Proposed EQS for Water Framework Directive Annex VIII substances: carbendazim *(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 members and partners.

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

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

Executive summary

The UK Technical Advisory Group (UKTAG) has commissioned a programme of work to derive Environmental Quality Standards (EQSs) for substances falling under Annex VIII of the Water Framework Directive (WFD). This report proposes predicted no-effect concentrations (PNECs) for carbendazim using the methodology described in Annex V of the Directive. There are existing EQSs for carbendazim, but the method used to derive these is not considered to comply with the requirements of Annex V and so is unsuitable for deriving Annex VIII EQSs.

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

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

Values of 5.0 to 12.9 mg l^{-1} indicate that carbendazim has low to moderate water solubility (EU DAR 2000, NPIC 2008). A log Kow of 0.40-1.52, suggests that carbendazim is unlikely to significantly partition to sediments and suspended matter and/or accumulate in biota (US EPA, 2005). In a study of distribution in water and sediment systems carbendazim was shown to partition into sediment, but this did not occur rapidly (EU DAR, 2003). Volatilisation is not expected to be an important environmental fate process for carbendazim, based on a Henry's Law constant of 3.5 $x10^{-8}$ $Pa.m^3.mole^{-1}$ (US EPA 2005).

The ready biodegradability of technical grade carbendazim in a closed bottle test according to OECD Guideline 301D was reported in the EU DAR (2000). Within 28 days 6% biodegradation was attained, and, therefore, technical grade carbendazim may not be classified as readily biodegradable.

Bioconcentration data (as BCF values) for carbendazim is low, with values for whole fish ranging from 23 to 159 (and 380 to 460 for viscera). The one BCF value over 100 for rainbow trout was an exception and values <100 were recorded in the same study for channel catfish and bluegill sunfish. Other studies have reported BCF values of 23 and 27.

Availability of data

Long-term freshwater toxicity data are available for five taxonomic groups, i.e. algae, crustaceans, fish, molluscs and platyhelminths with crustaceans being more sensitive than the other taxa. Short-term toxicity tests are available for eight taxonomic groups, i.e. algae, amphibians, crustaceans, fish, insects, molluscs, platyhelminths and protozoa, with crustaceans and fish being more sensitive than other taxa. For marine

organisms, single species short-term toxicity data are available for three different taxonomic groups; crustaceans, fish and molluscs. However, no long-term toxicity data are available for saltwater taxa. Information is available on the effects of carbendazim in freshwater microcosm and mesocosm studies.

Derivation of PNECs

Long-term PNEC for freshwaters

The lowest valid long-term toxicity value for freshwater invertebrates is a 21-day No Observed Effect Concentration (NOEC) of 1.5 µg active ingredient (a.i.) l^{-1} for effects on the reproduction of the waterflea *Daphnia magna*. Reliable long-term NOECs are available for algae, crustaceans, fish, molluscs and platyhelminths. Therefore, based on the EU Technical Guidance Document (TGD) methodology, an assessment factor of 10 could be applied to the lowest valid toxicity value. This results in a $PNEC_{freshwater\_lt}$ of 0.15 µg l^{-1}.

The existing EQS is 0.1 µg l^{-1} (rounded value) and was derived by applying assessment factor of 100 to the most reliable acute data (96-hour LC50 of 7 µg a.i. l^{-1}) obtained for channel catfish yolk sac fry (*Ictalurus punctatus*).

Short-term PNEC for freshwaters

Reliable short-term data are available for algae, amphibians, annelids, crustaceans, fish, insects, molluscs and protozoa. The lowest valid short-term toxicity value is a 96-hour LC50 of 7 µg a.i. l^{-1} in the channel catfish (*Ictalurus punctatus*) yolk sac fry. Based on the EU Technical Guidance Document (TGD) methodology and a large body of reliable acute data for carbendazim, an assessment factor of 10 can be applied to the lowest valid toxicity value. This results in a $PNEC_{freshwater\_lt}$ of 0.7 µg l^{-1}.

The current short-term EQS for freshwaters is 1.0 µg l^{-1} (rounded value) and was derived by applying assessment factor of 10 to the most reliable acute data (96-hour LC50 of 7 a.i. µg l^{-1}) obtained for channel catfish yolk sac fry (*Ictalurus punctatus*).

Long-term PNEC for saltwaters

No long-term single species toxicity data for marine organisms are available. The absence of long-term data means that it is not possible to generate a $PNEC_{saltwater\_lt}$ based on the saltwater data alone, and it is proposed that the combined freshwater and saltwater dataset is used for the PNEC generation. Reliable long-term NOECs are available for algae, crustaceans, fish, molluscs and platyhelminths, but no data are available for saltwater species and in particular additional marine taxa such as such as echinoderms. Therefore, based on the methodology outlined in the TGD, an assessment factor of 100 could be applied to the lowest valid freshwater chronic toxicity value. This results in a $PNEC_{saltwater\_lt}$ of 0.015 µg l^{-1}.

This existing EQS is 0.1 µg l^{-1} (rounded value) and was derived by applying assessment factor of 100 to the most reliable acute data (96-hour LC50 of 7 µg a.i. l^{-1}) obtained for channel catfish yolk sac fry (*Ictalurus punctatus*).

Short-term PNEC for saltwaters

Single species short-term toxicity data for marine organisms are available for three different taxonomic groups, i.e. crustaceans, molluscs and fish. Therefore, it is proposed that the $PNEC_{saltwater\_st}$ is based on the combined freshwater and saltwater dataset.

The lowest valid short-term toxicity value for freshwater fish is a 96-hour LC50 of 7 µg a.i. l^{-1} in the channel catfish yolk sac fry (*Ictalurus punctatus*). Since no data are available for marine taxa such as echinoderms, an additional assessment factor of 10 would also normally be applied, resulting in a total assessment factor of 100. However, there is a large body of short-term data in the combined freshwater and saltwater

dataset and there are toxicity data for saltwater molluscs. Therefore, a reduced assessment factor of 50 applied to the lowest valid toxicity value has been adopted resulting in a $PNEC_{saltwater\_st}$ of 0.14 µg a.i. l^{-1}.

The current short-term EQS for saltwaters is 1.0 µg l^{-1} (rounded value) and was derived by applying assessment factor of 10 to the most reliable acute data (96-hour LC50 of 7 µg a.i. l^{-1}) obtained for channel catfish yolk sac fry (*Ictalurus punctatus*).

PNECs for sediment

The log Kow of methiocarb is 0.4-1.52 and the log Koc is 2.30-2.39, so the derivation of PNECs for the protection of benthic organisms is not required according to the TGD since the log Kow/Koc trigger value of 3 is not exceeded.

PNECs for secondary poisoning

Bioconcentration data for carbendazim indicate low BCF values with values for whole fish ranging from 23 to 159 (and 380 to 460 for viscera). The one BCF value over 100 for rainbow trout was an exception and values <100 were recorded in the same study for channel catfish and bluegill sunfish. Other studies have reported BCF values of 23 and 27. Therefore, on a weight of evidence basis it is considered that the TGD BCF trigger of 100 has not been exceeded and the derivation of a PNEC in whole fish for secondary poisoning of predators is not required.

Summary of proposed PNECs

Receiving medium/exposure scenario	**Proposed PNEC (µg l^{-1})**	**Existing EQS (µg l^{-1})**
Freshwater/long-term	0.15	0.1
Freshwater/short-term	0.7	1.0
Saltwater/long-term	0.015	0.1
Saltwater/short-term	0.14	1.0
Sediment	Not required	-
Secondary poisoning	Not required	–

Analysis

For water, proposed PNECs derived for carbendazim range from 0.03 to 0.7 µg l^{-1}. The data quality requirements are that, at one third of the EQS, total error of measurement should not exceed 50 per cent. Using this criterion, it is evident that current analytical methodologies (non-standard) employing gas chromatography/mass spectrometry (GC-MS) and capable of achieving detection limits as low as 0.001 ng l^{-1} should offer adequate performance to analyse for carbendazim.

Implementation issues

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

- The analytical capability should be adequate for compliance assessment
- The assessment factors applied are within the range of 10-100 and therefore the PNECs derived are not subject to excessive uncertainty. The size of the assessment factors applied in the derivation of the saltwater standards could potentially be reduced if additional data on the toxicity to marine organisms was available.

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 carbendazim using the methodology described in Annex V of the Directive. There are existing EQSs for carbendazim, but the method used to derive these is not considered to comply with the requirements of Annex V and so is unsuitable for deriving Annex VIII EQSs.

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

This report provides a data sheet for carbendazim. The most recent available data have been used in this report where this is considered reliable.

1.1 Properties and fate in water

Values of 5.0 to 12.9 mg l^{-1} indicate that carbendazim has low to moderate water solubility (EU DAR 2000, NPIC 2008). A log Kow of 0.40-1.52 and log Koc of 2.30-2.39, suggests that carbendazim is unlikely to significantly partition to sediments and suspended matter and/or accumulate in biota (US EPA, 2005). However, a distribution in water and sediment systems study has shown that carbendazim will partition into sediment, but this did not occur rapidly (EU DAR, 2003). Volatilisation is not expected to be an important environmental fate process for carbendazim, based on a Henry's Law constant of 3.5 $x10^{-8}$ $Pa.m^3.mole^{-1}$ (US EPA 2005).The ready biodegradability of technical grade carbendazim in a closed bottle test according to OECD Guideline 301D was reported by EU DAR (2000). Within 28 days 6% biodegradation was attained, and, therefore, technical grade carbendazim may not be classified as readily biodegradable.

Bioconcentration data (as BCF values) for carbendazim indicate low BCF values with values for whole fish ranging from 23 to 159 (and 380 to 460 for viscera). The one BCF value over 100 for rainbow trout was an exception and values <100 were recorded in the same study for channel catfish and bluegill sunfish. Other studies have reported BCF values of 23 and 27. Therefore, on a weight of evidence basis it is considered that the TGD BCF trigger of 100 has not been exceeded and the derivation of a PNEC in whole fish for secondary poisoning of predators is not required.

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

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

2 Results and observations

2.1 Identity of substance

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

Table 2.1 Species covered by this report

Name	CAS Number
Carbendazim	10605-21-7

2.2 PNECs proposed for derivation of quality standards

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

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

Table 2.2 Proposed overall PNECs as basis for quality standard setting

PNEC	TGD deterministic approach (AFs)	TGD probabilistic approach (SSDs)	Existing EQS
Freshwater short-term	0.7 µg l^{-1}	-	1.0 µg l^{-1}
Freshwater long-term	0.15 µg l^{-1}	-	0.1 µg l^{-1}
Saltwater short-term	0.14 µg l^{-1}	-	1.0 µg l^{-1}
Saltwater long-term	0.03 µg l^{-1}	-	0.1 µg l^{-1}
Sediment	Not required	-	-
Secondary poisoning	Not required	-	-

AA = Annual Average
AF = Assessment Factor
SSD = Species Sensitivity Distribution
TGD = Technical Guidance Document
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2.3 Hazard classification

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

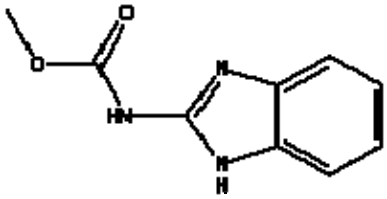
Table 2.3 Hazard classification

R-phrases and labelling	Reference
R46, 60, 61, 50/53 S53, 45, 60, 61	ECB (2009)

2.4 Physical and chemical properties

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

Table 2.4 Physical and chemical properties of carbendazim

Property	Value	Reference
CAS number	10605-21-7	Chemfinder 2007
Substance name	Methyl benzimidazol-2-ylcarbamate	Chemfinder 2007
Molecular formula	$C_9H_9N_3O_2$	Chemfinder 2007
Molecular structure		Chemfinder 2007
Molecular weight	191.2	Chemfinder 2007
Colour/form	Light grey powder White crystalline solid	Chemfinder 2007 WHO 1993
Odour	Odourless	HSDB 2005
Melting point (°C)	302-307	Chemfinder 2007, EU DAR 2000
Boiling point (°C)	N/D	
Vapour pressure	7.5 $x10^{-10}$ mmHg at 20°C (experimental) < 1 $x10^{-7}$ Pa (< 1 $x10^{-9}$ mbar) at 20°C 9 $x10^{-5}$ Pa (20°C); 1.5 $x10^{-4}$ Pa (25°C)	SRC 2007 WHO 1993 EU DAR 2000 EU DAR 2000
Density/specific gravity	0.25-0.35 $g.cm^3$ at 20°C 0.27 $g.cm^3$ (loose); 0.62 $g.cm^3$ (packed)	IUCLID 2000 WHO 1993
Henry's Law Constant	2.12 $x10^{-11}$ $atm.m^3.mol^{-1}$ at 20°C 1.02 $x10^{-9}$ $atm.m^3.mol^{-1}$ at 20°C 3.6 $x10^{-3}$ $atm.m^3.mol^{-1}$ at 24°C 3.5 $x10^{-8}$ $atm.m^3.mol^{-1}$ at 20°C	SRC 2007 WHO 1993 EU DAR 2000 US EPA 2005
Solubility	12.9 mg l^{-1} water at 20.0 ± 0.5°C 8 mg l^{-1} in water 28 - 36 mg l^{-1} at pH 4 and ambient temperature 5 - 7 mg l^{-1} at pH 7-8 and ambient temperature 29 mg l^{-1} in water at 24°C and pH 4 28 mg l^{-1} in water at 20°C and pH 4 8 mg l^{-1} in water at 20°C and pH 4 7 mg l^{-1} in water at 20°C and pH 4 300 mg l^{-1} in ethanol at 24°C 359 – 480 mg l^{-1} in methanol at 24°C	US EPA 2005 NPIC 2008 EU DAR, 2000 SRC 2007 WHO 1993 EU DAR 2000

	166 – 300 mg l^{-1} in acetone at 24°C	

2.5 Environmental fate and partitioning

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

Table 2.5 Environmental fate and partitioning of carbendazim

Property	Value	Reference
Abiotic fate	The second order rate constant for HO radicals with carbendazim is 2.2 ± 0.3 $x10^9$ $molecule^{-1}$ sec^{-1}. The second order rate constant for carbonate radicals with carbendazim is 6 ± 2 $x10^6$ $molecule^{-1}$ sec^{-1}.	Mazellier *et al.* 2003 Mazellier *et al.* 2003
Speciation	pKa 4.49 and pKb 10.62 at 25°C pKa 4.2	US EPA, 2005 EU DAR 2000
Hydrolytic stability	The hydrolysis half-life ($t_{1/2}$) of carbendazim is 1.56 x 10^3 hours at pH 9 (25 ± 1°C) with no breakdown products. No hydrolysis was observed in pH 5 and 7 buffer solutions after 24 days at 25 ± 1°C. DT_{50} at pH 5: > 350 days (22°C) DT_{50} at pH 7: > 350 days (22°C) DT_{50} at pH 9: 124 days (22°C)	US EPA, 2005 EU DAR 2000 EU DAR 2000 EU DAR 2000
Photostability	Carbendazim is photostable. There was no degradation of a 5 mg l^{-1} solution of carbendazim (technical grade) after exposure to simulated daylight for 17 days at 25 ± 1°C. Stable in water at pH 5	US EPA 2005 EU DAR 2000
Volatilisation	Volatilisation is not expected to be an important environmental fate process, based on a Henry's Law constant of 3.5 x 10^{-8} $Pa.m^3.mole^{-1}$.	US EPA 2005
Distribution in water/sediment systems (active substances)	Carbendazim is unlikely to significantly partition to sediments and suspended matter and/or accumulate in biota, based on a log Kow of 0.40-1.52 and log Koc of 2.30-2.39. However, a water/sediment distribution study in two aquatic test systems has shown that carbendazim will partition to sediment, but not rapidly.	EU DAR 2000, US EPA 2005 EU DAR 2003
Metabolites	The primary metabolite in soil and plants is 2-aminobenzimidazole (2-AB). In animal systems, carbendazim is metabolised to (5-hydroxy-lH-benzimidazol-2-yl)-carbamate (5-HBC) and other	WHO 1993

