Proposed EQS for Water Framework Directive Annex VIII substances: triclosan *(For consultation)*

by
Water Framework Directive - United Kingdom Technical Advisory Group (WFD-UKTAG)

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Use of this report

The development of UK-wide classification methods and environmental standards that aim to meet the requirements of the Water Framework Directive (WFD) is being sponsored by the UK Technical Advisory Group (UKTAG) for WFD on behalf of its member and partners.

This technical document has been developed through a project managed by the Environment Agency and has involved members and partners of UKTAG. It provides background information to support the ongoing development of the standards and classification methods.

While this report is considered to represent the best available scientific information and expert opinion available at the time of its completion, it does not necessarily represent the final or policy positions of UKTAG or any of its partner agencies.

Executive summary

The UK Technical Advisory Group (UKTAG) has commissioned a programme of work to derive Environmental Quality Standards (EQSs) for substances falling under Annex VIII of the Water Framework Directive (WFD). This report proposes predicted no-effect concentrations (PNECs) for triclosan using the methodology described in Annex V of the Directive. There are no existing EQSs for triclosan.

The PNECs described in this report are based on a technical assessment of the available ecotoxicity data for triclosan, along with any data that relate impacts under field conditions to exposure concentrations. The data have been subjected to rigorous quality assessment such that decisions are based only on scientifically sound data. Following consultation with an independent peer review group, critical data have been identified and assessment factors selected in accordance with the guidance given in Annex V of the WFD.

Where possible, PNECs have been derived for freshwater and saltwater environments, and for long-term/continuous exposure and short-term/transient exposure. If they were to be adopted as EQSs, the long-term PNEC would normally be expressed as an annual average concentration and the short-term PNEC as a 95th percentile concentration. The feasibility of implementing these PNECs as EQSs has not been considered at this stage. However, this would be an essential step before a regulatory EQS can be recommended.

Properties and fate in water

Triclosan is an antibacterial agent belonging to the chlorinated diphenyl ethers which is added to a wide range of consumer products to offer long lasting protection against bacteria, moulds and yeasts. The majority of its usage is associated with household and personal care products (e.g. toothpastes, mouthwashes, soaps and deodorants). These uses result in the substance being released to the sewerage system where there is the potential for release to the aquatic environment.

Triclosan has a pKa of 7.9-8.1 and readily ionises at environmental pH. The substance is predominantly in its neutral form at pH 7.0 but is predominantly in its ionised form at pH 8.5. Organic molecules have been shown to be less likely to cross lipid membranes when in their ionised state which is consistent with the greater bioaccumulation found in aquatic organisms exposed to triclosan at pH below the pKa (see below). In waters above pH 8.0 the predominance of the ionised form of triclosan results in lower levels of bioaccumulation.

Triclosan is transformed via direct photolysis and the pH-dependent dissociation of triclosan governs its susceptibility to photooxidation (Lindström *et al.* 2002). Half lives of 8 and 4 days respectively have been reported for the photolysis of triclosan (using a starting concentration of 9.4 mg l^{-1}) in freshwater and seawater (at pH of 7.0) under a low intensity artificial white light source. 2,8-Dichlorodibenzo-p-dioxin (at a level of 1%) was detected in both samples after 3 days of irradiation (Aranami and Readman, 2007). In contrast, a half-life of only 41 minutes for aqueous photolysis was found in drinking water at pH 7 and 25°C, with most of the triclosan being converted to 2,4-dichlorophenol (Spare, 1993).

In receiving waters with a pH <8.0 triclosan is expected to adsorb to suspended solids and sediment based on an estimated Koc value of 9200 (determined from a log Kow of 4.76). Volatilization of triclosan is not expected to be an important fate process given

an estimated Henry's Law constant of $2.4x10^{-7}$ Pa-m^3/mole (derived from its vapour pressure of $7x10^{-4}$ Pa, and a water solubility of 10 mg l^{-1}.

Triclosan is not readily or inherently degradable in standardised screening tests like OECD 301C (MITI I) or OECD 302C (MITI II). The negative results in these tests may be a consequence of the bacterial toxicity of triclosan at the high substrate concentration required for these biodegradability screening tests. However, removal rates up to 99% have been recorded in tests using a Continuous Activated Sludge (CAS) system. In a study at a sewage treatment works triclosan was found to be susceptible to biodegradation with over 79% of the substance being degraded during a one-week survey and only 6% of the triclosan entering the plant remaining in the treated effluent. Methyl triclosan and 2,7/2,8 dibenzodichloro-p-dioxin are potential biotransformation products following treatment of triclosan at a sewage treatment plant.

Bioconcentration factors are reported in the range 15 to 90 and 2.7 to 44 in carp (*Cyprinus carpio*) at concentrations of 3 and 30 µg l^{-1}, respectively, suggesting the potential for bioconcentration in aquatic organisms is low to moderate. However, the BCFs for zebra fish (*Danio rerio)*, assessed over a five-week test period were 4157 at 3 µg l^{-1} and 2532 at 30 µg l^{-1}, indicating that there is a significant likelihood for bioaccumulation. The pH of the test media used in the studies are not known but pH differences may explain the differences in BCF values between species. This is supported by a study which reported BCF values of 3700-8700 in zebrafish exposed to triclosan at pH in the range 6-9, with the higher BCFs being reported in fish exposed to the substance at pHs of 6-7. High concentrations of triclosan have also been reported in the bile of fish exposed to the substance.

Availability of data

Longer-term toxicity data are available for seven taxonomic groups namely; algae, crustaceans, fish, insects, macrophytes, protozoa and rotifers. Freshwater short-term toxicity data are available for six taxonomic groups (algae, crustaceans, fish, insects, macrophytes and protozoa). Freshwater unicellular algae are generally more sensitive to triclosan than other taxonomic groups which is consistent with the mode of action of the substance. For marine organisms, short-term toxicity data are only available for two different taxonomic groups (algae and bacteria) and there is only long-term toxicity data available for algae. Therefore the minimum of three saltwater taxa (algae, crustaceans and fish) required under by the EU Technical Guidance Document are not satisfied for saltwater species.

Derivation of PNECs

Long-term PNEC for freshwaters

The lowest valid long-term toxicity value for any freshwater species is a 3-day no observed effect concentration (NOEC) of 0.5 µg l^{-1} for effects on the growth rate of the algae *Scenedesmus subspicatus*. Reliable long-term NOECs are available for algae, invertebrates and fish including a large body of data for algae which are the most sensitive taxonomic group. Based on the data available and the combined weight of evidence it presents it is considered appropriate to apply a reduced assessment factor of 5 (based on the specific circumstances applying to triclosan in terms of its mode of action and the available dataset for target species) this results in a $PNEC_{freshwater\_lt}$ of 0.10 µg l^{-1} triclosan.

The PNEC value of 0.10 µg l^{-1} is supported by an HC5,50 value of 0.13 µg l^{-1} generated from an SSD based on the available long-term data for freshwater algae.

Short-term PNEC for freshwaters

Reliable short-term data are available for algal, invertebrate and fish species. The lowest valid short-term toxicity value is a 72-hour growth rate EC50 of 2.8 μg l^{-1} for the alga *Scenedesmus subspicatus*. There is a considerable short-term toxicity database for freshwater organisms, which shows that algae such as *Scenedesmus* are likely to be most sensitive to triclosan. An assessment factor of 10 was therefore applied, resulting in a $PNEC_{freshwater\_st}$ of 0.28 μg l^{-1} triclosan.

Long-term PNEC for saltwaters

The only long-term saltwater data available is a 4-day EC25 of >66 μg l^{-1} for effects of triclosan on the marine alga *Skeletonema costatum*. Since algae are the most sensitive freshwater taxa and marine algae are apparently not more sensitive to triclosan than freshwater species it is recommended that the freshwater PNEC is also adopted to protect saltwater taxa. It is proposed not to apply an additional assessment factor of 10 for marine species due to the antimicrobial mode of action of triclosan (i.e. by disruption of cell wall formation in micro-organisms) which indicates that organisms such as echinoderms and molluscs are likely to be less sensitive than algae. This results in a $PNEC_{saltwater\_lt}$ of 0.10 μg l^{-1}.

Short-term PNEC for saltwaters

There are limited short-term data available for saltwater species exposed to triclosan, but the data for marine algae (the most sensitive freshwater species) do not show greater sensitivity compared to freshwater species. It is therefore recommended that the freshwater PNEC is also adopted to protect saltwater taxa. This results in a $PNEC_{saltwater\_st}$ of 0.28 μg l^{-1} triclosan.

PNECs for sediment

Triclosan has a log Kow of 4.76 which exceeds the TGD trigger value of a log Kow of >3, therefore a sediment quality standard is necessary. However, data on direct toxicity to sediment-dwelling organisms are limited and are insufficient to derive a PNEC.

Secondary poisoning

Bioconcentration data (as BCF values) for triclosan for fish are variable ranging from 2.7 to 90 for carp (*Cyprinus carpio*) and 2532-8700 for zebrafish (*Danio rerio*). The BCF values are dependent on the pH of the exposure medium. As the trigger of BCF values >100 is met the derivation of a PNEC for secondary poisoning of predators is required. The PNEC based on the risks of secondary poisoning to mammals and birds (3.8 μg l^{-1}) is higher than those derived for the protection of aquatic life and so does not influence the development of aquatic EQSs for triclosan.

Summary of proposed PNECs

Receiving medium/exposure scenario	**Proposed PNEC (μg l^{-1})**
Freshwater/long-term	0.10
Freshwater/short-term	0.28
Saltwater/long-term	0.10
Saltwater/short-term	0.28
Sediment	Insufficient data
Secondary poisoning	3.8

Analysis

For water, proposed PNECs derived for triclosan range from 0.10 to 0.28 µg l^{-1}. The data quality requirements are that, at a third of the EQS, total error of measurement should not exceed 50 per cent. Using this criterion, it is evident that current analytical methodologies (non-standard) employing voltammetry, gas chromatography–mass spectrometry (GC-MS), or liquid chromatography–mass spectrometry (GC-MS),capable of achieving detection limits as low as 0.5 ng l^{-1}, should offer adequate performance to analyse for triclosan.

Implementation issues

Based on consideration of the information collated within the report and the proposed PNECs the following comments are made re: implementation:-

- Current analytical capability should be adequate for compliance assessment
- The assessment factors applied are within the range of 5-10 and therefore the PNECs derived are not subject to excessive uncertainty.

Contents

1 Introduction

The UK Technical Advisory Group (UKTAG) supporting the implementation of the Water Framework Directive (2000/60/EC)[1] is a partnership of UK environmental and conservation agencies. It also includes partners from the Republic of Ireland. UKTAG has commissioned a programme of work to derive Environmental Quality Standards (EQSs) for substances falling under Annex VIII of the Water Framework Directive (WFD). This report proposes predicted no-effect concentrations (PNECs) for triclosan using the methodology described in Annex V of the Directive. There are no existing EQSs for triclosan.

The PNECs described in this report are based on a technical assessment of the available ecotoxicity data for triclosan, along with any data that relate impacts under field conditions to exposure concentrations. The data have been subjected to rigorous quality assessment such that decisions are based only on scientifically sound data.[2] Following consultation with an independent peer review group ,critical data have been identified and assessment factors selected in accordance with the guidance given in Annex V of the WFD. The feasibility of implementing these PNECs as EQSs has not been considered at this stage. However, this would be an essential step before a regulatory EQS can be recommended.

1.1 Properties and fate in water

Triclosan is an antibacterial agent belonging to the chlorinated diphenyl ethers, which is added to a wide range of consumer products to offer long lasting protection against bacteria, moulds and yeasts. The majority of its usage is associated with household and personal care products (e.g. toothpastes, mouthwashes, soaps and deodorants). These uses result in the substance being released to the sewerage system where there is the potential for release to the aquatic environment.

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Triclosan is transformed via direct photolysis and the pH-dependent dissociation of triclosan governs its susceptibility to photooxidation (Lindström *et al.* 2002). Half lives of 8 and 4 days respectively have been reported for the photolysis of triclosan (using a starting concentration of 9.4 mg l^{-1}) in freshwater and seawater (at pH of 7.0) under a low intensity artificial white light source. 2,8-Dichlorodibenzo-p-dioxin (at a level of 1%) was detected in both samples after 3 days of irradiation (Aranami and Readman, 2007). In contrast, a half-life of only 41 minutes for aqueous photolysis was found in drinking water at pH 7 and 25°C, with most of the triclosan being converted to 2,4-dichlorophenol (Spare, 1993).

In receiving waters with a pH <8.0 triclosan is expected to adsorb to suspended solids and sediment based on an estimated Koc value of 9,200 (determined from a log Kow of 4.76). At these pH values the compound will partially exist in the dissociated form in the environment and

[1] *Official Journal of the European Communities*, **L327**, 1–72 (22/12/2000). Can be downloaded from http://www.eu.int/comm/environment/water/water-framework/index_en.html

[2] Data quality assessment sheets are provided in Annex 1.

anions generally do not adsorb more strongly to organic carbon and clay than their neutral counterparts

Volatilization of triclosan is not expected to be an important fate process given an estimated Henry's Law constant of $2.4x10^{-7}$ Pa-m^3/mole (derived from its vapour pressure of $7x10^{-4}$ Pa, and a water solubility of 10 mg l^{-1}.

Triclosan is not readily or inherently degradable in standardised screening tests like OECD 301C (MITI I) or OECD 302C (MITI II). The negative results in these tests may be a consequence of the bacterial toxicity of triclosan at the high substrate concentration required for these biodegradability screening tests (Danish EPA, 2003). However, removal rates up to 99% have been recorded in tests using a Continuous Activated Sludge (CAS) system. In a study at a sewage treatment works triclosan was found to be susceptible to biodegradation with over 79% of the substance being degraded during a one-week survey and only 6% of the triclosan entering the plant remaining in the treated effluent (Federle *et al.,* 2002). Methyl triclosan and 2,7/2,8 dibenzodichloro-p-dioxin are potential biotransformation products following waste water treatment of triclosan (Latch *et al*. 2003, Mezcua *et al*. 2004).

Bioconcentration factors are reported in the range 2.7 to 90 in carp (*Cyprinus carpio*) at concentrations of 3 and 30 µg l^{-1}, respectively, suggesting the potential for bioconcentration in aquatic organisms is low to moderate (Hansen and Källqvist, 2001, cited in Danish EPA, 2003). However, the BCFs for zebra fish (*Danio rerio)*, assessed over a five-week test period were 4157 at 3 µg l^{-1} and 2532 at 30 µg l^{-1}, indicating that there is a significant likelihood for bioaccumulation (Orvos *et al*. 2002). The pH of the test media used in the studies are not known but pH differences may explain the differences in BCF. This is supported by a study by Schettegen *et al.* (1999) which reported BCF values of 3700-8700 in zebrafish exposed to triclosan at pHs in the range 6-9, with the higher BCFs being reported in fish exposed to the substance at pHs of 6-7.

