Proposed EQS for Water Framework Directive Annex VIII substances: 3,4-dichloroaniline *(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 the Environment Agency, 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 3,4-dichloroaniline (3,4-DCA) using the methodology described in Annex V of the Directive.

The PNECs described in this report are based on a technical assessment of the available ecotoxicity data for 3,4-DCA, 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 – text to be incorporated once done. 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.

An EU Risk Assessment Report (RAR) has been compiled for 3,4-DCA, and the UK has already committed to the use of RAR PNECs for the derivation of the Water Framework Directive Annex X EQSs. Consequently, in this document, RAR PNECs are recommended as the proposed PNECs. At this stage, no consideration has been taken of the feasibility of implementing these PNECS as EQSs, but that would be an essential step before a regulatory EQS can be recommended.

Properties and fate in water

Few data are available on the mode of toxic action of 3,4-DCA in aquatic organisms. However, polar narcosis is generally accepted as the primary mode of action

3,4-DCA is not expected to volatilise from the water column and neither is it expected to undergo hydrolysis. However, it is likely to be susceptible to photolysis with half-lives ranging from 0.4 hours to 6 days. On release to the aquatic environment 3,4-DCA forms covalent bonds with the organic fraction of sediments and suspended matter, removing it from the water column.

3,4-DCA would not be regarded as readily biodegradable. In a 14-day MITI-I biodegradation test (OECD 301 C) and a 28-day closed bottle test (OECD 301 D), both using activated sludge, no degradation of 3,4-DCA was reported. Similarly, in a coupled units test with activated sludge (OECD 303 A), less than 5% degradation was reported after 29 days. Based on the available data the majority (>90%) of 3,4-DCA released to the environment is expected to partition to the organic fraction of sediments and soils where, due to its slow degradation, it is likely to accumulate over time.

Availability of data

Long-term freshwater toxicity data are available for eight taxonomic groups including algae, crustaceans, fish, insects, macrophytes, molluscs, planarians and rotifers. Freshwater short-term toxicity data are available for nine taxonomic groups including algae, annelids, bacteria, ciliates, crustaceans, fish, insects, molluscs and rotifers. Long-term saltwater toxicity data are available for six taxonomic groups: algae, annelids, crustaceans, fish, molluscs and plankton. Short-term saltwater toxicity data are available for seven different taxonomic groups: algae, annelids, bacteria, crustaceans, fish, molluscs and rotifers. Fish and crustaceans appear to be the most sensitive organisms to water column exposures of 3,4-DCA.

In addition, both freshwater and saltwater toxicity data are available for sediment dwelling organisms. Freshwater long and short-term data are available for sediment dwelling annelids and insects. Marine long and short-term toxicity data for sediment dwelling organisms are available only for annelids. Annelids appear to be the most sensitive organisms to sediment exposures of 3,4-DCA.

Both freshwater and saltwater mesocosm and field studies are available. However, each study used different exposure systems with varying exposure concentrations. Consequently, there are a range of different endpoints and effect concentrations. The lowest endpoints from the available studies are MATCs of 8-10 µg l^{-1} for zooplankton abundance and sediment invertebrate abundance in an outdoor stream experiment. Based on the available data 3,4-DCA concentrations below 10 µg l^{-1} should have little effect on field populations of aquatic organisms.

Based on limited data 3,4-DCA may have some effects on the endocrine systems of wildlife. However, endocrine effects appear to occur at concentrations higher than those causing effects on survival and growth in standard toxicity tests.

Derivation of PNECs

Long-term PNEC for freshwaters

Long-term freshwater toxicity data are available for eight taxonomic groups including algae, crustaceans, fish, insects, macrophytes, molluscs, planarians and rotifers. The lowest valid long-term freshwater data points are 42 and 48 day NOECs (for growth and survival) of 2 µg l^{-1} in guppies and zebra fish. Both values were generated in flow-through studies with measured exposure concentrations and were regarded by the 3,4-DCA RAR as fully valid for PNEC derivation. The long-term freshwater PNEC in the EU RAR for 3,4-DCA was therefore based on these data points with an assessment factor of 10 given the availability of long-term data for three or more trophic levels. This results in a **$PNEC_{freshwater\_lt}$ = 2 µg l^{-1}/AF (10) = 0.2 µg l^{-1}.**

A number of field and mesocosm studies are available for 3,4-DCA. The lowest endpoints from the available studies were MATCs of 8-10 µg l^{-1} for zooplankton abundance and sediment invertebrate abundance in an outdoor stream experiment. In addition, an MATC of 10 µg l^{-1} 3,4-DCA has been suggested to be protective of field populations of *Daphnia*. Therefore the proposed PNEC of 0.2 µg l^{-1} would be regarded as protective of long-term exposures to 3,4-DCA in the field.

There are no existing EQSs for 3,4-DCA for comparison with the proposed PNECs.

Short-term PNEC for freshwaters

Freshwater short-term toxicity data are available for nine taxonomic groups including algae, annelids, bacteria, ciliates, crustaceans, fish, insects, molluscs and rotifers. The lowest valid short-term freshwater data point is a 48-hour EC50 of 54 µg l^{-1} for the immobilisation of *D. magna*. This is a replicated, static study with measured exposure concentrations.

No short-term freshwater PNEC was derived in the EU RAR for 3,4-DCA. The lowest short-term value identified by the RAR was a 96-hour LC50 of 160 µg l^{-1} for *D. magna*. However, the lowest valid value identified in this report is the 48-hour EC50 of 54 µg l^{-1} for the immobilisation of *D. magna*. Consequently, it is proposed that the short-term freshwater PNEC be based on the lower EC50 of 54 µg l^{-1} and an assessment factor of

10. The assessment factor of 10 is felt justified due to the availability of reliable short-term data for at least three taxonomic groups. This results in a **$PNEC_{freshwater\_st}$ = 54 µg l^{-1} /AF (10) = 5.4 µg l^{-1}.**

There are no existing EQSs for 3,4-DCA for comparison with the proposed PNECs.

Long-term PNEC for saltwaters

Long-term saltwater toxicity data are available for six taxonomic groups: algae, annelids, crustaceans, fish, molluscs and plankton.

No PNEC specific to saltwater was derived in the EU RAR for 3,4-DCA. Instead the freshwater and saltwater datasets were combined and a $PNEC_{aqua}$ calculated without the addition of an additional assessment factor. The lowest reliable value in the saltwater data set is a 38-day NOEC (reproduction) of 3.2 µg l^{-1} for the polychaete worm (*Ophryotrocha diadema*). This datum is based on measured exposure concentrations and was regarded by the RAR as fully valid for PNEC derivation. However, in the combined dataset there are lower NOECs (42 and 48 day) of 2 µg l^{-1} for the growth/survival of guppies and zebra fish. Given the similarity in the sensitivity of freshwater and saltwater species of the same taxonomic group and the non-specific mode of action of 3,4-DCA as a polar narcotic it is proposed that the saltwater PNEC be based on the lower freshwater data. As a consequence of this and given that good quality data are available for a range of taxonomic groups the same assessment factor (10) applied to the freshwater PNEC is also applicable for the saltwater PNEC resulting in a **$PNEC_{Saltwater\_lt}$ = 2 µg l^{-1} /AF (10) = 0.2 µg l^{-1} .**

This is supplemented by a very similar PNEC of 0.32 µg l^{-1} that could be derived by applying an assessment factor of 10 to the 38-day NOEC (reproduction) of 3.2 µg l^{-1} for the polychaete worm (*Ophryotrocha diadema*).

There were only very limited field data for salt waters. In marine enclosures, effects on phytoplankton, zooplankton and bacteria occurred only at 3,4-DCA concentrations of >100 µg l^{-1}. These limited data suggest that the proposed long-term saltwater PNEC would be protective of field populations of saltwater phytoplankton, zooplankton and bacteria.

There are no existing EQSs for 3,4-DCA for comparison with the proposed PNECs

Short-term PNEC for saltwaters

Short-term saltwater toxicity data are available for seven different taxonomic groups: algae, annelids, bacteria, crustaceans, fish, molluscs and rotifers. The lowest reliable short-term saltwater value is a 72-hour EC50 (growth) of 1100 µg l^{-1} for *Phaeodactylum tricornutum*. However, in the combined data set a lower 48-hour EC50 of 54 µg l^{-1} is available for *D. magna*, based on immobilisation. This value was generated in a replicated, static study with measured exposure concentrations. Consequently, it is regarded as fully valid for PNEC derivation.

No short-term saltwater PNEC was derived in the 3,4-DCA RAR. Consequently, the lowest valid value identified in this report (48-hour EC50 (Immobilisation) of 54 µg l^{-1} for *D. magna*) is proposed as the critical data for the derivation of the short-term saltwater PNEC for 3,4-DCA. Given the similarity in the sensitivity of freshwater and saltwater species of the same taxonomic group and the mode of action of 3,4-DCA as a polar narcotic it is proposed that the same assessment factor (10) be used for the freshwater

short-term PNEC for the saltwater environment. Therefore the saltwater PNEC(s) for short term is:

$PNEC_{saltwater\_st}$ = 54 µg l^{-1}/AF (10) = 5.4 µg l^{-1}

There are no existing EQSs for 3,4-DCA for comparison with the proposed PNECs

PNECs for sediment

The log Kow for 3,4-DCA is 2.7 and in theory does not meet the EU TGD criterion for the assessment of sediment dwelling organisms. However, on release to the aquatic environment 3,4-DCA forms covalent bonds with the organic fraction of sediments and suspended matter, removing it from the water column. Consequently, sediments are one of the primary sinks for environmental releases of 3,4-DCA and sediment PNECs are required.

The EU RAR identifies the 28 day LOEC of 5 mg/kg dw of Oetken *et al.* (2000) for deformations of *L. variegatus* as the lowest reliable sediment data, but the effect was not statistically significant. However, this study did report 28-d NOECs of 5 mg/kg dw for effects of 3,4-DCA on the number and total biomass of worms. Consequently, the appropriate assessment factor for two long-term sediment values is 50 resulting in a **$PNEC_{freshwater\ sediment}$= 5 mg/kg/AF (50) = 0.1 mg/kg dw (0.04 mg/kg ww).**

There are no marine sediment exposure data available. However, based on the available data there appears to be no significant differences in the sensitivity of freshwater or saltwater species of the same taxonomic group. This may reflect the mode of toxic action of 3,4-DCA as a polar narcotic. Consequently, the freshwater and saltwater datasets for 3,4-DCA can be combined. As such it is proposed that the freshwater sediment PNEC be adopted as the marine sediment PNEC.

PNECs for secondary poisoning

The EU RAR identifies a NOAEL of 30 mg/kg body weight per day 2,5-DCA from a rat oral toxicity study as most suitable for the derivation of the $PNEC_{secpois.biota}$**.** The RAR proposes a conversion factor of 10 (to convert the dose to a concentration in food) and an assessment factor of 1000, giving:

$PNEC_{secpois.biota}$ = (NOAEL 30 mg/kg bw x 10)/AF 1000 = 0.3 mg/kg in food

(The 2,5-DCA NOAEL was chosen due to a lack of suitable data for 3,4-DCA. The EU RAR stated that due to the structural similarity of 2,5-DCA to 3,4-DCA it causes similar toxic effects such as haemolytic anaemia and methaemoglobinaemia. Consequently, 2,5-DCA was considered a suitable surrogate for 3,4-DCA (ECB 2006)).

Reported BCF values range from 4 to 800. However, the RAR identifies a BAF of 570 for *Lumbriculus variegatus* as most suitable for PNEC derivation. Consequently, the concentration in water preventing bioaccumulation in prey to levels >$PNEC_{secpois.biota}$ is:

$PNEC_{secpois.water}$ = 0.3 mg/kg prey/BAF (570) = 0.0005 mg l^{-1} (0.5 µg l^{-1})

This is the calculation made by the RAR. However, the AF (1000) used appears to be far too high and it could be that they have used the conversion factor incorrectly or duplicated it and added also to the AF. If this is correct the resulting $PNEC_{secpois.water}$ is overprecautionary and any reduction in the assessment factor would result in a higher value.

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

Summary of proposed PNECs

Receiving medium/exposure scenario	Proposed PNEC (µg l^{-1})	Existing EQS (µg l^{-1})
Freshwater/long-term	0.2	-
Freshwater/short-term	5.4	-
Saltwater/long-term	0.2	-
Saltwater/short-term	5.4	-
Sediment (freshwater and saltwater)	0.1 mg/kg dw (0.04 mg/kg ww)	-
Secondary poisoning	0.5	-

Analysis

Proposed PNECs derived for 3,4-DCA range from 0.2 to 5.4 µg l^{-1} in environmental waters and 0.1 mg/kg dw (0.04 mg/kg ww) in sediments. The data quality requirements are that, at a third of the EQS total error of measurement should not exceed 50 per cent. Using this criterion, current analytical methodologies should offer adequate performance to analyse for 3,4-DCA

Implementation issues

The proposed PNECs are consistent with those proposed in the EU Risk Assessment Report for 3,4-DCA (ECB, 2006). These PNECs are suitable for use as they are not subject to excessive uncertainty and current analytical capability should be adequate for compliance assessment purposes. Due to the potential for 3,4-DCA to adsorb to sediment and bioaccumulate consideration should be given as to the relevance of sediment and biota standards for this substance.

Contents

1	**Introduction**	**1**
1.1	Properties and fate in water	1
2	**Results and observations**	**3**
2.1	Identity of substance	3
2.2	PNECs proposed for derivation of quality standards	3
2.3	Hazard classification	3
2.4	Physical and chemical properties	4
2.5	Environmental fate and partitioning	4
2.6	Effects data	8
3	**Calculation of PNECs as a basis for the derivation of quality standards**	**34**
3.1	Derivation of PNECs by the TGD deterministic approach (AF method)	34
3.2	Derivation of PNECs by the TGD probabilistic approach (SSD method)	40
3.3	Derivation of existing EQSs	40
3.4	Derivation of PNECs for sediment	40
3.5	Derivation of PNECs for secondary poisoning of predators	42
4	**Analysis and monitoring**	**46**
5	**Conclusions**	**47**
5.1	Availability of data	47
5.2	Derivation of PNECs	47
5.3	Analysis	51
5.4	Implementation issues	51
References & Bibliography		**52**
List of abbreviations		**58**
ANNEX 1 Data quality assessment sheets		**60**

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 3,4-dichloroaniline (3,4-DCA) using the methodology described in Annex V of the Directive.

The PNECs described in this report are based on a technical assessment of the available ecotoxicity data for 3,4-DCA, 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. Critical data have been identified and assessment factors selected in accordance with the guidance given in Annex V of the WFD.

An EU Risk Assessment Report (RAR) has been compiled for 3,4-DCA (ECB 2006), and the UK has already committed to the use of RAR PNECs for the derivation of the WFD Annex X EQSs. Consequently, in this document, where RAR PNECs have been derived these are recommended as the proposed PNECs. At this stage, no consideration has been taken of the feasibility of implementing these PNECS as EQSs, but that would be an essential step before a regulatory EQS can be recommended.

1.1 Properties and fate in water

3,4-DCA is a chemical intermediate used in the production of 3,4-dichlorophenylisocyanate, herbicides, biocides and azo dyes. The chemical itself has no direct uses and so enters the environment through waste streams or as an impurity or degradation/transformation product of chemicals produced from 3,4-DCA (ECB 2006).

Few data are available on the mode of toxic action of 3,4-DCA in aquatic organisms. However, polar narcosis is generally accepted as the primary mode of action (Bearden and Schultz 1997 and Argese *et al.* 2001).

3,4-DCA is not expected to volatilise from the water column and neither is it expected to undergo hydrolysis. However, it is likely to be susceptible to photolysis with half-lives ranging from 0.4 hours to 6 days (ECB 2006). On release to the aquatic environment 3,4-DCA forms covalent bonds with the organic fraction of sediments and suspended matter, removing it from the water column. In water/sediment experiments using radio labelled 3,4-DCA, only 21% radioactivity remained in the water column after 8 days and only 1% remained after 90 days (Heim *et al.* 1994 cited in ECB 2006).

Based on the available data 3,4-DCA would not be regarded as readily biodegradable. In a 14-day MITI-I biodegradation test (OECD 301 C) and a 28-day closed bottle test (OECD 301 D), both using activated sludge, no degradation of 3,4-DCA was reported (CITI 1992 and Janicke and Hilge 1980 cited in ECB 2006). Similarly, in a coupled units test with activated sludge (OECD 303 A), less than 5% degradation was reported after 29 days (Janicke and Hilge 1980 cited in ECB 2006).

[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

In non-acclimated sediments, under anaerobic conditions, 90% of the applied 3,4-DCA was dechlorinated to 3-chloroaniline (44%) and 4-chloroaniline (33%) within 60 days (Struijs and Rogers 1989 cited in ECB 2006). However, dechlorination only started after 20 days and the products (3- and 4-chloroaniline) did not undergo additional degradation. In soils low mineralisation rates are reported with 3.9-11.9% mineralisation of 1 mg/kg radio labelled 3,4-DCA after 16 weeks in 4 different soil types. These degradation rates correspond to DT50 values of approximately 470-1500 days (Süß *et al*. 1978 cited in ECB 2006).

Based on the available data the majority (>90%) of 3,4-DCA released to the environment is expected to partition to the organic fraction of sediments and soils where, due to its slow degradation, it is likely to accumulate over time (ECB 2006).

2 Results and observations

2.1 Identity of substance

Table 2.1 gives the chemical name and Chemical Abstracts Service (CAS) number for 3,4-DCA.

Table 2.1 Chemical species covered by this report

Name	CAS Number
3,4-dichloroaniline (3,4-DCA)	95-76-1

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 3,4-DCA. 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	5.4 µg l^{-1}	_	-
Freshwater long-term	0.2 µg l^{-1}	Insufficient data	-
Saltwater short-term	5.4 µg l^{-1}	_	-
Saltwater long-term	0.2 µg l^{-1}	Insufficient data	-
Sediment (freshwater and saltwater)	0.1 mg/kg dw (0.04 mg/kg ww)	Insufficient data	-
Secondary poisoning	0.5 µg l^{-1}	–	-

AF = Assessment Factor
SSD = Species Sensitivity Distribution

2.3 Hazard classification

Table 2.3 gives the R-phrases (Risk-phrases) and labelling for 3,4-DCA.

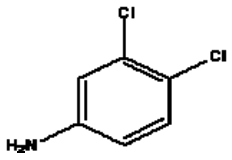
Table 2.3 Hazard classification

R-Phrases and Labelling	Reference:
R23/24/25, R41, R43, R50-53	ECB 2006

2.4 Physical and chemical properties

Table 2.4 summarises the physical and chemical properties of 3,4-DCA.

Table 2.4 Physical and chemical properties of 3,4-DCA

Property	Value	Ref.
Molecular formula	$C_6H_5Cl_2N$	Verschueren 1996
Molecular structure		Cambridgesoft 2006
Molecular weight	162 g/mol	ECB 2006
Appearance	Solid at 20°C	ECB 2006
Melting point (°C)	72°C	ECB 2006
Boiling point (°C)	272°C at 1013 hPa	ECB 2006
Vapour pressure	0.184 Pa at 20°C	ECB 2006
Henry's law constant	0.05 $Pa.m^3/mol$	ECB 2006
Water solubility	580 mg l^{-1} at 20°C	ECB 2006
Octanol-water partition coefficient (log K_{ow})	2.7 (shake-flask method)	ECB 2006

2.5 Environmental fate and partitioning

Table 2.5 summarises the information obtained from the literature on the environmental fate and partitioning of 3,4-DCA.

