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Proposed Environmental Quality Standards for 2-, 3- and 4-Chlorophenol and 2,4-Dichlorophenol in Water

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Statement of use
This report reviews the available data on the use, fate/behaviour and aquatic toxicity of the monochlorophenols and 2,4-dichlorophenol. Environmental Quality Standards have been proposed for the protection of aquatic life which will assist Agency staff in assessing the potential effects of these substances on water quality.

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FOREWORD

This report, which proposes Environmental Quality Standards (EQSs) for monochlorophenols and 2,4-Dichlorophenol for the protection of fresh and saltwater life and for abstraction to potable supply, is one of a series of eleven produced under the Environment Agency/SNIFFER co-funded Phase IV EQS contract.

The other reports establish EQSs for aluminium, cyanide, nonyl phenol, octyl phenol, dioxins, sheep dip chemicals, chlorine dioxide, fluoride, mevinphos and naphthalene.

CONTENTS

LIST OF TABLES

EXECUTIVE SUMMARY

This report, prepared for the Environment Agency and the Scotland and Northern Ireland Forum for Environmental Research (SNIFFER), reviews and critically assesses information taken from the open literature on the environmental fate and toxicity of 2-, 3- and 4-chlorophenol and 2,4-dichlorophenol (referred to as 2-CP, 3-CP, 4-CP and 2,4-DCP for simplicity). The information is used to derive Environmental Quality Standards (EQSs) for the protection of fresh and saltwater life and for abstraction to potable supply.

In fish, polychlorophenols appear to be Type II 'polar' narcotics. In other words, compounds that cause narcosis associated with a specific mode of action. This has been identified as an uncoupling of electron transport (oxidative phosphorylation) in mitochondria. However, the mechanism of toxicity in invertebrates and of the monochlorophenols appears to be less specific. 2-, 3- and 4-CP and 2,4-DCP are of moderate to high acute toxicity to fresh and saltwater organisms, with the majority of reported L(E)C50 data ranging from 8-85, 2.6-25 and 1.7-16 mg l^{-1} for algae, crustaceans and fish, respectively. No one group of organisms appears to be more sensitive than any other group and there appears to be little difference in toxicity following short or long-term exposure. In addition, chlorophenol toxicity increases with increasing degree of chlorination and substitution away from the *ortho-* (2-) position. The higher toxicity of the more highly chlorinated congeners can be ascribed to an increase in lipophilicity which leads to a greater potential for uptake into the organism, while the *ortho-*substituted congeners are generally of lower toxicity than the *meta-* and *para-* substituted compounds, as the close proximity of the *ortho-*substituted chlorine tends to 'shield' the OH group on the molecule, which interacts with the active site in aquatic organisms. So for the congeners reviewed in this report, toxicity generally increases in the order 2-CP<3-CP<4-CP<2,4-DCP, although the monochlorinated congeners and 2,4-DCP do not appear to vary greatly in toxicity (usually less than one order of magnitude).

Toxicity also depends on the extent to which the chlorophenol molecules are dissociated in the exposure medium. In general, an increase in toxicity is observed with a decrease in pH since the more toxic non-dissociated form exists at lower pH, while at higher pH the less toxic dissociated form is predominant. This suggests that transfer of chlorophenols from exposure media to biotic tissues is greatly increased when the molecules are in the undissociated form. Moreover, the pKa values (pH at which an acid compound is 50% dissociated) of 2-, 3- and 4-CP and 2,4-DCP indicate that at the pH range characterising most physiological and environmental conditions, these compounds will exist predominately in the more active undissociated form.

The vast majority of aquatic organisms do not readily accumulate monochlorophenols or 2,4-DCP to high levels. Bioconcentration Factors (BCFs) for these congeners are not, on the whole, particularly high, with the majority for fish ranging from 3.8-34 at neutral pH, with depuration half-lives in the order of hours to days. However, BCFs of 282-980 have been reported for leeches, although this has been attributed to a deficiency in these organisms of the enzyme necessary for the metabolism of chlorophenols.

The proposed EQSs for the protection of fresh and saltwater life are set in two parts (see Table S1); a Maximum Allowable Concentration (MAC) and an Annual Average concentration. The MAC is intended for the protection of aquatic life against the adverse

effects of short-term episodic inputs, whereas the annual average should be adopted for protection against the effects of continuous discharges. Given the apparent differences in the mechanism of toxicity of the mono- and polychlorinated phenols, separate EQSs have been proposed for the mono- congeners and for 2,4-DCP. In addition, since the monochlorinated phenols are essentially very similar in toxicity (despite the effect of substitution pattern) and are likely to show simple additivity when in a mixture, a single EQS has been set that is applicable for total monochlorophenols in a mixture or for each congener when in isolation.

The lowest reliable toxicity data are 85-day Lowest-Observed Effect Concentrations (LOECs, growth, development and mortality) of 0.5 and 0.18 mg l^{-1} reported for the larvae of rainbow trout (*Oncorhynchus mykiss*), following exposure to 4-CP and 2,4-DCP, respectively. Applying a safety factor of 10 to these values results in respective EQSs of 50 and 20 µg l^{-1}, for total MCPs and 2,4-DCP, expressed as Annual Average "total" concentrations. Corresponding MACs of 250 and 140 µg l^{-1} have been proposed by applying a safety factor of 10 to the lowest, most reliable, 48 hour EC_{50} values of 2.5 and 1.4 mg l^{-1}, reported for immobility of the water flea (*Daphnia magna*), following exposure to 4-CP and 2,4-DCP, respectively. The MACs therefore approximate the long-term NOECs for rainbow trout and are set factors of 5 and 7 higher than the corresponding Annual Average values for 4-CP and 2,4-DCP. This difference between the Annual Average and MAC values falls within the range of acute to chronic toxicity ratios (1:1 to 7:1). These safety factors are considered sufficient to account for any slight increase in toxicity that may occur in surface waters of low pH.

Given the limited dataset on the toxicity of chlorophenols to saltwater organisms, along with the absence of reliable long-term toxicity and bioaccumulation data, it is proposed that the EQSs set for the protection of freshwater life should also be adopted as tentative values for the protection of saltwater life. This can be justified by the fact that the toxicity, fate and behaviour of the monochlorinated phenols and 2,4-DCP are the same under both fresh and saltwater conditions, as suggested by the available data.

The limit of detection values for 2-CP, 3-CP, 4-CP and 2,4-DCP are around three orders of magnitude lower than the proposed EQSs. Therefore current analytical methods are adequate for monitoring purposes.

It is not appropriate to set health-based values for 3- or 4-CP due to limitations in the toxicity dataset. Conservative health-based guideline values of 40 µg l^{-1} and 9 µg l^{-1} may be set for 2-CP and 2,4-DCP, respectively despite limitations in the toxicity database. However, all of these chemicals have low taste and odour thresholds and Reference Levels of 0.1, 10 and 0.1 µg l^{-1} are proposed as MACs for abstraction to potable water supply, for 2-CP, 4-CP and 2,4-DCP, respectively. At these levels, public health should also be protected. There are insufficient data with which to propose a Reference Level for the protection of potable supply for 3-CP.

Table S1 Proposed EQSs for 2-CP, 3-CP, 4-CP and 2,4-DCP

Use/chemical	AA ($\mu g\ l^{-1}$)	MAC ($\mu g\ l^{-1}$)	Notes
Protection of freshwater life			
2-chlorophenol	50	250	1,4
3-chlorophenol	50	250	1,4
4-chlorophenol	50	250	1,4
2,4-dichlorophenol	20	140	4
Protection of saltwater life			
2-chlorophenol	50	250	1,2,4
3-chlorophenol	50	250	1,2,4
4-chlorophenol	50	250	1,2,4
2,4-dichlorophenol	20	140	2,4
Abstraction to potable supply			
2-chlorophenol	-	-	3
3-chlorophenol	-	-	
4-chlorophenol	-	-	3
2,4-dichlorophenol	-	-	3

Notes to Table S1:
AA: Annual average
MAC: Maximum Allowable Concentration

1 Total concentration for monochlorophenols i.e. 2-CP, 3-CP and 4-CP in a mixture or as each congener individually.
2 Tentative Values based on EQSs proposed for freshwater.
3 A Reference Level of 0.1 $\mu g\ l^{-1}$ has been proposed for 2-CP, 4-CP and 2,4-DCP on the basis of taste and odour considerations (see Section 6.5 for details). This value is expressed as a MAC.
4 These standards are derived on the basis of toxicity data. However, tainting of fish flesh can occur at concentrations lower than levels causing chronic toxicity. Therefore, where the prevention of fish tainting is a priority, values lower than the proposed EQSs may be adopted (see Section 6.3.1).

KEY WORDS

Environmental Quality Standards, EQS, monochlorophenols, 2,4-dichlorophenol, chlorophenols, aquatic toxicity, bioaccumulation, freshwater, saltwater.

1. INTRODUCTION

Chlorophenols are organic chemicals formed by the substitution of phenol with one or more atoms of chlorine. Nineteen congeners are possible ranging from the monochlorophenols to the fully substituted pentachlorophenol. The majority are used as intermediates in the production of many different products such as pesticides. This is particularly true of the higher chlorinated tri-, tetra- and pentachlorophenols. However, this review concentrates only on the three monochlorinated congeners and on 2,4-dichlorophenol (hereafter referred to as 2-CP, 3-CP, 4-CP and 2,4-DCP for simplicity). These compounds can enter the aquatic environment through either direct discharge or via the natural degradation of higher chlorinated congeners. 2,4-DCP is of particular concern as large quantities are used and released as a result of the manufacture of the phenoxy herbicide 2,4-D and its breakdown in the environment.

The purpose of this report is to derive Environmental Quality Standards (EQSs) for the protection of fresh and saltwater life against the adverse effects of the monochlorophenols and 2,4-DCP, and for water intended for human consumption through abstraction to potable supply. This has been achieved by critical assessment of available data on the aquatic and mammalian toxicity of these compounds, their fate and behaviour in the environment and reported environmental concentrations. Section 2 gives details of the physico-chemical properties of these compounds, their manufacture, uses, likely routes of entry to the aquatic environment and reported environmental concentrations; Section 3 outlines appropriate analytical methodologies, while Sections 4 and 5 summarise the fate and toxicity of these chemicals in the environment. The derivation of EQSs is outlined in Section 6, with overall conclusions given in Section 7. The fate and toxicity of 2-CP, 3-CP, 4-CP and 2,4-DCP are discussed in full detail in Appendices A, B, C and D.

2. 2-CP, 3-CP, 4-CP AND 2,4-DCP IN THE ENVIRONMENT

2.1 Physico-chemical properties

Physico-chemical characteristics of 2-CP, 3-CP and 4-CP and 2,4-DCP are listed in Table 2.1. Despite the high solubility of these compounds, some adsorption to the organic carbon content of aquatic sediments may occur as indicated by the moderate octanol-water (Kow) partition coefficients. However, the high solubilities and lower organic-carbon coefficients (Koc) for some soils, suggest that the lower chlorinated phenols may be susceptible to leaching to surface and ground waters. The low Henry's Law Constants for these compounds suggest that volatilisation from surface waters is not likely to be an important removal route.

Since chlorophenols are weak acids in aqueous solution, one of the major factors affecting environmental transport, degradation and toxicity is the degree to which the compounds are dissociated in natural waters. Under acidic conditions, chlorophenols exist primarily in the toxic molecular (undissociated) form, while under basic conditions the dissociated form predominates. The pKa values (pH at which an acid compound is 50% dissociated) of 2-, 3- and 4-CP and 2,4-DCP indicate that at the pH range characterising most physiological and environmental conditions, these compounds will exist predominately in the more active undissociated form. Furthermore, as pH decreases the proportion of molecules in the undissociated state will increase further, leading to yet higher activity as shown by parameters such as adsorption to suspended solids and sediments and toxicity.

