied to the dorsal skin of the rabbit, was tested at different concentrations (2, 20, 35, 50% w/v, i.e. 0.24, 2.4, 4.2 and 6 % available chlorine): The study showed slight irritation effects at the lowest concentration, moderate irritation at the other concentrations and corrosive effects at the highest concentration tested probably according to the Draize scale) (Loden *et al*., 1985). Based on poor reporting, the study protocol followed could not be verified, although the Draize scale appears to have been used.

The primary skin irritation score in the rabbit was found to be 5.08 using 0.5 ml of undiluted liquid. This score was an average of mean scores on intact and abraded skin. Contact time is not specified. The compound was considered as “corrosive” (Industrial Bio-Test Laboratories Inc., 1970). However, this study is considered of very limited value to assess the irritation properties of hypochlorite, as neither the test substance concentration in this study nor the exact protocol used are described.

The studies from Loden and Biotest had been given a validity 4 based on too little study details reported.

Eye

An eye irritation test was carried out in rabbits by Momma *et al*., using the Draize method (1986). The ocular irritation score was calculated from examinations up to 21 days post exposure of 5% solution. The eyes were either left unrinsed or rinsed with water after the application of sodium hypochlorite. Scoring at 24, 48 and 72 hours revealed slight – moderate eye irritation potential in both groups. In the group where rinsing was applied, eyes had returned to normal by day 14. More persistent and more pronounced injury to the cornea and the conjunctiva was observed in the group without washing. Day 21 was the end of

observation time in this study at which some effects were still noted. The study indicates that rinsing of the eyes either 4 or 30 seconds after instillation significantly reduced the degree of ocular irritation.

Osterberg also performed an eye irritation test using an unofficial ocular irritation classification method (with similarities to the FHSA and Draize methodology). A dose of 0.1 ml of an otherwise unspecified mixture containing hypochlorite at a conc. of 4.74 % available chlorine, was placed into the rabbit eyes and the score for alteration was followed up to 7 days post exposure. The compound was found to be severely irritant to the rabbit eye according to the specific grading scale used. Detailed scores observed however are not reported in the study. Recovery was not complete at day 7 (Osterberg *et al*., 1977). It has to be noted that the mixture was specified as laundry bleach by the author and contained other ingredients, which would have contributed to irritancy.

The standard Draize method was applied for the evaluation of eye irritation in the rabbit. In addition to the Draize mean average scores, a non-standard microscopic evaluation was used in the grading. Commercial sodium hypochlorite (12.5% available chlorine) and a ½ dilution (6.87%) were considered severe irritants with a Draize MAS score of 60 and 49, respectively. Complete recovery was observed in week 10 for the 12.5% solution and in week 4 for the 6.25% solution. ¼ Dilution (3.6%) of the solution was found to be moderately irritant with a Draize MAS of 11 and complete recovery at day 15 while a 1/8 dilution (1.85%) was found to be slightly irritant (Draize MAS 1) with complete recovery at day 4. Rinsing with 20 ml physiological saline was done at 10 sec, 1 min and 5 min after application of the test substance. Washing was also done using higher volumes of physiological saline (300 ml or 600 ml). The immediate rinse (at 10 sec with 20 ml physiological saline) was the most effective in reducing the irritant effect significantly (Duprat *et al*., 1974).

According to the Draize method, a quantity of 0.1 ml of sodium hypochlorite 12.7 % active chlorine was applied in rabbit eyes. At day 7, hypochlorite 12.7% was considered to be severely irritant (MAS of 64.75). Most effects had not cleared by the end of the observation period, day 14 (Colgate-Palmolive, unpublished data-1985).

Undiluted solutions at 5.25% or 8% were found to be low to moderate eye irritants in rabbits. Specifically, an 8% concentrated solution resulted in moderate irritant effects in a Draize test, with recovery being completed within 7 days. Low irritant effects were observed when the 8% solution was tested in an LVET study (Low Volume Eye Test, applied dose = 0.01 ml), with recovery being completed in 3 days. 5.25% sodium hypochlorite was tested according to the LVET protocol only and led to low irritant effects. Exact scores are not reported in the study. The authors emphasize that the LVET provides a better correlation with human eye irritancy experience than the Draize test. Dilutions of 1:10 of the described bleaches (0.55% and 0.8 % sodium hypochlorite) also had only a low eye irritant potential in an LVET study. It was observed that a rinse with water following the eye contact reduced the degree of irritation (Racioppi *et al*., 1994).

The instillation of 0.1 ml of undiluted sodium hypochlorite (unspecified concentration) into the rabbit eye gives a MAS score for irritation of 61.3 according to the Draize methodology. The compound was considered as “severe irritant“ (Industrial Bio-Test Laboratories Inc., 1970). However, this study is considered of very limited value to assess the irritation properties of hypochlorite, as the test substance concentration in this study was not described.

Griffith *et al*. (1980), studied hypochlorite amongst other chemicals using the Draize scale for grading of the effects. Different dose volumes were applied (0.01, 0.03 and 0.1 ml) with the purpose of comparing the observed effects to data from human experience (literature, occupational incidents and consumer accidental exposures). 0.01 ml was considered the volume leading to effects most consistent with human eye reactions. Therefore, application of 0.01 ml (as used in the LVET protocol) was proposed to be a much more realistic test of eye hazard than the Draize test. A 5 % solution of sodium hypochlorite (pH 11.1-11.6) produced only mild transient effects in a Draize test (MAS of 11 after 1 day) when rinsed out with water in the first 30 seconds. When the LVET protocol was used (0.01 ml), similar mild and transient effects were noted. Effects had cleared completely by day 7. If 0.1 ml was applied and the eyes were not rinsed, moderate irritation effects involving the cornea and the conjunctiva occurred (MAS of 31 after 1 day). Recovery was not entirely complete at the end of the observation period (day 21). At the intermediate dosing volume of 0.03 ml applied, also moderate effects were observed (MAS of 28 at day 1) which had cleared completely by day 18 (Griffith *et al*., 1980).

A 15% solution of sodium hypochlorite caused severe pain and damage and there were indications that healing was not complete 2-3 weeks after exposure (Grant, 1962).

Carter and Griffith (1965) summarized effects that Buehler and Newsman (1964) observed when comparing the eye irritation potential of a sodium hypochlorite aqueous solution 5.5 % in rabbits and monkeys. The eyes were not rinsed. The Draize methodology appears to have been used. No detailed scores apart from the recovery times are reported. The irritant response was much greater in rabbits (recovery between day 7 and day 35) than in monkeys (recovery at day 2). Data were also compared to recovery dates from human exposure in factory eye accidents. The author noted that the irritation response observed in the monkey seemed to be a better indicator of eye irritation following accidental exposure in workers than the response observed in the rabbit.

Pashley *et al*. (1985) reported rabbit eye experiments following a Draize-related methodology. Detailed scores are not reported. Sodium hypochlorite, applied to rabbit eyes at concentration of 5.25 % NaClO produced moderate to severe conjunctival palpebral edema and hyperemia within 30 minutes of exposure with the maximum severity observed at 2 h. The eyes exposed to 5.25 % revealed corneal pitting but no ulceration. Some conjunctival edema was observed, which had not fully cleared up at day 7 (and of observation period). With a 0.52 % NaClO solution, only moderate effects were observed and the

reaction was gone within 24 hours. The author pointed out that "the rabbit eye model tends to exaggerate the toxicity of agents since rabbits blink their eyes at a much lower frequency than humans" (Pashley, 1985).

Other Data (in-vitro)

Solutions of sodium hypochlorite were also tested *in vitro* using different cell systems. The principal aim of the studies was to compare the results obtained *in vitro* with the *in vivo* Draize score methods. The results obtained *in vitro* basically confirmed the irritation properties on the sodium hypochlorite solution obtained with the standard methodology (Chan, 1985; Borenfreund and Borrero, 1984, Borenfreund and Shopsis 1985; Shopsis and Sate, 1984). However, the use of *in vitro* methods for eye irritancy assessment of hypochlorite containing products at this point in time is questionable, as current *in vitro* methods are known not to realistically predict the irritant properties of oxidizing substances like hypochlorite.

Respiratory Tract

The respiratory (sensory) irritation potential of sodium hypochlorite has been assessed in the mouse and has been compared to that of chlorine. An aerosol of sodium hypochlorite was generated from a 10% v/v solution in distilled water using a glass concentric jet atomiser with cyclone. Three groups of mice (gender and age not defined) were exposed to sodium hypochlorite aerosols at atmospheric concentrations of 9.2, 5.7 or 2.6 ppm, expressed as chlorine. The particulate concentrations of each atmosphere were 24, 11 and 9 mg/m^3, respectively, and the mass median aerodynamic diameter of the three aerosol atmospheres ranged from 2.3 to 4.3 µm. The RD50 value, the exposure concentration causing a 50% reduction in the respiratory rate due to respiratory irritation, for sodium hypochlorite aerosol was estimated to be 4.11 ppm, expressed as chlorine. A similar test was conducted concurrently with chlorine for which an RD50 value of 5.7 ppm was estimated.

It was concluded that the similarity of the results showed that the degree of respiratory irritation seen after exposure to an aerosol of sodium hypochlorite is most likely related to its content of chlorine (Lewis, 1990).

B.6.2.2.2 Human data

Skin

The human skin irritation potential of hypochlorite bleaches has been investigated under occluded patch test conditions and /or prolonged contact times.

Nixon *et al*. (1975) reported that a hypochlorite solution at 5-5.25% available chlorine (pH 10.7) was found to be severely irritating to intact human skin after 4 h exposure under occluded patch conditions. In this study a clear evidence of irritating effects above 5% is identified.

Weak to moderate irritation was observed in 15 of 69 dermatitis patients patch tested (48 h, patch conditions not specified, reported as “covered contact”) with 2% NaClO. No irritation was observed in 20 persons from the same group after additional patch testing (48 h “covered contact”) with 1% NaClO (Habets *et al*., 1986).

A recent survey of accidental exposure to hypochlorite containing products during the years 2000 and 2001 is available from the Spanish Poison Control Center. Only 1.3% of all contacts on bleach products (total of 2924 contacts) are about skin contacts. Of these, 52.6% of persons remain asymptomatic and 47.4% (18 cases) of all skin contacts lead to some skin effects. Slight skin irritation or local burns are the prevailing symptoms (Instituto Nacional de Toxicologia, 2002).

Eye

In two cases where Clorox (containing 5.25% sodium hypochlorite) was accidentally splashed into the eyes, a burning sensation and slight damage to the cornea was reported: prompt rinsing of the eyes with water led to complete recovery within 48 h (Grant *et al*., 1974 cited in BIBRA, 1990).

With 5% NaClO solutions only very few human eye injuries have been reported. Apparently it causes burning discomfort, but only superficial disturbance of the corneal epithelium, which recovers completely in one or two days.

Poison Control Centres record relatively few cases of ocular exposure to bleach (in Italy e.g. this represents about 4% of the total number of bleach contacts), and return to normality is rapid (Racioppi *et al*., 1994).

The above mentioned survey of accidental exposures to hypochlorite containing products in 2000 and 2001 form the Spanish PCC cites 18% of all contacts on bleach products (total of 2924 contacts) being about eye contacts. 41.5% of all eye contacts lead to some symptoms (218 cases). Most of them report eye mucous membrane irritation and all were reversible within two to maximum three days (Instituto Nacional de Toxicologia, report Nr. 07168/02).

In conclusion, under typical use conditions, accidental spillage of hypochlorite bleach into the eyes is expected to cause slight, temporary discomfort, which subsides within a short period of time or after rinsing with water (BIBRA, 1990).

Other data

Usually sodium hypochlorite at low concentrations comes in contact with the eye in swimming pools where it is the most widely employed disinfectant. If the pH is kept at approximately 7-9, levels of up to 1.5 ppm of available chlorine in water are usually not irritating to the eyes. In susceptible individuals, slight irritant effects may occur. However, this effect is always transient. At lower pH there may be

smarting and redness of the conjunctiva, but no significant injury occurs. It is thought that products formed by the reaction of chlorine with nitrogenous compounds (such as urea and ammonia) and by the formation of chlorinated and non-chlorinated aldehydes in the water are responsible for the eye irritation; maintaining the pH above 7 reduces the formation of these products.

Erdiger *et al*. (1998) examined the mucous membrane irritating potential for compounds which can occur as disinfection-by-products in swimming pool water. The study reports that halogenated carboxyl compounds, which act as precursor during the formation of chloroform may be responsible for eye irritation. These compounds were found to have a significantly increased irritating effect when compared to a chlorine/chloramines mixture of the same concentration, and significantly enhanced effects when combined with aqueous chlorine. The result of this study suggests that the mucous membrane irritating potential is a consequence of the effects and synergistic action of a number of disinfection-by-products in the presence of chlorine.
A study by Héry *et al*. (1994) describes that swimming pool instructors exposed to the same agents reported irritation phenomena (acute ocular and upper respiratory irritation) at chloramines values of around 0.5 mg/m^3 in the atmosphere.

Massin *et al*. (1998) measured trichloramine levels in the atmosphere of indoor swimming pools and examined their relationship to irritant and chronic respiratory symptoms. They concluded that lifeguards working in indoor swimming pools can develop irritant eye, nose and throat symtomps.

Various authors have suggested that a number of changes and symptoms may be associated with exposure to the atmosphere in swimming pools, in particulare with nitrogen trichoride (Carbonelle *et al*., 2002; Thickett *et al*., 2002; Bernard *et al*, 2003), althought the studies were unable to confirm the specific chemicals that were cause of the symptoms experienced. Symptoms are likely to be particularly pronounced in those suffering from asthma (WHO, 2006).

Hecht *et al*. (1998) carried out a study on worker exposure to levels of chloramines when washing vegetables in 6 industrial facilities using chlorinated water. In all cases but one the levels in the atmosphere are below the comfort level advised by French INRS of 0.5 mg/m^3.

Another study was published by the same authors the same year (Hery *et al*.,1998), that reported cases of acute eye and upper respiratory irritation in one industrial facility processing green salads in water containing hypochlorite. These effects were related to chloramines resulting from reaction of hypochlorite and nitrogen compounds coming from the sap proteins released when the vegetables were cut. The exposure of workers determined by personal sampling ranged from 0.2 to 5 mg/m^3. The authors conclude that increased level of chloramines occur when the industrial facility uses recycled water.

B.6.2.3 Sensitisation

B.6.2.3.1 Studies in animals

A sample containing 8.0% of sodium hypochlorite was tested for delayed contact hypersensitivity in guinea pigs. The test material was administered undiluted at induction and as a 3.2% solution in distilled water at challenge. The test was preceded by an irritation test carried out at concentration up to 100% v/v (= 8% NaClO) in distilled water. Occluded application of the undiluted test material during the irritation screen resulted in increased incidence and severity of irritation (erythema) observed. Applications of test material at 40% (= 3.2% NaClO) were well tolerated. There were no erythematous responses to challenge with 40% v/v (= 3.2% NaOCl) in either the test group or the control group, hence the test material did not show any potential to cause delayed contact hypersensitivity (P&G unpublished data, 1982).

Mixtures of sodium hypochlorite and different surfactants were tested for skin sensitisation potential in two guinea pig sensitisation studies. A 50:50 (v/v) mixture of a sodium hypochlorite solution and surfactant was tested in one study and a mixture (50:50 v/v) of sodium hypochlorite and another surfactant was tested in the other study. The concentration of sodium hypochlorite in the test substance was in both cases 4.5 %. During induction, 4.5%, 3.35%, 2.25%, and 1.1% hypochlorite were patched. Only the two higher concentrations resulted in slight patchy erythema. During challenge, 2.25% hypochlorite was used. The tests were carried out according to the guinea pig sensitisation test modified by Ritz H.L. and Buehler E.V. There were no differences in skin alteration between test and control groups upon challenge, therefore both studies did not show any potential to cause delayed contact hypersensitivity for hypochlorite (P&G unpublished data, 1985).

B.6.2.3.2 Human data including case reports

Sensitisation tests conducted on human volunteers (H.R.I.P.T.: human repeated insult patch test) with hypochlorite bleach formulations have shown no evidence of potential allergic contact dermatitis.

A first test had been performed on 86 volunteers. The solutions (containing 0.034% solution hypochlorite) caused an acceptable level of irritation during both preliminary and main tests. There was no evidence of sensitisation observed on eighty-six subjects upon challenge.
The second test involved 90 volunteers. The material (sodium hypochlorite solution at a concentration of 0.076%) caused an acceptable level of irritation through both the preliminary irritation screen and main test. At challenge, one subject gave some evidence of skin sensitisation. This subject also reacted to another product patched in an adjacent site and a rechallenge on the same above mentioned other test article would be necessary to confirm the nature of this reaction. There was no evidence of skin sensitisation on the other eighty-nine subjects and the one positive reaction could

not be clearly attributed to the test substance hypochlorite (unpublished data from Procter & Gamble 1987 and 1989).

Habets *et al*. (1986) reported that two housewives with diagnosed dermatitis showed a positive reaction to sodium hypochlorite patched in different dilutions. In both patients, additional reactions to other standard allergens were found together with a positive reaction to sodium hypochlorite solutions of 2%, 1%, 0.5%, 0.1%. The specific test allergens showed a positive reaction. In a control study, 69 control patients (randomly selected with suspect allergic contact dermatitis) were patched with NaClO 2% in water; 15 of them showed a weak or moderate irritant reaction; 20 of the control were further tested with concentrations of 1% and 0.5%, but no reaction indicating an allergic response was seen.