Table 2.5 Environmental fate and partitioning of 3,4-DCA

Property	Value:	Ref.
Hydrolytic stability (DT_{50})	Based on the molecular structure of 3,4-DCA hydrolysis is not expected.	ECB 2006

Property	Value:	Ref.
Photostability	A half-life of 0.4 hours was calculated based on quantum yield of direct phototransformation in water (average yearly value at water surface at 40-50° latitude).	ECB 2006
	A half-life of 4.1-6.3 days has been determined for a model pond (1m depth, 52° latitude).	ECB 2006
	Half-lives of 6 ± 3.6 hours have been reported at water surfaces in natural sunlight at 40° latitude in the summer.	ECB 2006
	In the atmosphere, a half-life for the reaction with hydroxyl radicals of 0.4 days is reported.	Verschueren 1996
Volatility	Based on a Henry's Law constant of 0.05 $Pa.m^3/mol$ only low levels of volatilisation from water are expected.	ECB 2006
Readily biodegradable	Based on the available biodegradation data 3,4-DCA would not be regarded as readily biodegradable.	ECB 2006
	14-day MITI-I biodegradation test with activated sludge (OECD 301 C): 0% degradation.	ECB 2006
	28-day closed bottle test with activated sludge (OECD 301 D): 0% degradation.	ECB 2006
	29-day coupled units test with activated sludge (OECD 303 A): <5% degradation.	ECB 2006
	28-day closed bottle test (OECD 301 D), adapted industrial sludge: 82% degradation.	ECB 2006
	In a degradation test with water from the Rhine, 45% primary degradation occurred at a substrate concentration of 0.01 mg l^{-1} after 30 days and >95% primary degradation at a concentration of 1 mg l^{-1} after 50 days.	ECB 2006
	In anaerobic biodegradation tests with fresh water and pond sediments 90% of the applied 3,4-DCA was dechlorinated within 60 days.	ECB 2006
Degradation in Water/sediment - DT_{50} water - DT_{50} sediment	 - -	 ECB 2006
Mineralisation	In a 16-week study, radio labelled 3,4-DCA was added to four different soils at a concentration of 1 mg/kg. 3.9-11.9% mineralisation occurred, corresponding to DT50s for mineralisation of 470-1500 days.	ECB 2006
Bound residue	3,4-DCA forms covalent bonds with the organic fraction in soil and sediment. The extent of bonding is dependent on 3,4-DCA concentration and the nature of the soil, but is typically >90%.	ECB 2006

Property	Value:	Ref.
Distribution in water / sediment systems	In a laboratory sediment/water study, radiolabelled 3,4-dichloroaniline was applied to water containing three sediments collected from a creek, a pond and a drainage ditch of a fruit growing plantation. After 8 days, 21.8% of the applied radioactivity was present in the water phase of the creek sediment system. After 90 days, 10.4% of the applied radioactivity was present in the water phase of the pond sediment system and again after 90 days only 1% was present in the water phase of the drainage sediment system.	ECB 2006
Environmental residues	Irradiation (sun lamp) of a 0.8 µg l^{-1} solution of 3,4-DCA in distilled water resulted in the formation of 2-chloro-5-aminophenol (78%) and 3-chloroaniline (2%). In anaerobic biodegradation tests with fresh water and pond sediments 90% of the applied 3,4-DCA was dechlorinated to form 3-chloroaniline (44%) and 4-chloroaniline (33%) after 60 days. Microbial degradation of 3,4-DCA can result in the formation of 3,3',4,4'-tetrachloroazobenzene (TCAB) and 3,3',4,4'-tetrachloroazoxybenzene (TCAOB).	ECB 2006 ECB 2006 ECB 2006
Degradation in soil	In a 16-week study, radio labelled 3,4-DCA was added to four different soils at a concentration of 1 mg/kg. 3.9-11.9% mineralisation occurred, corresponding to DT50 for mineralisation of 470-1500 days.	ECB 2006
Partition coefficient (log K_{OW}) pKa Koc Sediment – water Suspended matter – water	2.7 - 344 l/kg (estimated from Kow) 190 l/kg (measured) 1900-10400 l/kg (measured) - -	ECB 2006 ECB 2006
BCF BAF	Carp (*Cyprinus carpio*) = 4.1-14.4 Zebra fish (*Danio rerio*) = 30-38 Rainbow trout (*Oncorhynchus mykiss*) = 45 Guppy (*Poecilia reticulata*) = 34 Hornwort (*Ceratophyllum demersum)* = 82 Water flea (*Daphnia magna*) = 9 Aquatic sowbug (*Asellus aquaticus*) = 10 Snail (*Planorbarius corneus*) = 12 Worm (*Tubifex tubifex*) = 18 Hornwort (*Ceratophyllum demersum)* = 113 Water flea (*Daphnia magna*) = 276 Aquatic sowbug (*Asellus aquaticus*) = 76 Snail (*Planorbarius corneus*) = 533 Worm (*Tubifex tubifex*) = 271 Worm (*Lumbriculus variegatus*) = 570	IUCLID 2000 ECB 2006 ECB 2006

3,4-DCA is a chemical intermediate used in the production of 3,4-dichlorophenylisocyanate and the herbicides propanil, linuron, diuron, and neburon (ECB 2006). It is also used in the production of azo dyes for polyester fabrics. The chemical itself has no direct uses and so enters the environment through waste streams or as an impurity or degradation / transformation product of chemicals produced from 3,4-DCA (ECB 2006).

Based on a Henry's law constant of 0.05 $Pa.m^3/mol$ 3,4-DCA is not expected to volatilise from the water column. Neither is it expected to undergo hydrolysis. However, it is likely to be susceptible to photolysis with half-lives ranging from 0.4 hours to 6 days (ECB 2006). On release to the aquatic environment 3,4-DCA forms covalent bonds with the organic fraction of sediments and suspended matter, removing it from the water column. In water/sediment experiments using radio labelled 3,4-DCA only 21% radioactivity remained in the water column after 8 days and only 1% remained after 90 days (Heim *et al.* 1994 cited in ECB 2006). Similar results have been reported in laboratory microcosms where 71% of the applied parent compound (based on radioactivity) was bound to sediment and 2.4% was bound to suspended matter in the water (Nagel 1997 cited in ECB 2006).

Based on the available data 3,4-DCA would not be regarded as readily biodegradable. In a 14-day MITI-I biodegradation test (OECD 301 C) and a 28-day closed bottle test (OECD 301 D), both using activated sludge, no degradation of 3,4-DCA was reported (CITI 1992 and Janicke and Hilge 1980 cited in ECB 2006). Similarly, in a coupled units test with activated sludge (OECD 303 A), less than 5% degradation was reported after 29 days (Janicke and Hilge 1980 cited in ECB 2006). Conversely, 82% degradation has been reported in a 28-day closed bottle test (Bayer 1987 cited in ECB 2006). However, adapted industrial sludge was used and as such the EU RAR for 3,4-DCA stated that this result was not relevant to the wider environment.

Degradation data for environmental waters are conflicting. In a river water (Rhine River) degradation test 45% primary degradation occurred at a substrate concentration of 0.01 mg l^{-1} within 30 days and >95% primary degradation occurred at a concentration of 1 mg l^{-1} within 50 days (Bayer 1992 cited in ECB 2006) . However, in contrast, when water from the North Sea was used as an innoculum no degradation was observed (Kuiper and Hanstveit 1984 cited in ECB 2006).

In non-acclimated sediments, under anaerobic conditions, 90% of the applied 3,4-DCA was dechlorinated to 3-chloroaniline (44%) and 4-chloroaniline (33%) within 60 days (Struijs and Rogers 1989 cited in ECB 2006). However, dechlorination only started after 20 days and the products (3- and 4-chloroaniline) did not undergo additional degradation. In soils low mineralisation rates are reported with 3.9-11.9% mineralisation of 1 mg/kg radio labelled 3,4-DCA after 16 weeks in 4 different soil types. These degradation rates correspond to DT50 values of approximately 470-1500 days (Süß *et al* 1978 cited in ECB 2006). The rate of mineralisation in soils is reported to decrease as the concentration of 3,4-DCA increases (ECB 2006).

Based on the available data the majority (>90%) of 3,4-DCA released to the environment is expected to partition to the organic fraction of sediments and soils where, due to its slow degradation, it is likely to accumulate over time (ECB 2006).

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. Critical data on freshwater and marine organisms (water column and sediments) were collected from the EU Risk Assessment Report (RAR) for 3,4-DCA (ECB 2006). Further data were then retrieved from the US Environmental Protection Agency (US EPA) ECOTOX database[2].

Further data sources used included:

- ScienceDirect®;[3]
- Hazardous Substances Data Bank (HSDB®) of the US National Library of Medicine;[4]

Toxicity data and other information on the inherent properties of 3,4-DCA taken from the EU risk assessment report were not subjected to additional quality assessment in this data sheet as the data were already quality assessed by the authors of the risk assessment. Where such data have been used in this report the quality criteria assigned by the RAR document have been reported and prefixed by the word RAR to identify it as an RAR assigned quality criteria. Studies identified in this report that were not covered by the RAR have been subject to quality assessment and suitable quality criteria assigned.

2.6.1 Toxicity to freshwater organisms

Freshwater toxicity data on 3,4-DCA are available for various taxonomic groups including algae, invertebrates and fish as required for the application of the assessment factor approach specified in the EU Technical Guidance Document (TGD) (ECB 2003). Long-term data are available for eight taxonomic groups including algae, crustaceans, fish, insects, macrophytes, molluscs, planarians and rotifers. Freshwater short-term toxicity data are available for nine taxonomic groups including algae, annelids, bacteria, ciliates, crustaceans, fish, insects, molluscs and rotifers.

Diagrammatic representations of the available freshwater data (cumulative distribution functions) for 3,4-DCA 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 3,4-DCA PNECs. The lowest critical freshwater data for 3,4-DCA are presented in Tables 2.6 and 2.7.

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

Figure 2.1 Cumulative distribution function of freshwater long-term data (µg l^{-1}) for 3,4-DCA

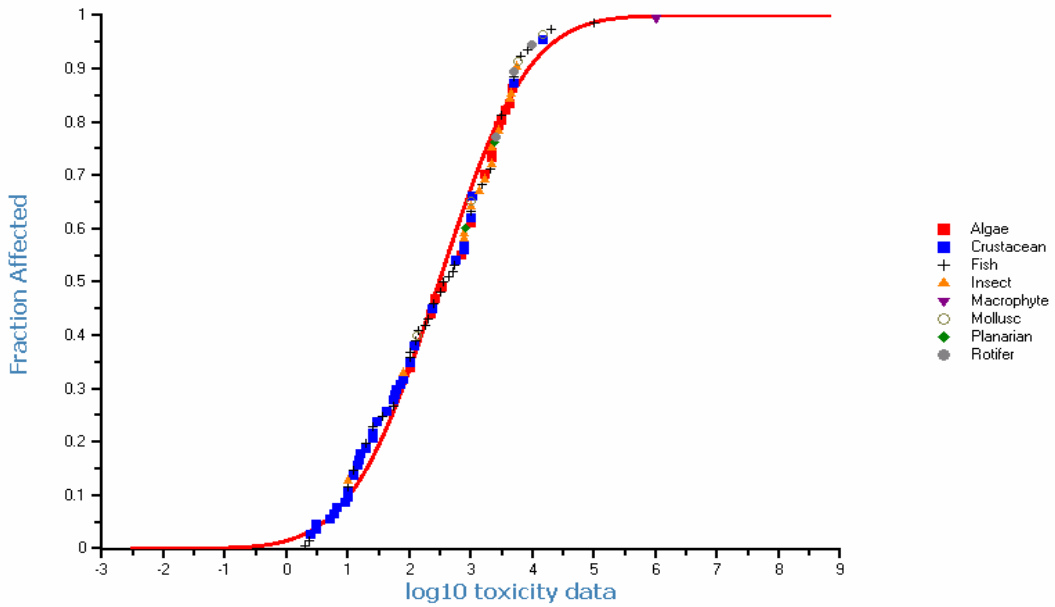


Figure 2.2 Cumulative distribution function of freshwater short-term data (µg l^{-1}) for 3,4-DCA

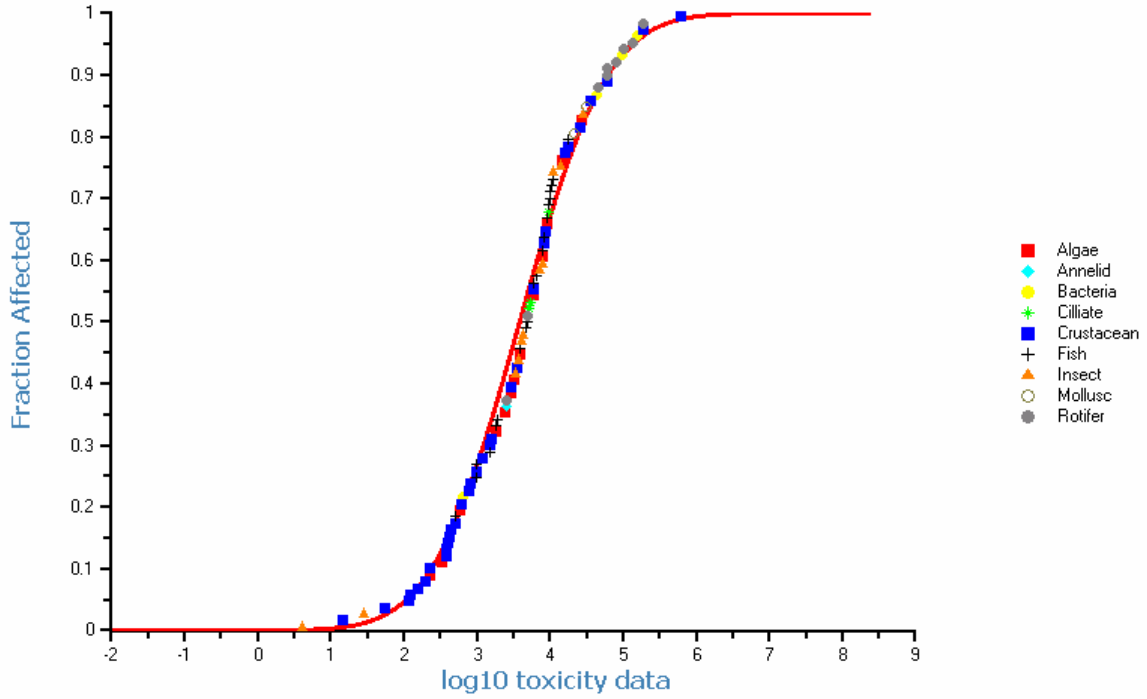


Table 2.6 Lowest available 3,4-dichloroaniline long-term aquatic toxicity data for freshwater organisms

Scientific Name	Common Name	Taxon. Grp.	End-point	Effect	Test Duration (hours)	Conc. (µg l^{-1})	Environmental conditions	Expo-sure[1]	Toxicant analysis[2]	Comment	Reliability (Klimisch code*)	Reference
Planarian												
Polycelis sp.	Planarian	PLAN	NOEC LOEC	Population abundance	28 day	800 2400	Temp. 10-22°C; DO 3-19 mg l^{-1}; pH 7.6-8.9 ; hardness 220-270 mg $CaCO_3$ l.	f	y	Outdoor mesocosm	NA	Girling *et al*. (2000)
Algae												
Algae	Algae	ALG	MATC	Population biomass	26 day	>100-<1000	Temp. 10-22°C; DO 3-19 mg l^{-1}; pH 7.6-8.9 ; hardness 220-270 mg $CaCO_3$ l.	f	y	Outdoor mesocosm	NA	Girling *et al.* (2000)
Chlamydomonas reinhardtii	Green algae	ALG	EC10	Population growth	96	225	Temp. 24 +/-1°C; illumination (7000 lux) for 14 hours/day; pH 6-7	f	y	OECD guideline 201.	2	Schafer *et al.* (1993)
Chlamydomonas reinhardtii	Green algae	ALG	NOEC	Population growth	96	260	Temp. 24 +/-1°C; illumination (7000 lux) for 14 hours/day.	f	y	OECD guideline 201. chemicals dissolved in acetone pa.	2	Schafer *et al.* (1994)
Chlamydomonas reinhardtii	Green algae	ALG	NOEC	Population growth	7 day	330	Temp. 24 +/-1°C; illumination (7000 lux) for 14 hours/day.	f	y	OECD guideline 201. chemicals dissolved in acetone pa.	2	Schafer *et al.* (1994)
Scenedesmus pannonicus	Green algae	ALG	NOEC	Population growth	96	1000		s			RAR (Valid)	Adema *et al.* (cited in ECB 2006) (1982)
Macrophytes												
Lemna minor	Duckweed	MAC	LOEC		28 day	>1000000	Temp. 10-22°C; DO 3-19 mg l^{-1}; pH 7.6-8.9 ; hardness 220-270 mg $CaCO_3$ l.	f	y	Outdoor mesocosm	NA	Girling *et al.* (2000)
Crustaceans												
Daphnia magna	Waterflea	CRU	NOEC LOEC	Reproduction	21 day	5 10		ss	y	Renewal 1/week	RAR (Valid)	UBA (cited in ECB 2006) (1994)
Daphnia magna	Waterflea	CRU	NOEC	Mortality/ Reproduction	21 day	6.5			y		RAR (Valid)	Adema and Vink (cited in ECB 2006) (1981)
Daphnia magna	Waterflea	CRU	NOEC LOEC	Reproduction (# offspring)	14 day	5 10	Temp. 20°C ; 16:8h light:dark cycle ; DO >75% ; pH 7.0-8.0.	f	n		RAR (Valid)	Diamantino *et al.* (cited in ECB 2006) (1997)
Daphnia magna	Waterflea	CRU	NOEC LOEC	Reproduction (# offspring)	14 day	2.5 5	Temp. 20°C ; 16:8h light:dark cycle ; DO >75% ; pH 7.0-8.0.	ss	n	Third brood neonates (<24h old).	RAR (Valid)	Diamantino *et al.* (cited in ECB 2006) (1997)
Daphnia magna	Waterflea	CRU	NOEC	Aborted	14 day	5	Temp. 20°C ; 16:8h	ss	n	Third brood neonates	RAR	Diamantino *et al.* (cited

Scientific Name	Common Name	Taxon. Grp.	End-point	Effect	Test Duration (hours)	Conc. (µg l^{-1})	Environmental conditions	Expo-sure[1]	Toxicant analysis[2]	Comment	Reliability (Klimisch code*)	Reference
			LOEC	reproduction		10	light:dark cycle ; DO >75% ; pH 7.0-8.0.			(<24h old).	(Valid)	in ECB 2006) (1997)
Daphnia magna	Waterflea	CRU	NOEC	Adult length	21 day	<3.1	Temp. 20°C ; 16:8h light:dark cycle	ss	n	OECD guideline 202.	2	Samel *et al.* (1999)
Daphnia magna	Waterflea	CRU	NOEC	live neonates	21 day	3.1	Temp. 20°C ; 16:8h light:dark cycle	ss	n	OECD guideline 202.	2	Samel *et al*. (1999)
Daphnia magna	Waterflea	CRU	LC50	Egg mortality	72	14.58	14/10 hour light/dark cycle; Temp. 20 +/- 1°C.	ss	y	Test solutions changed daily.	2	Barata and Baird (2000)
Gammarus pulex	Scud	CRU	NOEC	Precopula separation time	26 day	60	Temp. 10-22°C; DO 3-19 mg l^{-1}; pH 7.6-8.9 ; hardness 220-270 mg $CaCO_3$ l.	f	y	Outdoor mesocosm	NA	Girling *et al.* (2000)
Gammarus pulex	Scud	CRU	NOEC	Growth	25 day	760	Hardness 229 mg l^{-1}; Temp. 17°C; DO 10.16 mg l^{-1}: pH = 8.6	f	n	Mesocosm study	NA	Taylor *et al.* (1994)
Insects												
Baetis rhodani	Mayfly	INS	LOEC	Population abundance	28 day	>10-<100	Temp. 10-22°C; DO 3-19 mg l^{-1}; pH 7.6-8.9 ; hardness 220-270 mg $CaCO_3$ l.	f	y	Outdoor mesocosm	NA	Girling *et al.* (2000)
Chironomus riparius	Midge	INS	NOEC	Growth	25 day	80	Hardness 229 mg l^{-1}; Temp. 17°C; DO 10.16 mg l^{-1}; pH = 8.6	f	n	Mesocosm study	NA	Taylor *et al.* (1994)
Chironomus riparius	Midge	INS	NOEC	Growth	12 day	760	Hardness 229; mg l^{-1}; Temp. 17°C; DO 10.16 mg l^{-1}; pH = 8.6		y	Mesocosm study	NA	Taylor *et al.* (1994)
Rotifer												
Brachionus calyciflorus	Rotifer	ROT	ND	Population reduction	10 day	5000		ss	n		NA	Ferrnando *et al.* (1993)
Molluscs												
Lymnaea stagnalis	Great pond snail	MOL	NOEC	Morphology/ egg hatching	16 day	130	DO >70%; pH ~ 8		y		NA	Adema and Vink (1981)
Lymnaea stagnalis	Great pond snail	MOL	EC50	Development	16 day	1000	DO >70%; pH ~ 8		y		NA	Adema and Vink (1981)
Fish												
Pimephales promelas	Fathead minnow	FIS	NOEC	Growth	28 day	5.1	Lake water	f	y	Egg fry ELS	RAR (Valid)	Call *et al.* (cited in ECB 2006) (1987)
Poecilia reticulata	Guppy	FIS	NOEC LOEC	Growth	42 day	20 200	DO >70%; pH ~ 8	f	y	Females F0 generation	RAR (Valid)	Schafers and Nagel (cited in ECB 2006) (1991)

