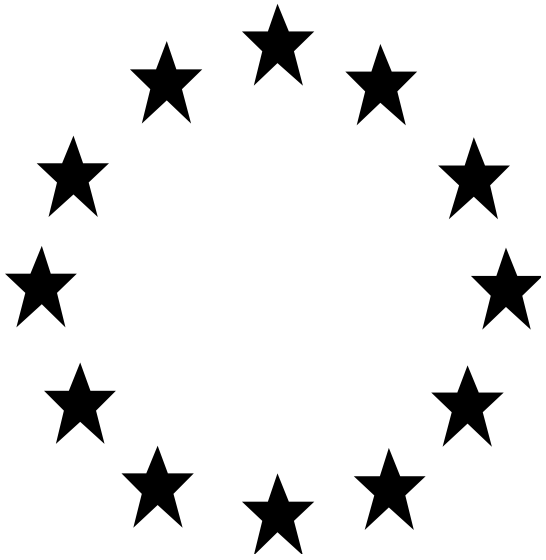

Draft Assessment Report (DAR)

- 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

Volume 3, Annex B, part 5, B9

February 2009

B.9 Ecotoxicology

Introduction

For risk assessment, the notifier only submitted the Risk Assessment Report (RAR) for sodium hypochlorite (Draft November 2007). The assessment in the RAR is based on an extensive literature review. The studies used in the RAR are not available to the RMS and for the acceptability is therefore relied on the summaries from the RAR, in which the following classification was made:

"The validity of the aquatic toxicity data was evaluated according to the criteria recommended by the European authorities and present in IUCLID. The evaluation criteria are defined as:
- Rated 1 = valid without restriction
- Rated 2 = valid with restriction
- Rated 3 = invalid (not reliable)
- Rated 4 = not assignable

The main parameters that need to be documented when describing aquatic toxicity tests for sodium hypochlorite are:

(a) the pH and temperature

The experimental pH and temperature have an important role in the evaluation of aquatic toxicity data as they determine the chemical species present. In water, the hypochlorite ion is in equilibrium with hypochlorous acid, the ratio of each species being pH and temperature dependent. The amount of hypochlorous acid decreases as pH and temperature increase (Hellawell, 1986). At pH 7.0 70% is HOCl whereas at pH 8.0 80% is ClO- when the temperature is 25oC (Taylor, 1993). Therefore in freshwater (characteristic pH range of 6.5-7.2) the dominant form is the more toxic HOCl while at estuarine pH's (7.5-8.2) the reverse is true (Gentile et al., 1976). At pH values above 4.0, Cl2 does not exist - hence when authors have reported that they used chlorine in aquatic toxicity studies conducted at a pH of about pH 7.0, the chemical species present are a mixture of HOCl and ClO-. Temperature also affects the equilibrium between hypochlorous acid and hypochlorite, but to a much lesser extent than pH, with the ionization content (pKa) for hypochlorous acid decreasing from 7.75 at 5oC to 7.63 at 15oC and 7.54 at 25oC. Aquatic organisms are generally more sensitive to chlorine at higher temperature; an inverse relationship between LC50 and temperature was found in fish (Brooks and Seegert, 1977; Scott and Middaugh, 1977). It has been demonstrated that a thermal stress combined with exposure to chlorinated compounds produces a synergetic effect (Thatcher et al., 1976).

(b) the characteristics of the test media

Some characteristics of the test media may also affect the evaluation of aquatic toxicity data e.g. presence of ammonia and organic compounds, hardness and salinity. In clean aqueous test media the chlorine present is likely to exist as hypochlorous acid/hypochlorite ion (i.e. detectable as FAC) decaying rapidly due to reduction and photolysis, e.g. half-life < 2 hours for both hypochlorous acid and the

hypochlorite ion (Taylor, 1993). In natural water there will be more interactions with nitrogen and organic compounds so that the hypochlorous acid/hypochlorite ion decays faster and the chlorine present is likely to be a mixture of FAC and chloramines (Ewell et al, 1986; Klerks and Fraleigh, 1991). The products formed from chlorination of natural water are also a function of salinity. Since sea water typically contains 60 mg/kg bromide, bromination rather than chlorination may predominate as salinity increases (Scott *et al.*, 1980). In sewage the decay of hypochlorous acid is very rapid, with nearly all the chlorine present being as chloramines. The effect of the hardness of the test media on the aquatic toxicity of sodium hypochlorite has not been systematically studied. However it is not considered to be substantial as the dissociation of calcium hypochlorite to the hypochlorite ion is reported as reversible suggesting that the availability of the hypochlorite ion in test media would not be reduced by the presence of calcium ions. Some authors report that variation caused by changes in hardness is less than a factor of 3 in some fish species.

(c) the measured test concentration

Sodium hypochlorite is rapidly hydrolyzed in water and hypochlorous acid/hypochlorite ion concentrations can decay over the duration of the test, by mechanisms shown in sections 2.4 to 2.6. Therefore the initial dosed concentration is not representative of the concentration to which the test organisms have been exposed for the duration of the test. For example Taylor (1993) has shown that the aquatic toxicity of hypochlorite to *Ceriodaphnia dubia* in a flow-through test was 24h LC50 = 0.006 mg/l compared to 0.048 mg/l in a static test - in the latter case the concentration of hypochlorite was believed to be poorly maintained. In freshwater, measured concentrations are usually expressed as Free Available Chlorine (FAC) or Total Residual Chlorine (TRC), which encompass free plus combined chlorine; in saltwater, they are often expressed as Total Residual Oxidant (TRO) or Chlorine-Produced Oxidant (CPO), which encompass free and combined chlorine as well, but also include other oxidative products such as brominated species).

For data to be considered:

* valid without restriction (i.e. rated 1)

test details should include the use of standard, or well-documented methodology, standard or known species, standard end-points for short and long-term toxicity, flow-through system, information on the pH, temperature, dissolved oxygen, number of concentrations, number of animals, duration of the test, measured concentrations of the test substance, performance of the control, clear concentration-effect relationship, statistical method used for calculating the endpoint.

* valid with restriction (i.e. rated 2)

test report should include a description of the methodology, information on the taxonomy of the species, number of concentrations, number of animals, pH, temperature, test duration, oxygen concentration (or the use of a flow-through method such to guarantee its adequate maintenance), measured test concentrations. A semi -static exposure regime can be considered satisfactory provided that the

chemical concentration has been adequately maintained and its fluctuations have properly been monitored and taken into account in the calculation of the endpoint.
* invalid (i.e. rated 3)
test methodology is poorly documented or does not include all the major information needed for 'valid with restriction' rating, or bad performance of the control(s).
* not assignable (i.e. rated 4)
test details reported are insufficient to give the test any rate.
* supportive information. The reason for introducing this additional rating class stems from the complexity and diversity of the multitude of the studies which were not specifically designed for a Risk Assessment procedure as the present one, still they are *per se* good and reliable studies well conducted and described. We didn't want to waste useful indication of toxicity from these studies which can support the discussion in the PNEC calculation. Examples are tests conducted under intermittent exposure, where effect are evaluated after a standard period (e.g. 96h) but refer to a much shorter and discontinuous exposure (in the order of minutes for few times a day), or cases when no clearly identified endpoint can be retrieved for short or long-term toxicity (e.g. LC50/NOEC can only be expressed as "smaller than" or "greater than"). These kind of data, although not adequate as such to derive a PNEC, can provide useful indication of toxicity and are retrieved as supportive information."

For the acceptability of the study, the classification from the RAR is adopted concerning the reliability of the study based on measurements, analytical data, guidelines, etc. However, since the defended uses in the RAR are considerably different from the proposed use in mushrooms and the risk assessment in the RAR (PNEC derivation) cannot be used for current risk assessment, the acceptability or usefulness of endpoints or set-up of the studies are reconsidered for the proposed use.

Although only the RAR was submitted and used for risk assessment (see Volume 2), summaries of the underlying studies are given, including the original reference in the RAR, in order to be able to refer to certain studies or endpoints.

B.9.1 Effects on birds (Annex IIA 8.1; Annex IIIA 10.1)

No studies were submitted

Risk assessment

Exposure to birds is negligible, only exposure via drinking water is expected. Since low concentrations in surface water of 0.0086 µg/L are calculated, it is not expected that birds are at risk via drinking water. Therefore the risk to birds is acceptable.