Houtman *et al.* (2004) reported bioaccumulation at up to 80 mg l^{-1} in the bile of bream (*Abramis brama*) from the Netherlands. Furthermore, its degradation product methyl triclosan is present at up to 35 µg/kg wet wt. in the muscle of roach (*Rutilus rutilus*) and whitefish (*Coregonus* sp.) from various sewage-contaminated Swiss lakes (Balmer *et al*. 2004).

2 Results and observations

2.1 Chemical species

Table 2.1 gives the chemical name and Chemical Abstracts Service (CAS) number for the species of interest.

Table 2.1 Species covered by this report

Name	CAS Number
Triclosan (2,4,4'-trichloro-2'-hydroxy-diphenyl-ether)	3380-34-5

2.2 PNECs proposed for derivation of quality standards

Table 2.2 lists proposed PNECs obtained using the methodology described in the Technical Guidance Document (TGD) issued by the European Chemicals Bureau (ECB) on risk assessment of chemical substances (ECB 2003).

Section 2.6 summarises the effects data identified from the literature for triclosan. The use of these data to derive the values given in Table 2.2 is explained in Section 3.

Table 2.2 Proposed overall PNECs as basis for quality standard setting

PNEC	TGD deterministic approach (AFs)	TGD probabilistic approach (SSDs)	Existing EQS
Freshwater short-term	0.28 µg l^{-1}	–	-
Freshwater long-term	0.10 µg l^{-1}	–	-
Saltwater short-term	0.28 µg l^{-1}	–	-
Saltwater long-term	0.10 µg l^{-1}	–	-
Sediment	Insufficient data	–	-
Secondary poisoning	3.8 µg l^{-1}	–	-

AF = assessment factor
SSD = species sensitivity distribution

2.3 Hazard classification

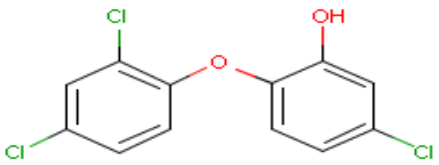
Table 2.3 gives the R-phrases (Risk-phrases) and labelling for the species of interest.

R-Phrases and Labelling	Reference:
R36/38, 50/53	ECB – ESIS (Accessed December 2006)

2.4 Physical and chemical properties

Table 2.4 summarises the physical and chemical properties of the species of interest.

Table 2.4 Physical and chemical properties of triclosan

Property	Triclosan	Reference
CAS number	3380-34-5	HSDB (2006)
Substance name	Triclosan	ChemID (2006)
Molecular formula	$C_{12}H_7Cl_3O_2$	ChemID (2006)
Molecular structure		ChemID (2006)
Molecular weight	289.55	HSDB (2006)
Colour/form	White to off-white crystalline powder	HSDB (2006)
Odour	Slight, faintly aromatic odour	HSDB (2006)
Melting point (°C)	54 – 57.3	HSDB (2006)
Boiling point (°C)	280 - 290	HSDB (2006)
Vapour pressure	7 x 10^{-4} Pa at 25°C	HSDB (2006)
Density/ specific gravity	No data located	-
Henry's Law constant	2.4 x 10^{-7} Pa-m3/mole at 25°C (estimated)	HSDB (2006)
Water solubility	10 mg l^{-1} at 20 °C 12 mg l^{-1}	HSDB (2006) Danish EPA (2003)
Solubility in organic solvents	No data located	-

2.5 Environmental fate and partitioning

Table 2.5 summarises the information obtained from the literature on the environmental fate and partitioning of triclosan.

Table 2.5 Environmental fate and partitioning of triclosan

Property	Value	Reference
Abiotic fate	The pKa of triclosan is 7.9-8.1, indicating that this compound will partially exist in the dissociated form in the environment and anions generally do not adsorb more strongly to organic carbon and clay than their neutral counterparts.	Orvos *et.al.* (2002) HSDB (2006)
Speciation	Not relevant	-
Hydrolytic stability	No data located	-
Photostability	When exposed to natural sunlight in fortified lake water at different pH values triclosan is transformed via direct photolysis with pH-dependent dissociation governing its susceptibility to photooxidation. A half-life of 41 minutes for aqueous photolysis was found in drinking water at pH 7 and 25°C, with most of the triclosan being converted to 2,4-dichlorophenol. In contrast, half lives of 8 and 4 days respectively have been reported for triclosan in freshwater and seawater under a low intensity artificial white light source. 2,8-Dichlorodibenzo-p-dioxin was detected in both samples after 3 days of irradiation.	Spare (1993) Lindström *et al.* (2002), Danish EPA (2003); Aranami and Reader (2007),
Volatilisation	Volatilization of triclosan is not expected to be an important fate process given an estimated Henry's Law constant of $2.4x10^{-7}$ Pa-m^3/mole (derived from its vapour pressure of $7x10^{-4}$ Pa, and a water solubility of 10 mg l^{-1}.	
Distribution in water/sediment systems (active substances)	Based on an estimated Koc value of 9200 (determined from a log Kow of 4.76) triclosan is expected to adsorb to suspended solids and sediment. This sorption is affected by water pH.	HSDB (2006)
Distribution in water/sediment systems (metabolites)	Methyl triclosan is expected to sorb to sediments based on a log Kow of 4.76	Balmer *et al.* (2006)
Degradation in soil	If released to soil, triclosan is not expected to be mobile based upon an estimated Koc of 9,200 (log Koc = 3.96). Volatilization from moist soil surfaces is not expected to be an important fate process based upon an estimated Henry's Law constant of $1.5x10^{-7}$ atm-cu m/mole. The pKa of triclosan is 7.9-8.1, indicating that this compound will partially exist in the dissociated form in the environment. Anions generally do not adsorb more strongly to organic carbon and clay than their neutral counterparts.	HSDB (2006)

	The biological half life of triclosan in an agricultural sludge amended soil spiked with 40 and 600 µg kg^{-1} (soil dry weight) occurred within the 577 day period (i.e. half lives were <577 days).	Danish EPA (2003)
Biodegradation	Triclosan is not readily or inherently degradable in standardised screening tests like OECD 301C (MITI I) or OECD 302C (MITI II). The negative results in these tests may be a consequence of the bacterial toxicity of triclosan at the high substrate concentration required for these biodegradability screening tests. However, removal rates up to 99% have been recorded in tests using a Continuous Activated Sludge (CAS) system. The presence of methyl triclosan, a biotransformation product, following waste water treatment indicates that triclosan is susceptible to biodegradation in sewage treatment works. Triclosan has also been shown to photodegrade in wastewater to 2,7/2,8-dibenzodichloro-*p*-dioxin	HSDB (2006) Danish EPA (2003) Federle *et al.* (2002) Latch *et al*. (2003) Mezcua *et al*. (2004)
Octanol-water coefficient (log Kow)	4.76	HSDB (2006)
Log Koc	3.96 4.3 in soil and 4.7 in sludge	HSDB (2006) Danish EPA (2003)
Bioaccumulation **BCF values**	BCF ranges of 2.7 to 90, 2532 to 8700 were measured in carp *(Cyprinus carpio)* and zebrafish *(Danio rerio)* respectively. The extent of triclosan accumulation in fish is affected by water pH, with greater BCFs measured in organisms exposed to pHs below 8.0.	HSDB (2006) Orvos *et al*. (2002) Schettegen *et al*. (1999)

BCF = bioconcentration factor

Triclosan has a pKa of 7.9-8.1 and readily ionises at environmental pH. The substance is predominantly in its neutral form at at pH 7.0 but is predominantly in its ionised form at pH 8.5. Organic molecules have been shown to be less likely to cross lipid membranes when in their ionised state which is consistent with the greater bioaccumulation found in organisms exposed at pHs below the pKa (see below). However in waters above pH 8.0 the predominance of the ionised form of triclosan results in lower levels of bioaccumulation.

Lindström *et al.* (2002) carried out an experiment in which triclosan and methyltriclosan were exposed to natural sunlight in fortified lake water at different pH values. Neither triclosan nor methyltriclosan were transformed at pH 5.6. Triclosan was, however, transformed at pH 8.0 whereas methyltriclosan was still not transformable. A parallel experiment performed at pH 8.0 in distilled water showed that the transformation of triclosan was independent of the presence of natural organic matter and other water constituents. The results indicate that triclosan is transformed via direct photolysis and that the dissociation of triclosan governs its susceptibility to photooxidation.

Aranami and Readman (2007) conducted a 12 day photolysis experiment with triclosan (at a starting concentration of 9.4 mg l^{-1}) in freshwater and seawater under a low intensity artificial light source. Half-lives of triclosan in freshwater and seawater (assuming a first-order reaction) were 8 and 4 days respectively. 2,8-Dichlorodibenzo-p-dioxin (DCDD) (at a level of 1%) was

detected in both samples after 3 days of irradiation. The photodegradation of triclosan and the production of DCDD indicated that triclosan could be less stable and DCDD may be more stable in seawater than freshwater, with the DCDD produced in seawater surviving for a longer duration. These findings contrast with the findings of Spare (1993) which reported a half-life of 41 minutes for the aqueous photolysis of triclosan in drinking water at pH 7 and 25°C, with most of the triclosan being converted to 2,4-dichlorophenol. However, the conditions under which the study was carried out are not known.

If released into water, triclosan is expected to adsorb to suspended solids and sediment based upon the log Koc values of 3.96 to 4.3. Hydrolysis is not expected to be an important environmental fate process due to the stability of triclosan against strong acids and bases. Volatilization from water surfaces is not expected based upon the estimated Henry's Law constant of 1.57×10^{-7} atm-cm/mole.

The Danish EPA (2003) reported that triclosan is not readily or inherently degradable in standardised screening tests like OECD 301C (MITI I) or OECD 302C (MITI II). The negative results in these tests may be a consequence of the bacterial toxicity of triclosan at the high substrate concentration required for these biodegradability screening tests. Federle *et al.* (2002) carried out various experiments using radiolabelled triclosan in a Continuous Activated Sludge (CAS) system to examine the degradation of triclosan and to study the effect of a shock loading with pulses of a high concentration of triclosan. Removal rates of up to 99% were found at concentrations up to 2000 µg l^{-1}. Depending on the study conditions, mineralisation was between 78% and 90%, the remainder being sorption to sludge or unchanged parent material. The inhibitory concentrations of triclosan varied by a factor of more than 10 times (20 to 239 mg l^{-1}) in these studies. The presence of methyl triclosan, a biotransformation product, following waste water treatment indicates that triclosan is susceptible to biodegradation in sewage treatment works (Danish EPA, 2003). Recent studies have demonstrated >90% removal in sewage treatment works (STWs), with up to 0.07 µg l^{-1} triclosan being measured in effluents (Paxeus 2004, Thomas and Foster 2005). Triclosan has also been shown to photodegrade in wastewater to 2,7/2,8-dibenzodichloro-*p*-dioxin (Latch *et al*. 2003, Mezcua *et al*. 2004).

Hansen and Källqvist (2001), cited in Danish EPA (2003), reported BCF ranges of 2.7 to 90 in carp (*Cyprinus carpio*) exposed to triclosan concentrations of 3 and 30 µg l^{-1}. However, BCF values of 2532 to 4157 in zebrafish (*Danio rerio*) exposed to triclosan concentrations of 3 and 30 µg l^{-1} suggest there is a significant potential for bioconcentration in aquatic organisms (Orvos *et al.* 2002). In the zebrafish study fish were exposed for five weeks in a flow-through system with methods modified from OECD TG 305C (OECD 1996). The pH of the test media used in the studies are not known but pH differences may explain the differences in BCF values. This is supported by a study by Schettegen *et al.* (1999) which reported BCF values of 3700-8700 in zebrafish exposed to triclosan at pH in the range 6-9, with the higher BCFs being reported in fish exposed to the substance at pH of 6-7. Orvos *et al.* (2002) used the triclosan log Kow of 4.76 and the model equation log bioconcentration factor = 0.76 log Kow – 0.23, the predicted BCF was calculated to be approximately 2500, suggesting that triclosan behaves similarly to non-polar organic compounds. Results from laboratory bioconcentration studies by Adolfsson-Erici *et al.* (2002) showed that 98% of accumulated triclosan is eliminated rapidly from fish and the amount remaining does not accumulate in edible portions of the fish. When exposure is reduced or stopped the accumulated triclosan is almost completely excreted. Houtman *et al.* (2004) reported bioaccumulation at up to 80 mg l^{-1} in the bile of bream (*Abramis brama*) from the Netherlands. Furthermore, its degradation product methyl triclosan is present at up to 35 µg/kg wet wt. in the muscle of roach (*Rutilus rutilus*) and whitefish (*Coregonus* sp.) from various sewage-contaminated Swiss lakes (Balmer *et al*. 2004).

If released to soil, triclosan is not expected to be mobile based upon an estimated Koc of 9200. This is consistent with German mobility studies in which there was limited leaching of triclosan in columns of soil over a period of 48 hours (Danish EPA, 2003). Approximately 50% mineralisation of triclosan was observed in an agricultural sludge-amended soil spiked at 40 and 600 µg kg^{-1} (soil dry weight) over the test period of 577 days. However, shorter half-lives in the range of 17.4-35.2 days were reported for the three other experimental soils amended with sludge from treatment facilities receiving triclosan in the waste stream. Volatilization from moist soil surfaces is not expected to be an important fate process (Danish EPA, 2003).

If released to air, a vapour pressure of $4x10^{-6}$ mm Hg at 20 ºC indicates triclosan will exist in both the vapour and particulate phases. It is expected that vapour-phase triclosan will be degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 8 hours (HSDB, 2006). Particulate-phase triclosan will be removed from the atmosphere by wet and dry deposition (HSDB, 2006).

2.6 Effects data

A summary of the mode of action of this substance can be found in Section 2.6.5. There are limited data available for triclosan and no UK EQS. The main data was retrieved from the US Environmental Protection Agency (US EPA) ECOTOX database[3] and the Danish Environmental Protection Agency which published a study in 2003 on the Fate and Effects of triclosan.