Scientific Name	Common Name	Taxon. Grp.	End-point	Effect	Test Duration (hours)	Conc. (µg l^{-1})	Environmental conditions	Expo-sure[1]	Toxicant analysis[2]	Comment	Reliability (Klimisch code*)	Reference
Poecilia reticulata	Guppy	FIS	NOEC LOEC	Growth	42 day	2 20	DO >70%; pH ~ 8	f	y	Females FI generation	RAR (Valid)	Schafers and Nagel (cited in ECB 2006) (1991)
Poecilia reticulata	Guppy	FIS	NOEC LOEC	Reproduction	42 day	20 200	DO >70%; pH ~ 8	f	y	F0 generation	RAR (Valid)	Schafers and Nagel (cited in ECB 2006) (1991)
Poecilia reticulata	Guppy	FIS	LOEC	Reproduction	42 day	2	DO >70%; pH ~ 8	f	y	FI generation	RAR (Valid)	Schafers and Nagel (cited in ECB 2006) (1991)
Brachydanio rerio	Zebrafish	FIS	NOEC	Growth	42 day	60		f	y	ELS, F0 generation	RAR (Valid)	Nagel and Schafers (1988) and Nagel (1991) (cited in ECB 2006)
Brachydanio rerio	Zebrafish	FIS	NOEC LOEC	Survival	42 day	20 200		f	y	ELS, FI generation	RAR (Valid)	Nagel and Schafers (1988) and Nagel (1991) (cited in ECB 2006)
Brachydanio rerio	Zebrafish	FIS	NOEC	Reproduction/ growth	42 day	20		f	y	ELS, FI generation	RAR (Valid)	Nagel and Schafers (1988) and Nagel (1991) (cited in ECB 2006)
Brachydanio rerio	Zebrafish	FIS	NOEC	Survival	42 day	2		f	y	ELS, FII generation	RAR (Valid)	Schafers and Nagel (cited in ECB 2006) (1991)
Brachydanio rerio	Zebrafish	FIS	LOEC	Survival	42 day	20		f	y	ELS, FII generation	RAR (Valid)	Nagel (cited in ECB 2006) (1988)
Brachydanio rerio	Zebrafish	FIS	NOEC LOEC	Survival	48 day	20 200	Temp. 23-28°C; pH 6.3-8.3	f	y	ELS, FI generation	RAR (Valid)	Nagel *et al.* (cited in ECB 2006) (1991)
Brachydanio rerio	Zebrafish	FIS	NOEC LOEC	Survival	48 day	2 20	Temp. 23-28°C; pH 6.3-8.3	f	y	ELS, FII generation	RAR (Valid)	Nagel *et al.* (cited in ECB 2006) (1991)
Oncorhynchus mykiss	Rainbow trout	FIS	EC25	Growth	28 day	100-390	DO >70%; Temp. 12.5-17.5°C; pH = 6.75-8.25	f	n		NA	Mallet *et al.* (1997)
Gasterosteus aculeatus	Threespine stickleback	FIS	MATC	Growth	26 days	>100-<1000	Temp. 10-22°C; DO 3-19 mg l^{-1}; pH 7.6-8.9 ; hardness 220-270 mg $CaCO_3$ l.	f	y	Outdoor mesocosm	NA	Girling *et al.* (2000)
Perca fluviatilis	Perch	FIS	LC50	Mortality	6 day	1500	Temp.18.8 +/-4°C; pH = 8.2 +/-0.2; DO > 60%	ss	n	Purity = 99.5% (Merck-Schuchart).	NA	Schafers and Nagel (1993)

[*] see Annex 1, For data where a numerical RI (1-4) is assigned a “Data Quality Assessment Sheet” is available in Annex 1, ‘RAR valid’ indicates that the respective study was already quality assessed in the EU risk assessment for 3,4-DCA and is considered valid for PNEC derivation, NA = As the data was not critical for PNEC derivation the reliability has not been assessed

[1] Exposure: f= flow-through; s = static; ss = semi static

[2] Toxicant analysis: y = measured, n = nominal

ALG = alga, CRU = Crustacea, FIS = Fish, INS = Insects, MAC = Macrophytes, MOL = Molluscs, PLAN = Planarians, ROT = Rotifers

NOEC = No Observed Effect Concentration, LOEC = Lowest Observed Effect Concentration, MATC = Maximum Acceptable Toxicant Concentration, EC10 = Concentration causing a 10% effect, EC25 = Concentration causing a 25% effect, EC50 = Concentration resulting in a 50% effect , LC50 = Concentration causing 50% mortality

Table 2.7 Lowest available 3,4-dichloroaniline short-term aquatic toxicity data for freshwater organisms

Scientific Name	Common Name	Taxon Grp	Endpoint	Effect	Test Duration (hours)	Conc. (µg l^{-1})	Environmental conditions	Exposure[1]	Toxicant analysis[2]	Comment	Reliability (Klimisch code*)	Reference
Bacteria												
Photobacterium phosphoreum	Bacteria	BAC	EC50	Light inhibition	0.5	650					NA	Ribo and Kaiser (1984)
Cilliates												
Tetrahymena pyriformis	Ciliate	CILL	EC50	Growth	24	9000	Temp. 24°C	s	n		NA	Schafer *et al.* (1994)
Tetrahymena pyriformis	Ciliate	CILL	NOEC	Physiology	96	5100	Temp. 24°C	s	n		NA	Schafer *et al.*(1994)
Algae												
Scenedesmus subspicatus	Green algae	ALG	EC50	Population biomass	48	6800	Temp. 24 +/-1°C; pH 8-9.1			Culture was still in logarithmic growth after 72 hours.	2	Kuhn and Pattard (1990)
Scenedesmus pannonicus	Green algae	ALG	EC50	Population growth	96	4800		s			RAR (Valid)	Adema *et al.* (cited in ECB 2006) (1982)
Scenedesmus quadricauda	Green algae	ALG	EC50	Growth	96	2200	DO >70%; pH ~ 8		y		2	Adema and Vink (1981)
Crustaceans												
Daphnia magna	Waterflea	CRU	LC50	mortality	96	160	DO >70%; pH ~ 8		y		RAR (Valid)	Adema and Vink (cited in ECB 2006) (1981)
Daphnia magna	Waterflea	CRU	EC50	Immobilisation	24	56		s	y		1	Pedersen *et al.* (1998)
Daphnia magna	Waterflea	CRU	EC50	Immobilsation	48	54		s	y		1	Pedersen *et al.* (1998)
Daphnia longispina	Waterflea	CRU	EC50	Immobilisation	48	440			y	Outdoor mesocosm	NA	Crossland and Hillaby (1985)
Gammarus pulex	Scud	CRU	LC50	Mortality	48	17400		ss	y		NA	Taylor *et al.* (1991)
Rotifers												
Brachionus calyciflorus	Rotifer	ROT	LT50	Mortality	81.6	2500	hard water (96 mg NaHCO3/l, 60 mg CaSO4/l.2H2O, 60 mg MgSO4/l and 4 mg KCl/l).	ss	y		1	Janssen *et al.* (1994)
Brachionus calyciflorus	Rotifer	ROT	LT50	Mortality	62	5000	hard water (96 mg NaHCO3/l, 60 mg CaSO4/l.2H2O, 60 mg MgSO4/l and 4 mg KCl/l).	ss	y		1	Janssen *et al.* (1994)

Scientific Name	Common Name	Taxon Grp	Endpoint	Effect	Test Duration (hours)	Conc. (µg l^{-1})	Environmental conditions	Exposure[1]	Toxicant analysis[2]	Comment	Reliability (Klimisch code*)	Reference
Brachionus calyciflorus	Rotifer	ROT	LC50	Mortality	24	61470		s	n		NA	Ferrando and Andreu-Moliner (1991)
Annelids												
Pristina longiseta	worm	ANN	LC50	Mortality	96	2500	Temperature = 26 +/- 1°C. Charcoal filtered tap water	s	y	Mesocosm study	NA	Schmitz and Nagel (1995)
Molluscs												
Lymnaea stagnalis	Pond snail	MOL	LC50	Mortality	48	32000			y		NA	Adema and Vink (1981)
Dreissena polymorpha	Zebra mussel	MOL	LC50	Mortality	96	22000	DO >70%; pH ~ 8		y		NA	Adema and Vink (1981)
Insects												
Aedes aegypti	Yellow fever mosquito	INS	LC50	Mortality	24	6600	Temp. 20 +/-1°C; 14:10h light:dark cycle. DO >80%; pH 5.9-10.5.	s	y		2	Ribeiro *et al.* (1995)
Aedes aegypti	Yellow fever mosquito	INS	LC50	Mortality	48	5200	Temp. 20 +/-1°C; 14:10h light:dark cycle. DO >80%; pH 5.9-10.5	s	y		2	Ribeiro *et al.* (1995)
Aedes aegypti	Yellow fever mosquito	INS	LC50	Mortality	72	4400	Temp. 20 +/-1°C; 14:10h light:dark cycle. DO >80%; pH 5.9-10.5	s	y		2	Ribeiro *et al.* (1995)
Aedes aegypti	Yellow fever mosquito	INS	LC50	Mortality	96	3400	Temp. 20 +/-1°C; 14:10h light:dark cycle. DO >80%; pH 5.9-10.5	s	y		2	Ribeiro *et al.* (1995)
Aedes aegypti	Yellow fever mosquito	INS	LC50	Mortality	96	6000	Temp. 20 +/-1°C; 14:10h light:dark cycle. DO >80%; pH 5.9-10.5	s	y		2	Ribeiro *et al.* (1995)
Chironomus riparius	Midge	INS	LC50	Mortality	96	4		s	n	95% CI = 2.52-6.35 µg/l	4	Hooftman *et al.* (1989)
Chironomus riparius	Midge	INS	LC50	Mortality	24	28		s	n	95% CI = 2.52-6.35 µg/l	4	Hooftman *et al.* (1989)
Chironomus riparius	Midge	INS	LC50	Mortality	96	7400	Temp. 20°C; DO >80%; pH6.8-7.2	ss	y	95% CI = 2.52-6.35 µg/l	2	Taylor *et al.* (1994)
Fish												
Oncorhynchus mykiss	Rainbow trout	FIS	LC50	Mortality	96	1940		s	n		RAR (Valid)	Hodson (cited in ECB 2006) (1985)
Poecilia reticulata	Guppy	FIS	LC50	Mortality	96	8700		f	y		RAR (Valid)	Adema and Vink (cited in ECB 2006) (1981)
Poecilia reticulata	Guppy	FIS	LC50	Mortality	48	9500	DO >70%; pH ~ 8	f	y			Adema and Vink (1981)
Pimephales promelas	Fathead minnow	FIS	LC50	Mortality	96	6990		f	y		RAR (Valid)	Call *et al.* (cited in ECB 2006) (1987)
Brachydanio rerio	Zebrafish	FIS	LC50	Mortality	96	8500					NA	Becker *et al.* (1990)
Leuciscus idus	Goldern orfe	FIS	LOEC	Locomotive	19.2	500		f	n	Minimum length	NA	Hendriks and Stouten

Scientific Name	Common Name	Taxon Grp	Endpoint	Effect	Test Duration (hours)	Conc. (µg l^{-1})	Environmental conditions	Exposure[1]	Toxicant analysis[2]	Comment	Reliability (Klimisch code*)	Reference
				behaviour						= 12 cm before use.		(1993)

* see Annex 1, For data where a numerical RI (1-4) is assigned a "Data Quality Assessment Sheet" is available in Annex 1, 'RAR valid' indicates that the respective study was already quality assessed in the EU risk assessment for 3,4-DCA and is considered valid for PNEC derivation, NA = As the data was not critical for PNEC derivation the reliability has not been assessed

[1] Exposure: f= flow-through; s = static; ss = semi static
[2] Toxicant analysis: y = measured, n = nominal
ALG = alga, ANN = Annelids, BAC = Bacteria, CIL = Ciliates, CRU = Crustacea, FIS = Fish, INS = Insects, MOL = Molluscs, ROT = Rotifers

EC50 = Concentration causing a 50% effect, LC50 = concentration causing 50% mortality, NOEC = No Observed Effect Concentration, LOEC = Lowest Observed Effect Concentration, LT50 = Lethal time to a 50% effect

2.6.2 Toxicity to saltwater organisms

Saltwater toxicity data for 3,4-DCA are available for various taxonomic groups including algae, invertebrates and fish. Long-term toxicity data are available for six taxonomic groups: algae, annelids, crustaceans, fish, molluscs and plankton. Short-term saltwater toxicity data are available for seven different taxonomic groups: algae, annelids, bacteria, crustaceans, fish, molluscs and rotifers.

Diagrammatic representations of all the available long and short-term saltwater data (cumulative distribution function) for 3,4-DCA are presented in Figures 2.3 and 2.4, respectively. These diagrams include all data regardless of quality and provide an overview of the spread of the available data. The diagrams are not species sensitivity distributions and have not been used to set the 3,4-DCA PNECs.

Long-term toxicity data for marine species are summarised in Table 2.8 and short-term toxicity data are summarised in Table 2.9.

Figure 2.3 Cumulative distribution function of saltwater long-term data (µg l^{-1}) for 3,4-DCA

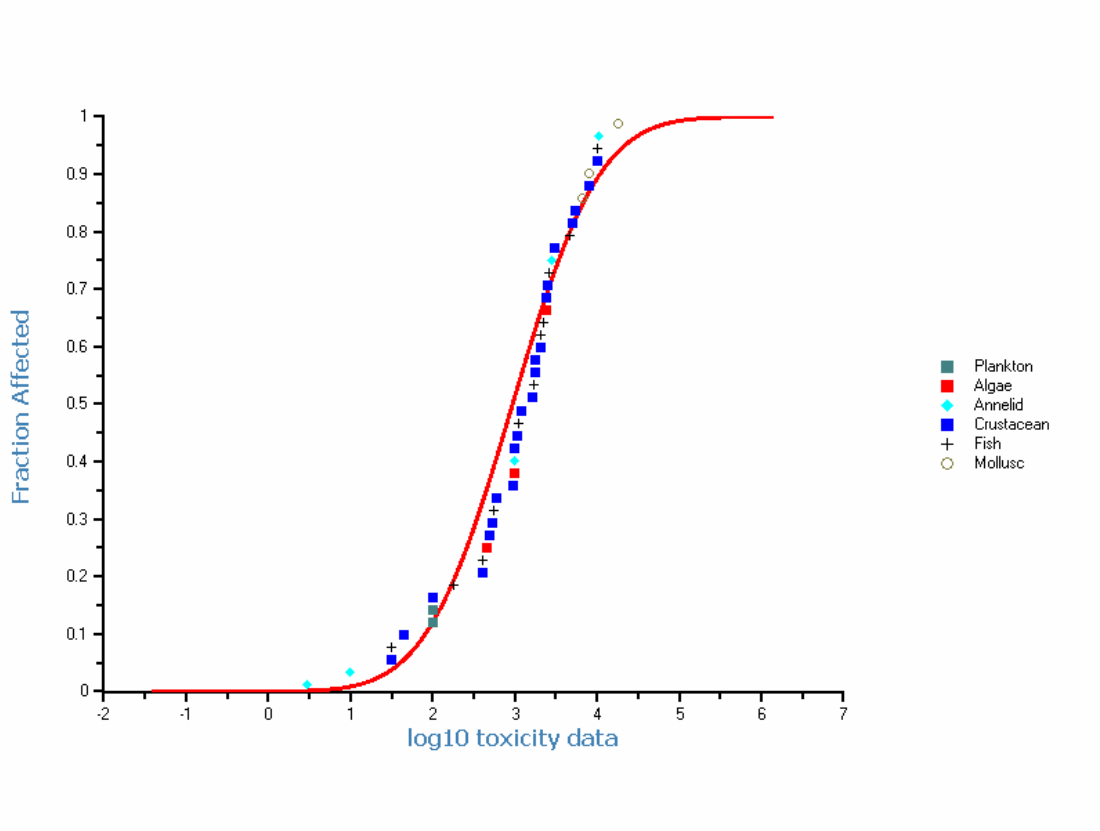


Figure 2.4 Cumulative distribution function of saltwater short-term data (µg l^{-1}) for 3,4-DCA

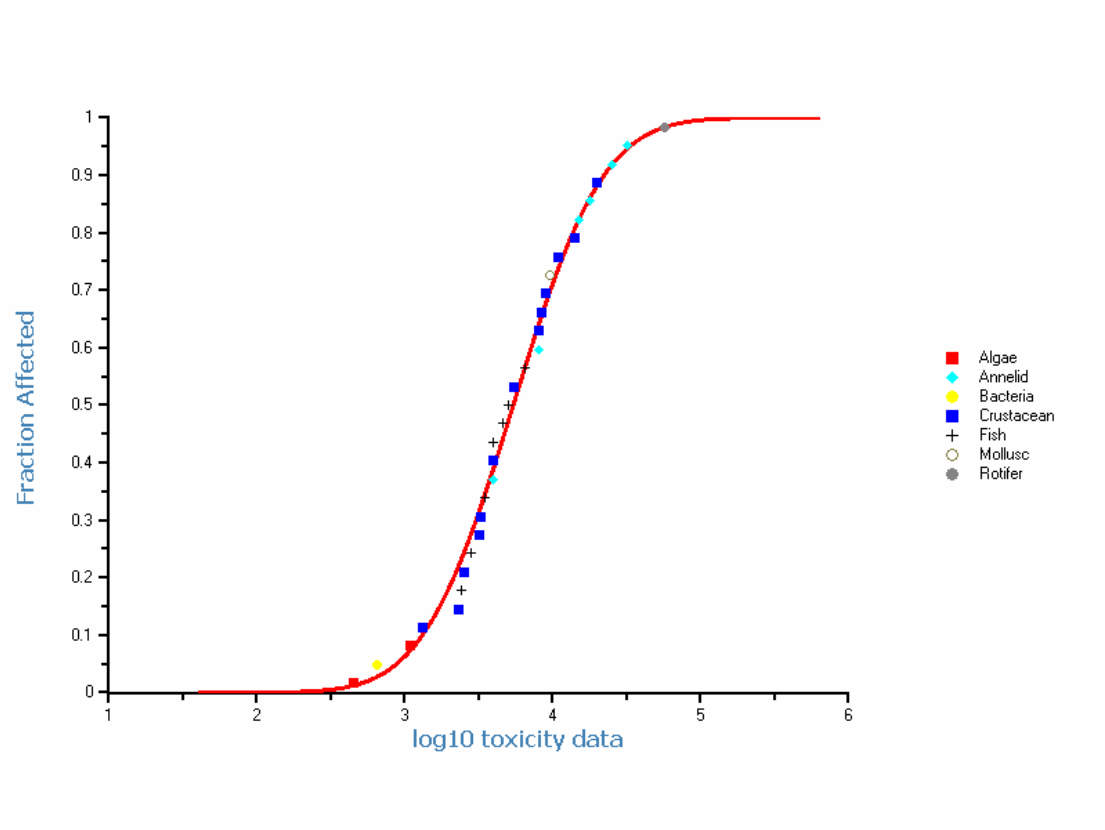


Table 2.8 Lowest available 3,4-dichloroaniline long-term toxicity data for marine organisms

Scientific Name	Common Name	Taxon Grp	Endpoint	Effect	Test Duration (hours)	Conc. (µg l^{-1})	Environmental conditions	Expo-sure[1]	Toxicant analysis[2]	Comment	Reliability (Klimisch code*)	Reference
Algae												
Phaeodactylum tricornutum	Diatom	ALG	NOEC	Growth	96	1000		s			RAR (Valid)	Adema *et al.* (cited in ECB 2006) (1982)
Plankton												
Plankton	Plankton	PLNK	LOEC	Sig. change in biomass/Sp. composition	37 days	100				Outdoor mesocosm	NA	Kuiper and Hanstveit (1984)
Crustaceans												
Artemia salina	Brine shrimp	CRU	NOEC	Mortality/ reproduction	28 day	32	DO >70%; pH ~ 8		y	3 day old larvae,1 mm length	RAR (Valid)	Adema and Vink (cited in ECB 2006) (1981)
Artemia salina	Brine shrimp	CRU	EC50	Reproduction	28 day	100	DO >70%; pH ~ 8		y		NA	Adema and Vink (1981)
Chaetogammarus marinus	Amphipod	CRU	EC50	Reproduction	213 day	45	DO >70%; pH ~ 8		y		NA	Adema and Vink (1981)
Palaemonetes varians	Grass or Atlantic ditch shrimp	CRU	LOEC	Development	21 day	1020	Kept in 100% seawater at 15°C	ss	n	Young adults (2 cm)	2	Van der Meer *et al.* (1988)
Palaemonetes varians	Grass or Atlantic ditch shrimp	CRU	NOEC	Development	21 day	486	Kept in 100% seawater at 15°C	ss	n	Young adults (2 cm)	2	Van der Meer *et al.* (1988)
Molluscs												
Mytilus edulis	Common bay mussel, blue mussel	MOL	LC50	Mortality	21 day	6500	DO >70%; pH ~ 8		y		NA	Adema and Vink (1981)
Mytilus edulis	Common bay mussel, blue mussel	MOL	LC50	Mortality	7 day	8000	DO >70%; pH ~ 8		y		NA	Adema and Vink (1981)
Annelids												
Ophryotrocha diadema	Polychaete	ANN	NOEC	Reproduction	38 day	3.2	Enriched seawater without sediment. DO >70%; pH ~ 8	f	y		RAR (Valid)	Adema and Vink (cited in ECB 2006) (1981)
Ophryotrocha diadema	Polychaete	ANN	EC50	Reproduction	31 day	10	DO >70%; pH ~ 8	f	y		NA	Adema and Vink (1981)

Scientific Name	Common Name	Taxon Grp	Endpoint	Effect	Test Duration (hours)	Conc. (µg l^{-1})	Environmental conditions	Expo-sure[1]	Toxicant analysis[2]	Comment	Reliability (Klimisch code*)	Reference
Fish												
Pleuronectes platessa	Plaice	FIS	LC50	Mortality	28 day	180	DO >70%; pH ~ 8		y		NA	Adema and Vink (1981)
Pleuronectes platessa	Plaice	FIS	NOEC	Mortality, growth, malformation	180 day	32	DO >70%; pH ~ 8		y	Egg to larvae.	2	Adema and Vink (1981)
Pleuronectes platessa	Plaice	FIS	LOEC	Mortality, growth, malformation	180 day	180	DO >70%; pH ~ 8		y	Egg to larvae.	2	Adema and Vink (1981)
Poecilia reticulata	Guppy	FIS	EC50	Growth	120 day	550	DO >70%; pH ~ 8		y		NA	Adema and Vink (1981)