B.9.2 Effects on aquatic organisms (Annex IIA 8.2; Annex IIIA 10.2)

In the summaries of the studies, the terms 'Free available chlorine (FAC), Total residual chlorine (TRC), Total Residual oxidated (TRO) and Chlorine-produced oxidant (CPO) are used, but not reported in every study. When not reported it is assumed to be TRC. For the risk assessment, the endpoints expressed in TRC (or TRO for salt water) are used for risk assessment. When a concentration is only expressed in FAC, this can be used as a worst-case, since this concentration is always equal to or lower than TRC.

B.9.2.1 Effects on algae (Annex IIA 8.4; Annex IIIA 10.2.2.3)

STUDY IIA 8.4/001

Reference/notifier	: Kott and Edlis (1969)	GLP statement	: no
Type of study	: algae, growth inhibition	Guideline	: -
Year of execution	: -	Acceptability	: 3
Test substance	: hypochlorite		

Summary from RAR

Kott and Edlis (1969) ran a short-term test with *Chlorella* to determine the concentration needed to inhibit its growth. One litre Chlorella solutions (containing 225 cells/mm3) were maintained at 28-32oC for a 20 hour exposure (in the dark) to two concentrations of hypochlorite The concentrations of chlorine were measured by amperometric titration and were initially 0.2 mg/l and 0.6 mg/l - these were checked and readjusted after 8 hours of contact (no information is given about the decay curve or concentration maintenance at the end of the test). The data were presented as % kill of algae after 20h: at 0.2 mg/l the % kill was 26.8%, whereas at 0.6 mg/l the % kill was 43.0%. The test report does not provide sufficient information and the test methodology does not meet the requirement for a valid test.

Remarks by RMS

The study is not valid for risk assessment

STUDY IIA 8.4/002

Reference/notifier	: Brooks and Liptak (1979)	GLP statement	: no
Type of study	: Chlorophyll a depletion	Guideline	: -
Year of execution	: -	Acceptability	: 3
Test substance	: chlorine		

Summary from RAR

The study by Brooks and Liptak (1979) reports the results of a 30 minutes static test. The endpoint measured was chlorophyll *a* depletion. The experimental conditions are not sufficiently described.

Remarks by RMS

The study is not valid for risk assessment

STUDY IIA 8.4/003

Reference/notifier	: Videau et al (1979)	GLP statement	: no
Type of study	: *Dunaliella primolecta*	Guideline	: -
Year of execution	: -	Acceptability	: 3
Test substance	: hypochlorite		

Summary from RAR

The effects of chlorine to three phytoplanktonic algae species were tested by Videau et al. (1979) in static tests with filtered sea water, aimed to evaluate a number of variables. The most sensitive species was *Dunaliella primolecta*, for which a initial dose of 400 µg/l TRO caused 50% mortality in 24 hours. After 3 hours from dosing, free chlorine had disappeared in chlorinated water below 500 µg/l. Based on their experimental results, the authors extrapolated that in a natural medium, having a population of 1 cell/ml, mortality would reach 65% after 24 h exposure at 200 µg/l.

Remarks by RMS

Sine the exposure is uncertain and no valid endpoint could be determined, this study is not valid for risk assessment

STUDY IIA 8.4/004

Reference/notifier	: Gentile et al (1976)	GLP statement	: no
Type of study	: pytoplankton	Guideline	: -
Year of execution	: -	Acceptability	: 3
Test substance	: chlorine		

Summary from RAR

Gentile et al. (1976) report the Cl2 concentrations causing 50% growth reduction in a series of 24 h static tests on 11 phytoplanctonic species; the LC50 ranged from 75 to 330 µg/l. Only the highly concentrated stock solution was measured. Other tests performed on the diatom *Thalassiosira pseudonana,* using different exposure times up to 20 minutes showed that, 48h after exposure to 200 µg/l chlorine, growth was reduced by about 60%.

Remarks by RMS

Sine the exposure is uncertain and no valid endpoint could be determined, this study is not valid for risk assessment

STUDY IIA 8.4/005

Reference/notifier	: Sanders et al (1981)	GLP statement	: no
Type of study	: pytoplankton	Guideline	: -
Year of execution	: -	Acceptability	: 3
Test substance	: hypochlorite	EC50	: 1-10 µg/L

Summary from RAR

Sanders et al (1981) studied the effects of prolonged chlorination on natural marine phytoplankton communities cultivated in large tanks under flow through conditions (semi-field test). To achieve

measurable concentration in the exposure tanks, HOCl was added by single daily additions directly to the tanks, where it degraded within 2 hours (an intermittent exposure was therefore resulting). A 50% reduction in cell density (the most sensitive endpoint) was observed in the 21 day test at concentrations as low as 1-10 µg/l TRC. These data provide evidence of the severe impact of free chlorine on phytoplankton at very low concentrations, even at intermittent exposure (data used as supportive information).

Remarks by RMS

The study can be used as supportive information.

STUDY IIA 8.4/006

Reference/notifier	: Cairns et al. (1990)	GLP statement	: no
Type of study	: Flow-through microcosm	Guideline	: -
Year of execution	:	Acceptability	: 2
Test substance	: Sodium hypochlorite	EC50	>6 µg/L

Summary from RAR

Cairns et al. (1990) used a laboratory multispecies microcosm to study the chronic effects of chlorine (alone or together with ammonia) to naturally derived periphytic communities exposed for 7 days to sodium hypochlorite in a flow-through system. Sodium hypochlorite concentration was expressed as TRC; FAC accounted for 73 ± 19.9%. Chlorine was tested at nominal concentration of 6 and 60 µgTRC/l. Mean measure TRC were 6.3 ± 3.9 µg/l and 56.6 ± 24.5 µg/l in the low and high treatment respectively. The reduction in protozoa species richness was statistically significant (LOEC) at 6 µgTRC/l, while for a reduction of 20%, considered biologically significant, a concentration of 2.7 µgTRC/l was calculated. At 6 µg/l the composition of protozoa communities (number of taxa) changed significantly; since the effect was about 10% we can calculate for this endpoint a NOEC (LOEC/2) = 3 µgTRC/l. The results from this study are interesting because protozoa represent a group with a great diversity in physiology and function; data were judged reliable and relevant but rated 2 because a non-standard test system was used. Non-taxonomic responses were also measured. In vivo fluorescence, used as an index of algal biomass, was significantly reduced (22%) at 6 µgTRC/l (the lowest tested concentration). This data can be used to calculate a NOEC = 3 µgTRC/l as an indication of long-term toxicity to algae.

Remarks by RMS

No NOEC could be derived. LOEC was 6 µg/L (TRC), with 22% effect. Therefore the EC50 can be considered > 6 µg/L

STUDY IIA 8.4/009

Reference/notifier	: Erickson and Foulk 91980)	GLP statement	: no
Type of study	: Flow-through microcosm	Guideline	: -
Year of execution	:	Acceptability	: 3
Test substance	: Sodium hypochlorite		

Summary from RAR

Erickson and Foulk (1980) used outdoor and indoor flow-through systems to evaluate the effects of continuous chlorination (1 year) on entrained estuarine plankton communities consisting of eggs, larvae, algae and juveniles (not better specified). NaOCl was continuously applied at dose levels of 125 to 1441 µg/l, which resulted in concentrations of residual chlorine in the systems below the detection limits of the amperometric analyzer used (10 µg/l). In all treatments a reduction of ATP, measured as indication of biomass, was observed (from 13% to 58%). This result is used as supportive information.

Remarks by RMS

Considering the uncertainty about organism groups tested, exposure and statistical analysis, this study is not acceptable for risk assessment.

B.9.2.2 Effects on aquatic species other than fish and aquatic species field testing

B.9.2.2.1 Acute toxicity to aquatic invertebrates (Annex IIA 8.3.1; Annex IIIA 10.2.2.2)

STUDY IIA 8.3.1.1/01

Reference/notifier	:	Taylor (1993)	GLP statement	:	no
Type of study	:	*Ceriodaphnia dubia*, acute toxicity (24h), flow-through	Guideline	:	-
Year of execution	:	-	Acceptability	:	2
Test substance	:	Sodium hypochlorite	24h LC50	:	5 µg/L

Summary from RAR

Taylor (1993) tested the acute toxicity of various forms of free and combined chlorine to *Ceriodaphnia dubia* in standard 24h toxicity tests, carried out under static and flow through conditions. Sodium hypochlorite was tested at pH 7 for HOCl (70% HOCl and 30% OCl-) and pH 8 for OCl- (80% OCl and 20% HOCl). In static tests the decay of free chlorine was very rapid (1 minute and 7 hours in tests with or without food, respectively) and the results were not considered valid. Flow-through tests (without food) were carried out to maintain a constant concentration over the exposure time.