Further data sources used included: ScienceDirect®;[4] Hazardous Substances Data Bank (HSDB®) database of the US National Library of Medicine;[5] Inchem, RIVM, Google, ECB-ESIS, PubMed, SETAC, Norwegian Pollution Control Authority, ENDS, TOXNET, British Library, Syress, ATSDR, EUROPA – Environment, WHO, EXTOXNET

2.6.1 Toxicity to freshwater organisms

Freshwater toxicity data on triclosan are available for various taxonomic groups (including algae, amphibians, fish, invertebrates, macrophytes and protozoa) required for the application of the approach specified in the EU Technical Guidance Document (TGD) (ECB, 2003). Longer-term toxicity data are available for seven taxonomic groups namely; algae, crustaceans, fish, insects, macrophytes, protozoa and rotifers. Short-term-term toxicity tests are available for six taxonomic groups namely algae, crustaceans, fish, insects, macrophytes and protozoa.

Overall, the available short-term and long-term toxicity test data indicate that for triclosan, algae are generally the most sensitive taxa of those tested. This is consistent with the use of the substance as an antimicrobial agent, designed specifically to kill bacteria and other unicellular microorganisms (see Section 2.6.5 where the mode of action is discussed in further detail).

Diagrammatic representations of the available freshwater data (cumulative distribution functions) for triclosan are presented in Figures 2.1 and 2.2. These diagrams include all data regardless of quality and provide an overview of the spread of the available data. However, they are not species sensitivity distributions and have not been used to derive triclosan PNECs.

The lowest critical freshwater data are presented in Tables 2.6 (for longer-term chronic data) and 2.7 (for short-term acute data). These tables do not contain all the available toxicity data but only those which are considered to be most relevant to the derivation of PNECs.

[3] http://www.epa.gov/ecotox/
[4] http://www.sciencedirect.com/
[5] http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB

As triclosan has a pKa of 7.9-8.1, it is predominantly in its neutral form at pH 7.0 but it is in its ionised form at pH 8.5. The unionised form of triclosan is considered to be more toxic as molecules are believed to be less likely to cross lipid membranes in their ionised state (Orvos *et al.* 2002).

Figure 2.1 Cumulative distribution function of freshwater long-term data (μg l^{-1}) for triclosan

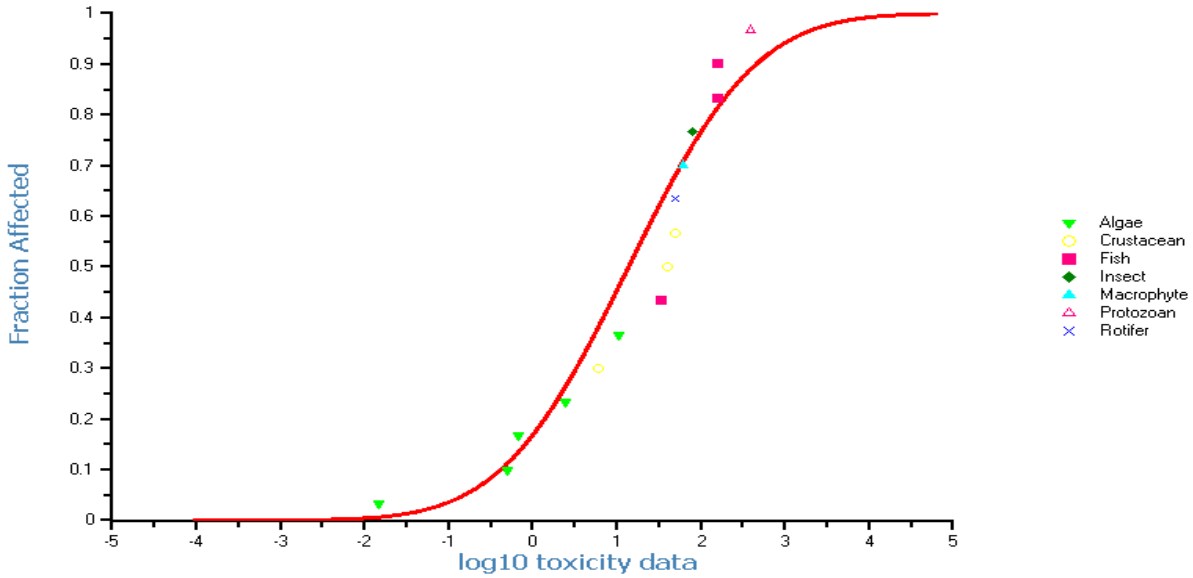


Figure 2.2 Cumulative distribution function of freshwater short-term data (μg l^{-1}) for triclosan

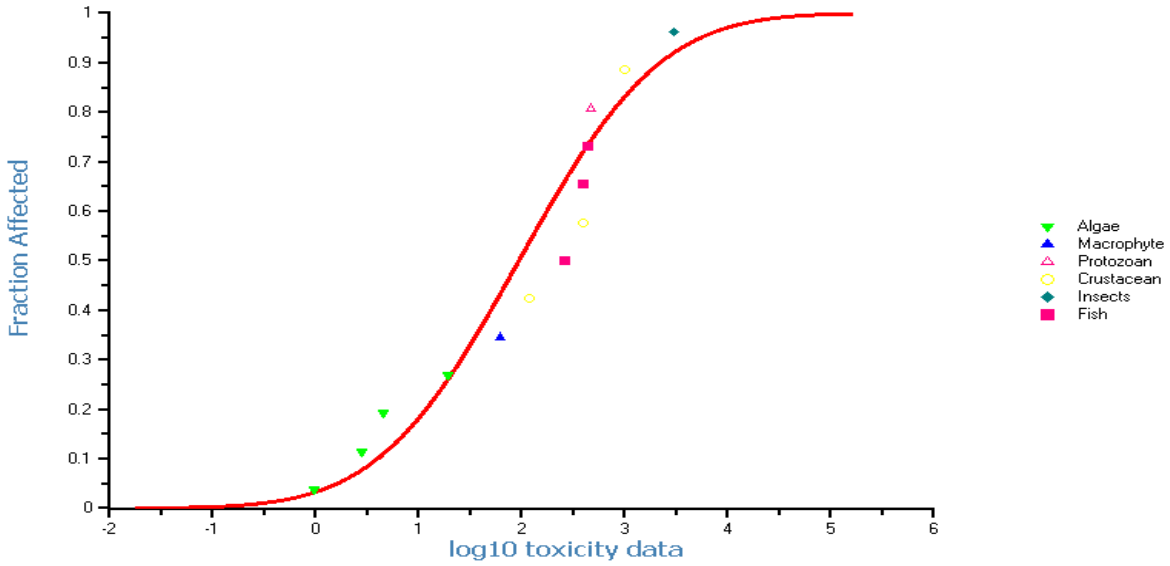


Table 2.6 Most sensitive long-term aquatic toxicity data for freshwater organisms exposed to triclosan

Chemical formulation	Scientific name	Common name	Taxonomic group	Effect	Endpoint	Test duration	Conc. ($\mu g\ l^{-1}$)	Exposure [1]	Toxicant analysis [2]	Comments	Reliability (Klimisch Code*)	Reference
Algae												
95% a.i.	*Mixed natural algal communities*	Green alga	ALG	Genus richness	LOEC	<=13 days	0.015	s	n	T =18 ^{o}C	3	Wilson *et al. (2003)*
99.5% a.i.	*Anabaena flos-aquae*	Blue/green alga	ALG	Biomass	EC25	4 days	0.67	s	y	T =24 $\pm$ 2^{o} C	2	Orvos *et al.* (2002)
99.5% a.i.	*Navicula pelliculosa*	Diatom	ALG	Biomass	EC25	4 days	10.7	s	y	T =24 $\pm$ 2^{o} C	2	Orvos *et al.* (2002)
99.5% a.i.	*Pseudokirchneriella subcapitata (formerly Selenastrum capricornutum*	Green algae	ALG	Growth rate and biomass	EC25	4 days	2.44	s	y	T =24 $\pm$ 2^{o} C	2	Orvos *et al.* (2002)
99.5% a.i.	*Scenedesmus subspicatus*	Green alga	ALG	Population growth rate and biomass	NOEC	3 days	0.5	s	y	T =24 $\pm$ 2^{o} C	2	Orvos *et al.* (2002)
Higher plants												
99.5% a.i.	*Lemna gibba*	Duckweed	MAC	Population biomass	EC25	7 days	>62.5	s	n	T = 25 $\pm$ 2^{o} C	2	Orvos *et al.* (2002)
Protozoa												
100% a.i.	*Paramecium caudatum*	Ciliate	PRO	Population abundance	IC50	5days	399 (reported as 1.38 μM)	s	n	T = 23 ^{o}C	2	Miyoshi *et al.* (2003)
Invertebrates												
Not known	*Brachionus calyciflorus*	Rotifer	ROT	Reproduction/ fecundity	NOEC	2 days	50	Not known	Not known	Not known	4	Ferrari *et al.* (2002)
>99.5% a.i.	*Ceriodaphnia dubia* (neonates <24h old)	Water flea	CRU	Survival	NOEC	7 days	50	ss	y	pH = 7.0 Hardness = 174 mg l^{-1}	2	Orvos *et al.* (2002)
>99.5% a.i.	*Ceriodaphnia dubia* (neonates <24h old)	Water flea	CRU	Survival	NOEC	7 days	339	ss	y	pH = 8.5 Hardness = 174 mg l^{-1}	2	Orvos *et al.* (2002)
>99.5% a.i.	*Ceriodaphnia dubia* (neonates <24h old)	Water flea	CRU	Reproduction/ fecundity	NOEC	7 days	6.0 (5.6 as unionised)	ss	y	pH = 7.0 Hardness = 174 mg l^{-1}	2	Orvos *et al.* (2002)

Chemical formulation	Scientific name	Common name	Taxonomic group	Effect	Endpoint	Test duration	Conc. ($\mu g\ l^{-1}$)	Exposure [1]	Toxicant analysis [2]	Comments	Reliability (Klimisch Code*)	Reference
>99.5% a.i.	*Ceriodaphnia dubia* (neonates <24h old)	Water flea	CRU	Reproduction/ fecundity	NOEC	7 days	182 (51.8 as unionised)	ss	y	pH = 8.5 Hardness = 174 mg l^{-1}	2	Orvos *et al.* (2002)
>99.5% a.i.	*Daphnia magna* (<24hrs old)	Water flea	CRU	Reproduction/fecundity	NOEC	21 days	40 (18 as unionised)	ss	y	pH = 8.2	2	Orvos *et al.* (2002)
≥97% a.i.	*Hyalella azteca*	Amphipod	CRU	Growth	EC10	10 days	50	ss	y	pH = 7.7 ± 0.30	2	Dussault *et al .* (2008)
≥97% a.i.	*Chirononomus tentans (larvae)*	Midge	INS	Growth	EC10	10 days	80	ss	y	pH = 8.4 ± 0.12	2	Dussault *et al .* (2008)
Fish												
Not known	*Brachydanio rerio*	Zebrafish	FIS	Hatchability and fry survival	IC25	14 days post hatch	160	Not known	Not known	Not known	4	Taratazako *et al.* (2004)
99.5% a.i.	*Oncorhynchus mykiss*	Rainbow trout	FIS	Survival	NOEC	70 days (35 days post-hatch	34.1 (15.1 as unionised)	f	y	pH = 8.2	2	Orvos *et al.* (2002)
99.5% a.i.	*Oncorhynchus mykiss*	Rainbow trout	FIS	Survival	NOEC	96 days (61 days post-hatch)	34.1 (15.1 as unionised)	f	y	pH = 8.2	2	Orvos *et al.* (2002)
>98.0% a.i.	*Oryzias latipes*	Japanese medaka	FIS	Hatchability and fry survival	NOEC	21 days	156	ss	y	T = 25 ± 1 ^{o} C	2	Ishibashi *et al.* (2004)

* See Annex 1.
[1] Exposure: s = static, ss = semi-static, f = flow-through
[2] Toxicant analysis: y = measured; n = not measured
ALG = alga, CRU = crustacean, INS = insects, FIS = fish, MAC = macrophyte, PRO = protozoa, ROT = rotifers
LOEC = lowest observed effect concentration
NOEC = no observed effect concentration
EC10 = concentration effective against 10 per cent of the organisms or animals tested
EC25 = concentration effective against 10 per cent of the organisms or animals tested
IC50 = concentration inhibitory against 50 per cent of the organisms or animals tested
a.i. = active ingredient

Table 2.7 Most sensitive short-term aquatic toxicity data for freshwater organisms exposed to triclosan

Chemical formulation	Scientific name	Common name	Taxonomic group	Effect	Endpoint	Test duration	Conc. ($\mu g\ l^{-1}$)	Exposure [1]	Toxicant analysis [2]	Comments	Reliability (Klimisch Code*)	Reference
Algae												
99.5% a.i.	*Anabaena flos-aquae*	Green alga	ALG	Biomass	EC50	96 hours	0.97	s	y	T =24 ± 2°C, pH = 7.5	2	Orvos *et al.* (2002)
99.5% a.i.	*Navicula pelliculosa*	Green alga	ALG	Biomass	EC50	96 hours	19.1	s	y	T =24 ± 2°C,	2	Orvos *et al.* (2002)
99.5% a.i.	*Pseudokirchnereilla subcapitata (formerly Selenastrum capricornutum*	Green alga	ALG	Biomass	EC50	96 hours	4.46	s	n	T =24 ± 2 °C, pH 7.5-7.8	2	Orvos *et al.* (2002)
99.5% a.i.	*Scenedesmus subspicatus*	Green alga	ALG	Biomass	EC50	72 hours	0.7	s	y	T =24 °C, pH 7.5-7.8	2	Orvos *et al.* (2002)
99.5% a.i.	*Scenedesmus subspicatus*	Green alga	ALG	Growth rate	EC50	72 hours	2.8	s	y	T =24 °C, pH 7.5-7.8	2	Orvos *et al.* (2002)
Higher plants												
99.5% a.i.	*Lemna gibba*	Duckweed	MAC	Population biomass	EC50	7 days	>62.5	s	n	T = 25 °C	2	Orvos *et al.* (2002)
Protozoa												
100% a.i.	*Paramecium caudatum*	Ciliate	PRO	Population abundance	IC50	48 hours	475 (reported as 1.64 μM)	s	n	T = 23 °C	2	Miyoshi *et al.* (2003)
Invertebrates												
99.5% a.i.	*Ceriodaphnia dubia* (neonates <24h old)	Water flea	CRU	Mortality	EC50	48 hours	120 (115 as unionised)	ss	y	pH = 6.8-7.0 Hardness = 174 mg l^{-1} Alkalinity =115 mg l^{-1}	2	Orvos *et al.* (2002)
99.5% a.i.	*Ceriodaphnia dubia* (neonates <24h old)	Water flea	CRU	Mortality	EC50	48 hours	182 (136 as unionised)	ss	y	pH = 7.4-7.6 Hardness = 174 mg l^{-1} Alkalinity =115 mg l^{-1}	2	Orvos *et al.* (2002)
99.5% a.i.	*Ceriodaphnia dubia* (neonates <24h old)	Water flea	CRU	Mortality	EC50	48 hours	236 (132 as unionised)	ss	y	pH = 8.0-8.2 Hardness = 174 mg l^{-1}	2	Orvos *et al.* (2002)