* see Annex 1, For data where a numerical RI (1-4) is assigned a "Data Quality Assessment Sheet" is available in Annex 1, 'RAR valid' indicates that the respective study was already quality assessed in the EU risk assessment for 3,4-DCA and is considered valid for PNEC derivation, NA = As the data was not critical for PNEC derivation the reliability has not been assessed

[1] Exposure: f= flow-through; s = static; ss = semi static
[2] Toxicant analysis: y = measured, n = nominal
ALG = alga, ANN = Annelids, CRU = Crustacea, FIS = Fish, MOL = Molluscs, PLNK = Plankton

NOEC = No Observed Effect Concentration, LOEC = Lowest Observed Effect Concentration, MATC = Maximum Acceptable Toxicant Concentration, EC50 = Concentration causing a 50% effect, LC50 = Concentration causing 50% mortality

Table 2.9 Lowest available 3,4-dichloroaniline short-term toxicity data for marine organisms

Scientific Name	Common Name	Taxon Grp	Endpoint	Effect	Test Duration (hours)	Conc. (µg l^{-1})	Environmental conditions	Expo-sure[1]	Toxicant analysis[2]	Comment	Reliability (Klimisch code*)	Reference
Bacteria												
Photobacterium phosphoreum	Bacteria	BAC	EC50	Light Inhibition	30 min	650	Temp. 15°C.	s		.	RAR (Valid)	Ribo and Kaiser (cited in ECB 2006) (1984)
Algae												
Phaeodactylum tricornutum	Diatom	ALG	EC50	Growth	72	1100	Natural seawater at 20% salinity ; Temp. 15 +/-1°C.	s	y	ISO 8692.	RAR (Valid)	Kusk andNyholm (cited in ECB 2006) (1992)
Phaeodactylum tricornutum	Diatom	ALG	EC50	Growth	96	450	DO >70%; pH ~ 8		y	OECD guideline 201.	2	Adema and Vink (1981)
Crustaceans												
Artemia salina	Brine shrimp	CRU	LC50	Mortality	96	5500	DO >70%; pH ~ 8		y	3 days old, 1 mm length.	RAR (Valid)	Adema and Vink (cited in ECB 2006) (1981)
Chaetogammarus marinus	Amphipod	CRU	LC50	Mortality	96	3200	DO >70%; pH ~ 8		y	4 mm larvae.		Adema and Vink (1981)
Crangon crangon	Common shrimp	CRU	LC50	Mortality	96	2300	DO >70%; pH ~ 8		y	Adult. 4 cm.	2	Adema and Vink (1981)
Palaemonetes varians	Grass or Atlantic ditch shrimp	CRU	LC50	Mortality	96	2500	DO >70%; pH ~ 8		y	Adult. 4 cm.		Adema and Vink (1981)
Palaemonetes varians	Grass or Atlantic ditch shrimp	CRU	LC50	Mortality	96	1310	Kept in 100% seawater at 15°C.	ss	n	Young adults (2 cm)	2	Van der Meer *et al.* (1988)
Rotifers												
Brachionus plicatilis	Rotifer	ROT	LC50	Mortality	96	57450		s	n		NA	Ferrando and Andreu-Moliner (1991)
Molluscs												
Mytilus edulis	Common bay mussel, blue mussel	MOL	LC50	Mortality	96	9500	DO >70%; pH ~ 8		y	Adult, size = 3 cm.	NA	Adema and Vink (1981)
Annelids												
Ophryotrocha diadema	Polychaete	ANN	LC50	Mortality	48	8000	Salinity 33 +/- 1%; pH 8.1 +/- 0.1; Temp. 21 +/- 1°C; DO >70%	ss	y	2-3 day old larvae Solutions changed once/day	2	Hooftman and Vink (1980)
Ophryotrocha diadema	Polychaete	ANN	LC50	Mortality	72	4000	Salinity 33 +/- 1%; pH 8.1 +/- 0.1; Temp. 21 +/- 1°C; DO >70%	ss	y	2-3 day old larvae. Solutions changed once/day.	2	Hooftman and Vink (1980)
Ophryotrocha diadema	Polychaete	ANN	LC50	Mortality	96	4000	Salinity 33 +/- 1%; pH	ss	y	2-3 day old larvae.	2	Hooftman and Vink

Scientific Name	Common Name	Taxon Grp	Endpoint	Effect	Test Duration (hours)	Conc. (µg l^{-1})	Environmental conditions	Expo-sure[1]	Toxicant analysis[2]	Comment	Reliability (Klimisch code*)	Reference
							8.1 +/- 0.1; Temp. 21 +/- 1°C; DO >70%			Solutions changed once/day.		(1980)
Fish												
Poecilia reticulata	Guppy	FIS	LC50	Mortality	96	3500	DO >70%; pH ~ 8	s	y	Adult fish.	2	Adema and Vink (1981)
Pleuronectes platessa	Plaice	FIS	LC50	Mortality	96	4600	DO >70%; pH ~ 8	s	y		2	Adema and Vink (1981)

*see Annex 1, For data where a numerical RI (1-4) is assigned a "Data Quality Assessment Sheet" is available in Annex 1, 'RAR valid' indicates that the respective study was already quality assessed in the EU risk assessment for 3,4-DCA and is considered valid for PNEC derivation, NA = As the data was not critical for PNEC derivation the reliability has not been assessed

[1] Exposure: s = static; ss = semi static

[2] Toxicant analysis: y = measured, n = nominal

ALG = alga, ANN = Annelids, BAC = Bacteria, CRU = Crustacea, FIS = Fish, MOL = Molluscs, ROT = Rotifers

EC50 = Concentration causing a 50% effect, LC50 = concentration causing 50% mortality

2.6.3 Toxicity to sediment dwelling organisms

Both freshwater and saltwater toxicity data are available for sediment dwelling organisms (Tables 2.10 and 2.11). Freshwater long-term data are available for sediment dwelling annelids and insects. Short-term freshwater data are also available for sediment dwelling annelids and insects. Marine long and short-term toxicity data for sediment dwelling organisms are available only for annelids.

2.6.4 Endocrine-disrupting effects

There are only limited data on the effects of 3,4-DCA on the endocrine system of organisms. The EU RAR identifies two studies one with fish and the other with rats. Concentrations of 200 and 400 µg l^{-1} 3,4-DCA have been shown to lower androgen synthesis in breeding male sticklebacks (*Gasterosteus aculeatus*) (Allner 1997 cited in ECB 2006). In addition, the changes in androgen metabolism are associated with changes in secondary sex characteristics, regression in courtship colouration and reduced courtship behaviour at 3,4-DCA concentration of 100, 200 and 400 µg l^{-1}.

In rats it has been reported that 3,4-DCA competes *in vitro* for binding sites on the androgen receptor. The IC50 for displacement of [^{3}H] testosterone on the androgen receptor was 110 mM (18 mg l^{-1}) (Cook *et al.* 1993 cited in ECB 2006)

The limited data for 3,4-DCA suggests that this chemical may have some effects on the endocrine systems of wildlife. However, endocrine effects appear to occur at concentrations higher than those causing effects on survival and growth in standard toxicity tests.

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Table 2.10 Lowest available 3,4-dichloroaniline short and long-term toxicity data for freshwater sediment organisms

Scientific Name	Common Name	Taxon Grp	Endpoint	Effect	Test Duration (hours)	Concentration mg/kg dw	Environmental conditions	Expo-sure[1]	Toxicant analysis[2]	Comment	Reliability (Klimisch code*)	Reference
Short-term data												
Annelids												
Pristina longiseta	Oligochaete worm	ANN	LC50	Mortality	96	2500 (µg l^{-1})		S	n	No sediment in test system	RAR (Valid)	Schmitz and Nagel (cited in ECB 2006) (1995)
Tubifex tubifex	Tubificid worm	ANN	LC50	Mortality	24	11000 (µg l^{-1})				No sediment in test system	RAR (not assignable)	Yoshioka *et al.* (cited in ECB 2006) (1986)
Tubifex tubifex	Tubificid worm	ANN	LC50	Mortality	48	11000 (µg l^{-1})				No sediment in test system	RAR (not assignable)	Yoshioka *et al.* (cited in ECB 2006) (1986)
Lumbriculus variegatus	Blackworm	ANN	LC50	Mortality	96	25200 (µg l^{-1})				No sediment in test system	RAR (Valid)	Oetken *et al.* (cited in ECB 2006) (2000)
Insects												
Chironomus riparius	Midge larvae	INS	LC50	Mortality	48	9200 (µg l^{-1})				No sediment in test system	RAR (Valid)	Oetken *et al.* (cited in ECB 2006) (2000)
Chironomus riparius	Midge	INS	NOEC LOEC	Dry weight	10 day	<250 250	Sediment = 69% sand, 20% clay, 10% peat. Temp. 20 +/- 1°C; 14:10 hour light: dark; pH = 7.0-7.5; DO >80%.	s	y	Early first and second instar. Interstitial water concentration of 3,4-DCA 1.39 mg l^{-1} and overlying water 1.35 mg l^{-1} (day 10)	RAR (Valid)	Naylor and Howcroft (cited in ECB 2006) (1997)
Chironomus riparius	Midge	INS	NOEC LOEC	Length	10 day	250 350	Sediment = 69% sand, 20% clay, 10% peat. Temp. 20 +/- 1°C; 14:10 hour light: dark; pH = 7.0-7.5; DO >80%.	s	y	Early first, late first and second instar. Interstitial water concentration of 3,4-DCA 1.39-2.2 mg l^{-1} and overlying water 1.35-3.1 mg l^{-1} (day 10)	RAR (Valid)	Naylor and Howcroft (cited in ECB 2006) (1997)

Scientific Name	Common Name	Taxon Grp	Endpoint	Effect	Test Duration (hours)	Concentration mg/kg dw	Environmental conditions	Expo-sure[1]	Toxicant analysis[2]	Comment	Reliability (Klimisch code)*	Reference
Long-term data												
Annelids												
Lumbriculus variegatus	Blackworm	ANN	NOEC LOEC	Worm number	28 day	5 25		s	n	Sediment pre-incubated with 3,4-DCA for 14 days	RAR (Valid)	Oetken *et al.* (cited in ECB 2006) (2000)
Lumbriculus variegatus	Blackworm	ANN	NOEC LOEC	Biomass	28 day	5 25		s	n	Sediment pre-incubated with 3,4-DCA for 14 days	RAR (Valid)	Oetken *et al.* (cited in ECB 2006) (2000)
Lumbriculus variegatus	Blackworm	ANN	NOEC LOEC	Deformation	28 day	1 5		s	n	Deformations were obvious but not statistically significant from solvent controls	RAR (Valid)	Oetken *et al.* (cited in ECB 2006) (2000)
Insects												
Chironomus riparius	Midge larvae	INS	NOEC LOEC	Emergence/ gender ratio	28 day	40 >40		s	n	Sediment pre-incubated with 3,4-DCA for 14 days	RAR (not valid)	Oetken *et al.* (cited in ECB 2006 (2000)
Chironomus riparius	Midge larvae	INS	NOEC LOEC	Emergence rate/eggs per clutch	28 day	<0.064 0.064		s	n	Sediment pre-incubated with 3,4-DCA for 14 days	RAR (Not valid)	Oetken *et al.* (cited in ECB 2006) (2000)
Chironomus riparius	Midge larvae	INS	EC10 EC15 EC50	Emergence rate (pooled sex)	14 day	219 223 239	Sediment = 75% sand, 20% clay, 2% peat. pH = 6.5-7.5	s	y	Sediment pre-incubated with 3,4-DCA for 14 days. Nominal results reported	RAR (Valid)	Bayer AG (cited in ECB 2006) (2001)
Chironomus riparius	Midge larvae	INS	EC10 EC15 EC50	Development rate (pooled sex)	14 day	129 165 >180	Sediment = 75% sand, 20% clay, 2% peat. pH = 6.5-7.5	s	y	Sediment pre-incubated with 3,4-DCA for 14 days. Nominal results reported	RAR (Valid)	Bayer AG (cited in ECB 2006) (2001)
Chironomus riparius	Midge larvae	INS	EC10 EC15 EC50	Development rate (male)	14 day	122 >180 >180	Sediment = 75% sand, 20% clay, 2% peat. pH = 6.5-7.5	s	y	Sediment pre-incubated with 3,4-DCA for 14 days. Nominal results reported	RAR (Valid)	Bayer AG (cited in ECB 2006) (2001)
Chironomus riparius	Midge larvae	INS	EC10 EC15 EC50	Development rate (female)	14 day	104 154 >180	Sediment = 75% sand, 20% clay, 2% peat. pH = 6.5-7.5	s	y	Sediment pre-incubated with 3,4-DCA for 14 days. Nominal results reported	RAR (Valid)	Bayer AG (cited in ECB 2006) (2001)

[*] *see Annex 1, For data where a numerical RI (1-4) is assigned a “Data Quality Assessment Sheet” is available in Annex 1, ‘RAR valid’ indicates that the respective study was already quality assessed in the EU risk assessment for 3,4-DCA and is considered valid for PNEC derivation

[1] Exposure: s = static
[2] Toxicant analysis: y = measured, n = nominal
ANN = Annelids, INS = Insects

NOEC = No Observed Effect Concentration, LOEC = Lowest Observed Effect Concentration, EC10 = Concentration causing a 10% effect, EC15 = Concentration causing 15% mortality, EC50 = Concentration causing a 50% effect

Table 2.11 Lowest available 3,4-dichloroaniline short and long-term toxicity data for marine sediment organisms

Scientific Name	Common Name	Taxon Grp	Endpoint	Effect	Test Duration (hours)	Concentration mg/kg dw	Environmental conditions	Exposure[1]	Toxicant analysis[2]	Comment	Reliability (Klimisch code)*	Reference
Short-term data												
Annelids												
Ophryotrocha diadema	polychaete worm	ANN	LC50	Mortality	24	25000 (µg l^{-1})			y	No sediment present	RAR (Valid)	Adema and Vink (cited in ECB 2006) (1981)
Long-term data												
Annelids												
Ophryotrocha diadema	polychaete worm	ANN	LC50	Mortality	7 day	2800 (µg l^{-1})			y	No sediment present	RAR (Valid)	Adema and Vink (cited in ECB 2006) (1981)
Ophryotrocha diadema	polychaete worm	ANN	NOEC	Reproduction	38 day	3.2 (µg l^{-1})			y	No sediment present	RAR (Valid)	Adema and Vink (cited in ECB 2006) (1981)

* see Annex 1, For data where a numerical RI (1-4) is assigned a "Data Quality Assessment Sheet" is available in Annex 1, 'RAR valid' indicates that the respective study was already quality assessed in the EU risk assessment for 3,4-DCA and is considered valid for PNEC derivation

[2] Toxicant analysis: y = measured, n = nominal

ANN - Annelids

LC50 = concentration causing 50% mortality, NOEC = No Observed Effect Concentration

2.6.5 Mode of action of 3,4-DCA

The standard view is that anilines such as 3,4-DCA act as polar narcotics (Bearden and Schultz 1997 and Argese *et al.* 2001).

2.6.6 Mesocosm and field studies

Freshwater mesocosm and field studies

Girling *et al.* (2000) investigated the effects of 3,4-DCA on various biological components of artificial streams. The streams consisted of five stainless steel channels (5m long, 0.35m wide and 0.25m deep) in a semi-recirculating system. Each channel was divided into an upstream slow flowing section (0.2 m depth, ~3 cm/s flow rate) and fast riffle section (0-0.02 m depth, ~30 cm/s flow rate). Test water was pumped from an adjacent natural calcareous stream at a rate of 10 l/hour. Water was recirculated by electronically operated pumps at a rate of 5500 l/hour. Shingle (10-20mm diameter) and sharp sand (1-2 mm grain size) provided a heterogenous stream bed and a woven polyester mesh canopy reduced the amount of direct sunlight, preventing overheating and excessive algal growth. In a second experiment four downstream ponds were incorporated into the system. Each pond consisted of a 1.04 metre, 510 litre plastic cylindrical tank with a polythene liner. Water (10 litres/hour) from the test streams was piped into the pond so that the mean residence time for water in the pond was approximately 2 days. Inlets and outlets were covered by a nylon mesh to prevent loss of macro-invertebrates. The base of each pond was covered with a 2 cm layer of clay which was covered with an 8 cm layer of natural pond sediment. Artificial plants (plastic mesh) were placed in each pond and leaf litter added to provide food for detritivores.

Stones from a natural stream provided an inoculum for introducing periphyton over a 7-10 day period. Invertebrates obtained from a natural stream were introduced into the artificial streams over a 4-6 week pre-treatment period. Macro-invertebrates, zooplankton and phytoplankton were collected from mature ponds and were used to establish downstream communities over a 6-week pre-treatment period. Rainbow trout (*Oncorhynchus* mykiss) and three spine stickleback (*Gasterosteus aculeatus*) were also introduced to the stream systems. Water was transferred between the ponds prior to treatment to ensure similarity of the communities. The studies were not replicated. Instead pseudo replicates were created by adding multiple enclosures of the various biological components to each stream or pond.

Water temperature and dissolved oxygen were monitored constantly. Conductivity, pH, alkalinity, nitrate, nitrite, reactive phosphorus, silica, suspended solids and total organic carbon concentrations were determined weekly. Streams were continuously dosed with 3,4-DCA via dosing pumps which delivered nominal concentrations of 0, 100, 300, 1000 and 3000 µg l^{-1} in the first experiment and 0, 1, 4.3, 18, 77, 330, 1400 and 6000 µg l^{-1} in the second experiment. In the second experiment, the control and streams treated with 4.3, 77 and 1400 µg l^{-1} were connected to the downstream ponds. Subsurface water samples (0.2-2.5 l) were taken 2-3 times/week from the slow flowing section of the downstream ponds.

Little difference in water quality was noted between the streams or between the streams and ponds. The concentrations of 3,4-DCA in the streams and ponds also remained constant with little variation from the nominal concentrations throughout the exposure period of 28 days.

The study monitored a wide range of biological parameters throughout the experiment, but highlighted a number of specific endpoints where effects were observed:

- In the first experiment *Gammarus pulex* populations were evenly distributed between the streams. Numbers were significantly reduced on day 28 in streams treated at 800 and 2400 µg l^{-1}. *Baetidae* (mayflies) were significantly reduced in all streams (70 µg l^{-1} and above) on day 28 and *Polycelis* (flatworms) were reduced in the 2400 µg l^{-1} treated stream.

- A significant increase in the number of drifting *G. pulex*, *Baetidae* and *Polycelis* was reported in the first 24 hours of treatment at 2400 µg l^{-1} in the first experiment. In the second experiment, numbers of drifting *G. pulex* were initially noted at 940 and 4700 µg l^{-1}. Drift of juveniles was reported in the first 5 days and drift of adults in the first 2 days. Numbers in drift samples collected on day 11 following treatment with 4700 µg l^{-1} were very low, which was believed to be due to population depletion.

- The percentage of *G. pulex* that were swimming in the water column was significantly increased at 4700 µg l^{-1} in the first 0.5-3 hours. Precopula separation times were significantly lower than the control at measured concentrations of 190, 630 and 2200 µg l^{-1}, but not at 60 µg l^{-1}. A reduction in growth rate of juvenile *G. pulex* was reported in the second experiment over 25 days at concentrations of 240, 800 and 2400 µg l^{-1}.

- In terms of *Asellus aquaticus* survival and reproduction, no treatment related effects were noted after 5 days at any exposure level. A 12-day LC50 of 58 µg l^{-1} was reported. High control mortality (44%) prevented calculation of a 19-day LC50.

- Reduced growth of 3rd instar *Chironomus riparius* (midge) was reported in the first experiment after 10 days exposure to 2200 µg l^{-1}.

- No effects were reported in the amount of chlorophyll-a extracted in the first experiment. In the second experiment, chlorophyll-a concentrations were increased in streams treated with 940 and 4700 µg l^{-1}. The highest increase in chlorophyll-a was noted in the stream treated with 940 µg l^{-1}

- Mean community feeding rates of leaf material were significantly reduced in the pool and riffle sections of streams treated with 940 and 4700 µg l^{-1}. Photosynthesis was decreased in all treated streams between days 0-10 and then rapidly increased in the stream treated at 2400 µg l^{-1} and gradually in the stream treated at 800 µg l^{-1}. Photosynthesis was low, compared to the control, throughout the whole experiment in streams treated with 70 and 240 µg l^{-1}.

- No rainbow trout sac-fry survived the full treatment period at 3,4-DCA concentrations of 210 µg l^{-1} or above. The calculated 18-day LC50 from eyed-

eggs to sac-fry was 37 µg l^{-1} and 12 µg l^{-1} by probit analysis and moving point average, respectively.

- Macro-invertebrate community diversity in downstream pond sediment was slightly reduced between days -2 to 26 in ponds treated with 37 and 820 µg l^{-1}. Numbers of extracted zooplankton varied widely, with the highest numbers in samples extracted on day 7. On day 26, the numbers were highest in the control and the pond treated with 1.7 µg l^{-1}. Treatment related effects were not noted. An absence of clear treatment related effects on invertebrates associated with plants were noted. However, there was an indication of a downward trend in the numbers of Chydoridae and Tardigrada and an increase in the numbers of Copepoda nauplii. Chlorophyll-a concentrations reached a maximum after 12 days of treatment, but returned to pre-treatment levels by day 25. A depression was noted in chlorophyll a concentration in the pond treated with 820 µg l^{-1}.