Inclusion of sodium hypochlorite at about 0.5% in water in a series of routine patch tests involving 225 patients in total showed three positive reactions (at gradings 48h and 96h after patch removal). From these 3 out of 225 patients who tested positively, one positive response was directly attributed to sodium hypochlorite by the author (Osmundsen *et al*., 1978).

A case of occupational allergic contact dermatitis to NaClO has been seen in a veterinary surgeon, who during his work has occasionally washed his hands and forearms with undiluted Halasol (containing 4-6% sodium hypochlorite and Betadine as antiseptic). As the patch test results suggested sodium hypochlorite allergy, the same concentration of Halasol and sodium hypochlorite have been tested on three normal healthy controls: all were negative except the undiluted Halasol closed patch test. (Eun *et al*., 1984). It should be noted that the short test report does not allow a conclusion whether the reactions of the 3 control subjects were of an irritant or contact dermatitis type.

Isolated cases of hypochlorite sensitivity of the delayed type (allergic contact dermatitis), as well as immediate-type reactions from inhalation or topical challenge of sensitised individuals have been observed according to Hostynek *et al.* (1990). Only one case where an immunologic component to the patient's reaction could not be ruled out is reported. The author also concludes that such rare hypersensitivity is usually preceded by either long-term or exaggerated skin exposure to hypochlorite (Hostynek *et al*., 1989).

A very short report is available on the case of a patient who had a hystory of allergic contact dermatitis from flavine. The patient had developed symptoms upon wound treatment with a hypochlorite-containing wound disinfectant. In a patch test, he reacted positive to flavine, potassium iodide as well as the hypochlorite containing disinfectant. Crossreactivity cannot be excluded. 20 volunteers also patched with the disinfectant did not show any reaction (Ng and Goh, 1989).

In a group of 40 housewives selected for suspected allergic hand dermatitis, sensitisation by patch test was established. Thirty-eight housewives gave a negative response to sodium hypochlorite, although

several had apparently used bleaching agents for longer periods. For two of them a sodium hypochlorite sensitisation was concluded but concomitantly with a nickel allergy in the first case and with Kathon allergy in the second case (van Joost *et al*., 1987). Therefore the two cases cannot be clearly interpreted as positive sensitisation reaction to hypochlorite.

Sodium hypochlorite does not induce contact sensitisation when tested according to standard skin sensitisation test protocols; however, there have been rare reports of alleged allergic contact sensitisation. Literature reports place NaClO in the category of non-sensitising chemicals that can, on rare occasions, be implicated in sensitisation reactions. It is important to underline that in the past hypochlorite solutions were associated with skin sensitisation owing to the presence of chromium salts added to the product as staining agent. However the voluntary decision taken by industry to remove chromium from the hypochlorite solutions more than 15 years ago was a major contribution to the solution of the problem (Raccioppi *et al*., 1994).

In conclusion, given the widespread use of sodium hypochlorite, the likelihood of allergic contact sensitisation due to NaClO in practice is negligible.

B.6.2.4 Summary

The results of the acute toxicity, irritation and sensitisation studies are presented in table 6.2.4.1, 6.2.4.2 and 6.2.4.3, respectively.

Table 6.2.4.1 Acute toxicity, LD_0/LD_{50} values for sodium hypochlorite as available Cl_2 (oral)

% available chlorine	Species	LD_0 g/kg bw (of the solution)	LD_{50} g/kg bw (of the solution)	LD_0 g/kg bw (as av. Cl_2)	LD_{50} g/kg bw (as av. Cl_2)	Reference	VAL*
10	mouse				0.36 (f) 0.42 (m)	Momma, 1986	
3.6	rat	>10.5 **		>0.378		CERB, 1985	1
12.5	rat	>5.8 **		>0.725		CERB, 1985	1
12.5	rat	5.01	8.83	0.626	1.1	Kaestner, 1981	2
3.6	rat	>11.8 **		>0.425		AISE, 1997	3
5.25	rat		Ca. 13		0.682	Chlorine Inst., 1982	3
4.74	rat		>5		>0.237	Osterberg, 1977	3

* VAL: validity of the study

** no death in the study

Table 6.2.4.2 Skin and eye irritation studies

Test substance	Test / Result	Species	Route	Reference	VAL*
Sol. 5-5.25% FAC **	FHSA method (equal to Draize) (semioccl. for 4 h on abraded and non-abraded skin Slightly irritant	guinea pig and rabbit	skin	Nixon, 1975	2
Sol. 12.5% and 6.25% FAC	Irritation test (abraded and non-abraded skin for 24 h) Severely irritant at 6.25%. Moderate – slight effects at lower concentrations	rabbit	skin	Duprat, 1974	2
Sol. 12.7% FAC	Moderatly irritant	rabbit	skin	Colgate-Palmolive, 1985	2
Sol. 4.74% FAC	FHSA method (equal to Draize) (semioccl. for 24 h) Not irritant (mixture containing other ingredients)	rabbit	skin	Osterberg, 1977	3
Sol. 5.25% FAC	irritant	rabbit	eye	Carter, 1965	2
Sol. 5.25% FAC	Irritant	monkey	eye	Carter, 1965	2
Sol. 4.76% FAC	Moderatly irritant	rabbit	eye	Momma, 1986	2
Sol. 5.25% FAC	Irritant	rabbit	eye	Pashley, 1985	2
Sol. 12.5% FAC Sol. 1.6% FAC	Severely irritant Slightly irritant	rabbit	eye	Duprat, 1974	2
Sol. 12.7% FAC	Severely irritant	rabbit	eye	Colgate-Palmolive, 1985	2
Sol. 4.74% FAC	Severely irritant, effects observed in mixture containing other ingredients	rabbit	eye	Osterberg, 1977	3
Sol. 4.76% FAC	Mild – moderatly irritant	rabbit	eye	Griffith, 1980	3
Sol. 0.52% FAC Sol. 5.25% FAC Sol. 8% FAC	Low irritant potential Low irritant potential Moderatly irritant	rabbit	eye	Racioppi, 1994	3
Sol. 15% FAC	Severely irritant	rabbit	eye	Grant, 1962	3

* VAL: validity of the study

** FAC: free available chlorine

Table 6.2.4.3 Sensitisation studies and case reports with sodium hypochlorite

Test	Result	Notes	Reference	VAL*
Sensitisation – animal data				
guinea pig test	Negative	Sodium hypochlorite	P&G, 1982	1
guinea pig test	Negative	Sodium hypochlorite + surfactant A	P&G, 1985	1
guinea pig test	Negative	Sodium hypochlorite+ surfactant B	P&G, 1985	1
Sensitisation – human data				
Hum. Patch test	Negative	Well conducted study – 86 subjects	P&G, 1987	2
Hum. Patch test	Negative	Well conducted study – 90 subjects	P&G, 1989	2
Sensitisation – human case reports from dermatologist practices				

Test	Result	Notes	Reference	VAL*
Sensitisation – animal data				
Diagnostic Hum. Patch test	1/225 alleged positive	225 control (CD) patients, 3 of which showed a reaction to hypchlorite, but only 1 was accepted as true reaction to hypochlorite	Osmundsen, 1978	4
Diagnostic Hum. Patch test	Positive	Case report	Habets, 1986	4
including a larger control group	Negative	No sensitisation potential identified in a control group of 69 contact dermatitis patients		
Diagnostic Hum. Patch test	Positive	Case report (1 patient, hypochlorite accepted as cause of contact dermatitis)	Eun, 1984	4
including a larger control group	Negative	No reactions to hypchlorite were seen in 3 control patients		
Diagnostic Hum. Patch test	Equivocal	Case report (1 patient). Reaction could be due to crossreactivity with iodide or flavine	Ng, 1989	4
Small negative control group	Negative	Negative patch test result in 20 volunteers		
Diagnostic Patch test	Equivocal	Case report (1 patient, no controls)	Hostynek, 1989	4
Diagnostic Hum. Patch test	Positive	Case report (2 patients) (doubtful positive, as 1 patient suffered from nickel allergy an the other from kathon allergy	Van Joost, 1987	4

* VAL: validity of the study

Acute toxicity

The acute toxicity of marketed hypochlorite solutions by the oral route is low. The LD_{50} values for solutions containing active chlorine concentrations up to 12.5% are greater than 5.8 g/kg bw. The data for dermal acute toxicity as well as inhalation toxicity indicate a low level of toxicity for these routes of administration.

Accidental human data are reported for ingestion and parenteral route: it can be concluded that the effects of accidental ingestion of domestic sodium hypochlorite bleaches are not expected to lead to severe or permanent damage of the gastro intestinal tract as recovery is rapid and without any permanent health consequences. This is also expected for small quantities of solutions accidentally injected in blood system or in the tissues.

Although the overall lowest LD_{50} available is from the Momma study (see table 6.2.4.1), it is preferred to use the value of the Kaestner study as the key reference study. The Osterberg study is poorly reported and the LD_{50} value is not exactly defined but indicated just as higher than 5000 mg/kg. The Momma study (see table 6.2.4.1) is poorly reported as well. Therefore the Kaestner data is considered of higher

reliability, and it is based on the rat, the standard animal, contrary to the Momma study which used mice. The LD_{50} and LD_0 values (of, theoretically, 100% sodium hypochlorite) were calculated as follows:

LD_{50}= 12.5% (% of available Cl_2 in sol.) x 8.83 (g/kg bw LD_{50} of the sol. to female rat) = 1.1 g/kg bw (LD_{50} as available Cl_2) = 1100 mg/kg bw NaClO as av. Cl_2

LD_0= 12.5% (% of available Cl_2 in sol.) x 5.01 (g/kg LD_{50} of the sol. to female rat) = 0.626 g/kg bw (LD_{50} as available Cl_2) = 626 mg/kg bw NaClO as av. Cl_2

Because the acute toxicity of corrosive substances is more related to concentration than to dose, extrapolation from data obtained from using a hypochlorite solution to a fictive 100% sodium hypochlorite is not relevant. As the highest concentrations of hypochlorite solutions as industrially produced and marketed are about 15%, and solutions marketed for consumer use are typically 5% or less, it can be concluded from the data presented that hypochlorite solutions are of low acute oral toxicity. This is confirmed by the available data from human accidents, where the few deaths that have occurred after hypochlorite ingestion are mostly attributable to aspiration pneumonia.
The information available shows that sodium hypochlorite has a very low dermal acute toxicity.
The only study on inhalation acute toxicity available did not show an effect on rat. This confirms that inhalation is not a route of exposure for sodium hypochlorite, except in case of aerosol formation.
Therefore, sodium hypochlorite does not need to be classified for acute oral, dermal, and inhalation toxicology.

Irritation/corrosivity

Concerning skin irritation, some irritant responses have been recorded at around 5% hypochlorite under exaggerated conditions. Nixon *et al*. (1975) observed only slightly irritating effects at 5 - 5.25% available chlorine solutions in a four-hour patch test where abraded skin results were also included in the evaluation. However given the fact that in the Osterberg study, no significant irritation is observed at 4.74 % concentration where the contact time was 24 hours, the findings in the Nixon study should not raise any concern. In addition, data on human skin do not contradict this conclusion as they clearly indicate an irritating effect at 5% and above. Altough in older studies some effects were seen at lower concentrations, these would not be considered irritant according to current classification criteria.

Concerning eye irritation, some irritant responses have been recorded below 5% available chlorine on rabbits. However, the study results cannot be compared on a common basis, because different protocol variations were used and the original data on which the scores were determined are not retrievable from the study reports. All data obtained using the LVET method or another method (e.g. monkey) that resulted in similar effects as seen from human experience, showed reversibility of effects within short time periods. The available data from human exposure (Poison Control Centers) support Pashley's

observation (1985), according to which irritant effects in the human eye are less severe than in rabbits. Rinsing with water shows a reduction in the irritant effects both in animals and humans.

As regards the studies by Héry *et al*. and Erdiger *et al*., these clearly report that the observed eye effects can be linked to the reaction products (found in swimming pools, e.g. chlorinated carboxyl compounds) and not to hypochlorite, therefore they are not considered in the hypochlorite assessment.

With respect to corrosivity, animal data show that a 12.7% hypochlorite solution produced severely irritant or corrosive effects to the eye. However, the same concentration applied to rabbit skin does not show corrosive effects there, as only moderate irritation was observed. For these concentrated products, data from accidents are also available (mostly from the French market). No serious or long-term consequences were associated with accidental skin and eye contact.

Sodium hypochlorite aerosols may be irritant to the respiratory tract.

Despite some difficulties in interpreting the older animal data, the overall evaluation of both animal and human data supports the current EU classification as irritant above 5% and as corrosive above 10%.

Sensitisation

Three separate animal tests carried out according to standard sensitisation test protocols indicate that there is no potential for skin sensitisation from hypochlorite in animals. Also standard sensitisation patch tests in healthy human volunteers do not indicate a potential for hypochlorite to induce contact sensitisation.

Reports from dermatological case studies indicate that there have been a few isolated cases of allergic contact sensitisation. However, these isolated cases are poorly reported and not fully conclusive. Events like the case studies reported are very scarce in view of the extensive use of hypochlorite in the marketplace. It is important to underline that in the past hypochlorite solutions were associated with skin sensitisation owing to the presence of chromium salts added to the product as staining agent. However the voluntary decision taken by industry to remove chromium from the hypochlorite solutions more than 15 years ago was a major contribution to the solution of the problem. Based on the systematic animal and human study data as well as on the scarcity of alleged sensitisation cases reported from the market it is concluded that sodium hypochlorite does not pose a skin sensitisation hazard.

B.6.3 SHORT-TERM TOXICITY (ANNEX IIA 5.3)

The toxicity of sodium hypochlorite has been studied in a number of species following repeated or continuous administration (by the oral route by adding it to drinking water or to milk) and by the dermal route. There are no studies available in which exposure has been by inhalation.

For the evaluation of repeated dose drinking water toxicity studies, the quantity of available chlorine as total concentration of hypochlorous acid and hypochlorite ions is described.

B.6.3.1 28-day oral studies

Groups of 5 male weanling Wistar rats were given sodium hypochlorite administered in their milk ad-libitum at concentrations of 0, 40, 200 and 1000 mg/L as av. Cl_2 for 9 days (only whole cow's milk, mixed daily with sodium hypochlorite, was available – no water). In addition, groups of 10 female Wistar rats were given either 0, 8, 40 or 200 mg available chlorine/kg bw/day by gavage, for 14 days. Finally, groups of 10 male rats were given either 0, 20, 40 or 80 mg/L available chlorine in their drinking water ad-libitum for 6 weeks. Observations were confined to the measurement of body weight and to the weights of the liver, kidneys, heart and brain. Weanling rats given sodium hypochlorite showed a non-statistically significant increase in body weight gain. There were no effects on organ-to-body weight ratios. Female rats receiving 8.0 mg/kg/bw sodium hypochlorite (as av. Cl_2) by gavage showed an increase in body weight gain when compared to controls. Those receiving 200 mg available chlorine/kg bw/day showed an increase in kidney weight compared to controls. No other effects were noted in treated female rats. Male rats receiving sodium hypochlorite in their drinking water at all dose levels showed an increase in body weight gain. No effects on organ weights were reported. The NOEL for sodium hypochlorite in the study was 1000 mg/L for weanling rats (9 days), 40 mg available chlorine/kg bw/day for females (14 days) and 80 mg/L available chlorine in drinking water for males (6 weeks), corresponding to a NOEL for sodium hypochlorite of 15.7 mg/kg/day as av. Cl_2 (Cunningham, 1980).

Four groups of 10 male albino rats were given 0, 30, 2500 or 7500 mg/L available chlorine for 28 days by mixing a sodium hypochlorite solution in corn oil with the normal laboratory diet (equivalent to 2.7, 221 and 683 mg available chlorine/kg bw/day). No deaths occurred during the study and no significant gross lesions were noted among treated rats when compared to controls. No differences in liver, kidney or testes weight were observed between the four groups. Adrenal weights in rats given 7500 mg/L available chlorine were statistically significantly increased (P>0.01) when compared to controls. The NOEL for sodium hypochlorite in this study was 2500 mg/L available chlorine (Industrial Bio-testlaboratories, 1970).

Groups of 20 male guinea pigs were given sodium hypochlorite dissolved in their drinking water at concentrations of either 0 or 50 mg available chlorine/l for 5 weeks. No adverse effects were observed (Cunningham, 1980).

B.6.3.2 (Sub-acute) dermal studies

The effect of sodium hypochlorite on epidermal hyperplasia was studied in female SENCAR mice exposed cutaneously to sodium hypochlorite at concentrations of 1000 mg/L for 10 minutes/day for four days. Whole body exposure, except the head, was performed using specially designed Plexiglass

chambers to prevent inhalation of any vapours or aerosols. Concentrations of 1000 mg/L sodium hypochlorite caused increases in epidermal thickness when exposed for four days. In a similar preliminary study using 1000 mg/L HClO with 10 minutes exposure for 8 consecutive days, the maximal response was observed after four daily 10 minute exposures. No effects were observed following treatment with concentrations of 1, 10 or 100 mg/L hypochlorous acid for four days.
Significantly increased numbers of both total and basal epithelial cells in the skin were observed following four daily 10-minute exposures to 300 mg/L hypochlorous acid and above (Robinson, 1986). The results of this study suggest that the threshold concentration for the local skin irritant effects of sodium hypochlorite (as hypochlorous acid) is 300 mg/L, and that the effects were seen following repeated exposure to 0.1% sodium hypochlorite solution. Such local effects are dependant on the concentration of the applied irritant and not on the total dose. The study only reported the effects of a single concentration of sodium hypochlorite solution, there were inconsistencies on the reported effects of different concentrations of hypochlorous acid, suggesting that the findings of the study may be unreliable. It is noted that the non-treated control mice showed an unexplained reduction in skin thickness that will also have impacted on the outcome of the study.