Chemical formulation	Scientific name	Common name	Taxonomic group	Effect	Endpoint	Test duration	Conc. ($\mu g\ l^{-1}$)	Exposure [1]	Toxicant analysis [2]	Comments	Reliability (Klimisch Code*)	Reference
										Alkalinity =115 mg l^{-1}		
99.5% a.i.	*Ceriodaphnia dubia* (neonates <24h old)	Water flea	CRU	Mortality	EC50	48 hours	409 (115 as unionised)	ss	y	pH = 8.2-8.5 Hardness = 174 mg l^{-1} Alkalinity =115 mg l^{-1}	2	Orvos *et al.* (2002)
99.5% a.i.	*Daphnia magna*	Water flea	CRU	Mortality	EC50	48 hours	390	s	n	Hardness = 160-180 mg l^{-1} $CaCO_3$	2	Orvos *et al.* (2002)
≥97% a.i.	*Hyalella azteca*	Amphipod	CRU	Mortality	LC50	10 days	1000	ss	y	pH = 7.7 ± 0.30	2	Dussault *et al.* (2008)
≥97% a.i.	*Chironomus tentans (larvae)*	Midge	INS	Mortality	LC50	10 days	3000	ss	y	pH = 8.4 ± 0.12	2	Dussault *et al.* (2008)
Fish												
99.5% a.i.	*Lepomis macrochirus*	Bluegill sunfish	FIS	Mortality	LC50	96 hours	440	s	n	Hardness = 40-48 mg l^{-1} $CaCO_3$	2	Orvos *et al.* (2002)
>98.0% a.i.	*Oryzias latipes*	Japanese medaka	FIS	Mortality	LC50	96 hours	399	s	n	T = 25 ± 1^{o} C	2	Ishibashi *et al.* (2004)
99.5% a.i.	*Pimephales promelas*	Fathead minnow	FIS	Mortality	LC50	96 hours	260	s	n	Hardness = 40-48 mg l^{-1} $CaCO_3$	2	Orvos *et al.* (2002)

* See Annex 1.
[1] Exposure: s = static; f = flow-through , ss = semi-static
[2] Toxicant analysis: y = measured; n = not measured
ALG = alga, CRU = crustacean, FIS = fish, , INS = insect, MAC = macrophytes, PRO = protozoa
LC50 = concentration lethal to 50 per cent of the organisms or animals tested
EC50 = concentration effective against 50 per cent of the organisms or animals tested
IC50 = concentration inhibitory against 50 per cent of the organisms or animals tested

2.6.2 Toxicity to saltwater organisms

Acute toxicity tests are available for two different taxonomic groups (algae and bacteria) and are given in Table 2.8, but long-term toxicity data is only available for marine algae. The saltwater algal toxicity data show lower sensitivity than corresponding freshwater species (Orvos *et al.* 2002, McHenry, 2006). Due to the limited marine toxicity data available, it is not possible to calculate a saltwater PNEC based on saltwater data alone.

A diagrammatic representation of the available short-term saltwater data (cumulative distribution functions) for triclosan is presented in Figures 2.3. This diagram includes all data regardless of quality and provides an overview of the spread of the available data. However, it is not a species sensitivity distribution and has not been used to derive triclosan PNECs.

The lowest critical long-term and short-term saltwater data are presented in Table 2.8.

Figure 2.3 Cumulative distribution function of saltwater short-term data (μg l^{-1}) for triclosan

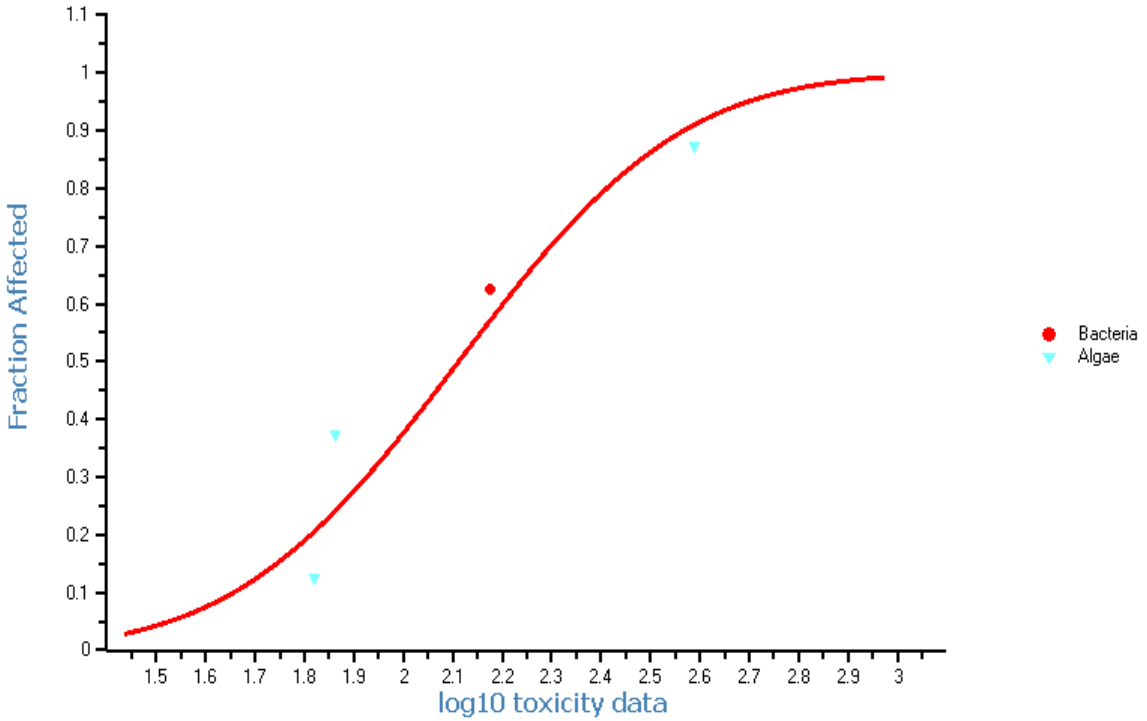


Table 2.8 Most sensitive long-term and short-term aquatic toxicity data for saltwater organisms exposed to triclosan

Chemical formulation	Scientific name	Common name	Taxonomic group	Effect	Endpoint	Test duration	Conc. ($\mu g\ l^{-1}$)	Exposure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
Long-term Algae												
99.5% a.i.	*Skeletonema costatum*	Diatom	ALG	Population biomass	EC25	4 days	>66	s	n	T = 20 ^{o}C,	2	Orvos *et al.* (2002)
Short-term Algae												
99.5% a.i.	*Skeletonema costatum*	Diatom	ALG	Population biomass	EC50	96 hours	>66	s	n	T = 20 ^{o}C,	2	Orvos *et al.* (2002)
Triclosan	*Tetraselmis chuii*	Algae	ALG	Population growth	EC50	72 hours	72.9	-	-	T =23-27 ^{o}C, Salinity 25 = $\%_{o}$	2	McHenry *et al.* 2006
Triclosan	*Nannochlorop-sis oculata*	Algae	ALG	Population growth	EC50	72 hours	388	-	-	T =23-27 ^{o}C, Salinity = 25$\%_{o}$	2	McHenry *et al.* 2006
Bacteria												
Triclosan	*Vibrio fisheri*	Microtox bacterium	BAC	Inhibition of light output	IC25	15 min	70	s	-	-	2	Tatarazako *et al*. (2004)
Triclosan	*Vibrio fisheri*	Microtox bacterium	BAC	Inhibition of light output	IC50	15 min	150	s	-	-	2	Tatarazako *et al*. (2004)

* See Annex 1.
[1] Exposure: s = static;
[2] Toxicant analysis: y = measured; n = not measured
ALG = alga, BAC = bacteria
EC25 = concentration effective against 25 per cent of the organisms or animals tested
EC50 = concentration effective against 50 per cent of the organisms or animals tested
IC25 = concentration inhibitory against 25 per cent of the organisms or animals tested
IC50 = concentration inhibitory against 50 per cent of the organisms or animals tested

2.6.3 Toxicity to sediment dwelling organisms

Triclosan has a log Kow of 4.76 which means that the substance is expected to partition significantly to sediments and suspended matter (HSDB, 2006). However, there are limited data on the potential risks that triclosan poses to sediment-dwelling organisms. Dussault *et al.* (2008) reported on the effects of triclosan on the midge *Chironomus tentans* and the freshwater shrimp *Hyalella azteca*, using standard 10 day water-only tests (though sand was added as a substrate in the *Chironomus* tests). In the tests the reported 10-day LC50 values for mortality were 400 µg l^{-1} for *C. tentans* and 200 µg l^{-1} for *H. azteca*. The corresponding 10-day EC50 values for the growth endpoint were 280 µg l^{-1} for *C. tentans* and 250 µg l^{-1} for *H. azteca*.

Dussault *et al.* (2004) reported on preliminary studies using sediments spiked with triclosan The study indicated lower toxicity relative to water-only exposures based on survival and growth of *C.tentans* but no values were quoted in the paper.

2.6.4 Endocrine-disrupting effects

The potential of triclosan to act as an endocrine disruptor was examined because its chemical structure closely resembles known non-steroidal oestrogens (e.g. diethylstilbene and bisphenol A). Foran *et al*. (2000) exposed japanese medaka fry (*Oryzias latipes*) were exposed to triclosan for 14 days at nominal concentrations of 1, 10 and 100 µg l^{-1} from 2 days post-hatch. An oestradiol positive control was also used in the study. Two months post-exposure, the phenotypic sex of each adult was assessed visually using sexually dimorphic fin shape and size. The proportion of females in each group was similar for the ethanol-treated solvent controls (53%) and triclosan exposed animals (1 µg l^{-1} = 58%, 10 µg l^{-1} = 45%, 100 µg l^{-1} = 36%) although the oestradiol treatment did result in 92% of adults being female. Sexually dimorphic fin traits were quantified to assess the potential effects of triclosan and oestradiol on the development of secondary sexual characters. These results do not support the hypothesis that triclosan is potently oestrogenic. However, changes in fin length and changes in sex ratio (which were not statistically significant) suggest triclosan is potentially weakly androgenic.

A study by Gross *et al.* (2004) investigated the endocrine disrupting effects of triclosan in captive largemouth bass (20 male and 20 female) exposed to low and high triclosan concentrations (values not given) for 28 days. Fish were sacrificed for the assessment of plasma sex steroids (oestradiol, testosterone, 11-ketotestosterone), thyroid hormones (T3 and T4), gonadosomatic index, vitellogenin, and sperm quality (motility and morphology). Water and tissues were analyzed for triclosan and its metabolites. Physiological effects were reported in both sexes, although the magnitude of the effects were more pronounced in male fish which showed decreased concentrations of sex steroids and decreased sperm quality. The data also indicated decreased thyroid hormone concentrations in fish exposed to the higher triclosan concentration. From this information it appears that triclosan acts as an endocrine disruptor though at higher exposure concentrations.

A study by Ishibashi *et al.* (2004) investigated the effects of triclosan on the early life stages and reproduction of Japanese medaka (*Oryzias latipes*). An assessment of the effects of a 21-day exposure period of triclosan on the reproduction of paired medaka showed no significant differences in the number of eggs produced and fertility among the control and 20, 100 and 200 µg l^{-1} triclosan treatment groups. However, concentrations of hepatic vitellogenin were increased significantly in males treated with triclosan at 20 and 100 µg l^{-1}, but not at 200 µg l^{-1}. In the F1 generations, although the hatching of embryos in the 20 µg l^{-1} treatment showed adverse effects, there was no concentration–response relationship between hatchability and triclosan exposure levels. The results showed that a metabolite of

triclosan (probably methyltriclosan) may be a weak oestrogen in male medaka but with no adverse effect on reproductive success (such as fecundity and fertility) or offspring health.

Matsumura *et al.* (2005) investigated the effects of triclosan and nonylphenol on production of vitellogenin, testosterone and hepatic cytochrome P450 1A and 2B activities in male South African clawed frogs (*Xenopus laevis*). In a 14 day waterborne exposure test, no significant differences in the level of plasma vitellogenin synthesis in male frogs were observed among the control and 20, 100, and 200 µg l^{-1} triclosan treatment groups.

Veldhoen *et al.* (2006) investigated the effects of triclosan on the thyroid mediated process of metamorphosis of the North American bullfrog (*Rana catesbeiana*). This involved assessing the extent to which triclosan alters the expression profile of thyroid hormone receptor (TR) α and β, basic transcription element binding protein (BREB) and profilerating nuclear cell antigen (PCNA) gene transcripts. Premetamorphic tadpoles were immersed in triclosan concentrations of $\geq$ 0.15 µg l^{-1} and injected with 1 x 10^{-11} mol g^{-1} body weight 3,5,3'-triiodothyronine (T_3)[6] or vehicle control. Morphometric and steady state mRNA levels obtained by quantitative polymerase chain reaction were determined. mRNA abundance was also examined in *Xenopus laevis* XTC-2 cells treated with triclosan and/or 10 nM T_3. Tadpoles pretreated with triclosan concentrations as low as 0.15 µg l^{-1} for 4 days showed increased hindlimb development and a decrease in total body weight following T_3 administration. Triclosan exposure also resulted in decreased T_3 mediated TRα mRNA expression in the tadpole tail fin and increased levels of PCNA transcript in the brain within 48 hours of T_3 treatment whereas TRα and BTEB were unaffected. Triclosan alone altered thyroid hormone receptor α transcript levels in the brain of premetamorphic tadpoles and induced a transient weight loss. *In vitro* studies using XTC-2 cells exposed to T_3 plus nominal triclosan concentrations as low as 0.03 µg l^{-1} for 24 hours resulted in altered thyroid hormone receptor mRNA expression. It was concluded in the paper that exposure to low levels of triclosan disrupts thyroid hormone associated gene expression and can alter the rate of thyroid hormone-mediated post-embryonic anuran development.