- 80% of the three-spine sticklebacks exposed to 820 µg l^{-1} died within 26 days. No mortalities occurred in the ponds treated with 1.7 and 37 µg l^{-1}. The 26-day LC50 was calculated to be 360 µg l^{-1}.

The lowest reported effects from the two studies were a population density LOEC of 10-100 µg l^{-1} for *Baetis rhodani* in the first study and MATCs of 8-10 µg l^{-1} for zooplankton abundance and sediment invertebrate abundance in the second study.

Crossland and Hillaby (1985) used nine outdoor ponds (10 m long, 5 m wide and 1 m deep) to investigate the chronic toxicity of 3,4-DCA on *Daphnia longispina* and various other planktonic crustaceans. Tests were conducted using three ponds at a 3,4-DCA concentration of 45 µg l^{-1}, three at a 450 µg l^{-1} and three controls. Concentrations were maintained for 28 days. However, no chemical analysis was carried out to confirm the exposure concentrations. Population densities were estimated from numbers collected in samples of pond water. Samples were collected twice per week.

In ponds treated with 450 µg l^{-1}, there was a reduction in numbers of *D. longispina* between days 3-7. Numbers were significantly lower than the control during days 7-65 and then quickly recovered. Ponds treated with 45 µg l^{-1} remained comparable to the control throughout days 0-10. Numbers were significantly lower than the control during days 14-24, but quickly recovered from days 31-38. The other species of Cladocera appeared to be affected in a similar manner, but they were fewer in number compared to *D. longispina*. The mean birth rate of *D. longispina* was significantly affected in both the high and low concentration ponds, with the most profound affect occurring in the high concentration pond. During days 15-28, population densities were at or near zero levels. The death rate in the pond treated with the low concentration was significantly increased during days 15-28. No significant effect was noted on two species of copepod, *D. gracilis* and *Cyclops sp.* at the low concentration at any time point or the high concentration until day 38, after which a statistically significant increase in numbers was reported. No adverse effects of the test concentrations on the various families of molluscs and insects in the ponds were reported and there was no indication of changes in the structure of macro-invertebrate communities in treated ponds.

Schmitz and Nagel (1995) investigated the effects of 3,4-DCA on the recolonisation of benthic organisms in two experimental streams. The streams consisted of an aquarium (2.5 x 0.5 x 0.5 m) with a pane in the middle and a pump at one edge,

giving a circulating current of 5 m length. Gravel substrate was profiled to give a zone of high current of 2 m/sec, with an average depth of 30 cm, slope zones with a depth of 5-30 cm and zones with no current with a depth of 5 cm. The streams were filled with tap water and loss by evaporation replaced with deionised water. The stream was inoculated with pond water. Two identical streams were produced. Temperature = 26.2 $\pm$ 0.6°C, light intensity at surface water = 30000 lux, 12:12 light:dark cycle, oxygen saturation = 106 $\pm$ 9%. pH in the first 32 weeks decreased to an average of 8.58 $\pm$ 0.04 in stream I and 8.42 $\pm$ 0.05 in stream II during the last 18 weeks. The biotic community in the test system had existed for more than 2.5 years prior to testing and also included 12 zebrafish (*Brachydanio rerio*). Fish were replaced for the experiment with 10 fish of the same age which were fed twice a day with pulverised dry feed.

A nominal exposure concentration of 200 µg l^{-1} 3,4-DCA was used in stream I and 1400 µg l^{-1} in stream II. The test concentrations in the streams were tested twice a week. The re-colonisation of introduced enclosures, devoid of biology, was examined four times during a 12 week pre-exposure and three times during the 9 weeks of exposure.

Diverse species of blue and green algae, the macrophytes *Anubias lanceolata* and *A. nana*, several species of protozoa and diverse species of Platyhelminths were found in the experimental streams. The nermertini *Prostoma graecense*, diverse species of nemathelminths, molluscs, annelids and *Hydrozete. lacustris* (Arthropod) were also identified.

In stream I at measured concentration 158 ± 66 µg l^{-1} (nominal concentration 200 µg l^{-1}), *Pristina longiseta* (an annelid) died out within three weeks of exposure. The number of immigrated *Pristina* was reduced significantly in all sample areas of both streams. *Aelosom variegatum* and *Aelosoma hempichi* (annelids) appeared to take the place of *P. longiseta* in high current areas of stream I. In stream II at a measured concentration of 901± 616 µg l^{-1} (nominal concentration 1400 µg l^{-1}), immigration of *H. lacustris* was significantly reduced.

Sherratt *et al.* (1999) also investigated the effects of 3,4-DCA on the recovery of freshwater invertebrate populations. The tests used eighteen mesocosms comprising of cylindrical fibreglass tanks (1.25 m diameter and 1.25 m depth) filled with 1m of water. To regulate temperature, tanks were maintained in two 5 x 5 x 1 m outdoor concrete ponds filled with water to a depth of 1 m. Water was taken from a pond on the test site. Sediment derived from an established pond on the site was added to a depth of 10 cm to aid in the establishment of the invertebrates and increase environmental realism. The mesocosms were left uncovered and open to natural colonisation between Autumn 1994 and June 1996. At the start of the experiment, mesocosms were covered with individual net hoods (0.45 x 0.78 mm mesh) to prevent entry of macro-invertebrates, but allow entry of water and 90% light penetration. Ten of the mesocosms were selected based on the range of aquatic invertebrates they supported. Four were dosed with 3,4-DCA at a concentration of 10000 µg l^{-1}. Macro-invertebrate samples were taken the day before 3,4-DCA was applied and on days 1, 3, 7 and 14 and then fortnightly until day 280. To simulate immigration, mesocosms were further divided into ponds into which selected invertebrate species would be added. From day 15 and at any subsequent sampling point, selected invertebrates from untreated mesocosms which were not part of the study were added to the study ponds.

Mortality of 11 invertebrate species or taxonomic groups (*Asellus asellus*, Chaoboridae, Coenagrionidae, *Crangonyx pseudogracilis*, *Helophorus* spp., *Lymnaea peregra*, *Lymnaea stagnalis*, *Notonecta* spp., Oligochaeta, *Planorbis carinatus* and *Polycelis tenuis*) was studied on day one of the test (one day after 3,4-DCA application). The highest mortalities were observed for *Asellus aquaticus* and *Crangonyx pseudogracilis*. No mortality was observed in Coenagrionidae, Chaoboridae or *Lymnea stagnalis*. Only three species exhibited a full recovery before the end of the 280 day exposure period.

Taylor *et al.* (1994) investigated the effects of 3,4-DCA on *Gammarus pulex* and *Chironomus riparius* in an artificial stream mesocosm. Mesocosms consisted of six partly recirculating, partly flow-through streams (5 m long, 0.35 m wide and 0.25 m deep), containing both pool and ripple sections. Forty four *G. pulex* neonates (<2 weeks old, approx. 1.5 mm body length, 200 µg ww) obtained from a laboratory culture were randomly allocated to each of five net cages which were suspended to a depth of 6 cm in the pool section of the streams. They were fed to excess with a mixture of faeces of adult *G. pulex* and macerated horse chestnut leaves. Sixty third-instar *C. riparius* from a laboratory culture were randomly allocated to each of the five net cages which were suspended in the streams. They were provided with a cellulose mulch substrate to a depth of 1 cm in each cage and fed to excess with finely ground synthetic fish food. Nominal test concentrations of 100, 300, 1000 and 3000 µg l^{-1} 3,4-DCA were used. Environmental conditions and chemical exposure concentrations were measured throughout the exposure period.

Mortality was less than 10%, except for *G. pulex* exposed to 780 and 2360 µg l^{-1}, which had mortality rates of 77 and 100%, respectively. Final wet weights of both species were found to be statistically significantly affected by 3,4-DCA. Twenty five and twelve day NOECs of 80 and 760 µg l^{-1} were determined for *G. pulex* and *C. riparius*, respectively.

In summary there are various freshwater mesocosm and field studies available for 3,4-DCA. Each study used different exposure systems with varying exposure concentrations. Consequently, there are a range of different endpoints and effect concentrations. The lowest endpoints from the available studies were the MATCs of 8-10 µg l^{-1} for zooplankton abundance and sediment invertebrate abundance in the outdoor stream experiment of Girling *et al.* (2000). Similarly, Crossland and Hillaby (1985) suggested that an MATC of 10 µg l^{-1} 3,4-DCA would be protective of field populations of *Daphnia*. Based on the available data, 3,4-DCA concentrations below 10 µg l^{-1} should have little effect on field populations of freshwater aquatic organisms.

Saltwater mesocosm and field studies

Kuiper and Hanstveit (1984) investigated the effects of 3,4- DCA in two separate studies on marine plankton communities in experimental enclosures. Three-metre depth bags were constructed and filled with 1.5 m^3 natural seawater collected from a few miles offshore. The bags were anchored in a sheltered location in the harbour of Den Helder, The Netherlands. In the first experiment, 3,4-DCA was added to the bags at initial concentrations of 2, 10 and 25 µg l^{-1}. Only the 10 µg l^{-1} exposure was replicated (duplicate bags). The experiment lasted 35 days. In the second experiment 3,4-DCA was added to two bags on day 5 at initial concentrations of 100 and 1000 µg l^{-1}, respectively. The second study lasted 42 days. Development of phytoplankton, zooplankton and bacteria were measured. Phosphate, ammonia, nitrate, nitrite, silicate, pH, light, temperature and concentrations of 3,4-DCA in the water and sediment were also measured.

The concentration of 3,4-DCA gradually decreased in the first experiment to about half the initial concentration after 4 weeks. In the second experiment, a gradual decrease was also noted in the bag treated with 100 µg l^{-1} and a rapid decrease was noted in the bag treated with 1000 µg l^{-1}. After 21 days, the concentration stabilised at 500 µg l^{-1}.

In the first experiment, after the bags were filled, chlorophyll concentrations reached a maximum after 5 days. This increase coincided with an increase in populations of *Leptocylindrus danicus*, *Phaeocystis poucheti* and other microflagellates. Chlorophyll concentrations decreased from days 5-10. After day 10, a slight increase due to an increase in microflagellates was reported. Overall the addition of 2-25 µg l^{-1} 3,4-DCA did not significantly affect the phytoplankton community.

In the second experiment, in the week before the experiment started, chlorophyll concentrations in the tidal inlet to the Wadden Sea from which the water was taken had decreased from 50 to 15 mg/m^3. This decrease continued in the bags and from day 5 (when 3,4-DCA was added) to day 10, chlorophyll concentrations were extremely low (0.01-0.05 mg/m^3). After day 10, chlorophyll concentrations in the controls increased due to growth of microflagellates and a maximum chlorophyll concentration of 30 mg/m^3 was reached by day 40. In the bag treated with 100 µg l^{-1}, the increase in chlorophyll concentration was delayed compared to the control and a maximum was reached 3-4 days after the control. In the 1000 µg l^{-1} system, the maximum chlorophyll concentration was reached 3-4 days later than the controls and was much higher. A chlorophyll peak was obtained around day 18, mainly produced by the growth of the diatom *Nitzschia longissima*. After day 18, chlorophyll concentrations decreased and reached a new maximum on day 28. After day 28, chlorophyll concentrations remained very low compared to the controls. Consequently, concentrations of 100 µg l^{-1} resulted in a slight inhibition of the resident phytoplankton, but exposure to 1000 µg l^{-1} resulted in very low phytoplankton concentrations by the end of the experiment.

In the first experiment, Calanoid copepods were initially dominant, but *Eurterpina acutifrons*, *Podon intermedius*, nauplii of barnacles and larval worms were also present in low numbers. At the end of the experiment, the calanoid copepod *Temora longicornis* had become the dominant species. The addition of 3,4-DCA concentrations of 2-25 µg l^{-1} did not result in significant differences between systems.

In the second experiment, *T. longicornis* was initially the dominant copepod species, followed by *Centropages hamatus*, *Acartia clausi* and *Paracalanus parvus*. Addition of 100 µg l^{-1} did not alter the biomass development in comparison to the controls. Addition of 1000 µg l^{-1} produced high mortality in all species during the first weeks. After this period, numbers remained low, but did not decrease further. The development of copepods was also inhibited.

In summary, there is only one marine field study available. Effects on marine phytoplankton, zooplankton and bacteria occurred only at 3,4-DCA concentrations >100 µg l^{-1} and no effects were evident at 10 µg l^{-1}.

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)

Based on the available data there appear to be no significant differences in the sensitivity of freshwater or saltwater species of the same taxonomic group. This may reflect the mode of toxic action of 3,4-DCA as a polar narcotic. Consequently the RAR for 3,4-DCA combined freshwater and saltwater datasets for the derivation of the $PNEC_{aqua}$. The same approach has been adopted for the derivation of PNECs in this report.

3.1.1 PNECs for freshwaters

PNEC accounting for the annual average concentration

Long-term freshwater data are available for eight taxonomic groups including algae, crustaceans, fish, insects, macrophytes, molluscs, planarians and rotifers. Consequently, data are available for the 'base set' of toxicity tests (i.e. tests with algae, crustaceans and fish) and the EU TGD assessment factor (AF) method can be applied (ECB 2003). Based on the available data fish and crustaceans appear to be the most sensitive organisms to long-term exposures of 3,4-DCA (see Table 2.6).

Studies are available for various fresh water algal species. Comparisons of the available NOEC/EC10 data indicate similar sensitivities of the species tested. The lowest available value is a 26-day MATC of >100-<1000 µg l^{-1} for algal populations of an outdoor mesocosm (Girling *et al.* 2000). This value was generated in a flow-through system with measured exposure concentrations. However, the study was not replicated (only pseudo replicates were used) and in addition only a range of concentrations for the MATC were reported (see Section 2.6.6 for further study details). Visual inspection of diagrammatic results published in the mesocosm study indicate that the MATC lies closer to 1000 µg l^{-1} than 100 µg l^{-1}. Taking these factors into consideration the algal mesocosm MATC of Girling *et al.* (2000) should be used in a supporting capacity only.

The next lowest algal value is a 96-hour EC10 for population growth of *Chlamydomonas reinhardtii* of 225 µg l^{-1} (Schafer *et al.* 1993). This value was generated in a flow-through system with measured exposure concentrations. However, the study duration of 96 hours exceeds that usually recommended for algal studies (72 hours as specified by the OECD 201 method). The same issues apply to the other algal data available (96-hour and 7 days NOECs of 260 and 330 µg l^{-1} for *Chlamydomonas reinhardtii* (Schafer *et al.* 1994) and a 96-hour NOEC of 1000 for

Scenedesmus pannonicus (Adema *et al.* 1982 cited in ECB 2006). Consequently, although the available algal data would not be regarded as of poor quality, issues with exposure duration mean that they should be used only to support the PNEC derivation.

Long-term freshwater data for crustaceans are available primarily for *Daphnia magna*. The lowest available value is a 14-day NOEC of 2.5 µg l^{-1} (LOEC of 5 µg l^{-1}) for the reproduction of *D. magna* (Diamantino *et al.* 1997 cited in ECB 2006). This was a semi-static study with nominal exposure concentrations and was regarded as valid by the RAR for 3,4-DCA. This NOEC is supported by two good quality 21 day NOECs of 3.1 µg l^{-1} (live neonates) (Samel *et al.* 1999) and 5 µg l^{-1} (reproduction) (UBA 1994, cited in ECB 2006) for *D. magna*. Both NOECs were generated in semi-static systems and would be regarded as suitable for PNEC derivation. The available data suggest that *D. magna* reproduction is particularly sensitive to 3,4-DCA.

Long-term data are also available for freshwater insects. In general these organisms appear to be less sensitive than crustaceans with 12 and 25 day NOECs of 760 and 80 µg l^{-1} reported for the midge (*Chironomus riparius*) (Taylor *et al.* 1994) (see Section 2.6.6 for further details). However, a LOEC of >10-<100 µg l^{-1} has been reported for the mayfly *Baetis rhodani* following exposure in an outdoor mesocosm (Girling *et al.* 2000) (see Section 2.6.6 for further details). This value was generated in a flow-through system with measured exposure concentrations. However, as with the algal study above the experiment was not replicated and only a range of concentrations for the LOEC were reported. Visual inspection of diagrammatic results published in the mesocosm study indicate that the LOEC lies closer to 100 µg l^{-1} than 10 µg l^{-1} (Girling *et al.* 2000). However, it was not possible to estimate the NOEC from the available data and as such this value should be used in a supporting capacity only.

There are a number of low no-effect concentrations for various fish species. The lowest available long-term NOECs were generated in life cycle studies with zebra fish (*Brachydanio rerio*) *and* guppies (*Poecilia reticulate*) (Schafers and Nagel 1991 and Nagel *et al.* 1991 cited in ECB 2006). Forty two day NOEC and LOEC values of 2 and 20 µg l^{-1}, respectively have been reported for the growth of FI generation guppies (Schafers and Nagel 1991 cited in ECB 2006) and 48 day NOEC and LOEC values of 2 and 20 µg/l, respectively have been reported for the survival of FII generation zebra fish (Nagel *et al.* 1991 cited in ECB 2006). Both studies were carried out using flow-through conditions with measured exposure concentrations. As such they were regarded as fully valid for PNEC derivation by the 3,4-DCA RAR (ECB 2006). These data are supported by a 28-day NOEC of 5.1 µg l^{-1} for growth of fathead minnow (*Pimephales promelas*) (Call *et al.* 1987 cited in ECB 2006) and a 42-day LOEC of 2 µg l^{-1} for the reproduction of FI generation guppies (Schafers and Nagel 1991 cited in ECB 2006). Both these supporting studies were conducted using flow-through conditions and measured exposure concentrations and as such were regarded as fully valid by the EU RAR.

Toxicity data available for other taxonomic groups (planarians, macrophytes, rotifers and molluscs) indicate lower sensitivity in these organisms than reported in algae, crustaceans, insects or fish.

The lowest valid long-term freshwater data points were the 42 and 48 day NOECs (for growth and survival) of 2 µg l^{-1} in guppies and zebra fish (Schafers and Nagel 1991 and Nagel *et al.* 1991 cited in ECB 2006). Both values were generated in flow-through studies with measured exposure concentrations and were regarded by the 3,4-DCA RAR as fully valid. The long-term freshwater PNEC in the EU RAR for 3,4-

DCA was therefore based on these data points with an assessment factor of 10 given the availability of long-term data for three trophic levels. This results in:

$PNEC_{freshwater\_lt}$ = 2 µg l^{-1} /AF (10) = 0.2 µg l^{-1}

A number of field and mesocosm studies are available for 3,4-DCA (See Section 2.6.6). The lowest endpoints from the available studies were MATCs of 8-10 µg l^{-1} for zooplankton abundance and sediment invertebrate abundance in an outdoor stream experiment (Girling *et al.* 2000). In addition, Crossland and Hillaby (1985) suggested that an MATC of 10 µg l^{-1} 3,4-DCA would be protective of field populations of *Daphnia*. Therefore the proposed PNEC of 0.2 µg l^{-1} would be regarded as protective of long-term exposures to 3,4-DCA in the field.

PNEC accounting for a maximum allowable concentration

Freshwater short-term toxicity data are available for nine taxonomic groups including algae, annelids, bacteria, ciliates, crustaceans, fish, insects, molluscs and rotifers (see Table 2.7). Consequently, data are available for the 'base set' of organisms and the EU TGD assessment factor (AF) method can be applied (ECB 2003). Crustaceans and insects appear to be the most sensitive organisms to short-term exposures of 3,4-DCA. However, there are issues with the reliability of the lowest insect data.

The lowest available short-term algal study is a 96-hour EC50 (growth) of 2200 µg l^{-1} for *Scenedesmus quadricauda* (Adema and Vink 1981). This datum was based on measured exposure concentrations and the study fulfilled the test validity criteria. However, the 96-hour study duration means that the value should be used in a supporting capacity only. The same issue applies to the 96-hour EC50 (population growth) of 4800 µg l^{-1} for *S. pannonicus* (Adema *et al.* 1982 cited in ECB 2006), which was regarded as valid by the EU RAR. The only short-term algal study conducted with a $\leq$72h hour exposure duration is the population biomass 48-hour EC50 of 6800 µg l^{-1} for *Scenedesmus subspicatus* reported by Kuhn and Pattard (1990). However, due to a lack of details on chemical analysis this study should be used in a supporting capacity only.

As with long-term exposures, data for crustaceans are available primarily for *Daphnia magna.* The lowest valid values are 24 and 48-hour EC50s (immobilisation) of 56 and 54 µg l^{-1}, respectively, reported for *D. magna* (Pedersen *et al.* 1998). These are based on measured exposure concentrations, static exposure and a standard methodology. Therefore the lower 48 hour EC50 of 54 µg l^{-1} was regarded as fully valid for PNEC derivation. The lowest reliable value identified by the EU RAR was a 96 hour LC50 of 160 µg l^{-1} for *D. magna* (Adema and Vink 1981 cited in ECB 2006).