The effects of the repeated administration of sodium hypochlorite solutions on the skin has been studied in the guinea-pig. 0.1% and 0.5% solutions of sodium hypochlorite (950 and 4765 mg/L as available chlorine - prepared daily by diluting a proprietary household bleach (Clorox), which was a 5.25% solution of sodium hypochlorite) were applied to female guinea pig skin for up to 14 days, using gauze bandages soaked at 8 hour intervals with the solutions. The pH of the freshly prepared solutions were 7.4 and 9.65 for the 0.1% and 0.5% solutions respectively. Toxicity was assessed in terms of basal epidermal cell viability (trypan blue exclusion), the growth of single-cell cultures of epidermal cells to confluence *in vitro*, and by histopathological examination. Tissue taken from areas of treated skin was compared to control adjacent tissue taken from the same animals. Basal epidermal cell viability was reduced from 85% to 65% in skin treated with the 0.5% solution for 14 days, but
not for 1, 4 or 7 days and was not affected by treatment with the 0.1% solution. Similarly, the growth of epidermal cells *in vitro* was affected only after treatment for 14 days with the 0.5% solution. Marked epidermal hyperplasia with an influx of inflammatory cells into the papillary dermis was observed in guinea-pigs treated with the 0.1% solution for 14 days, but not 1, 4 or 7 days and with the 0.5% solution for either 7 or 14 days, but not 1 or 4 days (Cotter *et al*., 1985). These results suggest that
the LOAEC (expressed as a concentration) for the local irritant effects following repeated administration of this proprietary bleach solution for 14 days is 0.1%. It is noted that the test solutions were dilutions of proprietary bleach, which may have contained components in addition to sodium hypochlorite and that no adequate control was used (eg. a solution of equal osmolarity and/or pH).

A 0.1 ml solution of 0.125% (1190 mg/L av. chlorine) sodium hypochlorite was applied daily to guinea pig skin of the dorsal site of the ear for 1, 2, 4 and 8 weeks using 10 animals per group. No effect on epidermal proliferation, development and differentiation was observed. In the same study, sodium

hypochlorite solution was blended with milk to assess the effects due to the formation of chloramines: no adverse effects were reported (Wohlab and Wozniak, 1982).

B.6.3.3 Inhalation studies

No repeat dose inhalation studies are available on sodium hypochlorite aerosol, either in animals or humans. It might be possible to use data that is available on chlorine gas as a surrogate, although it is recognised that this data will be derived following exposure to the gas and not to an aerosol. This difference may affect both the qualitative and quantitative aspects of the findings, although it is anticipated that the use of data on chlorine gas is likely to be a conservative assessment of the potential effects of sodium hypochlorite aerosol. The relevant data on chlorine is as follows:

Animal studies

Groups of 4 male and 4 female rhesus monkeys were exposed to either 0, 0.1, 0.5 or 2.3 ppm (0, 0.3, 1.5 or 6.8 mg/m^3) chlorine gas for 6 h/d, 5 d/week for 12 months. Eye irritation, mild focal hyperplasia and cilia loss in the nasal passages and trachea occurred in those exposed to 2.3 ppm. Only very mild hyperplasia of the nasal epithelium, a lesion also seen in some of the control animals, was observed in those exposed to lower concentrations. 0.5 ppm was considered to be a NOAEL in this study (Klonne *et al*, 1987).

An independent examination of these lesions confirmed that the responses were less severe in the monkey than in rodents and that the pathological effects in the rhesus monkey extended further into the respiratory tract than in the rodent species, in which the pathology was confined to the nasal passages. It was concluded that the rhesus monkey was a more appropriate model for the inhalation of chlorine gas in humans than rodents (Ibanes *et al*, 1996).

Human volunteer studies

There are three relevant studies reported in which human volunteers have been exposed to chlorine. A group of 8 volunteers were exposed on a single occasion to either 0.5 or 1.0 ppm (1.5 or 3.0 mg/m^3) chlorine gas for either 4 or 8 hours. Sensory irritation and a transient impairment in lung function were seen in those exposed to 1.0 ppm chlorine gas. No effects were reported in those exposed to 0.5 ppm. (Rotman *et al*, 1983).

A group of 8 male volunteers were exposed to either 0, 0.1, 0.3 or 0.5 ppm (0, 0.3. 0.9 or 1.5 mg/m^3) chlorine gas for 6 h/d on 3 consecutive days. Each individual was exposed to each of the four exposure scenarios in a double-blind fashion. A range of respiratory function parameters was measured and, in addition, nasal lavage fluid was analysed for a number of indicators of inflammatory cell damage. No significant effects were seen in parameters measured. 0.5 ppm was a NOAEL in the study (Schins *et al*, 2000).

The studies in rhesus monkeys are consistent with those in human volunteers and confirm that 0.5 ppm is a NOAEL for the effects of chlorine gas on the respiratory tract. This level of exposure could thus be used as an indicator of the NOAEL for sodium hypochlorite aerosol.

B.6.3.4 Semichronic oral studies

Groups of male and female F344 rats were given sodium hypochlorite dissolved in their drinking water at concentrations of 0, 0.05, 0.1, 0.2 or 0.4% for 92 days (corresponding to 0, 475, 950, 1900, 3800 mg/L available chlorine). Toxicity was assessed in terms of effects on body weight, organ weight, serum biochemistry and pathology. Both male and female rats given 3800 mg/L sodium hypochlorite showed a significant decrease in body weight gain (47% and 31% reduction in males and females respectively compared to controls). Significant reductions in the absolute weights of certain organs in both males and females given 0.4% sodium hypochlorite were reported. However, the organ weight to body weight ratios were not reported and so the true significance of these findings could not be established. No remarkable pathological changes were observed among the treated rats. In this study the daily intakes of NaClO in µl/day as av. Cl_2 are reported. The NOEL for sodium hypochlorite in the study was 0.2% for both males and females (44.4 and 97.1 mg/kg bw/d available chlorine, respectively considering the drinking water intakes reported in the study) (Furukawa *et al*., 1980).

Groups of 10 male and 10 female F344 rats were given sodium hypochlorite dissolved in their drinking water (distilled water) at concentrations of 0, 0.025, 0.05, 0.1, 0.2 or 0.4% (from a solution of 14% available chlorine, purity not specified) for 13 weeks to determine maximum tolerated doses for further long-term carcinogenicity studies. These concentrations correspond to 120, 240, 480, 950, 1900 and 3800 mg/L available chlorine respectively. Toxicity was assessed in terms of daily clinical observations, mortality, and changes in body and organ weights, haematology and blood biochemistry and histopathology of all major organs. The maximum tolerated dose of sodium hypochlorite given in the drinking water to F-344 rats was estimated to be between 0.1 and 0.2% for males and 0.2 and 0.4% for females No mortality occurred in any group. A dose-related decrease in body weight gain was observed in both sexes with a marked effect in both male and female rats given 0.4% sodium hypochlorite. Differences were statistically significant only for male rats given 0.2% and 0.4% sodium hypochlorite and female rats given 0.4% sodium hypochlorite. No histological changes attributable to treatment were reported. Biochemical examination of the blood sera was reported to show signs of slight liver damage in both male and female rats given 0.2% and 0.4% sodium hypochlorite (details not given). Absolute weights of the lung, liver and spleen of males and the salivary gland, lung, heart and brain of females were significantly lower in those given 0.4% sodium hypochlorite than in the controls. The NOEL for sodium hypochlorite in the study was 0.1% for both males and females (47.5 and 54 mg/kg bw/d available chlorine, respectively) (Hasegawa, 1986).

B.6.3.5 Summary

The results of the subacute and semichronic toxicity studies are summarised in Tables 6.3.5.1 and 6.3.5.2, respectively.

Table 6.3.5.1 Subacute studies

Duration	Species	Route	NOAEL (as av. Cl_2)	NOAEL (as av. Cl_2 in mg/kg bw/ day)	Note / Critical effects	Reference	Val*
9 days	Weanling rats	Oral, in milk	≥ 1000 mg/L	≥ 200 **	Non standard test. No adverse effects observed	Cunningham, 1980	3
14 days	Rat, female	Oral, gavage	40 mg/kg bw/day	40	Non standard test. Increased kidney weights.	Cunningham, 1980	3
28 days	Rat, male	Oral, in diet	2500 ppm	221	Limited data.	Ind. Bio-test, 1970	3
5 weeks	Guinea pig	Oral, in drinking water	≥ 50 mg/L		Non standard test. No adverse effects observed	Cunningham, 1980	3
6 weeks	Rat, male	Oral, in drinking water	≥ 80 mg/L	≥ 15.7 ***	Non standard test. No adverse effects observed	Cunningham, 1980	3
4 days	Mice, female	Dermal	LOAEL 950 mg/L		Tested substance: 0.1% sodium hypochlorite. Whole body 10 min/d, 8 d. Increased skin thickness	Robinson, 1986	3
14 days	Guinea pig	Dermal	950 mg/L		Tested substance: 0.1% and 0.5% sodium hypochlorite. Non standard test; continuous, 14 d.	Cotter, 1985	3
8 weeks	Guinea pig	Dermal	≥ 1190 mg/L		Tested substance: 0.125% sodium hypochlorite. 0.1 ml, daily, 8 wk; only dose tested.	Wohlab, 1982	3

* Validity of the study

** NOEL in mg/kg/day derived using the assumption that weanling rats weigh 50 g and consume 10 ml milk/day (conversion factor of 5)

*** NOEL in mg/kg bw/day taken from original paper

Table 6.3.5.2 Semichronic studies

Duration	Species	Route	NOAEL (as av. Cl_2 in mg/L)	NOAEL (as av. Cl_2 in mg/kg/day)	Note / Critical effects	Reference	Val*
13 weeks	Rat	Oral, drinking water	950 (m) 950 (f)	47.5 (m) 54 (f)	MTD study. Decreased body weight and specific organ weight, associated with some biochemical changes.	Hasegawa, 1986	2
92 days	Rat	Oral, drinking water	1900	44.4 (m) 97.1 (f)	Decreased body weight gain.	Furukawa, 1980	2

* Validity of the study

The values expressed as av. Cl_2 in mg/kg bw/day, reported in the tables above, are calculated using values of body weight and water consumption for rats and mice reported in the document NO_NL/01/99_Rev.1 (table below) and based on Gold *et al*., 1984 and Wilschut *et al*., 1998. The use of the values reported below is considered to be a conservative approach.

Experimental animal	Standard lifespan (years)	Sex	Body weight (kg)	Water per day (ml)
Mouse	2	Male	0.03	5
	2	Female	0.025	5
Rat	2	Male	0.5	25
	2	Female	0.35	20
Hamster	2	Male	0.125	15
	2	Female	0.110	15

Oral

No standard 28-day or 90-day repeated dose toxicity studies on sodium hypochlorite in animals by the oral route have been reported. However, the data available from non-standard studies are sufficient to derive a NOAEL for sodium hypochlorite by this route of exposure. The dermal exposure studies reflect the reversible irritant effects of sodium hypochlorite at the doses tested.

No systemic effects have been observed in any of the studies reported. A general decrease of body weight or body weight gain was usually observed following treatment with the highest doses used, most probably due to a secondary effect linked to low water consumption.

In male and female rats treated with 0.2% and 0.4% of sodium hypochlorite in drinking water for 13 weeks, a decrease in body weight and in specific organ weights, associated with some biochemical changes, were reported. A NOAEL of 0.1% of sodium hypochlorite (950 mg/L available chlorine or 47.5 mg/kg bw/d) can be derived from this study (Hasegawa, 1986).

Dermal

Three experimental studies are available to evaluate the dermal toxicity of sodium hypochlorite following repeated exposure, in which damage of viable basal skin cells has been evaluated. Daily exposure of mice to 0.1% sodium hypochlorite solution for 10 minutes on four consecutive days caused an increase in epidermal thickness (Robinson, 1986).

No effects were observed in the dermal studies except for specific skin toxicity in guinea pigs (marginally lowered *in vitro* basal cell viability) of uncertain toxicological relevance at 0.5% sodium hypochlorite solution which is related to the acute irritant effects of the substance (Cotter, 1985). In the same study, epidermal hyperplasia was observed following 14 days exposure (8 hours/day) to 0.1% sodium hypochlorite solution, but not following one, four or seven days of exposure. Exposure to 0.5% sodium hypochlorite solution for 8 hours/day for seven or fourteen days caused significant epidermal hyperplasia, but not after 1 or 4 days.
No effects were observed after exposure of guinea pigs to 0.125% sodium hypochlorite solution for up to 8 weeks (Wohlab, 1982).

The NOAEL for repeated dermal exposure to sodium hypochlorite solution is related to its cytotoxicity/ irritating properties and is dependant on the concentration of the applied solution. Therefore, irritation can be seen as a threshold for dermal toxicity. No dermal toxicity will occur at concentrations of sodium hypochlorite solution that do not cause irritation, either after single or repeated exposure.

A study in mice suggests that ten minutes exposure to 0.1% sodium hypochlorite solution for four days causes an increase in epidermal thickness. However, inconsistencies in the reporting of the study suggest that the finding might be unreliable.

Taking all of these comments into account, it is concluded from the available animal data (which is unreliable and might not adequately reflect human experience) that a very conservative NOAEL for repeated effects following dermal exposure to sodium hypochlorite solution is 0.1%.

There is no information on systemic toxicity following dermal application route. Fully dissociated in water, and immediate oxidising organic molecules, sodium hypochlorite is not expected to pass the skin to become systemic available. The amount of chlorinated substances passing the skin depends on the amount of mobile organochlorine substances being formed on and in the skin.

B.6.4 GENOTOXICITY (ANNEX IIA 5.4)

B.6.4.1 *In vitro*

Bacterial systems

Sodium hypochlorite has been examined for its potential mutagenicity in a number of *in vitro* studies using bacterial systems. In many cases the methodology is not fully valuable with only limited data presented.

Sodium hypochlorite was tested in a standard assay on mutagenicity in *Salmonella typhimurium* strain TA 1530, TA 1535 and TA 1538 at concentrations ranging from $1.4\ 10^{-1}$ to $1.4\ 10^{-4}$ µmole/plate without metabolic activation. Only a weak, not dose-related, effect was reported in TA 1530 strain. The high toxicity displayed by the agent was claimed for the negative result and a modified procedure was applied in the attempt to overcome this problem. Bacteria were treated in liquid medium and before plating, sodium hypochlorite was decomposed by addition of ascorbic acid. Data presented only in graphic form indicated a clear effect in TA 1530 strain but not in TA1538 (Wlodkowski and Rosenkranz, 1975). These results are consistent with a presumable oxidative activity of the agent.

In a Japanese study sodium hypochlorite was mutagenic in TA 100 strain in the presence of S9 mix but negative in TA 98 strain. No further information on doses and test procedures were provided (Kawachi *et al*., 1980).

In a study conducted under a project operated by Japanese Institution on chemical additives, sodium hypochlorite was tested in *Salmonella typhimurium* strain TA 92, TA 94, TA 98, TA 100, TA 1535 and TA 1537 with or without metabolic activation (S9 mix from the rat liver treated with polychlorinated biphenyls). In the presence of S9 mix, a 3.4 fold increase over the control was observed in TA 100 strain at 5 mg/plate (Ishidate *et al*., 1981) (Ishidate *et al*., 1984).

A negative result, based on the limited data presented, was reported for sodium hypochlorite in the Ames test using two *S. typhimurium* tester strains TA97 and TA102, with and without S9-mix, at concentrations of 0.01, 0.05, 0.1, 0.5 and 1 µl/plate, (Fujita and Sasaki, 1987).

A negative result in *S. typhimurium* by fluctuation test, a modified Ames test, was reported for sodium hypochlorite in strains TA 100, TA 98 and TA102 tested without metabolic activation at concentrations ranging from 0.1 to 100 µg/ml (Le Curieux *et al*., 1993). In the same study a negative result for sodium hypochlorite was reported in the SOS chromotest in *E. coli* (Le Curieux *et al*., 1993).

It has been reported that the activity of some known mutagens in the Ames test was reduced in the presence of sodium hypochlorite (Tsuda *et al*., 1983).

In a non-standard assay, sodium hypochlorite inhibited the growth of a DNA repair deficient strain of *Escherichia coli*, indicating DNA damage (Rosenkranz, 1973). Little methodology or data is provided and the presence or absence of metabolic activation is not specified. The significance of the positive response reported is questionable and is deemed to be of limited value. In two further studies, negative responses were obtained in the *Bacillus subtilis* rec assay with and without metabolic activation (Kawachi *et al*., 1980), and in the SOS chromotest (Klimm *et al*., 1989).
However, in both these studies, only limited data has been reported and the protocols are insufficient to enable full assessment.

Mammalian cells
Data on cytogenetic effects of sodium hypochlorite in mammalian cells are reported in a series of studies from Japanese groups.

Chromosome aberrations were analyzed in Chinese hamster cells treated for 24 or 48 hours with three different doses, in the absence of metabolic activation. A positive increase of chromosome aberrations (including gaps) was observed only in culture treated with 0.5 µg/ml for 48 hours. All the other experimental points reported were negative (Kawachi *et al*., 1980; Ishidate *et al*., 1981 and 1984).

Chinese hamster cells were treated for three hours with 0.5 µg/ml (6.7 mM) of the agent in the presence of metabolic activation with S9 mix from liver of PCB treated Wistar rats. A slight increase of chromosome aberration (including gaps) was observed (Matsuoka *et al*., 1979).
Because gaps were included in the evaluations and no other data are provided the results of both studies cannot be interpreted satisfactorily.