Fort *et al.* (2008) investigated the effects of triclosan on metamorphosis in the anuran *Xenopis laevis*. Standard NF stage 51 *X. laevis* larvae were exposed for 21 d via flow-through to four different concentrations of TCS: <0.2 [control], 0.6, 1.5, 7.2, or 32.3 µg l^{-1}. Primary endpoints were survival, hind limb length, body length (whole; snout-to-vent), developmental stage, wet whole body weight, and thyroid histology. Thyroid hormone (TH) concentrations were determined in whole thyroid and plasma in stage-matched exposure d 21. TH-receptor beta (TRβ) expression was measured in stage-matched tail fin tissue samples collected at exposure d 0 and 21. Reduced larval growth occurred at exposure d 21 with 1.5 µg l^{-1} treatment. Larval developmental stage at exposure d 21 was not significantly different from controls based on observed parameters; thyroid histology was not affected by TCS; and T4 levels in thyroid gland or plasma were not different from controls. A concentration-dependent increased in TRβ expression in exposure d 21 larvae was not detected. Increased expression was found in stage-matched larvae exposed to 1.5- or 7.2-µg l^{-1}, but not to the 0.6- or 32.3-µg l^{-1}. It was concluded in the paper that environmentally-relevant triclosan concentrations do not alter the normal course of thyroid-mediated metamorphosis in this standard anuran model. This information is only available from an abstract and the reliability of the data cannot currently be assessed.

[6] In the absence of the thyroid hormones 3,5,3'-triiodothyronine (T_3) or thyroxine (T_4) tadpoles fail to metamorphose into frogs

2.6.5 Mode of action of triclosan

Triclosan is a synthetic, broad-spectrum antimicrobial agent designed to destroy or suppress the growth of 'harmful' microorganisms. Triclosan inhibits the growth of micro-organisms such as bacteria and funguses by using an electro-chemical mode of action to penetrate and disrupt their cell walls. Once inside the cell, triclosan poisons a specific enzyme that micro-organisms need for survival. Triclosan blocks the active site of an enzyme called enoyl-acyl carrier-protein reductase (ENR for short), preventing the manufacture of fatty acids needed for building cell membranes and other vital functions or reproducing (GCO, 2006 and SpecialChem, 2006). Brain *et al*. (2004) have indicated that there may be conserved receptors and pathways in plants which would mean unicellular algae would be sensitive to triclosan exposure.

In algae the effects of triclosan have been shown to be algistatic rather than algicidal in nature. As a result the observed effects can be regarded as reversible if organisms are returned to uncontaminated conditions (Orvos *et al.* 2002). Reiss *et al.* (2003) have also stated that triclosan has transitory algicidal properties.

2.6.6 Mesocosm and field studies

Freshwater mesocosm and field studies

White *et al.* (2005) evaluated the growth−inhibiting effects of triclosan on periphytic algae at the White River, Northwest Arkansas. The periphytometer was deployed for one week with seven replicates of nine treatments, including control (deionized H_2O), methanol, low triclosan (50 µg l^{-1}), medium triclosan (100 µg l^{-1}), high triclosan (500 µg l^{-1}), nutrients (2000 µg PO4−P l^{-1} and 20000 µg NO3−N l^{-1}), low triclosan with nutrients, medium triclosan with nutrients, and high triclosan with nutrients. Relatively low stream nutrient concentrations were observed; maximum nitrate–nitrogen (NO_3−N), total N, ammonium–nitrogen (NH_4N−N), total organic carbon (TOC), and soluble reactive phosphorus (SRP) concentrations during the deployment were 230, 603, <50, 12500, and 15 µg l^{-1} , respectively. The Student−Newman−Kuels test identified three significantly different groups within the treatments. The nutrients and low TCS with nutrients treatments had chlorophyll−a means of 10.9 and 5.8 mg m^{-2}, respectively, which were significantly different from each other and all other treatments. Chlorophyll−a content means of the remaining treatments ranged from 1.8 to 3.5 mg m^{-2} and were not significantly different from each other. Exponential regression of chlorophyll−a contents in nutrient and TCS with nutrient treatments against TCS concentration produced a significant decreasing trend; however, no trend in chlorophyll−a content was observed in treatments without nutrients.

Saltwater mesocosm and field studies

No data from mesocosm or field studies using saltwater organisms were found.

3 Calculation of PNECs as a basis for the derivation of quality standards

3.1 Derivation of PNECs by the TGD deterministic approach (AF method)

3.1.1 PNECs for freshwater

PNEC accounting for the annual average concentration

For the freshwater environment, data are available for the 'base set' of toxicity tests (i.e. tests with algae, crustaceans and fish) and therefore the EU TGD assessment factor (AF) method can be applied (ECB 2003). Longer-term toxicity data are available for seven taxonomic groups namely; algae, crustaceans, fish, insects, macrophytes, protozoa and rotifers. These data indicate that algae are generally more sensitive than other taxa and this is consistent with the use of substance as an antimicrobial agent designed to be effective against unicellular organisms by disrupting cell wall formation. Table 2.6 summarises the most sensitive long-term freshwater toxicity data found for triclosan.

The lowest freshwater long-term result for algae is an estimated $\leq$ 13-day NOEC of 0.015 $\mu g\ l^{-1}$ for changes in the genus diversity of a mixed algal population collected from Cedar Creek (Kansas) in a microcosm study (Wilson *et al*. 2003). Studies were conducted on algal communities in water samples collected in April and May 2002 and exposed to nominal triclosan concentrations of 0.015, 0.15 and 1.5 $\mu g\ l^{-1}$. Triclosan had significant impacts on *Chlamydomonas* biomass in the April 2002 sample at exposure concentrations of 0.15 and 1.5 $\mu g\ l^{-1}$. In the May 2002 sample triclosan caused a significant increase in *Synedra* biomass and a significant reduction of *Chlamydomonas* biomass at 0.015 and 0.15 $\mu g\ l^{-1}$. The study used field collected organisms in a non-standardised methodology where certain key data were not provided. On this basis it is not considered appropriate to base the derivation of the PNEC on this data. However, the study can be taken to indicate that algal community productivity and probably algal genus richness are statistically affected at exposure concentrations of $\leq$1.5 $\mu g\ l^{-1}$.

Orvos *et al.* (2002) reported a 3-day NOEC value of 0.5 $\mu g\ l^{-1}$ for effects of triclosan on the growth of *Scenedesmus subspicatus* (based on both the preferred growth rate and biomass endpoints). Orvos *et al.* (2002) also reported a 4 day EC25 value of 0.67 $\mu g\ l^{-1}$ (95% confidence limits of 0.72 to 1.30 $\mu g\ l^{-1}$) for *Anabaena flos-aquae*. Both these studies can be considered reliable and appropriate for use in the derivation of the PNEC since they followed a standardised methodology and there was analytical confirmation of the exposure concentrations. However, it should be noted that the toxicity of triclosan may have been reduced during the study as it appears the pH of the exposure media increased from 7-8 at test initiation to around pH 10 at the end of the studies.

The lowest long-term result available for invertebrates is a 7-day NOEC for the crustacean *Ceriodaphnia dubia* of 6.0 μg l^{-1} triclosan (5.6 μg l^{-1} as unionised triclosan) at pH 7.0 (Orvos *et al*. 2002). Effects on *Ceriodaphnia* reproduction were found to be pH dependent with the 7-day NOEC at pH 8.5 being 182 μg l^{-1} triclosan (51.8 μg l^{-1} as unionised triclosan). The lowest NOEC for *Daphnia magna* is 40 μg l^{-1} (18 μg l^{-1} as unionised triclosan) for a 21-day study carried out by Orvos *et al*. (2002) at pH 8.2.

The lowest toxicity data for fish were from a study on the effects of triclosan on early-life stages of rainbow trout (*O. mykiss*) (Orvos *et al*. 2002). Fry survival was determined for the period from sac-fry release to 35 and 61-days after hatch. The NOEC and LOEC values after both exposure durations were determined as 34.1 and 71.3 μg l^{-1} triclosan (15.1 and 31.6 μg l^{-1} as unionised triclosan) at pH 8.2 based on measured concentrations. The growth (weight and length) of the surviving fry after 61 days (post-hatch) were not affected at 71.3 μg l^{-1}.

The lowest long-term result for macrophytes is a 7-day population biomass EC50 of 62.5 μg l^{-1} triclosan for *Lemna gibba* (Orvos *et al*. 2002). In terms of acute endpoints the multicellular macrophyte *Lemna* was markedly less sensitive than unicellular freshwater algae using *Selenastrum capricornutum, Scenedesmus subspicatus and Anaebaena flos-aquae* in shorter 3-4 day studies which showed EC50 values in the range 0.7-4.46 μg l^{-1} triclosan (see Table 2.7). The reduced sensitivity of *Lemna* could have been either due to this organism being capable of rapid triclosan metabolism or reduced uptake of the substance.

For protozoans a 5-day IC50 of 399 μg l^{-1} was reported for the ciliate *Paramecium caudatum* based on population abundance (*Miyoshi et al*. 2003).

The data for these standardised ecotoxicological endpoints have to be considered in the context of the potential endocrine disrupting effects of triclosan on amphibians (see Section 2.6.4). Veldhoen *et al.* 2006 indicate that exposure to low levels of triclosan (0.15 μg l^{-1}) can disrupt thyroid hormone associated gene expression and can alter the rate of thyroid hormone-mediated post-embryonic anuran development. However, a study by Fort *et al.* (2008) has shown that there was no effect of triclosan on the metamorphosis of the anuran *Xenopus laevis* at concentrations up to 32.3 μg l^{-1}.

Based on the available data a long-term freshwater PNEC for triclosan should be derived using the 3 day NOEC for effects on the growth of the algae *Scenedesmus subspicatus* (0.5 μg l^{-1}) and a reduced assessment factor of 5 (based on the specific circumstances applying to triclosan in terms of its mode of action and the available dataset for target species). This results in:

$PNEC_{freshwater\_lt}$ = 0.5 μg l^{-1}/AF (5) = 0.10 μg l^{-1} triclosan

In determining the reduction in the size of the assessment factor to be applied to derive the PNEC the following points have been used as a guide:

1. The relatively large body of data for algae which are the most sensitive taxonomic group
2. The overall quality of the database and the end-points covered,
3. The diversity and representativeness of the taxonomic groups covered by the database, including also the variation represented relating to differences in the life forms, feeding strategies and trophic levels of the organisms;
4. The mode of action of the chemical which for triclosan involves disrupting cell wall formation in target micro-organisms as represented by bacteria and unicellular algae;
5. The proposed deterministic-based PNEC is supported by HC5,50 values of 0.13 and 0.096 μg l^{-1} derived from SSDs of small algal datasets (see Section 3.2).

PNEC accounting for a maximum allowable concentration

Freshwater short-term toxicity data are available for six taxonomic groups (algae, crustaceans, fish, insects, macrophytes and protozoa). Table 2.7 summarises the most sensitive short-term freshwater toxicity data found for triclosan.

As expected, triclosan has the greatest effects on algae with Orvos *et al*. (2002) reporting a 72-hour growth rate EC50[7] of 2.8 μg l^{-1} (95% confidence limits of 2.3 to 3.7 μg l^{-1}) for the green alga *Scenedesmus subspicatus*. This is supported by a 96-hour population biomass EC50 of 0.97 μg l^{-1} (95% confidence limits of 0.72-1.3 μg l^{-1}) for *Anabaena flos-aquae* and a 96-hour EC50 of 4.46 μg l^{-1} (95% confidence limits of 2.06-9.66 μg l^{-1}) for *Selenastrum capricornutum* (Orvos *et al*. 2002).

Data are also available for crustaceans, fish, insects and protozoa, but these are generally less sensitive than algae, with toxicity values ranging from 120-3000 μg l^{-1} (Orvos *et al.* 2002, Miyoshi *et al.* 2003, Dussault *et al.* 2004).

Based on the guidance given in the TGD on effects assessment for intermittent releases [Section 3.3.2 of Part II of the TGD document (ECB 2003)] and the fact that there is a considerable acute toxicity database for freshwater organisms and particularly algae, the most sensitive taxonomic group, an assessment factor of 10 rather than 100 should be applied to the lowest reliable data for *Scenedesmus subspicatus*. This gives:

$PNEC_{freshwater\_st}$ = 2.8 μg l^{-1}/AF (10) = 0.28 μg l^{-1} triclosan

3.1.2 PNECs for saltwaters

The effects database for marine species is considerably smaller than that for freshwater organisms. Short-term toxicity data are available for two different taxonomic groups (algae and bacteria) and details are given in Table 2.8. Only long-term data for saltwater algae was located.

McHenry *et al.* (2006) reported 72 hour EC50 values of 72.9 μg l^{-1} and 388 μg l^{-1} for effects of triclosan on the growth of two marine phytoplankton algae species, *Tetraselmis chuii* and *Nannochloropsis oculata*. This data is consistent with the 96 hour EC50 value of >66 μg l^{-1} (nominal concentration) reported for the marine alga *Skeletonema costatum* (Orvos *et al*. 2002). These EC50s for marine species are considerably higher than corresponding 96-hour EC50 values (0.7 to 4.46 μg l^{-1}) reported for freshwater algae, *Scenedesmus* sp., *Anabaena flos-aquae* and *Selenastrum capricornutum.*

Data for the marine bacterium *Vibrio fischeri* are available with 15 minute IC25 and IC50 values of 70 and 150μg l^{-1} being reported (Tatarazako *et al*. 2004). These data are of relevance because they relate to a target organism, though the short exposure period of 15 minutes probably results in toxicity values indicating lower sensitivity than are measured over the longer-term in algal species. Furthermore, these data are not acceptable for deriving the PNEC under the TGD approach.

Although there are differences in the sensitivity of freshwater and saltwater species of the same taxonomic group, it is proposed that the TGD approach of using a combined freshwater and saltwater dataset for the marine effects assessment is used for deriving the

[7] The TGD specifies [Section 6.3.2.2, ECB 2003) that, in considering algal data, growth rate values (EC_r50) should be used in preference to population biomass values (E_bC50).

saltwater PNECs. This is because the mode of action of triclosan should be consistent across species from different media and the differences in toxicity values reflect *"differences in species sensitivity and not a change in the chemistry of the substance"*[8] (Orvos *et al.* 2002)

PNEC accounting for the annual average concentration

A long-term freshwater PNEC for triclosan of 0.10 μg l^{-1}. is based on the 3 day NOEC for effects on the algae *Scenedesmus subspicatus* (0.5 μg l^{-1}) and a reduced assessment factor of 5.

The only long-term saltwater data available is a 4-day EC25 of >66 μg l^{-1} for effects of triclosan on the marine alga *Skeletonema costatum*. Since algae are the most sensitive freshwater taxa and marine algae are apparently not more sensitive to triclosan than freshwater species it is recommended that the freshwater PNEC is also adopted to protect saltwater taxa. It is proposed not to apply an additional assessment factor of 10 for marine species due to the antimicrobial mode of action of triclosan which indicates that organisms such as echinoderms and molluscs are likely to be less sensitive than algae given the substances mode of action (i.e. disruption of cell wall formation). This results in:

$PNEC_{saltwater\_lt}$ = 0.5 μg l^{-1}/AF (5) = 0.10 μg l^{-1} triclosan

PNEC accounting for a maximum allowable concentration

The short-term freshwater PNEC for triclosan is based on a 72-hour growth rate EC50 of 2.8 μg l^{-1} for the green algae *Scenedesmus subspicatus* and an assessment factor of 10, leading to a proposed PNEC of 0.28 μg l^{-1}.

