# Proposed EQS for Water Framework Directive Annex VIII substances: chlorothalonil *(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.

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 chlorothalonil using the methodology described in Annex V of the Directive.

The PNECs described in this report are based on a technical assessment of the available ecotoxicity data for chlorothalonil, 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

Chlorothalonil is a broad spectrum, non-systemic, organochlorine fungicide used primarily in agriculture and to a much lesser extent on managed amenity turf. It is also used in approved antifoulant products.

Chlorothalonil is a polychlorinated aromatic compound, but it is atypical in that it does not have the high degree of persistence associated with many other chlorinated organic chemicals. This difference is attributed to the two nitrile groups which activate the molecule. Several of chlorothalonil’s primary metabolites are also polychlorinated, and they appear to be more persistent and more mobile than chlorothalonil.

Bioconcentration of chlorothalonil in aquatic organisms is considered to be low to moderate with reported Bioconcentration Factors (BCFs) of 9.4 to 264.

### Availability of data

Long-term exposure laboratory data are available for five different freshwater taxonomic groups: algae, crustaceans, fish, insects and macrophytes. Freshwater short-term toxicity data are available for 10 taxonomic groups (algae, amphibians, annelids, crustaceans, fish, insects, macrophytes, molluscs, platyhelminths and rotifers). Based on the information available, algae, crustaceans and fish appear to be similarly sensitive to chlorothalonil at very low concentrations. Macrophytes appear to be at least one order of magnitude less sensitive.

For marine organisms, single species short-term toxicity data are available for six different taxonomic groups (algae, ascidians, crustaceans, echinoderms, fish and molluscs). However, insufficient long-term toxicity data are available to fulfil the minimum requirement of three saltwater taxa (algae, crustaceans and fish) as required

under Annex V of the Water Framework Directive, hence the fresh and saltwater datasets were combined.

In addition, laboratory data are supplemented by freshwater mesocosm data, which suggest that under more natural conditions, toxicity is reduced due to dissipation of cholorothalonil, thus reducing the potential exposure of non-target organisms.

Chlorothalonil is not generally suspected of being an endocrine-disrupting chemical.

## Derivation of PNECs

Long-term PNEC for freshwaters
The lowest reliable long-term toxicity value for freshwater organisms is a NOEC of 0.35 $\mu g\ l^{-1}$ for rainbow trout, *Oncorhynchus mykiss*. Reliable long-term NOECs are available for algae, invertebrates and fish and therefore, an assessment factor of 10 has been applied, resulting in a $PNEC_{freshwater\_lt}$ of 0.035 $\mu g\ l^{-1}$.

This value is lower than the existing EQS of 0.1 $\mu g\ l^{-1}$ chlorothalonil which was derived by applying a safety factor of 100 to the lowest short-term effects concentration (an asymptotic LC50 for rainbow trout, *Oncorhynchus mykiss*, of 7.6 $\mu g\ l^{-1}$).

Short-term PNEC for freshwaters
Reliable short-term data are available for algal, invertebrate and fish species, which shows that acute sensitivity to chlorothalonil is comparable across taxa. The lowest reliable short-term result is the 5-day growth inhibition (using the biomass endpoint) EC50 of 8.8 $\mu g\ l^{-1}$ for the diatom, *Navicula pelliculosa* with a corresponding growth inhibition EC50 (using the more relevant growth rate endpoint) of 14 $\mu g\ l^{-1}$. Reliable 96-hour LC50 values of 12 $\mu g\ l^{-1}$ for effects of chlorothalonil on the survival of the freshwater lobster *Astacopsis gouldi* and rainbow trout *Oncorhynchus mykiss* have also been reported. To derive the short-term freshwater PNEC an assessment factor of 10 (given the large body of acute data) was therefore applied to the 96-hour LC50 values of 12 $\mu g\ l^{-1}$ for *A.gouldi* and *O.mykiss*, resulting in a $PNEC_{freshwater\_st}$ of 1.2 $\mu g\ l^{-1}$.

This value is higher than the existing EQS of 1.0 $\mu g\ l^{-1}$ chlorothalonil which was derived by applying a safety factor of 10 to the lowest reliable short-term effects data (an asymptotic LC50 for *Oncorhynchus mykiss* of 7.6 $\mu g\ l^{-1}$).

Long-term PNEC for saltwaters
Long-term single species saltwater toxicity data are only available for algae and crustaceans. The most sensitive result is a 28-day NOEC of 0.83 $\mu g\ l^{-1}$ for the mysid shrimp, *Americamysis bahia*, but this study is not considered reliable enough to be used as the basis of a PNEC. As the saltwater toxicity data values available do not appear to differ markedly from the range obtained for corresponding freshwater species, a combined freshwater and saltwater dataset for marine effects assessment was used to derive the long-term saltwater PNEC. Therefore, the freshwater PNEC is recommended to be adopted to protect saltwater taxa. It is proposed that an additional assessment factor of 10 is applied to account for the paucity of long-term toxicity data for marine species. This results in a $PNEC_{saltwater\_lt}$ of 0.0035 $\mu g\ l^{-1}$.

This value is lower than the existing guideline EQS of 0.1 $\mu g\ l^{-1}$ chlorothalonil which was based on the long-term PNEC for freshwaters because of insufficient marine data to set an EQS.

Short-term PNEC for saltwaters

Reliable short-term data are available for six different taxonomic groups (algae, ascidians, crustaceans, echinoderms, fish and molluscs) including the base set of algae, invertebrates and fish. The most sensitive short-term result for saltwater species is a 96-hour shell deposition EC50 of 3.6 μg $l^{-1}$ for *Crassostrea virginica*. This is an unpublished study, but a further unpublished study using the same species reported 96-h EC50 values of 5.0 μg $l^{-1}$ supporting this sensitive result. In addition, there is a 48-hour EC50 of 6.6 μg $l^{-1}$ for developmental effects in the echinoderm *Parcentrotus lividus*. It is therefore recommended that a short-term saltwater PNEC should be based on effects to the mollusc *C. virginica* (3.6 μg $l^{-1}$) and an assessment factor of 10. This results in a $PNEC_{saltwater\_st}$ = 0.36 μg $l^{-1}$.

This value is lower than the existing EQS of 1 μg $l^{-1}$ chlorothalonil which was derived by applying a safety factor of 10 to the lowest reliable short-term effects data (an asymptotic LC50 for *Oncorhynchus mykiss* of 7.6 μg $l^{-1}$).

PNECs for sediment

The TGD trigger value of a log Koc or log Kow of ≥3 is met, as reported log Kow and Koc values are in the range 2.91 – 3.05 and 2.9-3.84 respectively. However, there is only limited information with respect to experimental data on sediment toxicity for chlorothalonil and therefore no $PNEC_{sediment}$ can be derived.

PNEC for secondary poisoning

Fish bioconcentration data (as BCF values) for chlorothalonil range from 9.4 to 264, hence the trigger of a BCF >100 is exceeded and the derivation of PNECs for secondary poisoning of predators is required. The lowest relevant $NOEC_{food}$ is 120 mg $kg^{-1}$ derived from a 2-year study with dogs. Using the highest reported BCF of 264 for the calculation results in a corresponding water concentration of $PNEC_{secpois.water}$ = 4 mg $kg^{-1}$ prey / BCF (264) = 15 μg chlorothalonil $l^{-1}$.

This concentration is higher than the proposed long-term PNECs for the protection of freshwater and saltwater organisms. Therefore, if EQS are set on the basis of these PNECs the protection of predators from secondary poisoning would be included, and the derivation of additional quality standards for secondary poisoning is unnecessary.

**Summary of proposed PNECs**

| **Receiving medium/exposure scenario** | **Proposed PNEC (μg $l^{-1}$)** | **Existing EQS (μg $l^{-1}$)** |
|---|---|---|
| Freshwater/long-term | 0.035 | 0.1 |
| Freshwater/short-term | 1.2 | 1.0 |
| Saltwater/long-term | 0.0035 | 0.1 |
| Saltwater/short-term | 0.36 | 1.0 |
| Sediment | Insufficient data | None available |
| Secondary poisoning | 15 | None available |

## Analysis

For water, the lowest proposed PNECs derived for chlorothalonil is 0.007 μg $l^{-1}$. The data quality requirements are that, at a third of the EQS, total error of measurement should not exceed 50%. Using this criterion, it is evident that current analytical methodologies (non-standard) employing gas chromatography (GC) with electron capture detection or off-line solid-phase extraction followed by high performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry, are both capable of achieving detection limits of 0.001 μg $l^{-1}$. This should offer adequate performance to analyse for chlorothalonil.

## 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 methods are sensitive enough to assess compliance with the proposed PNECs in receiving waters.
- The freshwater long term and short term PNECs are not subject to excessive uncertainty with assessment factors of 10 being applied to derive the PNECs. The long term saltwater value was derived using an assessment factor of 100. This could be potentially reduced if additional reliable data was available for marine taxa.

# 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 chlorothalonil using the methodology described in Annex V of the Directive.

The PNECs described in this report are based on a technical assessment of the available ecotoxicity data for chlorothalonil, along with any data that relate impacts under field conditions to exposure concentrations. The data have been subjected to rigorous quality assessment so 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

Chlorothalonil is a broad spectrum, non-systemic, organochlorine fungicide. Chlorothalonil is used primarily as a fungicide in agriculture and, to a much lesser extent, on managed amenity turf. It is also used in approved antifoulant products.

Chlorothalonil is a polychlorinated aromatic compound, but it is atypical in that it does not have the high degree of persistence associated with many other chlorinated organics. This difference is attributed to the two nitrile groups which activate the molecule. Several of chlorothalonil’s primary metabolites are also polychlorinated, and they appear to be more persistent and more mobile than chlorothalonil (US EPA RED 1999).

Bioconcentration of chlorothalonil in aquatic organisms is considered to be low to moderate with reported Bioconcentration Factors (BCFs) of 9.4 to 264.

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[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 I.

# 2 Results and observations

## 2.1 Identity of substance

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

**Table 2.1 Species covered by this report**

| Name | CAS Number |
|---|---|
| Chlorothalonil | 1897-45-6 |

## 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 for chlorothalonil identified from the literature. 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 | 1.2 µg $l^{-1}$ | – | 1.0 µg $l^{-1}$ (MAC – tentative standard) |
| Freshwater long-term | 0.035 µg $l^{-1}$ | – | 0.1 µg $l^{-1}$ (AA – tentative standard) |
| Saltwater short-term | 0.36 µg $l^{-1}$ | – | 1.0 µg $l^{-1}$ (MAC – guideline standard) |
| Saltwater long-term | 0.0035 µg $l^{-1}$ | – | 0.1 µg $l^{-1}$ (AA – guideline standard) |
| Sediment | Insufficient data | – | - |
| Secondary poisoning | 15 µg $l^{-1}$ | – | - |

AA = Annual Average
AF = Assessment Factor
MAC = Maximum Allowable Concentration
SSD = Species Sensitivity Distribution
TGD = Technical Guidance Document

## 2.3 Hazard classification

Table 2.3 gives the R-phrases (Risk-phrases) and labelling for chlorothalonil.

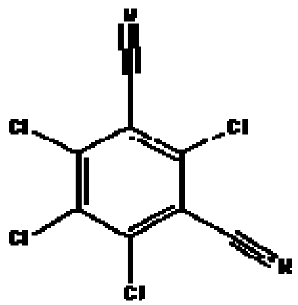
**Table 2.3 Hazard classification**

| R-phrases and labelling | Reference |
|---|---|
| R26, 37, 40, 41, 43, 50/53<br>S2, 28, 36/37/39, 46, 60, 61 | ECB 2005 |

## 2.4 Physical and chemical properties

Table 2.4 summarises the physical and chemical properties of chlorothalonil.

**Table 2.4 Physical and chemical properties of chlorothalonil**

| Property | Reference | |
|---|---|---|
| CAS number | ECB 2005 | 1897-45-6 |
| Substance name | ECB 2005 | Tetrachloroisophthalonitrile |
| Molecular formula | HSDB 2006 | $C_8Cl_4N_2$ |
| Molecular structure | Chemfinder 2005 |  |
| Molecular weight | EU DAR 2006<br>HSDB 2006 | 265.9 |
| Colour/form | HSDB 2006<br>EU DAR 2006 | White crystalline solid or powder (pure)<br>White or tan powder (technical) |
| Odour | HSDB 2006 | Odourless |
| Melting point (°C) | HSDB 2006<br>FAO 2005 | 250-251°C<br>252.5-254.5°C |
| Boiling point (°C) | HSDB 2006 | 350°C at 760 mmHg |
| Vapour pressure | HSDB 2006<br>EU DAR 2006<br>FAO 2005 | $5.7 \times 10^{-7}$ mm Hg at 25°C<br>$7.62 \times 10^{-5}$ Pa at 25°C<br>$2.2 \times 10^{-4}$ Pa at 25°C |
| Density/ specific gravity | HSDB 2006<br>EU DAR 2003 | 1.8 at 25°C |
| Henry's Law constant | HSDB 2006<br>EU DAR 2006 | $2.5 \times 10^{-7}$ atm $m^3$ $mol^{-1}$ at 25°C<br>$2.5 \times 10^{-2}$ Pa $m^3$ $mol^{-1}$ at 25°C |
| Water solubility | HSDB 2006/ EU DAR 2006<br>FAO 2005 | 0.81 mg $l^{-1}$ at 25°C<br>0.54 mg $l^{-1}$ at 25°C; pH7 (distilled water) |

| Property | Reference | |
|---|---|---|
| Solubility in organic solvents (in g $l^{-1}$ at 20°C) | EU DAR 2006 | acetone 20.6<br>dichloroethane 22.4<br>ethyl acetate 13.8<br>n-heptane 0.20<br>xylene 74.4<br>methanol 1.36 |

**Table 2.5 Environmental fate and partitioning of chlorothalonil**

| Property | |
|---|---|
| **Abiotic fate** | Chlorothalonil is expected to exist in both the vapour and particulate phases in the ambient atmosphere. Vapour-phase chlorothalonil is degraded slowly in the atmosphere by reaction with photochemically produced hydroxyl radicals with an estimated half-life of ~ 7 years at an atmospheric concentration of 5 x $10^5$ hydroxyl radicals per $cm^3$ (Meylan and Howard 1993). |
| **Hydrolytic stability** | Chlorothalonil is resistant to hydrolysis at pH 5 and 7; however, chlorothalonil hydrolyzed to 2,5,6-trichloro-4-hydroxy-isophtalonitrile and 3-cyano-2,4,5,6-tetrachlorobenzamide at pH 9 with a half-life of 38.1 d (HSDB 2006).<br><br>Half-life at 50°C - pH5 > 62 d; pH7 = 14 d; pH9 = 0.28 d; and half-life at 20°C - pH5 = stable; pH7 = stable; pH9 = 16.1 d (FAO 2005). |
| **Photostability** | Photolysis in sunlit surface waters may occur based on an aqueous photolysis half-life of 65 d (HSDB 2006).<br><br>Half-life = 10.5 h at equivalent of continuous summer sunlight, at 30°N (FAO 2005).<br><br>Half-life in water ($DT_{50}$) of 64.7 d at pH5, 25°C with 12 h sunlight per day (EU DAR 2006). |
| **Volatilisation** | Volatilisation from water surfaces is not expected to be an important fate process based upon this compound's Henry's Law constant (HSDB 2006). |
| **Distribution in water/ sediment systems** | Koc values of 900 to 7,000 indicate that chlorothalonil is expected to adsorb to suspended solids and sediment (HSDB 2006). |
| **Degradation in soil** | Aerobic biodegradation half-lives of chlorothalonil in four different soils ranged from 10 – 40 d (HSDB 2006). |
| **Biodegradation in water** | Chlorothalonil degrades under aerobic and anaerobic aquatic conditions. Anaerobic half-lives of chlorothalonil in two different flooded soils were ~ 5-15 d. Aerobic $DT_{50}$ in marine water reported as 8.1 and 8.8 d (HSDB 2006). Dissipation from water in the presence of sediment is rapid: 4 – 8 h (Hamer 2003) |
| **Octanol–water coefficient (log Kow)** | 3.05 (HSDB 2006)<br><br>2.91 at pH 4 and 25°C (FAO 2005)<br><br>2.94 at pH 7 and 9 and 25°C (FAO 2005) |

| Property | |
|---|---|
| **Log Koc** | 2.9 – 3.84 (USEPA RED 1999) |
| **Dissociation constant pKa** | No dissociation (EU DAR 2006) |
| **Bioconcentration (BCF) values** | BCF values of 9.4 to 264 measured in fish (HSDB 2006) suggest bioconcentration in aquatic organisms can be low to moderate.<br>BCF estimated <100 (EU DAR 2006) |

BCF = bioconcentration factor

## 2.5 Environmental fate and partitioning

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

The available data indicate that chlorothalonil is rapidly dissipated in aquatic environments. It is rapidly metabolised, with a half life (T½) of < 2 h in both fresh and saltwater-sediment test systems at 25°C. Initial dissipation is rapid and nonlinear, but residues persist, with approximately 1.6% (9.5 µg $l^{-1}$) and 2.6% (16 µg $l^{-1}$) of the originally applied concentration (615 µg $l^{-1}$) detectable as the parent compound after 30 d in freshwater and saltwater systems, respectively. $^{14}C$-residues could be divided into three fractions: organic soluble, polar water-soluble, and bound (Table 2.6). Two major and three minor metabolites were formed during one study and the pattern of these metabolites was similar in fresh and saltwater systems. The breakdown of chlorothalonil was described in a manufacturer's report as microbial in origin, involving an attack on chlorothalonil by glutathione (or other sulphur species) and it was suggested that this occurred at the sediment interface. It was also predicted that the two major metabolites would undergo further metabolism, resulting in the formation of bound and polar water-soluble residues (Manufacturer's unpublished study, cited in HSE 2002 and Caux 1996).