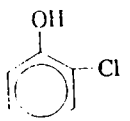
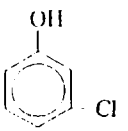
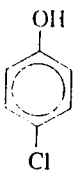
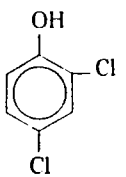
The fate and behaviour of these compounds in the environment is discussed in detail in Section 4 and Appendix A.

2.2 Manufacture

While most chlorophenols can be produced by several different procedures only a few methods appear to be used in commercial manufacture (WHO 1989). Most chlorophenols (including the monochlorinated congeners and 2,4-DCP) are produced by the stepwise chlorination of phenol or a lower chlorinated phenol at elevated temperature and pressure. For higher chlorinated congeners this can lead to the formation of microcontaminants such as dioxins and furans, although this does not appear to be a problem for the mono- or dichlorinated phenols, presumably due to the lower temperatures required for their manufacture (WHO 1989).

Reliable data on the production levels of chlorophenols other than pentachlorophenol are not available in the open literature. In 1975, the combined global production of all chlorophenols approached 200 million kilograms. Slightly more than half consisted of chlorophenols other than PCP, with 2,4-DCP, 2,4,5-TCP and 2,3,4,6-TCP predominating (WHO 1989). Krijgsheld and van der Gen (1986, cited in WHO 1989) reported European production levels of 4.5 and 9.1 million kilograms for total monochlorophenols and 2,4-DCP, respectively (year not stated), while in 1972, total chlorophenol production in the UK was reported to be 1.14 million kilograms (WHO 1989).

Table 2.1 Physical and chemical properties of 2-, 3- and 4-chlorophenol and 2,4-dichlorophenol

IUPAC CHEMICAL NAMES	2-Chlorophenol[1]	3-Chlorophenol	4-Chlorophenol	2,4-Dichlorophenol
SYNONYMS	2-monochlorophenol, 2-MCP, ortho-chlorophenol, o-chlorophenol, 1-chloro-2-hydroxybenzene, 2-hydroxychlorobenzene[1,2,3]	3-monochlorophenol, 3-MCP, meta-chlorophenol, m-chlorophenol, 1-chloro-3-hydroxybenzene, 3-hydroxychlorobenzene[1,2,3]	4-monochlorophenol, 4-MCP, para-chlorophenol, p-chlorophenol, 1-chloro-4-hydroxybenzene, 4-hydroxychlorobenzene[1,2,3]	2,4-DCP, DCP, 1-hydroxy-2,4-dichlorobenzene, Isobac[1,2,3]
CAS NUMBER	95-57-8[1]	108-43-0[1]	106-48-9[1]	120-83-2[1]
MOLECULAR FORMULA	C_6H_5ClO[1]	C_6H_5ClO[1]	C_6H_5ClO[1]	$C_6H_4Cl_2O$[1]
MOLECULAR STRUCTURE				
MOLECULAR WEIGHT	128.56[1]	128.56[1]	128.56[1]	163.01[1]
COMPOSITION	C=56.05%, H=3.92%, Cl=27.58%, O=12.44%[1]	C=56.05%, H=3.92%, Cl=27.58%, O=12.44%[1]	C=56.05%, H=3.92%, Cl=27.58%, O=12.44%[1]	C=44.21%, H=2.47%, Cl=43.5%, O=9.82%[1]
APPEARANCE	Colourless liquid[1,4]	Needles[1,4]	Crystals[1,4]	Needle-like crystals[1]
MELTING POINT (°C)	7.0-9.3[1,2,3,4]	32.8-33.5[1,2,3,4]	43.0-43.7[1,2,3,4]	42-45[1,2,3,4,5]
BOILING POINT (°C) (@ 760 mm Hg)	174.9-175.6[1,2,3,4]	214.0[1,2,3,4]	217.0-220.0[1,2,3,4]	209.0-211.0[1,2,3,4,5]

IUPAC CHEMICAL NAMES	2-Chlorophenol[1]	3-Chlorophenol	4-Chlorophenol	2,4-Dichlorophenol
VAPOUR PRESSURE (mm Hg)	1.0 @ 12.1 °C[3] 1.42 @ 25.0 °C[4]	1.0 @ 44.2 °C[3] 0.119 @ 25.0 °C[4]	1.0 @ 49.8 °C[3] 0.1 @ 20.0 °C[4]	1.0 @ 76.5 °C[3]
VAPOUR DENSITY	1.2634 (20/4)[3]	1.268 (25/4)[3]	1.2651 (30/4)[3]	1.38 (60/7)[3]
VOLATILITY	Slow from water[2]	Slow from water[2]	Slow from water[2]	Slow from water[3]
FLASH POINT (°C)	63.9[3]	No data	121.1[3]	62.0[3]
WATER SOLUBILITY (mg l-1)	28,500 @ 20.0-25.0 oC(2,4)	26,000 @ 20.0-25.0 oC(2,4)	27,000-27,100 @ 20.0-25.0 oC(2,4)	4,600 @ 20.0 °C[4]
pKa	8.52[2]	9.12[2]	9.41[2]	7.85[2]
HENRY'S LAW CONSTANT (atm m^3 mol^{-1})	5.6x10-7 (2)	5.6x10-7 (2)	5.6x10-7 (2)	$3.16x10^{-6}$ [2]
log K_{ow}	2.15[2,3,4]	2.50[2,3,4]	2.39[2,3,4]	3.06[3]
log K_{oc}	3.69 (Fine sediments)[2] 3.60 (Coarse sediments)[2] 1.70 (Clay-loam soil)[2]	1.71 (Clay-loam soil)[2]	1.85 (Clay-loam soil)[2]	2.54[6]

References:

1. Merck (1989)
2. Cited in Howard *et al* (1989)
3. Cited in WHO (1989)
4. Verschueren (1983)
5. Aldrich (1988)
6. Lagas (1988)

2.3 Uses

The principal use of the monochlorinated phenols is as intermediates in the synthesis of the higher chlorinated congeners and certain dyes and pesticides. The chief use of 2,4-DCP is as an intermediate in the production of 2,4-D and other herbicides. 2,4-DCP is also used as an ingredient in antiseptics. Table 2.2 displays the uses of these compounds more fully (cited in WHO 1989).

Table 2.2 Uses of the monochlorophenols and 2,4-dichlorophenol

Compound	Principal uses	Other uses
2-CP	Intermediate for further chlorination to 2,4-DCP, 2,4,6-TriCP, and PCP. Also in the production in dyes	Polymer intermediate for fire retardant varnishes, cotton fabric treatment to provide rot resistance, ingredient in coal processing
3-CP	Intermediate for production of higher chlorinated phenols	None
4-CP	Intermediate for production of higher chlorinated phenols and for dyes, fungicides and certain drugs	None
2,4-DCP	Intermediate for production of 2,4-D and other herbicides. Ingredient of anti-septics, starting material for higher chlorinated phenols	Intermediate for the production of Sesone, nitrofen, nemacide, Genite-EM-923. Raw material for polyester films. Also used in mothproofing agents and in miticides

2.4 Entry into the aquatic environment

The main route of entry of 2-, 3- and 4-CP and 2,4-DCP to the aquatic environment is likely to be as a result of discharges from plants manufacturing the compounds themselves or from plants using the compounds as intermediates in the production of higher chlorinated phenols and other products such as phenoxy herbicides.

Indirect sources include discharges from paper mills, where they are formed as by-products of the bleaching process, as a result of the disinfection of sewage, industrial wastes and drinking water with chlorine, and from the microbial breakdown of agricultural herbicides such as 2,4-D and subsequent run-off/leaching of the products.

2.5 Recorded levels in the environment

Both the Environment Agency in England and Wales and the Scottish Environment Protection Agency (SEPA) were contacted for details concerning reported environmental concentrations, either from routine monitoring or as a result of discharge consents. SEPA were unable to locate any data concerning mono- and dichlorophenols (I. Ridgeway, Pers. Comm. 1996).

However, the Environment Agency provided a very large quantity of data relating to routine monitoring of "background" concentrations and monitoring downstream of discharges from sewage treatment works, industrial/trade outfalls and mine workings/contaminated land drainage. Data for rivers in the Midlands and North East Regions are summarised in Tables 2.3 to 2.5 for ease of interpretation. The means, minima and maxima reported for 2-, 3- and 4-CP are for all sampling sites within a particular river or catchment associated with a typical type of discharge.

From the data given in Tables 2.3-2.5, it is difficult to detect a distinct pattern of monochlorophenol concentrations arising from the different types of discharge. However, high levels of 2-CP appear to be more often detected downstream of industrial/trade outfalls and contaminated land drainage than downstream of sewage treatment works and at routine monitoring sites, with the majority of upper means for these type of sites in the Severn-Trent catchment ranging from 4.7-131, 1.9-14.5, 0.9-2.4 and 1.2-3.4 $\mu g\ l^{-1}$, respectively. Data for 3-CP and 4-CP are not extensive enough to identify this pattern. For all monochlorophenols, concentrations at all sites are usually between 0.1 and 0.25 $\mu g\ l^{-1}$. The only data reported by the Agency for 2,4-DCP in the North East Region are concentrations of <0.2-0.4 and <0.2-330 $\mu g\ l^{-1}$ detected in the River Tyne at a routine monitoring site and downstream of an industrial discharge, respectively.

These data are broadly supported by the extremely large dataset provided by the South West Region of the Agency for 2-CP. Over the reported monitoring period (1992-1995), the great majority of concentrations at sites associated with routine monitoring in fresh and saltwaters, were less than 0.2 $\mu g\ l^{-1}$. Concentrations 1-2 orders of magnitude higher were reported for this congener downstream of discharges from sewage treatment works, while concentrations as high as 280 000 $\mu g\ l^{-1}$ were measured following pollution incidents, although associated effects on biota (if any) were not reported (H Wilkinson, Pers. Comm. 1997).

The above data are broadly supported by the limited information available in the published literature (see Sections 2.5.1 - 2.5.3).

2.5.1 Water

Residues of all chlorophenol isomers have been detected in aquatic systems (WHO 1989). Generally, residues are present at measurable concentrations in discharges from such sources as manufacturing plants, wood-treatment facilities, municipal waste discharges and in receiving waters adjacent to these sources. Concentrations in other surface waters are more sporadic and usually low, although some isomers have been detected in some of the cleanest waters in the world (WHO 1989).