Property	Value	Reference
	polar metabolites, which are rapidly excreted.	
Degradation in soil	A DT_{50} value of about 100 days was proposed for rate of degradation of carbendazim in soil. In laboratory studies under aerobic conditions DT_{50}s for carbendazim were reported to be 25-502 days and 28-36 days at 20°C and 15°C, respectively. Under anaerobic conditions at 20°C a DT_{50} of 250-302 days was reported. In field studies in Germany a DT_{50} and a DT_{90} of ≈ 18 days and 234 days, respectively.	EU DAR 2000 EC 2007
Biodegradation	The ready biodegradability of carbendazim technical is reported in a closed bottle test according to OECD Guideline 301D. After 28 days at 20 ± 2°C, 6% biodegradation was attained. Therefore carbendazim may not be classified as readily biodegradable. At day 14, only 31% of the total ThOD for the test and reference carbendazim was present, therefore it is not considered to have had an inhibitory effect on sewage bacteria under the conditions of this test.	EU DAR 2000
Octanol–water coefficient (Log Kow)	1.49 0.9 at pH 4 1.5 at pH-range 5 – 9 0.4	WHO 1993 EU DAR 2000 EU DAR 2000 US EPA 2005
Log Koc	A Koc of 400 has been reported in soil. Koc 200-246 (log Koc = 2.30-2.39).	NPIC 2008 EU DAR 2000
Bioaccumulation BCF	Peak whole body BCFs in *Lepomis macrochirus* (bluegill sunfish) at 0.018 and 0.17 mg l^{-1} after 4 weeks exposure were 27 and 23, respectively. In the same study the viscera tissue bioaccumulated more carbendazim than the edible tissue, remaining carcass and whole fish, the peak BCFs in the viscera were 460 and 380 for the low and high concentrations, respectively. However, during the 14 day depuration phase, >94% of the peak radioactivity (radiolabelled carbendazim concentrations) was lost from the whole fish, viscera and muscle indicating that the substance is not retained in the organism. This is consistent with the low Kow for carbendazim. In another test when *Oncorhynchus mykiss* (rainbow trout) were exposed to 45 µg carbendazim l^{-1} for 96 hours, followed by a 96 hour depuration phase, a bioconcentration factor of 159 was reported However, this value is higher than the values generated in the same study for other fish species, namely channel catfish (*Ictalurus punctatus*) and bluegill sunfish	WHO 1993 (Palawski and Knowles 1986).

Property	Value	Reference
	(*Lepomis macrochirus*) which showed lower levels of accumulation. The elimination rate constant was greater and the biological half-life shorter in channel catfish (13 hours) than in the other two species.	

The water solubility of carbendazim (technical grade) has been determined to be 12.9 mg l^{-1} at a temperature of 20.0 ± 0.5°C using a GLP and EPA Guideline 63-8 (based on OECD Method) (US EPA, 2005). Solubility is found to be pH dependant, being 28-36 mg l^{-1} at pH 4, but 5-7 mg l^{-1} at pH 7-8, all at ambient temperature (EU DAR, 2000). NPIC (2008) report a water solubility of 8 mg l^{-1}. These values indicate that carbendazim has low to moderate solubility.

A log Kow of 0.40-1.5 and log Koc of 2.30-2.39, suggests that carbendazim is unlikely to significantly partition to sediments and suspended matter and/or accumulate in biota (EU DAR 2000, US EPA, 2005). However, data reported from a water/sediment distribution study in two aquatic test systems (Bickenbach sand and Widdersheim sandy silty-loam) have shown that carbendazim will partition to sediment, but not rapidly (EU DAR, 2003). This assumption is based on the results from the study where following 3 days incubation, 13.0 and 36.7% of the total applied radioactivity was found in the sediment extractable radioactivity, in the two systems tested. A maximum peak value of 68% of the total applied radioactivity in the sediment extractable radioactivity was reported after 28 days incubation. At the end of the study (149 days) the total applied radioactivity in the extractable sediment decreased to <5% and 33.4% in the two systems (EU, DAR, 2003). Unknown metabolites only reached radioactivity levels of <5% of the applied radioactivity throughout the study. The metabolite 2-aminobenzimidazole reached a maximum peak of 6.3% of the applied radioactivity in the extractable sediment on day 76 (EU DAR, 2003). In the water/sediment distribution study reported in EU DAR (2003), carbendazim was found to be degraded in both aquatic test systems studied. The half-lives of the parent compound with their disappearance times (DT50 and DT-90) and their regression coefficients (r^2) in the water phases and the total systems are summarised in the Table 2.6 below.

Table 2.6 Disappearance times of carbendazim in two aquatic test systems (EU DAR, 2003)

System	Disappearance time					
	From total systems			From water		
	DT-50 (days)	DT-90 (days)	r^2	DT-50 (days)	DT-90 (days)	r^2
Bickenbach sand	16.1	53.5	0.99	10.8	36.0	0.99
Widdersheim sandy silty-loam	73.6	244.4	0.99	5.8	19.2	0.97

Volatilisation is not expected to be an important environmental fate process for carbendazim, based on a Henry's Law constant of 3.5 x10^{-8} Pa.m³.$mole^{-1}$ (US EPA (2005).

The hydrolysis half-life $t_{1/2}$ of carbendazim is calculated at 1.56 x 10^3 hours at pH 9 (25 ± 1°C) with an estimated rate constant ($hour^{-1}$) of 4.44 x 10^{-4} with no breakdown products. This rate constant and half-life at 25°C for pH 9 has been calculated from the graph of log10 concentration (g l^{-1}) against time (hours). The test was carried out under GLP and using EPA Guideline 161-1. No hydrolysis was observed in pH 5 and 7 buffer solutions after 24 days at 25 ± 1°C (US EPA 2005).

Carbendazim is photostable since there was 0% photochemical degradation of a 5 mg l^{-1} solution of carbendazim (technical grade) after exposure to simulated daylight for 17 days at 25 ± 1°C. The experiment was carried out using GLP and EPA Guideline 161-2 (US EPA 2005).

A DT_{50} value of about 100 days was proposed in the EU DAR (2000) for rate of degradation of carbendazim in soil. NPIC (2008) also report a similar value with a soil half life of 120 days. Carbendazim is decomposed in the environment with half-lives of 6 to 12 months on bare soil, 3 to 6 months on turf, and half lives in water of 2 and 25 months under aerobic and anaerobic conditions, respectively (WHO 1993). In laboratory studies under aerobic conditions DT_{50}s for carbendazim were reported to be 25-502 days and 28-36 days at 20°C and 15°C, respectively. Under anaerobic conditions at 20°C a DT_{50} of 250-302 days was reported. In field studies in Germany a DT_{50} and a DT90 of ≈ 18 days and 234 days, respectively were reported (EC, 2007).

The ready biodegradability of technical grade carbendazim in a closed bottle test according to OECD Guideline 301D was reported by EU DAR (2000). Sealed bottles containing the test substance and inorganic nutrient medium were inoculated with activated sewage sludge bacteria and incubated for up to 28 days at 20 ± 2°C. The percentage biodegradation values were calculated by comparing the extent of oxygen depletion (electrochemical measurement of dissolved oxygen) with the Theoretical Oxygen Demand (ThOD: 2.34 mg O_2 mg^{-1}). Within 28 days 6% biodegradation was attained, and, therefore, technical grade carbendazim may not be classified as readily biodegradable. At day 14 the inhibition check cultures containing carbendazim and a readily biodegradable standard substance (sodium benzoate) showed an oxygen depletion value which was 31% of the total ThOD values for the test and reference compounds. Consequently, technical grade carbendazim is not considered to have had an inhibitory effect on sewage bacteria under the conditions of this test.

Bioaccumulation data indicate that carbendazim is unlikely to bioaccumulate significantly (WHO, 1993). *Lepomis macrochirus* (bluegill sunfish) were exposed to radiolabelled carbendazim concentrations of 0.018 or 0.17 mg l^{-1} for 4 weeks in a dynamic study designed to measure the bioaccumulation of ^{14}C-labelled residues in edible tissue, viscera, remaining carcass and whole fish. A two-week depuration phase followed the exposure phase. Results were similar at the two exposure concentrations. The peak whole fish bioconcentration factors (BCFs) were reported as 27 and 23 at the low and high exposure levels, respectively. The viscera bioaccumulated most carbendazim compared to the other tissues tested with the peak viscera BCFs being 460 and 380 for the low and high exposure levels, respectively. Little bioconcentration occurred in the muscle tissue (BCF = <4) or the remaining carcass (BCF = <7). During the 14 day depuration phase, >94% of the peak level of radioactivity was lost from the whole fish, viscera and muscle indicating that the substance is not retained in the organism. This is consistent with the low Kow for carbendazim.

In another test when *Oncorhynchus mykiss* (rainbow trout) were exposed to 45 µg carbendazim l^{-1} for 96 hours, followed by a 96 hour depuration phase, a bioconcentration factor of 159 was reported (Palawski and Knowles 1986). However, this value is higher than the values generated in the same study for other fish species, namely channel catfish (*Ictalurus punctatus*) and bluegill sunfish (*Lepomis macrochirus*) which showed lower levels of accumulation. The elimination rate constant was greater and the biological half-life shorter in channel catfish (13 hours) than in the other two species.

Overall, on a weight of evidence basis it is considered that the TGD BCF trigger of 100 has not been exceeded and the derivation of a PNEC in whole fish for secondary poisoning of predators is not required.

2.6 Effects data

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

Data collation followed a tiered approach. First, critical freshwater and marine data were compiled from the existing EQS document. Further data published after derivation of the current UK EQS were then retrieved from:

- the US Environmental Protection Agency (US EPA) ECOTOX database[3];
- the Draft Assessment Report prepared under Council Directive 91/414/EEC and Addenda (EU DAR 1997, 2000 and 2003);
- sources such as ScienceDirect®[4] and the World Health Organization (WHO), i.e. Environmental Health Criteria (EHC) 90 (WHO 1993).

All relevant data has been quality assessed as part of the preparation of the report (see relevant Klimisch codes in data tables), even if it has already undergone prior quality assessment by other organisations.

2.6.1 Toxicity to freshwater organisms

Freshwater toxicity data on carbendazim are available for various taxonomic groups. Long-term toxicity data are available for five taxonomic groups (algae, crustaceans, fish, molluscs and platyhelminths), with crustaceans being more sensitive than other taxa. Short-term toxicity tests are available for eight taxonomic groups (algae, amphibians, crustaceans, fish, insects, molluscs platyhelminths and protozoa), with crustaceans and fish being more sensitive than other taxa. Overall, the available short-term and long-term toxicity test data indicate that crustaceans and fish are more sensitive than the other taxa to technical grade carbendazim.

Diagrammatic representations of the available freshwater data (cumulative distribution functions) for carbendazim are presented in Figures 2.1 and 2.2. These diagrams include all data regardless of quality and provide an overview of the spread of the available data. These diagrams are not species sensitivity distributions and have not been used to set the carbendazim PNECs. The lowest critical freshwater data for carbendazim are presented in Tables 2.7 (for long-term data) and 2.8 (for short-term data).

[3] http://www.epa.gov/ecotox/
[4] http://www.sciencedirect.com/

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

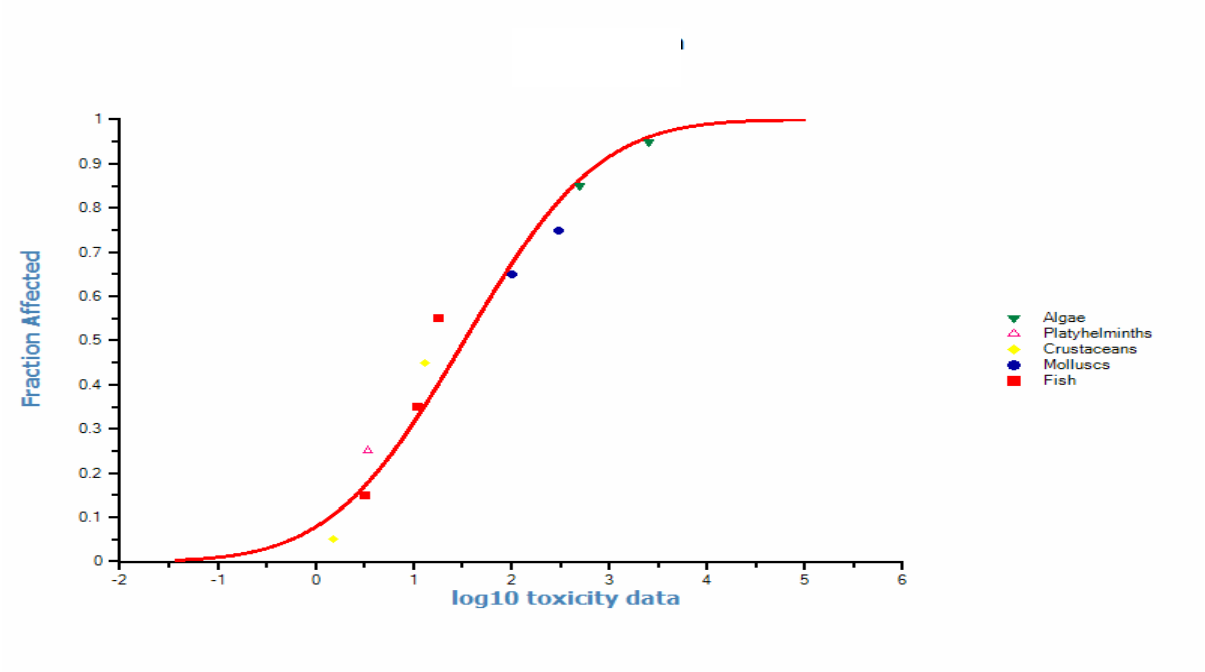


Figure 2.2 Cumulative distribution function of freshwater short-term data (µg a.i. l^{-1}) for carbendazim