**Table 2.6 Percentage distribution of $^{14}C$-residues**

| Type of sediment | % of Total Radioactive Residue | | |
|---|---|---|---|
| | Organic soluble | Polar, water-soluble | Bound |
| Saltwater – sediment | 39 -68 | 1- 9 | 28 – 62 |
| Freshwater - sediment | 56 -96 | 3 – 15 | 4 – 22 |

Davies (1988) carried out a series of experiments to investigate the route and rate of chlorothalonil disappearance in the aquatic environment at different temperatures with different stream substrates. Temperature had a marked effect on the degradation half-life of chlorothalonil: a 10 ºC decrease in temperature almost doubled the reported half-lives from 80 h at 15°C to 150 h at 5°C, supporting the conclusion that the degradation was biotic. The presence of a biotic component, in the form of fish or algae, together with aeration was found to increase dissipation, resulting in a half-life of 4.4 h. Aeration alone had no effect.

Hamer (2003) describes two studies, on chlorothalonil dissipation from water, carried out in indoor and outdoor microcosms. In both cases chlorothalonil was applied as a 720 g $l^{-1}$ soluble concentrate formulation. In the indoor microcosm, (nominal water concentration of 25 µg a.i. $l^{-1}$)

containing water, sediment and aquatic plants the half-life was 4 h at approximately 18°C. In replicated outdoor systems containing water, sediment, aquatic plants and invertebrates, two applications of chlorothalonil formulation one week apart both gave similar half-lives of 8 h at approximately 10°C.

Bioconcentration of chlorothalonil in aquatic organisms is considered to be low to moderate with reported Bioconcentration Factors (BCFs) of 9.4 to 264 (HSBD 2006).

## 2.6 Effects data

A summary of the mode of action of chlorothalonil can be found in Section 2.6.5.

Data collation followed a tiered approach. First, critical freshwater and saltwater data were compiled from existing EQS documents. Further data published after derivation of the current UK EQS were then retrieved from the US Environmental Protection Agency (US EPA) ECOTOX database.[3]

Further data sources used included:

- ScienceDirect®;[4]
- US EPA Re-registration Eligibility Report (RED) for chlorothalonil (US EPA 1999 – referred to in this report as US EPA RED 1999);
- The Health and Safety Executive Biocide and Pesticides Assessment Unit's evaluation report on chlorothalonil: use as a booster biocide in antifouling products (HSE 2002);
- European Commission Draft Assessment Report (Public Version) on chlorothalonil prepared under the Plant Protection Products Directive 91/414/EEC (EU DAR 2006);
- OPP Pesticide Ecotoxicity Database - an online US EPA database held by the Office of Pesticide Programs that summarises ecotoxicological data used by the EPA for ecotoxicological assessments. This consists primarily of the endpoint data submitted in support of registration and re-registration of pesticide products (OPP 2007)[5].

Many of the most sensitive toxicity results are reported in unpublished studies carried out in support of pesticide registration. As only summary information on the tests is provided in compendium reports the Pesticide Safety Directorate was asked to provide additional information where possible. Relevant extracts from the Draft Assessment Report (EU DAR 2006) prepared by the chlorothalonil Rapporteur Member State (RMS) (The Netherlands) under the Directive 91/414/EEC Review process were made available.

All concentrations of chlorothalonil in this report are expressed as active ingredient and all key data were checked for accuracy as far as practicable using the available data. All PNEC values have been derived from studies either using technical grade material or where the results are clearly expressed as active ingredient.

### 2.6.1 Toxicity to freshwater organisms

Freshwater toxicity data on chlorothalonil are available for various taxonomic groups including algae, invertebrates and fish as required for the application of the approach specified in the EU Technical Guidance Document (TGD) (ECB 2003). Long-term data are available for five taxonomic groups including algae, crustaceans, fish, insects and macrophytes. Freshwater short-term toxicity data are available for ten taxonomic groups including algae, amphibians, annelids, crustaceans, fish, insects, macrophytes, molluscs, platyhelminths and rotifers (Table 2.7).

[3] http://www.epa.gov/ecotox/
[4] http://www.sciencedirect.com/
[5] Http://www.ipmcenters.org/Ecotox/index.cfm

**Table 2.7 Summary of available freshwater data for chlorothalonil**

| Type of data | Taxonomic groups for which information is available |
|---|---|
| Long-term | Algae, crustaceans, fish, insects and macrophytes |
| Short-term | Algae, amphibians, annelids, crustaceans, fish, insects, macrophytes, molluscs, platyhelminths and rotifers |

Overall, the available short-term and long-term toxicity test data and that from mesocosm and field studies indicate that for chlorothalonil sensitivity is similar across taxa with the exception of macrophytes which appear to be significantly less sensitive.

The data were also evaluated to assess whether differences in toxicity were due to different physical formulation effects (e.g., the use of the same chemical formulation but including either technical grade material or an emulsifiable concentrate). Where comparative data are available the majority of species show small differences in effects after exposure to formulations or technical material, expressed as active ingredient (a.i.). A comparison of acute toxicity of chlorothalonil and chlorothalonil formulations to selected taxa is shown in Table 2.8.

**Table 2.8 Comparison of acute toxicity to different taxa of chlorothalonil and chlorothalonil formulations (data from EU DAR 2006)**

| Species | Endpoint | Value (µg a.i. $l^{-1}$) | | | |
|---|---|---|---|---|---|
| | | Technical grade material | Bravo 720 formulation | 75WG formulations | Daconil 2782 Extra |
| *Pseudokirchneriella subcapitata*[6] | 72-96 h EC50 | 120 | - | 83- 158 | - |
| *Daphnia magna* | 48 h EC50 | 54 - 117 | 97 | 38 - 84 | 344 |
| *Onchorhynchus mykiss* | 96 h EC50 | 17 - 76 | 33 | 25 - 30 | 80 |

Diagrammatic representations of the available freshwater data (cumulative distribution functions) for chlorothalonil are presented in Figure 2.1 for long-term data and Figure 2.2 for short-term data.

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 chlorothalonil PNECs.

The lowest critical freshwater data are presented in Table 2.9 for long-term toxicity data and Table 2.10 for short-term toxicity data. These tables do not contain all the available toxicity data but only those which are considered most relevant to the derivation of PNECs.

[6] Formerly known as *Selenastrum capricornutum*

The data in Tables 2.9 and 2.10 indicate that the toxicity of chlorothalonil and its formulations occurs over a fairly tight concentration range. The formulation of chlorothalonil shows a negligible difference in toxicity from the technical material.

**Figure 2.1 Cumulative distribution function of freshwater long-term data (µg a.i. $l^{-1}$) for chlorothalonil**

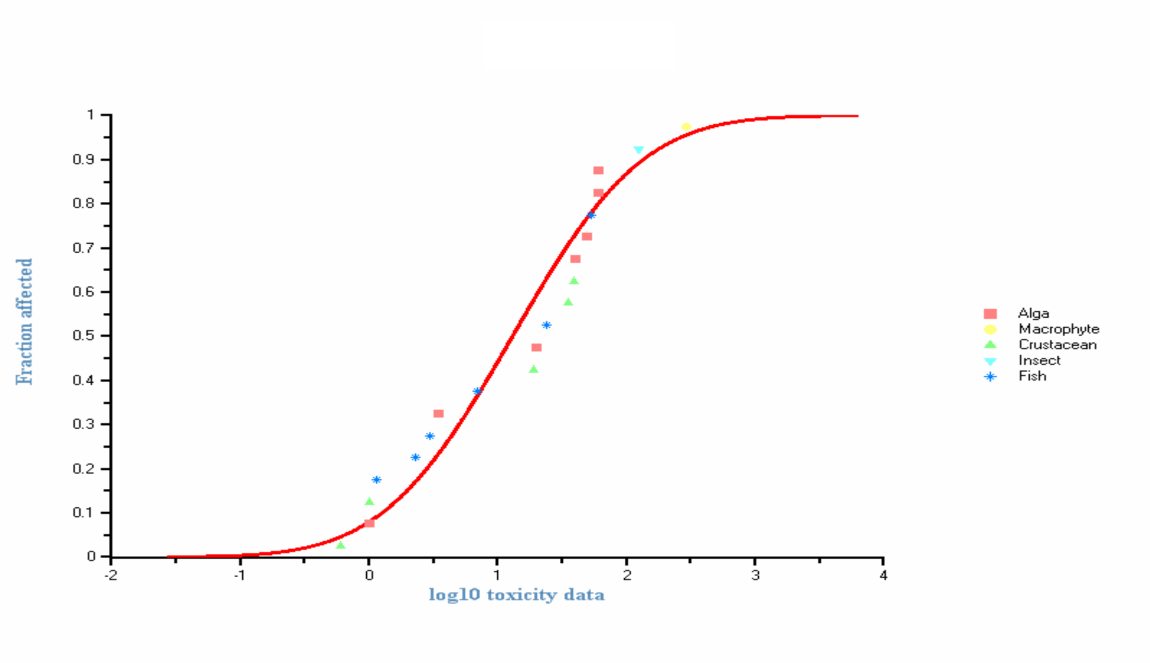



**Figure 2.2 Cumulative distribution function of freshwater short-term data (µg a.i. $l^{-1}$) for chlorothalonil**

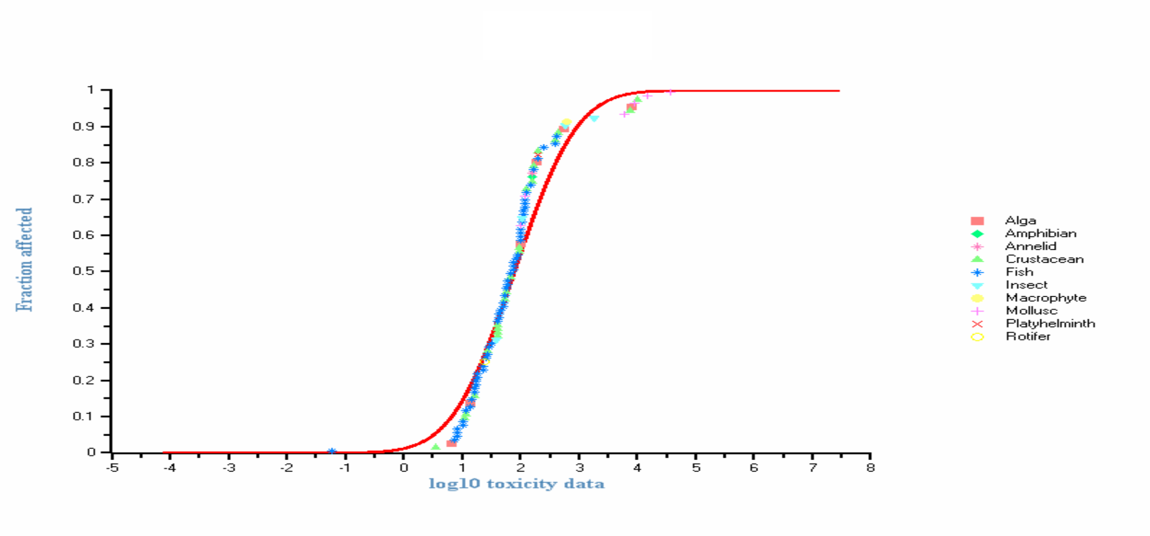


**Table 2.9 Most sensitive long-term aquatic toxicity data for freshwater organisms exposed to chlorothalonil**

| Chemical formulation (% purity) | Scientific name | Common name | Taxonomic group | Effect | Endpoint | Test duration | Conc. (µg a.i. $l^{-1}$) | Exposure[1] | Toxicant analysis[2] | Comments | Reliability (Klimisch Code*) | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Algae** | | | | | | | | | | | | |
| Technical grade | *Pseudokirchneriella subcapitata*[7] | Green alga | ALG | Growth inhibition | LOEC | 72 h | 1.0 | s | n | T=25°C | 3 | Fernandez-Alba *et al.* (2002) |
| Technical grade (98.1%) | *Navicula pelliculosa* | Diatom | ALG | Growth inhibition | NOEC | 5 d | 3.5 | s | u | | 1 | EU DAR (2006) |
| Technical grade (98.1%) | *Navicula pelliculosa* | Diatom | ALG | Growth inhibition | NOEC | 5 d | 3.9 | s | u | | C | OPP (2007) |
| Daconil 2787 extra (40%) | *Scenedesmus subspicatus* | Green alga | ALG | Growth inhibition | NOEC | 72 h | 6.5 | s | y | | 1 | EU DAR (2006) |
| Technical grade (98.1%) | *Anabaena flos-aquae* | Blue-green alga | ALG | Growth inhibition (growth rate) | NOEC | 5 d | 20 | s | n | pH 7 – 7.6 | 4 | Cited in RIVM (2001) and EU DAR (2006) |
| Bravo 500 (40.4%) | *Scenedesmus subspicatus* | Green alga | ALG | Growth rate | NOEC | 96 h | 40 | - | u | - | 4 | ISK Biotech (1993) cited in Crane *et al*. (1995) |
| **Higher plants** | | | | | | | | | | | | |
| Technical grade 98.1% | *Lemna gibba* | Duckweed | MAC | Growth inhibition | NOEC | 14 d | 290 | s | u | - | C | EU DAR (2006), OPP (2007) |
| **Invertebrates** | | | | | | | | | | | | |
| 75WG formulation (75%) | *Daphnia magna* | Waterflea | CRU | Reproduction | NOEC | 21 d | 0.5 | ss | y | | 1 | EU DAR (2006) |
| Technical grade (99.18%) | *Daphnia magna* | Waterflea | CRU | Parental generation survival | NOEC | 21 d | 0.6 | ss | y | pH 7-7.4 hardness 150 -200 mg $CaCO_3$ $l^{-1}$ | 4 | FAO (2005)/RIVM (2001), EU DAR (2006) |
| Daconil 2787 extra | *Daphnia magna* | Waterflea | CRU | Reproduction | NOEC | 22 d | <0.92 | ss | y | | 1 | EU DAR (2006) |

[7] Formerly known as *Selenastrum capricornutum*

| Chemical formulation (% purity) | Scientific name | Common name | Taxonomic group | Effect | Endpoint | Test duration | Conc. ($\mu$g a.i. $l^{-1}$) | Exposure[1] | Toxicant analysis[2] | Comments | Reliability (Klimisch Code*) | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (40%) | | | | | | | | | | | | |
| Technical grade (99.2%) | *Daphnia magna* | Waterflea | CRU | Reproduction | NOEC | 21 d | 19 | ss | y | pH 7-7.4 hardness 150 -200 mg $CaCO_3$ $l^{-1}$ | 4 | RIVM (2001) |
| Technical grade (98.8%) | *Daphnia magna* | Waterflea | CRU | Reproduction | NOEC | 21 d + 21 d | 35 | f | y | T=22°C pH 7.8-8.2 hardness 172 mg $CaCO_3$ $l^{-1}$ | 4 | FAO (2005), HSE (2002), RIVM (2001) |
| Technical grade (98.1%) | *Chironomus riparius* | Midge | INS | Reproduction | NOEC | 28 d | 125 | s | n | pH 6.2-8.4 | 4 | FAO (2005), RIVM (2001) |
| **Fish** | | | | | | | | | | | | |
| Commercial formulation | *Oryzias latipes* | Japanese medaka | FIS | Skewed sex ratio | Effect | 7 d | 0.06 | ss | n | T=25°C | 3 | Teather *et al.* (2005) |
| Daconil 2787 Extra (40%) | *Oncorhynchus mykiss* | Rainbow trout | FIS | Growth and survival | NOEC | 21 d | 0.35 | u | y | | 1 | EU DAR (2006) |
| Daconil 2787 (40.4%) | *Oncorhynchus mykiss* | Rainbow trout | FIS | Survival | NOEC | 21 d | 2.3 | ss | y | T=15°C hardness 88 mg $CaCO_3$ $l^{-1}$ | 4 | ISK Biotech (1989) cited in Caux (1996) |
| Technical grade (96%) | *Pimephales promelas* | Fathead minnow | FIS | Reproduction | NOEC<br>LOEC | § | 3<br>6.5 | f | y | T=25°C pH 6.5- 7.3 hardness 30 mg $CaCO_3$ $l^{-1}$ | C | Shults *et al.* (1980) cited in EU DAR 2006, USEPA RED (1999 |
| Technical grade (99.18%) | *Oncorhynchus mykiss* | Rainbow trout | FIS | Growth | NOEC | 21 d | 7 | f | y | pH 7.1 hardness 150 -200 mg $CaCO_3$ $l^{-1}$ | 4 | RIVM (2001) EU DAR (2006) |