Table 2.3 Reported concentrations of 2-Chlorophenol in English rivers (Environment Agency, Pers. Comm. 1997)

Catchment or river	Routine monitoring data (µg l^{-1})			Downstream of sewage treatment works (µg l^{-1})			Downstream of industrial /trade (µg l^{-1})			Downstream of mine workings/contaminated land/site drainage (µg l^{-1})		
	range of means	range of min	range of max	range of means	range of min	range of max	range of means	range of min	range of max	range of means	range of min	range of max
Midlands Region												
Upper Trent	0.25-2.17	<0.5	<0.5-<180	0.25-2.39	<0.5-1.4	<0.5-<10	0.25-131	<0.5	<0.5-1170	0.25-14.5	<0.5-<4	<0.5-<250
Lower Trent	0.25-25	<0.5-<2	<0.5-<50	0.25-1.37	<0.5	<0.5-<10	0.25-19.1	<0.5-<10	<0.5-<500	0.25-0.96	<0.5	<0.5-<10
Upper Severn	0.25-1.18	<0.5	<0.5-<10	0.25-0.9	<0.5	<0.5-<10	0.25-6.6	<0.1-<0.5	<0.5-<50	0.25-1.9	<0.5	<0.5-<50
Lower Severn	0.12-3.4	<0.1-<0.5	<0.5-10	0.25-8.5	<0.5	<0.5-<500	0.25-4.7	<0.5	<0.5-13.1	0.25-10	<0.5	<0.5-<20
North East Region												
Esk at Ruswarp	0.1	<0.2	<0.2									
Cover at Cover Bridge	0.1	<0.2	<0.2									
Hull at Drypool Bridge	0.1	<0.2	<0.2									
Don	0.01-0.19	<0.2	<0.2-7.3									

Catchment or river	Routine monitoring data (µg l^{-1})			Downstream of sewage treatment works (µg l^{-1})			Downstream of industrial /trade (µg l^{-1})			Downstream of mine workings/contaminated land/site drainage (µg l^{-1})		
	range of means	range of min	range of max	range of means	range of min	range of max	range of means	range of min	range of max	range of means	range of min	range of max
Bentley Mill stream	0.1	<0.2	<0.2									
Dearne	0.1	<0.2	<0.2									
Rother	0.1-2.84	<0.02-<0.2	<0.2-360									
Doe Lea	0.1-5.07	<0.02-<0.2	<0.2-450									
Spital	0.102	<0.2	0.2									
Totley brook	0.104	<0.2	0.2									
Aire	0.1-0.25	<0.2	<0.2-.2									
Calder	0.1-0.17	<0.2	<0.2-<2									
Carr Beck	0.11	<0.02	0.5									
Lupsett Beck	0.1	<0.2	<0.2									
Spen Beck	0.102-0.113	<0.2	0.3									
Bidgram Beck	0.1	<0.2	<0.2									

Catchment or river	Routine monitoring data (µg l^{-1})			Downstream of sewage treatment works (µg l^{-1})			Downstream of industrial /trade (µg l^{-1})			Downstream of mine workings/contaminated land/site drainage (µg l^{-1})		
	range of means	range of min	range of max	range of means	range of min	range of max	range of means	range of min	range of max	range of means	range of min	range of max
Derwent	0.1	<0.2	<0.2									
Wharfe	0.1	<0.2	<0.2									
Tyne	<0.2											
Tees	<0.2											
Wear	<0.2											
Unidentified river				0.1	<0.2	<0.2	0.24	<0.2	3	0.1	<0.2	<0.2
				0.14	<0.2	0.3	6.72	<0.2	200			
				0.18	<0.2	1	1.67	<0.2	17.8			
				0.64	<0.2	10	0.1	<0.2	<0.2			

Table 2.4 Reported concentrations of 3-Chlorophenol in English rivers (Environment Agency, Pers. Comm. 1997)

Catchment or river	Routine monitoring data(µg l^{-1})			Downstream of sewage treatment works (µg l^{-1})			Downstream of industrial /trade (µg l^{-1})			Downstream of mine workings/contaminated land/site drainage (µg l^{-1})		
	range of means	range of min	range of max	range of means	range of min	range of max	range of means	range of min	range of max	range of means	range of min	range of max
North East Region												
Foss	0.1	<0.2	<0.2									
Skell	0.1	<0.2	<0.2									
Burn	0.1	<0.2	<0.2									
Cover	0.1	<0.2	<0.2									
Wharfe	0.087	<0.02	<0.2									
Derwent	0.1	<0.2	<0.2									
Midgram Beck	0.096	<0.02	<0.2									
Colne	0.1	<0.2	<0.2									
Spen Beck	0.147	<0.2	0.8									
Hunsworth Beck	0.233	<0.2	6.5									
Clough Beck	0.1	<0.2	<0.2									
Sugden Beck	0.233	<0.2	2.1									
Calder	0.1-0.45	<0.02-<0.2	<0.2-13									

Catchment or river	Routine monitoring data(µg l^{-1})			Downstream of sewage treatment works (µg l^{-1})			Downstream of industrial /trade (µg l^{-1})			Downstream of mine workings/contaminated land/site drainage (µg l^{-1})		
	range of means	range of min	range of max	range of means	range of min	range of max	range of means	range of min	range of max	range of means	range of min	range of max
Aire	0.1-0.2	<0.2	<0.2-4.8									
Doe Lea	0.1-0.22	<0.2-<0.02	<0.2-5.4									
Rother	0.1-0.41	<0.1-<0.2	<0.2-7									
Dearne	0.1-0.1	<0.2	<0.2-0.5									
Don	0.1-0.21	<0.2	<0.2-8.3									
Hull	0.1	<0.2	<0.2									
Ouse	0.1	<0.2	<0.2									
Ure	0.1	<0.2	<0.2									
Humber	0.1	<0.2	<0.2									
Esk	0.1	<0.2	<0.2									
Unidentified river				0.5	<0.2	4	0.93	<0.2	9			
				0.1	<0.2	<0.2	0.1	<0.2	<0.2			
				0.27	<0.2	3.4	0.53	<0.2	3.6			

Table 2.5 Reported concentrations of 4-Chlorophenol in English rivers (Environment Agency, Pers. Comm. 1997)

Catchment or river	Routine monitoring data (µg l^{-1})			Downstream of sewage treatment works (µg l^{-1})			Downstream of industrial /trade (µg l^{-1})			Downstream of mine workings/contaminated land/site drainage (µg l^{-1})		
	range of means	range of min	range of max	range of means	range of min	range of max	range of means	range of min	range of max	range of means	range of min	range of max
North East Region												
Foss	0.1	<0.2	<0.2									
Skell	0.1	<0.2	<0.2									
Burn	0.1	<0.2	<0.2									
Cover	0.1	<0.2	<0.2									
Wharfe	0.1	<0.2	<0.2									
Derwent	0.1	<0.2	<0.2									
Midgram Beck	0.1	<0.2	<0.2									
Colne	0.109	<0.2	0.5									
Spen Beck	0.161	<0.2	0.9									
Hunsworth Beck	0.505	<0.02	11.1									
Clough Beck	0.14	<0.2	1.9									
Sugden Beck	0.1	<0.2	<0.2									

Catchment or river	Routine monitoring data (µg l^{-1})			Downstream of sewage treatment works (µg l^{-1})			Downstream of industrial /trade (µg l^{-1})			Downstream of mine workings/contaminated land/site drainage (µg l^{-1})		
	range of means	range of min	range of max	range of means	range of min	range of max	range of means	range of min	range of max	range of means	range of min	range of max
Calder	0.1-0.247	<0.2	<0.2-3.3									
Aire	0.1-0.4	<0.2	<0.2-0.5									
Doe Lea	0.1-3.79	<0.02-<0.2	<0.2-200									
Rother	0.1-0.95	<0.2-<0.02	<0.2-37									
Dearne	0.1-0.11	<0.2	<0.2-0.9									
Don	0.1-0.163	<0.2	<0.2-3.1									
Hull	0.1	<0.2	<0.2									
Ouse	0.1	<0.2	<0.2									
Ure	0.1	<0.2	<0.2									
Humber	0.1	<0.2	<0.2									
Esk	0.1	<0.2	<0.2									
Unidentified river				0.65	<0.2	2.9	0.24	<0.2	1.6			
				0.1	<0.2	<0.2	1.269	<0.2	52			
				0.11	<0.2	0.6	1.17	<0.2	14			

Most reports of chlorophenol levels in water are at sites associated with discharges from wood-treatment facilities and most of these are for the more highly chlorinated congeners. Paasivirta *et al* (1985) conducted various surveys on waters and biota of a Finnish lake which receives effluents from pulp mills. The authors reported 2,4-DCP concentrations of 2, 5 and 11 $\mu g\ l^{-1}$ in effluents discharged from kraft (birch run), kraft (pine run) and sulphite (birch run) mills, respectively. In lake water 5 km downstream from the mills, 2,4-DCP was below the detection limit.

These values are supported by the limited data reported in various other studies. For example, 2-CP, 3-CP and 4-CP have been detected at $\mu g\ l^{-1}$ levels in effluents from European sewage treatment plants and cooling water from power stations as a result of disinfection by chlorination (cited in WHO 1989), while in coastal areas and in rivers flowing through industrialised regions of the Netherlands, Piet and deGrunt (1975, cited in WHO 1989) reported that concentrations of monochlorophenols ranged from not-detected up to 20 $\mu g\ l^{-1}$ and dichlorophenols from not-detected up to 1.5 $\mu g\ l^{-1}$.

2.5.2 Sediments

Chlorophenol concentrations in sediments are for the most part higher than in overlying water. This may be as a result of adsorption onto suspended solids in the water column and subsequent sedimentation. However, very few data are available for mono- and dichlorophenols.

Wegman and Van den Broek (1983, cited in WHO 1989) have measured a range of mono-, di-, tri- and tetrachlorophenols in sediments of industrialised rivers in the Netherlands. A 3-CP concentration of 43 $\mu g\ kg^{-1}$ was detected in sediments of Lake Ketelmeer, while median and maximum 2,4-DCP concentrations of 4.4 and 10 $\mu g\ kg^{-1}$ were reported for the sediments of the same water body. The latter values are supported by a 2,4-DCP range of up to 3.8 $\mu g\ kg^{-1}$ reported for other surface waters in the Netherlands (WHO 1989).

At a site 2 km distant from a sulphate pulp mill, sediments in the Baltic sea were reported to contain a 2,4-DCP concentration of 0.9 $\mu g\ kg^{-1}$ (Xie 1983, cited in WHO 1989), while in a later survey, the same authors reported a sediment concentration of 16 $\mu g\ kg^{-1}$ 2 km from the discharge and 0 $\mu g\ kg^{-1}$ 5-10 km from the discharge.

2.5.3 Biota

Very few data are available concerning reported levels of mono- and dichlorophenols in aquatic biota. Bacon (1978, cited in WHO 1989) studied the levels of 2,4-DCP in fish sampled from a river in the US which receives pulp mill effluents. Residues were usually detected at several tens of $\mu g\ kg^{-1}$ (wet weight) in all tissues, including muscle, viscera, skin and liver. In some instances, liver concentrations of 2,4-DCP were as high as 242.9 $\mu g\ kg^{-1}$ (wet weight).

3. ANALYSIS

3.1 Analytical requirements for EQS monitoring

The adequate monitoring of EQSs requires a suitably accurate analytical method. The accepted approach for the derivation of the accuracy requirements of an analytical system (when monitoring for a particular water quality standard) is described in WRc Report NS30 (Cheeseman *et al* 1989).

For an EQS of X units, the error on a single analytical result should not be larger than X/10 concentration units or 20% of the concentration in the sample, whichever is the greater. Following the convention of dividing the tolerable error equally between random and systematic sources, this implies:

- a maximum tolerable standard deviation of X/40 concentration units or 5% of the concentration in the sample, whichever is greater; and
- a maximum tolerable bias of X/20 concentration units or 10% of the concentration in the sample, whichever is the greater.

It is recommended that the target limit of detection should be set at X/10 concentration units. For example, for a proposed EQS of 10-20 $\mu g\ l^{-1}$ for di- and mono-chlorophenols:

- the limit of detection should be 1-2 $\mu g\ l^{-1}$ or less;
- the total error should not exceed 1-2 $\mu g\ l^{-1}$ or 20% of the determinand concentration (whichever is greater);
- the systematic error or bias should not exceed 0.5-1 $\mu g\ l^{-1}$ or 10% of the determinand concentration (whichever is the greater); and
- the total standard deviation of individual results should not exceed 0.25-0.5 $\mu g\ l^{-1}$ or 5% of the determinand concentration (whichever is greater).

3.2 Analytical techniques

A method for the determination of phenols and chlorophenols in rivers and potable waters has been published as a 'Blue Book' by the Standing Committee of Analysts (SCA, HMSO 1988).

3.2.1 SCA method A

A sample of water is made alkaline and pre-extracted with hexane to reduce levels of interfering compounds. The sample is then buffered using sodium carbonate and chlorophenols reacted with a solution of pentafluorobenzoyl chloride in hexane, by shaking, to produce the corresponding pentafluorobenzoyl ester. The chlorophenol derivatives are then extracted into

hexane and determined using electron-capture gas chromatography (GC-ECD) with the following conditions:

GC column:	10M fused silica capillary - BP5 (SE54 equivalent) 0.33 id 0.5 μm coating thickness
Temperature:	Injector 230 °C, Detector - 250 °C
Column:	130 °C for 1 min. then 3 °C min^{-1} to 145 °C then 15 °C min^{-1} to 165 °C then 25 °C min^{-1} to 250 °C. Hold for 3 minutes.
Gases:	He carrier (0.40 bar)
	Nitrogen make up (30 ml min^{-1} total) to detector
Injection volume:	1.5 μl (0.15 min residence)

The limits of detection and average recoveries for chlorophenols using the above method are as follows in Table 3.1.