The toxicity of free chlorine in these tests was much higher: 5 and 6 µg/l for HOCl and OCl -, respectively. These data were judged valid with restriction (rated 2) because the test concentrations were calculated from measured chlorine concentration of the stock solution and dilution ratios, the number of concentrations/replicates are not specified, the performance of the controls not mentioned, and the 24h LC50s determined by graphical interpolation.

Remarks by RMS

Reliability of the study is questionable because of the above-mentioned reasons. The 24h LC50 of 5 µg/L (FAC) can be used as indication for toxicity. This is assumed to be worst-case for TRC.

STUDY IIA 8.3.1.1/02

Reference/notifier	:	Thatcher (1978)	GLP statement	:	no
Type of study	:	Invertebrate acute toxicity, flow-through, including thermal stress	Guideline	:	-
Year of execution	:	-	Acceptability	:	s
Test substance	:	Sodium hypochlorite	96h LC50	:	90 µg/l

Summary from RAR

Thatcher (1978) tested seven species, including shrimps, mysids, amphipods and crabs, in flow through apparatus where animals were simultaneously exposed to chlorinated unfiltered seawater and 5°C thermal stress to mimic a common scenario in the real environment. The most sensitive species was the shrimp *Pandalus goniurus* for which a 96h LC50= 90 µg/l (TRC) was calculated pooling data from different tests. These data have been rated 2 because of data pooling and, above all, because the additional thermal shock might have influenced the sensitivity to sodium hypochlorite.

Remarks by RMS

The toxicity to sodium hypochlorite increases with increasing temperature. A thermal shock could be expected for the uses proposed in the RAR, but not for the proposed use in mushrooms. Since it is very likely that the thermal shock increased the toxicity to sodium hypochlorite, this study is less reliable for the proposed use and can only be used as supportive information.

STUDY IIA 8.3.1.1/03

Reference/notifier	:	Capuzzo et al (1976, 1979, a, b)	GLP statement	:	no
Type of study	:	Invertebrate acute toxicity, flow through, including thermal stress	Guideline	:	-
Year of execution	:	-	Acceptability	:	s
Test substance	:	chlorite			

Summary from RAR

Very short-term exposure to pulse chlorination, with and without thermal stress, has been evaluated by Capuzzo and coworkers (1976, 1979a,b), who conducted flow-through bioassays with *Brachionus plicatilis*, *Acartia tonsa*, and larvae of *Crassostrea virginica* with exposure periods of 30-60 minutes at various temperatures. In bioassays performed at acclimation temperatures, lethality (measured after 48 h after exposure) was LC50= 820, 180 and **120 µg/l,** respectively. For all species tested, except the copepods, the thermal stress showed a synergistic effect with residual chlorine concentration, lowering LC50 values down to **10 µg/l** (ΔT=7.5°C) for *B. plicatilis* and 80µg/l (ΔT=5°C) for *C. virginica.*

Remarks by RMS

The toxicity to sodium hypochlorite increases with increasing temperature. A thermal shock could be expected for the uses proposed in the RAR, but not for the proposed use in mushrooms. Considering the

short exposure period and the uncertainty about the substance tested and the temperature at which was tested (not mentioned in the summary), the study can only be used as supportive information.

STUDY IIA 8.3.1.1/04

Reference/notifier	:	Roberts et al (1975)	GLP statement	:	no
Type of study	:	Invertebrate acute toxicity test	Guideline	:	-
Year of execution	:	-	Acceptability	:	s
Test substance	:	Calcium hypochlorite	96h LC50	:	23 µg/L

Summary from RAR

Four invertebrate species were tested by Roberts et al. (1975) using estuarine river water, chlorinated by addition of Ca(OCl)2 in flow through or static systems. When static systems were used the TRC concentrations were kept as stable as possible by constant addition of the chemical and average values were used to calculate the endpoints. The lowest lethal concentrations to be used as supportive information are extrapolated values for *Mercenaria mercenaria* larvae (48h TL50=1 µg/l TRC, in the static test) and *Crassostrea virginica* juveniles (96hEC50(shell deposition)=23 µg/l TRC, in the flow through test). The authors report that when oyster larvae were exposed intermittently (manual additions at 6-8h intervals) the 48h LT50 was 0.11 mg/l, i.e. two orders of magnitude higher.

Remarks by RMS

Test substance was calcium hypochlorite, not sodium hypochlorite. Expression of endpoints in the summary is confusing and effects are therefore unknown. The 96h LC50 of 23 µg/L can be used as supportive information.

STUDY IIA 8.3.1.1/05

Reference/notifier	:	Roberts and Gleeson (1978)	GLP statement	:	no
Type of study	:	Invertebrate acute toxicity test	Guideline	:	-
Year of execution	:	-	Acceptability	:	2
Test substance	:	Calcium hypochlorite	48 h LC50	:	26 µg/L

Summary from RAR

Roberts and Gleeson (1978) tested the toxicity in oyster and copepods when continuously exposed for 48 hours to Ca (OCl)2 in flowing estuarine river water. For *Crassostrea virginica* larvae the 48h EC50 was 26 µg/l (TRC) and for adults of *Acartia tonsa* the lowest 48h LC50 was 29 µg/l (TRC) as residual chlorine measured amperometrically. In the absence of other, more reliable studies with the relevant chemical species (sodium hypochlorite) it was decided to use these data, because they provide valuable information for the toxicity assessment of chlorinated natural water, and assigned these data the rate 'valid with restriction' because of the different parent compound.

Remarks by RMS

Conclusion from the RAR can be adopted.

B.9.2.2.2 Chronic toxicity to aquatic invertebrates (Annex IIA 8.3.2)

STUDY IIA 8.3.2/001

Reference/notifier	:	Martin et al (1993)	GLP statement	:	no
Type of study	:	*Dreissena polimorpha*, chronic toxicity, static test	Guideline	:	-
Year of execution	:	-	Acceptability	:	3
Test substance	:	Sodium hypochlorite			

Summary in RAR

Martin et al. (1993) measured mortality in *Dreissena polimorfa* exposed in a static test at 21 °C for 20 days in a darkened environment to minimize photolytic loss of sodium hypochlorite. The LC50 after 11 days was about 1 mg/l and remained practically unchanged at the end of the experiment. At 0.5 mg/l mortality was <10% after 20 d; this was the highest concentration with no significant effects, so that it can be considered as NOEC. The same authors (Martin et al., 1993) carried out a longer test at 12 °C, using the same experimental procedure. After 15d exposure at 1 mg/l 50% mortality occurred and at the end of the experiment (29d) it reached 70%. No NOEC has been calculated. In these studies the actual exposure chlorine concentrations are likely to be over-estimated: concentrations were measured and maintained by daily adjustments, but authors did not measure the decay curve and do not specify if initial or average concentrations were used in the endpoint calculation.

Remarks by RMS

Since exposure is uncertain, the study cannot be used for risk assessment.

STUDY IIA 8.3.2/002

Reference/notifier	:	Ramsay et al (1988)	GLP statement	:	no
Type of study	:	*Corbicula fluminea*, chronic toxicity, chronic	Guideline	:	-
Year of execution	:	-	Acceptability	:	3
Test substance	:	Sodium hypochlorite			

Summary in RAR

Ramsay et al. (1988) carried out a field study to measure the time required for continuous chlorination (with sodium hypochlorite) to produce 100% mortality in adult *Corbicula fluminea* (asiatic clam). The TRC was continuously monitored during the flow-through test. At the lowest concentration (0.05 mg/l), 100% of the clams died after 36d exposure. From the effect-time curve we estimated that 50% clams were dead after 8d.

Remarks by RMS

No NOEC is determined. Study only measured time to effect. The study is not valid for risk assessment.