* See Annex I and Annex II for explanation, C = Core data, equivalent to Klimisch code 1
[1] Exposure: s = static; ss = semi-static; f = flow-through
[2] Toxicant analysis: y = measured; n = not measured; u = unknown
ALG = alga, CRU = crustacean, FIS = fish, INS = insect, MAC = macrophyte

LOEC = lowest observed effect concentration
NOEC = no observed effect concentration
§ Reported duration time varies with report from 24 to 45 weeks. This was a two generation study. The NOEC for the first generation was also 3 µg $l^{-1}$

**Table 2.10 Most sensitive short-term aquatic toxicity data for freshwater organisms exposed to chlorothalonil**

| Chemical formulation (% purity) | Scientific name | Common name | Taxonomic group | Effect | Endpoint | Test duration | Conc. (µg a.i. $l^{-1}$) | Exposure[1] | Toxicant analysis[2] | Comments | Reliability (Klimisch Code*) | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Algae** | | | | | | | | | | | | |
| Technical grade | *Pseudokirch-neriella subcapitata* | Green alga | ALG | Growth inhibition | EC50 | 72 h | 6.8 | s | n | T=25°C | 3 | Fernandez-Alba *et al.* (2002) |
| Technical grade (98.1%) | *Navicula pelliculosa* | Diatom | ALG | Growth inhibition (Biomass) | EC50 | 5 d | 8.8 | s | y | | 1 | EU DAR (2006) |
| Technical grade 98.1% | *Navicula pelliculosa* | Diatom | ALG | Growth inhibition | EC50 | 5 d | 14 | s | u | | C | OPP (2007) |
| 30% formulation | *Chlorella pyrenoidosa* | Green alga | ALG | Growth inhibition (Growth rate) | EC50 | 96 h | 100 | s | n | T=25°C | 2 | Ma *et al.* (2002) |
| **Higher plants** | | | | | | | | | | | | |
| Technical grade (98.1%) | *Lemna gibba* | Duckweed | MAC | Growth inhibition | EC50 | 14 d | 510 | - | u | | 1 | EU DAR (2006) |
| Technical grade (98.1%) | *Lemna gibba* | Duckweed | MAC | Growth inhibition | EC50 | 14 d | 630 | s | u | | C | OPP (2007) |
| **Invertebrates** | | | | | | | | | | | | |
| Technical grade (≥ 98%) | *Astacopsis gouldi* | Freshwater lobster | CRU | Survival | LC50 | 96 h | 12 | f | y | T=12°C | 1 | Davies *et al.* (1994) |
| Technical grade (98.1%) | *Chironomus riparius* | Midge | INS | Immobility | EC50 | 48 h | 15 | u | y | | 1 | EU DAR (2006) |
| Technical grade (≥ 98%) | *Paratya australiensis* | Shrimp | CRU | Survival | LC50 | 96 h | 16 | f | y | T=12°C | 1 | Davies *et al.* (1994) |

| Chemical formulation (% purity) | Scientific name | Common name | Taxonomic group | Effect | Endpoint | Test duration | Conc. (μg a.i. $l^{-1}$) | Exposure[1] | Toxicant analysis[2] | Comments | Reliability (Klimisch Code*) | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Technical grade | *Brachionus calyciflorus* | Rotifer | ROT | - | EC50 | 24 h | 24 | - | u | - | 4 | Hamer and Gentle (1999) cited in EU DAR (2006) |
| Technical grade | *Daphnia magna* | Waterflea | CRU | Immobility | EC50 | 48 h | 28 | s | n | T=20°C | 2 | Fernandez-Alba *et al.* (2002) |
| Technical grade | *Leptocerus* sp | Caddis fly | INS | - | EC50 | 48 h | 38 | - | u | - | 4 | Hamer and Gentle (1999), cited in EU DAR (2006) |
| Technical grade | *Lymnaea stagnalis* | Pond snail | MOL | - | EC50 | 48 h | 100 | - | u | - | 4 | Hamer and Gentle (1999), cited in EU DAR (2006) |
| Technical grade | *Erpodella* sp. | Leech | ANN | - | EC50 | 48 h | 160 | - | u | - | 4 | Hamer and Gentle (1999), cited in EU DAR (2006) |
| Technical grade | *Planaria* sp. | Flatworm | PLA | - | EC50 | 48 h | 200 | - | u | - | 4 | Hamer and Gentle (1999), cited in EU DAR (2006) |
| **Vertebrates (fish and amphibians)** | | | | | | | | | | | | |
| Unknown | *Oncorhynchus mykiss* | Rainbow trout | FIS | Survival | LC50 | Asym-ptotic | 7.6 | f | n | T=16ºC DO= 5.1 mg $l^{-1}$ | 3 | Davies and White (1985) |
| Technical grade | *Oncorhynchus mykiss* | Rainbow trout | FIS | Survival | LC50 | 96 h | 12 | ss | u | GLP study | 4 | IUCLID (2000) |
| Technical grade (≥ 99%) | *Galaxias maculatus* | Common jollytail | FIS | Survival | LC50 | 96 h | 16.3 | f | y | T=16°C | 2 | Davies and White (1985) |
| Technical grade | *Oncorhynchus mykiss* | Rainbow trout | FIS | Survival | LC50 | 96 h | 17 | u | y | | 1 | EU DAR 2006 |

| Chemical formulation (% purity) | Scientific name | Common name | Taxonomic group | Effect | Endpoint | Test duration | Conc. (µg a.i. $l^{-1}$) | Exposure[1] | Toxicant analysis[2] | Comments | Reliability (Klimisch Code*) | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (99.18%) | | | | | | | | | | | | |
| Technical grade (≥ 99%) | *Oncorhynchus mykiss* | Rainbow trout | FIS | Survival | LC50 | 96 h | 17.1 | f | y | T=13°C | 2 | Davies and White (1985) |
| Dragon Daconil 2787 (12.9% a.i.) | *Pimephales promelas* | Fathead minnow | FIS | Survival | LC50 | 7 d | 22.6 | ss | y | T=25°C | 2 | Sherrard *et al.* (2003 |
| Bravo 720 (54% a.i.) | *Lepomis macrochirus* | Bluegill sunfish | FIS | Survival | LC50 | 96 h | 26.3 | f | u | - | C | OPP (2007) |
| Technical grade | *Bufo bufo japonicus* | Toad | AMP | Survival | LC50 | 48 h | 160 | s | n | T=25°C | 4 | Hashimoto and Nishiuchi (1981) cited in ECOTOX database |

* See Annex I and Annex II for explanation, C = Core data, equivalent to Klimisch code 1
[1] Exposure: s = static; ss = semi-static; f = flow-through
[2] Toxicant analysis: y = measured; n = not measured; u = unknown
ALG = alga, AMP = amphibian, ANN = annelid, CRU = crustacean, FIS = fish, INS = insect, MAC = macrophyte, MOL = mollusc, PLA = platyhelminth, ROT = rotifer
EC50 = Concentration effective against 50 per cent of the organisms or animals tested
LC50 = Concentration lethal to 50 per cent of the organisms or animals tested

### 2.6.2 Toxicity to saltwater organisms

Single species short-term toxicity data for chlorothalonil for saltwater organisms are available for six different taxonomic groups: algae, ascidians, crustaceans, echinoderms, fish and molluscs. Long-term toxicity data are available for algae and crustaceans. Long-term toxicity data for saltwater species are summarised in Table 2.11 and short-term toxicity data are summarised in Table 2.12. A diagrammatic representation of all the available long-term saltwater data (cumulative distribution function) for chlorothalonil is presented in Figure 2.3. A similar diagram for short-term data is presented in Figure 2.4. These diagrams include all data regardless of quality and provide an overview of the spread of the available data. The diagrams are not species sensitivity distributions and have not been used to set the chlorothalonil PNECs.

**Figure 2.3 Cumulative distribution function of saltwater long-term data (µg a.i. l$^{-1}$) for chlorothalonil**

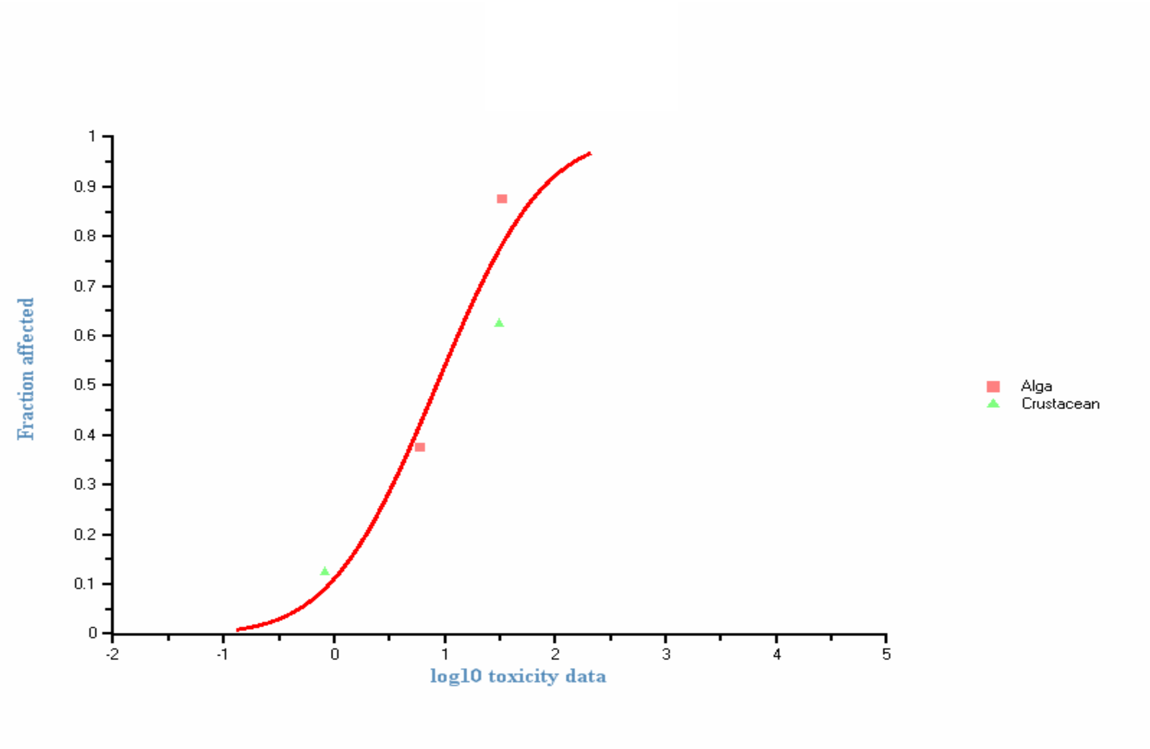


**Figure 2.4** **Cumulative distribution function of saltwater short-term data ($\mu$g a.i. $l^{-1}$) for chlorothalonil**

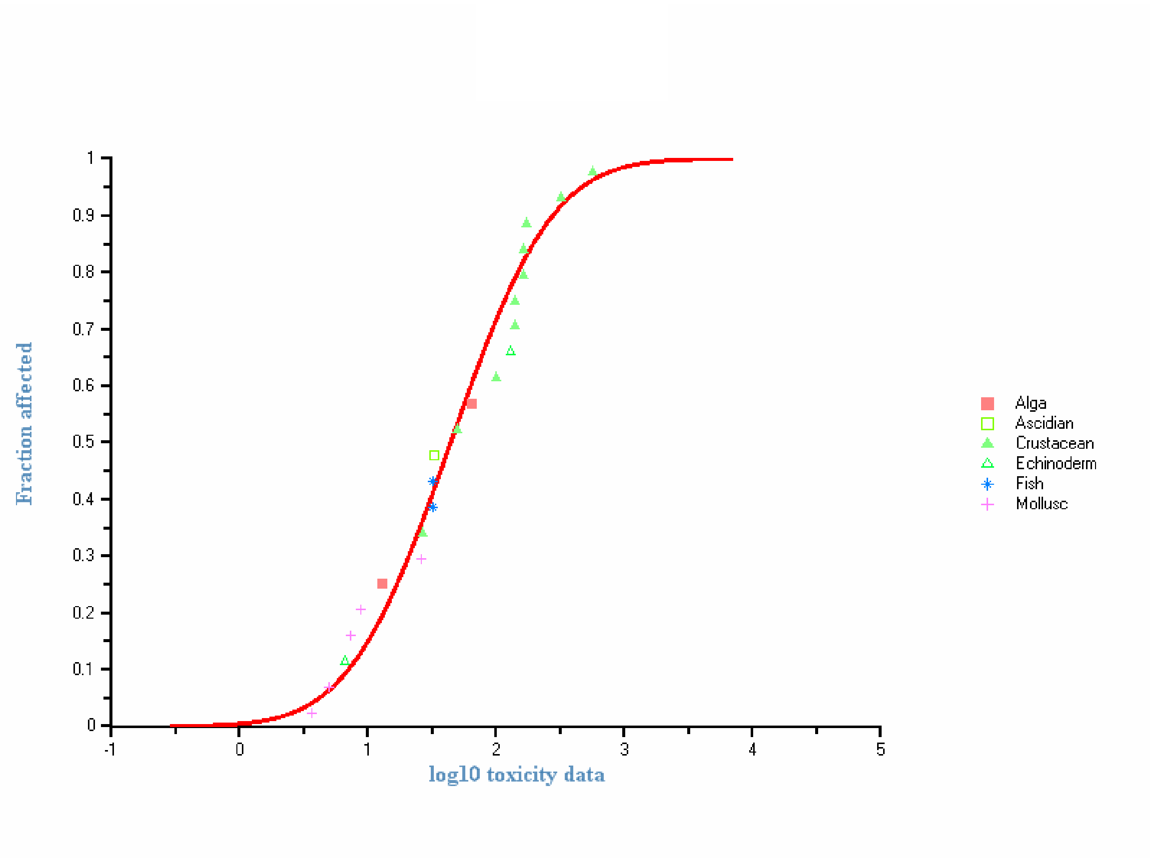


**Table 2.11 Most sensitive long-term aquatic toxicity data for saltwater organisms exposed to chlorothalonil**

| Chemical formulation (% purity) | Scientific name | Common name | Taxonomic group | Effect | Endpoint | Test duration | Conc. (μg a.i. $l^{-1}$) | Exposure[1] | Toxicant analysis[2] | Comments | Reliability (Klimisch Code*) | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Algae** | | | | | | | | | | | | |
| Technical grade (98.1%) | *Skeletonema costatum* | Diatom | ALG | Growth inhibition | NOEC | 14 d | 5.9 | s | u | - | C | OPP (2007) |
| Technical grade | *Dunaliella tertiolecta* | Green alga | ALG | Growth inhibition | NOEC | 96 h | 33.3 | s | n | T=25°C | 2 | DeLorenzo and Serrano (2003) |
| **Invertebrates** | | | | | | | | | | | | |
| Technical grade (98%) | *Americamysis bahia* | Mysid shrimp | CRU | Reproduction | NOEC<br>LOEC | 28 d | 0.83<br>1.1 | f | y | Salinity 31-33‰ | 3 | Shults and Hoberg (1991) cited in EU DAR (2006)<br>RIVM (2001)<br>HSE (2002) |
| Technical grade (98%) | *Palaemonetes pugio* | Grass shrimp | CRU | No. of moults | LOEC | 20 d pulsed exposure | 33.3 | ss | n | T=25.3°C<br>Salinity 20 ‰ | 2 | Key *et al.* (2003) |

* See Annex I and Annex II for explanation, C = Core data, equivalent to Klimisch code 1, S = supplemental data, equivalent to Klimisch code 2
[1] Exposure: s = static; ss = semi-static; f= flow-through
[2] Toxicant analysis: y = measured; n = not measured; u = unknown
ALG = alga, CRU = crustaceans
NOEC = no observed effect concentration