In human cells, a non-standard embryo fibroblast line (HE2144) was used for the analysis of chromosome aberrations and SCE. In these cells no increase of chromosome aberrations was reported at both 0.0744 (10^{-3} M) and 0.1488 µg/ml (2* 10^{-3} M). 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 by the highest (0.1488 µg/ml) and the lowest tested doses (0.0744 µg/ml), respectively (Sasaki *et al*. 1980).

No mammalian cell gene mutation studies have been conducted.

B.6.4.2 ***In vivo***

In a series of assays, sodium hypochlorite has been tested for its ability to induce genetic damage *in vivo*. Chlorine gas bubbled in water (leading to hypochlorite and hypochlorous acid solution) has been evaluated for induction of 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. In the mouse micronucleus assay, a small but statistically significant increase in the percentage of micronucleated polychromatic erythrocytes were observed in the combined male and female data but not separately. However the micronucleus frequency in the hypochlorite treated animals was in the range of other control groups present in the same study. The statistic 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 (Meier *et al*., 1985). No statistically or biologically significant increase in the frequency of either structural or numerical chromosome aberrations was observed in the mouse chromosomal aberration assay (Meier *et al*., 1985).
The methodology used in these studies is well described, however no rationale for dose level selection is reported and it cannot be ascertained whether a maximum tolerated dose (MTD) has been achieved because no information on bone marrow toxicity were reported.

In a 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 of sodium hypochlorite (6.6% Cl active) at dose levels from 312.5 to 2500 mg/kg. A reduction of polychromatic erythrocytes was observed at 1250 mg/kg and all animals died at 2500 mg/kg. 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 chromosome aberrations in rat bone marrow has been reported by Kawachi *et al*., 1980. No other information was provided.

In a non-standard assay, rats given 900 mg/kg orally showed no evidence of DNA damage, detected as 8-hydroxyguanosine adducts, in the kidney (Kasai *et al*., 1987).

Meier *et al*., 1985 have tested chlorine, as hypochlorite and hypochlorous acid, in a sperm head abnormality test in B6C3F1 mice. Sperm head abnormality tests are also indicative for germ-line genotoxicity (although of limited validity, if any) (see B.6.6.1)

B.6.4.3 Summary

The results from the *in vitro* and *in vivo* genotoxicity studies are summarized in table 6.4.3.1 and 6.4.3.2, respectively.

Table 6.4.3.1 *In vitro* genotoxicity studies

Test	Result	Notes	Reference	VAL*
Genetic toxicity *in vitro*: bacteria				
Ames / *S.typh*.	Positive TA1530 Negative TA1535 and TA1538	Positive only with modification of standard procedure	Wlodkowski and Rosenkranz, 1975	3
Ames / *S.typh*.	Positive TA100 (+S9) Negative TA98	Limited data presented	Kawachi, 1980	2
Ames / *S.typh*.	Positive TA100 (+S9) Negative TA92, TA94, TA98, TA1535 and TA1537	Limited data presented	Ishidate, 1981/1984	2
Ames / *S.typh*.	Negative TA97 and TA102 (±S9)	Limited data presented	Fujita, 1987	2
Ames / *S.typh*. SOS Chromotest	Negative TA100, TA98 and TA102 (-S9) Negative	Not standard assay	Le Curieux, 1993	2
Ames / *S.typh*.	Activity of some known mutagens was reduced in presence of sodium hypochlorite	Supplemental information	Tsuda, 1983	2
Pol. A / *E. coli*	Positive	Supplemental information	Rosenkranz, 1973	3
Rec A / *B. subt*.	Negative (±S9)	Supplemental information	Kawachi, 1980	3
SOS Chromotest	Negative	Supplemental information	Klimm, 1989	3
Genetic toxicity *in vitro*: mammalian cells				
CA / CHL cells	Positive (-S9)	Limited data presented	Ishidate, 1984	2
CA / CHL cells	Positive (+S9)	Limited data presented	Matsouka, 1979	2
CA / HEF cells	Negative	Limited data presented	Sasaki, 1980	2
SCE / HEF cells	Positive	Limited data presented	Sasaki, 1980	2
No mammalian cell gene mutation studys have been conducted				

* Val: validity of the study

Table 6.4.3.2 *In vivo* genotoxicity studies

Test	Result	Notes	Reference	VAL*
MN / Mouse BM	Negative	Data on bone marrow toxicity is missing	Meier, 1985	2
CA / Mouse BM	Negative	Data on bone marrow toxicity is missing	Meier, 1985	2
MN / Mouse BM	Negative	Data well presented	Hayashi, 1988	1
CA / Rat BM	Negative	No data presented	Kawachi, 1980	3
DNA adduct / rat kidney	Negative	Supplemental information	Kasai, 1987	2

* Val: validity of the study

Sodium hypochlorite has 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.
The positive results produced in bacteria assays and the induction of chromosome aberrations (including gaps) and SCE in mammalian cells suggest, even if mammalian cell gene mutation studies are lacking, that sodium hypochlorite may exert an *in vitro* genotoxic activity.

Sodium hypochlorite was without effect in a well-conducted mouse micronucleus assay suggesting that sodium hypochlorite is not genotoxic *in vivo*.

The available data are not conclusive with respect to genotoxicity. However, since sodium hypochlorite has shown lack of carcinogenicity effects (see B.6.5), no additional testing is required.

B.6.5 LONG-TERM TOXICITY AND CARCINOGENICITY (ANNEX IIA 5.5)

B.6.5.1 Chronic toxicity and carcinogenicity

Oral administration

Groups of 50 male and 50 female F344 rats (7 weeks old) were given sodium hypochlorite dissolved in their drinking water (distilled water) at concentrations of 0, 500 or 1000 ppm (0, 0.05 or 0.1% / 0, 29.3 or 58.7 mg/kg bw/day as available chlorine) for males and 0, 1000 or 2000 ppm (0, 0.1 or 0.2% / 0, 67.0, 133.9 mg/kg bw/day as available chlorine) for females, prepared from a commercial product (14% available chlorine, purity not specified) for 104 weeks. The control groups were given distilled water. All surviving rats were sacrificed at week 112. At week 112, the incidences of surviving rats were: control 30/50, low dose 26/50, high dose 31/50 for males and control 31/50, low dose 36/50, high dose 35/50 for females. A dose-related decrease in body weight gain was seen after 16 weeks treatment in all treated groups. This effect was accompanied by decreases in absolute organ weight that, in some cases, were reflected in decreases in relative organ weight (*eg* salivary glands in both groups of treated females; heart in males given 58.7 mg/kg bw/day NaClO expressed as available chlorine). The magnitude of the changes in both body and organ weights were small. 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. No treatment-related non-neoplastic lesions were reported.
All three treated groups demonstrated relatively high incidences of tumours of the testis, pituitary, thyroid, lung, pancreas, uterus, mammary gland, spleen and subcutaneous tissue. Histologically, chromophobic adenomas of the pituitary, adenomas and adenocarcinomas of c-cells of the thyroid, adenomas of the lung, insulomas of the pancreas, fibroadenomas of the mammary gland and mononuclear cell leukemias were identified in both males and females. Also, a high incidence of interstitial cell tumours of the testis and fibromas in subcutaneous tissues in males and endometrial polyps of the uterus in females were observed. However, the occurrence of tumours at any site was not significantly greater in rats receiving

sodium hypochlorite than in the controls. The proportions of low-dose and high-dose female rats with fibroadenomas of the mammary gland were significantly lower than that of controls. Similarly, the proportion of high-dose male rats with nodular hyperplasia of the liver was decreased.
The NOAEL for sodium hypochlorite in the study was 0.1% (1000 ppm) corresponding to 58.7 mg/kg bw/day as available chlorine for males and 67.0 mg/kg bw/day as av. Cl_2 for females (Hasegawa *et al.*, 1986; Kurokawa *et al.*, 1986).

Groups of 70 male and 70 female F344 rats were given sodium hypochlorite dissolved in their drinking water at concentrations corresponding to 0 mg/L av. Cl_2, 70 mg/L av. Cl_2 (3.5 mg/kg bw/d for male rats), 140 mg/L av. Cl_2 (7 mg/kg bw/d for male rats) and 275 mg/L av. Cl_2 (13.75 mg/kg bw/d for male rats) for two years. Groups of 10 rats of each sex were pre-selected for evaluation at weeks 14 or 15 and 66.
Survival at 2 years of rats receiving chlorinated water was similar to that of the controls.
Mean body weights for males and females were similar among treated and control groups at both the 14-week and 66-week interim evaluations. After 2 years of exposure, the mean body weights of dosed male rats and high-dose female rats were slightly lower than those of their respective control groups.
A dose-related decrease in water consumption was observed throughout the study in the treated groups from both sexes. Water consumption by high-dose rats during the second year of these studies was 21% lower than that of control males and 23% lower than that of control females. Food consumption was comparable among treated and control groups. There were no clinical findings attributable to the treatment at 14-week and 66-week interim evaluations. There were no alterations in haematological parameters and no biologically significant differences in relative organ weights between treated and control groups at the interim evaluations.
The incidence of mononuclear cell leukemia in mid-dose, but not high-dose, female rats was significantly higher than that in controls (control, 8/50; low-dose, 7/50; mid-dose 19/50; high-dose 16/50). The proportion of female rats that died of leukemia before the end of the study and the mean time for observation of animals dying with leukemia were similar among all treated groups and controls. Although the marginal increase in leukemia incidence in the mid- and highdose female rats suggested a possible association with the administration of chlorinated water, the incidence of leukemia was not clearly dose related. There was no indication of reduced latency of leukemia and the incidence of leukemia in concurrent controls was less than the mean for historical controls. Furthermore, there was no supporting evidence of an effect in male rats. Thus the marginal increase in leukemia incidence in female rats was considered equivocal evidence of carcinogenic activity. There were no neoplasms or non-neoplastic lesions in male rats that were clearly associated with the consumption of chlorinated water.
The NOAEL for sodium hypochlorite in the study was 275 mg/L available chlorine in the drinking water for both males and females (corresponding to a NOAEL of 13.75***** mg/kg bw/d for males and 15.7***** mg/kg bw/d for females) (NTP, 1992).
***** Note from the RMS: The NOAEL of 13.75 and 15.7 mg/kg bw/day in the RAR was calculated using the table reported in B.6.3.5, which is considered to be a conservative approach. The compound consumption was however reported in the NTP study report (in mg of available chlorine/kg bw/day) in

appendix L. The mean compound consumption (as available chlorine) for the 275 ppm dose group was 15 mg/kg bw/day for males and 16 mg/kg bw/day for females. These values will be used for the risk assessment in this DAR.

Four groups of 50 male and 50 female Sprague-Dawley rats each, 12 weeks old at the start of the study, received drinking water with added sodium hypochlorite, with concentrations of active chlorine of 750, 500 or 100 mg/L (treated groups) or tap water (active chlorine <0.2 mg/L) (control group), respectively, for 104 weeks. No group was treated with non-chlorinated water. In the male rats, a slight increase of total tumours was seen in all treated groups, compared to controls, but the effect was not dose related. Among the treated female rats, an increased incidence of lymphomasleukemias (not specified) was observed, but the effect was not clearly dose-dependant. The control group (at <2mg/L of active chlorine) showed an unusually low incidence of leukemia (0 cases against 4 cases in male control group). Some unusual tumours in Sprague-Dawley rats were observed randomly but were not treatment related. Considering the conclusions not definitive, the authors suggested further studies for a quantitative assessment of cancer risk (Soffritti *et al*., 1997).

Chlorinated water, containing available chlorine at a periodically controlled level of 100 mg/L was well tolerated when given daily as drinking water over the whole lifespan (maximum of 2 years) to 236 BDII (cPah albino) rats for five generations (excluding F3 and F4). Following haematological tests and final necropsy of all animals, the results indicated that there was no difference in survival and in malignant tumour incidence in any generation group when compared to the untreated controls (Druckrey, 1968).

Groups of 50 male and 50 female B6C3F1 mice, 4 to 6 weeks old, were given sodium hypochlorite prepared from a commercial product (14% available chlorine, purity not specified) dissolved in their drinking water at concentrations of 0, 500 or 1000 mg/L (0, 0.05 or 0.1% / 0, 477 or 954 mg/L as available chlorine) for 103 weeks. Groups of 73 male and 72 female mice were used as controls using distilled water. All surviving mice were sacrificed at week 106. At that time, the survival rates were for males: controls 48/73, low dose 39/50, high dose 37/50 and for females: controls 56/72, low dose 40/50, high dose 39/50. A dose-related reduction in body weight gain occurred in treated groups from both sexes. Water consumption, haematology, blood chemistry and histopathology were apparently recorded but no information is given about these parameters.
Combined tumour incidences of leukemias and malignant lymphomas and of adenomas and adenocarcinomas of the lung were very high in all groups (control and all treated) for both sexes. Also, a high incidence of hyperplastic nodules and hepatocellular carcinomas of the liver in males of all groups was noted. However, no statistically significant differences in tumour incidences were observed for any organ in treated animals. It was concluded that there was no effect upon tumour incidence in either male or female mice.
The NOAEL for sodium hypochlorite in the study was 477 mg/L available chlorine in the drinking water for both males and females (Kurokawa *et al*, 1986).

Groups of 70 male and 70 female B6C3F1 mice were given sodium hypochlorite dissolved in their drinking water at concentrations corresponding to 0, 70, 140 or 275 mg/L available chlorine for two years. Groups of 10 mice of each sex were preselected for evaluation at weeks 14 or 15 and week 66.
Survival at 2 years of mice receiving chlorinated water was similar to that of the controls.
Mean body weights were similar among treated and control groups after 15 weeks. After 66 weeks, the body weight of males given 275 mg/L available chlorine was significantly lower than that of the controls. At the end of the study, mean body weights of treated mice were slightly lower than those of their respective control groups.
A dose-related decrease in water consumption was observed throughout the study in treated groups from both sexes: water consumption by high-dose mice was 31% lower than that of controls for males and 26% lower for females. Mean food consumption of treated males was slightly higher than for the controls for the first 52 weeks. During the same period, mean food consumption of the treated females was slightly lower than for the controls. The mean food consumption for the remainder of the study was similar for both sexes and all groups. There were no clinical findings, no haematological changes and no changes in relative organ weights attributable to the consumption of chlorinated water at either the interim evaluations or the end of the experiment. No treatment-related neoplasms or non-neoplastic findings were reported in either sex.
The NOAEL for sodium hypochlorite in the study was 140 mg/L available chlorine in the drinking water for both males and females (corresponding to a NOAEL of 23.3* mg/kg bw/d for males and 28* mg/kg bw/d for females) (NTP, 1992).
* Note from the RMS: The NOAEL of 23.3 and 28 mg/kg bw/day in the RAR was calculated using the table reported in B.6.3.5. The compound consumption was however reported in the NTP study report (in mg of available chlorine/kg bw/day) in appendix L. The mean compound consumption (as available chlorine) for the 140 ppm dose group was 15.3 mg/kg bw/day for males and 17 mg/kg bw/day for females. These values will be used for the risk assessment in this DAR.

Summary of oral administration

The available animal studies are not sufficient to indicate a clear relationship between the oral administration of sodium hypochlorite in drinking water and cancer. Slight and equivocal evidence is reported in two studies (NTP, 1992, and Soffritti *et al*., 1997) for leukaemia in female rats. No other evidence is reported in other tests.

Inhalation exposure

No chronic or carcinogenicity study is available for inhalation exposure of sodium hypochlorite and the only information available concerns chlorine inhalation.

Groups of approximately 70 male and 70 female F344/N rats and B6C3F1 mice were exposed to either 0, 0.4, 1.0 or 2.5 ppm (0, 1.2, 3.0 or 7.4 mg/m^3) chlorine gas for 6 h/d, either for 5 d/week (male and

female mice and male rats) or for 3 alternate days/wk (female rats), for 2 years. An interim post-mortem of 10 rats/sex was performed at 12 months. Exposure did not affect survival and did not cause tumours. Exposure-related non-neoplastic lesions were confined to the nasal passage in all exposed groups. They were most severe in the anterior nasal cavity including 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 (Wolf *et al*, 1995). The LOAEL for respiratory irritation was 0.4 ppm. A NOAEL was not established in the study.

Human data

There are no case reports or epidemiological studies of human carcinogenicity directly linked to administration of sodium hypochlorite. The only human data relating with sodium hypochlorite are in connection with its use to disinfect drinking water.

Drinking water epidemiological studies

The addition of sodium hypochorite and chlorine gas gives the same chlorine species in solution - i.e., an equilibrium mixture of mainly hypochlorous acid and hypochlorite anion. In this way much of the general population is exposed to hypochlorite via drinking water.

Several epidemiology studies are available to evaluate the carcinogenicity of chlorinated drinking water, but their use in sodium hypochlorite risk assessment is questionable considering the great differences in water quality in diverse geographical areas and the contribution of the chemical species present in water supplies and the derived reaction products. For an overall assessment of the effect of chlorinated drinking water on the general population including sodium hypochlorite as the active chlorine source, specific activity should be assessed while the following text is only an initial contribution.

IARC reviewed in 1991 the available studies and gave prominence to the difficulties in the interpretation of the data for an evaluation of the carcinogenicity of chlorinated drinking water. In the studies performed there are several methodological inadequacies, many confounding variables, and no causal link between increased cancer risk and consumption of chlorinated drinking water. Some studies have reported a correlation between the higher risk of cancer of the urinary bladder and the long-term consumption of chlorinated drinking water. Evidence from other studies, although showing some degree of consistency, is severely compromised by the weaknesses outlined above.

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).