STUDY IIA 8.3.2/003

Reference/notifier	:	Klerk and Fleileigh (1991)	GLP statement	:	no
Type of study	:	*Dreissena polimorfa*, chronic toxicity,	Guideline	:	-
Year of execution	:	-	Acceptability	:	3
Test substance	:	Sodium hypochlorite			

Summary in RAR

Klerk and Freileigh (1991) investigated the long-term toxicity of NaOCl to zebra mussel (*Dreissena polimorfa*) in a number of tests (28d, static renewal and intermittent exposure; 28 and 56 d flow-through), carried out using natural water at different temperatures. In the longer flow-through test, after 56d exposure to the lowest tested concentration (0.08 mg/l as free chlorine), the morality reached 55%. At this concentration, 86% reduction in the filtering frequency was observed, but this effect was not concentration related. For the three treatments (0.08, 0.26, 1.35 mg/l of free Cl2) the LT50 values ranged from 16 to 54 d.

Remarks by RMS

No NOEC is determined. Study only measured time to effect. The study is not valid for risk assessment.

STUDY IIA 8.3.2/004

Reference/notifier	:	Kilgour and Baker (1994)	GLP statement	:	no
Type of study	:	*Dreissena polimorfa*, chronic toxicity,	Guideline	:	-
Year of execution	:	-	Acceptability	:	3
Test substance	:	Sodium hypochlorite			

Summary in RAR

The study by Kilgour and Baker (1994) was designed to evaluate the influence of many variables (season and place of animals' collection and experimental protocol) on the toxicity of sodium hypochlorite to *Dreissena polimorfa*. The test lasted 9 d, during which zebra mussels were exposed in a static system to 5 hypochlorite concentrations that were adjusted daily. The LC50 was calculated using average concentrations estimated on the basis of decay curves. The most sensitive mussels were those collected in late summer, whose 9d LC50 was about 0.5 mg/l (as TRO). It is worth noting that this concentration corresponds to the NOEC in the experiments of Martin et al (1993). The study by Kilgour and Baker is reliable but no NOEC can be derived; it can only be retrieved the LC50 as an indication of long term toxicity (supportive information).

Remarks by RMS

No NOEC is determined. Study only measured time to effect. The study is not valid for risk assessment.

STUDY IIA 8.3.2/005

Reference/notifier	:	Liden et al (1980)	GLP statement	:	no
Type of study	:	invertebrate chronic toxicity	Guideline	:	-
Year of execution	:	-	Acceptability	:	s
Test substance	:	Sodium hypochlorite	LOEC	:	14 µg/L

Summary in RAR

In a field study on chlorinated condenser cooling effluents using mollusk bivalves (Liden et al., 1980), the survival of oysters (*Crassostrea virginica*) and clams (*Rangia cuneata*) maintained at three TRO concentrations for 15 days was not affected at concentration as high as 62 µg/l, while oyster mean shell deposition was significantly reduced in the treated animals. At the lowest test concentration (14 µg/l) a 14% reduction in shell deposition was observed, so that following TGD the NOEC can be estimated as LOEC/2, i.e. 7 µg/l. This data is rated 2 because it was obtained from a non-standard test.

Remarks by RMS

No NOEC could be derived. LOEC after 15 days of exposure was 14 µg/L (TRO). This can be used as supportive information.

STUDY IIA 8.3.2/006

Reference/notifier	:	Scott and Middaugh (1977)	GLP statement	:	no
Type of study	:	*Crassostrea virginica,* chronic toxicity	Guideline	:	-
Year of execution	:	-	Acceptability	:	3
Test substance	:	Sodium hypochlorite		:	

Summary in RAR

Scott and Middaugh (1977) examined the lethal and sublethal effects of chlorine to adult oyster *(Crassostrea virginica*) during seasonal chronic exposures (45-75 days) to incoming estuarine sea water, whose temperature, pH and salinity was naturally fluctuating. Survival was reduced at relatively high concentrations of Chlorine- Produced Oxidants (CPO); a rough estimate is LC10 ~ 160 µg/l CPO on the basis of the concentration-effect curves. On the other hand, severe sublethal effects (mean condition index and gonadal index) were observed also at the lowest tested concentration (140 µg/l CPO), so that the NOEC for these effects would be expected to be <<140 µg/l CPO. Because of the lack of a clear endpoint, these data cannot be used in risk assessment.

Remarks by RMS

No NOEC could be derived. The study is not valid for risk assessment.

STUDY IIA 8.3.2/007

Reference/notifier	:	Scott et al (1979)	GLP statement	:	no
Type of study	:	*Crassostrea virginica,* chronic toxicity	Guideline	:	-
Year of execution	:	-	Acceptability	:	3
Test substance	:	Sodium hypochlorite		:	

Summary in RAR

In another experiment conducted in summer Scott. *et al*. (1979) found that mortality of *C. virginica* was not significantly reduced at 110 µg/l but the high mortality observed in the control make this result unreliable. At the same concentration marked sublethal effects were observed, but no direct concentration-effects relationship was found (not valid data).

Remarks by RMS

No NOEC could be derived. The study is not valid for risk assessment.

B.9.2.3 Effects on fish (Annex 8.2)

B.9.2.3.1 Acute toxicity (Annex IIA 8.2.1; Annex IIIA 10.2.2.1)

STUDY IIA 8.2.1.1/01

Reference/notifier	:	Bass et al (1977), Heath (1978)	GLP statement	:	no
Type of study	:	fish, acute toxicity, pulse exposure	Guideline	:	-
Year of execution	:	-	Acceptability	:	s
Test substance	:	Calcium hypochlorite	96h LC50	:	60 µg/L

Summary in RAR

In two studies (Bass *et al.*, 1977 and Heath, 1978) six fish species (*Salmo gairdneri, Oncorhynchus kisutch, Ictalurus punctatus, Notemigonus crysoleucas, Lepomis macrochirus, and Cyprinus carpio*) were exposed for different time intervals (ranging from 24 to 168 hours) to 40' calcium hypochlorite pulses (3 per day) in flow through tap water at different temperatures. *S. gairdneri* and *I. punctatus* were the most sensitive species in both studies, showing, respectively, an 120h LC50=50 µgTRC/l (at 12 °C) and an 120h**.** LC50= 33 µgTRC/l (at 24°C). At 96h, the LC50 for the trout was 60 µgTRC/l (at 5°C) and 64 µgTRC /l for the channel catfish (at 24°C).

Remarks by RMS

Test substance was calcium hypochlorite and no continuous exposure over 96h took place. FAC is > 50% of TRA. The study can therefore only be used as supportive information.

STUDY IIA 8.2.1.1/02

Reference/notifier	:	Brooks and Seegert (1977)	GLP statement	:	no
Type of study	:	fish, acute toxicity, pulse exposure	Guideline	:	-
Year of execution	:	-	Acceptability	:	s
Test substance	:	Sodium hypochlorite	24h LC50	:	430 µg/L

Summary in RAR

In the paper of Brooks and Seegert (1977) the effects of single (30 min) and multiple (5 min exposure at 3 hours interval) exposure to sodium hypochlorite in tap water with and without the addition of a thermal stress on two species of fish - *Perca flavescens* and *Salmo gairdneri* (juveniles) - have been described. A single 30' dose was more toxic than the triple exposure. Generally, *S. gairdneri* was more sensitive than *Perca flavescens*; after 24h recovery, the LC50 was 990 µg/l. With the addition of a thermal stress, the median lethal concentration lowered to 430 µg/l.

Remarks by RMS

No continuous exposure over 96h took place. The study can therefore only be used as supportive information.

STUDY IIA 8.2.1.1/03

Reference/notifier	:	Seegert and Brooks (1978)	GLP statement	:	no
Type of study	:	fish, acute toxicity, pulse exposure	Guideline	:	-
Year of execution	:	-	Acceptability	:	s
Test substance	:	Sodium hypochlorite	48h LC50	:	1260 µg/L

Summary in RAR

Seegert and Brooks (1978) exposed for 30 min. four fish species – *Oncorhyncus kisutch, Alosa pseudoharengus, Notropis hudsonius* and *Osmerus mordax* - to sodium hypochlorite to various temperatures. Coho salmon (*Oncorhyncus kisutch)* showed a sensitivity comparable with that of rainbow smelt (*Osmerus mordax)* and both were more sensitive than the other two species. As observed in other studies, the LC 50 values showed an inverse relationship with test temperature and a typical species dependent variability; at 10°C the LC50s, measured after 48 hrs, were in the range of 1.26 - 2.41 mg/l.

Remarks by RMS

No continuous exposure over 96h took place. The study can therefore only be used as supportive information.