Table 3.1 Limits of detection and average recoveries of 2-CP, 3-CP, 4-CP and 2,4-DCP adopting SCA Method A

	LoD ($\mu g\ l^{-1}$)	% Recoveries (0.2 $\mu g\ l^{-1}$ in river water)	SD %
2 - chlorophenol	0.030	92	6.1
3 - chlorophenol	0.031	90	8.9
4 - chlorophenol	0.036	96	7.8
2,4-dichlorophenol	0.043	90	10.0

3.2.2 Other methods

Although SCA Method D could be applied for the determination of substituted phenols by HPLC with UV detection, the limits of detection for this procedure are higher than for SCA Method A above and are not cited for 3- or 4-chlorophenol. It is therefore recommended that SCA Method A should be the favoured method.

4. SUMMARY OF FATE AND BEHAVIOUR IN THE ENVIRONMENT

This section summarises the fate and behaviour of 2-CP, 3-CP, 4-CP and 2,4-DCP in soil and aquatic ecosystems. The available data are discussed in more detail in Appendix A. The four chlorophenol congeners considered in this report are reviewed together to facilitate a comparison of their fate in the environment.

4.1 Soil

The relatively high solubility and low to moderate organic-carbon partition coefficients, indicate that 2-CP, 3-CP, 4-CP and 2,4-DCP will show low to moderate adsorption. In addition, degree of dissociation and hence pH affects the affinity with which chlorophenols bind to organic matter in soils, such that adsorption is stronger under acidic conditions (greater prevalence of un-ionised form) and less so under more basic conditions (cited in WHO 1989). Therefore, chlorophenols (especially the lower chlorinated congeners) may leach to surface and groundwaters from soils, particularly in soils of low organic carbon content and under conditions of neutral/basic pH.

Biodegradation is likely to be the most important removal route of chlorophenols in soils. As with surface waters, photolysis may be important on soil surfaces, although there are no data to confirm this. Biodegradation is relatively rapid in soils and often does not have the lag period associated with biodegradation in aquatic systems. This is probably as a result of the complex/active microbial communities and more favourable environmental conditions in soils (e.g. organic matter content, nutrient status, pH, etc). Near complete aerobic biodegradation (i.e to CO_2) of 2-CP, 3-CP, 4-CP and 2,4-DCP has been reported in a clay-loam soil over 1.5-6.5, 160, 12-20 and 12-40 days, respectively. These values indicate that, as in water and sediments, *meta-* (3-) and *para-* (4-) substitution and increased chlorination of chlorophenols leads to an increase in resistance to biodegradation.

The limited anaerobic data available indicate that chlorophenols are not biodegraded under these conditions in soil at all. However, this is in contrast to the biodegradation observed in anaerobic sediments. More data are therefore required to assess the biodegradation potential of chlorophenols in anaerobic zones of soils. Aspects of chlorophenol biodegradation are summarised more fully in the section on water below.

4.2 Water

It is a well established fact that the highly chlorinated chlorophenols adsorb strongly to suspended solids and sediments. However, there are no experimental data to indicate how important this process is for the lower chlorinated congeners. Theoretically, they should not be adsorbed as strongly as the tetra- and pentachlorophenols, although the moderate octanol-water partition coefficients (2.15-3.06) for 2-, 3- and 4-CP and 2,4-DCP indicate that some adsorption of these compounds to suspended solids may occur. Indeed, many studies have been conducted on the fate of these compounds in aquatic sediments.

Chlorophenols are susceptible to both photolysis and biodegradation. Photolysis is only expected to be an important process near the surface of water bodies (particularly in summer months). In deeper waters and sediments, aerobic and anaerobic biodegradation will be the main route of removal for chlorophenols. Photolysis of polychlorinated phenols appears to be higher than for monochlorinated congeners. Respective summer/winter half-lives for complete photomineralisation (i.e. breakdown to CO_2) of 6/14 and 53/334 days have been reported for 2,4-DCP and 4-CP in estuarine samples (Hwang and Hodson 1986). The higher rates in summer were attributed to higher irradiance in this season.

2-, 3-, 4-CP and 2,4-DCP all undergo microbial degradation under aerobic conditions, via oxidative dechlorination and hydroxylation. Biodegradation is more rapid in sediments (and soils) as a result of more complex/active microbial communities and more favourable environmental conditions in these media (e.g. organic matter content, nutrient status, pH, etc). The available data also suggest that aerobic biodegradation is less rapid for *meta-* (3-) and *para-* (4-) substituted compounds and for the highly chlorinated congeners. This pattern is more apparent on an observation of the whole chlorophenol series from the mono- compounds through the di-, tri- and tetrachlorophenols up to pentachlorophenol. In addition, there is often a lag period associated with chlorophenol biodegradation in water and sediment. Such lag periods are usually attributed to the period required by resident micro-organisms to become physiologically acclimated to toxic compounds or to the period in which small populations of resident DCP degraders increases to large enough numbers, such that DCP biodegradation becomes detectable. In the water column and sediments of aquatic ecosystems, aerobic biodegradation half-lives of 2-36 days have been reported for the monochlorinated congeners at ambient temperatures. Corresponding values of 70-100% biodegradation of 2,4-DCP in 10-30 days have been reported. Some of these values may also incorporate removal by photolysis. Indeed, respective half-lives in estuarine water samples that account for both photolysis and microbial degradation of 4-CP and 2,4-DCP, have been reported to be 10/95 and 4/17 days in summer/winter, respectively.

Under anaerobic conditions in aquatic sediments, chlorophenols are biodegraded by reductive dechlorination (progressive replacement of the chlorines by hydrogen) usually by a consortium of several different microbial species. Under these conditions, *meta-* (3-) and *para-* (4-) substituted congeners appear to be more resistant to biodegradation. Moreover, in the complete mineralisation (i.e. reductive dechlorination to methane) of the higher chlorinated congeners, the breakdown of 4-CP is the rate limiting step. This is shown by values for complete biodegradation of 28-30, 15-61, 61 and 90 days for 2-CP, 3-CP, 4-CP and 2,4-DCP, respectively. As with aerobic biodegradation, a lag period is usually associated with anaerobic biodegradation in aquatic sediments.

5. SUMMARY OF TOXICITY AND BIOACCUMULATION

This section summarises the toxicity and bioaccumulation of 2-CP, 3-CP, 4-CP and 2,4-DCP in aquatic and mammalian organisms. The available data are discussed in more detail in Appendices B, C and D. The four chlorophenol congeners considered in this report are reviewed together to facilitate a comparison of their toxicity and bioaccumulation.

5.1 Toxicity to freshwater organisms

5.1.1 Mode of action

Quantitative Structure-Activity Relationship (QSAR) studies in fish reveal chlorophenols to be Type II 'polar' narcotics in these organisms. In other words, compounds that cause narcosis associated with a specific mode of action as opposed to 'non-polar' narcotics which have no specific mechanism of toxicity. The specific mode of action has been identified as an uncoupling of electron transport (oxidative phosphorylation) in mitochondria. However, QSAR studies in saltwater crustaceans (Smith *et al* 1994) have shown the action of chlorophenols in these organisms to be different, with a non-specific, 'non-polar' narcotic mode of action. Moreover, LeBlanc *et al* (1988) found that the dose-response slopes of the monochlorinated congeners were markedly different to those of the polychlorinated congeners for the water flea (*Daphnia magna*), leading to the conclusion that the former must have a less specific mechanism of toxicity than the latter. It is likely that these mechanisms of toxicity will be common to both freshwater and saltwater organisms.

5.1.2 Level of toxicity

The available data generally indicate that no one group of organisms is more sensitive than any other group. In fact, the chlorophenols considered in this report all appear to be of moderate to high acute toxicity to freshwater organisms, with the majority of reported L(E)C50 data ranging from 8-85, 2.6-25 and 1.7-16 mg l^{-1} for algae, crustaceans (mainly *Daphnia* spp.) and fish, respectively. Moreover, there appears to be little difference in toxicity following short or long-term exposure. For example, LC_{50}s of 6 and 5 mg l^{-1} have been reported for the water flea (*Daphnia magna*) following exposure to 4-CP over 48 hours and nine days, respectively (Cowgill and Milazzo 1991), while 96 hour LC_{50}s of 10.7, 4.9 and 6.3 mg l^{-1} for the medaka (*Oryzias latipes*) exposed to 2-CP, 4-CP and 2,4-DCP, respectively, have been reported alongside EC_{50}s (hatching) of 10.7, 10.7 and 1 mg l^{-1} (Shigeoka *et al* 1988c). In fact, an observation of short and long-term data taken from consistent studies indicates that acute to chronic ratios generally range from approximately 1:1 to 7:1, with the majority in the 1:1 to 3:1 range.

The lowest reported toxicity data include 21-day reproductive No-Observed Effect Concentrations (NOEC, defined as the highest concentration in a bioassay at which effects are not significantly different to those observed in the control) of 0.3, 0.63 and 0.21 mg l^{-1} for the water flea (*D. magna*), following exposure to 2-CP, 4-CP and 2,4-DCP, respectively (Kuhn *et al* 1989b), and 85-day NOECs (growth, development and survival) of 0.25 and 0.1 mg l^{-1} for

embryo-larval stages of rainbow trout exposed to 4-CP and 2,4-DCP, respectively (Hodson *et al* 1991). In addition, 4-CP and 2,4-DCP concentrations as low as 0.03-0.05 mg l^{-1} have been reported to cause erratic swimming behaviour, irregular ventilation and coughing in rainbow trout (*Oncorhynchus* mykiss) after one hour exposure, although no effects were apparent following transfer to toxicant-free water (Kaiser *et al* 1995).

The lowest and most reliable toxicity data for freshwater organisms are shown in Table 5.1.

5.1.3 Effect of chlorination and substitution position on toxicity

There appears to be an increase in the toxicity of chlorophenols to aquatic organisms with increasing degree of chlorination and substitution away from the *ortho-* (2-) position. The higher toxicity of the more highly chlorinated congeners can be ascribed to an increase in lipophilicity (as measured by Kow), which leads to a greater potential for uptake into the organism and hence, transfer from the media to the site of action. *Ortho*-substituted congeners are generally of lower toxicity than the *meta-* and *para-* substituted compounds, as close proximity of the *ortho*-substituted chlorine to the OH group on the molecule appears to 'shield' the OH, which apparently interacts with the active site in aquatic organisms, causing the observed toxic effects.

For the congeners reviewed in this report, toxicity generally increases in the order 2-CP<3-CP<4-CP<2,4-DCP. This is clearly shown by LC_{50}s of 92.7, 50, 30.1 and 7.8 mg l^{-1} reported for the goldfish (*Carassius auratus*) following exposure to each of these congeners, respectively, over five hours (Kishino and Kobayashi 1996a). A similar pattern is shown in many other studies (see Table 5.1 and B1). Indeed, much of the data included in this report are taken from studies that assessed the toxicity of the whole chlorophenol series from 2-CP, through the tri- and tetra- chlorinated congeners, up to pentachlorophenol. An observation of the data reported in these studies strongly supports the hypothesis that toxicity increases with increasing degree of chlorination and substitution away from the *ortho-* position. In fact, while this report shows that the mono-chlorinated congeners and 2,4-DCP do not appear to vary greatly in toxicity (usually less than one order of magnitude), an observation of data for phenol and all the chlorophenols show that there is a marked difference in toxicity between phenol and the mono-chlorinated phenols, and between the mono-/di-chlorinated phenols and the higher chlorinated tetra- and pentachlorophenol.