Data are available for saltwater algae (e.g. *Tetraselmis chuii, Nannochloropsis oculata* and *Skeletonema costatum*. The available data for freshwater and saltwater species and the mode of action of the substance indicate that algae are generally the most sensitive taxa. Therefore, despite the absence of data on marine taxa such as echinoderms and molluscs, it is recommended that the freshwater PNEC is also adopted to protect saltwater taxa, without application of an additional assessment factor of 10. This results in:

$PNEC_{saltwater\_st}$ = 2.8 μg l^{-1}/AF (10) = 0.28 μg l^{-1} triclosan

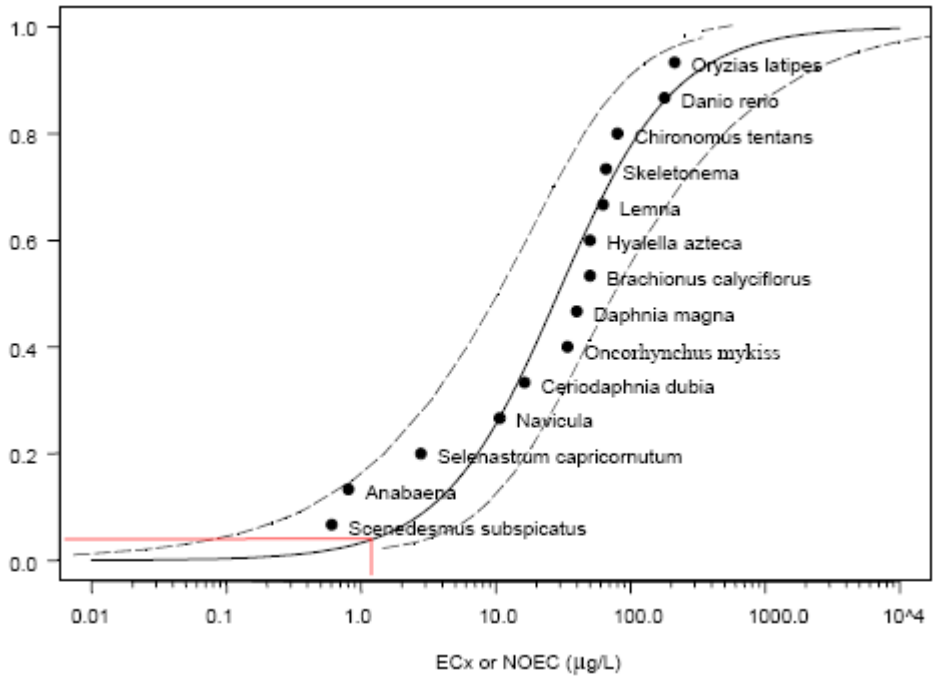
3.2 Derivation of PNECs by the TGD probabilistic approach (SSD method)

Capdevielle *et al.* (2007) have used a chronic toxicity dataset of for triclosan to construct a Species Sensitivity Distribution (SSD) and derive a probabilistic-based PNEC. The SSD was constructed based on either No Observed Effect Concentrations (NOECs) or various percentile adverse effect concentrations (EC10-25 values) for 14 aquatic species including algae (5 species), macrophytes (1 species), invertebrates (5 species) and fish (3 species)[9]. Figure 3.1 shows the SSD generated which involved a log-logistic nonlinear regression of the data. The study proposed a PNEC of 1.55 μg l^{-1} using the HC5,50 value with the application of an assessment factor of 1.

[8] Given the greater buffering capacity of seawater the exposure pH of saltwater studies should be less variable than freshwater studies and typically range from 7.5 to 8.5.

[9] In the Annex V Guidance the minimum number of long-term toxicity data for use of the species sensitivity distribution (SSD) approach is at least 10 NOECs from eight taxonomic groups).

Figure 3.1 Species sensitivity distribution constructed by Capdevielle *et al.* (2007) using long-term toxicity data



The derivation of this probabilistic PNEC value has been considered by an independent peer review group and concerns have been expressed that the resulting value may not be sufficiently protective of the receiving water community as required under the WFD. The concern results from a number of issues, namely:

1. The use of data for all taxonomic groups is inappropriate given that unicellular algae are shown by the available toxicity data to be more sensitive to triclosan exposure than aquatic plants, invertebrates or fish. This conclusion is consistent with Lepper (2005) which states that for substances with specific modes of action "*"it may in most instances be more appropriate to derive a quality standard on the basis of the SSD of the most sensitive group"*. This is appropriate because the group that is especially sensitive may well conform to a different distribution to the other groups (see below).

2. An assessment factor of 1 has been applied to the HC5,50 value derived from the SSD despite the fact that standard toxicological effects on growth and possible endocrine disrupting effects are evident in a number of studies below the proposed PNEC. It was considered that a larger assessment factor should have been applied in the derivation of the PNEC.

3.2.1 Use of data in the construction of the SSD and derivation of the HC5,50 value

On the basis that algae are the taxonomic group most sensitive to triclosan, different SSDs have been constructed using freshwater long-term toxicity data for all species, algal species only and non-algal species. From these SSDs HC5,50 values have been determined. Table 3.1 summarises the HC5,50 values derived for the different algal datasets and the following key points are evident:

1. The HC5,50 value derived from the SSD constructed using the toxicity values for the 13 freshwater long-term studies for all species (0.68 µg l^{-1}) was lower than the value of 1.55 µg l^{-1} given in Capdevielle *et al.* (2007), where the data for the marine algae *Skeletonema costatum* was included in the SSD and different NOEC/ECx values were used for certain species.

2. The HC5,50 value derived using all the freshwater long-term toxicity is higher than the value derived using the data for algal species alone. On this basis the HC5,50 value from the SSD for all the species tested would not be protective of effects on the sensitive algal species.

Table 3.1 Summary of the HC5,50 values derived for the different freshwater long-term toxicity datasets

All freshwater long-term toxicity data (see Table 2.6)		Freshwater long-term toxicity data for algal species (see Table 2.6)		Freshwater long term toxicity data for non-algal species (see Table 2.6)	
Species	Toxicity values[1]	Species	Toxicity values[1]	Species	Toxicity values[1]
A. flos-aquae	0.67	*A. flos-aquae*	0.67	*C.dubia*	6.0
S.subspicatus	0.5	*S.subspicatus*	0.5	*O.mykiss*	34
P.subcapitata	2.4	*P.subcapitata*	2.4	*D.magna*	40
C.dubia	6.0	*N.pelliculosa*	10.7	*B.calyciflorus*	50
N.pelliculosa	10.7			*H.azteca*	50
O.mykiss	34			*Lemna*	62.5
D.magna	40			*C.tentans*	80
B.calyciflorus	50			*O.latipes*	156
H.azteca	50			*B.rerio*	160
Lemna	62.5				
C.tentans	80				
O.latipes	156				
D.rerio	160				
Tests for normality of the data[2] Andersen-Darling test – accepted at 0.1% Kolmogorov-Smirnov test – accepted at 0.025%		**Tests for normality of the data[2]** Andersen-Darling test – accepted at 0.1% Kolmogorov-Smirnov test – accepted at 0.1%		**Tests for normality of the data[2]** Andersen-Darling test – accepted at 0.1% Kolmogorov-Smirnov test – accepted at 0.1%	
HC5,50 value = 0.68 µg l^{-1}		HC5,50 value = 0.13 µg l^{-1}		HC5,50 value = 9.7 µg l^{-1}	
Lower estimate of HC5,50 = 0.10 µg l^{-1} Upper estimate of HC5,50 = 2.20 µg l^{-1}		Lower estimate of HC5,50 = 0.0012 µg l^{-1} Upper estimate of HC5,50 = 0.61 µg l^{-1}		Lower estimate of HC5,50 = 2.7 µg l^{-1} Upper estimate of HC5,50 = 19.6 µg l^{-1}	

Note: [1] – NOEC, EC10 or EC25 values, [2] – If a test statistic is above the 5% critical value, normality is rejected at the 5% critical value, indicating doubts about normality. If a test statistic is below the 5% critical value, normality is accepted (not rejected) at the 5% critical value. If a higher critical value is accepted (e.g. at 2.5% significance level), then the probability that these data derive from a normal distribution is smaller than at 5%, but it is not impossible that the sample derives from a normal distribution.

If the HC5,50 value is calculated from an SSD which comprises all algal data (i.e. also the data for the marine algae *Skeletonema costatum*) then the resulting value is 0.096 µg l^{-1}

3.2.2 Magnitude of the application factor

In determining the size of the additional assessment factor to be applied to derive a PNEC based on the 5th percentile, the following points should be used as a guide:

1. The overall quality of the database and the end-points covered, e.g., if all the data are generated from ”true” chronic studies (e.g., covering all sensitive life stages);
2. The diversity and representativeness of the taxonomic groups covered by the database, including also the variation represented relating to differences in the life forms, feeding strategies and trophic levels of the organisms;
3. The mode of action of the chemical;
4. Statistical uncertainties around the 5th percentile estimate, e.g., reflected in the goodness of fit or the size of confidence interval around the 5th percentile;
5. Comparisons between field and mesocosm studies and the 5th percentile and mesocosm/ field studies to evaluate the laboratory to field extrapolation.

3.2.3 Derivation of a PNEC

Given that algae have been shown to be more sensitive than other taxonomic groups to triclosan exposure any long-term freshwater PNEC derived using a probabilistic approach should ideally be generated from an SSD for this taxonomic group. The available long-term freshwater toxicity data for algae is limited to four values and is not considered appropriate for deriving a probabilistic based PNEC. Instead a tentative probabilistic PNEC derived from an SSD using algal data can be used to support the PNEC derived using a deterministic approach (see Section 3.1.1).

Using an HC5,50 value of 0.13 µg l^{-1} derived from the SSD for algal toxicity dataset the resulting PNEC[10] would be 0.13 µg l^{-1} by applying the smallest assessment factor of 1. An AF of 1 would be considered appropriate since the HC5, 50 is from an SSD for the most sensitive taxonomic group.

This value supports a proposed PNEC of 0.1 µg l^{-1} derived using a deterministic approach with a reduced assessment factor (see Section 3.1.1).

3.3 Derivation of existing EQSs

There are no existing EQSs for triclosan.

3.4 Derivation of PNECs for sediment

Triclosan has a log Kow of 4.76 which exceeds the TGD trigger value of a log Kow of >3, therefore a sediment quality standard is necessary. However, data on direct toxicity to sediment-dwelling organisms are limited and are insufficient to derive a PNEC.

[10] If a PNEC were derived using the HC5,50 derived from the entire dataset (0.68 µg l^{-1}) along with an assessment factor of 5 to account for the uncertainty associated with the dataset then a value of 0.14 µg l^{-1} would result.

3.5 Derivation of PNECs for secondary poisoning of predators

3.5.1 Mammalian and avian toxicity data

There are limited toxicity data published on triclosan and the two reviews located (CEC 2002, NSCFS 2004) have been used as the primary sources (see Table 3.2). Additional literature searches were performed from 2002 to the present day in an attempt to locate any lower effect data since 2002, however none were located.

Limited avian data were located in a comprehensive literature review from 2000 to the present day.

Table 3.2 Mammalian and avian oral toxicity data for the assessment of non-compartment specific effects relevant for the food chain (secondary poisoning)

Type of study	Details
Sub-chronic toxicity to mammals	
Ciba (2001) Cited in CEC (2002) **Sub-chronic NOAEL = 100 mg/kg bw/day**	Rats received triclosan orally for 13 weeks at doses of 0, 100, 300 or 600 mg/kg bw/day. The NOAEL was based on hepatic changes at the top two doses and changes in haematology and clinical chemistry at the top dose. The types of changes observed were unspecified.
Ciba (2001) Cited in NSCFS (2004) and CEC (2002) **Sub-chronic NOAEL = 75 mg/kg bw/day**	Hamsters received triclosan orally for 13 weeks at doses of 0, 75, 200, 750 or 900 mg/kg bw/day. The NOAEL was based on unspecified kidney effects at 200, 750 and 900 mg/kg bw/day. Dose-related changes in clinical chemistry were also observed. The types of changes observed were unspecified.
Ciba (2001) Cited in NSCFS (2004) and CEC (2002) **Sub-chronic NOAEL ≥25 mg/kg bw/day**	Beagle dogs received triclosan orally for 13 weeks at doses of 0, 5, 12.5 or 25 mg/kg bw/day. The NOAEL was based on the absence of any clinical, pathological or histopathological effects at any dose.
Chronic toxicity to mammals	
Borzella *et al*. (1992) Cited in NSCFS (2004) and CEC (2002) **Chronic NOAEL = 52 mg/kg bw/day in males and 67 mg/kg bw/day in females**	Sprague-Dawley rats (60/sex/group) received triclosan orally for 2 years at doses of 0, 16, 52, 168 or 418 mg/kg bw/day in males and 0, 20, 67, 218 and 532 mg/kg bw/day in females. The NOAEL was based on dose-related changes in mean body weight gain, as well as changes in haematology and urinalysis parameters. Toxicity was observed as hepatic centrilobular hypertrophy and associated clinical chemistry parameters. The

	types of changes observed were unspecified.
Ciba (2001) Cited in NSCFS (2004) and CEC (2002) **Chronic NOAEL = 30 mg/kg bw/day**	Baboons received triclosan orally for 12 months at doses of 0, 30, 100 or 300 mg/kg bw/day. The NOAEL was based on the intermittent occurrence of diarrhoea at the top two doses.
Effects on reproduction of mammals	
No studies were available regarding the potential effects of triclosan on mammalian reproduction, development or potential carcinogenicity.	
Toxicity to birds	
The available toxicity studies with birds include two 14-day acute oral toxicity studies with mallard duck and bobwhite quail and an 8-day acute dietary study with bobwhite quail. All the studies appear to have been carried out according to standardised test guidelines in contract laboratories during the 1990s (Reiss *et al.* 2001). The mallard duck study showed no significant effects on body weight, feed consumption or gross pathology at doses up to 2150 mg/kg body weight. Therefore, the reported NOEC was 2150 mg/kg body weight. The acute oral study with bobwhite quail resulted in a LD 50 of 862 mg/kg body weight and diarrhoea was noted in the lowest concentration test group (147 mg/kg body weight) and therefore no NOEL could be established. The 8-day acute dietary study with bobwhite quail indicated no mortality up to 1250 mg/kg food. However, at 2500 mg/kg food, one death and, at 5000 mg/kg food, 4 deaths (10 birds/group) were recorded. The LC50 was > 5000 mg/kg food but no conclusion was drawn regarding the significance and interpretation of the mortality recorded at 2500 and 5000 mg/kg food.	