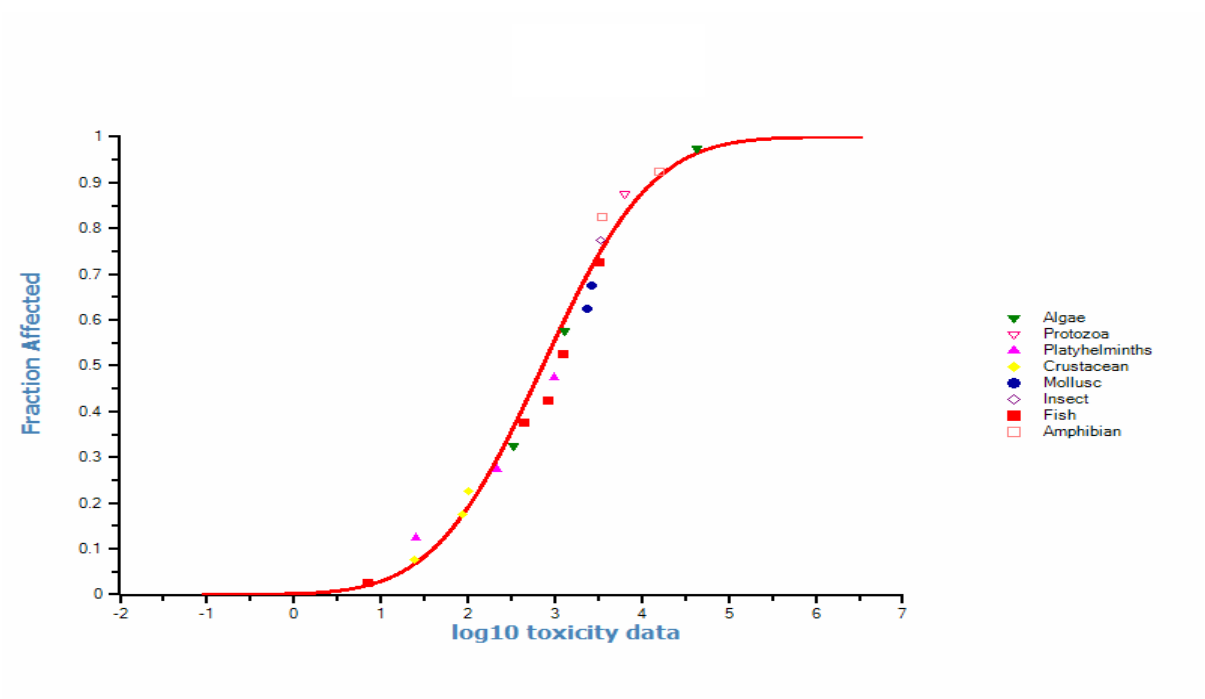


Table 2.7 Most sensitive long-term aquatic toxicity data for freshwater organisms exposed to carbendazim

Form of substance	Scientific name	Common name	Taxonomic group	End-point	Effect	Test duration	Conc. (µg a.i. l^{-1})	Exposure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
Algae												
Technical grade (99-100% purity)	*Pseudokirchnereilla subcapitata* (*Selenastrum capricornutum)*	Unicelluar green algae (log phase culture)	ALG	NOEC	Growth	3 days	500	s	n	Temperature = 24 ± 1°C, pH = 7.6-8.9	3	Douglas and Handley (1987) cited in EU DAR (1997)
Technical grade (99-100% purity)	*Pseudokirchnereilla subcapitata* (*Selenastrum capricornutum)*	Unicelluar green algae (log phase culture)	ALG	NOEC	Growth	3 days	2500	s	y	Temperature = 24 ± 1°C, pH = 7.6-8.9	1	Undisclosed (Ref SNG 44(a) 960463) cited in EU DAR (2000)
Invertebrates												
Technical grade (99.5%)	*Daphnia magna*	Water flea	CRU	NOEC	Reproduction	21 days	1.5	ss	y	Conducted in accordance with OECD Guideline No. 202	1	Kelly *et al*. (1997) cited in EU DAR (2000)
Derosal (a.i. carbendazim, 511g l^{-1})	*Dugesia lugubris*	Planarian, vortex worm	PLA	NOEC	Reproduction	21 days	3.4	ss	y	Temperature = 18 ± 1°C.Total hardness:= 71.2-89.2 mg.l^{-1} as $CaCO_3$ at pH 8	2	van Wijngaarden *et al*., (1998)
Derosal (a.i. carbendazim, 511g l^{-1})	*Bithynia tentaculata*	Snail	MOL	NOEC	Reproduction	28 days	103	ss	y	Temperature = 18 ± 1°C.Total hardness:= 71.2-89.2 mg.l^{-1} as $CaCO_3$ at pH 8	2	van Wijngaarden *et al*., (1998)
Fish												
Technical grade	*Oncorhynchus mykiss*	Rainbow trout (10 month old)	FIS	NOEC	Behaviour	21 days	3.2	f	n	ND	1	Fischer (1988[b]) cited in EU DAR (1997)
Technical grade	*Oncorhynchus mykiss*	Rainbow trout (10 month old)	FIS	NOEC	Mortality	21 days	18	f	n	ND	1	Fischer (1988[b]) cited in EU DAR (1997)

Form of substance	Scientific name	Common name	Taxonomic group	End-point	Effect	Test duration	Conc. (µg a.i. l^{-1})	Exposure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
Technical grade	*Oncorhynchus mykiss*	Rainbow trout (early life stage)	FIS	NOEC	Embryo survival	79 days	11	f	y	ND	1	Baer (1993) cited in EU DAR (1997)

* See Annex 1.
[1] Exposure: s = static; ss = semi-static; f = flow-through.
[2] Toxicant analysis: y = measured; n = nominal.
ALG = algae; CRU = crustaceans; FIS = fish; MOL = molluscs, PLA = platyhelminth
ND = no data, NOEC = No Observed Effect Concentration

Table 2.8 Most sensitive short-term aquatic toxicity data for freshwater organisms exposed to carbendazim

Form of substance	Scientific name	Common name	Taxonomic group	End-point	Effect	Test duration (hours)	Conc. (µg a.i. l^{-1})	Exposure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
Algae												
Formulation (97.4%purity)	*Chlorella pyrenoidosa*	Unicellular green algae (log phase)	ALG	EC50	Growth	48	340	s	n	Temperature = 24ºC	2	Canton (1976) cited in EU DAR (1997)
Formulation Technical grade (99-100%purity)	*Pseudokirchnereilla subcapitata* (*Selenastrum capricornutum)*	Unicellular green algae (log phase)	ALG	EC50	Growth	72	1300	s	n	Temperature = 24 ± 1ºC, pH = 7.6-8.9	3	Douglas and Hanley (1987) cited in EU DAR (1997)
Protozoa												
Analytical grade MBC	*Tetrahymena pyriformis*	Ciliate	PRO	EC50	Growth	36	6380	s	n	Temperature = 23 ± 1ºC	2	Rankin *et al*. (1977)
Invertebrates												
Technical grade	*Daphnia magna*	Water flea	CRU	EC50	Immobility	48	150	s	n	-	1	Fischer (1988[a]) cited in EU DAR (1997)
Technical grade	*Daphnia magna*	Water flea	CRU	EC50	Immobility	48	87	s	n	-	1	Hutton (1988) cited in EU DAR (1997)
Derosal (a.i. carbendazim, 511 g l^{-1})	*Dugesia lugubris*	Planarian, vortex worm	PLA	EC50	Response to stimuli	96	25	ss	y	Temperature = 18 ± 1°C.Total hardness:= 71.2-89.2 mg.l^{-1} as $CaCO_3$ at pH 8	2	van Wijngaarden *et al*. 1998
Derosal (a.i. carbendazim, 511 g l^{-1}	*Chaoborus obscuripes*	Midge	INS	EC10	Behaviour	96	>3435	s	y	Temperature = 18 ± 1°C.Total hardness:= 71.2-89.2 mg.l^{-1} as $CaCO_3$ at pH 8	2	van Wijngaarden *et al*. 1998

Form of substance	Scientific name	Common name	Taxonomic group	End-point	Effect	Test duration (hours)	Conc. (µg a.i. l^{-1})	Exposure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
Derosal (a.i. carbendazim, 511 g l^{-1}	*Bithynia tentaculata*	Snail	MOL	LC50	Mortality	168	>2391	ss	y	Temperature = 18 ± 1°C.Total hardness:= 71.2-89.2 mg.l^{-1} as $CaCO_3$ at pH 8	2	van Wijngaarden *et al*., (1998)
Fish												
Technical grade	*Cyprinus carpio*	Common carp	FIS	LC50	Lethality	96	440	s	n	-	1	Fischer (1988[d]) cited in EU DAR (1997)
Technical grade (99-100% purity)	*Ictalurus punctatus*	Channel catfish (yolk-sac fry)	FIS	LC50	Lethality	96	7 (6-9)[3]	s	n	Hardness = 40-48 mg.l^{-1} as $CaCO_3$; Temperature = 10ºC; pH = 7.4	2	Palawski and Knowles (1986)
Technical grade (99-100% purity)	*Ictalurus punctatus*	Channel catfish (fry-0.2 g)	FIS	LC50	Lethality	96	10 (8-13)[3]	s	n	Hardness = 40-48 mg.l^{-1} as $CaCO_3$; Temperature = 10ºC; pH = 7.4	2	Palawski and Knowles (1986)
Technical grade (99-100% purity)	*Ictalurus punctatus*	Channel catfish (swim-up fry)	FIS	LC50	Lethality	96	12 (9-15)[3]	s	n	Hardness = 40-48 mg.l^{-1} as $CaCO_3$; Temperature = 10ºC; pH = 7.4	2	Palawski and Knowles (1986)
Technical grade (99-100% purity)	*Ictalurus punctatus*	Channel catfish (fingerlings)	FIS	LC50	Lethality	96	14 (11-18)[3]	s	n	Hardness = 40-48 mg.l^{-1} as $CaCO_3$; Temperature = 10ºC; pH = 7.4	2	Palawski and Knowles (1986)
Technical grade (99-100% purity)	*Ictalurus punctatus*	Channel catfish (fingerlings)	FIS	LC50	Lethality	96	18 (11-28)[3]	s	n	Hardness = 40-48 mg.l^{-1} as $CaCO_3$; Temperature = 10ºC; pH = 7.4	2	Palawski and Knowles (1986)

Form of substance	Scientific name	Common name	Taxonomic group	End-point	Effect	Test duration (hours)	Conc. (µg a.i. l^{-1})	Exposure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
Technical grade	*Oncorhynchus mykiss*	Rainbow trout	FIS	LC50	Lethality	96	830	s	n	-	1	Fischer (1988[c]) cited in EU DAR (1997)
Amphibians												
Formulation 50% active ingredient (wettable powder)	*Rana hexadactyla*	Frog (tadpole)	AMP	LC50	Lethality	96	16020 (12620-19900)[3]	s	n	Hardness = 15.0-35.0 mg.l^{-1} as $CaCO_3$; Temperature = 12.0-17.0ºC; pH = 6.0-6.4	2	Khangarot *et al*. (1985)
Technical grade	*Bufo bufo japonicus*	Toad (tadpole)	AMP	LC50	Lethality	72	3500	s	n	Temperature = 25.0ºC	4	Nishiuchi and Yoshida (1974)

* See Annex 1;
[1] Exposure: s = static; ss = semi-static.
[2] Toxicant analysis: y = measured; n = nominal.
[3] 95% confidence intervals
ALG = algae; AMP = amphibians; CRU = crustaceans; FIS = fish; INS = insects, MOL = mollusc, PLA = platyhelminths, PRO = protozoa
MBC = 1H-Benzimidazol-2-yl carbamic acid, Methyl ester
EC50 = concentration effective against 50% of the organisms tested
LC50 = concentration lethal to 50% of the organisms tested
ND = no data

2.6.2 Toxicity to saltwater organisms

Single species short-term toxicity data referring to marine organisms are available for three different taxonomic groups (crustaceans, molluscs and fish), with crustaceans apparently being the most sensitive group. No long-term toxicity data were located for saltwater taxa.

The lowest critical short-term toxicity data for marine species are summarised in Table 2.9. A diagrammatic representation of the available short-term saltwater data (cumulative distribution function) for carbendazim is presented in Figure 2.3. This diagram includes all data regardless of quality and provides an overview of the spread of the available data. The diagram is not a species sensitivity distribution and has not been used to set carbendazim PNECs.

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

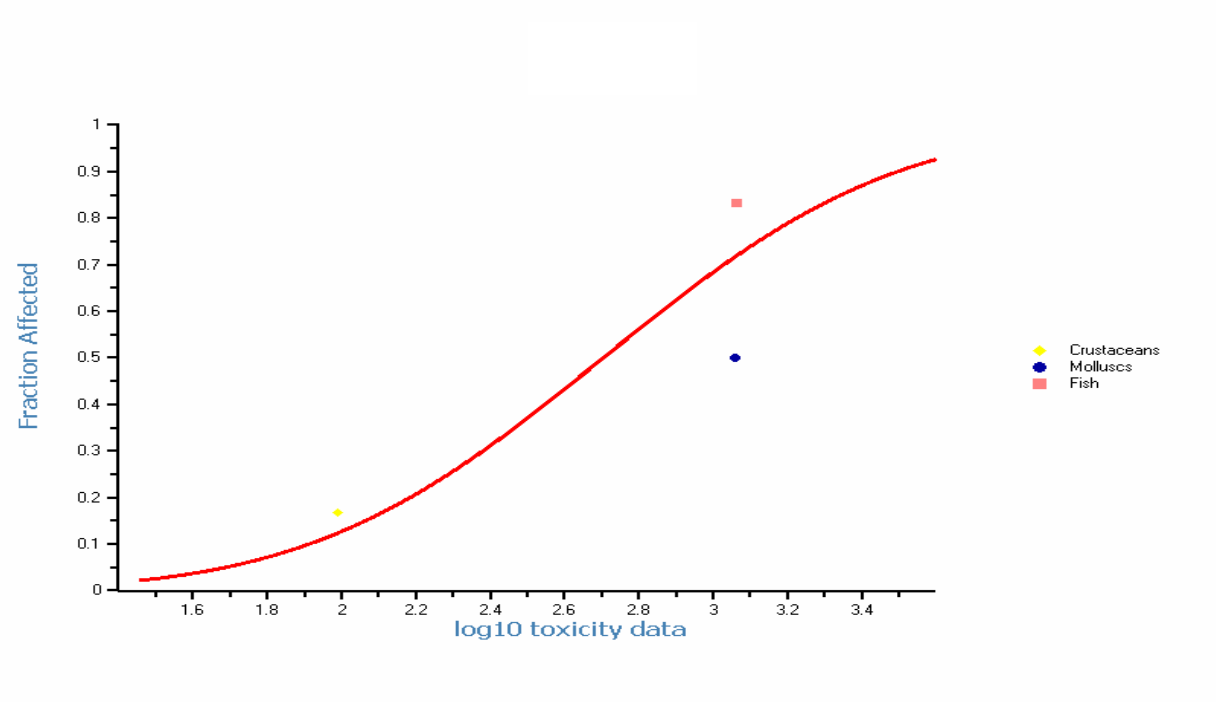


Table 2.9 Most sensitive short-term aquatic toxicity data for saltwater organisms exposed to carbendazim

Form of substance	Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration (hours)	Conc. (µg a.i. l^{-1})	Exposure[1]	Toxicant analysis[2]	Comment	Reliability (Klimisch Code*)	Reference
Invertebrates												
Technical grade (purity unspecified)	*Americamysis bahia (Mysidopsis bahia)*	Mysid shrimp	CRU	LC50	Lethality	96	98	s	y	-	2	Proprietary data (1995) cited in Bates *et al*. (1998)
Technical grade (purity unspecified)	*Crassostrea virginica*	Eastern Oyster	MOL	EC50	Shell deposition	ND	>1145 (highest concentration tested)	f	y	-	2	Proprietary data (1995) cited in Bates *et al*. (1998)
Technical grade (purity unspecified)	*Crassostrea virginica*	Eastern Oyster	MOL	EC50	Survival	ND	>1145 (highest concentration tested)	f	y	-	2	Proprietary data (1995) cited in Bates *et al*. (1998)
Fish												
Technical grade (purity unspecified)	*Cyprinodon variegatus*	Sheepshead minnow	FIS	LC50	Lethality	96	> 1158 (highest concentration tested)	ND	y	-	1	Boeri (1988) cited in EU DAR (1997)

* See Annex 1.
[1] Exposure: s = static; f = flow-through.
[2] Toxicant analysis: y = measured; n = nominal.
CRU = crustaceans; MOL = molluscs, FIS = fish
EC50 = concentration effective against 50% of the organisms tested
LC50 = concentration lethal to 50% of the organisms tested

2.6.3 Toxicity to sediment-dwelling organisms

The log Kow of carbendazim is reported as between 0.4 and 1.5 (see Section 2.5), therefore it is unlikely to undergo adsorption to sediment and suspended solids and the TGD log Kow trigger value of 3 is not exceeded. An investigation of the distribution of carbendazim in water and sediment systems has shown that it will partition into sediment, but this did not occur rapidly (EU DAR, 2003). There are limited toxicity data available for carbendazim to sediment dwelling organisms but these have been included for information.