**Table 2.12 Most sensitive short-term aquatic toxicity data for saltwater organisms exposed to chlorothalonil**

| Chemical formulation (% purity) | Scientific name | Common name | Taxonomic group | Effect | Endpoint | Test duration | Conc. (μg a.i. $l^{-1}$) | Exposure[1] | Toxicant analysis[2] | Comments | Reliability (Klimisch Code*) | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Algae** | | | | | | | | | | | | |
| Technical grade (98.1%) | *Skeletonema costatum* | Diatom | ALG | Growth inhibition | EC50 | 14 d | 13 | s | u | - | C | OPP (2007) |
| Technical grade | *Dunaliella tertiolecta* | Green alga | ALG | Growth inhibition | EC50 | 96 h | 64 | s | n | T=25°C | 2 | DeLorenzo and Serrano (2003) |
| **Invertebrates** | | | | | | | | | | | | |
| Technical grade | *Crassostrea virginica* | Eastern oyster | MOL | Shell deposition | EC50 | 96 h | 3.6 | - | u | - | 4 | US EPA RED (1999) |
| Technical grade (96%) | *Crassostrea virginica* | Eastern oyster | MOL | Shell deposition | EC50 | 96 h | 5 | f | y | T= 14-16°C Salinity 22‰ | 4 | Shults *et al.* (1983) cited in EU DAR (2006) |
| Technical grade | *Paracentrotus lividus* | Sea urchin | ECH | Development | EC50 | 48 h | 6.6 | s | n | T=20°C | 2 | Bellas (2006) |
| Technical grade | *Mytilus edulis* | Edible blue mussel | MOL | Development | EC50 | 48 h | 8.8 | s | n | T=15°C | 2 | Bellas (2006) |
| Technical grade (98%) | *Amphiascus tenuiremis* | Copepod | CRU | Survival | LC50 | 96 h | 27 | s | y | T=20°C Salinity 30‰ males only | 1 | Bejarano *et al.* (2005) |
| Technical grade | *Ciona intestinalis* | Sea squirt | ASC | Settlement | EC50 | 48 h | 33 | s | n | T=15°C | 2 | Bellas (2006) |
| Technical grade (98%) | *Palaemonetes pugio* | Grass shrimp | CRU | Survival | LC50 | 96 h | 49.5 | ss | n | T=25.3°C Salinity 20‰ | 2 | Key *et al.* (2003) |
| **Fish** | | | | | | | | | | | | |
| Technical grade | *Cyprinodon variegatus* | Sheepshead minnow | FIS | Survival | LC50 | 96 h | 32 | s | n | - | C | HSE (2002), OPP (2007) |
| Technical grade | *Leiostomus xanthurus* | Spot | FIS | Survival | LC50 | 48 h | 32 | f | n | T=11°C Salinity 22‰ | 4 | Mayer (1987) cited in Caux (1996) |

* See Annex I and Annex II for explanation, C = Core data, equivalent to Klimisch code 1
[1] Exposure: s = static; ss = semi-static; f= flow-through
[2] Toxicant analysis: y = measured; n = not measured; u = unknown
ALG = alga, ASC = ascidian, CRU = crustacean, ECH = echinoderm, FIS = fish, MOL = mollusc
EC50 = Concentration effective against 50 per cent of the organisms or animals tested, LC50 = Concentration lethal to 50 per cent of the organisms or animals tested

### 2.6.3 Toxicity to sediment dwelling organisms

Reliable data on the toxicity of chlorothalonil in sediment to sediment-dwelling organisms were not found in the open literature. FAO (2005) report a NOEC of 125 µg $l^{-1}$ (initial water concentration) or 0.95 mg/kg (sediment concentration at end of study) from a 28-day static long-term toxicity test with *Chironomus riparius*, a sediment-dwelling midge. No other details are available and therefore it is not possible to assess the quality or relevance of this result.

### 2.6.4 Endocrine-disrupting effects

The list of purported endocrine disruptors compiled by the Institute of Environment and Health (IEH 2005) does not list chlorothalonil. Andersen *et al.* (2002) found that chlorothalonil was very cytotoxic to both CHO and MCF-7 cells, even at low concentrations, rendering cellular assays unsuitable for evaluating the potential hormone-disrupting effects of this substance. The US EPA does not class chlorothalonil as a known or suspected endocrine disruptor, based on developmental and reproductive toxicity studies in rats and rabbits (USEPA 2004).

Teather *et al.* (2005) report a skewed sex ratio in favour of females in Japanese medaka (*Oryzias latipes)* after exposure to 0.06 µg $l^{-1}$ chlorothalonil for 7 days, however, there are issues with the reliability of this study. The test substance used was an un-named commercial formulation and the test was carried out at a single concentration with no analytical confirmation of the exposure concentration.

### 2.6.5 Mode of action of chlorothalonil

*Plants*

The target organisms for chlorothalonil are fungi. The fungitoxic mode of action is to bind and deplete cellular glutathione (GSH), effectively inhibiting glucose oxidation. Glutathione is a peptide that occurs widely in plant tissues and plays an important role in biological oxidation-reduction processes and the activation of some enzymes.

*Aquatic animals*

Glutathione also occurs widely in animal tissues. Tests on channel catfish (*Ictalurus punctatus*) found that enhanced toxicity resulted from GSH depletion (Gallagher *et al.* 1992). The exact mechanism of action is unknown, but the authors suggested that once GSH stores were depleted, chlorothalonil was free to react with sulfhydryl groups of critical functional proteins. Other studies have shown that chlorothalonil inhibits essential thiol-dependent cellular enzymes including GAPDH and NADPH oxidase, which are involved in glycolysis and superoxide production respectively (Baier-Anderson and Anderson 1998a, 1998b, 2000).

### 2.6.6 Mesocosm and field studies

## *Freshwater mesocosm and field studies*

Ernst *et al.* (1991) studied the cumulative effects of three direct aerial applications of formulated chlorothalonil (Bravo 500 at a rate of 875 g a.i. $ha^{-1}$) on aquatic organisms in a 0.2 ha pond. The applications were made at weekly intervals. Measured concentrations sampled just below the water surface immediately after each spray event ranged from 150 – 2900 µg $l^{-1}$. Acute toxicity was monitored using caged water boatman (*Sigara alternata*), caddisfly larvae (*Limnephilus* sp.), freshwater clam (*Pisidium* sp.), crawling water beetle (*Haliplus* sp.), scud (*Gammarus* spp.), midge larvae (Chironomidae), threespine stickleback and one year old trout rainbow trout. Water boatman and threespine stickleback suffered mortality from exposure to chlorothalonil. Mortality in the other invertebrates could not be attributed to chlorothalonil, and no mortality was observed in rainbow trout. This result was unexpected as the initial concentrations in the water were well above LC50 values for rainbow trout. The trout in this study were described as one year old (no other measurements given), whereas trout used in laboratory tests are described as fingerlings weighing 3 – 4 g. Therefore the larger size of the fish used could have contributed to the lower sensitivity. In addition, it is possible that the dissipation rate in the pond was sufficient to rapidly reduce the exposure concentration to below the laboratory LC50 value.

An outdoor aquatic mesocosm study looked at the effect of chlorothalonil (as a 720 g $l^{-1}$ SC formulation) on aquatic invertebrates, algae and aquatic plants. Application was at 3, 10, 30, 100 and 300 µg a.i. $l^{-1}$ to replicated systems at weekly intervals (though the actual duration was not stated). The NOEC was 10 µg $l^{-1}$, with short-term effects on the phytoplankton community apparent at 30 µg $l^{-1}$ and above. There was recovery of phytoplankton at all concentrations within the duration of the study. At 100 and 300 µg $l^{-1}$ there were effects on the zooplankton populations and although recovery was apparent, significant differences in the communities remained at the end of the study. The authors concluded that concentrations up to 30 µg $l^{-1}$ would have no significant impact on aquatic invertebrate and algal/plant communities, which could be taken to represent a LOEC (Ashwell *et al.*, 2002 cited in Hamer 2003).

An unpublished aquatic field study (Hutchinson *et al.*, 1982 cited in US EPA RED 1999) reported exposure concentrations of 0.6 µg $l^{-1}$ and 1.1 µg $l^{-1}$ for water and 31 kg $l^{-1}$ and 51 kg $l^{-1}$ for sediment in two ponds adjacent to a soybean site. No mortality of pond organisms was observed in the study. This submission was considered supplemental, in part because only one site was studied and the conditions did not represent a reasonable worst case high runoff scenario, however this does not invalidate the findings.

In summary, these mesocosm data suggest that under more natural conditions chlorothalonil toxicity is lower than under laboratory conditions, probably due to dissipation, which reduces the potential exposure of non-target organisms.

## *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 freshwaters

*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). Long-term data were available for five taxonomic groups (algae, crustaceans, fish, insects and macrophytes) for chlorothalonil. Based on the information available algae, crustaceans and fish appear to be of similar sensitivity to chlorothalonil. Table 2.9 summarises the most sensitive long-term freshwater toxicity data that were found.

The lowest long-term result for algae is a 72-hour growth LOEC of 1.0 µg $l^{-1}$ for *Pseudokirchneriella subcapitata* (Fernandez-Alba *et al.* 2002; Table 2.9). The algal growth inhibition test was reported to be performed in accordance with OECD Guideline 201 using a commercially available algal toxicity test kit, but the publication suggests a number of deviations. The inoculum level was 2 orders of magnitude higher than that recommended in the guideline and details of the methodology are limited. For example, there is no mention of agitation/aeration of the vessels and there is no indication whether the effect is based on growth rate or biomass. In addition the result reported in the Fernandez-Alba *et al.* (2002) study is lower than other values reported for this species. A 5-day NOEC of 50 µg $l^{-1}$ is reported in USEPA RED (1999), and a geometric mean for 72-96 h NOECs for this species is reported as 33 µg $l^{-1}$ in EU DAR (2006) but no further details are given.

The remaining long-term results available for algal species are all unpublished studies provided by manufacturers in support of their products and are reported in various regulatory reviews (USEPA RED 1999, RIVM 2001, HSE 2002, EU DAR 2006 shown in Table 2.9). The next lowest long-term result is a 5-day growth inhibition NOEC of 3.5 µg $l^{-1}$ for *Navicula pelliculosa* which has been evaluated in the Draft Assessment Report (EU DAR, 2006). As part of this process the study was peer reviewed and considered reliable for use in the risk assessment. However, no further details of the study were obtained.

The one available long-term study with macrophytes is also an unpublished study and reports a 14-day growth NOEC of 290 µg $l^{-1}$ for *Lemna gibba* (Table 2.9). This would suggest that macrophytes are not as sensitive as other taxa. This study has also been evaluated in the EU DAR (2006) and was considered reliable for use in the risk assessment. However, no further details of the study were ascertained.

The six long-term results available for the invertebrate *Daphnia magna* are all unpublished studies reported in various compendium documents (USEPA RED 1999, RIVM 2001, HSE 2002, EU DAR 2006 shown in Table 2.9). These indicate that this species is sensitive to chlorothalonil, with 21-day NOEC values ranging from 0.5 – 39 µg $l^{-1}$. The 21-day NOEC for effects on reproduction of 0.5 µg $l^{-1}$ was derived from results with a chlorothalonil 75WG formulation and was the value selected for the initial risk evaluation of chlorothalonil under the 91/414/EEC review process.

For fish Teather *et al.* (2005) reported high toxicity to the Japanese medaka (*Oryzias latipes)* in the form of reduced activity and skewed sex ratio. The fish were exposed for 7 days to a single test concentration of 0.06 µg $l^{-1}$ and at this concentration no effects were seen on survival, time to hatch or foraging ability. These tests were of intermediate duration, were non standard concentration-response studies, and there was no analytical confirmation of the test concentration. Therefore the results are not readily interpretable and not appropriate for use in the derivation of the PNEC.

Four other long-term studies are available in Table 2.9. The most sensitive result is a 21-day NOEC of 0.35µg $l^{-1}$ (mean measured value) for effects on the growth/survival of rainbow trout (*Oncorhynchus mykiss)* exposed to the commercial chlorothalonil formulation Daconil 2787. This study has been evaluated in the EU DAR (2006) and was considered to be a key endpoint in the chronic risk assessment for fish.

The long-term *D. magna* results and the comparison of acute toxicity data in Table 2.8 suggest that there is no significant difference in toxicity after exposure to technical grade chlorothalonil or formulations when the data is expressed on an a.i. basis. Therefore, it is proposed that the long-term freshwater PNEC for chlorothalonil should be based on the NOEC for effects on the fish *O. mykiss* (0.35 µg $l^{-1}$) and an assessment factor of 10 because of the availability of long-term data for three trophic levels. This results in:

**$PNEC_{freshwater\_lt}$ = 0.35 µg $l^{-1}$/AF (10) = 0.035 µg $l^{-1}$ chlorothalonil**

Since chlorothalonil has been shown to dissipate rapidly it is unlikely that aquatic habitats would be exposed to chlorothalonil for sufficiently long to result in chronic toxicity unless field application is repeated, allowing a continuous release of herbicide into the water body, or there is a continuous discharge from a point source. The PNEC calculated above is, therefore, likely to be conservative under most natural conditions.

### *PNEC accounting for a maximum allowable concentration*

Freshwater short-term toxicity data are available for ten taxonomic groups (algae, amphibians, annelids, crustaceans, fish, insects, macrophytes, molluscs, platyhelminths and rotifers). Table 2.10 summarises the most sensitive short-term freshwater toxicity data found for chlorothalonil and its formulations.

Sensitivity to chlorothalonil is comparable across taxa, with the exception of macrophytes, which appear to be the least sensitive group (Table 2.10). The lowest reported result is the 72-hour growth EC50 of 6.8 µg $l^{-1}$ for the green alga *P. subcapitata* but is not considered reliable for the reasons described for the corresponding NOEC value. The lowest short-term result considered reliable is the 5-day growth inhibition (using the biomass endpoint) EC50 of 8.8 µg $l^{-1}$ for the diatom *Navicula pelliculosa*. The corresponding growth inhibition EC50 (using the more relevant growth rate endpoint) is 14 µg $l^{-1}$. This study has been evaluated in the EU DAR (2006) and as part of this process the study was peer reviewed and considered reliable for use in the risk assessment. However, no further details of the study were ascertained.

Reported short-term values for invertebrates range from 12 to 200 µg $l^{-1}$ (see Table 2.10).The lowest reliable value is considered to be 96-hour LC50 values of 12 µg $l^{-1}$ for effects of chlorothalonil on the survival of the freshwater lobster *Astacopsis gouldi* (Davies *et al.* 1994).

Fish studies which have produced reliable 96-h LC50 values indicate that fish species are sensitive to acute exposure to chlorothalonil with values ranging from 12.0 to 26.3 µg $l^{-1}$ for various species (see Table 2.10). It should be noted that the lowest value reported of 10.6 µg $l^{-1}$ (Davies and White 1985) was seen in a study carried out at low dissolved oxygen concentrations and is therefore not considered appropriate for setting the PNEC. However, the 96-hour LC50 value of 12 µg $l^{-1}$ for effects on the survival of rainbow trout (*Oncorhynchus mykiss)* is considered to be the lowest reliable value (EU DAR 2006).

To derive the short-term freshwater PNEC it is proposed that the 96-hour LC50 values of 12 µg $l^{-1}$ for *A.gouldi* and *O.mykiss* are used. Based on guidance in the TGD on effects assessment for intermittent releases [Section 3.3.2 of Part II of the TGD (ECB 2003)] and the fact that there is a considerable acute toxicity database for freshwater organisms, an assessment factor of 10 rather than 100 should be applied to the lowest reliable data for *A.gouldi* and *O.mykiss* . This results in:

**$PNEC_{freshwater\_st}$ = 12 µg $l^{-1}$/AF (10) = 1.2 µg $l^{-1}$ chlorothalonil**

### 3.1.2 PNECs for saltwaters

The effects database for saltwater species is considerably smaller than that for freshwater organisms. Short-term toxicity data are available for six different taxonomic groups (algae, ascidians, crustaceans, echinoderms, fish and molluscs) and long-term data are available for algae and crustaceans. The limited saltwater toxicity data do not differ markedly from the range of values obtained for corresponding freshwater species (Tables 2.9 and 2.10). Therefore, since there are no obvious differences in the sensitivity of freshwater or saltwater species of the same taxonomic group, the TGD approach of using a combined freshwater and saltwater dataset for the saltwater effects assessment can be used. Therefore, proposed freshwater PNECs should be considered in deriving corresponding values for saltwater bodies.