Short-term freshwater data are also available for insects. The lowest reported value is a 96-hour LC50 of 4 µg l^{-1} for the midge (*Chironomus riparius*) (24-hour LC50 = 28 µg l^{-1}) (Hooftman *et al.* 1989). It was not possible to obtain the original documentation for this study and as such no quality assessment could be made. The 96 hour LC50 is the lowest value in the short-term freshwater database. However, Taylor *et al.* (1991) also assessed the effects of 3,4-DCA on *C. riparius* over 96 hours and reported an LC50 of 7400 µg l^{-1}. The study by Taylor *et al.* (1991) was conducted using semi-static exposures with measured exposure concentrations. Consequently, this study is regarded as valid for PNEC derivation and suggests that the LC50 of 4 µg l^{-1} of Hooftman *et al.* (1989) is unlikely to be reliable (and it is possible that the units of the Hooftman *et al.* (1989) study have been reported incorrectly).

Short-term freshwater fish data were available for various species. The lowest effect concentration is a 96-hour LC50 of 1940 µg l^{-1} for the rainbow trout (*Oncorhynchus mykiss*) (Hodson 1985 cited in ECB 2006). This value was regarded as valid for PNEC derivation by the EU RAR.

Toxicity data available for other taxonomic groups (bacteria, ciliates, rotifers, annelids and molluscs) indicate similar or lower sensitivity than reported for algae, insects or fish. However, crustaceans are significantly more sensitive than the other organisms tested.

The lowest valid short-term freshwater data point is the 48-hour EC50 of 54 µg l^{-1} for the immobilisation of *D. magna* (Pedersen *et al*. 1998). This was a replicated, static study with measured exposure concentrations. Consequently, it was regarded as fully valid for PNEC derivation. The lowest short-term value identified by the RAR was the 96-hour LC50 of 160 µg l^{-1} for *D. magna* (Adema and Vink 1981 cited in ECB 2006). However, the lowest overall reliable value identified in this report is the 48-hour EC50 (immobilisation) of 54 µg l^{-1} for *D. magna.*

No short-term freshwater PNEC was actually derived in the EU RAR for 3,4-DCA (ECB 2006). Consequently, it is proposed that the short-term freshwater PNEC be based on the 48-hour EC50 of 54 µg l^{-1} for *D. magna* and an assessment factor of 10. The assessment factor of 10 is felt justified due to the availability of reliable short-term data for at least three taxonomic groups. This results in:

$PNEC_{freshwater\_st}$ = 54 µg l^{-1}/AF (10) = 5.4 µg l^{-1}

3.1.2 PNECs for saltwaters

The effects database for marine species is slightly smaller than that for freshwater organisms. Long-term toxicity data are available for six taxonomic groups: algae, annelids, crustaceans, fish, molluscs and plankton (Table 2.8). Short-term saltwater toxicity data are available for seven different taxonomic groups: algae, annelids, bacteria, crustaceans, fish, molluscs and rotifers (see Table 2.9). Both data sets include data for the base set of organisms (i.e. algae, crustaceans and fish) and so the EU TGD assessment factor approach can be applied.

Based on the available data there appears to be no significant differences in the sensitivity of freshwater or saltwater species of the same taxonomic group. This may reflect the mode of toxic action of 3,4-DCA as a polar narcotic. Consequently the RAR for 3,4-DCA combined freshwater and saltwater datasets for the derivation of PNECs. The same approach has been adopted for the derivation of PNECs in this report.

PNEC accounting for the annual average concentration

Only limited long-term saltwater algal toxicity data were available. The lowest long-term algal data point is a 96-hour NOEC (growth) of 100 µg l^{-1} in the diatom *Phaeodactylum tricornutum* (Adema *et al.* 1982 cited in ECB 2006). Very few details were available with which to assess this study. The study duration would not be

regarded as standard (96 hours as opposed to the 72 hours recommended by the OECD 201 methodology). However, the EU RAR classified the study as valid.

In addition to the algal data a 37-day LOEC of 100 µg l^{-1} is available for biomass and species composition changes in a marine plankton community exposed in field mesocosms (Kuiper and Hanstveit 1984) (see Section 2.6.6 for details). The LOEC was the lowest concentration tested and as such it is not possible to estimate the NOEC. The study was replicated and exposure concentrations were measured. Consequently this study would be regarded as valid.

The lowest available saltwater long-term crustacean datum is a 28-day NOEC (for reproduction and mortality) of 32 µg l^{-1} reported for the brine shrimp (*Artemia salina*) (Adema and Vink 1981 cited in ECB 2006). This datum was based on measured exposure concentrations and was regarded by the RAR as fully valid for PNEC derivation.

Long-term saltwater data are also available for annelids. The lowest value is a 38-day NOEC (for reproduction) of 3.2 µg l^{-1} for the polychaete worm (*Ophryotrocha diadema*) (Adema and Vink 1981 cited in ECB 2006). This datum was based on measured exposure concentrations and was regarded by the RAR as fully valid for PNEC derivation. The value is supported by a 31-day EC50 (reproduction) of 10 µg l^{-1} for the same species, which would also be regarded as valid for PNEC derivation (Adema and Vink 1981).

Long-term saltwater data are available for a number of fish species. The most sensitive species is the plaice (*Pleuronectes platessa*) with a 180-day NOEC of 32 µg l^{-1} (LOEC 180 µg l^{-1}) for mortality, growth and malformation. (Adema and Vink 1981 cited in ECB 2006). This datum was based on measured exposure concentrations and the study fulfilled the test validity criteria and as such is regarded as valid for PNEC derivation.

The available data for marine molluscs suggest that these organisms are less sensitive than saltwater algae, plankton, crustaceans, annelids or fish.

No specific saltwater PNEC was derived in the EU RAR for 3,4-DCA (ECB 2006). Instead the freshwater and saltwater datasets were combined and a single PNEC calculated based on the lowest reliable value in the whole data set. This PNEC was then termed a $PNEC_{aqua}$. The same approach of combining the freshwater and saltwater datasets and deriving a PNEC based on the lowest available value in the combined database is also adopted in this report.

The lowest reliable value in the saltwater data set is a 38-day NOEC (reproduction) of 3.2 µg l^{-1} for the polychaete worm (*Ophryotrocha diadema*) (Adema and Vink 1981 cited in ECB 2006). This datum is based on measured exposure concentrations and was regarded by the RAR as fully valid for PNEC derivation. However, in the combined dataset there are lower NOECs (42 and 48 day) of 2 µg l^{-1} for the growth and survival of guppies and zebra fish (Schafers and Nagel 1991 and Nagel *et al.* 1991 cited in ECB 2006). Given the similarity in the sensitivity of freshwater and saltwater species of the same taxonomic group and the non-specific mode of action of 3,4-DCA as a polar narcotic it is proposed that the saltwater PNEC be based on the lower freshwater data. As a consequence of this and given that good quality data are available for a range of taxonomic groups the same assessment factor (10) applied to the freshwater PNEC is also applicable for the saltwater PNEC resulting in:

$PNEC_{Saltwater\_lt}$ = 2 µg l^{-1}/AF (10) = 0.2 µg l^{-1}

This is supported by a very similar PNEC of 0.32 µg l^{-1} that could be derived by applying an assessment factor of 10 to the 38-day NOEC (reproduction) of 3.2 µg l^{-1} for the polychaete worm (*Ophryotrocha diadema*).

There were only very limited field data for salt waters. In marine enclosures, effects on phytoplankton, zooplankton and bacteria occurred only at 3,4-DCA concentrations of 100 µg l^{-1} or greater (Kuiper and Hanstveit 1984). These limited data suggest that the proposed long-term saltwater PNEC would be protective of field populations of saltwater phytoplankton, zooplankton and bacteria.

PNEC accounting for a maximum allowable concentration

In its assessment of the effects of 3,4-DCA on waste water treatment plants (WWTPs) the EU RAR identified and regarded as valid a single short-term marine bacterial data point for *Photobacterium phosphoreum* (renamed *Vibrio fischeri*) (30 min EC50 of 650 µg l^{-1}) (Ribo and Kaiser 1984 cited in ECB 2006). This was a static test based on nominal concentrations. The suitability of this value for saltwater PNEC derivation is questionable and the value was used by the RAR only in the assessment of WWTPs and was not included in the derivation of the aquatic PNEC.

The lowest short-term saltwater algal data point is a 96-hour EC50 (growth rate) of 450 µg l^{-1} for the diatom *Phaeodactylum tricornutum* (Adema and Vink 1981). Although based on measured exposure concentrations the duration of this study would not be regarded as standard, despite the OECD 201 methodology being used to carry out the study (96 hours as opposed to the 72 hours recommended by the OECD 201 methodology). As such this study should be used in a supporting capacity only. A 72-hour EC50 (growth) of 1100 µg l^{-1} is available for the same species (Kusk and Nyholm 1992 cited in ECB 2006). This study was conducted with measured exposure concentrations and was regarded as valid by the RAR.

Short-term saltwater crustacean data are available for various species. The most sensitive species is the grass shrimp (*Palaemonetes varians*) with a 96-hour LC50 of 1310 µg l^{-1} (Van der Meer *et al.* 1988). Although based on nominal concentrations this study was conducted with a semi-static exposure regime. Consequently, it could be regarded as supporting data for the derivation of the PNEC. The lowest crustacean data point regarded as valid for PNEC derivation is a 96-hour LC50 of 2300 µg l^{-1} for *Crangon crangon* (Adema and Vink 1981). This value was based on measured exposure concentrations and the study was reported to have satisfied all the test validity criteria.

Only limited short-term saltwater fish data are available. The lowest LC50 is a 96 hour value of 3500 µg l^{-1} for the guppy *Poecilia reticulata* exposed in salt water[5] (Adema and Vink 1981). This was a static test with measured exposure concentrations. The study was reported to have satisfied all the test validity criteria and was regarded as valid for PNEC derivation.

The available data for annelids, molluscs and rotifers indicate that these species are of lower sensitivity than saltwater bacteria, algae, crustaceans and fish.

Not including the 30 min EC50 of 650 µg l^{-1} for *Vibrio fischeri* (Ribo and Kaiser 1984 cited in ECB 2006) and the 96-hour (as opposed to 72-hour) EC50 of 450 µg l^{-1} for

[5] Adema and Vink (1981) investigated the effects of 3,4-DCA on various species and in some cases tested fish in both fresh and salt waters. The corresponding freshwater 96 hour EC50 for the guppy was 8700 µg l^{-1} (Table 2.7)

Phaeodactylum tricornutum (Adema and Vink 1981), the lowest short-term saltwater value suitable for PNEC derivation is the 72-hour EC50 (growth) of 1100 µg l^{-1} for *Phaeodactylum tricornutum* (Kusk and Nyholm 1992 cited in ECB 2006). However, in the combined data set a lower 48-hour LC50 of 54 µg l^{-1} is available for *D. magna* (Pedersen *et al.* 1998). This value is based on measured exposure concentrations, static exposure and a standard methodology.

No short-term saltwater PNEC was derived in the 3,4-DCA RAR (ECB 2006). Consequently, the lowest overall value identified in this report (48-hour EC50 of 54 µg l^{-1} for immobilisation of *D. magna*) is proposed as the critical data for the derivation of the short-term saltwater PNEC for 3,4-DCA. Given the similarity in the sensitivity of freshwater and saltwater species of the same taxonomic group and the mode of action of 3,4-DCA as a polar narcotic it is proposed that the same assessment factor (10) used for the freshwater short-term PNEC also be adopted for the saltwater environment. Therefors the saltwater PNEC(s) is:

$PNEC_{saltwater\_st}$ = 54 µg l^{-1}/AF (10) = 5.4 µg l^{-}

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

The minimum number of long-term toxicity data (at least 10 NOECs from eight taxonomic groups) is not available. Therefore, the species sensitivity distribution (SSD) approach cannot be used for PNEC derivation.

3.3 Derivation of existing EQSs

No existing EQSs are available for 3,4-DCA.

3.4 Derivation of PNECs for sediment

The log Kow for 3,4-DCA is 2.7 and in theory does not meet the EU TGD criterion for the assessment of sediment dwelling organisms. However, on release to the aquatic environment 3,4-DCA forms covalent bonds with the organic fraction of sediments and suspended matter, removing it from the water column. Consequently, sediments are one of the primary sinks for environmental releases of 3,4-DCA and sediment PNECs are required.

3.4.1 PNECs for freshwater sediments

Acute and chronic freshwater toxicity data are available for both sediment dwelling annelids and insects (see Table 2.10). However, a number of the studies were conducted in water alone, with no sediment exposure:

- 96-hour LC50 of 2500 µg l^{-1} for *Pristina longiseta* (Schmitz and Nagel 1995)
- 24- and 48-hour LC50s of 11000 µg l^{-1} for *Tubifex tubifex* (Yoshioka *et al.* 1986)
- 96-hour LC50 of 25200 µg l^{-1} for *Lumbriculus variegatus* (Oetken *et al.* 2000)

- 48 hour LC50 of 9200 µg l^{-1} for *Chironomus riparius* (Oetken *et al.* 2000)

Although providing an indication of the relative sensitivities of the various organisms, these data only predict effects through the uptake of 3,4-DCA contaminated water. Given the binding properties of 3,4-DCA and the potential uptake via organic matter contact/ingestion (see Section 3.5) it is essential to have data on the effects of 3,4-DCA via sediment exposures. Consequently, the water-only exposure data above would not be regarded as suitable for PNEC derivation.

One short-term and two long-term freshwater sediment exposure studies are available (Table 2.10). Naylor and Howcroft (1997) (cited in ECB 2006) studied the effects of sediment exposures of 3,4-DCA on the growth of midge larvae (*C. riparius*). Various growth stages of midge were exposed to 250-550 mg/kg dw 3,4-DCA over a 10 day period. The most sensitive endpoints were dry weight and length with NOECs of <250 mg/kg dw (LOEC 250 mg/kg dw) and 250 mg/kg (LOEC 350 mg/kg dw), respectively (Table 2.10). Early first instar midges appeared to be most sensitive to 3,4-DCA exposures. This was a replicated study with measured exposure concentrations and as such was regarded as valid for PNEC derivation by the EU RAR.

Oetken *et al.* (2000) (cited in ECB 2006) exposed, in two separate studies, *Lumbriculus variegatus* (annelid worm) and *C. riparius* (midge) to sediment spiked with 3,4-DCA in the ranges of 1 to 625 mg/kg dw and 0.064 to 40 mg/kg dw, respectively for 28 days. The survival, deformations and morphallaxis of worms and the total emergence, rate of emergence, gender ratio and eggs per clutch of midges were monitored. The most sensitive endpoints for the two species were a NOEC of 1 mg/kg (LOEC 5 mg/kg dw) for the deformations of *L. variegatus* and a NOEC of <0.064 mg/kg (LOEC 0.064 mg/kg dw) for emergence rate and eggs per clutch of *C. riparius* (Table 2.10). The *C. riparius* NOEC of <0.064 mg/kg dw is the lowest available sediment data point. However, the EU RAR for 3,4-DCA reports that this result should be treated with caution as no clear concentration-response curve was observed with these endpoints. Consequently, as part of the EU RAR the *C. riparius* study was repeated (see below) in order to clarify the above results. The EU RAR states that the *L. variegatus* data in this study are valid for PNEC derivation.

Bayer AG (2001) (cited in ECB 2006) repeated the *C. riparius* study exposing midge larvae to sediment spiked with 3,4-DCA in the range of 10 to 1000 mg/kg dw over 14 days. The emergence rate, development rate (pooled sex), development rate (male) and development rate (female) of midges were monitored (Table 2.10). The lowest endpoint was a 14 day EC10 (development rate in females) of 104 mg/kg dw (EC50 >180 mg/kg).This was a replicated study with measured exposure concentration (radioactive measurement) and as such was regarded by the RAR as fully valid for PNEC derivation.

The EU RAR identifies the 28 day LOEC of 5 mg/kg dw of Oetken *et al.* (2000) for deformations of *L. variegatus* as the lowest reliable sediment data, but the effect was not statistically significant. However, this study did report 28-d NOECs of 5 mg/kg dw for effects of 3,4-DCA on the number and total biomass of worms. Consequently, the appropriate assessment factor for two long-term sediment values is 50 resulting in:

$PNEC_{freshwater\ sediment}$= (5 mg/kg)/AF (50) = 0.1 mg/kg dw (0.04 mg/kg ww)

3.4.2 PNECs for saltwater sediments

Acute and chronic saltwater toxicity data are available only for sediment dwelling annelids (see Table 2.11), although all of the studies were conducted in water alone, with no sediment exposure:

- 24-hour LC50 of 25000 µg l^{-1} for *Ophryotrocha diadema* (Adema and Vink 1981)
- 7-day LC50 of 2800 µg l^{-1} for *Ophryotrocha diadema* (Adema and Vink 1981)
- 38-day NOEC (reproduction) of 3.2 µg l^{-1} for *Ophryotrocha diadema* (Adema and Vink 1981)

The above water-only exposure data would not be regarded as suitable for PNEC derivation as contact/ingestion of 3,4-DCA bound to organic matter is likely to be an important exposure mechanisms which is not incorporated in these studies.

There are no marine sediment exposure data available. However, based on the available data there appear to be no significant differences in the sensitivity of freshwater or saltwater species of the same taxonomic group. This may reflect the mode of toxic action of 3,4-DCA as a polar narcotic. Consequently the freshwater and saltwater datasets for 3,4-DCA can be combined. As such it is proposed that the freshwater sediment PNEC be adopted as the marine sediment PNEC:

$PNEC_{saltwater\ sediment}$= (5 mg/kg)/AF (50) = 0.1 mg/kg dw (0.04 mg/kg ww)

3.5 Derivation of PNECs for secondary poisoning of predators

3.5.1 Mammalian and avian toxicity data

Only two reviews have been published regarding 3,4-dichloroaniline (ECB 2006 and IUCLID, 2000). As the most recent, the EU RAR (ECB 2006) has been assumed to contain the most sound and complete mammalian data. For this reason, this was the primary source used. However, the IUCLID review was also consulted. Additional literature searches were performed from 2006 to the present day to locate any lower effect data published since 2006. However, none were found. Very limited data are available for 3,4-dichloroaniline. No sub-chronic or chronic oral mammalian studies are available (only an inhalation and dermal study). However, a suitable oral study is available for the structurally similar chloroaniline compound, 2,5-dichloroaniline, which the EU RAR used as the basis for their risk assessment.

For avian data, due to the lack of relevant data in the EU RAR, the IUCLID review was assumed to contain the most sound and complete data. However, due to the limited data, a comprehensive literature search was also performed to locate any relevant data, although none were found.

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	
Hoechst AG (1989) Cited in ECB (2006) **Sub-chronic NOAEL = 30 mg/kg bw/day (2,5-dichloroaniline)**	Male and female Wistar rats (number of animals per group unspecified) received 2,5-dichloroaniline orally (route unspecified) for 28 days at doses of 0, 30, 150 or 750 mg/kg bw/day. Non-specific clinical symptoms (reduced spontaneous activity, hunched posture and retracted flanks), respiratory distress, uncoordinated movements, trembling, salivation, increased water consumption, retarded growth (males only), brown-yellow discolouration of urine, decreased red blood cell counts, decreased haemoglobin, increased reticulocyte counts, increased relative spleen weight and extramedullary erythropoietic activity in the spleen were observed at the top dose. The NOAEL was based on decreased total bilirubin levels (females only), increased erythropoiesis in bone marrow and haemosiderosis in the spleen that occurred at the top two doses.
Chronic toxicity to mammals	
No chronic or carcinogenicity data are available for 3,4-dichloroaniline. Overall, the evidence for genotoxicity indicates that 3,4-dichloroaniline is unlikely to be genotoxic *in vivo* (ECB, 2006).	
Effects on reproduction of mammals	
No data on potential fertility effects of 3,4-dichloroaniline were available.	
Embryotoxicity and teratogenicity	
Clemens and Hartnagel (1990) Cited in ECB (2006) **Maternal NOAEL = 5 mg/kg bw/day** **Developmental NOAEL = 25 mg/kg bw/day**	Pregnant Charles River Crl:CD BR rats (28 inseminated dams/dose) received 3,4-dichloroaniline orally via gavage as a suspension at a volume of 10 ml/kg bw between gestation days 6 and 15 at doses of 0, 5, 25 or 125 mg/kg bw/day. Animals were sacrificed on gestational day 20. The number of dams with live progeny, corpora lutea, implantations, resorptions, litter size, placental weights and pre- and post-implantation loss were examined, as well as the weight, sex and gross external, visceral and skeletal dysmorphogenic changes of live foetuses. The maternal NOAEL was based on significantly reduced food consumption and average body weight gain that occurred at the top two doses. The developmental NOAEL was based on slightly, but not significantly, increased resorptions and post-implantation loss, and significantly delayed ossification of a few skeletal elements that occurred at the top dose.

Sub-chronic toxicity to birds	
Schafer *et al.* (1983) Cited in IUCLID (2000) **LD50 = 237 mg/kg bw**	Red-winged blackbirds (sex, age and number of birds per dose unspecified; *Agelaius phoenicus*) received 3,4-dichloroaniline orally (route unspecified) at unspecified doses. They were subsequently observed for 18 hours.
Schafer *et al.* (1983) Cited in IUCLID (2000) **LD50 = 562 mg/kg bw**	European starling (sex, age and number of birds per dose unspecified; *Sturnus vulgaris*) received 3,4-dichloroaniline orally (route unspecified) at unspecified doses. They were subsequently observed for 18 hours.
No reproductive, developmental or chronic studies were available regarding the potential effects of 3,4-dichloroaniline in birds.	