5.1.4 Effect of molecular dissociation and pH on toxicity

Bioaccumulation and hence toxicity also appear to depend on the extent to which the chlorophenol molecules are dissociated in the exposure medium. In general, an increase in toxicity is observed with a decrease in pH since the more toxic non-dissociated form predominates at lower pH, while at higher pH the less toxic dissociated form is predominant. Moreover, accumulation potential and toxicity of chlorophenols is little affected by pH where pH is less than the dissociation constant (pKa; pH at which 50% of a substance is dissociated), but there is a sharp decrease in accumulation and toxicity where pH is greater than the pKa. This suggests that the transfer of chlorophenols from exposure media to fish tissues primarily occurs in the undissociated form. The effect of pH on toxicity is shown by several authors, including Konneman and Musch (1981, cited in WHO 1989), who reported 24 hour LC_{50}s of

5.9 and 3.3 mg l^{-1} for the guppy (*Poecilia reticulata*) exposed to 2,4-DCP at pH of 7.8 and 6.1, respectively, and Kishino and Kobayashi (1995) who reported respective 5-hour LC_{50}s of 5-7, 7-10 and >100 mg l^{-1} for the goldfish (*C. auratus*), following exposure to 2,4-DCP at pH 6, 8 and 10. Since toxicity appears to be higher at a pH of 6 than at 8 or 10, there are clear implications for the toxicity of these substances under field conditions, where pH is lower than the norm (e.g. due to natural environmental factors, or as a result of industrial discharges of low pH). Under such conditions, toxicity might be higher than would otherwise be expected, although data are required to confirm this.

5.1.5 Effect of other parameters on toxicity

The only other parameter affecting the toxicity of chlorophenols to freshwater organisms that has been measured in the laboratory, is the presence of dissolved humic material (DHM). There is evidence to suggest that the presence of DHM may affect the bioavailability and hence toxicity of chlorophenols. The toxicity of 4-CP in the laboratory to the water flea (*Daphnia magna*) appears to be lower in the presence of DHM, while toxicity of 2,4-DCP is apparently increased (if only slightly) in the presence of DHM. The reduction in 4-CP toxicity has been shown by 24-hour EC_{50}s (immobility) of 6.8 and 8.78 mg l^{-1} in the presence of 0 and 5 mg l^{-1} (as total organic carbon). Corresponding values of 2.84 and 2.0 mg l^{-1} have been reported for 2,4-DCP (Steinberg *et al* 1992). In general terms, the reduction in 4-CP toxicity can be ascribed to a reduction in toxicant bioavailability due to preferential binding to the DHM. However, the apparent increase in 2,4-DCP toxicity (albeit slight) is less easy to explain and it has been suggested that DHM may cause photo-chemical changes in the toxicant leading to the production of daughter compounds of greater toxicity than the parent molecule. However, no evidence is available to substantiate this conclusion. Furthermore, a range of other substituted phenols tested by the authors all decreased in toxicity in the presence of DHM. Given this and the fact that there is little difference between the two EC_{50}s for 2,4-DCP, it seems likely that there is little concern that the presence of DHM will increase the toxicity of chlorophenols. If anything, toxicity may be reduced under field conditions where DHM is in abundance, as a result of reduced bioavailability.

Table 5.1 Lowest and most reliable freshwater toxicity data for 2-CP, 3-CP, 4-CP and 2,4-DCP

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
2-Chlorophenol										
ALGAE										
Scenedesmus subspicatus (Green unicell)	-	S	-	24±1	-	8 ± 0.3	48 h	50.0	EC_{50} (Percentage inhibition of cell multiplication)	3
ARTHROPODS -CRUSTACEANS										
Daphnia magna (Water flea)	<24 h	S	-	-	-	-	24 h	9.0	EC_{50} (immobility)	5
							14 d	0.18	MATC (total offspring produced)	5
Daphnia magna (Water flea)	<24 h	SS	m	25	-	7.7	21 d	0.3	NOEC (Reproduction)	18
			n				24 h	6.3	EC_{50} (immobility)	
Daphnia magna (Water flea)	<24 h	S	n	22±1	173±13.8	8± 0.2	48 h	2.6	LC_{50}	28
FISH (non-salmonid)										
Pimephales promelas (Fathead minnow)	28 d	F	m	25.4	42.6	7.8	96 h	9.4	LC_{50}	17
Lepomis macrochirus (Bluegill sunfish)	0.32-1.2 g	S	n	21-23	32-48	7.9-6.5	96 h	6.6	LC_{50}	33

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
3-Chlorophenol										
ALGAE										
Selenastrum capricornutum (Green unicell)	Exp gro phase	S	-	21±1	-	-	96 h	29.0	EC_{50} (Growth inhibition)	1
ARTHROPODS -CRUSTACEANS										
Daphnia pulex (Water flea)	12 h	SS	n	20	-	-	96 h	5.6	LC_{50}	21
FISH (non-salmonid)										
Poecilia reticulata (Guppy)	-	-	n	-	-	-	24 h	3.5	LC_{50}	24
4-Chlorophenol										
ALGAE										
Selenastrum capricornutum (Green unicell)	-	S	-	-	-	-	96 h	5.01	EC_{50}	27
ARTHROPODS -CRUSTACEANS										
Daphnia magna (Water flea)	6-24 h	S	n	20	2.4 mmol l^{-1}	8± 0.2	48 h	2.5	EC_{50} (immobility)	4

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
Daphnia magna (Water flea)	<24 h	SS	n	25	-	7	24 h	8.6	EC_{50} (Immobilisation)	18
			n				21 d	0.63	NOEC (Reproduction)	
Daphnia magna (Water flea)	<12 h	SS	m	25±2	160-180	8.2±.2	48 h	6.0	LC_{50}	10
							216 h	5.0	LC_{50}	
							264 h	0.6	NOEC (Total progeny)	
							264 h	0.3	NOEC (Mean brood size)	
Ceriodaphnia dubia (Water flea)	<12 h	SS	m	25±2	90-110	8.2±.2	240 h	0.2	NOEC (Survival)	10
FISH (non-salmonid)										
Pimephales promelas (Fathead minnow)	27 d	F	m	25.5	44.8	7.8	96 h	6.1	LC_{50}	17
Lepomis macrochirus (Bluegill sunfish)	0.32-1.2 g	S	n	21-23	32-48	7.9-6.5	96 h	3.8	LC_{50}	33
Fish (salmonid)										
Oncorhynchus mykiss (Rainbow trout)	Larvae	F	m	9.6-9.8	135	8.1	85 d	0.25	NOEC (Growth and development)	25
								0.5	LOEC (Growth and development)	

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
2,4-Dichlorophenol										
ALGAE										
Chlorella vulgaris (Green unicell)	Exp gro phase	S	-	21±1	-	-	96 h	9.2	EC_{50} (Growth inhibition)	1
MACROPHYTES										
Lemna gibba (Duck weed)	Fronds	S	-	27.8	-	-	10 d	1.50[3]	EC_{50} (Percentage frond increase)	2
ARTHROPODS -CRUSTACEANS										
Daphnia magna (Water flea)	6-24 h	S	n	20	2.4 mmol l^{-1}	8± 0.2	48 h	1.4	EC_{50} (immobility)	4
Daphnia magna (Water flea)	<24 h	SS	n	25	-	7	24 h	3.9	EC_{50} (Immobilisation)	18
			m				21 d	0.21	NOEC (Reproduction)	
FISH (non-salmonid)										
Lepomis macrochirus (Bluegill sunfish)	0.32-1.2 g	S	n	21-23	32-48	7.9-6.5	96 h	2.0	LC_{50}	33
FISH (salmonid)										
Salmo trutta (Brown trout)	4.5 g	S	-	5	-	-	24 h	1.7	LC_{50}	34

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
Oncorhynchus mykiss (Rainbow trout)	Embry o-fry	F	m	9.6-9.8	135	7.9	85 d	0.1	NOEC (Mortality)	25
								0.18	LOEC (Mortality)	

Notes:
- d Days
- EC_{50} Median effect concentration
- F Flow-through bioassay
- h Hours
- LC_{50} Median lethal concentration
- MATC Maximum Acceptable Toxicant Concentration (usually defined as the geometric mean of the NOEC and LOEC)
- m Measured concentration (i.e. analysed)
- n Nominal concentration (i.e. not analysed)
- S Static bioassay
- SS Semi-static (renewal) bioassay
- \- Information unavailable or not reported
- 3 Author reports data as µM. These figures have been converted to mg l^{-1}

References:

1. Shigeoka *et al* (1988a)
2. Ensley *et al* (1994)
3. Kuhn and Pattard (1990)
4. Kuhn *et al* (1989a)
5. Shigeoka *et al* (1988b)
8. Gersich and Milazzo (1988)
10. Cowgill and Milazzo (1991)
17. Geiger *et al* (1988)
18. Kuhn *et al* (1989b)
21. Trabalka and Burch (1978)
24. Benoit-Guyod *et al* (1984)
25. Hodson *et al* (1991)
27. LeBlanc (1984)
28. LeBlanc (1980)
33. Buccafusco *et al* (1981)
34. Hattula et al (1981)

5.2 Toxicity to saltwater organisms

Chlorophenols appear to have the same mode of toxic action in saltwater organisms as in freshwater organisms. Moreover, the effects of molecular chlorination, substitution position and dissociation on the toxicity of these compounds to saltwater organisms appear to be essentially the same as for freshwater organisms. Therefore, since these issues have already been discussed in some detail in the previous section, they are not re-iterated here.

5.2.1 Level of toxicity

Saltwater data are considerably more limited than for freshwater organisms, but also indicate that no one group of organisms is more sensitive than any other group, with the majority of reported $L(E)C_{50}$ data ranging from 0.6-19.5, 2.55-29.7 and 5-7 mg l^{-1} for algae, crustaceans and fish, respectively, indicating moderate to high acute toxicity. These data mainly represent 4-CP and 2,4-DCP, with 2- and 3-CP data for fish only. The only long-term exposure data available for saltwater organisms relate to a mesocosm study conducted under field conditions in which a 4-CP and 2,4-DCP concentration of 1.0 mg l^{-1} was found to cause severe inhibition of growth and biomass of natural phytoplankton communities (mixed species) (Kuiper and Hanstevit 1984). This suggests that, as with freshwater organisms, acute to chronic ratios may be somewhat less than 10 for some algal species, although further laboratory data are required to confirm this.

The lowest reported toxicity data for 4-CP include 5-day NOECs, of 0.39 (total cell volume) and 1.08 (total cell count) mg l^{-1} for the diatom, *Skeletonema costatum* (Cowgill *et al* 1989), and a 96 hour NOEC (mortality) of 3.2 mg l^{-1} for juveniles of sheepshead minnow (*Cyprinodon variegatus*) (Heitmuller 1981), while the lowest reported data for 2,4-DCP include a 96-hour LC_{50} and a 72-hour EC_{50} (growth) of 2.55 and 0.6 mg l^{-1} for the grass shrimp (*Palaemonetes pugio*) and diatom (*Phaeodactylum tricornutum*), respectively (Rao *et al* 1981, cited in WHO 1989 and Kusk and Nyholm 1992). Toxicity data for 2- and 3-CP are limited to just one or two fish values, with the lowest being 96-hour LC_{50}s of 6.6 and 4.0 mg l^{-1} for sole (*Solea solea*) and flounder (*Platichthys flesus*), respectively (Smith *et al* 1994).

The lowest and most reliable toxicity data for saltwater organisms are shown in Table 5.2.