Chronic toxicity of carbendazim in a water miscible suspension to the sediment dwelling chironomid larvae *Chironomus riparius* was investigated by Sowig and Gosch (2002), cited in EU DAR (2003). A formulation of carbendazim (41.6%) was used and the test was GLP compliant and followed a valid OECD Draft Guideline No 219 (Draft February 2001) method. The exposure method used spiked water at nominal concentration levels (as a.i.) of 0, 0.001, 0.0018, 0.0032, 0.0056, 0.010, 0.018 and 0.032 mg l^{-1} with measured concentration levels of 69.4 - 74.8%. The study reported a 28 day NOEC (emergence) of 0.032 mg l^{-1} and a 28-day EC50 (emergence) of >0.032 mg l^{-1} based on nominal concentrations. No corresponding sediment carbendazim concentrations were reported.

2.6.4 Endocrine-disrupting effects

No evidence has been found for carbendazim-induced endocrine disrupting effects in aquatic organisms. However, carbendazim has been shown to disrupt the production of sperm and damage testicular development in adult rats (Lu et al, 2004, Yu *et al* 2009a,b).

Lu *et al* (2004) investigated the endocrine-disrupting activity of carbendazim-induced reproductive and developmental toxicity in Sprague-Dawley rats treated orally with the fungicide. Co-treatment of male rats with 675mg/kg carbendazim and 50 or 100mg/kg flutamide, an androgen receptor antagonist, once daily for 28d blocked decrease of testis weight induced by treatment with carbendazim alone. The co-treatment prevented losses of spermatozoa and cell morphology and decreases of sperm concentration induced by carbendazim alone. Premating treatment of male and female rats with 200mg/kg carbendazim for 28days produced androgenic effects including incomplete development of the uterine horn, enlargement of the uretha, absence of the vagina, and induction of seminal vesicles in female offspring, without marked effects in male offspring. Premating treatment with 100mg/kg benomyl, the parent compound of carbendazim, resulted in incomplete development of the uterine horn and absence of vagina's in female offspring and produced testis and epidydimis atrophy in male offspring. Treatment of male rats with 25, 50, 100, 200, 400, and 800mg/kg carbendazim for 56days produced dose-dependent increases of androgen receptor concentrations in testis and epididymis. Additions of 5, 50, and 500 µM (956, 9560 and 95600 µg l^{-1}) carbendazim to testis extract from untreated rats replaced binding of [3 H]-5α-dihydrotestosterone to androgen receptor in a concentration-dependent manner. The study demonstrated that reproductive toxicity induced by carbendazim is blocked by an androgen receptor antagonist in male rats and developmental toxicity of the fungicide shows androgenic properties in female offspring. These results suggest that androgen- and androgen receptor-dependent mechanisms are possibly involved in carbendazim-induced toxicity in mammals.

Yu *et al* (2009a) investigated the effects of subchronic exposure to carbendazim on spermatogenesis and fertility in male rats. Ninety-eight healthy male rats were divided into four groups: three exposure groups and a control group. Carbendazim was administered orally to male rats at 0, 20, 100 and 200 mg/kg for 80 days prior to mating. Each male was cohabited with an unexposed female for a maximum of 5 days. In 100 and 200 mg/kg groups, the mating index was relatively increased, the fertility index was decreased, and the testis weight, the sperm counts and motility were also decreased. The levels of luteinizing hormone (LH) showed a decreasing tendency and there was a statistical difference between the 200 mg/kg group and the control group. There were no obvious effects on the levels of follicle stimulating hormone (FSH) and testosterone (T). Histopathological evaluation showed atrophic seminiferous tubules, decreased germ cells, and increased sloughing of germ cells. Flow cytometric analysis of the testicular tissue revealed that carbendazim inhibited meiotic transformation and interfered with the spermatogenic process. These results suggest that carbendazim has adverse effects on spermatogenesis, resulting in reduced fertility in male rats.

Yu *et al* (2009b) also investigated whether Sertoli cells are involved in spermatogenic failure induced by carbendazim. A total of 40 rats were treated with carbendazim by oral gavage at dosages of 0, 20, 100 and 200 mg/kg for 60 days. The testis weight, sperm counts, sperm motility and Sertoli cell morphology and secretions including vimentin, amyloid beta protein (ABP), inhibin B, stem cell factors (SCF)s and SCFm in testis were examined. In the 100 and 200 mg/kg groups, the testis weight, the sperm counts and motility and SCFm levels were significantly decreased, the sloughing of germ cells and ABP levels were increased, and the vimentin filaments and Sertoli cell morphology were altered. Inhibin B and SCFs levels were unchanged. The results suggested that alterations of Sertoli cell morphology and function were involved in spermatogenic failure induced by carbendazim.

The results of a study by Mantovani *et al* 1998 in rats indicate that carbendazim is a developmental toxicant in laboratory animals with an increased resorption rate and foetal oedema and this could be an endocrine effect. The substance is also teratogenic and exposure to carbendazim in the uterus has been shown to lead to deformities such as hydrocephalus (increased fluid in the brain), neural tube defects and anophthalmia/micophthalmia (lack of one or both eyes or small eyes). This teratogenic effect is probably not endocrine induced but is probably due to its effects on mitotic spindle formation (see Section 2.6.5).

2.6.5 Mode of action of Carbendazim

Carbendazim is a systematic benzimidazole fungicide and these compounds have been found to be positive in assays for numerical chromosomal aberrations due to the inhibition of tubulin preventing microtubule formation. As a consequence cytokinesis (the process in which the cytoplasm of a single eukaryotic cell is divided to form two daughter cells) is inhibited and aneuploidy (i.e. an error in cell division that results in the "daughter" cells having the wrong number of chromosomes) is produced above a threshold concentration required for tubulin binding. Unlike primary mutagens, carbendazim does not interfere with DNA and does not cause gene mutations or structural chromosome aberrations (EU DAR 2000).

Carbendazim inhibits the development of fungi probably by interfering with spindle formation at mitosis (cell division) during reproduction (WHO 1993).

2.7 Mesocosm and field studies

2.7.1 Freshwater mesocosm and field studies

Information on the effects of carbendazim on freshwater organisms from mesocosm and field studies was located and is summarised below.

Hunt *et al*. (2001) developed *in situ* feeding bioassay methods for the crustacean, *Gammarus pulex* using carbendazim. Fourteen mesocosms (2.25 m diameter) containing 90 cm depth of water overlying a 10 cm depth of sediment were allocated to one of four treatments (0.28, 2.16, 20.8, 226 µg l^{-1} carbendazim). Groups of *Gammarus pulex*, were deployed for 6 days post treatment to quantify effects on detritivore feeding. There was significant mortality of the crustaceans exposed to the highest test concentration (i.e. 226 µg l^{-1}) and a significant inhibition of feeding at 20.8 µg l^{-1} carbendazim.

Van Wijngaarden *et al*. (2002) undertook outdoor freshwater mesocosms tests in experimental ditches to study the impact of a single treatment with the fungicide Derosal (containing 511 g carbendazim l^{-1}). Carbendazim was applied at nominal concentrations of 330 µg l^{-1}. The ditches were designed to simulate a community of Dutch drainage ditches and a flow-through water regime was used. Residence time of the water was 8 days, except for a static period of 7 days after application. The water supplied contained propagules of several resident populations, permitting the study of recovery. The fate of carbendazim and responses of functional and structural endpoints were investigated. Macroinvertebrate populations were the most sensitive, especially water column dwelling oligochaetes and benthic detritivores. Sticklebacks were not influenced in contrast to pike, which had been introduced as a top-predator to control sticklebacks. Recovery of sensitive zooplankton and macroinvertebrate populations was suppressed, most probably because of predation by sticklebacks. Compared to an indoor microcosm study, treatment-related direct and indirect effects were less explicit. This can be attributed to feed-back mechanisms that may be more prominent in complex outdoor ecosystems.

Cuppen *et al*. (2002) studied the effects of chronic application of the fungicide Derosal (containing 511 g carbendazim l^{-1}) using indoor macrophyte-dominated freshwater microcosms. The concentrations (0, 3.3, 33, 100, 330 and 1000 µg l^{-1}) were kept at a constant level for 4 weeks. The study assessed the fate of carbendazim and its effects on water quality parameters, breakdown of particulate organic matter (POM), and responses of macroinvertebrates. Carbendazim proved very persistent in the water layer. Half-life values varied between 6 and 25 weeks, and decreased with the treatment level. Significant effects on water quality parameters (DO, pH, alkalinity, and conductivity) could not be demonstrated. After 4 weeks of incubation, the breakdown of poplar leaves was significantly slower at the two highest carbendazim concentrations, presumably due to its fungicidal action. The macroinvertebrate community was seriously affected by carbendazim application, with Oligochaeta, Turbellaria, Hirudinea and some Crustacea as the most sensitive groups. The snail *Bithynia* decreased in numbers, but other gastropods increased in numbers. The major effects on the macroinvertebrate community structure were evident at 33 µg l^{-1} and the calculated NOEC for the macroinvertebrate community as a total was 3.3 µg l^{-1}.

Van den Brink *et al.* (2000) also reported on the work described in Cuppen *et al*. (2002) above. In the paper it was reported that the zooplankton community was found to be negatively affected by the three highest treatment levels of carbendazim ($NOEC_{community}$ = 33 µg l^{-1}). At higher treatment levels Cladocera were completely eliminated, while copepod numbers were reduced. Rotatoria taxa decreased (e.g. *Keratella quadrata*

and *Lecane* sp.) or increased (e.g. *Testudinella parva*) in abundance at the highest treatment level only. Due to the reduced grazing pressure, the abundance of some phytoplankton taxa increased at the three highest treatment levels ($NOEC_{community}$ = 33 µg l^{-1}). This effect was not observed for the periphyton, most probably because the reduced grazing pressure was compensated by the increased abundance of some snail species such as *Lymnaea stagnalis* and *Physella acuta*. At the end of the experimental period the biomass of the macrophyte *Elodea nuttallii* was significantly elevated at the two highest treatment levels. It was hypothesised that carbendazim might have caused, directly or indirectly, the removal of pathogenic organisms from the macrophytes.

Slijkerman *et al*. (2003) conducted a mesocosm experiment to investigate the ability of an *in situ Daphnia magna* feeding bioassay to determine impairment of ecosystem function. Animals were deployed in mesocosms dosed with different concentrations of carbendazim (2.1, 21 and 221 µg l^{-1}) and effects on the post-exposure feeding rate of *D. magna* were evaluated along with effects on zooplankton species richness (ecosystem structure) and development of phytoplankton biomass (ecosystem function). In the medium-dosed systems (21 µg l^{-1}) a structural change was observed within the zooplankton community but no indirect effects on phytoplankton development were detected. It appears that at this treatment level functional redundancy was sufficient to prevent functional impairment despite species loss. The feeding assay did not show any response at this concentration. In the high-dosed systems (221 µg l^{-1}), structural changes in the zooplankton community resulted in an increase in the development of phytoplankton biomass. The feeding bioassay also showed a significant response at this concentration. At the high treatment level species loss resulted in functional impairment, indicating that at this level, functional redundancy could not compensate for loss of individuals. The *D. magna* feeding bioassay matched well with the functional response, i.e. the indirect effects on phytoplankton, in the dosed systems, but not with more subtle effects on zooplankton community structure.

Overall, the freshwater microcosm and mesocosm studies indicate that no effects on community structure and/or function were evident at concentrations below 21-33 µg l^{-1}.

2.7.2 Saltwater mesocosm and field studies

No information on the effects of carbendazim on saltwater organisms from mesocosm and field studies was located.

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 Technical Guidance Document assessment factor (AF) method can be applied. Long-term toxicity data are available for five taxonomic groups (algae, crustaceans, fish, molluscs and platyhelminths), with crustaceans being more sensitive than the other taxa.

The lowest toxicity value for effects of carbendazim on algae is a study by Douglas and Handley (1987) cited in EU DAR (1995) which reported a 72 NOEC of 500 µg l^{-1} for growth inhibition in green algae *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*). The study was carried out to GLP, adopted a static exposure design and did not involve analytical analysis of exposure concentrations. However, it cannot be considered valid since the test validity criterion of a 16x increase in cell number in the controls was not achieved. Therefore, the lowest valid toxicity value for algae is a 72-hour NOEC of 2500 µg l^{-1} for growth inhibition in the green algae *Pseudokirchneriella subcapitata* cited in the EU DAR (2000).

The lowest reliable toxicity value for crustaceans was in a study by Kelly *et al*. (1997) cited in the EU DAR (2000) that reported a 21 day NOEC of 1.5 µg l^{-1} based on effects on reproduction in the waterflea (*Daphnia magna*). This study was conducted according to OECD Guideline 202 and was carried out to GLP. A semi-static exposure system was used and there was analytical confirmation of the exposure concentrations.

The lowest most reliable toxicity value for platyhelminths was in a study by van Wijingaarden *et al*. (1998) that reported a 21 day NOEC of 3.4 µg l^{-1} based on effects on reproduction (number of neonates) in the vortex worm (*Dugesia lugubris*). This study also reported a 28 day NOEC for reproduction of 103 µg l^{-1} for the snail *Bithynia tentaculata*. The study used carbendazim in a formulation, but adopted a semi-static exposure design, with analytical confirmation of the exposure concentrations.