#### *PNEC accounting for the annual average concentration*

The lowest reported long-term NOEC of 0.83 µg $l^{-1}$ for reproduction in the mysid shrimp, *Americamysis bahia* is from an unpublished study. Mortality in the control group was 23% (both replicates) and 23 and 53% in the solvent control. The number of offspring/female/reproductive day was 0.51 and 0.96 in the control group, and 0.30 and 0.31 in the solvent control, but was not statistically significantly different (Student t-test). In the analysis of the results from the treatment concentrations data were compared to pooled control groups. This study was evaluated in the EU DAR (2006) and the result was considered unreliable. Other long-term data given in Table 2.11 range from a 14-day NOEC of 5.9 µg $l^{-1}$ for effects on algal (*Skeletonema costatum*) growth inhibition to a 20-day LOEC of 33.3 µg $l^{-1}$ for effects on moulting in the grass shrimp *Palaemonetes pugio*.

It is proposed that the derivation of the long-term saltwater PNEC for chlorothalonil is based on the 21-day NOEC for the effects on the growth and survival of rainbow trout *Oncorhynchus mykiss* (0.35 µg $l^{-1}$). This involves the use of an assessment factor of 10 because of the availability of long-term data for three trophic levels. It is also proposed that an additional assessment factor of 10 is applied to account for the paucity of long-term toxicity data for marine species. This results in:

**$PNEC_{saltwater\_lt}$ = 0.35 µg $l^{-1}$/AF (100) = 0.0035 µg $l^{-1}$ chlorothalonil**

### *PNEC accounting for a maximum allowable concentration*

Saltwater short-term toxicity data are available for six different taxonomic groups: algae, ascidians, crustaceans, echinoderms, fish and molluscs. Table 2.12 summarises the most sensitive short-term saltwater toxicity data found for chlorothalonil and its formulations.

The most sensitive short-term result for saltwater species is a 96-hour shell deposition EC50 of 3.6 µg $l^{-1}$ for *Crassostrea virginica*. This is an unpublished study, but another unpublished study using the same species reports a 96-h EC50 value of 5.0 µg $l^{-1}$ supporting this sensitive result. This latter study was evaluated in the EU DAR (2006) and the result was a key value in the risk assessment. The functional importance of this endpoint is not necessarily clear but it is considered useful for determining concentrations at which certain chemicals affect physiological functions in oysters. The next most sensitive result is a 48-hour developmental EC50 of 6.6 µg $l^{-1}$ for *Parcentrotus lividus*, a representative of the echinoderms, a key taxonomic group which is exclusively saltwater in distribution (Bellas 2006), Overall, when the other short-term studies that are available are considered, the weight of evidence suggests that a PNEC based on the oyster shell deposition endpoint should be sufficiently protective.

It is therefore recommended that a short-term saltwater PNEC should be based on effects on the mollusc *C. virginica* (3.6 µg $l^{-1}$) and an assessment factor of 10. This results in:

**$PNEC_{saltwater\_st}$ = 3.6 µg $l^{-1}$/AF (10) = 0.36 µg $l^{-1}$ chlorothalonil**

Since data is available for exclusively marine species such as echinoderms then it is not proposed to apply an additional assessment factor of 10.

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

The minimum number of long-term toxicity data (at least 10 NOECs from eight taxonomic groups) required by the TGD is not available. Therefore, the species sensitivity distribution (SSD) approach cannot be used for PNEC derivation. The EU DAR (2006) derived an HC5 value of 1.0 µg $l^{-1}$ based on an SSD for NOEC data for 9 species from 5 taxonomic groups (algae, crustaceans, fish, insects and macrophytes).

## 3.3 Derivation of existing EQSs

Some of the critical data used to derive PNECs in Section 3.1 were not available to Crane *et al.* (1995). The freshwater annual average (AA) for chlorothalonil was derived by applying a safety factor of 100 to the lowest reliable LC50 for rainbow trout, an asymptotic LC50 of 7.6 µg $l^{-1}$, resulting in an EQS of 0.1 µg $l^{-1}$. A tentative maximum allowable concentration (MAC) of 1.0 µg $l^{-1}$ was derived by applying a safety factor of 10 to this same LC50 value.

Based on the few data available on the toxicity of chlorothalonil to saltwater organisms, the view was taken by Crane *et al.* (1995) that saltwater and freshwater species have similar sensitivities. Therefore, the EQS proposed for the protection of freshwater organisms were considered adequate for the protection of saltwater life and the same values were proposed as guideline EQS.

## 3.4 Derivation of PNECs for sediment

The TGD trigger value of a log Koc or log Kow of ≥3 is met, as the reported log Kow is 2.91 – 3.05 (FAO2005, HSDB 2006) and log Koc 2.9 – 3.84 (equivalent to Koc of 900 to 7000) (USEPA RED 1999). However, there is only limited information with respect to experimental data on sediment toxicity for chlorothalonil and therefore no $PNEC_{sediment}$ can be derived.

## 3.5 Derivation of PNECs for secondary poisoning of predators

### 3.5.1 Mammalian and avian toxicity data

Several reviews have been published on chlorothalonil toxicity to mammals (JMPR 1992, EHC 1996, IUCLID 2000, ESR Reports 2000, EU DAR 2006). As the most recent, the EU DAR 2006, ESR Reports and the IUCLID reviews have been assumed to contain the most sound and comprehensive mammalian data. For this reason, these were the primary sources used. However, the two other reviews were also consulted. Additional literature searches were performed from 2006 to the present day to locate any lower effects data published since 2006, and one further study was located.

For avian data, due to the lack of relevant data in the aforementioned reviews, a comprehensive literature search was performed to locate any relevant data.

**Table 3.1 Most sensitive mammalian and bird oral toxicity data relevant for the assessment of secondary poisoning**

| Type of study, reference & result | Details |
|---|---|
| **Sub-chronic toxicity to mammals** | |
| Shults *et al.* (1983)<br>Cited in EHC (1996)<br>**Sub-chronic NOAEL = 15 mg $kg^{-1}$ diet (stated to be 3 mg $kg^{-1}$ bw $d^{-1}$)** | Male and female CD-1 mice (15/sex/group) received chlorothalonil via their diet for 13 weeks at doses of 0, 7.5, 15, 50, 275 or 750 mg $kg^{-1}$ diet (approximately 0, 1.5, 3, 10, 55 and 150 mg $kg^{-1}$ bw $d^{-1}$). There were no effects on clinical condition, mortality, body weight gain and food consumption. The NOAEL was based on increased kidney weights in females at the top three doses. |
| Wilson *et al.* (1985)<br>Cited in ESR Report (2000)<br>**Sub-chronic NOAEL = 50 mg $kg^{-1}$ bw $d^{-1}$ (mice)**<br>**Sub-chronic NOAEL = 10 mg $kg^{-1}$ bw $d^{-1}$ (rats)** | Rats and mice (strain unspecified) received chlorothalonil orally (route of administration unspecified) for 90 days at unspecified doses. Hyperplasia of fore-stomach mucosa and in the S2 segment of the proximal renal tubule was observed. The NOAEL was based on unspecified changes to renal pathology. No further details about this study were available. |
| Anon Cited in EU DAR (2006)<br>**Sub-chronic NOAEL = 1.5 mg $kg^{-1}$ bw $d^{-1}$** | Rats (strain unspecified) received chlorothalonil orally (route of administration unspecified) for 90 days at unspecified doses. The NOAEL was based on unspecified histopathological changes in stomach and kidneys and increased organ weights in kidneys. The original reference was not stated. |
| **Chronic toxicity studies in mammals** | |
| Anon Cited in ESR Report (2000)<br>**Chronic NOAEL = 150 mg $kg^{-1}$ bw $d^{-1}$** | Dogs (strain unspecified) received chlorothalonil for 1 year at doses up to 500 mg $kg^{-1}$ bw $d^{-1}$ (route of administration unspecified). Significantly decreased body weight gain and increased absolute liver and kidney weights were observed. The NOAEL was based on decreased body weight gain at the top dose of 500 mg $kg^{-1}$ bw $d^{-1}$. The original reference was not stated. |
| Holsing and Voelker (1970)<br>Cited in JMPR (1992)<br>**Chronic NOAEL = 120 mg $kg^{-1}$ diet (stated to be 3 mg $kg^{-1}$ bw $d^{-1}$)** | Male and female Beagle dogs (8/sex/group) received chlorothalonil in their diet for 2 years at doses of 0, 60 or 120 mg $kg^{-1}$ diet (approximately 0, 1.5 and 3 mg $kg^{-1}$ bw $d^{-1}$). The NOAEL was based on no treatment-related effects being observed at the highest dose employed. |
| Paynter and Busey (1966)<br>Cited in JMPR (1992)<br>**Chronic LOAEL = 1500 mg $kg^{-1}$ diet (stated to be 37.5 mg $kg^{-1}$ bw $d^{-1}$) (lowest dose employed)** | Male and female Beagle dogs received chlorothalonil via their diet for 2 years at doses of 0, 1500, 15000 or 30000 mg $kg^{-1}$ diet (stated to be 0, 37.5, 375 and 750 mg $kg^{-1}$ bw $d^{-1}$). Absolute and relative thyroid and kidney weights and liver to body weight ratios were increased at the top two doses. Histopathological changes occurred in the liver, thyroid, kidney and stomach at the top two doses. The LOAEL was based on these histopathological changes, irregular body weight reduction and borderline anaemia and was the lowest dose level employed. |

| Carcinogenicity studies in mammals | |
|---|---|
| NTP (1978)<br>**Carcinogenic NOEL = 10 126 mg kg$^{-1}$ diet (approximately 1220 mg kg$^{-1}$ bw d$^{-1}$) (highest dose employed)** | Male and female B6C3F1 mice (50/sex/group) received chlorothalonil via their diet for 80 weeks at doses of 0, 5063 or 10126 mg kg$^{-1}$ diet (approximately 0, 610 and 1220 mg kg$^{-1}$ bw d$^{-1}$) followed by observation for 11 to 12 weeks. No tumours were observed at any dose level. |
| Wilson *et al.* (1987)<br>Cited in EHC (1996)<br>**Carcinogenic NOEL = 15 mg kg$^{-1}$ diet (stated to be 1.6 mg kg$^{-1}$ bw d$^{-1}$) for hyperplasia/hyperkeratosis in the fore-stomach (not relevant for humans)**<br>**Carcinogenic NOEL = 40 mg kg$^{-1}$ diet (stated to be 4.5 mg kg$^{-1}$ bw d$^{-1}$) for tubular hyperplasia.** | Male Charles River CD-1 mice (60/group) received chlorothalonil via their diet for 2 years at doses of 0, 15, 40, 175 or 750 mg kg$^{-1}$ diet (approximately 0, 1.6, 4.5, 19.4 and 83.3 mg kg$^{-1}$ bw d$^{-1}$). At the highest dose employed, kidney weights and incidences of tubular hyperplasia were increased, which were only slightly increased at 175 mg kg$^{-1}$ diet (approximately 19.4 mg kg$^{-1}$ bw d$^{-1}$). The lower NOEL was based on the increased incidence of hyperplasia and hyperkeratosis of the fore-stomach between 40 and 750 mg/kg diet (approximately 4.5 and 19.4 mg kg$^{-1}$ bw d$^{-1}$) (this effect is not considered to be relevant for humans). The higher NOEL was based on hyperplasia of the renal tubules. |
| Wilson *et al.* (1983)<br>Cited in EHC (1996)<br>**Sub-chronic NOAEL = 3 mg kg$^{-1}$ bw d$^{-1}$** | Male and female rats (strain unspecified) (25/sex/group) received chlorothalonil in their diet for 13 weeks at doses of 0, 1.5, 3, 10 or 40 mg kg$^{-1}$ bw d$^{-1}$. Increased kidney weight was observed at doses >3 mg kg$^{-1}$ bw d$^{-1}$, increased liver weight was observed at the top dose and the incidence of hyperplasia and hyperkeratosis of the fore-stomach squamous epithelium occurred at the top two doses; the latter of which returned to normal on cessation of treatment. |
| Wilson *et al.* (1989)<br>Cited in EHC (1996); ESR Report (2000); EU DAR (2006)<br>**Carcinogenic NOEL = 1.8 mg kg$^{-1}$ bw d$^{-1}$ based on effects on the fore-stomach**<br>**Carcinogenic NOEL = 3.8 mg kg$^{-1}$ bw d$^{-1}$ based on effects on the kidneys** | Male and female Fischer 344 rats (65/sex/group) received chlorothalonil in their diet for 23 and 26 months (males) and 29 months (females) at doses of 0, 1.8, 3.8, 15 or 175 mg kg$^{-1}$ bw d$^{-1}$. A small increase in kidney weight was observed at 3.8 mg kg$^{-1}$ bw d$^{-1}$ and at ≥3.8 mg kg$^{-1}$ bw d$^{-1}$ there was an increased incidence and severity of epithelial hyperplasia in the proximal convoluted tubules. The lower NOEL was based on treatment-related increases in the incidence of renal tubular adenoma and carcinoma. The higher NOEL was based on squamous cell adenomas and carcinomas of the forestomach occurring in both sexes. |
| NTP (1978)<br>**Carcinogenic LOEL = 5063 mg kg$^{-1}$ diet (approximately 253 mg kg$^{-1}$ bw d$^{-1}$) (the lowest dose employed)** | Male and female Osborne-Mendel rats (50/sex/group) received chlorothalonil via their diet for 80 weeks at doses of 0, 5063 or 10126 mg kg$^{-1}$ diet (approximately 0, 253 and 506 mg kg$^{-1}$ bw d$^{-1}$) followed by observation for 30 to 31 weeks. The LOEL was based on the occurrence of adenomas and carcinomas of the renal tubular epithelium that occurred at all dose levels and was the lowest dose employed. |
| | |

| Reproductive and developmental toxicity in mammals | |
|---|---|
| Farag *et al.* (2006)<br>**Reproductive NOAEL = 100 mg $kg^{-1}$ bw $d^{-1}$** | Pregnant ICR (CD-1) mice received sublethal doses of chlorothalonil via oral gavage on gestation days 6 to 15 at doses of 0, 100, 400 or 600 mg $kg^{-1}$ bw $d^{-1}$. Maternal effects at the top two doses included weakness and depression in maternal activity and reductions in body weight and weight gain. Foetal effects included reduced number of live foetuses, early resorption and decreased mean foetal weight in the top two doses. |
| Cited in EU DAR (2006)<br>**Reproductive NOAEL = 22.6 mg $kg^{-1}$ bw $d^{-1}$** | Rats (strain unspecified) received chlorothalonil orally (route of administration unspecified) at unspecified doses for an unspecified duration. The NOAEL was based on decreased pup weight and unspecified histopathological changes in the stomach at parentally toxic doses. Original reference not stated. |
| Lucas and Benz (1990)<br>Cited in JMPR (1992)<br>**Reproductive NOAEL = 1500 mg $kg^{-1}$ diet (stated to be 75 mg $kg^{-1}$ bw $d^{-1}$)** | In a two-generation reproduction study, male and female Charles River CD rats orally received (route of administration unspecified) chlorothalonil for 10 (F0) and 14 (F1) weeks prior to mating and continually from mating at doses of 0, 500, 1500 or 3000 mg $kg^{-1}$ diet. At the time of mating, low-dose males and females consumed approximately 25 and 32 mg $kg^{-1}$ bw $d^{-1}$, respectively; mid-dose males and females consumed approximately 75 and 100 mg $kg^{-1}$ bw $d^{-1}$, respectively; and high-dose groups consumed approximately 156 and 205 mg $kg^{-1}$ bw $d^{-1}$, respectively. Body weight depression occurred in the parents of both generations. Decreased body weight was observed in F0 mid- and high-dose males, and F0 high-dose females, while in the F1 parents, it occurred in the high dose groups of each sex. The NOAEL was based on hyperkeratosis and squamous epithelial hyperplasia of the fore-stomach, and epithelia hyperplasia, tubular hypertrophy and clear cell hyperplasia of the kidney that occurred in both sexes at the top dose, but were more pronounced in males. |
| De Castro *et al.* (2000)<br>**Reproductive NOAEL = 200 mg $kg^{-1}$ bw $d^{-1}$ Developmental LOAEL = 200 mg $kg^{-1}$ bw $d^{-1}$** | Rats (strain unspecified) received chlorothalonil orally (route of administration unspecified) during gestation days 1 to 6 at doses of 0 or 200 mg $kg^{-1}$ bw $d^{-1}$. No effects were observed on body weight gain of dams and offspring. Weights of gravid uterus, foetus, placenta and ovary also remained unaffected. At this dose level, physical and maturational development of offspring according to age was interrupted. No further details were provided. |
| **Embryo- and teratotoxicity in mammals** | |
| Mizens *et al.* (1983)<br>Cited in EHC (1996)<br>**Teratogenic NOAEL = 25 mg $kg^{-1}$ bw $d^{-1}$ (lowest dose employed)** | Female Sprague-Dawley rats received chlorothalonil orally via gavage on days 6 to 15 of gestation at doses of 0, 25, 100 or 400 mg $kg^{-1}$ bw $d^{-1}$ and animals were killed on day 20. Unspecified clinical signs of maternal toxicity were evident at the highest dose level. The NOAEL was based on the absence of compound-related incidences of external, internal or skeletal malformations in the foetuses of treated animals and was the lowest dose employed. |