3.5.2 PNECs for secondary poisoning of predators

Bioconcentration data (as BCF values) for triclosan for fish are variable ranging from 2.7 to 90 for carp (*Cyprinus carpio*) and 2532-8700 for zebrafish (*Danio rerio*). Hence the trigger of BCF values >100 is met and the derivation of a PNEC for secondary poisoning of predators is required.

The lowest dietary NOEC, based on the lowest mammalian NOAEL for effects on reproduction in 13 week studies with dogs is ≥25 mg kg bw^{-1} day^{-1} which corresponds to ≥1000 mg kg^{-1} diet (see Table 3.1).

The appropriate assessment factor to derive a PNEC based on a chronic $NOEC_{food}$ from a mammalian study is 30 (Table 23 of ECB 2003)

$PNEC_{secpois.biota}$ = $NOEC_{food}$ (1000 mg/kg) /AF 30 = 33.0 mg/kg prey (wet weight)

The highest reported BCF value for fish is 8700 but no information is available for other organisms such as crustaceans and molluscs. Information on biomagnification of triclosan is not available.

The corresponding safe concentration in water (preventing bioaccumulation in prey to levels >$PNEC_{secpois.biota}$) can therefore be calculated as follows:

$PNEC_{secpois.water}$ = (33.0 mg/kg prey)/BCF (8700) = 3.8 µg l^{-1} triclosan

This concentration is higher than the proposed long-term PNECs for the protection of pelagic communities in both freshwaters and saltwaters. Therefore, if quality standards are set on the basis of these PNECs, the protection of predators from secondary poisoning will be covered.

4 Analysis and monitoring

Safavi *et al*. (2003) reported on a voltammetric method for the determination of trace amounts of triclosan in wastewater. The determination method is based on the adsorption of triclosan on hanging mercury drop electrode and desorption of triclosan at the negative tensammetric peak. The peak current was proportional to the concentration of triclosan over the range 2.5-60 µg l^{-1}. Under optimum experimental conditions (pH = 7, accumulation potential of -450 mV and accumulation time of 90 s), the detection limit (LOD) obtained was 1.9 µg 1^{-1} and the relative standard deviation (R.S.D) was lower than 3%.

Analytical methods, based on GC-MS and LC-MS, for the determination of trace amounts of triclosan in urban wastewater and marine sediments were developed and reported by Aguera *et al.* (2003). These methods involve the use of diverse analytical techniques, such as solid phase extraction (SPE) and pressurized liquid extraction for sample preparation, and GC-negative chemical ionization MS and LC-electrospray ionization (ESI) MS-MS for identification and quantification. The recoveries of triclosan were 84 % in wastewater and 100 % in sediments. Detection limits obtained were in the range of µg 1^{-1} to mg l^{-1}. To validate their applicability to real samples and as part of a more extensive monitoring program, the developed methods were applied to the analysis of wastewater samples coming from an urban wastewater treatment plant (UWWTP), and of marine sediment samples collected at the outflow of two UWWTPs to the sea. Results obtained revealed the presence of triclosan in all the samples at concentrations that ranged from 0.8 to 37.8 µg l^{-1} in wastewater and from 0.27 to 130.7 µg kg^{-1} in sediments.

Hua and Letcher (2003) reported on a method developed for the determination of triclosan in sewage treatment plant (STP) effluents and surface waters based on solid-phase extraction (SPE) coupled to reverse phase liquid chromatography and electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS). The instrument detection limit was 0.5 ng l^{-1}. The method was successfully applied for the detection of triclosan in sewage effluent and surface water with method detection limits of 4 and 2 ng l^{-1}, respectively. The mean recovery was 104% ± 8% for sewage effluent and 91% ± 10% for surface water. Triclosan was detected at concentrations of 174 ± 10% ng l^{-1} in sewage effluent and 15 ± 25% ng l^{-1} in the surface waters from the Detroit River.

For water, the proposed PNECs derived for triclosan range from 0.10 to 0.28 µg l^{-1}. The data quality requirements are that, at a third of the EQS, total error of measurement should not exceed 50 per cent. Using this criterion, it is evident that current analytical methodologies should offer adequate performance to analyse for triclosan.

5 Conclusions

5.1 Availability of data

Longer-term toxicity data are available for seven taxonomic groups namely; algae, crustaceans, fish, insects, macrophytes, protozoa and rotifers. Freshwater short-term toxicity data are available for six taxonomic groups (algae, crustaceans, fish, insects, macrophytes and protozoa). Freshwater unicellular algae are more sensitive to triclosan than other taxonomic groups which is consistent with the mode of action of the substance. For marine organisms short-term toxicity data are available for two different taxonomic groups (algae and bacteria) and there are ony long-term data for algae. Without the minimum of three saltwater taxa (algae, crustaceans and fish) required by Annex V of the Directive a separate marine PNEC cannot be derived.

5.2 Derivation of PNECs

The proposed PNECS are described below and summarised in Table 5.1.

5.2.1 Long-term PNEC for freshwaters

The lowest valid long-term toxicity value for freshwater algae is a 3-day no observed effect concentration (NOEC) of 0.5 μg l^{-1} for effects on the growth rate of the alga *Scenedesmus subspicatus*. Reliable long-term NOECs are available for algae, invertebrates and fish including a large body of data for algae which are the most sensitive taxonomic group. Based on the data available and the combined weight of evidence it presents it is considered appropriate to apply a reduced assessment factor of 5 (based on the specific circumstances applying to triclosan in terms of its mode of action and the available dataset for target species) this results in a $PNEC_{freshwater\_lt}$ of 0.10 μg l^{-1} triclosan.

The PNEC value of 0.10 μg l^{-1} is supported by an HC5,50 value of 0.13 μg l^{-1} generated from an SSD based on the available long-term data for freshwater algae.

5.2.2 Short-term PNEC for freshwaters

Reliable short-term data are available for algal, invertebrate and fish species. The lowest valid short-term toxicity value is a 72 hour growth rate EC50 of 2.8 μg l^{-1} for the alga *Scenedesmus subspicatus*. There is a considerable short-term toxicity database for freshwater organisms, which shows that algae such as *Scenedesmus* are likely to be among the most sensitive to triclosan. An assessment factor of 10 was therefore applied, resulting in a $PNEC_{freshwater\_st}$ of 0.28 μg l^{-1}.

5.2.3 Long-term PNEC for saltwaters

The only long-term saltwater data available is a 4-day EC25 of >66 μg l^{-1} for effects of triclosan on the marine alga *Skeletonema costatum*. Since algae are the most sensitive freshwater taxa and marine algae are apparently not more sensitive to triclosan than

freshwater species it is recommended that the freshwater PNEC is also adopted to protect saltwater taxa. It is proposed not to apply an additional assessment factor of 10 for marine species due to the antimicrobial mode of action of triclosan (i.e. by disruption of cell wall formation in micro-organisms) which indicates that organisms such as echinoderms and molluscs are likely to be less sensitive than algae.

This results in a $PNEC_{saltwater\_lt}$ of 0.10 μg l^{-1}.

5.2.4 Short-term PNEC for saltwaters

There are limited saltwater data for short-term available for triclosan.

Since there should be no obvious differences in the sensitivity of freshwater or saltwater species of the same taxonomic group given the mode of action of triclosan, the TGD approach of using a combined freshwater and saltwater dataset for the marine effects assessment can be used. Therefore, proposed freshwater PNECs should be considered in deriving corresponding values for saltwater bodies. It is therefore recommended that the freshwater PNEC is also adopted to protect saltwater taxa. This results in a $PNEC_{saltwater\_st}$ of 0.28 μg l^{-1}.

5.2.5 PNEC for sediments

Triclosan has a log Kow of 4.76 which exceeds the TGD trigger value of a log Kow of >3, therefore a sediment quality standard is necessary. However, data on direct toxicity to sediment-dwelling organisms are limited and are insufficient to derive a PNEC.

5.2.6 PNEC for secondary poisoning

Bioconcentration data (as BCF values) for triclosan for fish are variable ranging from 2.7 to 90 for carp (*Cyprinus carpio*) and 2532-8700 for zebrafish (*Danio rerio*). Hence the trigger of BCF values >100 is met and the derivation of PNECs for secondary poisoning of predators is required. The PNEC based on the risks of secondary poisoning to mammals and birds (3.8 μg l^{-1}) is higher than those derived for the protection of aquatic life and so does not influence the development of aquatic EQSs for triclosan.

Table 5.1 Summary of proposed PNECs

Receiving medium/exposure scenario	Proposed PNEC (μg l^{-1})	Existing EQS (μg l^{-1})
Freshwater/long-term	0.10	-
Freshwater/short-term	0.28	-
Saltwater/long-term	0.10	-
Saltwater/short-term	0.28	-
Sediment	Insufficient data	-
Secondary poisoning	3.8	-

5.3 Analysis

For water, the proposed PNECs derived for triclosan range from 0.10 to 0.28 µg l^{-1}. The data quality requirements are that, at a third of the EQS, total error of measurement should not exceed 50 per cent. Using this criterion, it is evident that current analytical methodologies (non-standard) employing voltammetry, gas chromatography–mass spectrometry (GC-MS) or liquid chromatography–mass spectrometry (GC-MS),capable of achieving detection limits as low as 0.5 ng l^{-1}, should offer adequate performance to analyse for triclosan.

5.4 Implementation issues

Based on consideration of the information collated within the report and the proposed PNECs the following comments are made re: implementation:-

- Current analytical capability should be adequate for compliance assessment
- The assessment factors applied are within the range of 5-10 and therefore the PNECs derived are not subject to excessive uncertainty.

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List of abbreviations

AA	annual average
AF	assessment factor
a.i.	active ingredient
BCF	bioconcentration factor
bw	body weight
CAS	Chemical Abstracts Service
DO	dissolved oxygen
EC50	Concentration effective against 50 per cent of the organisms or animals tested
EQS	Environmental Quality Standard
GC	gas chromatography
GLP	Good Laboratory Practice (OECD)
LC50	Concentration lethal to 50 per cent of the organisms or animals tested
LC-ESI	liquid chromatography – electrospray ionisation
LOAEL	lowest observed adverse effect level
LOEC	lowest observed effect concentration
lt	long-term
MAC	maximum allowable concentration
MS	Mass spectrometry
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
OECD	Organization for Economic Co-operation and Development
PNEC	predicted no-effect concentration
SPE	Solid phase extraction
SSD	species sensitivity distribution
st	short-term
TGD	Technical Guidance Document
UKTAG	UK Technical Advisory Group
US EPA	US Environmental Protection Agency
WFD	Water Framework Directive

ANNEX 1 Data quality assessment sheets

Identified and ordered by alphabetical order of references.

Data relevant for PNEC derivation were quality assessed in accordance with the so-called Klimisch Criteria (Table A1).

Table A1 Klimisch Criteria*

Code	Category	Description
1	Reliable without restrictions	Refers to studies/data carried out or generated according to internationally accepted testing-guidelines (preferably GLP**) or in which the test parameters documented are based on a specific (national) testing guideline (preferably GLP), or in which all parameters described are closely related/comparable to a guideline method.
2	Reliable with restrictions	Studies or data (mostly not performed according to GLP) in which the test parameters documented do not comply totally with the specific testing guideline, but are sufficient to accept the data or in which investigations are described that cannot be subsumed under a testing guideline, but which are nevertheless well-documented and scientifically acceptable.
3	Not reliable	Studies/data in which there are interferences between the measuring system and the test substance, or in which organisms/test systems were used that are not relevant in relation to exposure, 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 is not convincing for an expert assessment.
4	Not assignable	Studies or data which do not give sufficient experimental details and which are only listed in short abstracts or secondary literature.

* Klimisch H.-J, Andreae M and Tillmann U, 1997 A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*, **25**, 1–5.
** OECD Principles of Good Laboratory Practice (GLP). See: http://www.oecd.org/department/0,2688,en_2649_34381_1_1_1_1_1,00.html

Author	Dussault *et al.* 2008
Information on the test species	
Test species used	*Chironomus tentans*
Source of the test organisms	In house laboratory cultures
Holding conditions prior to test	Reconstituted water
Life stage of the test species used	12 day old organisms

Information on the test design	
Methodology used	The study was carried out according to the US EPA Guidelines[111]
Form of the test substance	Triclosan (>97% purity)
Source of the test substance	Sigma Aldrich
Type and source of the exposure medium	Reconstituted water with a sand substrate
Test concentrations used	Eight or nine concentrations (seven test concentrations, a control and a 0.2% methanol control)
Number of replicates per concentration	4-5
Number of organisms per replicate	9-10
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Semi-static (with renewal every 48 hours), 10 days, no feeding
Measurement of exposure concentrations	Yes (Samples collected prior to water renewal)
Measurement of water quality parameters	Yes (dissolved oxygen, pH, temperature conductivity, hardness, alkalinity and ammonia)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	-

Reliability of study	**Reliable with restrictions**
Relevance of study	**Relevant**
Klimisch Code	**2**

[11] U.S. Environmental Protection Agency. (2000) Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. EPA/600/R-99/064. Office of Water, Washington, DC.

Author	Dussault *et al.* 2008

Information on the test species	
Test species used	*Hyalella azteca*
Source of the test organisms	Environment Canada laboratory (Bulrington, Ontario, Canada)
Holding conditions prior to test	Reconstituted standard artificial medium following US EPA Guidelines[12]
Life stage of the test species used	7-14 day old organisms

Information on the test design	
Methodology used	The study was carried out according to the US EPA Guidelines[8]
Form of the test substance	Triclosan (>97% purity)
Source of the test substance	Sigma Aldrich
Type and source of the exposure medium	Reconstituted standard artificial medium following US EPA Guidelines[8]
Test concentrations used	Eight or nine concentrations (seven test concentrations, a control and a 0.2% methanol control)
Number of replicates per concentration	4-5
Number of organisms per replicate	8-15
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Semi-static, 10 days, no feeding
Measurement of exposure concentrations	Yes (Samples collected prior to water renewal)
Measurement of water quality parameters	Yes (dissolved oxygen, pH, temperature conductivity, hardness, alkalinity and ammonia)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	-

Reliability of study	**Reliable with restrictions**
Relevance of study	**Relevant**
Klimisch Code	**2**

[12] U.S. Environmental Protection Agency. (2000) Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. EPA/600/R-99/064. Office of Water, Washington, DC.