The lowest reported chronic toxicity data for fish was obtained using the rainbow trout (*Onchorhynchus mykiss*) in a study carried out by Fischer (1998) cited in the EU DAR 1997). The study reported a 21-day NOEC of 3.2 µg l^{-1} for behaviour and a 21-day NOEC of 18 µg l^{-1} for mortality. The study was conducted according to OECD Guidelines) and was carried out to GLP. It used a flow-through exposure system, but did not involve analytical analysis of exposure concentrations.

Reliable long-term NOECs are available for algae, crustaceans and fish, and therefore an assessment factor of 10 could be applied to the lowest valid toxicity value based on

the TGD approach. Using the 21-day NOEC of 1.5 µg l^{-1} for effects of the carbendazim on the reproduction of the waterflea *Daphnia magna* this results in:

$PNEC_{freshwater\_lt}$ = 1.5 µg l^{-1}/AF (10) = 0.15 µg l^{-1}

The resulting PNEC is protective of the effects observed in freshwater mesocosm studies (see Section 2.7.2) in which no-effects concentrations tend to be an order of magnitude higher than the laboratory NOEC for *Daphnia* derived from the reproduction study.

PNEC accounting for transient concentration peaks

Short-term toxicity tests are available for eight taxonomic groups (algae, amphibians, crustaceans, fish, insects, molluscs, platyhelminths and protozoa), with crustaceans and fish being more sensitive than other taxa.

The lowest reliable toxicity value for algae was a 48-hour EC50 (growth inhibition) of 340 µg l^{-1} reported by Canton (1976), cited in EU DAR (2000) for the green algae (*Chlorella pyrenoidosa*). This study was conducted in static conditions and did not involve analytical analysis of exposure concentrations.

A study of the effects of carbendazim on protozoa by Rankin *et al.* (1977) reported a 36-hour EC50 (growth inhibition) of 6380 µg l^{-1} in *Tetrahymena pyriformis*. The study was conducted in static conditions and did not involve analytical analysis of exposure concentrations.

The lowest reliable toxicity value for crustaceans was in a study by Hutton (1988) cited in the EU DAR (1997) that reported a 48-hour EC50 of 87 µg l^{-1} based on mobility in the waterflea (*Daphnia magna*). This study was conducted according to an OECD guideline and was carried out to GLP. A static exposure system was used and did not involve analytical analysis of exposure concentrations. This value is supported by a 48-hour EC50 (immobility) of 150 µg l^{-1} reported by Fischer (1988) (cited in EU DAR 1997)

The lowest most reliable value for platyhelminths was in a study by van Wijingaarden *et al*. (1998) that reported a 96 hour EC50 of 25 µg l^{-1} based on response to stimuli in the vortex worm (*Dugesia lugubris*). This study used a semi-static exposure regime with analytical measurements of the exposure concentrations.

The lowest reliable toxicity value reported in fish is from a study by Palawski and Knowles (1986) that reported a 96-hour LC50 of 7 µg l^{-1} (95% Confidence intervals = 6-9 µg l^{-1}) for yolk-sac fry of the channel catfish (*Ictalurus punctatus*) exposed to carbendazim at 22°C. The study also reported 96-hour LC50 values of 10 µg l^{-1} (95% Confidence intervals = 8-13 µg l^{-1}) and 12 µg l^{-1} (95% Confidence intervals = 9-15 µg l^{-1}) for 0.2g fry and swim-up fry of the same species. In the study early life stages of rainbow trout and bluegill sunfish were reported to be less sensitive to carbendazim. This study was carried out to a standardised methodology and used a static exposure system. The study did not involve analytical confirmation of the exposure concentrations but marked losses were not expected during the study given the physico-chemical properties of carbendazim (see Section 2.5).

The lowest reliable value for amphibians was in a study by Khangarot *et al*. (1985) that reported a 96-hour LC50 of 16020 µg l^{-1} (12620-19900 µg l^{-1}) in frog tadpoles (*Rana hexadactyla*). This study used static exposure system and did not involve analytical exposure concentrations.

Overall the lowest reliable toxicity value is a 96-hour LC50 of 7 µg l^{-1} for channel catfish, *Ictalurus punctatus* measured in a static exposure regime (Palawski and

Knowles 1986). Although this species appears markedly more sensitive to short-term carbendazim exposure than other fish species the data is consistent with the longer-term mortality data for rainbow trout in Table 2.7. Based on the guidance given in the TGD on effects assessment for intermittent releases, and the large body of acute toxicity data for carbendazim it is proposed to apply an assessment factor of 10 resulting in:

$PNEC_{freshwater\_st}$ = 7 µg l^{-1}/AF (10) = 0.7 µg l^{-1}

3.1.2 PNECs for saltwaters

The effects database for marine species is considerably smaller than that for freshwater organisms. Short-term toxicity data are available for three different taxonomic groups (crustaceans, molluscs and fish). However, no long-term data are available.

The short-term toxicity data of the marine taxa do not show markedly lower values from the range of values obtained for freshwater species (see Tables 2.7 and 2.8). However, the marine database is too small to draw firm conclusions on possible differences, particularly due to the absence of long-term effects data.

Based on the available data, it is proposed that:

- the TGD approach of using freshwater data within the marine effect assessment is used;
- suggested freshwater PNECs for setting of quality standards should be considered in deriving corresponding values for marine water bodies.

PNEC accounting for the annual average concentration

No long-term single species toxicity data for marine organisms are available. The absence of long-term data means that it is not possible to generate a $PNEC_{saltwater\_lt}$ based on the saltwater data alone and it is proposed that the combined freshwater and saltwater dataset is used for the PNEC generation (see Section 3.1.1). This approach is consistent with that described in the TGD (ECB 2003).

Long-term NOECs are available for freshwater algae, invertebrates and fish, but no toxicity data are available for marine taxa such as echinoderms. On the basis of the available data an additional assessment factor of 10 is proposed to account for the uncertainty due to the absence of data for additional marine taxa eg. echinoderms, resulting in a total assessment factor of 100. This results in the following value using the 21-day NOEC of 1.5 µg a.i. l^{-1} for effects of the carbendazim on the reproduction of the waterflea (*Daphnia magna*):

$PNEC_{saltwater\_lt}$ = 1.5 µg l^{-1}/AF (100) = 0.015 µg l^{-1}

PNEC accounting for transient concentration peaks

Single species short-term toxicity data for marine organisms are available for three different taxonomic groups (crustaceans, fish and molluscs), with crustaceans apparently being the most sensitive group.

Proprietary data (1995) cited in Bates *et al*., (1998) reported the lowest acute toxicity value for a saltwater species to be a 96-hour LC50 of 98 µg l^{-1} in the mysid shrimp

Americamysis bahia (formerly *Mysidopsis bahia*) under static conditions and involving analytical measurement of the exposure concentrations.

As there are limited saltwater data and no toxicity values available for marine taxa such as echinoderms, it is proposed that the $PNEC_{saltwater\_st}$ is based on the combined freshwater and saltwater dataset. In the combined dataset the lowest reliable value is a 96-hour LC50 of 7 µg l^{-1} for channel catfish, *Ictalurus punctatus* measured in a static exposure regime (Palawski and Knowles 1986).

The calculation of the PNEC following short-term exposure to carbendazim has been based of the general guidance given in the TGD on the effects assessment for intermittent releases [Section 3.3.2 of Part II of the TGD (ECB 2003)]. This would normally result in application of an assessment factor of 10 being applied.

Since no data are available for marine taxa such as echinoderms, an additional assessment factor of 10 would also normally be applied, resulting in a total assessment factor of 100. However, there is a large body of short-term data in the combined freshwater and saltwater dataset and there are toxicity values for saltwater molluscs. Therefore, it is proposed that a reduced assessment factor of 50 is applied to the lowest valid toxicity value from the combined dataset. This results in:

$PNEC_{saltwater\_st}$ = 7 µg l^{-1}/AF (50) = 0.14 µg l^{-1}

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

There are insufficient data to construct a species sensitivity distribution (SSD) based upon long-term exposure data.

3.3 Derivation of existing EQSs

The derivation of the proposed EQSs for carbendazim was described in a 1998 report for the Department of the Environment (Bates *et al.* 1998). An EQS of 0.1 µg l^{-1}, expressed as an annual average concentration for the protection of freshwater (and saltwater) life against long-term exposure to carbendazim was derived by applying a safety factor of approximately 100 to the lowest reliable 96-hour LC50 of 0.007 mg l^{-1} for channel catfish yolk sac fry (*Ictalurus punctatus*).

Comparison of the acute and chronic data available data for *Daphnia magna* and for *Oncorhynchus mykiss* suggested an approximate acute:chronic ratio of 10. Therefore, a maximum allowable concentration (MAC) of 1.0 µg l^{-1} was proposed by applying a safety factor of approximately 10 to the LC50 for channel catfish yolk sac fry.

3.4 Derivation of PNECs for sediment

Since the log Kow of carbendazim is 0.4-1.52 and log Koc is 2.30-2.39, the derivation of PNECs for the protection of benthic organisms is not required according to the TGD, since the log Kow/Koc trigger value of 3 is not exceeded.

3.5 Derivation of PNECs for secondary poisoning of predators

3.5.1 Mammalian and avian toxicity data

Several reviews have been published regarding carbendazim (JMPR, 1973; JMPR; 1976; JMPR, 1977; JMPR, 1978; JMPR, 1983; JMPR, 1985; ACP, 1992; WHO, 1993; HSG, 1993; JMPR, 1995a; JMPR, 1995b; PDS, 1996; IUCLID, 2000; EC PPP, 2006). As the most recent, the EC PPP, IUCLID, PDS and JMPR (1995a) have been assumed to contain the most sound and scientifically accurate mammalian data. For this reason, these were the primary sources used (see Table 3.1). However, the other reviews were also consulted. Additional literature searches were performed from 2006 to the present day to locate any lower effect data since 2006, however, none were located.

For avian data, due to the lack of relevant data in the EC PPP review, the other reviews were consulted. However, similarly to the mammalian data, a comprehensive literature search was also performed from 1995 to the present day to locate any lower effect data since 1995.

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	
Til *et al.* (1972) Cited in JMPR (1995a) & presumed to be cited in EC PPP (2006) **Sub-chronic NOAEL = 7.5 mg.kg bw.d^{-1} (300 mg.kg diet^{-1})**	Male and female Beagle dogs (4/sex/group) were administered carbendazim in their diet for 13 weeks at doses of 0, 100, 300 or 1000 mg.kg diet^{-1}. The NOAEL was based on minor unspecified changes in clinical chemistry and organ weights (including increased liver weight and inhibition of spermatogenesis in males) that occurred at higher doses. Although not entirely clear, this study is presumed to be the one discussed in the EC PPP review, but with a stated NOAEL of 10 mg.kg bw.day (300 mg.kg diet^{-1}).
Hoffman and Kirsch (1987) Cited in JMPR (1995a) **Sub-chronic NOAEL = 12.5 mg.kg bw.d^{-1} (500 mg.kg diet^{-1})**	Male and female Beagle dogs (3/sex/group) were administered carbendazim in their diet for 13 weeks at doses of 0, 500, 1500 or 4500 mg.kg diet^{-1}. The NOAEL was based on increased liver weight that occurred at higher doses.
Hunter *et al.* (1973) Cited in JMPR (1995a) **Sub-chronic NOAEL = 35 mg.kg bw.d^{-1} (450 mg.kg diet^{-1})**	Male and female Sprague-Dawley rats (number per group unspecified) were administered carbendazim in their diet for 13 weeks at doses up to 1350 mg.kg diet^{-1}. The NOAEL was based on hepatomegaly that occurred at higher doses.
Chronic toxicity to mammals	
Sherman (1972) Cited in JMPR (1995a) & presumed to be cited in EC PPP (2006)	Male and female Sprague-Dawley rats (36/sex/group) were administered carbendazim in their diet for 104 weeks at doses of 0, 100, 500 or 2500 mg.kg diet^{-1} (the highest dose was increased to 10 000 mg/kg diet after 20 weeks). The NOAEL was based on decreased bodyweight gain and slightly increased incidences of

Sub-chronic NOAEL = 15 mg.kg bw.d^{-1} (500 mg.kg diet^{-1})	diffuse testicular atrophy and prostatitis in males (as well as increased liver weight and inhibition of spermatogenesis in males) that occurred at higher doses. Although it is not entirely clear, this study is presumed to be the one discussed in the EC PPP review, but with a stated NOAEL of 22 mg.kg bw.day^{-1} (500 mg.kg diet^{-1}).
Stadler (1986) Cited in JMPR (1995a) **Sub-chronic NOAEL = 5 mg.kg bw.d^{-1} (200 mg.kg diet^{-1})**	Male and female Beagle dogs (5/sex/group) were administered carbendazim in their diet for 1 year at doses of 0, 100, 200 or 500 mg.kg diet^{-1}. The NOAEL was based on increased serum cholesterol levels that occurred at higher doses.
Sherman (1972) Cited in JMPR (1995a) **Sub-chronic NOAEL = 2.5 mg.kg bw.d^{-1} (100 mg.kg diet^{-1})**	Male and female Beagle dogs (4/sex/group) were administered carbendazim in their diet for 2 years at doses of 0, 100, 500 or 2500 mg.kg diet^{-1}. The NOAEL was based on liver effects (hepatic cirrhosis, swollen, vacuolated hepatic cells, mild chronic hepatitis, and increased levels of cholesterol, blood urea nitrogen, total protein and serum alanine aminotransferase) that occurred at higher doses.
Effects on reproduction of mammals	
Koeter *et al.* (1976) Cited in JMPR (1995a) & presumed to be cited in EC PPP (2006) **Reproductive NOAEL = 120 mg.kg bw.d^{-1} (2000 mg.kg diet^{-1})**	Male and female Wistar rats (10 males and 20 females/group) were administered carbendazim in their diet for 3 generations at doses of 0, 150, 300 or 2000 mg.kg diet^{-1}. The NOAEL was based on the lack of adverse reproductive and teratogenic effects. Although it is not entirely clear, this study is presumed to be the one discussed in the EC PPP review, but with a stated NOAEL of 100 mg.kg bw.day^{-1} (2000 mg.kg diet^{-1}).
Gray *et al.* (1988, 1990) Cited in JMPR (1995a) **Reproductive LOAEL = 50 mg.kg bw.d^{-1} (300 mg.kg diet^{-1})**	Male and female rats (8-12/sex/group; strain unspecified) were administered carbendazim orally via gavage at doses of 0, 50, 100, 200 or 400 mg.kg bw.day^{-1}. Animals were dosed from weaning, they were mated at 84 days old and dosed until termination at 104-106 days for males and day 27 post partum for females. The LOAEL was based on decreased caudal epipidymal sperm count that occurred at all doses.
Embryotoxicity and teratogenicity	
Anon Cited in EC PPP (2006) & PDS (1996) **Developmental NOAEL = 10 mg.kg bw.d^{-1}**	Rats and rabbits (strain, sex and numbers per group unspecified) were administered carbendazim in their diet on days 7-16 and days 7-19 of gestation, respectively, at doses of >10 mg/kg bw.day^{-1}. The NOAEL in rats was based on increased malformations and unspecified anomalies. The NOAEL in rabbits was based on slightly decreased implantation rate and increased incidence of resorption that occurred at higher doses. It was also reported that teratogenicity occurred at doses that were maternally toxic.