| | |
|---|---|
| Sirasu and Teramoto (1975)<br>Cited in EHC (1996)<br>**Teratogenic NOAEL = 5 mg kg$^{-1}$ bw d$^{-1}$ (lowest dose employed)** | Female rabbits (strain unspecified) received chlorothalonil during days 6 to 18 of pregnancy at doses of 0, 5 or 50 mg kg$^{-1}$ bw d$^{-1}$ and animals were killed on day 29. The NOAEL was based on the absence of compound-related incidences of external, internal or skeletal malformations in the foetuses of treated animals and was the lowest dose employed. |
| **Sub-chronic toxicity to birds** | |
| OPP (2000) in ECOTOX database<br>**Sub-chronic LC50 = >21500 mg kg$^{-1}$ diet** | Juvenile mallard ducks (*Anas platyrhnchos*) received chlorothalonil via their diet at unspecified doses for 8 days. No further details were provided. |
| OPP (2000) in ECOTOX database<br>**Sub-chronic LC50 = >10 000 mg kg$^{-1}$ diet** | Fourteen day old mallard ducks (*Anas platyrhnchos*) received chlorothalonil via their diet at unspecified doses for 8 days. No further details were provided. |
| OPP (2000) in ECOTOX database<br>**Sub-chronic LC50 = 2000 mg kg$^{-1}$ diet** | Eight day old mallard ducks (*Anas platyrhnchos*) received chlorothalonil via their diet at unspecified doses for 9 days. No further details were provided. |
| OPP (2000) in ECOTOX database<br>**Sub-chronic LD50 = 158 mg kg$^{-1}$ bw** | Eight day old mallard ducks (*Anas platyrhnchos*) received chlorothalonil via their diet at unspecified doses for 14 days. No further details were provided. |
| OPP (2000) in ECOTOX database<br>**Sub-chronic LD50 = >4640 mg kg$^{-1}$ bw** | Fourteen day old mallard ducks (*Anas platyrhnchos*) received chlorothalonil via their diet at unspecified doses for 14 days. No further details were provided. |
| OPP (2000) in ECOTOX database<br>**Sub-chronic LC50 = >10 000 mg kg$^{-1}$ diet** | Fourteen day old Northern bobwhite quail (*Colinus virginianus*) received chlorothalonil via their diet at unspecified doses for 8 days. No further details were provided. |
| OPP (2000) in ECOTOX database<br>**Sub-chronic LC50 = 1746 mg kg$^{-1}$ diet** | Juvenile Northern bobwhite quail (*Colinus virginianus*) received chlorothalonil via their diet at unspecified doses for 8 days. No further details were provided. |
| No data could be located for chronic, reproductive and developmental avian toxicity for chlorothalonil. | |

### 3.5.2 PNECs for secondary poisoning of predators

Fish bioconcentration data (as BCF values) for chlorothalonil range from 9.4 to 264, hence the trigger of a BCF > 100 is met and derivation of PNECs for secondary poisoning of predators is required.

No data could be located for chronic effects on birds. The lowest relevant NOAEL is from a 2-year study with dogs. This NOAEL is conservative since it is based on the highest dose employed in the study where no adverse effects were observed (Holsing and Voelker 1970). This study is supported by the results of the carcinogenicity studies by Wilson et al. (1987, 1983, 1989).

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

**$PNEC_{secpois.biota}$ = $NOEC_{food}$ (120 mg $kg^{-1}$) / AF 30 = 4 mg $kg^{-1}$ prey (wet wt)**

Information on biomagnification of chlorothalonil is not available, but due to the normally rapid metabolisation of the compound the occurrence of this effect is not anticipated. The corresponding safe concentration in water (preventing bioaccumulation in prey to levels > $PNEC_{secpois.biota}$) can therefore be calculated as follows:

$PNEC_{secpois.water}$ = $PNEC_{secpois.biota}$ (4 mg $kg^{-1}$) / BCF

If the highest reported BCF of 264 is used for the calculation, this would result in a corresponding water concentration of:

**$PNEC_{secpois.water}$ = 4 mg $kg^{-1}$ prey / BCF (264) = 15 µg chlorothalonil $l^{-1}$**

This concentration is higher than the proposed long-term PNECs for the protection of freshwater and saltwater organisms. Therefore, if quality standards are set on the basis of these PNECs the protection of predators from secondary poisoning will be included, and derivation of additional EQS to protect against secondary poisoning is unnecessary.

# 4 Analysis and monitoring

The most common analytical method for measuring residues of chlorothalonil is gas chromatography (GC) with electron capture detection (ECD) (EHC 1996). GC with thermal conductivity detection (TCD) and flame ionization detection (FID) have also been used (Caux 1996).

Brief descriptions of analytical methods are given in Crane *et al.* (1995) with detection limits of 0.001 µg $l^{-1}$ quoted for water samples. The summary of analytical methods given by WHO (EHC 1996) has a lowest detection limit of 0.05 µg $l^{-1}$ for water samples.

Martinez *et al.* (2000) describe an analytical method for simultaneous determination of several antifouling pesticides, including chlorothalonil, in seawater samples using off-line solid-phase extraction followed by high performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. The limit of detection is given as 0.001 µg $l^{-1}$.

For water, the lowest proposed PNECs derived for chlorothalonil is 0.0035 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 chlorothalonil.

# 5 Conclusions

## 5.1 Availability of data

Long-term laboratory data are available for five different freshwater taxonomic groups including algae, crustaceans, fish, insects and macrophytes. Freshwater short-term toxicity data are available for 10 taxonomic groups (algae, amphibians, annelids, crustaceans, fish, insects, macrophytes, molluscs, platyhelminths and rotifers). Based on the information available, algae, crustaceans and fish appear to be equally sensitive to chlorothalonil at very low concentrations. Macrophytes appear to be at least one order of magnitude less sensitive. For saltwater organisms, single species short-term toxicity data are available for six different taxonomic groups (algae, ascidians, crustaceans, echinoderms, fish, and molluscs).

Short-term toxicity data for both freshwater and saltwater species are adequate to derive PNEC values, as are long-term data for freshwater species. However, insufficient long-term toxicity data are available to fulfil the minimum requirement of three saltwater taxa (algae, crustaceans and fish) as required under Annex V of the Water Framework Directive, hence the fresh and saltwater datasets were combined.

In addition, laboratory data are supplemented by freshwater mesocosm data, which suggest that under more natural conditions, toxicity is reduced due to dissipation of chlorothalonil, thus reducing the potential exposure of non-target organisms.

Chlorothalonil is not generally suspected of being an endocrine-disrupting chemical.

## 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 reliable long-term toxicity value for freshwater organisms is a NOEC of 0.35 $\mu g\ l^{-1}$ for rainbow trout, *Oncorhynchus mykiss*. Reliable long-term NOECs are available for algae, invertebrates and fish, and, therefore, an assessment factor of 10 has been applied, resulting in a $PNEC_{freshwater\_lt}$ of 0.035 $\mu g\ l^{-1}$.

### 5.2.2 Short-term PNEC for freshwaters

Reliable short-term data are available for algal, invertebrate and fish species, which shows that acute sensitivity to chlorothalonil is comparable across taxa. The lowest reliable short-term result is the 5-day growth inhibition (using the biomass endpoint) EC50 of 8.8 $\mu g\ l^{-1}$ for the diatom, *Navicula pelliculosa* with a corresponding growth inhibition EC50 (using the more relevant growth rate endpoint) of 14 $\mu g\ l^{-1}$. Reliable 96-hour LC50 values of 12 $\mu g\ l^{-1}$ for effects of chlorothalonil on the survival of the freshwater lobster *Astacopsis gouldi* and rainbow trout *Oncorhynchus mykiss* have also been reported. To derive the short-term freshwater PNEC is proposed that an assessment factor of 10 (given the large body of acute data) was therefore applied to the 96-hour LC50 values of 12 µg $l^{-1}$ for *A.gouldi* and *O.mykiss*, resulting in a $PNEC_{freshwater\_st}$ of 1.2 µg $l^{-1}$.

### 5.2.3 Long-term PNEC for saltwaters

Long-term single species saltwater toxicity data are only available for algae and crustaceans. The most sensitive result is a 28-day NOEC of 0.83 µg $l^{-1}$ for the mysid shrimp, *Americamysis bahia,* but this study is not considered reliable enough to be used as the basis of a PNEC. As the saltwater toxicity data values available do not appear to differ markedly from the range obtained for corresponding freshwater species, a combined freshwater and saltwater dataset for marine effects assessment was used to derive the long-term saltwater PNEC. Therefore, the freshwater PNEC is recommended to be adopted to protect saltwater taxa. It is also proposed that an additional assessment factor of 10 is applied to account for the paucity of long-term toxicity data for marine species. This results in a $PNEC_{saltwater\_lt}$ of 0.0035 µg $l^{-1}$.

### 5.2.4 Short-term PNEC for saltwaters

Reliable short-term data are available for six different taxonomic groups (algae, ascidians, crustaceans, echinoderms, fish and molluscs) including the base set of algae, invertebrates and fish. The most sensitive short-term result for saltwater species is a 96-hour shell deposition EC50 of 3.6 µg $l^{-1}$ for *Crassostrea virginica*. This is an unpublished study, but a further unpublished study using the same species reported 96-h EC50 values of 5.0 µg $l^{-1}$ supporting this sensitive result. In addition, there is a 48-hour EC50 of 6.6 µg $l^{-1}$ for developmental effects in the echinoderm *Parcentrotus lividus*. It is therefore recommended that a short-term saltwater PNEC should be based on effects to the mollusc *C. virginica* (3.6 µg $l^{-1}$) and an assessment factor of 10. This results in a $PNEC_{saltwater\_st}$ = 0.36 µg $l^{-1}$.

### 5.2.5 PNEC for sediments

The TGD trigger value of a log Koc or log Kow of ≥3 is met, as reported log Kow and Koc values are in the range 2.91 – 3.05 and 2.9-3.84 respectively. However, there is only limited information with respect to experimental data on sediment toxicity for chlorothalonil and therefore no $PNEC_{sediment}$ can be derived.

### 5.2.6 PNEC for secondary poisoning

Bioconcentration data (as BCF values) for chlorothalonil for fish range from 9.4 to 264, hence the trigger of a BCF >100 is exceeded and derivation of PNECs for secondary poisoning of predators is required. The lowest relevant $NOEC_{food}$ is 120 mg $kg^{-1}$ derived from a 2-year study with dogs. Using the highest reported BCF of 264 for the calculation results in a corresponding water concentration of $PNEC_{secpois.water}$ = 4 mg $kg^{-1}$ prey / BCF (264) = 15 µg chlorothalonil $l^{-1}$.

This concentration is higher than the proposed long-term PNECs for the protection of freshwater and saltwater organisms. Therefore, if quality standards are set on the basis of these PNECs the protection of predators from secondary poisoning will be included, and derivation of additional EQS to protect against secondary poisoning is unnecessary.

**Table 5.1 Summary of proposed PNECs**

| Receiving medium/exposure scenario | Proposed PNEC ($\mu g\ l^{-1}$) | Existing EQS ($\mu g\ l^{-1}$) |
|---|---|---|
| Freshwater/long-term | 0.035 | 0.1 |
| Freshwater/short-term | 1.2 | 1.0 |
| Saltwater/long-term | 0.0035 | 0.1 |
| Saltwater/short-term | 0.36 | 1.0 |
| Sediments | Insufficient data | - |
| Secondary poisoning | 15 | - |

# 5.3 Analysis

For water, the lowest proposed PNECs derived for chlorothalonil is 0.0035 $\mu g\ l^{-1}$. The data quality requirements are that, at a third of the EQS, total error of measurement should not exceed 50%. Using this criterion, it is evident that current analytical methodologies (non-standard) employing gas chromatography (GC) with electron capture detection or off-line solid-phase extraction followed by high performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry, are both capable of achieving detection limits of 0.001 $\mu g\ l^{-1}$. This should offer adequate performance to analyse for chlorothalonil.

# 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 methods are sensitive enough to assess compliance with the proposed PNECs in receiving waters.
- The freshwater long term and short term PNECs are not subject to excessive uncertainty with assessment factors of 10 being applied to derive the PNECs. The long term saltwater value was derived using an assessment factor of 100. This could be potentially reduced if additional reliable data was available for marine taxa.

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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 |
| d | Day |
| DO | Dissolved oxygen |
| EC50 | Concentration effective against 50 per cent of the organisms or animals tested |
| ECD | Electron Capture Detection |
| EHC | Environmental Health Criteria |
| EQS | Environmental Quality Standard |
| FID | Flame Ionization Detection |
| FIFRA | Federal Insecticide, Fungicide and Rodenticide Act [US] |
| GC | Gas chromatography |
| GLP | Good Laboratory Practice (OECD) |
| h | Hour |
| LC50 | Concentration lethal to 50 per cent of the organisms or animals tested |
| LD50 | Dose lethal to 50 per cent of the organisms or animals tested |
| LOEL | Lowest observed effect level |
| LOAEL | Lowest observed adverse effect level |
| LOEC | Lowest observed effect concentration |
| Lt | Long-term |
| MAC | Maximum allowable concentration |
| NOEL | No observed effect level |
| NOAEL | No observed adverse effect level |
| NOEC | No observed effect concentration |
| OECD | Organization for Economic Co-operation and Development |
| PNEC | Predicted no-effect concentration |
| RED | Reregistration Eligibility Document |
| SSD | Species sensitivity distribution |
| st | Short-term |

| | |
|---|---|
| TCG | Thermal conductivity detection |
| TGD | Technical Guidance Document |
| UKTAG | UK Technical Advisory Group |
| US EPA | US Environmental Protection Agency |
| WFD | Water Framework Directive |

# ANNEX I 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

| Reference | Bejarano *et al*. 2005 |
|---|---|

| Information on the test species | |
|---|---|
| Test species used | *Amphiascus tenuiremis* |
| Source of the test organisms | Laboratory culture |
| Holding conditions prior to test | Contaminant free muddy, flow-through microcosms, salinity 30 ‰; pH 8; temp 23 ± 2°C |
| Life stage of the test species used | Adults + stage 1 juveniles (life cycle test) |

| Information on the test design | |
|---|---|
| Methodology used | ASTM guidelines (E1192-88) 1988 adult test<br>ASTM E2317-04 2004 juvenile test (life cycle) |
| Form of the test substance | 98% purity dissolved in acetone |
| Source of the test substance | Chem Service, West Chester, PA, USA |
| Type and source of the exposure medium | Filtered seawater or filtered seawater containing salt-marsh DOM (TOC 11.6 mg $l^{-1}$) |
| Test concentrations used | 5 + carrier control (adult) (1 life-cycle) |
| Number of replicates per concentration | 4 |
| Number of organisms per replicate | 20 males and 20 non-gravid females (adult)<br>16 (life cycle) |
| Nature of test system (Static, Semi-static or, Flow- through, duration, feeding) | 96 h static<br>16 d life cycle test, semi-static renewal every 72 h, fed every 6 d |
| Measurement of exposure concentrations | Yes – ranged from 22% below to 10% above nominals. Pesticide loss over 96 h ≤ 15% |
| Measurement of water quality parameters | Yes: means - salinity 30.14‰; pH 8.16; DO 85.64%; temp = 20°C |
| Test validity criteria satisfied | Yes |
| Water quality criteria satisfied | Yes |
| Study conducted to GLP | Not stated |
| Comments | Significant increase in toxicity in absence of DOM<br>Males more sensitive than females (96 h LC 50s - M=27 and F=53 µg $l^{-1}$ and with DOM M=59 and F=90 µg $l^{-1}$<br>16 d life-cycle test carried out at 24 µg $l^{-1}$ ( no DOM) reduced reproductive success of exposed pairs by 26% |

| **Reliability of study** | **Reliable** |
|---|---|
| **Relevance of study** | **Relevant** |
| **Klimisch Code** | **1** |

| Reference | Bellas 2006 |
|---|---|

| Information on the test species | |
|---|---|
| Test species used | *(1) Mytilus edulis*<br>*(2) Paracentrotus lividus*<br>*(3) Ciona intestinalis* |
| Source of the test organisms | (1) and (3) field collected from gullmarsfjord area (SW Sweden)<br>(2) Ría de Vigo (Galicia, NW Spain) |
| Holding conditions prior to test | Maintained in aquaria with running seawater for at least 4 d prior to testing. Gametes from (2) and (3) obtained by dissection, (1) induced to spawn |
| Life stage of the test species used | Fertilized eggs |

| Information on the test design | |
|---|---|
| Methodology used | Non standard but well described |
| Form of the test substance | Analytical grade, dissolved in DMSO |
| Source of the test substance | Riedel-de Haën, Sigma Aldrich |
| Type and source of the exposure medium | 0 22 µm filtered sea water |
| Test concentrations used | Not stated, range chosen based on literature toxicity and solubility |
| Number of replicates per concentration | 5 |
| Number of organisms per replicate | (1) 15 -20 eggs $ml^{-1}$<br>(2) ~ 20 eggs $ml^{-1}$<br>(3) ~ 10 eggs $ml^{-1}$ |
| Nature of test system (static, semi-static or flow-through, duration, feeding) | static |
| 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 GLP | Not stated |
| Comment | (1) EC10 = 4.5 µg $l^{-1}$<br>(2) EC10 = 4.3 µg $l^{-1}$ ; $EC10_{growth}$ = 0.5 µg $l^{-1}$<br>(3) EC10 = 12 µg $l^{-1}$ |