More recent ecological studies reported a weak association between consumption of chlorinated drinking water and cancer of the colon and rectum in men and women (Flaten, 1992 in IPCS EHC 2000); no associations between chlorinated surface water supplies and bladder or stomach cancer were found in the Valencia Province, Spain (Suarez-Varela *et al*., 1994 in IPCS EHC 2000).

A study in Taiwan reported associations between use of chlorinated drinking water and cancer of the rectum, lung, bladder and kidney (Yang *et al*., 1998 in IPCS EHC 2000).

Some population-based case-control studies were conducted for bladder, colon, pancreatic, brain and cancer risk by different authors: King and Marrett (1996 cited in IPCS EHC 2000) conducted in residents between 25 and 74 years of age with a histologically confirmed primary cancer or carcinoma in situ of the bladder; they found higher relative risk estimats for a group of nonsmokers associated with many years of exposure to chlorinated surface water.

In a Colorado residents case-control study, McGeehin *et al*. (1993, cited in IPCS EHC 2000) suggested the hypothesis that prolonged (+30 years) exposure to chlorinated surface water is associated with an increased risk of bladder cancer, while Freedman *et al*. (1997, cited in IPCS EHC 2000) found a weak association between bladder cancer risk and the use of municipal chlorinated water, limited to cigarette smokers, in a population based case-control study in Maryland.

In a population-based case-control study for bladder, colon and rectal cancer, conducted by Cantor *et al*. (1998) in Iowa, an increased risk of bladder cancer in current and past male smokers and an association between brain cancer among men and increased duration of exposure to chlorinated surface water was found.

Hildesheim *et al*. (1998, cited in IPCS EHC 2000) suggested in a another, similar study in Iowa, the same association with bladder cancer and found a larger relative risk for rectal cancer among persons with low dietary fiber intake and longer duration exposure to chlorinated surface water sources compared to persons with high fiber diets and no exposure to chlorinated surface water.

In a population-based case-control for pancreatic cancer conducted by Ijsselmuiden *et al*. (1992, cited in IPCS EHC 2000) in Maryland, no increased risk of pancreatic cancer was associated with the consumption of chlorinated drinkink water.

Summary of epidemiological studies

Although some studies reported small relative risks for colon and bladder cancer incidence for population consuming chlorinated drinking water for long periods of time, they are equivocal or insufficent to establish a causal relationship, considering the quality and the completeness of the studies and the interpretation of the available data and of the confounding factors.

B.6.5.2 Summary

In Table 6.5.2.1 the results of the chronic toxicity and carcinogenicity studies with the test substance are summarised.

Table 6.5.2.1 Chronic toxicity and carcinogenicity studies

Duration	Species	NOAEL (as av. Cl_2)	NOAEL (as av. Cl_2 in mg/kg bw/day)	Notes / Results	Reference	Val*
Oral, drinking water						
104 weeks	Rat	1000 ppm (0.1%)	≥ 58.7 (m) 67.0 (f)	Well conducted study / negative	Hasegawa and Kurokawa, 1986	1
2 years	Rat	≥ 275 ppm	≥ 15 (m) ≥ 16 (f)	Well conducted study / equivocal evidence in females	NTP, 1992	1
104 weeks	Rat	**	**	Well conducted study / Not dose-related increase in leukemia-lymphomas in female group	Soffritti, 1997	1
2 years (5 genera-tions)	Rat	**	**	Sample analysis, necropsy / negative	Druckrey, 1968	3
103 weeks	Mouse	477 mg/L	78.3 (m) 95.4 (f)	Well conducted study / negative	Kurokawa, 1986	1
2 years	Mouse	140 ppm	15.3 (m) 17.2 (f)	Well conducted study / negative	NTP, 1992	1
Inhalation, chlorine gas						
2 years	Rat	***		Supplemental information / negative	Wolf, 1995	3
2 years	Mouse	***		Supplemental information / negative	Wolf, 1995	3

* Validity of the study

** In the RAR, no NOEL was derived for this study (carcinogenicity study).

*** The LOAEL for respiratory irritation was 0.4 ppm chlorine gas

In long term carcinogenicity studies, sodium hypochlorite administered in the drinking water did not increase the proportion of F344 rats and B6C3F1 mice with tumours. Under the conditions of the 2 year NTP drinking water study there was no evidence of carcinogenic activity of chlorinated water in male rats or in male and female mice. However, the study concluded that there was equivocal evidence of carcinogenic activity of chlorinated water in female rats based on a marginal statistical increase in the incidence of mononuclear cell leukemia. Similarly non-dose dependant increases in lymphoma/leukemia were found in female Sprague-Dawley rats in another long term rodent bio-assay with chlorinated drinking water. This study was deemed suggestive but inconclusive by its authors. Drinking water

containing 100 mg/L chlorine was tested for carcinogenicity in a multigeneration study in male and female BDII rats. No increase in the incidence of tumours was seen in the treated animals relative to controls through five generations (Druckrey). Taking into account all the available information, it can be concluded that carcinogenicity is not a relevant endpoint for the oral route.

No human data are available on carcinogenicity and the only data are related to chlorinated drinking water for which the epidemiological data are not sufficient to suggest a causal relationship between the use of chorinated drinking water and increased cancer risk.

The International Agency for Research on Cancer (IARC, 1991) has concluded that there is inadequate evidence for the carcinogenicity of sodium hypochlorite in animals and that sodium hypochlorite is not classifiable as to its carcinogenicity in humans (Group 3). This conclusion is still valid, taken into account the more recent available data.

B.6.6 REPRODUCTIVE TOXICITY (ANNEX IIA 5.6)

Animal data

There are no specific data on sodium hypochlorite itself. However, sodium hypochlorite exists as a solution in which the composition of ions depends on the pH. Considering the biological systems, the active chemical species available are HOCl and ClO^- in equilibrium in the pH range 6-8, and Cl_2 at pH below 4. As a consequence, when sodium hypochlorite is administered orally, it is transformed to chlorine in the stomach and can be transformed back to hypochlorous acid and hypochorite depending on the pH of the substrate. For this reason, the toxicological profile of sodium hypochlorite is linked to that of chlorine and hypochlorous acid. Available data on these species are therefore relevant for the toxicological assessment of sodium hypochlorite.

The potential developmental and reproductive effects of chlorine have been examined, mainly in 3 studies:

- in Long-Evans rats following oral administration of chlorine by gavage (Carlton *et al*., 1986)
- in BDII rats given chlorinated drinking water in a "multigeneration study" (Druckrey, 1968).
- in Sprague-Dawley rats given chlorinated drinking water prior to and throughout gestation (Abdel-Rahman *et al*., 1982)

B.6.6.1 Reproductive toxicity

In the Carlton study (1986), potential reproductive effects were assessed in Long- Evans rats. The protocol was in good compliance with actual current standards. Males (12 per group) were administered doses of 0, 1, 2, and 5 mg/kg body weight of aqueous chlorine, (as HOCl). Administration by gavage started 56 days prior to breeding and continued throughout the 10 day breeding cycle. Female rats (24

per group) received the same concentrations by gavage for 14 days prior to breeding and throughout breeding, gestation and lactation, until the pups were weaned on day 21. Following the breeding period, males were bled for complete blood count and thyroid hormone levels determination and then were subject to a complete gross necropsy and histopathological examination of the reproductive tract.

Dams were observed for fertility, gestation duration, body weight gain, maternal behaviour and at day 21 of lactation bled for complete blood count and then sacrificed for gross necropsy and histopathology of the reproductive tract.
Litters were observed for viability, litter size, day of eye opening (when all pups of a same litter had open eyes, this day was considered as day of eye opening for the whole litter), bodyweight gain, gross external abnormalities, day of vaginal opening (for selected pups).

No clinical signs of toxicity, haematological changes or body weight depression were observed in the treated adult male and female rats. No alterations in sperm count, sperm motility or sperm morphology were seen and there were no histopathological lesions in the reproductive tract of adult male and female rats exposed to 1 to 5 mg/kg/d of aqueous chlorine. There were no dose-related effects on fertility, foetal viability, litter size, foetal body weight, day of eye opening or day of vaginal patency.

It is noteworthy that the doubtful effect observed in the sperm-head abnormalities assay in mice (Meier *et al*., 1985, see below) was not found in the Carlton study in rats at similar doses and with treatment duration of 56 days against 5 days in the Meier study.
The NOAEL of the Carlton study is 5 mg/kg bw of aqueous chlorine (expressed as HOCl - maximum dose tested). Even if the value is expressed as HOCl, it is equivalent to available chlorine since the measure method used detects all the chlorine species in water. Therefore, the value of 5 mg/kg bw available chlorine is used in the risk characterisation as NOAEL for reproductive toxicity.

In the Druckrey study (1968), 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 (fresh preparation was repeated each week, in compliance with stability data). The animals were mated and the treatment was continued for life span through the 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 (Parental, F1, F2, F5 and F6). Two control groups were used (sex and age not specified), one starting in 1955 (n = 20) and the other in 1962 (n = 36).
The results are poorly documented but no toxic effects were reported on fertility, growth, survival or at histological examination of the main organs.

Meier *et al*., 1985 have tested chlorine, as hypochlorite and hypochlorous acid, in a sperm head abnormality test in B6C3F1 mice. In this assay, a chlorine solution at pH 8.5, where hypochlorite anion predominates, was administered orally at dose levels equivalent to 1.6, 4 and 8 mg/kg/day as

hypochlorite anion for 5 days. No abnormalities were detected for sampling times of 1 and 5 weeks. However, statistically significant increases in the frequency of sperm head abnormalities, at 3 weeks post-treatment, were observed at the two highest dose levels without dose related increase. The mean percentage of abnormalities was 2.12, 4.07 and 3.68 for the negative control, the 4 mg/kg and the 8 mg/kg dose levels respectively. This effect was reproduced in an independent repeated experiment and, in addition, a small statistically significant increase was observed at 1.6 mg/kg/day (1.41% compared to 0.91% for the negative control).

Although this study is validated as category 2 because it is well documented and meets generally accepted scientific principles, the toxicological significance of the findings is doubtful for several reasons:

- Concerning the assay itself, the authors pointed out that the major drawback of this assay was a low specificity with high number of false positives.
- Moreover, in the two experiments, the increases were small, (less than or about 2 fold) and without dose-dependency (the highest dose of 8 mg/kg had a lower rate of abnormality than the 4 mg/kg dose).
- Furthermore, this report showed that the background incidence of sperm abnormalities was very variable ranging from 0.91 to 3.3%. In the light of this observation, all of the statistically significant increases described by the authors in the treated groups fall in the background incidence of sperm abnormalities except for the 4 mg/kg dose which was slightly outside of this range in the first experiment only (4.07%).
- In the same study, at the same doses and under the same conditions, administration of chlorine solution at pH 6.5 did not cause any increase in the number of sperm abnormalities. Taking into account the acid pH of the stomach and the reaction with hypochlorite, there is no plausible mechanism which can explain the difference in terms of effect between a solution administered orally at pH 8.5 and pH 6.5. It is also expected that sodium hypochlorite will never reach the testes due to rapid decomposition (see B.6.1.1)
- In a rat reproductive toxicity study performed by Carlton *et al* (1986; see above), no sperm head abnormalities were found at similar doses and with treatment duration of 56 days against 5 days in this study.

In conclusion, the Meier *et al*. study, although well reported, cannot be considered for the risk characterisation of hypochlorite. The findings are of statistical significance but they are not supported by a biological and mechanistic point of view.

In a study by Abdel-Rahman and Suh (1984), a statistically significant increase of incorporation of ^{3}H-Thymidine was observed in testes of rats receiving 100 mg/L HClO in drinking water for three consecutive months. Concentrations of 1 and 10 mg/L were also tested and the small number of animals included in the assay (only four animals per group) lead to consider this finding as not valid for the Risk Assessment procedure.

B.6.6.2 Teratogenicity studies

In the Abdel-Rahman study (1982), female SD rats (6 per group) were administered chlorine, as hypochlorous acid (HOCl), in drinking water at concentrations of 0, 0.1, 10 and 100 mg/L per day for 2.5 months prior to conception and throughout gestation. Doses expressed in mg/kg bw of hypochlorous acid can be estimated as about 0.1, 1 and 10 mg/kg bw/day according to the specified rat body weight (225-250 g) and a default value of 20 ml/day/animal for water consumption (actual consumption not specified in the publication). Rats were killed on day 20 of gestation and the foetuses were examined, half for soft tissue abnormalities and half for skeletal abnormalities. No information was given on possible maternotoxic effects at any exposure concentration.

All foetuses were found viable and normal in external appearance. There was no statistical difference between control and treated groups for the number of resorptions and foetal weights. There was no statistical difference between control and treated groups in % of skeletal defects and in % of soft tissue anomalies.
There were no statistically significant changes in incidences of skeletal variants observed in treated groups versus controls. Incompletely ossified or missing sternebrae or rudimentary ribs changes that were not dose-related cannot be considered as attributable to treatment.

Some rare soft tissue defects were observed in the high dose group of 100 mg HOCl /l and in the control group while the lower concentrations of hypochlorous acid (1 and 10 mg/L) did not produce any defects. In the 100 mg/L group, the authors found 3 cases of adrenal agenesis, 1 case of dextrocardia, 1 case of improper orientation of the apex and 1 case of atrio ventricular valve enlargement. The distribution of these cases among the litters and foetuses were not presented but the authors reported similar defects in the control group although they do not specify the number of cases.
Furthermore, the difference in incidence between the treated group and the control group was not statistically significant using the foetus as the unit for the statistical analysis. The choice of the foetus as unit rather than the litter certainly maximizes the power of the statistical analysis and thus counterbalances the low number of dams and subsequent litters per group. Because similar tissue defects were observed in the control and high dose groups and none in the lower treated groups and because the difference in incidence was not statistically significant, these findings strongly indicate that these soft tissue defects were spontaneous in origin rather than treatment-related.

Moreover, no indication of toxic developmental effects was seen in the Carlton study (no abnormal offspring, no effect on litter size, perinatal mortality, pup growth and neonatal weight, see B.6.6.1) which was supported by the US-EPA.

Two other studies were performed on CD1 mice given municipal tap water (Durham, North Carolina) and compared with mice given the same but purified tap water (disinfectant was removed via organic removal cartridge, demineralizer and still). No indication about the dose of chlorine or HClO in tap water was provided:

- In a study by Chernoff *et al*. (1979) several groups of mice (unspecified number per group) were bred, successively over a period of 8 consecutive months, observed until gestational day 18 for body weight gain, and then sacrificed for pregnancy data and litter examination (external, visceral and skeletal examination). Finally, 236 pregnant female mice (in tap water group) and 257 (in control purified water group) were examined. There were no significant water-related effects on maternal data (% pregnant females, body weight gain and % implants) or any foetal parameter (% live/dead foetuses, body weight, external, visceral or skeletal anomalies).
- In a study by Staples (1979), a similar protocol was used for a 11 months period. An overall amount of 247 (tap water) and 217 (control) pregnant females were involved. No significant differences were noted between the 2 groups in the reproductive status and in the foetal parameters including malformations.

Human data

Several exploratory epidemiological studies assessing possible adverse developmental outcomes associated with chlorinated water are available. However, the results of these studies should be cautiously interpreted because of limitations of study design and likely bias.

Aschengrau *et al*., 1993, conducted a case-control study in Massachussetts to determine the possible relationship between community drinking water quality and adverse pregnancy outcomes. This study included infants from mothers delivering at Brigham and Women's Hospital between 1977 and 1980. There was no statistically significant increased risk of neonatal death or total congenital anomalies in women exposed to chlorinated drinking water. The stillbirth risk was no longer significantly increased after adjustment for appropriate confounding factors.

In a case-control study (Savitz *et al*., 1995), assessment of miscarriage, pre-term delivery and low birth weight in central North Carolina did not show any association between these parameters and chlorinated drinking water.

A cross-sectional study (Kanitz *et al*., 1996) was conducted in Italy comparing two groups of pregnant women/newborns during 1988-89. Assignment to a group (water source and type of disinfectant) was based on the mother's address. The treated group included 548 births at Galliera Hospital (Genoa) from mothers living in an area where drinking water was disinfected either by chlorine (108), chlorine dioxide (277) or both (163). Control group included 128 births at Chiavari Hospital from mothers living in Chiavari where well water was untreated.

Information was collected about mother's age, smoking and alcohol habits, education level and also about family income. Assessment of birth outcomes included birth weight (<2500 g or > 2500 g), pregnancy duration (< 37 weeks or > 37 weeks), body length (< 49.5 cm or > 49.5 cm), cranial circumference (< 35 cm or > 35 cm) and neonatal jaundice.

Statistically significant increased risks of smaller cranial circumference and smaller body length were associated with drinking water disinfected with chlorine. The adjusted odd ratios and 95 % confidence intervals were: 2.3 (1.3 - 4.2) for body length and 3.5 (2.1 - 8.5) for cranial circumference respectively.

The results of this study should be interpreted very cautiously (as rightly pointed out by the authors themselves), because of lack of information about real exposure, and likely bias in assessment of pregnancy outcomes. No information was collected to assess the mothers' tap water consumption (or bottled water consumption). Exposures to surface and ground water sources are being compared, and no information is presented about other possible water quality differences. There is also concern about whether the population may be different in respects other than the water system differences studied. Concerning pregnancy outcomes, parity was astonishingly not taken into account by the authors. Moreover, body length and cranial circumference measurements are subject to possible differences between hospitals. It is noteworthy that only two hospitals were involved in that study, one for the control group (Chiavari hospital) and the other one for the treated groups (Galliera hospital in Genoa). So, the reported differences in body length and cranial circumference might be due to differences in measurement methodology between hospitals, irrespective of the exposure.