STUDY IIA 8.2.1.1/04

Reference/notifier	:	Wilde et al (1981a, b)	GLP statement	:	no
Type of study	:	fish, acute toxicity, pulse exposure	Guideline	:	-
Year of execution	:	-	Acceptability	:	s
Test substance	:	Sodium hypochlorite	96h LC50	:	80 µg/L

Summary in RAR

The toxicity of hypochlorite following intermittent exposure was studied by Wilde et al. (1983a,b) in two studies conducted at two different temperatures (27.7 and 21.1 °C). Acute toxicity tests (96h) have been carried out on juvenile and adult fathead minnows (*Pimephales promelas*) and young-of-the-year bluegills (*Lepomis macrochirus*) in a mobile laboratory. For each test, sodium hypochlorite was dosed intermittently (for 1 hour at the beginning of each 24h interval) into a flow-through system using water from a reactor cooling reservoir as dilution water. The six test concentrations, as TRC and FAC, were measured at 10-minute intervals for the 2 hr per day that toxicant residuals were measurable. In the two studies, in the chamber receiving 100% biocide solution**,** FRC (the FAC measured after the reaction) contributed 50-70 and 83.2% to TRC during the 30-minutes period of maximum exposure. The lowest 96h LC50s, determined as intermittent exposure mean, was calculated as 0.08 mg/l for juvenile fathead minnow exposed to 27.7°C.

Remarks by RMS

No continuous exposure over 96h took place. Temperature was high, which increased toxicity. The study can therefore only be used as supportive information.

STUDY IIA 8.2.1.1/05

Reference/notifier	:	Tsai et al (1990)	GLP statement	:	no
Type of study	:	fish, acute toxicity, pulse exposure	Guideline	:	-
Year of execution	:	-	Acceptability	:	s
Test substance	:	Sodium hypochlorite	48h LC50	:	260 µg/L

Summary in RAR

Tsai *et al.* (1990) exposed three species of fish (*Cyprinus carpio, Dorosoma petenense and Gambusia affinis*) of different age classes to sodium hypochlorite in a flow - through exposure system for one hour and evaluated the toxic effects 48h after exposure (fish) and 3 days after fertilization (eggs). *C. carpio* fertilized eggs were far less sensitive than later development stages (one-d-old eggs LC50 = 140 mg/l; 1 week-old prolarvae LC50 = 0.33 mg/l) but hardened fertilized eggs were much less sensitive than 1-h soft eggs (10-h-old eggs LC50 = 158 mg/l; 1-h-old (soft) eggs LC50 = 54 mg/L). Sensitivity of *D. petenense* was slightly lower than that of *C. carpio*: LC50=0.260 mg/l for prolarvae. For *G. affinis* the sensitivity varied over the first year of life: the LC50 was 0.61 mg/l and 1.28 mg/l for 1 week-old postlarvae and 1-year-old young, respectively.

Remarks by RMS

No continuous exposure over 96h took place. The study can therefore only be used as supportive information.

STUDY IIA 8.2.1.1/06

Reference/notifier	:	Mattice et al (1981a)	GLP statement	:	no
Type of study	:	fish, acute toxicity, pulse exposure	Guideline	:	-
Year of execution	:	-	Acceptability	:	s
Test substance	:	Sodium hypochlorite	48h LC50	:	840 µg/L

Summary in RAR

A toxicity testing system was developed by Mattice et al. (1981a) to test the effects of sodium hypochlorite, dosed for 30 and 60 minutes, to the fish *Gambusia affinis*. The LC50, determined after 48 hours, was 1.59 and 0.84 mg/l, respectively.

Remarks by RMS

No continuous exposure over 96h took place. The study can therefore only be used as supportive information.

STUDY IIA 8.2.1.1/07

Reference/notifier	:	Bellanca and Bailey (1977)	GLP statement	:	no
Type of study	:	fish, acute toxicity	Guideline	:	-
Year of execution	:	-	Acceptability	:	1
Test substance	:	Sodium hypochlorite	96h LC50	:	90 µg/L

Summary in RAR

Bellanca and Bailey (1977) evaluated the short-term toxicity of chlorine to the estuarine fish *Leiostomus xanthurus* (ocean spot) in a flow through laboratory experiment, using a continuous flow serial diluter fed with river water. The authors calculated a 96h-TLm (equivalent to an LC50)= 0.090 mg/l of TRC, which consisted principally of free chlorine. This data is rated 1.

Remarks by RMS

Study can be used for risk assessment.

STUDY IIA 8.2.1.1/08

Reference/notifier	:	Thatcher (1978)	GLP statement	:	no
Type of study	:	Fish acute toxicity, flow-through, including thermal stress	Guideline	:	-
Year of execution	:	-	Acceptability	:	s
Test substance	:	Sodium hypochlorite	48h LC50	:	32 µg/L

Summary in RAR

Thatcher (1978) conducted many laboratory flow-through bioassays on 8 species of estuarine and marine fish, belonging to different families including salmonidae, clupeidae and percidae. Since the main purpose of the study was to investigate the impact of chlorinated effluents from power plants, fish were simultaneously exposed to sodium hypochlorite and to a 5°C thermal stress. The 96h LC50 ranged from 0.032 mg/l (as TRO), for the most sensitive species (*Oncorhynchus kisutch*), to 0.167 mg/l (*Gasterosteous aculeatus*). These data were considered relevant for the assessment because heat is

usually associated to chlorine in power plants effluents, but they were rated 2 because the authors report that in a previous study the addition of thermal stress resulted in a toxicity higher than chlorine alone and, moreover, LC50 was calculated pooling data from different tests.

Remarks by RMS

The toxicity to sodium hypochlorite increases with increasing temperature. A thermal shock could be expected for the uses proposed in the RAR, but not for the proposed use in mushrooms. Since it is very likely that the thermal shock increased the toxicity to sodium hypochlorite, this study is less reliable for the proposed use and can only be used as supportive information.

STUDY IIA 8.2.1.1/09

Reference/notifier	:	Roberts et al (1975)	GLP statement	:	no
Type of study	:	fish acute toxicity test	Guideline	:	-
Year of execution	:	-	Acceptability	:	s
Test substance	:	Calcium hypochlorite	96h LC50	:	37 µg/L

Summary from RAR

Roberts et al. (1975) tested the toxicity of continuous exposure of chlorinated river water (salinity 20‰) to three estuarine fish species. The lowest LC50 was calculated for *Menidia menidia*: 96h LC50= 37 µg/l (as TRC). This data was judged valid with restriction because Ca(OCl2) was applied as chlorine source and some information on the experimental procedure is lacking.

Remarks by RMS

Test substance was calcium hypochlorite, not sodium hypochlorite. Expression of endpoints in the summary is confusing and effects are therefore unknown. The 96h LC50 of 37 µg/L can be used as supportive information.

STUDY IIA 8.2.1.1/10

Reference/notifier	:	Middaugh et al (1977)	GLP statement	:	no
Type of study	:	Fish egg acute toxicity test	Guideline	:	-
Year of execution	:	-	Acceptability	:	s
Test substance	:	Calcium hypochlorite	48h EC50	:	8 µg/L

Summary from RAR

Middaugh et al. (1977) tested the toxicity of chlorinated brackish pond water to early life stages of *Morone saxatilis* in a flow-through test. Only data relative to eggs hatchability could be retrieved**.** A rough estimate of the 48h LC50 = 8 µg/l TRC was calculated by us using the authors' raw data relative to percentages of hatched eggs per test concentration. This data can be used as indicative information of eggs sensitivity**.**

Remarks by RMS

Study can only be used as supportive data for egg sensitivity, since no information on exposure is available (substance, TRC or FAC) and endpoints are only about relative hatchability of eggs. No statistical analysis could be performed.

B.9.2.3.2 Chronic toxicity (Annex IIA 8.2.2.)