Table 5.2 Lowest and most reliable saltwater toxicity data for 2-, 3- and 4-chlorophenol and 2,4-dichlorophenol

Species	Life stage	Test type	Analysis	Temp (°C)	Salinity (o/oo)	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
2-Chlorophenol										
FISH										
Solea solea (Sole)	45 g	SS	m	6	22	8	96 h	6.6	LC_{50}	7
3-Chlorophenol										
FISH										
Platichthys flesus (Flounder)	56 g	SS	m	6	5	8	96 h	3.99	LC_{50}	7
4-Chlorophenol										
ALGAE										
Skeletonema costatum (Diatom)	-	S	n	19.5-20.6	-	7.7-9.0	5 d	11.6	EC_{50} (Total cell count)	5
								0.39	NOEC (Total cell volume)	
Mixed phytoplankton	-	S[1]	m	-	'seawater'	-	18 d	1.0	Severe inhibition of growth (biomass) and productivity	2
								0.1	No effect	

Species	Life stage	Test type	Analysis	Temp (°C)	Salinity (o/oo)	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
ARTHROPODS -CRUSTACEANS										
Tisbe battagliai (Copepod)	-	S	m	20	30	8	24 h	21.0[4]	LC_{50}	7
Mixed copepod species	-	S[1]	m	-	'seawater'	-	17 d	1.0	Severe inhibition of biomass and production	2
								0.1	No effect	
FISH										
Platichthys flesus (Flounder)	56 g	SS	m	6	5	8	96 h	5.0	LC_{50}	7
2,4-Dichlorophenol										
ALGAE										
Phaeodactylum tricornutum (Diatom)	-	S	m	15±1	20	-	72 d	0.6[3]	EC_{50} (Growth)	6
Mixed phytoplankton	-	S[1]	m	-	'seawater'	-	25 d	1.0	Severe inhibition of growth (biomass) and productivity	2
								0.1	No effect	
ARTHROPODS -CRUSTACEANS										
Tisbe battagliai (Copepod)	-	S	m	20	30	8	24 h	16.0[4]	LC_{50}	7

Species	Life stage	Test type	Analysis	Temp (°C)	Salinity (o/oo)	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
Mixed copepod species	-	S[1]	m	-	'seawater'	-	17 d	1.0	Severe inhibition of biomass and production	2
								0.1	No effect	
Palaemonetes pugio (Grass shrimp)	'Inter-moult'	SS	n	20±1	10	7.6-7.7	96 h	2.55	LC_{50}	8
FISH										
Solea solea (Sole)	45 g	SS	m	6	22	8	96 h	5.13	LC_{50}	7

Notes:

d	Days
EC_{50}	Median effect concentration
F	Flow-through bioassay
h	Hours
LC_{50}	Median lethal concentration
m	Measured concentration (i.e. analysed)
n	Nominal concentration (i.e. not analysed)
S	Static bioassay
SS	Semi-static (renewal) bioassay
-	Information unavailable or not reported

1. Study conducted in outdoor mesocosms consisting of plastic bags holding 1.5 m^3 of natural seawater
3. EC_{50} values inferred from authors graphs
4. Calculated from authors data which are given in µM

References:

2. Kuiper and Hanstveit (1984)
5. Cowgill *et al* (1989)
6. Kusk and Nyholm (1992)
7. Smith *et al* (1994)
8. Rao *et al* (1981)

5.3 Bioaccumulation in fresh and saltwater organisms

Most available bioaccumulation data for 2-, 3-and 4-CP and 2,4-DCP relate to fish, although information is also available concerning the uptake of chlorophenols by leeches. There appear to be no available data on the bioaccumulation of these compounds in saltwater organisms, although it is likely that they will be accumulated to the same extent as in freshwater biota.

5.3.1 Level of bioaccumulation in aquatic organisms

Bioconcentration Factors (BCFs) for the four congeners under study are not particularly high, with the majority for fish ranging from 3.8-34 at neutral pH. However, BCFs as high as 1600-8500 have been reported for various species of leech (Metcalfe *et al* 1988), although these values should be treated with caution as they are based on "instantaneous" concentrations measured in water and biota under field conditions, where the exposure history is not known.

Some laboratory data are available to support this information (Hall and Jacob 1988) in which BCFs of 282-980 were reported for the leech, *Nephelopsis obscura*. Leeches apparently accumulate polychlorinated chlorophenols (including 2,4-DCP) to levels of up to two orders of magnitude greater than other macrofauna. This has been attributed to a deficiency in leeches of the enzyme necessary for the metabolism of these compounds, a theory supported by long 2,4-DCP depuration half-lives of up to 46 days or no significant depuration at all (Metcalfe *et al* 1984). Such characteristics may make leeches good organisms for biomonitoring chlorinated phenols in the aquatic environment.

5.3.2 Effect of chlorination and dissociation on bioaccumulation

Since toxicity is directly linked to bioaccumulation, all the issues concerning the effects of degree of chlorination and dissociation on the toxicity of chlorophenols to fresh and saltwater organisms, also relate to the uptake and bioaccumulation of these compounds (see Sections 5.1.3 and 5.1.4). Thus, higher BCFs are reported for congeners with a higher degree of chlorination (increased lipophilicity) and where pH is relatively low (greater proportion of compound in bioavailable undissociated form). For example, BCFs of 2.96, 5.34, 5.64 and 19.5 have been reported for goldfish (*Carassius auratus*) exposed to 2-CP, 3-CP, 4-CP and 2,4-DCP, respectively over five hours (Kishino and Kobayashi 1996b). Corresponding LC_{50}s of 92.7, 50, 30.1 and 7.8 mg l^{-1} were also reported in this study. As with toxicity, this pattern is shown more strongly on observing bioaccumulation data for the whole chlorophenol range from the mono-chlorophenols right up to pentachlorophenol. So while reported BCFs for the monochlorophenols and 2,4-DCP are generally low (10^1), BCFs for the higher chlorinated congeners are in the 10^2-10^3 range.

A point worthy of note is that while a degree of chlorination and dissociation affects the level of uptake and toxicity of chlorophenols, the actual concentrations accumulated in tissues of fish appear to be relatively constant for different congeners. Kishino and Kobayashi (1996b) noted that although LC_{50}s and BCFs varied with degree of chlorination and dissociation, corresponding tissue concentrations were more constant at 274, 267, 170 and 152 mg kg^{-1} for 2-CP, 3-CP, 4-CP and 2,4-DCP, respectively. In another study conducted by these authors

(Kishino and Kobayashi 1995) 2-CP, 3-CP, 4-CP and 2,4-DCP residues in goldfish (*C. auratus*) were around 230-350 mg kg^{-1}, regardless of molecular structure and pH of exposure media. It is probable that these chlorophenol congeners are ultimately accumulated *to* a level that is more or less similar, it is just that the lower chlorinated congeners take longer to reach a steady state equilibrium.

5.3.3 Persistence in biota and elimination

With the exception of leeches (see Section 5.3.1), elimination of chlorophenols from most aquatic organisms appears to be relatively rapid. Metcalfe *et al* (1988) cites half-lives of a few hours to a few days for tri- and pentachlorophenol in a range of aquatic organisms. However, the only data relevant to this EQS report are a half-life of less than one day reported for 2-CP in bluegill sunfish (*Lepomis macrochirus*) (Barrows 1980, cited in WHO 1989), although the *mono*-chlorinated phenols and 2,4-DCP are expected to be eliminated relatively rapidly from the majority of aquatic organisms. The presence of these compounds in biota under field conditions is therefore likely to be due to long-term exposure to chlorophenols in the water column, rather than as a result of a strong tendency to persist within tissues.

6. DERIVATION OF EQSs

6.1 Standards in other countries

Standards for 2-CP, 3-CP, 4-CP and 2,4-DCP have not been adopted in the UK or set by the EU. However, a standard of 2.0 $\mu g\ l^{-1}$ has been set by the EU for the protection of aquatic life against pentachlorophenol (PCP) as a result of the List I status of this compound (Dangerous Substances Directive 76/464/EEC). Only the Canadian Council of Ministers for the Environment (CCME) has set standards for the protection of freshwater life against the adverse effects of these compounds, with values of 7.0 and 0.2 $\mu g\ l^{-1}$ set for total monochloro- and dichlorophenols, respectively. However, these values are based on concentrations reported to impair the flavour of edible fish portions and are set well below levels which are toxic to aquatic organisms in the long-term.

6.2 Derivation of EQSs for monochlorophenols and 2,4-dichlorophenol

As discussed in Section 5.1.3, there is an increase in the bioaccumulation and hence toxicity of chlorophenols with increasing degree of chlorination and substitution away from the *ortho-* (2-) position. However, these parameters do not cause large differences between the monochlorinated phenols and 2,4-DCP and so are not important considerations in the EQS derivation *per se* for these compounds. The effect of increased chlorination on bioaccumulation and toxicity becomes more apparent on observing the whole chlorophenol series from the monochlorinated phenols, through the di-, tri- and tetrachlorinated congeners, up to pentachlorophenol.

The available information suggests that all chlorophenols display the same mode of action, namely specific polar narcosis in fish and perhaps non-specific, non-polar narcosis in lower life-forms such as crustaceans (see Section 5.1.1). However, while the polychlorinated congeners (including 2,4-DCP) appear to exert a very specific toxic effect, as shown by the slopes of dose-response plots, the slopes for the monochlorinated phenols indicate that these congeners exert a different, less specific, mechanism of toxicity (albeit the same for each congener).

Therefore, it is proposed that separate EQSs should be set for the mono- congeners and for 2,4-DCP. In addition, since the monochlorinated phenols are essentially very similar in toxicity (despite the effect of substitution pattern) and are likely to show simple additivity when in a mixture, it is proposed that a single EQS should be set that is applicable for total monochlorophenols in a mixture or for each congener when in isolation.

6.3 Protection of freshwater life

Data on the toxicity and bioaccumulation of 2-CP, 3-CP, 4-CP and 2,4-DCP are available for freshwater algae, macrophytes, crustaceans and fish (lowest and most reliable toxicity values are given in Table 5.1). The available data generally indicate that no one group of organisms is more sensitive than another group. In fact, the chlorophenols considered in this report all appear to be of moderate to high acute toxicity to freshwater organisms, with the majority of

reported L(E)C_{50} data ranging from 8-85, 2.6-25 and 1.7-16 mg l^{-1} for algae, crustaceans (*Daphnia* spp. only) and fish, respectively. Moreover, there appears to be little difference in toxicity following short or long-term exposure. An observation of short and long-term data taken from consistent studies (see tables in Appendix B) indicates that acute to chronic ratios generally range from approximately 1:1 to 7:1, with the majority in the 1:1 to 3:1 range.

Bioconcentration Factors (BCFs) for the four congeners under study are not particularly high, with the majority ranging from 3.8-34 at neutral pH, with depuration half-lives of 1-3 days expected for most aquatic organisms. However, BCFs as high as 282-980 have been reported for the leech, *Nephelopsis obscura* in the laboratory, although leeches apparently accumulate chlorophenols to levels of up to two orders of magnitude greater than other macroinvertebrates, fish and amphibia. This has been attributed to a deficiency in leeches of the enzyme necessary for the metabolism of these compounds (Hall and Jacob 1988).

For the monochlorophenols, the lowest reliable toxicity data is an 85-day LOEC (growth and development) of 0.5 mg l^{-1} for larval rainbow trout (*Oncorhynchus mykiss*), following exposure to 4-CP in a flow-through study with analytical confirmation of exposure concentrations (Hodson *et al* 1991). In this study, a corresponding NOEC of 0.25 mg l^{-1} was also reported. Applying a safety factor of 10 to the LOEC obtained in this study results in an EQS of 50 µg l^{-1}, expressed as an Annual Average (AA) concentration. Similar values can be derived by applying the same factor to other long-term data. For example, values of 20 and 30 µg l^{-1} can be derived by applying a safety factor of 10 to NOECs reported in chronic studies conducted on *Daphnia magna* and *Ceriodaphnia dubia*, respectively.