Swan, S.H. *et al*. (1992) presented an overview of 5 retrospective studies, reported in the same issue of "Epidemiology", in which the risk of spontaneous abortion was examined in relation to the source and amount of drinking water consumed during the early pregnancy (period 1982-1987). At first, in December 1981, a drinking water well in Santa Clara County, California, was found to be contaminated by organic solvents. Although two epidemiology studies conducted by the California Department of Health Services found associations between location of residence and spontaneous
abortion, uncertainties about the exposure characterisation precluded any firm inferences. A subsequent study, including a hydrogeologic model of the water distribution system, showed clearly that the solvent leak was not causally related to the observed cluster of spontaneous abortions. Unexpectedly, women who reported drinking tap water appeared at extra risk of spontaneous abortion, regardless of location residence. Consequently, the California Dept of Health Services included questions on prenatal water consumption in all reproductive studies conducted between 1982 and 1988 (about 5000 cases). Results from four of the five studies suggest that women drinking tap (or mostly tap) water compared with those drinking no tap water were at extra risk of spontaneous abortion. However, even with the large body of available data, it was not possible to decide whether the apparent differences in risk of spontaneous abortion can be accounted for by artifacts of the study design (especially reporting bias).

Next, a prospective study was conducted to extend the previous investigation in Santa Clara County to three different water systems (about 1600 cases per region), to later time period, as well as to eliminate recall bias by a prospective design. Regions I (mixed ground and surface water) and II (surface water) are in northern California, the region III (ground water) is in southern California. Region I is located within the Santa Clara County previously studied (see above). The results of the prospective study, reported by Swan, S.H., *et al*. 1998 showed that neither tap or bottled water consumption altered the risk of spontaneous abortion in regions II and III. However, the study confirmed the association between cold tap water consumption (but only for high consumption) and risk of spontaneous abortion previously seen in region I. Adjusted odds ratios comparing high (> 6 glasses /day) consumption of cold tap water with none was 2.17 (with a 95% confidence interval of1. 22 - 3. 87). The adjusted odd ratio for 0.5 to 5.5 glasses cold tap water per day was 1.10 (95% confidence interval 0.76-1.59). Taking into account total tap water consumption (cold and heated tap
water), a very marginal increase of risk (if any) was observed for a high tap water consumption. The adjusted odds ratios were respectively: 1.66 (95% confidence interval 0.99-2.78) for > 6 glasses and 1.03 (95% confidence interval 0.7-1.52) for consumption of 0.5 to 5.5 glasses per day.

These findings, indicating an apparent decrease in risk of spontaneous abortion when tap water is heated, could evoke a possible link with volatile by-products. This hypothesis was studied (by Waller, K. *et al*. 1998) taking into account the estimated total trihalomethanes (THMs) levels in tap water in the three regions considered in the prospective study cited above. The additional analysis of the data indicates a slight increase of risk of spontaneous abortions for a high consumption of cold tap water (> 5 glasses per day) containing > 75 µg/l of total THMs (adjusted odd ratio = 2.0; 95% confidence limit 1.1-3.6). However, as pointed out by the authors themselves, (Swan *et al*. 1998 ; Waller, K. *et al*. 1998), it is noteworthy that :

- a THMs level > 75 µg/l is not uncommon. The maximum contaminant level permissible for THMs is 100 µg/l according to Federal Law. The mean THMs in Region I in the high tap water consumption group was 93 µg/l (range 78-105 µg/l).
  For Region II, where women drinking tap water were not at extra risk for spontaneous abortion (see above, Swan, S.H. *et al*. 1998) the mean THMs level of 92 µg/l (range 75-123 µg/l) was comparable to that of Region I.
- the practice of letting water stand before drinking it, which allows chlorination byproducts to volatilise, should decrease the risk. On the contrary, the association in Region I appeared stronger in heavily exposed women who followed that practice (although numbers were small).

For the above reasons, Swan *et al*. believed that the association between spontaneous abortion and extensive cold tap water drinking recorded only in Region I cannot be explained by chlorination by-products.

A similar interpretation of the data was done by Williams, A. and Weiss, N.S. 1998, who concluded "since exposure to high levels of THMs in drinking water was more common in Region II than Region I, THMs would seem to be exonerated as a basis for the association in Region I."

B.6.6.3 Summary

The results of the reproduction toxicity and teratogenicity studies are summarised in table 6.6.3.1.

Table 6.6.3.1 Summary of reproduction toxicity and teratogenicity studies

Type of study	Result	Notes	Reference	VAL*
Reproduction toxicity				
Gavage, rat, 1 generation	No effects observed NOAEL ≥ 5 mg/kg bw/d as av. chlorine	a.s.: Aq. chlorine Well conducted study NOAEL determined	Carlton, 1986	2
Drinking water, rat, 2 years, 5 generations	No effects observed	German paper, only limited data available	Druckrey, 1968	3
Sperm head assay, mutagenicity mice	Doubtful positive	Data well presented	Meier, 1985	2
^{3}H-thymidine incorporation tests	Equivocal result	Limited protocol and data	Abdel-Rahman, 1984	4
Embryo/fetal toxicity and teratogenicity				
Drinking water, developmental effects, rat foetus	Minor effects at 100 mg/L	Study well conducted	Abdel-Rahman, 1982	2
Tap water, foetal devel. mice	No evidence	Limited available data	Chernoff, 1979	3
Tap water, foetal devel. mice	No evidence	Limited available data	Staples, 1979	3

* Val: validity of the study

There are no relevant studies of sodium hypochlorite per se looking at its reproductive toxicity potential in animals. However, relevant studies have been conducted using chlorine as the test substance, administered in solution by gavage or in drinking water. In a teratogenicity study, in which exposure was confined to the gestation period, no significant differences in the incidence of skeletal or soft tissue abnormalities were observed in treated groups when compared to controls. A small, but statistically significant increase in sperm head abnormalities was seen in mice, although the effect was not dose-dependant. However, no effects were seen in a well conducted one-generation reproductive toxicity study in rats up to a concentration of 5 mg/kg bw of available chlorine (maximum dose tested) (Carlton, 1986). Therefore, the value of 5 mg/kg bw available chlorine is used in the risk characterisation as NOAEL for reproductive toxicity. Long-term toxicity studies provide also additional assurance that the substance is not a reproductive toxicant as they did not identify the testes or ovaries as target organs.

The Carlton study appears relatively more updated and reliable with a number of animals more suitable for statistical analysis.
There are no studies performed at dose levels able to induce systemic toxicity.
A pragmatic approach is to accept the NOAEL derived from the Carlton study because it is the best study available even if no severe effects are shown in all dosed groups.

Although limited data are available in animals, the available studies are sufficient in their design and quality to draw the conclusion that there is no evidence to suggest that sodium hypochlorite would present adverse effects on development or fertility. Similarly, no such evidence is forthcoming from epidemiological studies on populations consuming chlorinated drinking water.

B.6.7 NEUROTOXICITY / DELAYED NEUROTOXICITY (ANNEX IIA 5.7)

No specific studies are available. In the summaries of all other studies described in the RAR no neurotoxic effects were reported.

B.6.8 FURTHER TOXICOLOGICAL STUDIES (ANNEX IIA 5.8)

In a mouse skin two-stage carcinogenesis model study, a control group of 40 ddN female mice, 5 weeks old, were given 60 topical applications of sodium hypochlorite (10% effective chlorine solution) (purity, vehicle and frequency of application unspecified). Another group of 40 female mice (group 2) were given 20 applications of 4-nitroquinoline 1-oxide, a potent carcinogen, (purity, vehicle and frequency of application unspecified); and a third group of 40 mice (group 1) were first given the carcinogen, painted on in 20 applications of 0.05 mg in 0.25% (w/v) benzene solution over the course of 50 days (1mg/mouse total), followed, six days later, by 45 applications of approx. 0.05 ml sodium hypochlorite solution (10% available chlorine), during a period of 245 days (frequency of application unspecified). No skin tumours occurred in the mice given sodium hypochlorite alone. However, skin
tumours were seen in 9/32 mice given applications of sodium hypochlorite following initiating doses of 4-nitroquinolene 1-oxide and included one fibrosarcoma, three squamous cell carcinomas and five papillomas. No skin tumours occurred in mice given applications of 4-nitroquinolene 1-oxide only. The results of this study suggest that sodium hypochlorite might have the potential for co-carcinogenicity or tumour promoting (Hayatsu *et al*., 1971).

A 1% solution of sodium hypochlorite applied to the skin twice weekly for 10 weeks prior to, or after treatment with 750 and 1500 µg of 3,4-benzopyrene, a potent carcinogen, reduced the number of NMRI mice developing skin tumours when compared with those receiving dermal applications of two doses of the carcinogen alone (Pfeiffer, 1978).

In another mouse skin two-stage carcinogenesis model study, a group of 20 female Sencar mice, 6 weeks old, were given a single topical application of 20 nmol (5 µg) dimethylbenzanthracene (DMBA) in acetone, followed by applications of 0.2 ml of 1% sodium hypochlorite solution in acetone twice weekly for 51 weeks. A group of 15 female mice given a single application of DMBA followed by applications of acetone served as controls. The effective number of mice was 20; the number of survivors was not reported. A squamous cell carcinoma of the skin occurred in 1/20 mice treated with DMBA and sodium hypochlorite, whereas none occurred in the initiated controls. In the same test to verify the complete carcinogenic activity, another group of 20 female Sencar mice, 6 weeks old were given topical applications of 0.2 ml of a solution of 1% (10 g/l) sodium hypochlorite in acetone twice weekly for 51 weeks at which time the study was terminated. A group of 15 female mice given applications of acetone were used as controls. All animals survived to the end of the study. No skin tumours were observed in the treated or control groups.
The NOAEL is 9500 (1% sodium hypochlorite) as av. Cl_2 in mg/L (Kurokawa, 1984).

The immunotoxic potential of sodium hypochlorite was assessed in male Sprague- Dawley rats from weaning to 12 weeks of age given drinking water containing 0, 5, 15 or 30 mg/L sodium hypochlorite for 9 weeks. No significant effects were observed on body weights, thymus weight, antibody response, natural killer cell cytotoxicity, interleukin production and phagocytic activity. Reductions in spleen weight and delayed-type hypersensitivity reactions were observed in the rats given 30 mg/L sodium hypochlorite (about 1.5 mg/kg bw/day), while in those given 15 and 30 mg/L, a reduction in macrophage oxidative metabolism and a statistically significant increase in prostaglandin E2 production were observed. No information on the reversibility of the effects was provided (Exon, 1987).

Effects on delayed-type hypersensitivity, hemagglutination titres and reticuloendothelial clearance were studied in two groups of 30 CR1: CD-1 mice each given sodium hypochlorite in their drinking water at concentrations of 15 and 30 mg/L as available chlorine for 120 days. Other groups of mice were exposed to distilled water, tap water or other water treatment chemicals. No significant differences in antibody titres, reticulo-endothelial clearance or spleen weight were observed in the treated groups compared with the control group, suggesting that sodium hypochlorite does not significantly affect the immune function of mice (Hermann *et al.*, 1982).

In a pharmacodynamics and toxicity study, groups of 4 Sprague-Dawley rats each were exposed to HClO as a single dose of 10, 20 and 40 mg/L (30, 60 and 120 µg, respectively) to control the rat blood glutathione after 15, 30, 60 and 120 minutes after the treatment. Other groups of 4 Sprague-Dawley rats, including a control group, were each treated daily for a period of 1 year by drinking water with 1, 10 and 100 mg/L of hypochlorous acid. Data collected were: the heparinized blood, collected at 3 and 6 months after HClO administration to control hematological parameters, chloroform content (at month 4, 6, 9 and 12), blood glutathione, and ^{3}H-thymidine incorporation (both at month 2, 3, 4, 6, 8, 10 and 12). For the acute exposure a maximum decrease in blood GSH was observed 60 min after dosing. In the long term

study a decrease of blood glutathione and increase of osmotic fragility after 6 months were observed. In animals exposed for 3 months, the ^{3}H-thymidine incorporation into nuclei of rat kidney and testis in the 100 mg/L group was increased. There was no dose-related effect and no information on the reversibility after treatment, no significant changes were observed relating the hematological parameters. Blood chloroform levels were without change during 1 year of treatment with hypoclorous acid (Abdel-Rahman and Suh, 1984).

A group of five C57BL/6N mice were given sodium hypochlorite in their drinking water at a concentration of 25-30 mg/L available chlorine for 4 weeks. A second group of mice served as controls. A decrease in the number of peritoneal exudate cells (suggestive of an effect on macrophage function) along with an overall depression of macrophage function was observed (Fidler, 1977).

Summary and conclusion

Table 6.8.1 Summary of further toxicological studies

Duration	Species	Dose	Results	Notes	Reference	Val*
Dermal application						
450 days	female ddN mouse	NaClO 100 g/L ; NQ-oxide 0.25% - combined	Skin cancers in combined groups	Skin two-stage model promoting test; the strain mouse is unusual	Hayatsu, 1971	3
51 weeks	Female Sencar mouse	NaClO 10000 mg/L ; DMBA (initiator) 20 nM - combined	Negative NOAEL 1% NaClO solution	Skin two-stage model promoting test	Kurokawa, 1984	2
104 weeks	NMRI mouse	NaClO 10000 mg/L ; BP (initiator) 750, 1500 µg - combined	Cancer decrease in combined group	Promoting test, lack of data	Pfeiffer, 1978	3
Oral						
1 year	Rats, oral	0, 10, 20, or 40 mg/L HClO		Specefic endpoints	Abdel-Rahman, 1984	3
9 weeks	Rats, drinking water	0, 5, 15, or 30 mg/L sodium hypochlorite		Immune system endpoint	Exon, 1987	3
4 weeks	Mice, drinking water	0, 25 - 30 mg/L as available chlorine		Specific endpoint	Fidler, 1977	3
120 days	Mice, drinking water	0, 15, or 30 mg/L as available chlorine		Immune system, no standard test	Hermann, 1982	3

* Val: validity of the study

The available data show no carcinogenic effect due to topical application of sodium hypochlorite solution at different concentrations. There is an indication, supported by a poorly described study, of co-carcinogenicity using NaClO as a promoting agent. In the same study, 4-nitroquinolene 1-oxide alone did

not show carcinogenicity, suggestive of methodological problems or unusual responses of the mouse strain used.
Two other studies using different initiators did not show promoting effects of NaClO, although the doses employed were lower. In one of these studies, a dermal two-stage carcinogenicity study, a clear no effect level was observed in female mice treated twice weekly for 51 weeks with 1% sodium hypochlorite solution. No animals died and no epidermal hyperplasia was observed in the treated group (Kurokawa, 1984).

Some incidental effects related to the immune system were reported in rats and mice administered with low doses of NaClO in chlorinated water for 12 or 17 weeks. These effects were observed in rats receiving chlorinated water containing 15-30 mg/L available chlorine (about 0.75-1.5 mg/kg bw/day) (Exon, 1987). However, in long term studies, no differences were reported between treated and controls for haematological analysis or thymus weight in rats given higher doses of NaClO in water (275 mg/L available chlorine or 15 mg/kg bw/d) (NTP, 1992). It is not possible to derive a no effect level for this specific end-point.

B.6.9 MEDICAL DATA AND INFORMATION (ANNEX IIA 5.9)

B.6.9.1 Medical surveillance on manufacturing

No data available.

B.6.9.2 Clinical cases and poisoning incidents

Although indirect exposure to sodium hypochlorite solution by aerosol or mist is possible, no cases on human exposure have been reported.

One case is reported about a child who sucked clothing that had been heavily bleached with NaClO solutions, although there was no information about the residual amount of sodium hypochlorite in the bleached cloth. Intermittent vomiting, stomach pains and inflammation of the lungs were reported, the effects disappearing after some time (Loeb and King, 1974).

A further case reported the continued use of 500 ml aqueous sodium hypochlorite solution (ca. 1%) as a wound dressing, 2-3 times a day for 1-2 weeks. Hypernatraemia due to poor kidney function was reported, along with the patient's inability to complain of thirst or to drink (Thorp *et al*., 1987).

See also the human data presented in B.6.2.

B.6.9.3 Observations on exposure of the general population

No indications of chronic toxicity in humans following exposure to sodium hypochlorite are reported in the literature: some case studies refer to specific effects and epidemiological studies designed for the

evaluation of the potential carcinogenicity of chlorinated drinking-water have been evaluated (see more details in B.6.5.1).

Several epidemiological studies of the effects of the consumption of chlorinated drinking water on the health of the general population have been reported. No causal link between any long term health effect (including increased cancer risk) and consumption of chlorinated drinking water was established in these studies (IARC, 1991).

B.6.9.4 Clinical signs and symptoms of poisoning and details of clinical tests

See B.6.9.2 and the human data presented in B.6.2.

B.6.9.5 First aid measures and therapeutic regimes (taken from the MSDS from IPCS)

Have the product container, label or MSDS with you when calling an emergency number, a poison control centre or physician, or going for treatment.

Inhalation	Fresh air, rest. Half-upright position. Refer for medical attention.
Ingestion	Rinse mouth. Do NOT induce vomiting. Give water or milk. Refer for medical attention.
Skin contact	First rinse with plenty of water, then remove contaminated clothes and rinse again. Refer for medical attention.
Eye contact	First rinse with plenty of water for several minutes (remove contact lenses if easily possible), then take to a doctor.