STUDY IIA 8.2.2/01

Reference/notifier	:	Hermanutz et al (1990)	GLP statement	:	no
Type of study	:	Fish chronic toxicity test	Guideline	:	-
Year of execution	:	-	Acceptability	:	s
Test substance	:	chlorite	NOEC		5 µg/L

Summary from RAR

The long-term toxicity to four standard fish species has been investigated by Hermanutz *et al.* (1990) in two field studies under flow-through conditions, lasting up to 134 days. In the first study, 3 chlorine concentrations were tested with one or two replicate fish pools; in the second study, only two concentrations with no replicates were tested. In all experiments, no effect on survival was observed in any species up to 183 µg/l TRC. In the first experiment, a concentration-effect relationship, although partial, was observed only for the growth endpoint in channel catfish. At the highest concentration tested (52 and 183 µg/l), the mean weight decreased by 25% and 34%, respectively, whereas at the immediately lower concentration (5 µg/l) it was equal to that of the control group. In the second experiment, growth reduction in the same species was observed only at 162 µg/l (37% reduction) but no effect at 53 µg/l. The experiment was not conducted under standard conditions, no statistical analysis of data was carried out by the authors to identify the NOEC or LOEC, and raw data do not allow the estimation of any endpoint useful for the assessment. Therefore we cannot derive any valid endpoint, but considering that 25% effect is biologically significant we can consider the NOEC for growth 5 µg/l and use it as an indication of the long term toxicity.

Remarks by RMS

Experiment was not conducted under standardized conditions, no statistical analysis could be performed and the FAC could not be determined. Also test substance was not mentioned in the RAR. Therefore the study can only be used as supportive information.

STUDY IIA 8.2.2/02

Reference/notifier	:	Liden et al (1980)	GLP statement	:	no
Type of study	:	Fish prolonged toxicity test	Guideline	:	-
Year of execution	:	-	Acceptability	:	3
Test substance	:	Calcium hypochlorite			

Summary from RAR

One prolonged field study has been carried out by Liden et al. (1980) on two different estuarine fish species *Brevoortia tyrannus* and *Leiostomus xanthurus* exposed to chlorinated condenser cooling effluents for 19 and 20 days, respectively. All experiments were carried out in long troughs system where discharge waters, at three levels of chlorination, were continuously pumped, with inflow rates maintained at 0.038 l/s to simulate cooling water retention times observed in the plant's discharge canal. Up to the highest concentration (62 µg/l as TRO) a negligible mortality occurred among *B. tyrannus*, whereas at all TRO concentrations, the survival of *L. xanthurus* was significantly lower than that of control fish. At the

lowest concentration (14 µg/l) mortality occurred in 26% of animals but, because of the lack of a clear concentration-effect relationship, no NOEC or EC10 can be calculated or extrapolated. These data are therefore not usable for assessment.

Remarks by RMS

Study cannot be used for risk assessment.

B.9.2.3.3 Early life stage toxicity test. Analytical data on concentrations in the test media (Annex IIA 8.2.4; Annex IIIA 10.2.5.2)

STUDY IIA 8.2.4/01

Reference/notifier	: Goodman et al (1983)	GLP statement	: no
Type of study	: fish, early life stage toxicity	Guideline	: -
Year of execution	: -	Acceptability	: 1
Test substance	: Sodium hypochlorite	NOEC	40 µg/L

Summary in RAR

Goodman et al. (1983) developed a method for testing the early-life stages of *Menidia peninsulae*, an estuarine fish of the Atherinidae family. They carried out a 28d test starting with 36h old eggs, under flow through conditions using natural seawater diluted with freshwater to a 20%° salinity, and measured the effects of sodium hypochlorite on eggs survival and fry survival and growth. Fry were the most sensitive stage. The authors calculated a NOEC (fry survival)= 0.04 mg CPO/l (CPO is to be considered analogous to TRC measured by other authors in saline waters), concentration at which only 5% of fish died. At this concentration no sublethal effects were evident. The results of this test are considered fully valid (rated 1).

Remarks by RMS

Study is acceptable for risk assessment.

B.9.2.4 Bioconcentration potential in fish (Annex IIA 8.2.6; Annex IIIA 10.2.3)

No study is available. No secondary poisoning exposure is thought to occur with hypochlorite as it is destroyed rapidly in contact with organic as well as inorganic species.

B.9.2.5 Effects on sediment-dwelling organisms (Annex IIA 8.5)

No data were submitted. Since no exposure to sediment is expected, this is not required.

B.9.2.6 Effects on aquatic plants. Analytical data on concentrations in the test media (Annex IIA 8.6)

No data were submitted.

B.9.2.7 Aquatic field testing (Annex IIA, 8.3.3)

STUDY IIA, 8.3.3/001

Reference/notifier	:	Pratt et al (1988)	GLP statement	:	No
Type of study	:	outdoor mesocosm,	Guideline	:	-
Year of execution	:	-	Acceptability	:	2/s
Test substance	:	hypochlorite	NOEC	:	1.5 µg/L

The study concerns a 28 day laboratory microcosm study and a 24 day outdoor mesocosm study in freshwater to determine the long-term toxicity of hypochlorite to freshwater organisms. In the laboratory study the effects of chlorine on the structure and function of a microbial community developed on artificial substrates and derived from naturally colonized substrates was investigated. Six test solutions were delivered continuously to polyethylene test chambers, where the TRC was determined by titration three times a week (virtually all chlorine was present in its free form i.e. FAC = 100%). Nominal chlorine concentrations were 3, 10, 30, 100, 300 µgTRC/l; the corresponding analytical measurements were 2.1, 6.1, 25, 100, 308 µgTRC/l. The measured taxonomic parameter was the number of protozoan species and the non-taxonomic responses included chlorophyll *a* (expression of algal biomass*)*, ATP, total protein, extracellular alkaline phosphatase activity, and potassium. The results from both types of parameter were analyzed using standard statistics.
The lowest NOEC was calculated for the number of protozoan species and for depression of alkaline phosphatase activity (28d NOEC = 2.1 µg TRC/l). The latter response likely reflects the toxicity to bacteria. This study is rated 2 because the test is non-standard.

As far as chlorophyll *a* is concerned, a reliable NOEC cannot be derived because a clear concentration-effect relationship is lacking. However, this appeared to be the most sensitive endpoint, as a significant reduction of chlorophyll *a* (about 50%) was recorded at 2.1 µg/l of TRC (the lowest tested concentration). Since no –dose response relationship was observed; this is considered as supportive information.

In the outdoor study, each enclosure consisted of a 130L polyethylene bag containing lake water and littoral sediment, which provided immigrating pelagic and benthic microorganisms for the colonization of the artificial substrates added. In the enclosures chlorine was introduced as a daily pulse, and decay curves were used to estimate the average chlorine concentration over a 24 h period. The substrates were examined once a week for protozoan species and algal genera. At the end of the exposure period (day 24) the water was sampled for zooplankton enumeration (filtered with a Wisconsin plankton net n. 10) and substrates were sampled for non-taxonomic measures (chlorophyll *a*, total protein, alkaline phosphatase activity) and microscopic examination.
In the 24 d field test, both taxonomic and non-taxonomic parameters showed lower sensitivity than in the laboratory test, likely due to differences in test design (water, source of species and, most important, method or chlorine application). The authors comment that “Possibly the timing of dosing could have maintained communities in a constant state of recovery and therefore made them appear less sensitive to chlorine stress”. At 79 µg TRC/l, neither chlorophyll *a* nor the number of algal genera was reduced

(NOEC). Protozoan species number was not significantly reduced at the lower test concentration, i.e. NOEC = 24 µgTRC/l. The most sensitive endpoint was the zooplankton density (24d NOEC = 1.5 µgTRC/l). Anyhow, the authors report only the number of zooplankton/ml of water without providing any other information about the effects on taxonomic composition of zooplankton community, so that it is not possible to draw any conclusion about the eventual elimination of taxa from the system. Also the potential for system recovery was not evaluated. In this study FAC concentration accounted for 100% of the measured TRC so that the above endpoint can be expressed also as µgFAC/l.
Because of the uncertainty associated to the most sensitive endpoint and likely underestimation of toxicity due to the pulse dosing system, the results of this test should be interpreted with caution. This conclusion is supported by the comparison of the long term zooplankton NOEC from this test (24d NOEC = 1.5 µgFAC(or TRC)/l) with the laboratory short-term toxicity to *ceriodaphnia* (24h LC50 = 5 µgFAC/l) which suggests that a continuous long term exposure of 1.5 µgFAC/l might dramatically affect invertebrate populations. For these reasons data from this study are used as supportive information (rated s).

Remarks by RMS

From this study it appears than zooplankton is the most sensitive group. However, no information on species composition is available. Therefore the results of these study can only be used as supplemental data.