Alverez (1987) Cited in JMPR (1995a) **Maternal NOAEL = 20 mg.kg bw.d-^{1}** **Developmental NOAEL = 10 mg.kg bw.day^{-1}**	Male and female Sprague-Dawley rats (25/sex/group) were administered carbendazim in a 0.5% aqueous suspension of carboxymethylcellulose orally via gavage on days 7-16 of gestation at doses of 0, 5, 10, 20 or 90 mg/kg bw/day. The developmental NOAEL was based on decreased foetal weight that occurred at higher doses. The maternal NOAEL was based on increased incidences of foetal malformations such as hydrocephaly, microphthalmia, anophthalmia, malformed scapulae and axial skeletal malformations that occurred at higher doses.
Christian *et al.* (1985) Cited in JMPR (1995a) **Maternal NOAEL = 20 mg.kg bw.d^{-1}** **Developmental NOAEL = 10 mg.kg bw.day^{-1}**	Artificially inseminated female New Zealand white rabbits (20/sex/group) were administered carbendazim in a 0.5% aqueous suspension of carboxymethylcellulose orally via gavage on days 7-19 of gestation at doses of 0, 10, 20 or 125 mg.kg bw.day^{-1}. The developmental NOAEL was based on decreased implantation rates that occurred at higher doses. The maternal NOAEL was based on increased foetal malformations (cervical vertebrae, ribs and proximate thoracic vertebrae) that occurred at higher doses.
Carbendazim has been found to be positive in assays for numerical chromosomal aberrations due to the inhibition of tubulin preventing microtubule formation. As a consequence cytokinesis is inhibited and aneuploidy is produced above a threshold concentration required for tubulin binding. Unlike primary mutagens, carbendazim does not interact with DNA and does not cause gene mutations or structural chromosome aberrations. This was supported by the Advisory Committee on Pesticides (PSD, 1997), which considered the available *in vitro* aneuploidy data was consistent with a threshold for carbendazim-induced aneuploidy. These data indicated that it is possible to determine Average Daily Intake (ADI) and Acceptable Operator Exposure Level (AOEL) values for carbendazim. Further *in vitro* investigations confirmed clear thresholds for carbendazim induced aneuploidy. These new studies suggested a threshold for *in vitro* aneuploidy at 0.6 μg/ml carbendazim (NOEL = 0.5 μg/ml). The blood level of 0.5 μg/ml for carbendazim corresponded to a single oral administration of approximately 2 mg/kg bw. However, excretion of this dose was 46-63 % within 0-6 h. During the 10-day administration of 2 mg/kg bw, no significant cumulative effect was observed. *In vivo* studies have shown, that carbendazim-induced micronuclei in polychromatic erythrocytes of NMRI mice when doses higher than 50 mg/kg bw were administered orally. Thus it was concluded, that there is a sufficient safety margin with regard to aneuploidy to the proposed ADI value for carbendazim of 0.02 mg/kg bw. It has been reported that two strains of mice developed enhanced liver tumours (specific pathogen-free Swiss mice and CD-1 mice), but a third mouse strain (NMRKf mice) and two rat studies (Sprague-Dawley and Wistar rats) did not show increased incidences of tumours (EC PPP, 2006; JMPR, 1995a).	
Sub-chronic toxicity to birds	
ECOTOX (2007) & EC PPP (2006) **8 day LC50 = >4200, >5000, >10 000 mg.kg diet^{-1}** **14 day LD50 = >2100, >2250 mg.kg bw.day^{-1}**	Northern bobwhites (*Colinus virginanus*) (sex and number unspecified) were administered carbendazim either via their diet (8 day LC50) or orally via an unspecified route (14 day LD50). The LC50/LD50 is the concentration required to kill 50% of the study population.

ECOTOX (2007) **8 day LC50 = 2278, >4467, >10 000 mg.kg diet^{-1}**	Mallard ducks (*Anas platyrchynchos*) (sex and number unspecified) were administered carbendazim via their diet.
Reproductive toxicity to birds	
Anon Cited in JMPR (1995b) **Sub-chronic NOEL = 160 mg.kg diet^{-1} (20 mg.kg bw.day^{-1})** **Reproductive NOEC = 400 mg.kg diet^{-1} (50 mg.kg bw.day^{-1})**	Japanese quail (*Coturnix japonica*) (sex and number unspecified) were administered carbendazim via their diet. It was not stated what the NOEL/C were based on.
Chronic toxicity to birds	
Singhal *et al.* (2003) **Chronic LOEL = 200 mg.kg diet^{-1}**	Chickens (species, sex and strain unspecified; 16/group) were administered carbendazim via their diet for 6 months at doses of 0 or 200 mg/kg diet. The birds had previously been vaccinated against New Castle Disease. The LOEL was based on decreased B-lymphocyte proliferation, serum IgG, IgM and IgA levels, total serum protein, serum gamma-globulins and serum globulins.
No histological evidence of delayed neuropathy has been reported in hens, although some acute clinical signs (such as slight leg weakness and ataxia) occurred (EC PPP, 2006; JMPR, 1995a). No studies were available regarding the potential effects of carbendazim on avian carcinogenicity.	

3.5.2 PNECs for secondary poisoning of predators

Bioconcentration data (as BCF values) for carbendazim indicates low BCF values with values for whole fish ranging from 23 to 159 (and 380 to 460 for viscera)(see Section 2.5). The one BCF value over 100 for rainbow trout was an exception and values <100 were recorded in the same study for channel catfish and bluegill sunfish. Other studies have reported BCF values of 23 and 27. Therefore, on a weight of evidence basis it is considered that the TGD BCF trigger of 100 has not been exceeded and the derivation of a PNEC in whole fish for secondary poisoning of predators is not required.

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4. Analysis and monitoring

A number of methods for the analysis of carbendazim in river and drinking waters have been produced as “Blue Books” by the Standing Committee of Analysts (SCA) (HMSO, 1987). They are based on either solid phase extraction (SPE) using C18 cartridges or solvent extraction using dichloromethane (DCM) followed by determination using thermospray liquid chromatography-mass spectrometry (LC-MS) or high performance liquid chromatography with an ultraviolet detector (HPLC-UV).

Using LCMS analysis after any of the extraction methods produced a limit of detection of 0.001 µg l^{-1} with a range of application up to 0.125 µg l^{-1}. In contrast, using HPLC-UV after liquid-liquid extraction with DCM, a limit of detection of 0.014 µg l^{-1} was obtained with a range of application up to 5 µg l^{-1}.

For water, proposed PNECs derived for carbendazim range from 0.03 to 0.7 µg l^{-1}. The data quality requirements are that, at one-third of the EQS, total error of measurement should not exceed 50 per cent. Using this criterion, it is evident that current analytical methodologies (non-standard) employing GC-MS, which are capable of achieving detection limits as low as 0.001 ug l^{-1}, should offer adequate performance to analyse for carbendazim.

5. Conclusions

5.1 Availability of data

Long-term freshwater toxicity data are available for five taxonomic groups, i.e. algae, crustaceans, fish, molluscs and platyhelminths with crustaceans being more sensitive than the other taxa. Short-term toxicity tests are available for eight taxonomic groups, i.e. algae, amphibians, crustaceans, fish, insects, molluscs, platyhelminths and protozoa, with crustaceans and fish being more sensitive than other taxa. For marine organisms, short-term toxicity data are available for three taxonomic groups, i.e. crustaceans, fish and molluscs. No long-term toxicity data are available for saltwater taxa.

5.2 Derivation of PNECs

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

5.2.1 Long-term PNEC for freshwaters

The lowest valid long-term toxicity value for freshwater invertebrates is a 21-day No Observed Effect Concentration (NOEC) of 1.5 µg active ingredient (a.i.) l^{-1} for effects on the reproduction of the waterflea *Daphnia magna*. Reliable long-term NOECs are available for algae, annelids, crustaceans, fish and molluscs. Therefore, based on the EU Technical Guidance Document (TGD) methodology, an assessment factor of 10 could be applied to the lowest valid toxicity value. This results in a $PNEC_{freshwater\_lt}$ of 0.15 µg l^{-1}.

The existing EQS is 0.1 µg l^{-1} and was derived by applying an assessment factor of 100 to the lowest most reliable acute data (96-hour LC50 of 7 µg l^{-1}) obtained for channel catfish yolk ac fry (*Ictalurus punctatus*).

5.2.2 Short-term PNEC for freshwaters

Reliable short-term data are available for algal, amphibian, annelids, crustaceans, fish, insects, molluscs and protozoa. The lowest valid short-term toxicity value is a 96-hour LC50 of 7 µg l^{-1} in the channel catfish yolk sac fry (fry (*Ictalurus punctatus*). Based on the EU Technical Guidance Document (TGD) methodology and a large body of acute data for carbendazim, an assessment factor of 10 can be applied to the lowest valid toxicity value. This results in a $PNEC_{freshwater\_lt}$ of 0.7 µg l^{-1}.

The current short-term EQS for saltwaters is 1.0 µg l^{-1} and was derived by applying an assessment factor of 10 to the most reliable acute data (96-hour LC50 of 7 µg l^{-1}) obtained for channel catfish yolk ac fry (*Ictalurus punctatus*).

5.2.3 Long-term PNEC for saltwaters

No long-term single species toxicity data for marine organisms are available. Long-term NOECs are available for freshwater algae, crustaceans, fish, molluscs and platyhelminths, but no toxicity data are available for marine taxa such as echinoderms. On the basis of the available data an additional assessment factor of 10 is proposed to account for the uncertainty due to the absence of chronic data for saltwater species and lack of data for additional marine taxa eg echinoderms, resulting in a total assessment factor of 100. The lowest reliable reported result is a 21-day NOEC of 1.5 µg a.i. l^{-1}, based on the reproduction, in the waterflea (*Daphnia magna*). This results in a $PNEC_{saltwater\_lt}$ of 0.015 µg l^{-1}.

The existing EQS is 0.1 µg l^{-1} and was derived by applying assessment factor of 100 to the lowest most reliable acute data (96-hour LC50 of 7 µg l^{-1}) obtained for channel catfish yolk ac fry (*Ictalurus punctatus*).

5.2.4 Short-term PNEC for saltwaters

Single species short-term toxicity data for marine organisms are available for three different taxonomic groups, i.e. crustaceans, molluscs and fish. Therefore, it is proposed that the $PNEC_{saltwater\_st}$ is based on the combined freshwater and saltwater dataset.

The lowest valid short-term toxicity value for freshwater fish is a 96-hour LC50 of 7 µg a.i. l^{-1} in the channel catfish yolk sac fry (*Ictalurus punctatus*). Since no data are available for marine taxa such as. echinoderms, an additional assessment factor of 10 would also normally be applied, resulting in a total assessment factor of 100. However, there is a large body of short-term data in the combined freshwater and saltwater dataset and there is toxicity data available for saltwater molluscs. Therefore, a reduced assessment factor of 50 applied to the lowest valid toxicity value has been adopted resulting in a $PNEC_{saltwater\_st}$ of 0.14 µg a.i. l^{-1}.

The current short-term EQS for saltwaters is 1.0 µg l^{-1} and was derived by applying an assessment factor of 10 to the lowest most reliable acute data (a 96-hour LC50 of 7 µg l^{-1}) obtained for channel catfish yolk ac fry (*Ictalurus punctatus*).

5.2.5 PNEC for sediments

The log Kow of carbendazim ranges from 0.4-1.52 and the log Koc ranges from 2.30-2.39, so the derivation of PNECs for the protection of benthic organisms is not required according to the TGD, since the log Kow/Koc trigger value of 3 is not exceeded.

5.2.6 PNEC for secondary poisoning

Bioconcentration data (as BCF values) for carbendazim indicated that the BCF values are low with values for whole fish ranging from 23 to 159 (and 380 to 460 for viscera). The one BCF value over 100 for rainbow trout was an exception and values <100 were recorded in the same study for channel catfish and bluegill sunfish. Other studies have reported BCF values of 23 and 27. Therefore, on a weight of evidence basis it is considered that the TGD BCF trigger of 100 has not been exceeded and the derivation of a PNEC in whole fish for secondary poisoning of predators is not required.

Table 5.1 Summary of proposed PNECs

Receiving medium/exposure scenario	Proposed PNEC (µg l^{-1})	Existing EQS (µg l^{-1})
Freshwater/long-term	0.15	0.1
Freshwater/short-term	0.7	1.0
Saltwater/long-term	0.015	0.1
Saltwater/short-term	0.14	1.0
Sediment	Not required	–
Secondary poisoning	Not required	–

5.3 Analysis

For water, proposed PNECs derived for carbendazim range from 0.03 to 0.7 µg l^{-1}. The data quality requirements are that, at one third of the EQS, total error of measurement should not exceed 50 per cent. Using this criterion, it is evident that current analytical methodologies (non-standard) employing GC-MS and capable of achieving detection limits as low as 0.001 ug l^{-1} should offer adequate performance to analyse for carbendazim.

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:-

- The analytical capability should be adequate for compliance assessment
- The assessment factors applied are within the range of 10-100 and therefore the PNECs derived are not subject to excessive uncertainty. The size of the assessment factors applied in the derivation of the saltwater standards could potentially be reduced if additional data on the toxicity to marine organisms was available.

References & Bibliography

ACP (1992). Advisory Committee on Pesticides. Issue No. 58. Evaluation of Fully Approved or Provisionally Approved Products. Carbendazim. Department for Environment, Food and Rural Affairs, Pesticides Safety Directorate.

Alverez L. (1987). Teratogenicity study of INE-965 (carbendazim) in rats. Unpublished report from E.I. DuPont de Nemours and Co., Haskell Laboratory, Newark, Delaware, USA.

Bates, K., Williams, D., Marscarenhas, R and Sutton, A. (1998). Proposed Environmental Quality Standards for Carbendazim in Water. Report No: DoE 3920/1 for the Department of the Environment.

Baer, K. (1993). Early life-stage toxicity test of DPX-E965-299 (Carbendazim, MBC) with Rainbow trout (*Oncorhynchus mykiss*). Report No. A52478. Registration No. WAT94-01358 cited in EUDAR 1997

Boeri, R. (1988). Static acute toxicity of carbendazim technical to the Sheepshead minnow (*Cyprinodon variegates*). Report No. A52017. Registration No. WAT95-50458. cited in EUDAR 1997

Canton, J. (1976). The toxicity of benomyl thiophanate-methyl and BCM to four freshwater organisms. *Bulletin of Environmental Contamination*, 16 (2), 214-218

Chemfinder (2007). Available from http://chemfinder.cambridgesoft.com

Christian N., Hoberman A. and Feussner E. (1985). Developmental toxicity study of carbendazim administered via gavage to New Zealand white rabbits. Unpublished report from Argus Research Laboratories, Inc., Horsham, Pennsylvania, USA. Submitted to WHO by E.I. DuPont de Nemours and Co., Inc.