B.6.9.6 Expected effects of poisoning (taken from the MSDS from IPCS)

Inhalation	Burning sensation. Cough. Laboured breathing. Shortness of breath. Sore throat. Symptoms may be delayed.
Ingestion	Abdominal pain. Burning sensation. Shock or collapse. Unconsciousness. Vomiting.
Skin contact	Redness. Skin burns. Pain. Blisters.
Eye contact	Redness. Pain. Severe deep burns.

B.6.10 SUMMARY OF MAMMALIAN TOXICOLOGY AND PROPOSED ADI, AOEL, ARFD AND DRINKING WATER LIMIT (ANNEX IIA 5.10)

B.6.10.1 Toxicokinetics

Animal data suggest that after exposure via oral route, HOCl is absorbed and excreted mainly through urine as chloride (36.43% of the administered dose after 96h); a lesser extent of $HO^{36}Cl$-derived radioactivity not necessarily associated with absorption was detectable in the faeces 96h after exposure (14.8%). Plasma levels peaked after 4 hours. Elimination half-life was 88.5 hours.
Although this will be an underestimation, oral absorption is at least 40% (rounded value) based on urinary excretion only, as the RAR-summary did not quantify the amount recovered from tissues, organs and residual carcass.
Once in the body, HOCL is not enzymatically metabolised and it reacts directly with organic molecules to form some organochlorinated compounds, characterised by their own toxicity.
Human data are very scant and indirect. Absorption is suggested by some transient and not severe systemic symptoms following ingestion, although the possibility they are secondary to a local effect could not be ruled out with certainty.

B.6.10.2 Toxicodynamics

Acute toxicity

The acute toxicity of marketed hypochlorite solutions by the oral route is low. The LD_{50} values for solutions containing active chlorine concentrations up to 12.5% are greater than 5.8 g/kg bw. The data for dermal acute toxicity as well as inhalation toxicity indicate a low level of toxicity for these routes of administration. Therefore, sodium hypochlorite does not need to be classified for acute oral, dermal, and inhalation toxicology.
A considerable number of skin and eye irritation tests were performed with various sodium hypochlorite solutions. Depending on the concentration, sodium hypochlorite solutions appeared to be irritating to skin and eyes or even corrosive. Despite some difficulties in interpreting the older animal data, the overall evaluation of both animal and human data supports the current EU classification as irritant above 5% (R36/38) and as corrosive above 10% (R34).
Based on the systematic animal and human study data as well as on the scarcity of alleged sensitisation cases reported from the market (also taking into account that in the past hypochlorite solutions were associated with skin sensitisation owing to the presence of chromium salts) it is concluded that sodium hypochlorite does not pose a skin sensitisation hazard.

Short-term toxicity

No standard 28-day or 90-day repeated dose toxicity studies on sodium hypochlorite in animals by the oral route have been reported. However, the data available from non-standard studies are sufficient to derive a NOAEL for sodium hypochlorite by this route of exposure. The dermal exposure studies reflect the reversible irritant effects of sodium hypochlorite at the doses tested.

Oral

No systemic effects have been observed in any of the studies reported. A general decrease of body weight or body weight gain was usually observed following treatment with the highest doses used, most probably due to a secondary effect linked to low water consumption.

In male and female rats treated with 0.2% and 0.4% of sodium hypochlorite in drinking water for 13 weeks, a decrease in body weight and in specific organ weights, associated with some biochemical changes, were reported. A NOAEL of 0.1% of sodium hypochlorite (950 mg/L available chlorine or 47.5 mg/kg bw/d) can be derived.

Dermal

Daily exposure of mice to 0.1% sodium hypochlorite solution for 10 minutes on four consecutive days caused an increase in epidermal thickness.

No effects were observed in the dermal studies except for specific skin toxicity in guinea pigs (marginally lowered *in vitro* basal cell viability) of uncertain toxicological relevance at 0.5% sodium hypochlorite solution which is related to the acute irritant effects of the substance. In the same study, epidermal hyperplasia was observed following 14 days exposure (8 hours/day) to 0.1% sodium hypochlorite solution, but not following one, four or seven days of exposure. Exposure to 0.5% sodium hypochlorite solution for 8 hours/day for seven or fourteen days caused significant epidermal hyperplasia, but not after 1 or 4 days.
No effects were observed after exposure of guinea pigs to 0.125% sodium hypochlorite solution for up to 8 weeks.

The NOAEL for repeated dermal exposure to sodium hypochlorite solution is related to its cytotoxicity/ irritating properties and is dependant on the concentration of the applied solution. Therefore, irritation can be seen as a threshold for dermal toxicity. No dermal toxicity will occur at concentrations of sodium hypochlorite solution that do not cause irritation, either after single or repeated exposure.

A study in mice suggests that ten minutes exposure to 0.1% sodium hypochlorite solution for four days causes an increase in epidermal thickness. However, inconsistencies in the reporting of the study suggest that the finding might be unreliable.

Taking all of these comments into account, it is concluded from the available animal data (which is unreliable and might not adequately reflect human experience) that a very conservative NOAEL for repeated effects following dermal exposure to sodium hypochlorite solution is 0.1%.

There is no information on systemic toxicity following dermal application route. Fully dissociated in water, and immediate oxidising organic molecules, sodium hypochlorite is not expected to pass the skin to become systemic available. The amount of chlorinated substances passing the skin depends on the amount of mobile organochlorine substances being formed on and in the skin.

Genotoxicity

Sodium hypochlorite has 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.
The positive results produced in bacteria assays and the induction of chromosome aberrations (including gaps) and SCE in mammalian cells suggest, even if mammalian cell gene mutation studies are lacking, that sodium hypochlorite may exert an *in vitro* genotoxic activity.
Sodium hypochlorite was without effect in a well-conducted mouse micronucleus assay suggesting that sodium hypochlorite is not genotoxic *in vivo*.
The available data are not conclusive with respect to genotoxicity. However, since sodium hypochlorite has shown lack of carcinogenicity effects (see B.6.5), no additional testing is required.

Long-term toxicity and carcinogenicity

In long term carcinogenicity studies, sodium hypochlorite administered in the drinking water did not increase the proportion of F344 rats and B6C3F1 mice with tumours. Under the conditions of the 2 year NTP drinking water study there was no evidence of carcinogenic activity of chlorinated water in male rats or in male and female mice. However, the study concluded that there was equivocal evidence of carcinogenic activity of chlorinated water in female rats based on a marginal statistical increase in the incidence of mononuclear cell leukemia. Similarly non-dose dependant increases in lymphoma/leukemia were found in female Sprague-Dawley rats in another long term rodent bio-assay with chlorinated drinking water. This study was deemed suggestive but inconclusive by its authors. Drinking water containing 100 mg/L chlorine was tested for carcinogenicity in a multigeneration study in male and female BDII rats. No increase in the incidence of tumours was seen in the treated animals relative to controls through five generations. Taking into account all the available information, it can be concluded that carcinogenicity is not a relevant endpoint for the oral route.

No human data are available on carcinogenicity and the only data are related to chlorinated drinking water for which the epidemiological data are not sufficient to suggest a causal relationship between the use of chorinated drinking water and increased cancer risk.

The International Agency for Research on Cancer (IARC, 1991) has concluded that there is inadequate evidence for the carcinogenicity of sodium hypochlorite in animals and that sodium hypochlorite is not classifiable as to its carcinogenicity in humans (Group 3). This conclusion is still valid, taken into account the more recent available data.

Reproductive and developmental toxicity

There are no relevant studies of sodium hypochlorite per se looking at its reproductive toxicity potential in animals. However, relevant studies have been conducted using chlorine as the test substance, administered in solution by gavage or in drinking water. In a teratogenicity study, in which exposure was confined to the gestation period, no significant differences in the incidence of skeletal or soft tissue abnormalities were observed in treated groups when compared to controls. A small, but statistically significant increase in sperm head abnormalities was seen in mice, although the effect was not dose-dependant. However, no effects were seen in a well conducted one-generation reproductive toxicity study in rats up to a concentration of 5 mg/kg bw of available chlorine (maximum dose tested). Long-term toxicity studies provide also additional assurance that the substance is not a reproductive toxicant as they did not identify the testes or ovaries as target organs.

The Carlton study appears relatively more updated and reliable with a number of animals more suitable for statistical analysis. There are no studies performed at dose levels able to induce systemic toxicity. A pragmatic approach is to accept the NOAEL derived from the Carlton study because it is the best study available even if no severe effects are shown in all dosed groups.

Although limited data are available in animals, the available studies are sufficient in their design and quality to draw the conclusion that there is no evidence to suggest that sodium hypochlorite would present adverse effects on development or fertility. Similarly, no such evidence is forthcoming from epidemiological studies on populations consuming chlorinated drinking water.

(Delayed) neurotoxicity

No specific studies are available. In the summaries of all other studies described in the RAR no neurotoxic effects were reported.

Further toxicological studies

The available data show no carcinogenic effect due to topical application of sodium hypochlorite solution at different concentrations. There is an indication, supported by a poorly described study, of co-carcinogenicity using NaClO as a promoting agent. In the same study, 4-nitroquinolene 1-oxide alone did not show carcinogenicity, suggestive of methodological problems or unusual responses of the mouse strain used.

Two other studies using different initiators did not show promoting effects of NaClO, although the doses employed were lower. In one of these studies, a dermal two-stage carcinogenicity study, a clear no effect

level was observed in female mice treated twice weekly for 51 weeks with 1% sodium hypochlorite solution. No animals died and no epidermal hyperplasia was observed in the treated group.

Some incidental effects related to the immune system were reported in rats and mice administered with low doses of NaClO in chlorinated water for 12 or 17 weeks. These effects were observed in rats receiving chlorinated water containing 15-30 mg/L available chlorine (about 0.75-1.5 mg/kg bw/day). However, in long term studies, no differences were reported between treated and controls for haematological analysis or thymus weight in rats given higher doses of NaClO in water (275 mg/L available chlorine or 15 mg/kg bw/d). It is not possible to derive a no effect level for this specific end-point.

The studies which are relevant for the derivation of the ADI, ARfD and AOEL are summarised below. The NOAELs are expressed in mg/kg bw/day as available chlorine. The LOAELs are in most cases estimated based on the NOAELs, since the exact values are not reported in the limited study summaries in the RAR.

Short-term toxicity studies

Duration	Species	Route	NOAEL (as av. Cl_2 in mg/kg/day)	LOAEL (as av. Cl_2 in mg/kg/day)	Note / Critical effects	Reference	Val*
13 weeks	Rat	Oral, drinking water	47.5 (m) 54 (f)	≈ 95 (m) ≈ 108 (f)	Decreased body weight and specific organ weight, associated with some biochemical changes.	Hasegawa, 1986	2
92 days	Rat	Oral, drinking water	44.4 (m) 97.1 (f)	≈ 88 (m) ≈ 194 (f)	Decreased body weight gain.	Furukawa, 1980	2

* Validity of the study

Long-term toxicity studies

Duration	Species	NOAEL (as av. Cl_2 in mg/kg bw/day)	LOAEL (as av. Cl_2 in mg/kg bw/day)	Notes / Results	Reference	Val*
Oral, drinking water						
104 weeks	Rat	≥ 58.7 (m) 67.0 (f)	- (NOAEL highest dose) ≈ 134 (f)	Well conducted study / negative	Hasegawa and Kurokawa, 1986	1
2 years	Rat	≥ 15 (m) ≥ 16 (f)	- (NOAEL is the highest tested dose)	Well conducted study / equivocal evidence in females	NTP, 1992	1
104 weeks	Rat	**		Well conducted study / Not dose-related increase in leukemia-lymphomas in female group	Soffritti, 1997	1

Duration	Species	NOAEL (as av. Cl_2 in mg/kg bw/day)	LOAEL (as av. Cl_2 in mg/kg bw/day)	Notes / Results	Reference	Val*
Oral, drinking water						
2 years (5 genera-tions)	Rat	**		Sample analysis, necropsy / negative	Druckrey, 1968	3
103 weeks	Mouse	78.3 (m) 95.4 (f)	≈ 156 (m) ≈ 190 (f)	Well conducted study / negative	Kurokawa, 1986	1
2 years	Mouse	15.3 (m) 17 (f)	26.5 (m) 29 (f)	Well conducted study / negative	NTP, 1992	1
Inhalation, chlorine gas						
2 years	Rat	***	***	Supplemental information / negative	Wolf, 1995	3
2 years	Mouse	***	***	Supplemental information / negative	Wolf, 1995	3

* Validity of the study

** In the RAR, no NOEL was derived for this study (carcinogenicity study).

*** The LOAEL for respiratory irritation was 0.4 ppm chlorine gas

Reproduction and teratogenicity studies

Type of study	Result	Notes	Reference	VAL*
Reproduction toxicity				
Gavage, rat, 1 generation	No effects observed NOAEL ≥ 5 mg/kg bw/d as av. chlorine	a.s.: Aq. chlorine Well conducted study NOAEL determined	Carlton, 1986	2
Drinking water, rat, 2 years, 5 generations	No effects observed	German paper, only limited data available	Druckrey, 1968	3
Sperm head assay, mutagenicity mice	Doubtful positive	Data well presented	Meier, 1985	2
^{3}H-thymidine incorporation tests	Equivocal result	Limited protocol and data	Abdel-Rahman, 1984	4
Embryo/fetal toxicity and teratogenicity				
Drinking water, developmental effects, rat foetus	Minor effects at 100 mg/L	Study well conducted	Abdel-Rahman, 1982	2
Tap water, foetal devel. mice	No evidence	Limited available data	Chernoff, 1979	3
Tap water, foetal devel. mice	No evidence	Limited available data	Staples, 1979	3

* Val: validity of the study

Further toxicological studies

Duration	Species	Dose	Results	Notes	Reference	Val*
Dermal application						
450 days	female ddN mouse	NaClO 100 g/L ; NQ-oxide 0.25% - combined	Skin cancers in combined groups	Skin two-stage model promoting test; the strain mouse is unusual	Hayatsu, 1971	3
51 weeks	Female Sencar mouse	NaClO 10000 mg/L ; DMBA (initiator) 20 nM - combined	Negative NOAEL 1% NaClO solution	Skin two-stage model promoting test	Kurokawa, 1984	2
104 weeks	NMRI mouse	NaClO 10000 mg/L ; BP (initiator) 750, 1500 µg - combined	Cancer decrease in combined group	Promoting test, lack of data	Pfeiffer, 1978	3

* Val: validity of the study

B.6.10.3 ADI

The calculation of the ADI is based on the highest dose at which no adverse effect is observed in the most appropriate study in the most sensitive species. Sodium hypochlorite was tested in several subacute, semi-chronic, and chronic toxicity studies in rats and mice, providing the basis for the establishment of the ADI. The results show that only relatively slight systemic effects were observed (decreased body weight (gain) and specific organ weights) and that the semi-chronic and chronic NOAELs are in the same order of magnitude.

The NTP (1992) study is used as the key study for deriving a NOAEL for risk characterisation. From this study a NOAEL of 15 mg/kg bw/d (or 275 mg/L available chlorine) administered in drinking water can be identified for repeated oral exposure in the rat following exposure to sodium hypochlorite. Taking the NOAELs from the semi-chronic and chronic studies into account, it is considered too conservative to use the NOAEL of 5 mg/kg bw/day from the 1-generation reproduction study, since no effects at all were observed in this study and the 'true' NOAEL is likely to be higher.

The NOAEL of 15 mg/kg bw/day is used as a starting point for the establishment of the ADI. Application of a safety factor for inter- and intraspecies differences of 100 results in an ADI of 0.15 mg/kg bw/day (as available chlorine).

B.6.10.4 ARfD (acute reference dose)

For sodium hypochlorite no ARfD is derived. No classification is required based on the acute oral toxicity studies and there are no indications for acute effects from the repeated dose toxicity studies.

B.6.10.5 AOEL

For establishing an AOEL for sodium hypochlorite, chronic studies are considered to be the most relevant, since sodium hypochlorite can be used frequently during the whole year in mushrooms (see B.6.14).

The NTP (1992) study is used as the key study for deriving a NOAEL for risk characterisation.

From this study a NOAEL of 15 mg/kg bw/d (or 275 mg/L available chlorine) administered in drinking water can be identified for repeated oral exposure in the rat following exposure to sodium hypochlorite, i.e. the same dose level used as starting point for the establishment of the ADI. Using the same safety factor, but correcting for oral absorption (approximately 40%), results in an AOEL for sodium hypochlorite of 0.06 mg/kg bw/day (as available chlorine).

Inhalation exposure

The operator and worker will not be exposed to vapours, see B.6.14, but for reasons of completeness, the available information on the exposure limit in air will be presented here.

Although there is no occupational exposure limit (OEL) for sodium hypochlorite, most countries adopted the OEL (or MAK or TLV) for chlorine in Germany, i.e. 1.5 mg/m^3 in air. The table below shows the occupational exposure limits for chlorine in European countries. In almost all countries, the limit for long term exposure (8 hours TWA – Time Weight Average) is 0.5 ppmV or 1.5 mg/m^3, with the only exception of The Netherlands, where limits are higher. In some cases, a short term exposure limit (15 minutes STEL - Short-Term Exposure Limit) of 1 ppmV is applied. A draft proposal of European Directive on occupational exposure limits establishes as indicative limit for short term exposure (15 minutes) to chlorine a value of 0.5 ppmV.