For 2,4-DCP, the lowest reliable toxicity data is an 85-day LOEC (mortality) of 0.18 mg l^{-1} reported in the same study along with a corresponding NOEC of 0.1 mg l^{-1} (Hodson *et al* 1991). Applying a safety factor of 10 to the LOEC results in an EQS of around 20 µg l^{-1}, expressed as an Annual Average (AA) concentration.

The Annual Average values should be adopted for the protection of freshwater organisms against the long-term effects of these compounds as a result of continuous input to the aquatic environment. However, in surface waters monochlorophenols and 2,4-DCP are readily removed by photolysis and microbial degradation, with half-lives ranging from 2-36 days. Therefore, for the protection against short-term episodic inputs EQSs expressed as Maximum Allowable Concentrations (MACs) should be adopted. These may be derived by applying a safety factor of 10 to the lowest, most reliable 48 hour EC_{50}s of 2.5 and 1.4 mg l^{-1}, reported for immobility of the water flea (*Daphnia magna*), following exposure to 4-CP and 2,4-DCP, respectively (Kuhn *et al.* 1989a). This results in respective MACs of 250 and 140 µg l^{-1} for total monochlorophenols and 2,4-DCP. The proposed MACs therefore approximate the long-term NOECs reported for rainbow trout and are set factors of 5 and 7 higher than the corresponding Annual Average values for 4-CP and 2,4-DCP. This difference between the Annual Average and MAC values falls within the range of acute to chronic toxicity ratios (1:1 to 7:1, see Section 5.1.2).

These EQSs are based on studies conducted at pH 8-8.1. It is well established that as pH decreases, toxicity of chlorophenols increases (see Section 5.1.4), with available data suggesting that a reduction in pH of the exposure medium from 7.8 to 6.1 leads to an increase in the toxicity of monochlorophenols and 2,4-DCP by a factor of up to 2. However, the application of a safety factor of 10 to the chronic LOECs is considered sufficient to ensure

protection of freshwater life against the slight toxicity that may occur in surface waters of lower pH.

Since there are no reliable data concerning the extent to which chlorophenols are removed from the aqueous phase by adsorption to suspended solids or if such adsorption reduces bioavailability, it is proposed that the EQSs should be expressed as 'total' concentrations.

The limit of detection values for 2-CP, 3-CP, 4-CP and 2,4-DCP are around three orders of magnitude lower than the proposed EQSs. Therefore current analytical methods are adequate for monitoring purposes.

6.3.1 Tainting of fish flesh

The above standards are derived on the basis of toxicity data to protect the health of aquatic organisms. However, it is well established that chlorophenols can lead to tainting of fish flesh, following exposure to concentrations that are lower than levels required to cause long-term toxic effects. Threshold concentrations of 60, 25, 45 and 1 $\mu g\ l^{-1}$ have been reported to impair the flavour of rainbow trout following exposure to 2-CP, 3-CP, 4-CP and 2,4-DCP, respectively (Shumway and Palensky 1973, cited in WHO 1989). Therefore, while the EQSs derived in this report are intended to protect aquatic ecosystems against ecotoxicological effects, where the prevention of tainting is a priority (e.g. commercial and sports fisheries) lower values may be adopted if required. Indeed, CCME have adopted values for the protection of freshwater life which are based on concentrations reported to impair the flavour of edible fish portions. These are 7 and 0.2 $\mu g\ l^{-1}$ for total monochlorophenols and dichlorophenols, respectively (see Section 6.1).

6.4 Protection of saltwater life

Saltwater data are considerably more limited than for freshwater organisms, but also indicate that no one group of organisms is more sensitive than any other, with the majority of reported $L(E)C_{50}$ data ranging from 0.6-19.5, 2.55-29.7 and 5-7 $mg\ l^{-1}$ for algae, crustaceans and fish, respectively, indicating moderate to high acute toxicity (lowest and most reliable toxicity values are given in Table 5.2). These data mainly represent 4-CP and 2,4-DCP, with 2- and 3-CP data available for fish only. The only long-term exposure data available relate to a field mesocosm study in which a 4-CP and 2,4-DCP concentration of 1.0 $mg\ l^{-1}$ was found to cause significant inhibition of growth and biomass of natural phytoplankton communities (mixed species) (Kuiper and Hanstevit 1984). There appear to be no available data on the bioaccumulation of these compounds in saltwater organisms, although it is likely that this will be similar to accumulation in freshwater biota.

Given the limited dataset on the toxicity of chlorophenols to saltwater organisms and the absence of reliable chronic data, it is suggested that the EQSs proposed for the protection of freshwater life should be adopted as tentative values for the protection of saltwater life. The available data indicate that toxicity, fate and behaviour of the monochlorinated phenols and 2,4-DCP is apparently the same under both fresh and saltwater conditions. Therefore, for the protection of saltwater life against adverse effects resulting from long-term, continuous exposure to these compounds, tentative Annual Average (AA) values of 50 and 20 $\mu g\ l^{-1}$ are

proposed for total monochlorophenols and 2,4-DCP, respectively. Corresponding tentative Maximum Allowable Concentrations (MACs) of 250 and 140 $\mu g\ l^{-1}$ are proposed for protection against short-term episodic inputs. As with the freshwater standards, the tentative saltwater EQSs should be expressed as 'total' concentrations.

6.5 Abstraction of water for potable supply

This section outlines the derivation of Reference Levels for abstraction of water to potable supply. These are not intended to become statutory standards but are a benchmarking tool to enable regulators to assess whether EQSs proposed for the protection of aquatic life are also adequate for the protection of surface water abstraction points. As such, Reference Levels may be based on either mammalian toxicology to determine a level protective of human health or on the taste and odour properties of a substance.

2-Chlorophenol

The USEPA have calculated a Reference dose of 5 mg kg^{-1} body weight per day based upon the reproductive study by Exon and Koller (1982) which identified a NOAEL of 5 mg kg^{-1} body weight per day, and allocating of an uncertainty factor of 1000 to account for inter-,and intra-species variation and the use of a subchronic rather than chronic study. Assuming a 70 kg adult drinking 2 litres of water per day and allocating 20% of the Reference dose to drinking water, the USEPA have proposed a draft lifetime Health Advisory of 0.04 mg l^{-1} However, the USEPA have given a low confidence rating to their Reference Dose because the study employed in their calculation only evaluated reproductive and haematological effects in the rats. This draft Health Advisory is higher than the taste and odour threshold of 0.1 $\mu g\ l^{-1}$ for 2-chlorophenol. Therefore, A Reference Level of 0.1 $\mu g\ l^{-1}$ based on taste and odour is proposed which should also be protective of public health.

3-Chlorophenol

Due to the limitations of the toxicity database, no Reference Level can be proposed.

4-Chlorophenol

Due to the limitations of the toxicity database, it is not appropriate to propose a Reference Level based on toxicity. However, a Reference Level of 10 $\mu g\ l^{-1}$ can be proposed based on taste and odour and in view of the similarity in structure to 2-chlorophenol, this is also likely to be protective of public health.

2,4-Dichlorophenol

Using the No Observed Adverse Effect Level of 0.3 mg kg^{-1} body weight per day identified in a 147-day study in rats, a Tolerable Daily Intake (TDI) for humans of 3 $\mu g\ kg^{-1}$ body weight per day can be calculated by applying an uncertainty factor of 1000 to account for inter- and intra-species variation and the use of a sub-chronic rather than chronic study. Assuming a 60 kg

adult drinking 2 litres of water per day and allocating 10% of the TDI to drinking water, a health-based guideline value of 9 $\mu g\ l^{-1}$ can be calculated. This is however, considerably greater than the taste and odour threshold for 2,4-dichlorophenol of 0.1 $\mu g\ l^{-1}$. Therefore, a Reference Level of 0.1 $\mu g\ l^{-1}$ is proposed as an MAC based upon taste and odour and this should also be protective of public health.

7. CONCLUSIONS

1. Chlorophenols are organic chemicals formed by the substitution of phenol with one or more atoms of chlorine. The higher chlorinated congeners (including 2,4-DCP) are principally used in the manufacture of various pesticidal products. For instance, large quantities of 2,4-DCP are used in the production of the phenoxy herbicide, 2,4-D. The only real use of the monochlorophenols (2-CP, 3-CP and 4-CP) is as intermediates in the production of the more highly chlorinated congeners. The main route of entry of 2-, 3- and 4-CP and 2,4-DCP is therefore likely to be as a result of discharges from plants manufacturing the compounds or from plants using the compounds as intermediates in the production of higher chlorinated phenols and other products such as phenoxy herbicides. However, input to the aquatic environment may also occur via a number of indirect routes such as discharges from paper mills (where they are formed as by-products of the bleaching process), as a result of the chlorination of sewage, industrial wastes and drinking water, and from the microbial breakdown of higher chlorinated congeners and of agricultural herbicides such as 2,4-D.

2. Most reported chlorophenol concentrations in water have been measured at sites associated with discharges from wood-treatment facilities and most of these are for the more highly chlorinated congeners. 2,4-DCP concentrations in surface waters and sediments generally appear to be in the 10^0-10^1 $\mu g\ l^{-1}$ range.

3. Removal of chlorophenols from the environment is primarily through a combination of photolysis and biodegradation, although photolysis is only an important process near the surface of water bodies (particularly in summer months). Photolysis rates of the polychlorinated phenols appear to be higher than for the monochlorinated congeners, with respective half-lives for complete photomineralisation of 6/14 and 53/334 days, reported for 2,4-DCP and 4-CP in estuarine samples monitored in the summer/winter, respectively. In deeper waters and sediments, aerobic and anaerobic biodegradation will be the main route of removal for chlorophenols, with rates decreasing with increasing degree of chlorination and substitution in the *meta-* (3-) and *para-* (4-) positions. Aerobic biodegradation half-lives of 2-36 days have been reported for the monochlorinated congeners at ambient temperatures in water and sediments, while 70-100% biodegradation has been reported over 10-30 days for 2,4-DCP. These values may also incorporate removal by photolysis. Half-lives in estuarine water samples that account for both photolysis and microbial degradation of 4-CP and 2,4-DCP, have been reported to be 10/95 and 4/17 days in summer/winter, respectively. Under anaerobic conditions values of 28-30, 15-61, 61 and 90 days have been reported for the complete biodegradation of 2-CP, 3-CP, 4-CP and 2,4-DCP, respectively.

4. In fish, polychlorophenols appear to be Type II 'polar' narcotics. In other words, compounds that cause narcosis associated with a specific mode of action. This has been identified as an uncoupling of electron transport (oxidative phosphorylation) in mitochondria. However, the mechanism of toxicity in invertebrates and of the monochlorophenols appears to be less specific. 2-, 3- and 4-CP and 2,4-DCP are of moderate to high acute toxicity to freshwater organisms, with the majority of reported $L(E)C_{50}$ data ranging from 8-85, 2.6-25 and 1.7-16 mg l^{-1} for algae, crustaceans and fish,

respectively; no one group of organisms appears to be more sensitive than any other group. Moreover, there appears to be little difference in toxicity following short or long-term exposure.

5. The toxicity of chlorophenols to aquatic organisms increases with increasing degree of chlorination and substitution away from the *ortho-* (2-) position. The higher toxicity of the more highly chlorinated congeners can be ascribed to an increase in lipophilicity which leads to a greater potential for uptake into the organism. *Ortho*-substituted congeners are generally of lower toxicity than the *meta-* and *para-* substituted compounds, as the close proximity of the *ortho*-substituted chlorine to the OH group on the molecule appears to 'shield' the OH, which apparently interacts with the active site in aquatic organisms, causing the observed toxic effects. So for the congeners reviewed in this report, toxicity generally increases in the order 2-CP<3-CP<4-CP<2,4-DCP, although the monochlorinated congeners and 2,4-DCP do not appear to vary greatly in toxicity (usually less than one order of magnitude). However, an observation of the whole chlorophenol series from 2-CP, through the tri- and tetra- chlorinated congeners, up to pentachlorophenol would show these patterns more clearly.