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

| **Reference** | Davies and White 1985 |
|---|---|

| **Information on the test species** | |
|---|---|
| Test species used | *(1) Oncorhynchus mykiss (S. gairdneri)*<br>*(2) Galaxias maculates*<br>*(3) Galaxias truttaceus*<br>*(4) Galaxias auratus* |
| Source of the test organisms | (1) Sevrup fisheries, Bridport, Tasmania<br>(2) and (3) field collected from small coastal streams in SE Tasmania<br>(4) field collected from Lake Crescent, central Tasmania |
| Holding conditions prior to test | Acclimated in 40 l flow-through aquaria for minimum 7 – 10 d prior to tests. Feeding stopped 24 h prior to testing |
| Life stage of the test species used | |

| **Information on the test design** | |
|---|---|
| Methodology used | Brief methodology given |
| Form of the test substance | Purified from 'Bravo' formulation by xylene extraction, purity ≥ 99% by GC |
| Source of the test substance | Not stated |
| Type and source of the exposure medium | Kingborough tap-water, filtered through activated charcoal |
| Test concentrations used | Not stated |
| Number of replicates per concentration | Not stated |
| Number of organisms per replicate | 15 |
| Nature of test system (Static, Semi-static or, Flow- through, duration, feeding) | Flow-through |
| Measurement of exposure concentrations | Yes |
| Measurement of water quality parameters | Yes Temp 13 - 16°C depending on species; DO 8-9 mg $l^{-1}$; and 5.12 mg $l^{-1}$ for low DO test (fish acclimated) |
| Test validity criteria satisfied | Not stated |
| Water quality criteria satisfied | yes |
| Study conducted to GLP | Not stated |
| Comments | *O. mykiss* 96 h LC50 17.1 µg $l^{-1}$ and 10.5 µg $l^{-1}$ (low DO)<br>*G. maculates* 96 h LC50 16.3 µg $l^{-1}$<br>*G. truttaceus* 96 h LC50 18.9 µg $l^{-1}$<br>*G. auratus* 96 h LC50 29.2 µg $l^{-1}$ |

| **Reliability of study** | **Reliable with restrictions** |
|---|---|
| **Relevance of study** | **Relevant** |
| **Klimisch Code** | **2 (study at low DO – 3)** |

| Reference | Davies *et al*. 1994 |
|---|---|

| Information on the test species | |
|---|---|
| Test species used | *(1) Oncorhynchus mykiss*<br>*(2) Galaxias maculates*<br>*(3) Paratya australiensis*<br>*(4) Astacopsis gouldi* |
| Source of the test organisms | (1)commercial trout hatchery<br>(2)electrofishing Mersey & Don Rivers, Tasmania<br>(3) field collected Lake Crescent, Tasmania<br>(4) Lab culture |
| Holding conditions prior to test | All animals were acclimated for 5 – 10 d, in a flow-through tank system before exposure. |
| Life stage of the test species used | (1) juveniles: 1.1 – 2.5 g; 45-60 mm or 10 – 30 g; 100-150 mm<br>(2) adults: 2.5 – 11 g; 80 – 135 mm<br>(3) 0.05 – 0.15 g<br>(4) juveniles (~ 0.1 g) |

| Information on the test design | |
|---|---|
| Methodology used | Non standard but well described |
| Form of the test substance | ≥ 98% purity |
| Source of the test substance | Not stated |
| Type and source of the exposure medium | Natural water from Ouse River, Tasmania (pH 6.5 -7) |
| Test concentrations used | (1) and (2) 5 + control range 0.3 – 8.2 µg $l^{-1}$<br>(3) and (4) 5 + control range 0.12 – 42.1 µg $l^{-1}$ |
| Number of replicates per concentration | 2 |
| Number of organisms per replicate | (1) and (2) = 10<br>(3) and (4) = 25 |
| Nature of test system (Static, Semi-static or, Flow- through, duration, feeding) | Flow-through, organisms fed |
| Measurement of exposure concentrations | yes |
| Measurement of water quality parameters | yes |
| Test validity criteria satisfied | yes |
| Water quality criteria satisfied | yes: DO > 95%; pH 6.5-7; 12°C or 12-15°C |
| Study conducted to GLP | Not stated |
| Comments | LC50 for fish species >than highest test concentration. 7 d LC50 *A. gouldi* 3.6 µg $l^{-1}$ and *P. australiensis* 10.9 µg $l^{-1}$ |

| Reliability of study | Reliable |
|---|---|
| **Relevance of study** | **Relevant** |
| **Klimisch Code** | **1** |

| Reference | DeLorenzo and Serrano 2003 |
|---|---|

| Information on the test species | |
|---|---|
| Test species used | *Dunaliella tertiolecta* |
| Source of the test organisms | University of Texas Culture Collection |
| Holding conditions prior to test | F/2 marine media; 25°C |
| Life stage of the test species used | Log phase growth |

| Information on the test design | |
|---|---|
| Methodology used | ASTM 1996 (Vol 11.05 pp 575-586) |
| Form of the test substance | Analytical grade dissolved in acetone |
| Source of the test substance | Chemservice, Westchester, PA , USA |
| Type and source of the exposure medium | As culture medium |
| Test concentrations used | 3.7, 11.1,33.3,100,300 µg $l^{-1}$ + control |
| Number of replicates per concentration | 3 |
| Number of organisms per replicate | Initial 50,000 cells $ml^{-1}$ |
| Nature of test system (Static, Semi-static or, Flow- through, duration, feeding) | Static |
| Measurement of exposure concentrations | No |
| Measurement of water quality parameters | N/A |
| Test validity criteria satisfied | Yes |
| Water quality criteria satisfied | N/A |
| Study conducted to GLP | Not stated |
| Comments | - |

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

| Reference | Fernández-Alba *et al*. 2002 |
|---|---|

| Information on the test species | |
|---|---|
| Test species used | *(1) Pseudokirchneriella subcapitata*<br>*(2) Daphnia magna* |
| Source of the test organisms | (1) provided with test kit immobilized in algal beads<br>(2) dormant eggs supplied with test kit |
| Holding conditions prior to test | Not stated |
| Life stage of the test species used | (1)<br>(2) < 24 h |

| Information on the test design | |
|---|---|
| Methodology used | (1) OECD Guideline 201 using Algaltoxkit (Creasel, Belgium)<br>(2) OECD Guideline 202 / ISO 6341 protocol using Daphtoxkit (Creasel, Belgium) |
| Form of the test substance | Technical grade chlorothalonil (purity not stated) |
| Source of the test substance | Various substances tested and several sources cited therefore not able to identify individually |
| Type and source of the exposure medium | (1) culture medium provided with test kit |
| Test concentrations used | Not stated |
| Number of replicates per concentration | (1) 5<br>(2) 6 |
| Number of organisms per replicate | (1) $10^6$ cells $ml^{-1}$<br>(2) not stated but 5 is norm with this test. |
| Nature of test system (static, semi-static or flow-through, duration, feeding) | Static |
| Measurement of exposure concentrations | No |
| Measurement of water quality parameters | (1) Temp 25°C<br>(2) Temp 20 ± 1°C; |
| Test validity criteria satisfied | Not stated |
| Water quality criteria satisfied | N/A |
| Study conducted to GLP | Not stated |
| Comments | The inoculum level was 2 orders of magnitude higher than that recommended in the guideline |

| **Reliability of study** | **Reliable with restrictions** |
|---|---|
| **Relevance of study** | **Relevant** |
| **Klimisch Code** | **3** |

| | |
|---|---|
| **Reference** | Key *et al*. 2003 |

| **Information on the test species** | |
|---|---|
| Test species used | *Palaemonetes pugio* |
| Source of the test organisms | Field collected from Leadenwah Creek, North Edisto River Estuary, SC |
| Holding conditions prior to test | Acclimated in 76 l tanks; 25°C; salinity 20‰ |
| Life stage of the test species used | Adults, larvae and stage 5 embryos |

| **Information on the test design** | |
|---|---|
| Methodology used | Methodology well described 96h acute assay. Pulsed exposure life cycle assay conducted from newly hatched larvae to juvenile stage. Pulse exposures were 6h/day every 5 d for 20 d administered in 10‰ salinity seawater. At end of 6h exposure animals placed in clean 20 ‰ seawater, refreshed every 24h. |
| Form of the test substance | 98% purity dissolved in acetone |
| Source of the test substance | Chemservice (West Chester, PA, USA) |
| Type and source of the exposure medium | seawater |
| Test concentrations used | 0, 31.3, 62.5, 125, 250, 500 µg $l^{-1}$ (adults, larvae and life-cycle tests)<br>0, 62.5, 125, 250, 500, 1000 µg $l^{-1}$ embryos |
| Number of replicates per concentration | 3 |
| Number of organisms per replicate | 10 (adults and larvae)<br>For embryo test one per well in 24-well plate |
| Nature of test system (Static, Semi-static or, Flow- through, duration, feeding) | Static renewal every 24 h – adults fed |
| Measurement of exposure concentrations | No |
| Measurement of water quality parameters | Yes: mean temp 25.6°C, pH 8; salinity 20.7‰; DO 6.2 mg $l^{-1}$ (adults): temp 25.3°C, pH 8.1; salinity 20.1‰; DO 6.1 mg $l^{-1}$ (larvae): temp 27°C and 24h darkness (embryo) |
| Test validity criteria satisfied | Yes |
| Water quality criteria satisfied | Yes |
| Study conducted to GLP | Not stated |
| Comments | Adult 96 h LC50 = 152.9 µg $l^{-1}$<br>Larval 96 h LC50 = 49.5 µg $l^{-1}$<br>Embryo 96 h LC50 = 396 µg $l^{-1}$ |

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

| Reference | Ma *et al*. 2002 |
|---|---|

| Information on the test species | |
|---|---|
| Test species used | *(1) Scenedesmus obliqnus*<br>*(2) Chlorella pyrenoidosa* |
| Source of the test organisms | Institute of Wuhan Hydrobiology, Chinese Academic of Science |
| Holding conditions prior to test | Cells propagated photoautotrophically in liquid HB-4 medium at 25$^{o}$C under continuous illumination |
| Life stage of the test species used | Not stated |

| Information on the test design | |
|---|---|
| Methodology used | Standard algal test methodology described |
| Form of the test substance | 30% suspension concentrate formulation |
| Source of the test substance | People's Republic of China |
| Type and source of the exposure medium | HB-4 medium |
| Test concentrations used | Range between 0 and 150 mg l-1 + control (several chemicals tested therefore range applicable not clear) |
| Number of replicates per concentration | 3 |
| Number of organisms per replicate | 6 x $10^{5}$ cells $ml^{-1}$ |
| Nature of test system (static, semi-static or flow-through, duration, feeding) | Static |
| Measurement of exposure concentrations | No |
| Measurement of water quality parameters | Algal medium |
| Test validity criteria satisfied | Yes |
| Water quality criteria satisfied | - |
| Study conducted to GLP | Not stated |
| Comment | EC50 *S.obliqnus* 8,069 µg $l^{-1}$ |

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

| Reference | Sherrard *et al*. 2003 |
|---|---|

| Information on the test species | |
|---|---|
| Test species used | *Pimephales promelas* |
| Source of the test organisms | Culture from Dept of Environmental Toxicology, Clemson University |
| Holding conditions prior to test | |
| Life stage of the test species used | < 24 h |

| Information on the test design | |
|---|---|
| Methodology used | 5 range-finding 96 h static non-renewal exposures, followed by a modified static renewal 7 d exposure |
| Form of the test substance | Dragon Daconil 2787 (12.9% chlorothalonil) |
| Source of the test substance | Local vendor |
| Type and source of the exposure medium | Moderately hard well water |
| Test concentrations used | 0, 5, 10, 12.5, 15, 17.5, 20. 22.5, 25 and 30 $\mu g\ l^{-1}$ |
| Number of replicates per concentration | 3 |
| Number of organisms per replicate | 10 |
| Nature of test system (static, semi-static or flow-through, duration, feeding) | Semi-static daily preparation of renewal solutions from stock solutions prepared at test initiation, anticipating that concentration would decrease over time as in the field. Fed daily |
| Measurement of exposure concentrations | Yes, samples from high, median and low concentrations. Measured concentrations at start of experiment were all below target levels. Levels decreased 13 – 19% over 7d test period. |
| Measurement of water quality parameters | Yes: pH7.8; temp 25 ± 1°C; hardness 80 mg $CaCO_3\ l^{-1}$ |
| Test validity criteria satisfied | Yes |
| Water quality criteria satisfied | Yes |
| Study conducted to GLP | Not stated |
| Comments | Reported results based on initial targeted concentrations. |

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

| Reference | Schults and Hoberg 1991 |
|---|---|

| Information on the test species | |
|---|---|
| Test species used | *Mysidopsis bahia (Americamysis bahia)* |
| Source of the test organisms | - |
| Holding conditions prior to test | - |
| Life stage of the test species used | < 24 h |

| Information on the test design | |
|---|---|
| Methodology used | Test according to EPA Guideline |
| Form of the test substance | 98% technical chlorothalonil |
| Source of the test substance | - |
| Type and source of the exposure medium | - |
| Test concentrations used | 5 (0.63 - 10 µg $l^{-1}$) + solvent control (acetone, max 23 µl $l^{-1}$) and control. Mean measured concs: 0.065, 0.83, 1.2, 3.0, and 5.7 µg $l^{-1}$ |
| Number of replicates per concentration | 2 |
| Number of organisms per replicate | 30 |
| Nature of test system (static, semi-static or flow-through, duration, feeding) | Flow-through<br>Daily feeding |
| Measurement of exposure concentrations | Yes, measured by GC with electron capture detection (recovery 109%). Actual concentrations were 34 – 103% of nominal during test. |
| Measurement of water quality parameters | Yes: pH7.7 - 8; temp 23 -26°C; salinity 31-33‰ |
| Test validity criteria satisfied | No |
| Water quality criteria satisfied | Yes |
| Study conducted to GLP | Not stated |
| Comments | High mortality in control and solvent controls |