Metabolites

Several reaction products can be formed after exposure to sludge. The most common groups of reaction products are:

1)Trihalomethanes (chloroform, bromoform)
2) Haloacetic acids (monochloracetic acid, monobromoacetic acid)
3) N-chloroamino compounds

In the RAR some information based on studies and QSAR-estimations is available. From the available data it appears that Haloacetic acids are the most toxic ones, with EC50 values for algae (growth) of 70 µg/L for monochloracetic acid and 5.96 µg/L for dibromoacetic acid (OECD 201, GLP study).

With this is formation is it expected that the toxicity of these formation products is not higher than the toxicity of sodium hypochlorite.

B.9.2.8 Summary and risk assessment

B.9.2.8.1 Summary of aquatic toxicity data

Accepted and supportive data on the toxicity of sodium hypochlorite are summarized in table B.9.2.8-1. In the table, the species tested, the endpoint, the rating and the reference from the RAR is given. If possible, it is indicated if the endpoint is expressed in TRC/TRO or FAC.

For algae one laboratory study, one field study and a micro-mesocosm study is available, all indicating toxicity in the range of 1-10 µg/L. The study of Cairns et al (1990) indicate an EC50 of > 6 µg/L (TRC), corresponding to > 4.38 µg/L (FAC). In the microcosm an 28d NOEC of 2.1 µg/L was determined, however, at this concentration a significant reduction in chlorophyll a was found (about 50%). In the mesocosm study, no effects on algae or algae dependent parameters were found at 1.5 µg/L. were found at 1.5 µg/L. However, considering the pulse exposure, this study will be used as supportive information. The most reliable endpoint therefore is based on the study of Cairns et al (1990). All other studies support the findings from this study. Since this study was based on a microcosm study, with continuous exposure for 7 days, the endpoint and a standard assessment factor of 10 will be used for risk assessment

Several acute toxicity studies with invertebrates are available, among which the study of Taylor (1993) obtained the lowest endpoint with *C. dubia*. Although a considerable about of species are tested which show a lower sensitivity to hypochlorite, the assessment factor will not be lowered in the risk assessment, since all studies are rates 'valid with restriction' or 'supportive information. No valid chronic test is available for invertebrates. In the mesocosm of Pratt et al (1988), zooplankton was included, which was considered to be the most sensitive group. However, no further details are available. Sodium hypochlorite is a highly reactive substance with a low DT50 (< 1hour) in water. It is expected to cause mainly acute effects. This is supported by the available (mainly supportive) acute and chronic toxicity data for both invertebrates and fish. A separate chronic endpoint for invertebrates is therefore not required, since the acute endpoint with an assessment factor of 100 will set the risk assessment.

For fish, two valid studies are available (Bellanca and Bailey (1977) and Goodman et al (1983)). The other studies do not contradict these findings. In cases lower endpoints were found, a thermal stress was often included in the test, increasing the toxicity of sodium hypochlorite. For studies in which this was not the case, the assessment factor of 100 (acute) is protective enough to cover for differences in species sensitivity.

Table B.9.2.8-1. The toxicity of sodium hypochlorite to aquatic life. Bold data are the effect concentrations that are chosen for risk assessment.

Species	Parameter	Criterion	Value (µg/L)	Rating	reference
Algae					
Pytoplankton	biomass	21d EC50	1-10 (TRC)	s	Sanders et al (1981_
Algae (multispecies)	Algae biomass	7d EC50	**> 6 (TRC)** > 4.38 (FAC)	2	Cairns et al (1990)

Invertebrates					
Ceriodapnia dubia	mortality	24h LC50	**5 (FAC)**	2	Tylor (1993)
Pandalus goniurus	mortality	96h LC50	90 (TRC)	s	Thatcher (1978)
Crassostrea virginica (larvae)	mortality	48h LC50	80-120 (TRC)	s	Capuzzo et al (1976, 1979 a,b,)
Brachionus plicatilis	mortality	48h LC50	10-820 (TRC)	s	Capuzzo et al (1976, 1979 a,b,)
Acartia tonsa	mortality	48h LC50	180 (TRC)	s	Capuzzo et al (1976, 1979 a,b,)
Crassostrea virginica (juveniles)	Shell deposition	96 h EC50	23 (TRC)	s	Roberts et al (1975)
Crassostrea virginica (larvae)		48h EC50	26 (TRC)	2	Roberts and Gleeson (1978)
Acartia tonsa	mortality	48h LC50	29 (TRC)	2	Roberts and Gleeson (1978)
Crassostrea virginica	Shell deposition	15d LOEC	14 (TRO)	s	Liden et al (1980)
Rangia cuneata	mortality	15d NOEC	62 (TRO)	s	Liden et al (1980)
Fish					
Salmo gairdneri	mortality	96h LC50	60 (TRC) 30 (FAC)	s	Brass et al (1977) Heath (1978)*
Ictalurus punctatus	mortality	96h LC50	64 (TRC) 32 (FAC)	s	Brass et al (1977) Heath (1978)*
Salmo gairdneri (juveniles)	mortality	24h LC50	430	s	Brooks and Seegert (1977)**
Oncorhynchus kisutch *Alosa pseudoharengus* *Notropis hudsonius* *Osmerus mordax*	mortality	48h LC50	1260-2410	s	Seegert and Brooks (1978)
Pimephales promelas (juveniles)	mortality	96h LC50	80 (TRC) >40 (FAC)	s	Wilde et al (1981, a,b)***
Cyprinus caprio (prolarvae)	mortality	48h LC50	260	s	Tsai et al (1990)****
Gambusia affinis (prolarvae)	mortality	48h LC50	610	s	Tsai et al (1990)****
Gambusia affinis	mortality	48h LC50	840	s	Mattice et al (1981)
Leiostomus xanthurus	mortality	96h LC50	**90 (TRC and FAC)**	1	Bellanca and Bailey (1977)
Oncorhynchus kisutch	mortality	48h LC50	32 (TRO)	s	Thatcher (1978)
Gasterosteous aculeatus	mortality	48h LC50	167 (TRO)	s	Thatcher (1978)
Menidia menidia	mortality	96h LC50	37 (TRC)	s	Roberts et al (1975)

Morone saxatilis	hatchability	48h EC50	8 (TRC)	s	Middaugh et al (1977)
Ictalurus punctatus	growth	134d NOEC 134d LOEC	5 (TRC) 52 (TRC)	s	Hermanutz et al (1990)
Menida peninsula	ELS, fry survival	28d NOEC	**40 (TRC)**	1	Goodman et al (1983)
Outdoor mesocosm					
In a 28 d laboratory microcosm toxicity of hypochlorite to a microbial community was tested. The measured taxonomic parameter was the number of protozoan species and the non-taxonomic responses included chlorophyll *a* (expression of algal biomass*)*, ATP, total protein, extracellular alkaline phosphatase activity, and potassium. The lowest NOEC was calculated for the number of protozoan species and for depression of alkaline phosphatase activity (28d NOEC = 2.1 µg TRC/l). As far as chlorophyll *a* is concerned, a reliable NOEC cannot be derived because a clear concentration-effect relationship is lacking. However, this appeared to be the most sensitive endpoint, as a significant reduction of chlorophyll *a* (about 50%) was recorded at 2.1 µg/l of TRC (the lowest tested concentration). Additionally an 24d outdoor mesocosm study with freshwater biota was conducted. In the enclosures chlorine was introduced as a daily pulse. At 79 µg TRC/l, neither chlorophyll *a* nor the number of algal genera was reduced (NOEC). Protozoan species number was not significantly reduced at the lower test concentration, i.e. NOEC = 24 µgTRC/l. The most sensitive endpoint was the zooplankton density (24d NOEC = 1.5 µgTRC/l). Only the number of zooplankton/ml of water without providing any other information about the effects on taxonomic composition of zooplankton community, so that it is not possible to draw any conclusion about the eventual elimination of taxa from the system. This study can only be used for supportive information.					

*_Onchorhynchus kisutch_, _Notemigonus crysoleucas, Lepomis macrochirus_ and _Cyprinus caprio_ were also tested, but less sensitive (no toxicity data reported).

**_Perca flavescens_ was also tested, but less sensitive (no toxicity data reported)

**** _Lepomis macrochirus_ was also tested, but less sensitive (no toxicity data reported).

****eggs and 1-year old young were less sensitive, sensitivity of _Dorosoma patenense_ was slightly lower than _C. caprio._

B.9.2.8. Risk assessment

For the proposed treatments contamination of surface water is possible following discharge of use solutions into the sewage system.