Cuppen, J., Van den Brink, P., Camps, E., Uil, K. and Brock, T. (2002). Impact of the fungicide carbendazim in freshwater microcosms. I. Water quality, breakdown of particulate organic matter and responses of macroinvertebrates. *Aquatic Toxicology*, **48(2-3)**, 233-250.

Douglas, M. and Hanley, J. (1987). The algistatic activity of carbendazim tech. Report No. A52909. Registration No. WAT94-01344 cited in EUDAR 1997

EC (European Commission) (2007). Review report for the active substance carbendazim finalised in the Standing Committee on the Food Chain and Animal Health at its meeting on 3 March 2006 in view of the inclusion of carbendazim in Annex I of Directive 91/414/EEC. 5032/VI/98 final.

ECB (European Chemicals Bureau) (2003) Technical Guidance Document in Support of Commission Directive 93/67/EEC on risk assessment for new and notified substances: Commission Directive (EC) No. 1488/94 on Risk Assessment for Existing Substances and Directive 98/8/EC of the European Parliament and of the Council Concerning the Placing of Biocidal Products on the Market. Parts I–IV. Luxembourg: Office for Official Publications of the European Communities. Available from: http://ecb.jrc.it/tgdoc.

ECB (European Chemicals Bureau) (2009). ESIS (European chemical Substances Information System). Available from: http://ecb.jrc.ec.europa.eu/esis-pgm/esis_reponse.php

ECOTOX (2007). US Environmental Protection Agency. ECOTOX Database.

EC PPP (2006). European Commission. Plant Protection Products. Carbendazim. 5032/VI//98 Final.

EU DAR (1997). European Union Draft Assessment Report – Public Version. Carbendazim. Prepared by the Member State United Kingdom by the Pesticides Safety Directive. Available at: http://www3.efsa.europa.eu/DAR/displaySubstance.cfm?provision=1.

EU DAR (2000). Addendum to the Monograph of 13 November 1997 – Carbendazim. Addendum 2 - 26 January 2000

EU DAR (2003). Addendum to the Monograph of 13 November 1997 – Carbendazim. Addendum 6 – 31 March 2003.

Fischer, R. (1988[a]). The effect of Carbendazim – substance, technical (identification code Hoe 017411 0F ZD99 0010) to *Daphnia magna* (water flea) in a static-acute toxicity test (method OECD). Report No. A39285. Registration No. WAT94-01331 cited in EUDAR 1997

Fischer, R. (1988[b]). The effect of Carbendazim – substance, technical (identification code: Hoe 017411 0F ZD99 0010) to *Salmo gairdneri* (Rainbow trout) in a 21 day prolonged toxicity test (method OECD). Report No. A40788. Registration No. WAT94-01335. cited in EUDAR 1997

Fischer, R. (1988[c]). The effect of carbendazim – substance technical (identification code: Hoe 017411 0F ZD99 0010) to *Salmo gairdneri* (Rainbow trout) in static-acute toxicity test (method OECD) Report No. A40135. Registration No. WAT94-01334. cited in EUDAR 1997

Fischer, R. (1988[d]). The effect of carbendazim – substance technical (identification code: Hoe 017411 0F ZD99 0010) to *Cyprinus carpio* (Mirror carp) in static-acute toxicity test (method OECD) Report No. A40032. Registration No. WAT94-01333. cited in EUDAR 1997

Gray L., Ostby J., Sigmon R., Ferrell J., Rehnberg G., Linder R., Cooper R., Goldman J. and Laskey J. (1988). The development of a protocol to assess reproductive effects of toxicants in the rat. Reproductive Toxicology, 2, 281-287.

Gray L., Ostby J., Linder R., Goldman J., Rehnberg G. and Cooper R. (1990). Carbendazim induced alterations of reproductive development and function in the rat and hamster. Fundam Appl Toxicol 15: 281-297.

HMSO (1987) Phenyluren herbicides (urons), dinocap, dinosek, benomyl, carbendazim and metanitron in waters. Method for the Examination of Water and Associated Materials. HM Stationary Office.

Hoffman H. and Kirsch P. (1987). Report on the subchronic toxicity of methyl benzimidazole-2-carbamate in beagle dogs on oral administration. Unpublished report from Hoechst AG, Frankfurt, Germany. Cited in JMPR 1995a

HSDB (2005). Carbendazim CAS RN: 10605-21-7. Hazardous Substances Data Bank. Availble from: http://toxnet.nlm.gov/cgi-bin/sis/search/f?./temp/~yuCePt:1:FULL

HSF (1993). International Programme on Chemical Safety. Health and Safety Guide No. 82. Carbendazim

Hunt, J., Maltby, L., Wood, R., Slijkerman, D. and Jak, R. (2001). In situ bioassays for assessing the ecological quality of fresh waters: detritus processing and detritivore feeding. Presented at SETAC 2001 Europe 7/5/2001. Available at: http://abstracts.co.allenpress.com/pweb/setaceu2001/document/?ID=22888

Hunter B., Benson H., Street A., Heywood R. & Newman A. (1973). Carbendazim toxicity to rats during dietary administration for 13 weeks followed by a recovery period of 6 weeks. Unpublished report from Huntingdon Research Centre, United Kingdom. Submitted to WHO by Hoechst AG, Frankfurt, Germany cited in JMPR 1995a

Hutton, D. (1988). Static acute 48 hour EC50 of Carbamic acid. 1H-benzimidazol-2-yl-,methyl ester to fed *Daphnia magna*. Report No. A52904. Registration No. WAT95-50227. cited in EUDAR 1997

IUCLID (2000). IUCLID Dataset Carbendazim. European Commission – European Chemicals Bureau.

JMPR (1973). Joint Meeting on Pesticide Residues. World Health Organization (WHO) Pesticide Residues Series 3. 263. Carbendazim.

JMPR (1976). Joint Meeting on Pesticide Residues. Pesticide Residues in Food. 359. Carbendazim.

JMPR (1977). Joint Meeting on Pesticide Residues. Pesticide Residues in Food. 388. Carbendazim.

JMPR (1978). Joint Meeting on Pesticide Residues. Pesticide Residues in Food. 429. Carbendazim.

JMPR (1983). Joint Meeting on Pesticide Residues. Pesticide Residues in Food. 613. Carbendazim.

JMPR (1985). Joint Meeting on Pesticide Residues. Pesticide Residues in Food. 718. Part II Toxicological Evaluations. Carbendazim.

JMPR (1995a). Joint Meeting on Pesticide Residues. Pesticide Residues in Food. 892 Part II Toxicological Evaluations. Carbendazim.

JMPR (1995b). Joint Meeting on Pesticide Residues. Pesticide Residues in Food. 907 Part II Toxicological Evaluations. Carbendazim, Benomyl and Thiophanate-methyl.

Kelly, C., Thirkettle, K., Smith, B., and Graham, F. (1997). Carbendazim technical prolonged toxicity to *Daphnia magna*. Report No. SNG 80/970692. Registration No. WAT 2000-565 cited in EUDAR 1997

Khangarot, B., Sehgal, A. and Bhasin, M. (1985). "Man and Biosphere" - Studies on the Sikkim Himalayas. Part 6: Toxicity of Selected Pesticides to Frog Tadpole *Rana hexadactyla* (Lesson). *Acta Hydrochim.Hydrobiol*. **13(3)**, 391-394

Koeter *et al*. (1976). Cited in JMPR (1995a). The full reference was not listed in the review.

Lu, S-Y, Liao, J-W, Kuo, M-L, Wang, S-C, Hwang, J-S and Ueng, T-H. (2004) Endocrine-disrupting activity in carbendazim-induced reproductive and developmental toxicity in rats. Journal of Toxicology and Environmental Health, Part A, 67 (19), 1501-1515.

Mantovani, A., Maranghi, F., Ricciardi, C., Macri, C., Stazi, A.V., Attias, L and Zapponi, G.A. (1998) Developmental toxicity of Carbendazim: Comparison of No-Observed-Adverse-Effect Level and Benchmark Dose Approach. Food and Chemical Toxicology, 36, 37-45.

Mazellier, P., Mazellier É., De Laat , L.J. and Legube, B (2003). Degradation of carbendazim by UV/H_2O_2 investigated by kinetic modelling. *Environmental Chemistry Letters*, **1(1**), 68-72.

Nishuchi, Y. and Yoshida, K. (1974) Toxicity of new agricultural chemicals to tadpoles. *Bulletin of Agricultural Chemistry Insp. Stn*., **14**, 66-68.

NPIC (2008). National Pesticide Information Center. Available at: http://npic.orst.edu/index.html

Office of Pesticide Programs (2000). Pesticide Ecotoxicity Database (Formerly: Environmental Effects Database (EEDB)) Environmental Fate and Effects Division, U.S.EPA, Washington, D.C.

Palawski, D.U. and Knowles, C.O. (1986) Toxicological studies of benomyl and carbendazim in rainbow trout, channel catfish and bluegills. *Environmental Toxicology and Chemistry*, **5**, 1039-1046.

PDS (1996). World Health Organization/Food and Agriculture Organization Data Sheets on Pesticides. Carbendazim PDS 89.

PSD (1997) U.K. Review of Methyl benzimidazole carbamate (MBC) fungacides, benomyl and carbendazim. Evaluation of Fully Approved or Provisionally Approved Products. Advisory Committee on Pesticides Issue 170, Pesticides Safety Directorate.

Rankin, P., Surak, J. and Thompson, N. (1977). Effect of Benomyl and Benomyl Hydrolysis Products on *Tetrahymena pyriformis*. *Food Cosmet. Toxicol*. **15(3)**, 187-193

Sherman H. (1972). Long-term feeding studies in rats and dogs with 2-benzimadazole carbamic acid, methyl ester (INE-965) (50% and 70% MBC wettable powder formulations). Unpublished report from E.I. DuPont de Nemours and Co., Inc., Haskell Laboratory, Newark, Delaware, USA. Cited in JMPR 1995a

Slijkerman, D., Baird, D., Conrad, A., Jak, R. and van Straalen, N., (2003). Assessing structural and functional plankton responses to carbendazim toxicity (WEP/152). Presented at SETAC 2003 Europe 30/4/2003. Available at: http://abstracts.co.allenpress.com/pweb/setaceu2003/document/?ID=22401

Singhal L., Bagga S., Kumar R. and Chauhan R. (2003). Down regulation of humoral immunity in chickens due to carbendazim. Toxicol In Vitro 17(5-6): 687-92.

Sowig, P. and Gosch, H. (2002). Chronic toxicity to the sediment dwelling chironomid larvae *Chironomus riparius* - Carbendazim; water miscible suspension. Cited in EU DAR (2003).

SRC (2007). Interactive Physprop Database. Syracuse Research Corporation Available from http://www.syrres.com/esc/physdemo.htm

Stadler J. (1986). One-year feeding study in dogs with carbendazim. Unpublished report from E.I. DuPont de Nemours and Co., Inc., Haskell Laboratory, Newark, Delaware, USA cited in JMPR 1995a.

Til H., van den Muelen H., Feron V., Seinen W. and de Groot A. (1972). Sub-chronic (90 day) toxicity study with W17411 in beagle dogs. Unpublished report from Central Institute for Nutrition and Food Research (TNO), The Hague, Netherlands. Submitted to WHO by Hoechst AG, Frankfurt, Germany cited in JMPR 1995a

US EPA (2005). Robust summaries for carbamic acid, 1H-benzimidazol-2-yl-, methyl ester (CAS No. 10605-21-7). Prepared January 11, 2005. Available at: http://epa.gov/chemrtk/pubs/summaries/carbam1h/c15800rs.pdf

Van den Brink, P., Hattink, J., Bransen, F., Van Donk, E. and Brock, T. (2000). Impact of the fungicide carbendazim in freshwater microcosms. II. Zooplankton, primary producers and final conclusions. *Aquatic Toxicology*, **48(2-3)**, 251-264.

Van Wijngaarden, R., Crum, S., Decraene, J., Hattink, J. and van Kammen, A. (1998). Toxicity of Derosal (active ingredient Carbendazim) to Aquatic Invertebrates. *Chemosphere*. **37(4)**, 673-683.

Van Wijngaarden, R., Arts, G., Cuppen, J. and Brock, T. (2002). The impact of a single treatment with the fungicide Derosal (a.i. carbendazim) on the freshwater community in realistic model ecosystems (59-08). Presented at SETAC 2002 Europe 15/5/2002. Available at: http://abstracts.co.allenpress.com/pweb/setaceu2002/document/?ID=1223

WHO (1993) Environmental Health Criteria, Vol 149, Carbendazim. International Programme on Chemical Safety. 132p

Yu, G., Guo, Q., Xie, L., Liu, Y and Wang, X. (2009a) Effects of subchronic exposure to carbendazim on spermatogenesis and fertility in male rats. Toxicology and Industrial Health, 25 (1), 41-47.

Yu, G., Liu, Y., Xie, L. and Wang, X. (2009b) Involvement of sertoli cells in spermatogenic failure induced by carbendazim. Environmental Toxicology and Pharmacology, 27(2), 287-293.

List of abbreviations

AA	Annual average
AF	Assessment factor
a.i.	Active ingredient
BCF	Bioconcentration factor
Bw	body weight
CAS	Chemical Abstracts Service
DAR	Draft Assessment Report
ECb	Effective concentration (biomass)
EC50	concentration effective against 50% of the organisms tested
EHC	Environmental Health Criteria
EQS	Environmental Quality Standard
FAO	Food and Agricultural Organization
GC-MS	gas chromatography/mass spectrometry
GLP	Good Laboratory Practice (OECD)
LC50	concentration lethal to 50% of the organisms tested
LOAEL	lowest observed adverse effect level
LOEC	lowest observed effect concentration
LOQ	limit of quantification
lt	long term
MAC	maximum allowable concentration
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
OECD	Organization for Economic Co-operation and Development
PNEC	predicted no-effect concentration
ppm	parts per million
SSD	species sensitivity distribution
st	short term
TGD	Technical Guidance Document
UKTAG	UK Technical Advisory Group
WFD	Water Framework Directive
w/w	weight/weight

ANNEX 1 Data quality assessment sheets

Identified and ordered by alphabetical order of references.

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

Table A1 Klimisch Criteria*

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

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

Reference	Baer (1993) cited in the EU DAR (1997)

Information on the test species	
Test species used	*Oncorhynchus mykiss*
Source of the test organisms	Not stated in EU DAR, 1997
Holding conditions prior to test	Not stated in EU DAR, 1997
Life stage of the test species used	Early life stage

Information on the test design	
Methodology used	Not stated in EU DAR, 1997
Form of the test substance	Technical grade (purity unspecified)
Source of the test substance	Not stated in EU DAR, 1997
Type and source of the exposure medium	Not stated in EU DAR, 1997
Test concentrations used	Not stated in EU DAR, 1997
Number of replicates per concentration	Not stated in EU DAR, 1997
Number of organisms per replicate	Not stated in EU DAR, 1997
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Flow-through, 79 days, fed
Measurement of exposure concentrations	Yes (Exposure concentrations measured)
Measurement of water quality parameters	Not stated in EU DAR, 1997
Test validity criteria satisfied	Not stated in EU DAR, 1997
Water quality criteria satisfied	Not stated in EU DAR, 1997
Study conducted to GLP	Yes
Overall comment on quality	Limited detail available.