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

| Reference | Schults *et al.* 1980 |
|---|---|

| Information on the test species | |
|---|---|
| Test species used | *Pimephales promelas* |
| Source of the test organisms | - |
| Holding conditions prior to test | - |
| Life stage of the test species used | - |

| Information on the test design | |
|---|---|
| Methodology used | An early life-stage test extended over 2 generations. Exposure 4 d hatching period, 280 d $F_0$ generation and 34 d $F_1$ generation. Test according to EPA guidelines |
| Form of the test substance | 96% technical chlorothalonil |
| Source of the test substance | - |
| Type and source of the exposure medium | - |
| Test concentrations used | 1.5, 3.1, 6.2, 12.5 and 25 µg $l^{-1}$ + solvent control (acetone, max 5 µl $l^{-1}$) and control. Mean measured concs: 0.6, 1.4, 3.0, 6.5 and 16 µg $l^{-1}$ |
| Number of replicates per concentration | - |
| Number of organisms per replicate | - |
| Nature of test system (static, semi-static or flow-through, duration, feeding) | Flow-through, no aeration |
| Measurement of exposure concentrations | Yes, actual concentrations measured weekly (recovery 84%) by GC with $^{63}$Ni-electron capture detection. Actual concentrations were 40 – 64% of nominal during test. |
| Measurement of water quality parameters | Yes: pH6.5 – 7.3; temp 25°C; hardness 30 mg $l^{-1}$ as $CaCO_3$ |
| Test validity criteria satisfied | Yes |
| Water quality criteria satisfied | Yes |
| Study conducted to GLP | Not stated |
| Comments | |

| **Reliability of study** | **Reliable** |
|---|---|
| **Relevance of study** | **Relevant** |
| **Klimisch Code** | **1** |

| Reference | Schults *et al.* 1983 |
|---|---|

| **Information on the test species** | |
|---|---|
| Test species used | *Crassostrea virginica* |
| Source of the test organisms | - |
| Holding conditions prior to test | - |
| Life stage of the test species used | Mean weight 2.6 g; mean height 5.3 cm |

| **Information on the test design** | |
|---|---|
| Methodology used | Test according to EPA Guidelines |
| Form of the test substance | 96% technical chlorothalonil |
| Source of the test substance | - |
| Type and source of the exposure medium | - |
| Test concentrations used | 1, 2, 4, 8, 16 and 32 µg $l^{-1}$ + solvent control (acetone, max 100 µl $l^{-1}$) and control. Mean measured concs: 0.6, 1.6, 3.2, 7.4, 15.2 and 30.8 µg $l^{-1}$ |
| Number of replicates per concentration | Not stated |
| Number of organisms per replicate | 10 (per concentration) |
| Nature of test system (static, semi-static or flow-through, duration, feeding) | Flow-through |
| Measurement of exposure concentrations | Yes, actual concentrations measured daily (recovery 80%) by GC with electron capture detection. Actual concentrations were 80 – 96% of nominal during test, except in 1 ug $l^{-1}$ (60%). |
| Measurement of water quality parameters | Yes: pH8 – 8.2; temp 14-16°C; salinity 22‰ |
| Test validity criteria satisfied | Yes |
| Water quality criteria satisfied | Yes |
| Study conducted to GLP | Not stated |
| Comments | EPA Guideline 1985 requires at least 20 oysters per conc. Nominal EC50 = 7.3 ug $l^{-1}$ (95% cf 5 – 12 ug $l^{-1}$) calculated with moving average angle method; slope 2.55. NOEC 2 ug $l^{-1}$ (Student t-test). EC50 using mean measured concs, with log-logistic regression = 0.5 ug $l^{-1}$ (no reliable conf. interval) |

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

| Reference | Teather *et al*. 2005 |
|---|---|

| Information on the test species | |
|---|---|
| Test species used | *Oryzias latipes* |
| Source of the test organisms | University of Prince Edward Island breeding stock |
| Holding conditions prior to test | 15 individuals per 10 gallon tank in dechlorinated tap water at 20°C |
| Life stage of the test species used | Fertilized eggs |

| Information on the test design | |
|---|---|
| Methodology used | Non standard well described. Fry were removed to clean water 7 days post hatch. Activity levels were monitored 3 w post hatching following removal to clean water. Fish sexed at 5 m. |
| Form of the test substance | Commercial formulation |
| Source of the test substance | Obtained through Environment Canada (Moncton) |
| Type and source of the exposure medium | Autoclaved distilled embryo-rearing solution |
| Test concentrations used | 0.06 µg $l^{-1}$ |
| Number of replicates per concentration | 4 |
| Number of organisms per replicate | 25 |
| Nature of test system (static, semi-static or flow-through, duration, feeding) | Semi-static, renewal every 24 h |
| Measurement of exposure concentrations | No |
| Measurement of water quality parameters | Temp = 25 ± 1°C |
| Test validity criteria satisfied | No |
| Water quality criteria satisfied | Not stated |
| Study conducted to GLP | Not stated |
| Comments | Overall survival of eggs to hatching in controls 81%. Control survival lower than some treatments (other pesticides and combinations). With respect to chlorothalonil, no statistical effect on survivorship, time to hatch or fry size. Activity level, as measured by swimming distance was reduced. Sex ratio 1:1 in controls and 1:2 in favour of females in chlorothalonil treatment. |

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

# ANNEX II Summary data from OPP 2007

This annex contains information taken from:

- Ecotoxicity Database – an online US EPA database held by the Office of Pesticide Programs that summarises ecotoxicological data used by the EPA for ecotoxicological assessments. Consists primarily of the endpoint data submitted in support of registration and reregistration of pesticide products (OPP 2007).

Data in the summary tables below are classified by the US EPA as ‘core’ if all essential information was reported and the study was performed according to recommended US EPA or American Society for Testing Materials (ASTM) methodology. Minor inconsistencies with standard recommended procedures may be apparent, but the deviations do not detract from the study's soundness or intent. Studies within this category fulfil the basic requirements of current FIFRA guidelines and are acceptable for use in a risk assessment (equivalent Klimisch code 1). Data not meeting this requirement are classified as either supplemental (Klimisch code 2) or invalid (Klimisch code 3). Supplemental studies are considered scientifically sound, however they were performed under conditions that deviated substantially from recommended protocols. Examples of the conditions that may place a study in this category include: non-native species, tested organisms were older/younger than guideline recommendations, deviations from recommended water quality characteristics (this list is not exhaustive). Where this data has been reported in Tables 2.9 to 2.12 the following notation has been used to identify the US EPA classification: c = core and s = supplemental.

**Table A1 Summary of most sensitive chronic data taken from OPP 2007 with additional information from other compendium reports**

| Chemical | Species | Test | Age | Duration | Additional information | LOEC (µg $l^{-1}$) | NOEC (µg $l^{-1}$) | Reference as cited in OPP 2007 |
|---|---|---|---|---|---|---|---|---|
| **Freshwater – algae (Table 29)** | | | | | | | | |
| 97% | *Pseudokirch-neriella subcapitata* | [123-2] static | | 5 days | | 100 | 50 | Ref No 42432801: Malcolm Pernie Inc, New York, USA 1992 |
| 98.1% | *Navicula pelliculosa* | [123-2] static | | 5 days | | - | 3.9 | EPA Identification 44908105: Brixham Laboratory, Brixham UK 1998 |
| **Freshwater – higher plants (Table 2.9)** | | | | | | | | |
| 98.1% | *Lemna gibba* | [123-2] static | | 14 days | | - | 290 | EPA Identification 44908102:Brixham Laboratory, Brixham, UK 1998 |
| **Freshwater – invertebrates (Table 2.9)** | | | | | | | | |
| 99.8% | *Daphnia magna* | [72-4b] static renewal | | 21 days | Endpoint survival, cumulative numbers of offspring per female | 79 | 39 | Ref No 00115107: EG & G Corp (Diamond Shamrock) 1981 |
| **Freshwater – fish (Table 2.9)** | | | | | | | | |
| 96% | *Pimephales promelas* | [74-4a] flow-through | ErlyLf | 168 days | Mean measured test concentrations: 0.6, 1.4, 3.0, 6.5, 16 µg a.i. $l^{-1}$ plus dilution water-only and a solvent control (acetone) ( HSE 2002) | 6.5 | 3 | Ref 00030391: EG & G Corp (Diamond Shamrock) 1980 |
| **Saltwater – algae (Table 2.11)** | | | | | | | | |
| 98.1% | *Skeletonema costatum* | [123-2] static | | 14 days | | - | 5.9 | EPA Identification: Brixham Laboratory, Brixham, UK 1998 |
| **Saltwater – invertebrates (Table 2.11)** | | | | | | | | |

| Chemical | Species | Test | Age | Duration | Additional information | LOEC ($\mu g\ l^{-1}$) | NOEC ($\mu g\ l^{-1}$) | Reference as cited in OPP 2007 |
|---|---|---|---|---|---|---|---|---|
| 98% | *Americamysis bahia* | Life cycle-flow-through | < 24 h | 28 days | 2 replicates per conc; 30 organisms per replicate. Mean measured test concentrations 0.65, 0.83, 1.2, 3.0, 5.7 $\mu g\ l^{-1}$+ dilution water-only control and a solvent control (acetone). No treatment related effects on adult, survival, behaviour or growth. Signif. reduction in no. of offspring per female at ≥ 1.2 $\mu g\ l^{-1}$ ( HSE 2002) | 1.2 | 0.83 | Ref No 42433807: Springborn Laboratory Inc, MA 1991 |

(m) = measured; AM = artificial medium; NR = not reported
MATC = maximum acceptable toxicant concentration
[72-4a] freshwater or marine/estuarine fish early life stage chronic toxicity using TGAI or TEP (FIFRA 158.490)
[72-4b] freshwater invertebrate life cycle chronic toxicity using TGAI or TEP (FIFRA 158.490)
[123 –2] Tier II Aquatic Plant Growth – multi-dose (FIFRA 158.540)
CI = confidence interval
TEP = typical end use product
TGAI = technical grade of the active ingredient

**Table A2 Summary of most sensitive acute data taken from OPP (2007)**

| Chemical a.i.% | Species | Test | Organism age/ size | Duration | LC/EC50 ($\mu g\ l^{-1}$) (95% CI) | NOEC ($\mu g\ l^{-1}$) | Curve slope | Reference (as cited in OPP 2007) |
|---|---|---|---|---|---|---|---|---|
| **Freshwater – algae (Table 2.10)** | | | | | | | | |
| 97.9% | *Pseudokirc-neriella subcapitata* | [123-2] static | | 5 days | 190 (180 – 210) | 50 | NR | Ref No 42432801: Malcolm Pernie Inc, New York, USA 1992 |
| 98.1% | *Navicula pelliculosa* | [123-2] static | | 5 days | 14 (12 -17) | 3.9 | 4.49 | EPA Identification 44908105: Brixham Laboratory, Brixham UK 1998 |
| **Freshwater – higher plants (Table 2.10)** | | | | | | | | |
| 98.1% | *Lemna gibba* | [123-2] static | | 14 days | 630 (550 – 730) | 290 | 5.3 | EPA Identification 44908102:Brixham Laboratory, Brixham, UK 1998 |
| **Freshwater – invertebrate (Table 2.10)** | | | | | | | | |
| 96% Technical grade | *Daphnia magna* | [72-2] static; not GLP, unmeasured concentrations 22°C; pH 7.2-7.4; DO 7.9-8.8 mg $l^{-1}$; hardness 60 mg $CaCO_3$ $l^{-1}$(HSE 2002) | < 24 h | 48 hours | 70 (34.2 – 143) | 6.8 | NR | Ref No 00068754: EG & G Corp (Diamond Shamrock) 1977 |
| 54% (Bravo 720) | *Daphnia magna* | [72-2b] flow-through; GLP, measured concs. 20°C; pH 8.1-8.3; hardness170- 180 mg CaCO3 l-1; DO 93 -105% saturation (HSE 2002) | 1st instar | 48 hours | 97 (86 – 108) | 49 | 7.9 | Ref No 42433806: Springborn Laboratory Inc., MA 1992 |
| **Freshwater – fish (Table 2.10)** | | | | | | | | |
| 96% | *Oncorhynchus mykiss* | [72-1] static | Juv | 96 hours | 42 (32 – 68) | 32 | NR | Ref No 00056486: EG & G Corp (Diamond Shamrock 1980 (supplemental) |

| Chemical a.i.% | Species | Test | Organism age/ size | Duration | LC/EC50 ($\mu g\ l^{-1}$) (95% CI) | NOEC ($\mu g\ l^{-1}$) | Curve slope | Reference (as cited in OPP 2007) |
|---|---|---|---|---|---|---|---|---|
| 96% | *Ictalurus punctatus* | [72-1] static | Juv | 96 hours | 43 (26 – 70) | 26 | NR | Ref No 00030390: Diamond Shamrock Agricultural Laboratory 1980 |
| 98% | *Lepomis macrochirus* | [72-1] static | 0.39 g | 96 hours | 51 (45 -57) | 0.37 | NR | Ref No 00127862 |
| 96% | *Lepomis macrochirus* | [72-1] static | Juv | 96 hours | 60 (52 – 78) | 28 | NR | Ref No 00041439: EG & G Corp (Diamond Shamrock) 1980 |
| 99.7% | *Lepomis macrochirus* | [72-1] static | 0.9 g | 96 hours | 84 (71 – 99) | 49 | NR | Ref No 00029410: Diamond Shamrock Agricultural Laboratory 1979 |
| 54% (Bravo 720) | *Oncorhynchus mykiss* | [72-1] flow-through; GLP; mean measured concentrations 6.5, 10.4, 15.8, 26.7,48.5 $\mu g\ l^{-1}$. pH 7; 12°C; hardness 39 mg $CaCO_3\ l^{-1}$; DO 89-94% saturation (HSE 2002) | 0.56 g | 96 hours | 33 (26 – 48) | 16 | NR | Ref No 43302101: Springborn Laboratory Inc., MD 1994 |
| 54% (Bravo 720) | *Lepomis macrochirus* | [72-1] flow-through | 0.39 g | 96 hours | 26 (22.1 – 32) | 15 | NR | Ref No 42433804: Springborn Laboratory Inc., MA 1992 |
| 75% (Bravo W-75) | *Oncorhynchus mykiss* | [72-1] static | 1.4 g | 48 hours | 152 (134 – 173) | < 140 | NR | Ref No 00087304: Agricultural Research Center, USDA, Beltsville, MD.1972 (supplemental) |
| 75% (Bravo W-75) | *Oncorhynchus mykiss* | [72-1] static | 0.76 g | 96 hours | 77 (73 – 81) | 65 | NR | Ref No 00087303: Agricultural Research Center, USDA, Beltsville, MD. 1972 |

| Chemical a.i.% | Species | Test | Organism age/ size | Duration | LC/EC50 ($\mu g\ l^{-1}$) (95% CI) | NOEC ($\mu g\ l^{-1}$) | Curve slope | Reference (as cited in OPP 2007) |
|---|---|---|---|---|---|---|---|---|
| 75% (Bravo W-75) | *Lepomis macrochirus* | [72-1] static | 35+ mm | 24 hours | 125 | 65 | NR | Ref No 00087258: Agricultural Research Center, USDA, Beltsville, MD 1973 (supplemental) |
| **Saltwater – algae (Table 2.12)** | | | | | | | | |
| 98.1% | *Skeletonema costatum* | [123-2] static | | 14 days | 13 (12 – 14) | 5.9 | NR | EPA Identification: Brixham Laboratory, Brixham, UK 1998 |
| **Saltwater – invertebrates (Table 2.12)** | | | | | | | | |
| Technical grade | *Crassostrea virginica* | [72-3b] flow-through Shell deposition 27‰ salinity; T=29°C (EHC 1996) | Spat | 96 hours | 26 | NR | NR | Ref No 00138143 (RED) EPA Identification 40228401 EPA Labs |
| Technical grade | *Penaeus duorarum* | [72-3c] static; unmeasured concentrations 75, 100, 150, 200, 300 $\mu g\ l^{-1}$ + dilution water-only & solvent (acetone) controls. 6 replicates; 2 organisms per replicate (HSE 2002) | Juv | 96 hours | 165 (100 -270) | 75 | NR | Ref No 00127864: EG & G Corp (Diamond Shamrock) 1982 |
| Technical grade | *Penaeus duorarum* | [72-3] flow-through | Juv | 48 hours | 320 | | | EPA identification 40228401: EPA Labs, Beltsville, Md or Gulfbreeze, Florida USA 1986 (supplemental) |
| 75% (Bravo) | *Cancer magister* | [72-3c] semi –static (24 hr renewal); T=13°C 25 ‰ salinity (ECH 1996) | larvae | 96 hours | 140 | - | - | EPA Identification 00127865: study date 1976 supplemental |

| Chemical a.i.% | Species | Test | Organism age/ size | Duration | LC/EC50 ($\mu g\ l^{-1}$) (95% CI) | NOEC ($\mu g\ l^{-1}$) | Curve slope | Reference (as cited in OPP 2007) |
|---|---|---|---|---|---|---|---|---|
| **Saltwater – fish (Table 2.12)** | | | | | | | | |
| Technical grade | *Cyprinodon variegatus* | [72-3a] static; unmeasured concentrations 20, 30, 45, 65, 90 $\mu g\ l^{-1}$ + dilution water-only and solvent controls. 2 replicates, 10 organisms per replicate (HSE 2002) | 3 -7 d | 96 hours | 32 (30 – 36) | 20 | NR | Ref No 00127863: Diamond Shamrock Agricultural Laboratory 1982 |

[72-1] Freshwater fish acute-warm and coldwater species with TGAI or TEP (FIFRA 158.490)
[72-2] Freshwater invertebrate acute TGAI or TEP
[72-3] Estuarine/marine fish, shellfish, shrimp acute using TGAI or TEP
[122-2] Tier I Aquatic Plant Growth – single dose (FIFRA 158.540)
[123 –2] Tier II Aquatic Plant Growth – multi-dose (FIFRA 158.540)
CI = confidence interval
TEP = typical end use product
TGAI = technical grade of the active ingredient
m = measured, n = nominal