The EU RAR identified the NOAEL of 30 mg/kg body weight per day 2,5-DCA from the rat oral toxicity study of Hoechst AG (1989) as most suitable for the derivation of the **$PNEC_{secpois.biota}$**. The 2,5-DCA NOAEL was chosen due to a lack of suitable data for 3,4-DCA. The EU RAR stated that due to the structural similarity of 2,5-DCA to 3,4-DCA it causes similar toxic effects such as haemolytic anaemia and methaemoglobinaemia. Consequently, 2,5-DCA was considered a suitable surrogate for 3,4-DCA (ECB 2006).

3.5.2 PNECs for secondary poisoning of predators

Bioconcentration data

The log Kow for 3,4-DCA is 2.7 and in theory does not meet the EU TGD criterion for the assessment of secondary poisoning. This corresponds with the reported BCF values for fish exposed to 3,4-DCA of between 4 and 45 (ECB 2006). However, in single species tests, water column and sediment dwelling invertebrate BCF values of up to 82 but higher BAF values of up to 572 have been reported (Nagel 1997 cited in ECB 2006). Consequently, the TGD BCF/BAF trigger of 100 is exceeded for 3,4-DCA and there is a need to derive PNECs for secondary poisoning.

The RAR for 3,4-DCA identifies a number of bioconcentration and bioaccumulation studies with invertebrates carried out by Nagel (1997). Based on ^{14}C activity bioconcentration factors (BCFs) of 113 (*Ceratophyllum demersum*), 79 (*Elodea canadensis*), 29 (*Daphnia magna*), 28 (*Asellus aquaticus*), 15 (*Planorbarius corneus*), 35 (*Tubifex tubifex*), 30 (*Limnodrilus hoffmeisteri*), and 800 (*Lumbriculus variegatus*) were reported. However, when the same BCFs were calculated based on chemical analysis of the parent compound, rather than ^{14}C, lower values of 82 (*C. demersum*), 11 (*E. canadensis*), 9 (*D. magna*), 10 (*A. aquaticus*), 12 (*P. corneus*), 18

(*T. tubifex*), and 16 (*L. hoffmeisteri*) were reported (Nagel 1997 cited in ECB 2006). The difference in the values based on ^{14}C activity and the parent compound are likely to be due to the fact that the radioactivity measurement will also include transformation/degradation products in the system.

In addition, Nagel (1997) (cited in ECB 2006) also estimated the bioaccumulation (rather than bioconcentration) of 3,4-DCA in the same species. When based on ^{14}C activity bioaccumulation factors (BAFs) were: 113 (*C. demersum*), 139 (*E. canadensis*), 78 (*D. magna*), 106 (*A. aquaticus*), 73 (*P. corneus*), 158 (*T. tubifex*), and 566 (*L. variegatus*). When these values were recalculated based on chemical analysis of the parent compound values were 210 (*C. demersum)*, 198 (*E. canadensis*), 276 (*D. magna*), 76 (*A. aquaticus*), 533 (*P. corneus*), 271 (*T. tubifex*) and 570 (*L. variegatus*).

The available data suggests a relatively low uptake of 3,4-DCA via the water column. However, much higher accumulation occurs when 3,4-DCA exposure occurs through the food (including sediment ingestion). Therefore, for the assessment of secondary poisoning the EU RAR for 3,4-DCA identified the BAF of 570 for *L. variegatus* as most suitable value for PNEC derivation.

PNECs for secondary poisoning

The EU RAR identified the NOAEL of 30 mg/kg body weight per day 2,5-DCA from the rat oral toxicity study of Hoechst AG (1989) as most suitable for the derivation of the $PNEC_{secpois.biota}$. The RAR proposes a conversion factor of 10 (to convert the dose to a concentration in food) and an assessment factor of 1000, giving:

$PNEC_{secpois.biota}$ = (NOAEL 30 mg/kg bw x 10)/AF 1000 = 0.3 mg/kg in food

This is the calculation made by the RAR. However, the AF (1000) appears to be far too high and it could be that they have used the conversion factor incorrectly or duplicated it and added also to the AF. If this is correct the resulting $PNEC_{secpois.water}$ is overprecautionary and any reduction in the assessment factor would result in a higher value.

The RAR identified the BAF of 570 for *L. variegatus* as most suitable for PNEC derivation. Consequently, the concentration in water preventing bioaccumulation in prey to levels >$PNEC_{secpois.biota}$ can be calculated as follows:

$PNEC_{secpois.water}$ = (0.3 mg/kg prey)/BAF (570) = 0.0005 mg l^{-1} (0.5 µg l^{-1})

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

4 Analysis and monitoring

There are numerous methods available for the analysis of 3,4-DCA in various environmental media. The US EPA (1996) proposes a method for the analysis of aniline and selected derivatives by gas chromatography (Method 8131). The method uses solvent extraction with Florisil or Gel Permeation Chromatography (GPC) clean-up and analysis by Gas Chromatography with a Nitrogen Phosphorus Detector (GC/NPD) or Gas Chromatography Mass Spectrometry (GCMS).Detection limits of 3.2 µg l^{-1} are reported for environmental waters and 2144 µg/kg for soils.

However, lower detection limits have been reported by a number of authors. In environmental waters detection limits of 7 ng l^{-1} (river water) and 11.1 ng l^{-1} (lake water) have been reported using Solid Phase Extraction (SPE) and High Performance Liquid Chromatography coupled with a UV-Diode Array Detector (HPLC-DAD) (Boti *et al.* 2007a). In seawater detection limits of 4.3 ng l^{-1} and 7 ng l^{-1} have been reported using SPE with HPLC-DAD (Gatidou *et al.* 2005 and Boti *et al.* 2007a).

In sediments limits of detection of 1.9, 4.8 and 63 µg/kg (dry weight) have been reported for marine sediments, river sediments and lake sediments, respectively using HPLC-DAD (Boti *et al.* 2007b). Using the same analytical method Gatidou *et al.* (2004) reported detections limits of 2.1 µg/kg (dry weight) in marine sediments.

Proposed PNECs derived for 3,4-DCA range from 0.2 to 1.5 µg l^{-1} in environmental waters and 0.1 mg/kg dw (0.04 mg/kg ww) in sediments. The data quality requirements are that, at a third of the EQS total error of measurement should not exceed 50 per cent. Using this criterion, current analytical methodologies should offer adequate performance to analyse for 3,4-DCA.

5 Conclusions

5.1 Availability of data

Long-term freshwater toxicity data are available for eight taxonomic groups including algae, crustaceans, fish, insects, macrophytes, molluscs, planarians and rotifers. Freshwater short-term toxicity data are available for nine taxonomic groups including algae, annelids, bacteria, ciliates, crustaceans, fish, insects, molluscs and rotifers. Long-term saltwater toxicity data are available for six taxonomic groups: algae, plankton, crustaceans, annelids, molluscs and fish. Short-term saltwater toxicity data are available for seven different taxonomic groups: bacteria, algae, crustaceans, fish, annelids, rotifers and molluscs. Fish and crustaceans appear to be the most sensitive organisms to water column exposures of 3,4-DCA.

In addition, both freshwater and saltwater toxicity data are available for sediment dwelling organisms. Freshwater long and short-term data are available for sediment dwelling annelids and insects. Marine long and short-term toxicity data for sediment dwelling organisms are available only for annelids. Annelids appear to be the most sensitive organisms to sediment exposures of 3,4-DCA.

Both freshwater and saltwater mesocosm and field studies are available. However, each study used different exposure systems with varying exposure concentrations. Consequently, there are a range of different endpoints and effect concentrations. The lowest endpoints from the available studies are MATCs of 8-10 µg l^{-1} for zooplankton abundance and sediment invertebrate abundance in an outdoor freshwater stream experiment. Based on the available data 3,4-DCA concentrations below 10 µg/l should have little effect on field populations of aquatic organisms.

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

Long-term freshwater toxicity data are available for eight taxonomic groups including algae, crustaceans, fish, insects, macrophytes, molluscs, planarians and rotifers. The lowest valid long-term freshwater data points are 42 and 48 day NOECs (for growth and survival) of 2 µg l^{-1} in guppies and zebra fish. Both values were generated in flow-through studies with measured exposure concentrations and were regarded by the 3,4-DCA RAR as fully valid for PNEC derivation. The long-term freshwater PNEC in the EU RAR for 3,4-DCA was therefore based on these data points with an assessment factor of 10 given the availability of long-term data for three or more trophic levels. This results in a **$PNEC_{freshwater\_lt}$ = 2 µg l^{-1}/AF (10) = 0.2 µg l^{-1}.**

A number of field and mesocosm studies are available for 3,4-DCA. The lowest endpoints from the available studies were MATCs of 8-10 µg l^{-1} for zooplankton abundance and sediment invertebrate abundance in an outdoor stream experiment. In addition, an MATC of 10 µg l^{-1} 3,4-DCA has been suggested to be protective of

field populations of *Daphnia*. Therefore the proposed PNEC of 0.2 µg l^{-1} would be regarded as protective of long-term exposures to 3,4-DCA in the field.

There are no existing EQSs for 3,4-DCA for comparison with the proposed PNECs.

5.2.2 Short-term PNEC for freshwaters

Freshwater short-term toxicity data are available for nine taxonomic groups including algae, annelids, bacteria, ciliates, crustaceans, fish, insects, molluscs and rotifers. The lowest valid short-term freshwater data point is a 48-hour EC50 (immobilisation) of 54 µg l^{-1} for *D. magna* which was considered a more suitable study for derivation of the PNEC. The lowest short-term value identified by the RAR was a 96 hour LC50 of 160 µg l^{-1} for *D. magna*.

No short-term freshwater PNEC was derived in the EU RAR for 3,4-DCA. Consequently, it is proposed that the short-term freshwater PNEC be based on the 48-hour EC50 of 54 µg l^{-1} and an assessment factor of 10. The assessment factor of 10 is felt justified due to the availability of reliable short-term data for at least three taxonomic groups. This results in a **$PNEC_{freshwater\_st}$ = 54 µg l^{-1}/AF (10) = 5.4 µg l^{-1}.**

Based on the available data the acute to chronic ratio (ACR) for crustaceans, algae and rotifers would be approximately 10. Based on the proposed PNECs the ratio between the short-term and long-term PNECs is 7.5 which is in line with the available ACRs.

There are no existing EQSs for 3,4-DCA for comparison with the proposed PNECs.

5.2.3 Long-term PNEC for saltwaters

Long-term saltwater toxicity data are available for six taxonomic groups: algae, plankton, crustaceans, annelids, molluscs and fish.

No specific saltwater PNEC was derived in the EU RAR for 3,4-DCA. Instead the freshwater and saltwater datasets were combined and a $PNEC_{aqua}$ calculated (presumably termed a $PNEC_{aqua}$ because both water compartments were covered). The lowest reliable value in the saltwater data set is a 38-day NOEC (reproduction) of 3.2 µg l^{-1} for the polychaete worm (*Ophryotrocha diadema*). This datum is based on measured exposure concentrations and was regarded by the RAR as fully valid for PNEC derivation. However, in the combined dataset there are lower NOECs (42 and 48 day) of 2 µg l^{-1} for the growth/survival of guppies and zebra fish. Given the similarity in the sensitivity of freshwater and saltwater species of the same taxonomic group and the non-specific mode of action of 3,4-DCA as a polar narcotic it is proposed that the saltwater PNEC be based on the lower freshwater data. As a consequence of this and given that good quality data are available for a range of taxonomic groups the same assessment factor (10) applied to the freshwater PNEC is also applicable for the saltwater PNEC resulting in a **$PNEC_{Saltwater\_lt}$ = 2 µg l^{-1}/AF (10) = 0.2 µg l^{-1}**

This is supported by a very similar PNEC of 0.32 µg l^{-1} that could be derived by applying an assessment factor of 10 to the 38-day NOEC (reproduction) of 3.2 µg l^{-1} for the polychaete worm (*Ophryotrocha diadema*).

There were only very limited field data for salt waters. In marine enclosures, effects on phytoplankton, zooplankton and bacteria occurred only at 3,4-DCA concentrations

of >100 µg l^{-1}. These limited data suggest that the proposed long-term saltwater PNEC would be protective of field populations of saltwater phytoplankton, zooplankton and bacteria.

There are no existing EQSs for 3,4-DCA for comparison with the proposed PNECs

5.2.4 Short-term PNEC for saltwaters

Short-term saltwater toxicity data are available for seven different taxonomic groups: bacteria, algae, crustaceans, fish, annelids, rotifers and molluscs. The lowest reliable short-term saltwater value is a 72 hour EC50 (growth) of 1100 µg l^{-1} for *Phaeodactylum tricornutum*. However, in the combined data set a lower 72-hour LC50 of 14.5 µg l^{-1} is available for *D. magna* eggs. This value was generated in a replicated, semi-static study with measured exposure concentrations. However, this was disregarded, as it represented an accelerated reproduction study and was not considered an appropriate acute end point. The next lowest short-term freshwater data point is a 48-hour EC50 (immobilisation) of 54 µg l^{-1} for *D. magna* which was considered a more suitable study for derivation of the PNEC.

No short-term saltwater PNEC was derived in the 3,4-DCA RAR. Consequently, the lowest overall value identified in this report (48-hour LC50 of 54 µg l^{-1} for *D. magna*) is proposed as the critical data for the derivation of the short-term saltwater PNEC for 3,4-DCA. Given the similarity in the sensitivity of freshwater and saltwater species of the same taxonomic group and the mode of action of 3,4-DCA as a polar narcotic it is proposed that the same assessment factor (10) used for the freshwater short-term PNEC also be adopted for the saltwater environment. Therefore the saltwater PNEC(s) for short term is:

$PNEC_{saltwater\_st}$ = 54 µg l^{-1}/AF (10) = 5.4 µg l^{-1}

There are no existing EQSs for 3,4-DCA for comparison with the proposed PNECs

5.2.5 PNEC for sediments

The log Kow for 3,4-DCA is 2.7 and in theory does not meet the EU TGD criterion for the assessment of sediment dwelling organisms. However, on release to the aquatic environment 3,4-DCA forms covalent bonds with the organic fraction of sediments and suspended matter, removing it from the water column. Consequently, sediments are one of the primary sinks for environmental releases of 3,4-DCA and sediment PNECs are required.

The EU RAR identifies the 28 day LOEC of 5 mg/kg dw of Oetken *et al.* (2000) for deformations of *L. variegatus* as the lowest reliable sediment data, but the effect was not statistically significant. However, this study did report 28-d NOECs of 5 mg/kg dw for effects of 3,4-DCA on the number and total biomass of worms. Consequently, the appropriate assessment factor for two long-term sediment values is 50 resulting in a **$PNEC_{freshwater\ sediment}$= (5 mg/kg)/AF (50) = 0.1 mg/kg dw (0.04 mg/kg ww).**

There are no marine sediment exposure data available. However, based on the available data there appears to be no significant differences in the sensitivity of freshwater or saltwater species of the same taxonomic group. This may reflect the mode of toxic action of 3,4-DCA as a polar narcotic. Consequently the freshwater

and saltwater datasets for 3,4-DCA can be combined. As such it is proposed that the freshwater sediment PNEC be adopted as the marine sediment PNEC.

5.2.6 PNEC for secondary poisoning

The EU RAR identifies a NOAEL of 30 mg/kg body weight per day 2,5-DCA from a rat oral toxicity study as most suitable for the derivation of the $PNEC_{secpois.biota}$. The RAR proposes a conversion factor of 10 (to convert the dose to a concentration in food) and an assessment factor of 1000, giving:

$PNEC_{secpois.biota}$ = (NOAEL 30 mg/kg bw x 10)/AF 1000 = 0.3 mg/kg in food

(The 2,5-DCA NOAEL was chosen due to a lack of suitable data for 3,4-DCA. The EU RAR stated that due to the structural similarity of 2,5-DCA to 3,4-DCA it causes similar toxic effects such as haemolytic anaemia and methaemoglobinaemia. Consequently, 2,5-DCA was considered a suitable surrogate for 3,4-DCA (ECB 2006)).

The RAR identifies a BAF of 570 for *Lumbriculus variegatus* as most suitable for PNEC derivation. Consequently, the concentration in water preventing bioaccumulation in prey to levels >$PNEC_{secpois.biota}$ is:

$PNEC_{secpois.water}$ = (0.3 mg/kg prey)/BAF (570) = 0.0005 mg l^{-1} (0.5 µg l^{-1}

This is the calculation made by the RAR. However, the AF (1000) used appears to be far too high and it could be that they have used the conversion factor incorrectly or duplicated it and added also to the AF. If this is correct the resulting $PNEC_{secpois.water}$ is overprecautionary and any reduction in the assessment factor would result in a higher value.

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

Table 5.1 Summary of proposed PNECs

Receiving medium/exposure scenario	Proposed PNEC (µg l^{-1})	Existing EQS (µg l^{-1})
Freshwater/long-term	0.2	-
Freshwater/short-term	5.4	-
Saltwater/long-term	0.2	-
Saltwater/short-term	5.4	-
Sediment (freshwater and saltwater)	0.1 mg/kg dw (0.04 mg/kg ww)	-
Secondary poisoning	0.5	-

5.3 Analysis

Proposed PNECs derived for 3,4-DCA range from 0.2 to 1.5 µg l^{-1} in environmental waters and 0.1 mg/kg dw (0.04 mg/kg ww) in sediments. The data quality requirements are that, at a third of the EQS total error of measurement should not exceed 50 per cent. Using this criterion, current analytical methodologies should offer adequate performance to analyse for 3,4-DCA

5.4 Implementation issues

The proposed PNECs are consistent with those proposed in the EU Risk Assessment Report for 3,4-DCA (ECB, 2006). These PNECs are suitable for use as they are not subject to excessive uncertainty and current analytical capability should be adequate for compliance assessment purposes. Due to the potential for 3,4-DCA to adsorb to sediment and bioaccumulate consideration should be given as to the relevance of sediment and biota standards for this substance.

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List of abbreviations

AA	Annual Average
AF	Assessment Factor
BAF	Bioaccumulation Factor
BCF	Bioconcentration Factor
3,4-DCA	3,4-Dichloroaniline
bw	body weight
CAS	Chemical Abstracts Service
d	days
DAD	Diode Array Detector
DO	Dissolved Oxygen
DT50	Time at which 50 per cent of the organisms or animals tested died at a specific concentration
dw	Dry weight
EC50	Concentration effective against 50 per cent of the organisms or animals tested
ECB	European Chemicals Bureau
EHC	Environmental Health Criteria
EQS	Environmental Quality Standard
EU	European Union
GC	Gas Chromatography
GC-MS	Gas Chromatography Mass Spectrometry
GLP	Good Laboratory Practice (OECD)
GPC	Gel Permeation Chromatography
h	hours
HPLC	High Performance Liquid Chromatography
LC50	Concentration lethal to 50 per cent of the organisms or animals tested
LD50	Dose lethal to 50 per cent of the organisms or animals tested
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration
lt	long-term
MAC	Maximum Allowable Concentration
MATC	Maximum Allowable Toxic Concentration
NOAEL	No Observed Adverse Effect Level
NOEC	No Observed Effect Concentration

NPD	Nitrogen Phosphorus Detector
OECD	Organization for Economic Co-operation and Development
PNEC	Predicted No-Effect Concentration
SPE	Solid Phase Extraction
SSD	Species Sensitivity Distribution
RAR	Risk Assessment Report
st	short-term
TGD	Technical Guidance Document
UKTAG	UK Technical Advisory Group
US EPA	US Environmental Protection Agency
WFD	Water Framework Directive
ww	Wet 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

Author	Adema *et al.* (1982)

Information on the test species	
Test species used	*Phaeodactylum tricornutum* *Scenedesmus pannonicus*
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	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Marine water
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)	Static
Measurement of exposure concentrations	Not stated
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	DCA was stable throughout the test
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Very few study details provided with which to assess the quality of the study. However, the RAR classified the study as valid

Reliability of study	**-**
Relevance of study	**-**
Klimisch Code	**RAR (valid)**

Author	Adema and Vink (1981)

Information on the test species	
Test species used	*Scenedesmus quaricauda*
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	Conducted according to method described in "Degradability, Ecotoxicity and Bioaccumulation; the determination of possible effects of chemicals and wastes on the aquatic environment".
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	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	Measured concentration (>70% of nominal)
Measurement of water quality parameters	Oxygen concentration was never less than 70% of saturation. pH was always about 8
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Although details are lacking in this study, The paper states that all data are based on measured concentrations and fulfil the quality and performance criteria of the tests employed. Other data in this paper were considered valid by the RAR.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2 (Reliable with restriction)**

Author	Adema and Vink (1981)

Information on the test species	
Test species used	*Phaeodactylum tricornutum*
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	Conducted according to method described in "Degradability, Ecotoxicity and Bioaccumulation; the determination of possible effects of chemicals and wastes on the aquatic environment".
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	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	Measured concentration (>70% of nominal)
Measurement of water quality parameters	Oxygen concentration was never less than 70% of saturation. pH was always about 8
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Although details are lacking in this study, the paper states that all data are based on measured concentrations and fulfil the quality and performance criteria of the tests employed. Other data in this paper were considered valid by the RAR.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2 (Reliable with restriction)**