Reliability of study	**Reliable without restrictions**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	Boeri (1988) cited in EU DAR (1997)

Information on the test species	
Test species used	*Cyprinodon variegatus*
Source of the test organisms	Not stated in EU DAR, 1997
Holding conditions prior to test	Not stated in EU DAR, 1997
Life stage of the test species used	Not stated in EU DAR, 1997

Information on the test design	
Methodology used	Not stated in EU DAR, 1997
Form of the test substance	Technical grade (purity unspecified)
Source of the test substance	Not stated in EU DAR, 1997
Type and source of the exposure medium	Not stated in EU DAR, 1997
Test concentrations used	Not stated in EU DAR, 1997
Number of replicates per concentration	Not stated in EU DAR, 1997
Number of organisms per replicate	Not stated in EU DAR, 1997
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Static, 96 hours, not fed
Measurement of exposure concentrations	Yes (Exposure concentrations measured)
Measurement of water quality parameters	Not stated in EU DAR, 1997
Test validity criteria satisfied	Not stated in EU DAR, 1997
Water quality criteria satisfied	Not stated in EU DAR, 1997
Study conducted to GLP	Yes
Overall comment on quality	Limited data available, however conducted to GLP and conducted analytical measurements of the exposure concentrations

Reliability of study	**Reliable without restrictions**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	Canton (1976) cited in EU DAR (1997)

Information on the test species	
Test species used	*Chlorella pyrenoidosa*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Taken from a culture in log-phase

Information on the test design	
Methodology used	Koch (1953); after 2 days the number of cells were determined by measuring the absorbance at 540 nm in a Beckham spectrophotometer model B.
Form of the test substance	Methyl benimidazole-2-yl carbamate purity 97.4%
Source of the test substance	BASF
Type and source of the exposure medium	According to procedure of Wanka F. (1965). Archives of Microbiology, 52, 305-318.
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Approximately 10^7 cells
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Static, 48 hours
Measurement of exposure concentrations	No (Nominal concentrations used)
Measurement of water quality parameters	Yes (Temperature = 24 ± 1°C)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	Limit study detail, there was a lack of suitable analytical methods so the exposure concentration was not measured and static conditions were used.

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

Reference	Douglas and Handley (1987) cited in the EU DAR (1997)

Information on the test species	
Test species used	*Selenastrum capricotnutum*
Source of the test organisms	Not stated in EU DAR, 1997
Holding conditions prior to test	Not stated in EU DAR, 1997
Life stage of the test species used	Not stated in EU DAR, 1997

Information on the test design	
Methodology used	Not stated in EU DAR, 1997
Form of the test substance	Technical grade (99-100% purity)
Source of the test substance	Not stated in EU DAR, 1997
Type and source of the exposure medium	Not stated in EU DAR, 1997
Test concentrations used	Not stated in EU DAR, 1997
Number of replicates per concentration	Not stated in EU DAR, 1997
Number of organisms per replicate	Not stated in EU DAR, 1997
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Static, 72 hours
Measurement of exposure concentrations	No (Nominal concentrations used)
Measurement of water quality parameters	Yes (Temperature = 24 ± 1°C, pH = 7.6-8.9)
Test validity criteria satisfied	Cell concentration in the control cultures have not increased by a factor of at least 16 within three days.
Water quality criteria satisfied	Not stated in EU DAR, 1997
Study conducted to GLP	Yes
Overall comment on quality	Limited data available.

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

Reference	Fischer (1988) cited in EU DAR (1997)

Information on the test species	
Test species used	*Daphnia magna*
Source of the test organisms	Not stated in EU DAR, 1997
Holding conditions prior to test	Not stated in EU DAR, 1997
Life stage of the test species used	Not stated in EU DAR, 1997

Information on the test design	
Methodology used	Unspecified OECD methodology.
Form of the test substance	Technical grade (purity unspecified)
Source of the test substance	Not stated in EU DAR, 1997
Type and source of the exposure medium	Not stated in EU DAR, 1997
Test concentrations used	Not stated in EU DAR, 1997
Number of replicates per concentration	Not stated in EU DAR, 1997
Number of organisms per replicate	Not stated in EU DAR, 1997
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Static, 48 hours, not fed
Measurement of exposure concentrations	No (Nominal concentrations used)
Measurement of water quality parameters	Not stated in EU DAR, 1997
Test validity criteria satisfied	Not stated in EU DAR, 1997
Water quality criteria satisfied	Not stated in EU DAR, 1997
Study conducted to GLP	Yes
Overall comment on quality	Limited study detail available and static exposure was employed and no analytical measurement of the exposure concentration was conducted. However OECD methodology was stated to have been used and the study was conducted to GLP.

Reliability of study	**Reliable without restrictions**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	Fischer (1988) cited in the EU DAR (1997)

Information on the test species	
Test species used	*Oncorhynchus mykiss*
Source of the test organisms	Not stated in EU DAR, 1997
Holding conditions prior to test	Not stated in EU DAR, 1997
Life stage of the test species used	10 months old

Information on the test design	
Methodology used	Unspecified OECD methodology
Form of the test substance	Technical grade (purity unspecified)
Source of the test substance	Not stated in EU DAR, 1997
Type and source of the exposure medium	Not stated in EU DAR, 1997
Test concentrations used	Not stated in EU DAR, 1997
Number of replicates per concentration	Not stated in EU DAR, 1997
Number of organisms per replicate	Not stated in EU DAR, 1997
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Flow-through, 96 hours, not fed
Measurement of exposure concentrations	No (Nominal concentrations used)
Measurement of water quality parameters	Not stated in EU DAR, 1997
Test validity criteria satisfied	Not stated in EU DAR, 1997
Water quality criteria satisfied	Not stated in EU DAR, 1997
Study conducted to GLP	Yes
Overall comment on quality	Limited of study detail available, nominal concentrations used, However an OECD standardised method was used.

Reliability of study	**Reliable without restrictions**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	Kelly *et al*. (1997) cited in EU DAR (2000)

Information on the test species	
Test species used	*Daphnia magna*
Source of the test organisms	Not stated in EU DAR, 1997.
Holding conditions prior to test	Not stated in EU DAR, 1997.
Life stage of the test species used	Not stated in EU DAR, 1997.

Information on the test design	
Methodology used	OECD Guideline No. 202.
Form of the test substance	Technical grade carbendazim, purity 99.5%.
Source of the test substance	Not stated in EU DAR, 1997.
Type and source of the exposure medium	Not stated in EU DAR, 1997.
Test concentrations used	0, 1.8, 5.6, 18, 56 and 180 nominal. 0, 1.5, 4.6, 15, 45 and 190 measured.
Number of replicates per concentration	Not stated in EU DAR, 1997.
Number of organisms per replicate	Not stated in EU DAR, 1997.
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Semi-static (solutions were renewed three times each week), 21 days, fed at renewals
Measurement of exposure concentrations	Yes (Exposure concentrations measured)
Measurement of water quality parameters	Not stated in EU DAR, 1997.
Test validity criteria satisfied	Not stated in EU DAR, 1997.
Water quality criteria satisfied	Not stated in EU DAR, 1997.
Study conducted to GLP	Not stated in EU DAR, 1997.
Overall comment on quality	Accepted as valid by RMS, measured concentrations used and OECD standardised test guidelines were followed, but lack of study detail reported in the EU DAR.

Reliability of study	**Reliable without restrictions**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	Khangarot *et al.* (1985)

Information on the test species	
Test species used	*Rana hexadactyla*
Source of the test organisms	Natural breeding ground.
Holding conditions prior to test	Acclimatised to laboratory conditions prior to exposure, no artificial food except for some water plant (detail not specified).
Life stage of the test species used	20 mm (15 to 25 mm) length and 500 mg (350 to 800 mg) (w/w)

Information on the test design	
Methodology used	Not stated.
Form of the test substance	Commercial grade 2-(methoxy-carbamyol)-benzimidazole 50% w/w
Source of the test substance	BASF India Ltd.
Type and source of the exposure medium	Not stated.
Test concentrations used	Not stated.
Number of replicates per concentration	3
Number of organisms per replicate	10
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Static. Test water was renewed after every 24 hours.
Measurement of exposure concentrations	No (Nominal concentrations used).
Measurement of water quality parameters	Yes (temperature = 12-17°C, pH = 6.0-6.4, acidity = 16-28 mg/l as $CaCO_3$, alkalinity = 20-40 mg/l as $CaCO_3$, total hardness = 15-35 mg/l as $CaCO_3$, dissolved oxygen = 5.5-8.0 mg/l)
Test validity criteria satisfied	Not stated.
Water quality criteria satisfied	Not stated.
Study conducted to GLP	Not stated.
Overall comment on quality	A 50% formulation of carbendazim was used and nominal measurements of the test concentrations were used.

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

Reference	Nishiuchi and Yoshida (1974)

Information on the test species	
Test species used	*Bufo bufo japonicus*
Source of the test organisms	Not stated.
Holding conditions prior to test	Not stated.
Life stage of the test species used	Not stated.

Information on the test design	
Methodology used	Not stated.
Form of the test substance	2-(Methoxycarbonylamino) benimidazole.
Source of the test substance	Not stated.
Type and source of the exposure medium	Not stated.
Test concentrations used	Not stated.
Number of replicates per concentration	Not stated.
Number of organisms per replicate	Not stated.
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Not stated.
Measurement of exposure concentrations	Not stated.
Measurement of water quality parameters	25°C
Test validity criteria satisfied	Not stated.
Water quality criteria satisfied	Not stated.
Study conducted to GLP	Not stated.
Overall comment on quality	Poorly reported study, no study details were included.

Reliability of study	**Not assignable**
Relevance of study	**Relevant**
Klimisch Code	**4**

Reference	Palawaski and Knowles (1986)

Information on the test species	
Test species used	*Ictalurus punctatus*
Source of the test organisms	Eggs were from Tishomingo (Oklahoma) National Fish Hatchery. Eggs were cultured at the Columbia National Fisheries Research Laboratory (Columbia, MO).
Holding conditions prior to test	Fish gradually acclimated to test conditions over 3 days. Fish placed in chamber 24 hours before exposure and were no fed during this time or the acclimatisation.
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	ASTM Standards, 1980 E729-80 and Committee on Methods for Toxicity Tests with Aquatic Organisms, 1975 EPA-660/3-75-009
Form of the test substance	Technical grade benomyl (99%) and carbendazim (99%)
Source of the test substance	Not stated.
Type and source of the exposure medium	Reconstituted soft water (water hardness 10 to 48 mg/l as CaCO3)
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	10
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Static, 96 hours, not fed.
Measurement of exposure concentrations	No (Nominal concentrations used)
Measurement of water quality parameters	Yes (temperature = 22°C, pH = 7.4; hardness = 40-48 mg/l as $CaCO_3$, alkalinity = 35 mg/l $CaCO_3$)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	Generally well reported, no mention of if the study was conducted to GLP, nominal concentrations and static exposure were used, but a standardised method was followed.

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

Reference	Proprietary data (1995) cited in Bates *et al*., (1998)

Information on the test species	
Test species used	*Mysidopsis bahia*
Source of the test organisms	Not stated in Bates *et al*. (1998)
Holding conditions prior to test	Not stated in Bates *et al*. (1998)
Life stage of the test species used	Not stated in Bates *et al*. (1998)

Information on the test design	
Methodology used	Not stated in Bates *et al*. (1998)
Form of the test substance	Carbendazim (form unspecified)
Source of the test substance	Not stated in Bates *et al*. (1998)
Type and source of the exposure medium	Not stated in Bates *et al*. (1998)
Test concentrations used	Not stated in Bates *et al*. (1998)
Number of replicates per concentration	Not stated in Bates *et al*. (1998)
Number of organisms per replicate	Not stated in Bates *et al*. (1998)
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Static, 96 hours, not fed
Measurement of exposure concentrations	Yes (Exposure concentrations measured)
Measurement of water quality parameters	Not stated in Bates *et al*. (1998)
Test validity criteria satisfied	Not stated in Bates *et al*. (1998)
Water quality criteria satisfied	Not stated in Bates *et al*. (1998)
Study conducted to GLP	Not stated in Bates *et al*. (1998)
Overall comment on quality	Limited data and original source was not located; however, it was reported in the previous EQS Report.

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

Reference	Rankin *et al*. (1977)

Information on the test species	
Test species used	*Tetrahymena pyriformis*
Source of the test organisms	Not stated.
Holding conditions prior to test	*T*. *pyriformis* strain E was incubated at 23 ± 1°C in dark in stationary 1 litre Roux flasks containing 200 ml autoclaved medium.
Life stage of the test species used	1% inoculum of early log growth cells, 20 hour old 200 ml cultures.

Information on the test design	
Methodology used	Not stated.
Form of the test substance	Analytical grade methylbenzimidazol-2-yl carbamate (MBC).
Source of the test substance	E.I. du Pont de Nemours & Co., Wilmington, Del.
Type and source of the exposure medium	Dimethysulphoxide (DMSO) (1% in the medium) stated not to effect cell growth. Medium consisted of 2% (w/v) proteose peptone and 0.1% (w/v) yeast extract.
Test concentrations used	0, 5, 10, 15, 20 or 25 $mg.l^{-1}$
Number of replicates per concentration	200 ml culture
Number of organisms per replicate	4
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Static, 36 hours
Measurement of exposure concentrations	Nominal
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.
Overall comment on quality	Well reported, however no analytical measurements were conducted.

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

Reference	Van Wijnagaarden *et al.* (1998)

Information on the test species	
Test species used	*Dugesia lugubris* (Planarian, vortex worm) *Bithynia tentaculata* (Snail) *Chaoborus obscuripes* (Midge larvae)
Source of the test organisms	Ponds at SC-DLO and ditches in agricultural area
Holding conditions prior to test	Acclimatised for a week to experimental conditions
Life stage of the test species used	*Dugesia lugubris* - Half to fully grown *Bithynia tentaculata* – Sub-adults *Chaoborus obscuripes* - Larvae

Information on the test design	
Methodology used	The methodology is reasonably well described
Form of the test substance	Emulsifiable formulation Derosal® (active ingredient, 511 g/l)
Source of the test substance	Not stated
Type and source of the exposure medium	Test media were prepared in copper-free tapwater (total hardness 71.2-89.2 mg/l as $CaCO_3$, pH 8)
Test concentrations used	Not stated
Number of replicates per concentration	Acute and chronic: 2
Number of organisms per replicate	Acute and chronic: 10
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Acute: Semi-static, 96 hours, fed Chronic: Semi-static, 21 days, fed
Measurement of exposure concentrations	Yes (Exposure concentrations measured)
Measurement of water quality parameters	Yes (temperature = 18 ± 1°C)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	Generally well reported, but test species were not bred in a laboratory so their condition is uncertain

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