Country	Limit		Reference
	Long-term exposure	Short-term exposure	
Austria	MAK: 0.5 ppmV; 1.5 mg/m3	Peak limit value is maximum 2x MAK, for up to 5 min.; permitted 8x per shift.	MAK Liste. 1994 Amtliche Nachrichten Arbeit-Gesundheit-Soziales, 48. Jahrgang, Sondernummer 2/1993
Belgium	8-hour TWA: 0.5 ppmV; 1.5 mg/m^3	15-minute STEL: 1 ppmV, 2.9 mg/m^3	Moniteur Belge/Belgisch Staatsblad 14.06.1995
Denmark	TWA: 0.5 ppmV, 1.5 mg/m^3		National Labour Inspectorate. Exposure Limit Values For Substances and Materials. Instruction No. 3.1.0.2. Dec 1996
Finland	8-hour limit: 0.5 ppmV, 1.5 mg/m^3	15-minute limit: 1 ppmV, 2.9 mg/m^3	HTP-Arvot 1996, Turvallisuustiedote 25, Työministeriö, Kemian Työsuojeluneuvottelukunta, Tampere 1996, as amended
France		VLE (valeur limite d'exposition): 1 ppmV, 3 mg/m^3	INRS, Valeurs limites d'exposition professionelle aux agents chimiques en France 1996 - Cahiers de Notes Documentaires ND 1945-153-93

Country	Limit		Reference
	Long-term exposure	Short-term exposure	
Germany	TRGS 900 limit value: 0.5 ppmV, 1.5 mg/m^3	MAK value should never be exceeded. AGS (Commission on Hazardous Substances) can establish other peak limitations for individual substances.	TRGS 900, Limit Values in the Ambient Air at the Workplace -- MAK and TRK values (Bundesarbeitsblatt 10/1996 as last amended in 5/1998)
Ireland	8-hour OEL (TWA): 0.5 ppmV, 1.5 mg/m^3	15-minute OEL (STEL): 1 ppmV, 3 mg/m^3	Code of Practice for the Safety, Health and Welfare at Work [Chemical Agents] Regulations, 1997
The Netherlands	MAC TWA (TGG): 1 ppmV, 3 mg/m^3		National MAC List 1997-98
Norway	threshold limit value: 0.5 ppmV, 1.5 mg/m^3	ceiling value: 1 ppmV, 3 mg/m^3	Administrative Norms for Contaminants in the Workplace 1996 (Arbeidstilsynet best. nr. 361)
Spain	VLA-ED: 0.5 ppmV, 3 mg/m^3	VLA-EL: 1 ppmV, 3 mg/m^3	Instituto nacional de seguridad e de higiene en el trabajo
Sweden	Level Limit Value (NGV) 0.5 ppmV, 1.5 mg/m^3	Ceiling Limit Value (TGV) 1 ppmV, 3 mg/m^3	National Board of Occupational Safety and Health. Occupational Exposure Limit Values. 1996 (AFS 1996:2)
Switzerland	TWA 0.5 ppmV, 1.5 mg/m^3	STEL 1 ppmV, 3 mg/m^3	SUVA. Grenzwerte am Arbeitsplatz 1997 (Limit Values at the Workplace 1997]) SUVA-Publikation 1903.d, Jan 1997
United Kingdom	TWA 0.5 ppmV, 1.5 mg/m^3	STEL 1 ppmV, 2.9 mg/m^3	Health and Safety Executive. EH40/98; Occupational Exposure Limits 1998

Note: Italy, Greece and Portugal have adopted the limit values published by ACGIH (American Conference of Governmental Industrial Hygienists), corresponding, for chlorine, to TLV-TWA of 0.5 ppmV or 1.5 mg/m^3 (for long term exposure) and TLV-STEL of 1 ppmV or 3 mg/m^3 (for short term exposure).

B.6.10.6 Drinking water limit

According to Council Directive 97/57/EC, exposure to sodium hypochlorite through drinking water should account for not more than 10% of the ADI. If it is assumed that the average daily consumption of water amounts to 2 litre per person of 60 kilogram, a drinking water limit of ((60 x 0.14)/10)/2 mg/L, i.e. 0.42 mg/L can be established. According to Document 8064/VI/79 of the European Commission, the EU drinking water limit for pesticides of 0.1 μg/l would be applicable for sodium hypochlorite.
However, sodium hypochlorite is also used for water disinfection and the legally permissible quantity of available chlorine in the water is set at values between 0.1-0.5 mg/l in many European countries. These values are well below the concentration of 5 mg/l of available chlorine indicated by the WHO as a guideline value. Actual figures are not available for Europe but are estimated to be below 0.1 mg/l (for comparison, the maximum content of active chlorine in swimming pools is e.g. in Italy 1.2 ppm and in the Netherlands 1.5 ppm).

B.6.11 ACUTE TOXICITY INCLUDING SKIN SENSITISATION OF PREPARATIONS (ANNEX IIIA 7.1)

No Annex III dossier was submitted. The plant protection product is the same as the active substance.

B.6.12 DERMAL ABSORPTION (ANNEX IIIA 7.3)

Dermal exposure to solutions of sodium hypochlorite might also lead to dermal absorption. The concentrate and spray dilution contain predominantly hypochlorite ions (see B.2.1.18). There are no data to indicate the degree of absorption of hypochlorite ions. However, the potential of hypochlorite solutions to penetrate the skin is low given its reactivity to proteinaceus material. The absorption has therefore been assessed by assuming a default fraction of 10% that is penetrating the skin. This is considered to be a conservative assumption based on the indicated low potential for dermal penetration given the reactivity and polarity of the substance.

B.6.13 TOXICOLOGICAL DATA ON NON ACTIVE SUBSTANCES (ANNEX IIIA 7.4 AND POINT 4 OF THE INTRODUCTION)

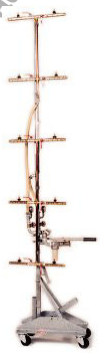
Not applicable.

B.6.14 EXPOSURE DATA (ANNEX IIIA 7.2)

Sodium hypochlorite is applied in the cultivation of mushrooms. For a good understanding of operator, bystander and worker exposure, it is important to know how mushrooms are cultivated and how sodium hypochlorite is applied. Therefore, some background information is presented below.

A mushroom farm has several growing rooms. A growing room is a closed room with two racks, carrying about 5 shelves with growing beds, one above the other, see pictures below. In each growing room, the cultivation of mushrooms is in a different stage of the cultivation process.

Water is essential for mushroom growing. Watering is possible from both sides of the bed as well as from just one side. Sodium hypochlorite can be added to the water used to irrigate the mushroom crop. The sodium hypochlorite treated water is applied to mushrooms with a watering tree (See below).



Watering trees for mushrooms are constructed from a hot-dip galvanized frame with swivel castors. The frame is hinged to allow the upper part to fold down. This allows the watering tree to be easily manoeuvred in and out of the growing rooms. The watering tree is supplied with two types of spray lances, one to water from two sides and the other to water from one side. Depending on the required capacity, the number of spray lances per growing bed can be adjusted, and various types of nozzles used. The concentrate is diluted using a diluter or large volume tank and pump.

Manually applying sodium hypochlorite with a watering tree is a worst-case scenario. Depending on the size of the mushroom farm, application will be mechanically or manually, but for example in the Netherlands all mushroom farms perform the watering of the mushrooms and the application of e.g. sodium hypochlorite mechanically without an operator present in the growing room (using fully

automated equipment). Since the cultivation of mushrooms can differ between the member states (e.g. longer cultivation cycle), it is described in detail below what the assumptions have been for the risk assessment in the DAR (using as worst-case scenario the manual application with a watering tree), and, if necessary, each member state can adjust the risk assessment for national authorisations.

The harvesting period in the cultivation of mushrooms is called flush. The first harvest is the first flush, starting about 14-18 days after casing. A farmer will harvest 2-3 flushes per growing room per cultivation cycle of about 5 weeks. At the end of a cultivation cycle, the growing room is steamed for 8 hours at about 70 °C and emptied. On average there are about 10 cultivation cycles a year.
The decision to water is usually based on the casing moisture. Especially after casing as much water as possible will be added to the growing beds. Farmers try not to water mushrooms any larger than pea size i.e. they water in between flushes on 2-4 consecutive days and then go without for 7/8 days until the flush has been picked off. Sodium hypochlorite can be added to the irrigation water from appearance of mushrooms on the beds until the mushrooms are pea size. Sodium hypochlorite will not be applied before the first flush ('problems' with Pseudomonas depend on e.g. temperature and humidity and application of sodium hypochlorite will not be necessary in each cultivation cycle and therefore sodium hypochlorite is not applied preventatively). Sodium hypochlorite can be applied between the first and second flush (the third flush will result in a significantly lower amount of mushrooms/m^2 than the first and second flush. Pseudomonas will grow there where the mushrooms touch each other and this will happen infrequently during the third flush). This means that as worst-case, sodium hypochlorite can be applied 3-4 times per cultivation cycle. Since a mushroom farm has several growing rooms, each in a different stage of the cultivation process, and there are about 10 cultivation cycles a year in a growing room, the operator can be chronically exposed to sodium hypochlorite.

A mushroom farm where manual application is performed will be relatively small and the operator will apply sodium hypochlorite only in one growing room per day (because each growing room is in a different stage of the cultivation process). A growing room has a cultivation surface of about 200 m^2. Since there are about 5 shelves with growing beds, the actual cultivation surface is about 1000 m^2. In other words, the treated area is about 1000 m^2. The operator can treat this area in about half an hour (based on information from the mushroom practice in the Netherlands). Since, as worst-case, it is possible that the operator applies sodium hypochlorite in the morning and in the afternoon, the risk assessment will be based on a treated area of 2000 m^2.

The sodium hypochlorite solution (10-12% available chlorine) is diluted 330 – 400 times. Sodium hypochlorite (NaClO) and hypochlorite ion (ClO^-) will not evaporate from water; only chlorine (Cl_2) or hypochlorous acid (HOCl) can potentially evaporate. The pH of the concentrate is >12 and the only species effectively present is ClO^- (see B.2.1.18). The pH of the spray dilution will still be >9 and also in the spray dilution ClO^- is the species present. This means that the operator will not be exposed to evaporated species.

Inhalation exposure is only possible in case of formation of sodium hypochlorite aerosols, which could lead to local irritation effects of the respiratory tract. Since sodium hypochlorite is sprayed with a relatively large droplet size, inhalation exposure will be relatively small and will be estimated with the Dutch greenhouse model, see below.

B.6.14.1 Operator exposure (IIIA 7.2.1)

The application on mushrooms is an indoor application. For indoor applications, no adequate module is available in UK-POEM and in the German model. Therefore, the operator exposure will be estimated with the Dutch greenhouse model. For risk assessment purposes, the 90th percentile of the Dutch model (NL-90th) is used.

Indoor application on mushrooms

Application technique : upward and downward spraying

Input data

Application rate : 3 kg a.s./ha (as available chlorine)
Treated area : 2000 m^2 (see explanation above)
PPE : reduction factor 0.1

B.6.14.1.1 Exposure estimates with the Dutch model

The model is based on studies published in the scientific literature and on studies performed in The Netherlands and indicative 90th percentiles are deduced from the exposure databases. In the Dutch model a default reduction factor of 0.1 for the use of PPE is used, which is common practice in the EU.

OPERATOR EXPOSURE			**DUTCH GREENHOUSE MODEL**	
form	Sodium hypochlorite solution (10-12% as av. chlorine)		Application including mixing and loading	
a.s.	sodium hypochlorite			
Parameter		**Value**	**Unit**	**References, comments**
MANUAL SPRAYING in greenhouses				
AR	Application rate	3	kg a.s./ha	summary of intended uses
A	Area treated	0.2	ha/ day	Information from practice (default in Dutch model: 1 ha)
Inhalation Exposure				without PPE
SV	Surrogate Exposure Value	1	mg a.s./ kg a.s.	Dutch model
Inhalation Exposure (without PPE)		**0.6**	mg a.s./ day	IE = SV x AR x A

Inhalation Exposure (with PPE)			with PPE
PPE-factor	10		default: 10
Inhalation Exposure (with PPE)	**0.06**	mg a.s./ day	IE(PPE) = (1/PPE factor) x IE
Dermal Exposure			without PPE
SV Surrogate Exposure Value	200	mg a.s./ kg a.s.	Dutch model
Dermal Exposure	**120**	mg a.s./ day	DE = SV x AR x A
Dermal Exposure (with PPE)			with PPE
PPE-factor	10		default (gloves & coverall): 10
Dermal Exposure (with PPE)	**12**	mg a.s./ day	DE(PPE) = (1/PPE-factor) x DE

Internal exposure			
IA Inhalation Absorption	100	%	
DA Dermal Absorption	10	%	
AOEL	4.2	mg a.s./ day	based on 70 kg bw

	Without PPE	**With PPE**	
Internal exposure	[mg a.s. / day]	[mg a.s. / day]	
Inhalation	0.6000	0.0600	IE(int) = IE x (IA/100)
Dermal	12.000	1.200	DE(int) = DE x (DA/100)
Total	**12.600**	**1.260**	**sum**
% AOEL			
Inhalation	14	1	%AOEL = 100 x IE(int) / AOEL
Dermal	286	29	%AOEL = 100 x DE(int) / AOEL
Total	**300**	**30**	**sum**

B.6.14.1.2 Local effects

The possibility of local effects due to repeated dermal exposure to (diluted) hypochlorite solutions is taken into account. It was concluded from the available data that a very conservative NOAEL for repeated effects following dermal exposure to sodium hypochlorite solution is 0.1%. No systemic effects were seen following long-term (51 weeks) dermal exposure to 10000 mg/L (1%) sodium hypochlorite. From the information available, it is not possible to calculate a NOAEL as a dose in mg/kg bw/day. Therefore, the approach chosen is to compare the concentration of sodium hypochlorite solutions to which operators can be repeatedly exposed with the NOAEL of 0.1%.

For repeated dermal exposure, a concentration of 0.1% hypochlorite is chosen as a NOAEL to evaluate local dermal effects. No additional safety margin seems to be required when comparing exposure to the NOEL:

- Local dermal effects, irritation, is the critical endpoint, which does not require a high margin of safety.
- Uncertainty and intra- and interspecies variation: Irritation levels have also been evaluated in humans. Assuming that 1% is the NOAEL at which no irritation was observed, there is an adequate margin of safety and no additional safety margin is needed.
- Although the animal test data is not optimal in design nor conducted to GLP, the confidence in the database is increased by the fact that sodium hypochlorite solutions have been in use for many years with relevant human exposure, without indication of problems.
- Chronic systemic effects through dermal exposure are not expected. No systemic effects have been observed in any of the oral dose studies reported.

The concentrate (10-12% as available chlorine) is classified as corrosive, so it is clear that operators should use PPE when handling the concentrate. Therefore the safety phrase S36/37/39 “Wear suitable protective clothing, gloves and eye/face protection” is proposed in B.4.
The dilution however, contains 30 g available chlorine/hL, which is a solution of 0.03% available chlorine. For the unprotected operator, no local effects are expected when handling the spray dilution.

B.6.14.1.3 Risk assessment for operators

Risk assessment was performed using the 90th percentile from the Dutch model (NL-90th).

Table 6.14.1.4-1 Operator internal exposure and risk assessment

Model	Route	Estimated internal exposure (mg a.s./day)		AOEL Systemic * (mg a.s/day)	% AOEL	
		without PPE	with PPE		without PPE	with PPE
Manual spraying on mushrooms, indoors **						
Dutch-90th	Respiratory	0.60	0.06	4.2	14	1
	Dermal	12.0	1.20	4.2	286	29
	Total	**12.6**	**1.26**	**4.2**	**300**	**30**

* Assuming a body weight of 70 kg
** No suitable module available in UK-POEM and / or German model

B.6.14.2 Bystander exposure (IIIA 7.2.2)

Indoors, no bystander exposure during application is considered, as Good Agricultural Practice requires that the presence of bystanders should be prohibited.

B.6.14.3 Worker exposure (IIIA 7.2.3)

Sodium hypochlorite is stated to be quickly inactivated once it makes contact with the highly organic casing layer. In the spray dilution, the species actually present is the hypochlorite ion and this will react immediately. On contact with organic material, hypochlorite is rapidly degraded and will result in HCl, HClO, ClO_2^- and ClO_3^- and finally Cl^-.

Sodium hypochlorite can be applied until the mushrooms are pea size. From this growth stage until harvest takes about a week. Since sodium hypochlorite degrades rapidly and re-entry activities will be performed at least a week after the last application, worker exposure will be negligible. It should furthermore be taken into account that the growing rooms are continuously ventilated, so inhalation exposure, if this is relevant at all (see B.6.14), during re-entry activities is also negligible.

B.6.14.4 Conclusions on risk assessments for operators, bystanders and workers

Operator

Using the Dutch-90th greenhouse model, a safe use was identified for operators, with PPE (gloves and coverall), for manual spraying on mushrooms, indoors. It should be taken into account that this is a worst-case scenario, since application of sodium hypochlorite will in many cases be performed mechanically without an operator present in the growing room.

Bystander

Indoors, no bystander exposure during application is considered, as Good Agricultural Practice requires that the presence of bystanders should be prohibited.

Worker

No adverse health effects are expected for workers without PPE after application of sodium hypochlorite in mushrooms.

B.6.15 REFERENCES RELIED ON

Section B.6 Toxicology and metabolism (Annex IIA, point 5, Annex IIIA, point 7)

The data/studies/evaluations with regard toxicology and metabolism presented in this DAR are taken from the RAR (Risk Assessment Report) for sodium hypchlorite (November 2007), which was written by Italy under the Existing Substances Regulation. For this DAR, the references referred to in the RAR have not been individually evaluated by the RMS, neither were the references submitted by the notifier.

Annex point/ reference no.	Author(s)	Year	Title Company, report no. Source (where different from company) GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
IIA 5	RAR (unknown)	2007	RISK ASSESSMENT REPORT FOR SODIUM HYPOCHLORITE, DRAFT November 2007 Italy non GLP Published (via ECB website)	N	ECB