Acute risk

PECsw values were taken from section B.8.6.1. The table below shows the results.

Table B.9.2.8-01: Acute TERs of hypochlorite for fish, invertebrates, and algae following discharge of use solutions into the sewage system.

	L(E)C50 (µg a.s./L)			PECsw	TERa		
application	**Fish**	**invertebrates**	**Algae**	**(µg a.s./L)**	**Fish**	**invertebrates**	**Algae**
Mushrooms	90	5.0	>6.0	0.0086	10465	581	>698

The acute TER for algae is above the Annex VI trigger of 10 and the acute TER for fish and invertebrates is above the ANNEX VI trigger of 100

Long-term risk

Sodium hypochlorite is a highly reactive substance with a low DT50 of <1 hour in water. It is expected to cause mainly acute effects. No acceptable chronic endpoint is available for invertebrates, however a long-term risk assessment is performed for fish.

The long-term TER based on the above PECsw and the NOEC value of the active substance (40 μg a.s./L for fish) is shown in the Table below.

Table B.9.2.8-02: Long-term TERs for fish following discharge of use solutions into the sewage system.

application	NOEC (μg as/L)	PECsw	TER
	fish	(μg a.s./L)	Fish
Mushrooms	40	0.0086	4651

The long-term TER for fish is above the Annex VI trigger of 10 and the long-term risk is considered to be acceptable.

Risks of metabolites

Several reaction products can be formed after exposure to sludge. The most common groups of reaction products are:

1)Trihalomethanes (chloroform, bromoform)
2) Haloacetic acids (monochloracetic acid, monobromoacetic acid)
3) N-chloroamino compounds

In the RAR some information based on studies and QSAR-estimations is available. From the available data it appears that Haloacetic acids are the most toxic ones, with EC50 values for algae (growth) of 70 μg/L for monochloracetic acid and 5.96 μg/L for dibromoacetic acid (OECD 201, GLP study).

With this is formation is it expected that the toxicity of these formation products is not higher than the toxicity of sodium hypochlorite.

Since no information about formation fraction or persistence of these reaction products is available, no PECs can be calculated. However, it is not expected that higher concentrations of these reaction products are found in water. Since the toxicity of these reaction products are also considered to be comparable or less than toxicity of sodium hypochlorite, the risk for aquatic organisms to these products is considered to be lower than to the parent.

Risk assessment for sediment-dwelling organisms

No exposure to sediment is expected.

Assessment of bioconcentration potential

No data on hypochlorite effects on secondary poisoning are available but as mentioned previously (B.9.2.4) no hypochlorite residue is thought to be present or to accumulate in the food chain.

B.9.3 Effects on other terrestrial vertebrates (Annex IIIA 10.3)

Based on the input from section B.6, the following endpoints are considered relevant for mammals:

Table B.9.3-01: Long-term TERs for fish following discharge of use solutions into the sewage system.

Species	Criterion	Value	Rating	Reference section
Rat	LD50 oral	>5800 mg/kg bw (based on a 12.5% solution)	1	B.6.2.1.1
Rat	Short-term NOAEL (drinking water)	47.5 mg a.s./kg/d	2	B.6.3.4
Rat	1- generation NOAEL	≥ 5 mg a.s./kg bw/d	2	B.6.6.1

Risk assessment

Exposure to mammals is negligible, only exposure via drinking water is expected.

The acute risk assessment is based on a small mammal weighing 10 g, with a daily water intake (DWI) of 1.6 mL/day. The acute oral LD50 is >5800 mg /kg bw (Based on a 12.5% solution. This is considered equivalent with > 725 mg a.s./kg bw). The estimated theoretical exposure (ETE) for the acute time scale is calculated as PECsw*DWI/BW. The PECsw is the worst-case initial PECsw value taken from Section B.8.6.1. Residue values in water, ETE and TERa values are presented in the table below.

Table B.9.3-02 Acute Toxicity Exposure Ratios for exposure of mammals to sodium hypochlorite due to consumption of contaminated drinking water

Application	LD50 (mg/kg bw)	Route	PEC_{FEED} or PEC_{WATER} (mg/kg wwt or mg/L)	ETE (mg/kg bw/d)	TERa
Mushrooms	>725	Water	0.0000086	0.0000014	> 5.2×10^8

The TERa value is far above the Annex VI 91/414 EEC trigger of 10. Hence, the acute risk to mammals is considered to be acceptable.

B.9.4 Effects on bees (Annex IIA 8.7; Annex IIIA 10.4)

No data were submitted. This is acceptable, since for the proposed treatment (use in mushroom crops in containers) exposure of bees to sodium hypochlorite will not occur.

B.9.5 Effects on other arthropod species (Annex IIA 8.8; Annex IIIA 10.5)

No data were submitted. This is acceptable, since for the proposed treatment (use in mushroom crops in containers) exposure of arthropods to sodium hypochlorite will not occur.

B.9.6 Effects on earthworms (Annex IIA 8.9; Annex IIIA 10.6)

No data were submitted. This is acceptable, since for the proposed treatment (use in mushroom crops in containers) exposure of earthworms to sodium hypochlorite will not occur.

B.9.7 Effects on other soil non-target macro-organisms (Annex IIIA 10.6.6 to 10.6.7)

No studies submitted.

B.9.8 Effects on soil microbial activity(Annex IIA 8.10; Annex IIIA 10.7)

No data were submitted. This is acceptable, since for the proposed treatment (use in mushroom crops in containers) exposure of soil micro-organisms to sodium hypochlorite will not occur.

B.9.9 Effects on other non-target organisms (flora and fauna) believed to be at risk (Annex IIA 8.6)

No data were submitted. This is acceptable, since for the proposed treatment (use in mushroom crops in containers) exposure of earthworms to sodium hypochlorite will not occur.

B.9.10 Effects on biological methods of sewage treatment (Annex IIA 8.7)

B.9.10.1 Study descriptions and results

STUDY IIA, 8.7/01

Reference/notifier	:	Raff et al, (1987)	GLP statement	:	no
Type of study	:	activated sludge respiration	Guideline	:	-
Year of execution	:		Acceptability	:	2
Test substance	:	hypochlorite	EC50		>375 µg/L

Summary from RAR

The EC50 for the inhibition of activated sludge respiration is situated around 3000 µg/l for a continuous hypochlorite dosage, with a LOEC of ca. 375 µg/l. The sludge concentration used and the pH were not

mentioned in the paper. The result obtained is very much dependent on the sludge concentration used (i.e. the organic content in the test vessel). Therefore the figure mentioned is just a figure and different data will be obtained using different sludge concentrations. Also different pH values will probably generate different data. The tentative LOEC of 375µg/l derived by the authors should only be used as an indication for potential effects on a sewage treatment plant with the limitations mentioned before.

Remarks by RMS

Although no conclusive endpoint can be determined, it appears that sludge microorganisms are not very sensitive to hypochlorite. Because of the uncertainties mentioned in the RAR, the EC50 cannot be set on 3000 µg/L, however it seems safe to assume that the EC50 > 375 µg/L.

B.9.10.2 Summary and assessment

For the proposed treatment exposure of activated sludge is possible following discharge of use solutions into the sewage system.

Risk assessment is based on the 3-hour EC50 of sodium hypochlorite for inhibition of the bacterial respiratory rate of >375 µg/L. The table below shows the results.

Table B.9.10.2-01 TERs of sodium hypochlorite for micro-organisms in activated sludge following discharge of use solutions into the sewage system.

application	EC50 (µg a.s./L)	PEC_{STP} (µg a.s./L)	TER
mushrooms	>375	0.033	>11364

Annex VI of Directive 91/414/EC does not specify a trigger for exposure of micro-organisms in the STP. Micro-organisms in activated sludge have a very short reproductive cycle similar to green algae, and it is reasonable therefore to apply the trigger of 10 for exposure of algae also to exposure of micro-organisms in activated sludge. The TER for micro-organisms in activated sludge is above the trigger of 10 and the risk is considered to be acceptable.

B.9.11 References relied on

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 8	RAR (unknown)	2007	RISK ASSESSMENT REPORT FOR SODIUM HYPOCHLORITE, DRAFT November 2007 Italy non GLP Published (via ECB website)	N	ECB