Author	Adema and Vink (1981)

Information on the test species	
Test species used	*Daphnia magna*
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	Conducted according to method described in "Degradability, Ecotoxicity and Bioaccumulation; the determination of possible effects of chemicals and wastes on the aquatic environment".
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	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	Measured concentration (>70% of nominal)
Measurement of water quality parameters	Oxygen concentration was never less than 70% of saturation. pH was always about 8
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Although details are lacking in this study, it was considered valid by the RAR

Reliability of study	**-**
Relevance of study	**-**
Klimisch Code	**RAR (valid)**

Author	Adema and Vink (1981)

Information on the test species	
Test species used	*Artemia salina* *Cheaetogammarus marinus*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	3 day old, 1 mm length

Information on the test design	
Methodology used	Conducted according to method described in "Degradability, Ecotoxicity and Bioaccumulation; the determination of possible effects of chemicals and wastes on the aquatic environment".
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	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	Measured concentration (>70% of nominal)
Measurement of water quality parameters	Oxygen concentration was never less than 70% of saturation. pH was always about 8
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Although details are lacking in this study, it was considered valid by the RAR

Reliability of study	**-**
Relevance of study	**-**
Klimisch Code	**RAR (valid)**

Author	Adema and Vink (1981)

Information on the test species	
Test species used	*Crangon crangon*
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	Conducted according to method described in "Degradability, Ecotoxicity and Bioaccumulation; the determination of possible effects of chemicals and wastes on the aquatic environment".
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	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	Measured concentration (>70% of nominal)
Measurement of water quality parameters	Oxygen concentration was never less than 70% of saturation. pH was always about 8
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Although details are lacking in this study, the paper states that all data are based on measured concentrations and fulfil the quality and performance criteria of the tests employed. Other data in this paper were considered valid by the RAR.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2 (Reliable with restriction)**

Author	Adema and Vink (1981)

Information on the test species	
Test species used	*Ophryotrocha diadema*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Larvae and adults

Information on the test design	
Methodology used	Conducted according to method described in "Degradability, Ecotoxicity and Bioaccumulation; the determination of possible effects of chemicals and wastes on the aquatic environment".
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	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)	Flow-through
Measurement of exposure concentrations	Measured concentration (>70% of nominal)
Measurement of water quality parameters	Oxygen concentration was never less than 70% of saturation. pH was always about 8
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Although details are lacking in this study, it has been considered valid by the RAR

Reliability of study	**-**
Relevance of study	**-**
Klimisch Code	**RAR (valid)**

Author	Adema and Vink (1981)

Information on the test species	
Test species used	*Poecilia reticulata*
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	Conducted according to method described in "Degradability, Ecotoxicity and Bioaccumulation; the determination of possible effects of chemicals and wastes on the aquatic environment".
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	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)	Static
Measurement of exposure concentrations	Measured concentration (>70% of nominal)
Measurement of water quality parameters	Oxygen concentration was never less than 70% of saturation. pH was always about 8
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Although details are lacking in this study, the paper states that all data are based on measured concentrations and fulfil the quality and performance criteria of the tests employed. Other data in this paper were considered valid by the RAR.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2 (Reliable with restriction)**

Author	Adema and Vink (1981)

Information on the test species	
Test species used	*Pleuronectes platessa*
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	Conducted according to method described in "Degradability, Ecotoxicity and Bioaccumulation; the determination of possible effects of chemicals and wastes on the aquatic environment".
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	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)	Static
Measurement of exposure concentrations	Measured concentration (>70% of nominal)
Measurement of water quality parameters	Oxygen concentration was never less than 70% of saturation. pH was always about 8
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Although details are lacking in this study, The paper states that all data are based on measured concentrations and fulfil the quality and performance criteria of the tests employed. Other data in this paper were considered valid by the RAR.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2 (Reliable with restriction)**

Author	Barata and Baird (2000)

Information on the test species	
Test species used	*Daphnia magna*
Source of the test organisms	Not stated
Holding conditions prior to test	Maintained in ASTM hard water with the addition of standard organic extract and fed daily. 14:10 hour light:dark cycle, temperature = 20 +/-1°C
Life stage of the test species used	8-9 day old

Information on the test design	
Methodology used	Not stated
Form of the test substance	98% pure
Source of the test substance	Aldrich, Gillingham, UK
Type and source of the exposure medium	ASTM water
Test concentrations used	Fed and starved controls plus seven concentrations (2.5 – 100 µg l^{-1}) of DCA
Number of replicates per concentration	10 replicates of both fed and starved controls and 7 test concentrations (2.5 – 100 µg l^{-1}) of DCA
Number of organisms per replicate	Not stated
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Semi-static
Measurement of exposure concentrations	Measured concentration (70-80% of nominal)
Measurement of water quality parameters	14:10 hour:light dark cycle, temperature = 20 +/-1°C. Test solutions changed daily
Test validity criteria satisfied	Test concentrations at t_0 and t_{24} differed from nominal concentrations by 20 and 30%, respectively
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Generally well documented study. Semi-static study with measured exposure concentrations.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2 (Reliable with restriction)**

Author	Call *et al.* (1987)

Information on the test species	
Test species used	*Pimephales promelas*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Eggs and fry

Information on the test design	
Methodology used	Not stated
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	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)	Flow-through
Measurement of exposure concentrations	Measured concentrations
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Study considered valid by RAR

Reliability of study	**-**
Relevance of study	**-**
Klimisch Code	**RAR (valid)**

Author	Diamantino *et al.* (1997)

Information on the test species	
Test species used	*Daphnia magna*
Source of the test organisms	Author's laboratory, single clone cultured for more than 5 years.
Holding conditions prior to test	Not stated
Life stage of the test species used	Third neonates

Information on the test design	
Methodology used	Not stated, but method described
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	One control and five toxicant concentrations (2.5, 5, 10, 25 and 50 µg l^{-1}).
Number of replicates per concentration	Eight
Number of organisms per replicate	Not stated
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Flow-through and semi-static tests were performed
Measurement of exposure concentrations	Nominal concentrations
Measurement of water quality parameters	Oxygen concentrations and pH were tested twice weekly
Test validity criteria satisfied	Concentration of stock solution was measured on days 9 and 14 and was not statistically different from initial concentration.
Water quality criteria satisfied	Oxygen always remained above 75% saturation, pH was always 7.0-8.0
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Well documented study, considered valid by RAR

Reliability of study	**-**
Relevance of study	**-**
Klimisch Code	**RAR (valid)**

Author	Hodson (1985)

Information on the test species	
Test species used	*Oncorhynchus mykiss*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	1-5 g

Information on the test design	
Methodology used	Not stated
Form of the test substance	99+% pure, dissolved in either pharmaceutical cod liver oil or in 5% ethanol in saline
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)	Static
Measurement of exposure concentrations	Nominal concentrations
Measurement of water quality parameters	Standardised water conditions (temperature = 15°C, alkalinity = 90 mg/l, pH = 7.8)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Despite the lack of details, it is considered valid by the RAR

Reliability of study	**-**
Relevance of study	**-**
Klimisch Code	**RAR (valid)**

Author	Hooftman *et al.* (1989)

Information on the test species	
Test species used	*Chironomus riparius*
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	Not stated
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)	Static
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 Good Laboratory Practice	Not stated
Overall comment on quality	Toxicity data summary obtained from US EPA AQUIRE data base. It was not possible to locate original study. Consequently, it was not possible to quality assess these data

Reliability of study	**Unknown**
Relevance of study	**Unknown**
Klimisch Code	**4 (Not assignable)**

Author	Hooftman and Vink (1980)

Information on the test species	
Test species used	*Ophryotrocha diadema*
Source of the test organisms	Originates from the harbour of Los Angeles
Holding conditions prior to test	Artificially prepared seawater (salinity = 33 +/-1%, pH8.1 +/-0.1, temperature = 21 +/-1°C, oxygen content = >70% saturation)
Life stage of the test species used	2-3 day old larvae which had started feeding

Information on the test design	
Methodology used	Not stated
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Dosed from stock solutions in distilled water made acidic
Test concentrations used	Not stated
Number of replicates per concentration	4
Number of organisms per replicate	10
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Semi-static
Measurement of exposure concentrations	Measured concentration
Measurement of water quality parameters	Artificially prepared seawater (salinity = 33 +/-1%, pH8.1 +/-0.1, temperature = 21 +/-1°C, oxygen content = >70% saturation)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Lacking some specific study details, but a replicated study with measured exposure concentrations

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2 (Reliable with restriction)**

Author	Janssen *et al.* (1994)

Information on the test species	
Test species used	*Brachionus calyciflorus*
Source of the test organisms	Collected in Gainesville, Florida, USA and maintained in laboratory cultures by T.W. Snell, Georgia Institute of Technology, Atlanta, GA, USA
Holding conditions prior to test	Cysts were produced in mass cultures under rigorously controlled conditions, collected, dried and stored at 6°C in the dark.
Life stage of the test species used	0-2 hour old neonates collected 16-18 hours after initiation of hatching

Information on the test design	
Methodology used	Not stated
Form of the test substance	Dissolved in acetone
Source of the test substance	Not stated
Type and source of the exposure medium	Synthetic freshwater medium
Test concentrations used	Three concentrations, control and solvent control
Number of replicates per concentration	Four
Number of organisms per replicate	12
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Semi-static
Measurement of exposure concentrations	Measured concentrations. Results reported as nominal with 15% error
Measurement of water quality parameters	Temperature = 25 +/-1°C
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Well documented study. Semi-static exposure with measured concentrations

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**1 (Reliable without restriction)**

Author	Kuhn and Pattard (1990)

Information on the test species	
Test species used	*Scenedesmus subspicatus*
Source of the test organisms	Maintained by authors over several decades
Holding conditions prior to test	Cultivated three days prior to preparation of test solution in 50 ml of nutrient solution. Cell number = 10E5 cells/ml
Life stage of the test species used	In a state of logarithmic growth

Information on the test design	
Methodology used	DIN 38 312
Form of the test substance	Dissolved in double distilled water using a magnetic stirrer
Source of the test substance	Not stated
Type and source of the exposure medium	Algal medium
Test concentrations used	Dilution series of 1:2, each had 1 part pollutant solution in 2^0-2^8 volumes of mixture.
Number of replicates per concentration	Not stated
Number of organisms per replicate	10^4 cells per ml
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Not stated
Measurement of exposure concentrations	Not stated
Measurement of water quality parameters	Temperature = 24 +/-1°C and a relative humidity of 50%
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Some study details lacking, but information given on the validity criteria of the test

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2 (Reliable with restriction)**

Author	Kusk and Nyholm (1992)

Information on the test species	
Test species used	*Phaeodactylum tricornutum*
Source of the test organisms	Norwegian Institute of Water Research
Holding conditions prior to test	Grown on a culturing medium based on seawater at 20 o/oo salinity and 15 +/-1°C
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	ISO 8692
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	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)	Static
Measurement of exposure concentrations	Measured concentration
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Concentration of DCA deviated by less than 10% of nominal
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Many key study details are lacking. However, the RAR considered the study to be valid

Reliability of study	**-**
Relevance of study	**-**
Klimisch Code	**RAR (valid)**

Author	Nagel (1998)

Information on the test species	
Test species used	*Brachydanio rerio*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Larvae and fry

Information on the test design	
Methodology used	Not stated
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	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)	Flow-through
Measurement of exposure concentrations	Measured concentration
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Unable to obtain original text (published in German), but the EU RAR considered the study to be valid

Reliability of study	**-**
Relevance of study	**-**
Klimisch Code	**RAR (valid)**

Author	Nagel *et al.* (1991)

Information on the test species	
Test species used	*Brachydanio rerio*
Source of the test organisms	With one exception (Hoechst AG), fish were obtained from West Aquarium Co., Bad Lauterberg. Hoechst AG obtained fish from in-company breeding
Holding conditions prior to test	Not stated
Life stage of the test species used	Larvae and fry

Information on the test design	
Methodology used	Not stated
Form of the test substance	99.5% pure
Source of the test substance	Merck Schuchardt in Hohenbrunn
Type and source of the exposure medium	Not stated
Test concentrations used	0, 2, 20 and 200 µg l^{-1} (one lab also used 100 µg l^{-1}).
Number of replicates per concentration	2 replicates per concentration, each run at 7 labs
Number of organisms per replicate	100 fertilised ova
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Flow-through
Measurement of exposure concentrations	Measured concentration
Measurement of water quality parameters	Several types of water, pH, temperature and oxygen concentrations were used. 12:12 hour light:dark cycles (except in two labs which operated 16:8 hour light:dark cycles and one lab with a 14:10 hour light:dark cycle).
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Well documented. RAR considered the study to be valid

Reliability of study	**-**
Relevance of study	**-**
Klimisch Code	**RAR (valid)**

Author	Pedersen *et al.* (1998)

Information on the test species	
Test species used	*Daphnia magna*
Source of the test organisms	In house VKI culture
Holding conditions prior to test	The animals were cultured in water from Lake Brådebæk at 20°C and fed three times every day with *Selenastrum* or *Chlorella* and a supply of yeast once or twice every week. Every week, a new culture was started with young animals.
Life stage of the test species used	<24h old juveniles

Information on the test design	
Methodology used	The standard procedure described in the EPA *Daphnia acute* toxicity test. 40 CFR Ch. 1 (7-1-92 Edition), § 797.1300 /1/ was used . This standard procedure also fulfils the requirements in the ISO /2/, OECD /3/, and EU /4/ standard methods.
Form of the test substance	>98% purity
Source of the test substance	Merck (Lot No. 2308664)
Type and source of the exposure medium	Freshly prepared ISO medoum
Test concentrations used	Control, 0.0125, 0.025, 0.05, 0.1, 0.2, 0.4 and 0.8 mg l^{-1}
Number of replicates per concentration	Six for the controls and 4 per treatment
Number of organisms per replicate	5
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Static and semi static, 48 hours, no feeding
Measurement of exposure concentrations	Measured concentration
Measurement of water quality parameters	Yes (pH and dissolved oxygen)
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	-

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

Author	Ribeiro *et al.* (1995)

Information on the test species	
Test species used	*Aedes aegypti*
Source of the test organisms	Not stated
Holding conditions prior to test	Larvae were reared at 25+/-1°C in M7 synthetic media and fed a Tetramin based diet.
Life stage of the test species used	Larvae

Information on the test design	
Methodology used	Not stated
Form of the test substance	Dissolved M7 media and nanopure water
Source of the test substance	Not stated
Type and source of the exposure medium	Various test media
Test concentrations used	Six concentrations plus control
Number of replicates per concentration	3
Number of organisms per replicate	Not stated
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Static
Measurement of exposure concentrations	Measured concentrations
Measurement of water quality parameters	Experiment performed at 20 +/-1°C, 14:10h light:dark cycle. Oxygen saturation was measured at beginning and end of experiment. pH was measured at beginning and end of experiment
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Oxygen saturation was always above 80%. pH ranged between 5.9-10.5.
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Reasonable well documented study. Measured exposure concentrations, but some mistakes in paper (the same data being reported as µg/l and mg/l in different figures). Based on methods section results should be mg l^{-1}

Reliability of study	**Questionable reliability**
Relevance of study	**Relevant**
Klimisch Code	**2 (Reliable with restriction)**

Author	Ribo and Kaiser (1984)

Information on the test species	
Test species used	*Photobacterium phosphoreum*
Source of the test organisms	Not stated
Holding conditions prior to test	Reagent prepared by reconstitution of freeze-dried bacteria with ultrapure water and kept at 2-4°C until use.
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Microtox test
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	4 concentrations plus control
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Static
Measurement of exposure concentrations	Not stated
Measurement of water quality parameters	Temperature = 15°C, 15 minute equilibrium period
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not reported
Overall comment on quality	Despite the lack of details, the study is considered valid by the RAR

Reliability of study	**-**
Relevance of study	**-**
Klimisch Code	**RAR (valid)**

Author	Samel *et al.* (1999)

Information on the test species	
Test species used	*Daphnia magna*
Source of the test organisms	Not stated
Holding conditions prior to test	Clones grown on LGWs and acclimated to Elendt M4, Elendt M7 or COMBO media at 20 +/-2°C with a 16:8 hour light:dark cycle.
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	OECD guideline 202
Form of the test substance	98% pure
Source of the test substance	Aldrich Chemicals Co. Ltd.
Type and source of the exposure medium	Not stated
Test concentrations used	Control, 3.1, 8.8 and 25µg l^{-1}.
Number of replicates per concentration	Not stated
Number of organisms per replicate	15
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Semi-static
Measurement of exposure concentrations	Results based on nominal concentrations. Measured stock concentrations 83-109% of nominal. However, test solutions were only 22-90% of nominal
Measurement of water quality parameters	Yes
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Reasonably well documented. Tests compared impacts of different culture medium and tests carried out at various laboratories. Issues with measured test concentrations being only 22-90% of nominal so results based on nominal concentrations. Study should be used in a supporting capacity only.

Reliability of study	**Questionable reliability**
Relevance of study	**Relevant**
Klimisch Code	**2 (Reliable with restriction)**

Author	Schafer *et al.* (1994)

Information on the test species	
Test species used	*Scenedesmus subspicatus* *Chlamydomonas reinhardtii*
Source of the test organisms	Sammlung von Algenkulturen (SAG), University of Gottingen D-3400 Gottingen
Holding conditions prior to test	A preculture was prepared from stock algae 3 days prior to the test.
Life stage of the test species used	Exponentially growing

Information on the test design	
Methodology used	OECD guideline 201
Form of the test substance	Dissolved in acetone p.a.
Source of the test substance	Shell Research Ltd, Sittingbourne Research Centre, Sittingbourne, England
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Tests were run in triplicate and each test was counted threefold.
Number of organisms per replicate	Initial concentration of 6x10E4 cells/ml
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Flowthrough and static
Measurement of exposure concentrations	Measured and nominal concentration
Measurement of water quality parameters	Temperature and light exposure reported, but no mention of water quality analysis.
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Generally good study. Critical data generated in a replicated, flowthrough study with measured exposure concentrations. However, study duration (96 hours) exceeds that recommended by OECD 201 (72 hours)

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2 (Reliable with restriction)**

Author	Schafers and Nagel (1991)

Information on the test species	
Test species used	*Poecilia reticulata*
Source of the test organisms	Westerwalder Fischzucht Stahler GmbH, Hadamar-Niederzeuzheim, FRG
Holding conditions prior to test	Aclimated to experimental conditions 2 weeks prior to test
Life stage of the test species used	1+ year old

Information on the test design	
Methodology used	Not stated
Form of the test substance	99.5% pure
Source of the test substance	Merck-Schuchart
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)	Flowthrough
Measurement of exposure concentrations	Measured
Measurement of water quality parameters	Yes
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	O2 saturation remained above 70%
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Generally well documented. RAR regarded the study as valid.

Reliability of study	**-**
Relevance of study	**-**
Klimisch Code	**RAR (valid)**

Author	Schafer *et al.* (1993)

Information on the test species	
Test species used	*Chlamydomonas reinhardtii*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Exponential growth phase

Information on the test design	
Methodology used	OECD guideline 201
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Control, vehicle control and 5 or 6 concentrations
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)	Flow-through
Measurement of exposure concentrations	Measured concentrations
Measurement of water quality parameters	Incubation vessels with a volume of 0.9l were illuminated with 7000 lux. Temperature = 24 +/- 2°C. pH = 6.2. CO2 used to stabilise pH if it approached 7. C02 supply and removal of gas accumulating beneath the membrane filters were managed by hollow fibre membranes.
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Reasonable well documented, but some details missing. Flowthrough study with measured exposure concentrations. No reason to reject study.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2 (Reliable with restriction)**

Author	Taylor *et al.* (1991)

Information on the test species	
Test species used	*Chironomus riparius*
Source of the test organisms	Laboratory culture
Holding conditions prior to test	Not stated
Life stage of the test species used	Larvae

Information on the test design	
Methodology used	Not stated
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	3.2-70 mg l^{-1}
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)	Semi-static
Measurement of exposure concentrations	Measured (78% of nominal)
Measurement of water quality parameters	Yes
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Reasonably well documented study. Semis-static study with measured exposure concentrations, but no mention of replication

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2 (Reliable with restriction)**

Author	UBA (1994)

Information on the test species	
Test species used	*Daphnia magna*
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	Not stated
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)	Semi-static
Measurement of exposure concentrations	Measured
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not reported
Overall comment on quality	Despite the lack of details, the study is considered valid by the RAR

Reliability of study	**-**
Relevance of study	**-**
Klimisch Code	**RAR (valid)**

Author	Van der Meer *et al.* (1988)

Information on the test species	
Test species used	*Palaemonetes varians*
Source of the test organisms	Caught in brackish ditches in Zeeland, The Netherlands
Holding conditions prior to test	Kept in 100% seawater at 15°C and fed mashed shrimps and *Enchytraeus albidus* Henle.
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Not stated
Form of the test substance	Dissolved in ethanol
Source of the test substance	Not stated
Type and source of the exposure medium	Artificial sea water
Test concentrations used	1.6-16 mg l^{-1}
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)	Semi-static
Measurement of exposure concentrations	Nominal concentrations
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Few study details, but a semi-static exposure regime.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2 (Reliable with restriction)**