Author	Ishabashi *et al. 2004*

Information on the test species	
Test species used	*Oryzias latipes*
Source of the test organisms	Embryos produced from sexually mature fish obtained from a local fish and held in the laboratory
Holding conditions prior to test	Dechlorinated tap water with adults fed a diet of *Artemia* nauplii once daily and a commercial diet three times daily for 21 days
Life stage of the test species used	Embryos (<24 hour post-fertilisation)

Information on the test design	
Methodology used	The methodology is well described in the paper
Form of the test substance	Triclosan (>98% purity)
Source of the test substance	Wako Pure Chemical Industries Ltd, Tokyo, Japan
Type and source of the exposure medium	Dechlorinated tap water
Test concentrations used	Control, solvent control (0.1% DMSO), 78, 156, 313, 625, 1250 and 2500 µg l^{-1}
Number of replicates per concentration	2
Number of organisms per replicate	60 (30 per replicate)
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Semi-static (renewal every 24 hours), 14 days, no feeding
Measurement of exposure concentrations	Yes (once per week)
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	-

Reliability of study	**Reliable with restrictions**
Relevance of study	**Relevant**
Klimisch Code	**2**

Author	McHenry *et al.* 2006

Information on the test species	
Test species used	*Nannochloropsis oculata*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	The methodology is reasonably well described in the paper
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	0 - 250 µg l^{-1} (seven concentrations)
Number of replicates per concentration	3
Number of organisms per replicate	100,000 algae/ml
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Not stated
Measurement of exposure concentrations	Not stated
Measurement of water quality parameters	Yes (salinity = 25 ‰, temperature = 23-27ºC, light intensity 4,000 lux)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	-

Reliability of study	**Reliable with restrictions**
Relevance of study	**Relevant**
Klimisch Code	**2**

Author	McHenry *et al.* 2006

Information on the test species	
Test species used	*Tetraselmis chuii*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	The methodology is reasonably well described in the paper
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	0 - 250 µg l^{-1} (seven concentrations)
Number of replicates per concentration	3
Number of organisms per replicate	100,000 algae/ml
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Not stated
Measurement of exposure concentrations	Not stated
Measurement of water quality parameters	Yes (salinity 25 = ‰, temperature = 23-27ºC, light intensity 4,000 lux)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	-

Reliability of study	**Reliable with restrictions**
Relevance of study	**Relevant**
Klimisch Code	**2**

Author	Miyoshi *et al*. 2003

Information on the test species	
Test species used	*Paramecium caudatum (SJ-4 strain)*
Source of the test organisms	Cultured in lettuce infusion at 23 ºC
Holding conditions prior to test	Flask
Life stage of the test species used	30 day sub culture

Information on the test design	
Methodology used	The methodology is well described in the paper
Form of the test substance	Triclosan (100 % purity)
Source of the test substance	Ciba-Geigy Ltd (Basel, Switzerland).
Type and source of the exposure medium	Water (not specified)
Test concentrations used	2.896 – 28955 µg l^{-1} (reported as 0.01 – 100 µM)
Number of replicates per concentration	Not stated
Number of organisms per replicate	10 *Paramecium* cells per well and 12 wells used
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Static, 5 days, no feeding
Measurement of exposure concentrations	Not stated
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	-

Reliability of study	**Reliable with restrictions**
Relevance of study	**Relevant**
Klimisch Code	**2**

Author	Orvos *et al.* 2002

Information on the test species	
Test species used	*Anabaena flos-aquae*
Source of the test organisms	University of Texas (Austin, TX, USA) culture collection
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Conducted to American Society for Testing and Materials (ASTM) Method "Standard Guide for Conducting Static 96-h toxicity tests with microalgae. E 1218-90. In Annual Book of ASTM Standards, Vol 11.04, Philadelphia
Form of the test substance	Triclosan (99.5% pure)
Source of the test substance	Ciba Speciality Chemicals (Basal, Switzerland)
Type and source of the exposure medium	Test media
Test concentrations used	Control solvent control (0.068 ml l^{-1} dimethyl-formanide) , 0.41, 0.81, 1.6, 3.3, 6.5 and 13.0 µg l^{-1} (measured)
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Static, 96 hours, -
Measurement of exposure concentrations	Yes
Measurement of water quality parameters	Yes (pH = 7.5 $\pm$ 0.1 at test initiation, temperature 24 $\pm$ 2 ºC, continuous illumination of 2150 $\pm$ 323 lux)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Good quality study

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Author	Orvos *et al.* 2002

Information on the test species	
Test species used	*Ceriodaphnia dubia*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Neonates (<24 h old)

Information on the test design	
Methodology used	Adapted from US EPA-600/4-85/013 and EPA-600/4-85/041
Form of the test substance	Triclosan (99.5% pure)
Source of the test substance	Ciba Speciality Chemicals (Basal, Switzerland)
Type and source of the exposure medium	Aged hard well water
Test concentrations used	Control, 6, 12, 24, 50 and 108 µg l^{-1} (measured)
Number of replicates per concentration	10
Number of organisms per replicate	1
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Semi-static, 7 day, -.
Measurement of exposure concentrations	Yes (frequency not stated)
Measurement of water quality parameters	Yes (pH = 7.0 or 8.5, hardness = 174 mg l^{-1} and alkalinity = 115 mg l^{-1} as $CaCO_3$)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Study conformed to GLP procedures
Overall comment on quality	Good quality study

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Author	Orvos *et al*. 2002

Information on the test species	
Test species used	*Ceriodaphnia dubia*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Neonates (<24 h old)

Information on the test design	
Methodology used	Adapted from US EPA-600/4-85/013 and EPA-600/4-85/041
Form of the test substance	Triclosan (99.5% pure)
Source of the test substance	Ciba Speciality Chemicals (Basal, Switzerland)
Type and source of the exposure medium	Aged hard well water
Test concentrations used	0, 6, 12, 24, 50 and 108 µg l^{-1}.
Number of replicates per concentration	1
Number of organisms per replicate	10
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Static renewal, 48 h, -.
Measurement of exposure concentrations	Yes (frequency not stated)
Measurement of water quality parameters	Yes (pH = 7.0, 7.5, 8.0 or 8.5, hardness = 174 mgl^{-1} and alkalinity = 115 mgl^{-1} as $CaCO_3$)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Study conformed to GLP procedures
Overall comment on quality	Good quality study

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Author	Orvos *et al.* 2002

Information on the test species	
Test species used	*Daphnia magna*
Source of the test organisms	Not stated
Holding conditions prior to test	Kept in beakers
Life stage of the test species used	Neonates (<24 h old)

Information on the test design	
Methodology used	Conducted to OECD Guideline 202
Form of the test substance	Triclosan (99.5% pure)
Source of the test substance	Ciba Speciality Chemicals (Basal, Switzerland)
Type and source of the exposure medium	Aged hard well water
Test concentrations used	Control, 10, 40, 200, 1000 and 5000 µg l^{-1} (nominal)
Number of replicates per concentration	1
Number of organisms per replicate	10
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Semi static, 21 day, feeding
Measurement of exposure concentrations	Yes (reverse-phase HPLC)
Measurement of water quality parameters	Yes (pH = 8.2)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Study conformed to GLP procedures
Overall comment on quality	Good quality study

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Author	Orvos *et al.* 2002

Information on the test species	
Test species used	*Lemna gibba*
Source of the test organisms	Not reported in paper
Holding conditions prior to test	Not stated
Life stage of the test species used	Fronded plants

Information on the test design	
Methodology used	Adapted from ASTM G3.E 1415-91
Form of the test substance	Triclosan (99.5% pure)
Source of the test substance	Ciba Speciality Chemicals (Basal, Switzerland)
Type and source of the exposure medium	Not stated
Test concentrations used	Control, 0.5, 2.5, 12.5 and 65 µg l^{-1} (nominal)
Number of replicates per concentration	2
Number of organisms per replicate	Three plants consisting of 4 fronds per replicate vessel
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Static, 7 day, -.
Measurement of exposure concentrations	No
Measurement of water quality parameters	Yes (temperature = 25ºC ± 2ºC, illuminated to 5,000 ± 750 lux, continuous warm white lights)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Study conformed to GLP procedures
Overall comment on quality	Good quality study

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Author	Orvos *et al*. 2002

Information on the test species	
Test species used	*Oncorhynchus mykiss*
Source of the test organisms	Mt. Lassen Trout Farms (Red Bluff, CA, USA).
Holding conditions prior to test	Individual testing chambers
Life stage of the test species used	Unfertilised eggs and milt.

Information on the test design	
Methodology used	American Society for Testing and Materials Standard E1241-88
Form of the test substance	Triclosan (99.5% pure)
Source of the test substance	Ciba Speciality Chemicals (Basal, Switzerland)
Type and source of the exposure medium	Well water
Test concentrations used	Control, 5, 10, 20, 40 and 80 µg l^{-1} (nominal)
Number of replicates per concentration	4
Number of organisms per replicate	10
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Flow through (82 litres per day but increased to 133 litres during the last 14 days), 61 days, fed from 14 d after hatching until 59 d after hatching.
Measurement of exposure concentrations	Yes (days -5, 0, 1 and 7 and at least weekly thereafter)
Measurement of water quality parameters	Yes (pH = 8.17 to 8.21)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Study conformed to GLP procedures
Overall comment on quality	Good quality study

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Author	Orvos *et al.* 2002

Information on the test species	
Test species used	*Pimephales promelas*
Source of the test organisms	In-house laboratory culture
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Adapted from EPA-600/4-85/013 and American Public Health Association Standard Method for the Examination of Water and Wastewater.
Form of the test substance	Triclosan (99.5% pure)
Source of the test substance	Ciba Speciality Chemicals (Basal, Switzerland)
Type and source of the exposure medium	Soft blended water
Test concentrations used	Control, 0.056, 0.10, 0.18, 0.32 and 0.56 mg l^{-1} (nominal)
Number of replicates per concentration	Not stated
Number of organisms per replicate	10
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Static, 96 hours,-.
Measurement of exposure concentrations	No (nominal - owing to previous experiments demonstrating solution stability)
Measurement of water quality parameters	Yes (hardness = 40 to 48 mg l^{-1} as $CaCO_3$)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Study conformed to GLP procedures
Overall comment on quality	Good quality study

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Author	Orvos *et al.* 2002

Information on the test species	
Test species used	*Scenedesmus subspicatus*
Source of the test organisms	University of Texas (Austin, TX, USA) culture collection
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Conducted to OECD Guideline 201
Form of the test substance	Triclosan (99.5% pure)
Source of the test substance	Ciba Speciality Chemicals (Basal, Switzerland)
Type and source of the exposure medium	Well water
Test concentrations used	Control, 0.50, 1.0, 2.0, 4.0 and 8.0 µg l^{-1} (measured)
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Static, 96 hours, -
Measurement of exposure concentrations	Yes
Measurement of water quality parameters	Yes (pH = 7.5 to 7.8 at test initiation and 7.8 to 10.2 at test termination, temperature 24 ºC, continuous illumination of approximately 8,000 to 9,000 lux)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Study conformed to GLP procedures
Overall comment on quality	Good quality study

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Author	Orvos *et al.* 2002

Information on the test species	
Test species used	*Selenastrum capricornutum (now Pseudokirchneriella subcapitata)*
Source of the test organisms	University of Texas (Austin, TX, USA) culture collection
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Adapted from US EPA-600/9-78/018 and ASTM E 1218-90 and E 1415-91
Form of the test substance	Triclosan (99.5% pure)
Source of the test substance	Ciba Speciality Chemicals (Basal, Switzerland)
Type and source of the exposure medium	Well water
Test concentrations used	Control, 0.5, 2.5, 12.5 and 65μl^{-1} (nominal)
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Static, 96 hours, -
Measurement of exposure concentrations	Yes
Measurement of water quality parameters	Yes (temperature 24 + 2 ºC, continuous illumination of 4306 $\pm$ 646 lux)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Study conformed to GLP procedures
Overall comment on quality	Good quality study

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Author	Orvos *et al.* 2002

Information on the test species	
Test species used	*Skeletonema costatum*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Adapted from US EPA-600/9-78/018 and ASTM E 1218-90 and E 1415-91
Form of the test substance	Triclosan (99.5% pure)
Source of the test substance	Ciba Speciality Chemicals (Basal, Switzerland)
Type and source of the exposure medium	Not stated
Test concentrations used	Control, 0.5, 2.5, 12.5 and 65μl^{-1} (nominal)
Number of replicates per concentration	Not stated
Number of organisms per replicate	77,000 cells/ml – initial inoculum concentration
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Static, 96 hours, -.
Measurement of exposure concentrations	No (nominal - owing to previous experiments demonstrating solution stability)
Measurement of water quality parameters	Yes (temperature = 20ºC ± 2ºC, Illumination 4,306 ± 646 lux cool white light with 14:10 h light:dark photoperiod)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Study conformed to GLP procedures
Overall comment on quality	Good quality study

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Author	Tatarazako *et al.* 2004

Information on the test species	
Test species used	*Vibrio fisheri*
Source of the test organisms	Azur Environmental (Carlsbad, CA, USA)
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Test conditions and operating protocols of the Microtox acute toxicity test method
Form of the test substance	Triclosan
Source of the test substance	Sigma Chemicals (St Louis, MO, USA)
Type and source of the exposure medium	Not stated
Test concentrations used	0.9, 0.45, 0.23, 0.11, 0.056, 0.028, 0.014, 0.007, 0.0035 and 0 mg l^{-1}.
Number of replicates per concentration	2
Number of organisms per replicate	Not stated
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Static, 15 minutes,-
Measurement of exposure concentrations	No
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Study conformed to GLP procedures
Overall comment on quality	Good quality study

Reliability of study	**Reliable with restrictions**
Relevance of study	**Relevant**
Klimisch Code	**2**

Author	Wilson *et al.* 2003

Information on the test species	
Test species used	*Chlamydomonas*
Source of the test organisms	Natural population in freshwater stream
Holding conditions prior to test	Sterile glass screw-capped test tubes
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Not stated
Form of the test substance	Triclosan (95% pure).
Source of the test substance	Rita Corporation
Type and source of the exposure medium	Freshwater -collected from Cedar Creek, KS, USA
Test concentrations used	Control, 0.015, 0.15 and 1.5 µg l^{-1} (nominal)
Number of replicates per concentration	2
Number of organisms per replicate	Not stated
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Static, <=13 days,-
Measurement of exposure concentrations	No
Measurement of water quality parameters	Yes (temperature = 18ºC, tubes aerated by leaving caps slightly loose to allow air exchange, 12/12 light cycle at 500 E $\mu m^{-2}s^{-1}$)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	The absence of measured exposure concentrations along with the use of field collected organisms in a non-standard methodology mean the data should not be used to derive the PNEC

Reliability of study	**Unreliable**
Relevance of study	**Relevant**
Klimisch Code	**3**