6. Toxicity also depends on the extent to which the chlorophenol molecules are dissociated in the exposure medium, with increased toxicity observed with a decrease in pH. This is because the more toxic non-dissociated form predominates at lower pH, while at higher pH the less toxic dissociated form is predominant. Moreover, the pKa values of 2-, 3- and 4-CP and 2,4-DCP indicate that at the pH range characterising most environmental conditions, these compounds will exist predominately in the more active non-dissociated form.

7. The vast majority of aquatic organisms do not readily accumulate monochlorophenols or 2,4-DCP to high levels, with BCFs in fish ranging from 3.8-34.0 at neutral pH, and depuration half-lives in the order of hours to days. However, BCFs of 282-980 have been reported for the leech, although this has been attributed to a deficiency in these organisms of the enzyme necessary for the metabolism of chlorophenols. Since toxicity is directly linked to bioaccumulation, all the issues concerning the effects of degree of chlorination and dissociation on the toxicity of chlorophenols, also relate to the uptake and bioaccumulation of these compounds. Therefore, an observation of bioaccumulation data for the whole chlorophenol series would show that the higher chlorinated congeners (e.g. tri-, tetra- and PCP) are accumulated to higher levels, with BCFs ranging from 10^2-10^3.

8. Given the apparent differences in the mechanism of toxicity of the mono and polychlorinated phenols, separate EQSs have been proposed for the mono- congeners and for 2,4-DCP. In addition, since the monochlorinated phenols are essentially very similar in toxicity (despite the effect of substitution pattern) and are likely to show simple additivity when in a mixture, a single EQS has been set that is applicable for total monochlorophenols or for each congener in isolation. EQSs of 50 and 20 $\mu g\ l^{-1}$, expressed as 'total' annual average concentrations have been proposed for total monochlorophenols and 2,4-DCP, respectively. These values are set for the protection of freshwater life against the effects of long-term exposure to these compounds. However, in surface waters monochlorophenols and 2,4-DCP are readily removed by photolysis and microbial degradation. Therefore, for the protection against short-term episodic

inputs, EQSs of 250 and 140 $\mu g\ l^{-1}$ expressed as Maximum Allowable Concentrations (MACs) are proposed. These values are also considered sufficient for surface waters of low pH where the potential exists for a slight increase in chlorophenol toxicity.

9. Saltwater data are considerably more limited than freshwater data, but also indicate that no one group of organisms is more sensitive than any other, with the majority of reported $L(E)C_{50}$ data ranging from 0.6-19.5, 2.55-29.7 and 5-7 $mg\ l^{-1}$ for algae, crustaceans and fish, respectively. The toxicity, fate and behaviour of the monochlorinated phenols and 2,4-DCP are apparently the same under both fresh and saltwater conditions. Therefore, given the limited dataset on the toxicity of chlorophenols to saltwater organisms and the absence of reliable chronic toxicity and bioaccumulation data, it is proposed that the EQSs set for the protection of freshwater life should also be adopted as tentative values for the protection of saltwater life.

10. It is not appropriate to set health-based values for 3- or 4-CP due to the limitations in the toxicity database. Conservative health-based guideline values of 40 $\mu g\ l^{-1}$ and 9 $\mu g\ l^{-1}$ may be set for 2-CP and 2,4-DCP, respectively despite limitations in the toxicity database. However, all of these chemicals have low taste and odour thresholds and Reference Levels of 0.1, 10 and 0.1 $\mu g\ l^{-1}$, are proposed as MACs for abstraction to potable water supply for 2-CP, 4-CP and 2,4-DCP, respectively. At these levels, public health should also be protected. There are insufficient data with which to propose a Reference Level for the protection of potable supply for 3-CP.

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APPENDIX A FATE AND BEHAVIOUR IN THE ENVIRONMENT

This section discusses the most pertinent studies relating to 2-CP, 3-CP, 4-CP and 2,4-DCP. The four congeners are reviewed together to facilitate a comparison of their fate and behaviour in the environment.

A1. FATE AND BEHAVIOUR IN SOIL

A1.1 Volatility

There appear to be no available data concerning the rate of volatilisation of chlorophenols from soil surfaces, although it is not expected to be an important removal route in this media (WHO 1989).

A1.2 Partitioning and mobility in soils

Organic-carbon and octanol-water partition coefficients (Koc and Kow) indicate that 2-CP, 3-CP, 4-CP and 2,4-DCP will show low to moderate adsorption to the organic matter content of soils. It follows that adsorption will be stronger in soils of high organic matter content than in mineral soils. In addition, degree of dissociation and hence pH affects the affinity with which chlorophenols bind to organic matter in soils, such that adsorption is stronger under acidic conditions (greater prevalence of un-ionised form) and less so under more basic conditions (WHO 1989). Therefore, it can be concluded that chlorophenols (especially the lower chlorinated congeners) are most likely to leach to surface and groundwaters in soils of low organic carbon content and under conditions of neutral/basic pH.

A1.3 Abiotic degradation processes

A1.3.1 Photolysis

Although there appear to be no available data on the photolytic degradation of chlorophenols in soil, given the potential for photolysis in aquatic systems it seems likely that photolysis will be the main abiotic route of degradation in soils.

A1.3.2 Chemical degradation processes

Since phenols and halogenated aromatics are generally resistant to hydrolysis, this process is not expected to be an important removal process for chlorophenols in moist soils (Howard *et al* 1989). There is one study in which losses of 2-CP, 4-CP and 2,4-DCP were observed in sterile silica sand, sterile aerobic soils and sterile/non-sterile anaerobic soils (Baker and Mayfield 1980). Since microbial degradation, photolysis and volatilisation were eliminated as causes, the authors concluded that some other mechanism of degradation was responsible. This was ascribed to catalytic reactions on the surface of the silica, or by chemical auto-oxidation in the soil. However, it is difficult to conclude this with certainty in the absence of supporting information. Details of this study are given more fully in Section A1.4.1.

A1.4 Biodegradation

A1.4.1 Aerobic processes

Biodegradation is relatively rapid in soils as a result of complex/active microbial communities and more favourable environmental conditions in these media (e.g. organic matter content, nutrient status, pH, etc). This is shown in a study conducted by Baker *et al* (1980), who found that 2- and 4-CP and 2,4-DCP at a concentration of 100 mg kg^{-1} were more readily biodegraded in a clay-loam soil than in water and sediments sampled from a freshwater stream, following incubation at 0 and 4 °C in the laboratory. At 4 °C, 94%, 84% and 82% of the added 2-CP, 4-CP and 2,4-DCP were completely biodegraded (i.e. to carbon dioxide) over periods of 6.5, 12 and 12 days, respectively, while removal rates at 0 °C were only slightly lower. Given that biodegradation was fairly rapid even at these low temperatures, it is reasonable to assume that at ambient temperatures, more complete and rapid biodegradation would be observed. In this study, no lag phase was apparent, suggesting the presence of an active chlorophenol degrading population of soil micro-organisms or rapid adaptation to the presence of these compounds. Of the compounds tested, 2-CP was the least resistant to biodegradation, indicating preferential dechlorination at the *ortho*-position.

These data are supported by the findings of Baker and Mayfield (1980) who studied the degradation of 2-, 3- and 4-CP and 2,4-DCP in a clay-loam soil under aerobic and anaerobic conditions. Under aerobic conditions, the authors found that there was no lag phase and that all four congeners were biodegraded on incubating a concentration of 100 mg kg^{-1} at 23 °C, with 100%, 87%, 83% and 81% removal of 2-CP, 3-CP, 4-CP and 2,4-DCP over 1.5, 160, 20 and 40 days, respectively. This indicates that the *meta*-substituted congener (3-CP) was biodegraded at a much slower rate. The authors also studied the removal of tri-, tetra- and pentachlorophenols and found that biodegradation rates also decreased with increasing chlorination. In this study, there were also considerable losses of the four congeners in the sterile cultures, particularly of 2-CP and 2,4-DCP. This finding was supported by similar results obtained on repeating the study in sterile soil over nine months and in sterilised silica sand over 10-32 days. However, these results could not be explained by microbial activity, photolysis or volatilisation, since the flasks were sealed, incubated in the dark and plating investigations ruled out microbial contamination. The authors concluded that, while microbial degradation was a major route of removal under aerobic conditions, another mechanism of degradation was clearly apparent. This was ascribed to catalytic reactions on the surface of the silica (see Section A1.3.2).

A1.4.2 Anaerobic processes

In the study conducted by Baker and Mayfield (1980) (see above) a concentration of 100 mg kg^{-1} was apparently not biodegraded under anaerobic conditions over periods of up to 160 days, as there were no significant differences between removal rates in sterile and non-sterile soils. This is in contrast to the chlorophenol biodegradation observed in anaerobic sediments (see Section A2.4.2). The authors suggest that the absence of anaerobic biodegradation in this study was due to a poorly developed microbial community. As with soils incubated under aerobic conditions in this study (see above), some removal of the chlorophenols occurred, apparently as a result of some chemical process such as auto-oxidation.

A2. FATE AND BEHAVIOUR IN WATER

A2.1 Volatility

Henry's Law constants for 2-, 3- and 4-CP and 2,4-DCP suggest that volatilisation potential of these compounds from water is very low. This is supported by estimated half-lives for volatilisation of 73 days for 2-, 3- and 4-CP, from a model river 1 m deep (Howard *et al* 1989). Volatilisation is therefore not expected to be an important removal route from aquatic ecosystems.

A2.2 Adsorption and partitioning in aquatic ecosystems

Despite the relatively high solubility and low to moderate organic-carbon and octanol-water partition coefficients (Koc and Kow), it is apparent that some adsorption of chlorophenols in sediments of aquatic ecosystems occurs, although data specific to 2-, 3- and 4-CP and 2,4-DCP are not available. As with soils, affinity for organic carbon in aquatic sediments decreases with increasing degree of dissociation and hence is stronger at acidic pH than under neutral/basic conditions (WHO 1989, Howard *et al* 1989).

A2.3 Abiotic degradation processes

A2.3.1 Photolysis

Photolysis appears to be the main abiotic route of degradation, most often involving an oxidation reaction in which the molecule is dechlorinated (WHO 1989). Photodecomposition of the mono- chlorinated congeners and 2,4-DCP generally leads to the formation of catechol and other hydroxybenzenes (cited in Hwang and Hodson 1986).

Photolysis rates of polychlorinated phenols (including 2,4-DCP) were found to be high in estuarine waters as measured in flasks suspended in outdoor circulation tanks, and low for 4-CP (Hwang and Hodson 1986). Respective half-lives in summer (25 °C) and winter (14 °C) for a concentration of 0.025 mg l^{-1}, were reported to be 6 and 14 days and 53 and 334 days, for 2,4-DCP and 4-CP in estuarine samples sterilised with formaldehyde (i.e. photolysis only; no microbial activity). These values are for complete mineralisation to carbon dioxide as opposed to simple transformation to an intermediate metabolite. Corresponding summer and winter transformation half-lives of 1.9 and 2.6 and 0.02 and 0.08 days were reported for 4-CP and 2,4-DCP, respectively. The authors also reported corresponding half-lives for the compounds subjected to both photo- and microbial degradation and to microbial degradation only. Half-lives for complete mineralisation of the compounds as subjected to both abiotic and biotic processes in summer and winter, were 4 and 17 days for 2,4-DCP and 10 and 95 days for 4-CP. The relative importance of photolysis and microbial degradation as removal routes for these compounds is discussed in Section A2.4.1. For all the chlorophenols tested, photolysis rates were higher in the summer than in the winter. This was ascribed to the higher irradiance in the summer which was of greater importance than the inhibiting effects of increased light attenuation as a result of higher suspended solids in this season. Moreover, photolysis of 2,4-DCP was higher in estuarine water than in distilled water indicating a photo-sensitised reaction by the presence of humic substances in the natural water. However, in deeper waters

(below 20 cm) photolysis is likely to be less important due to the increase in light attenuation (particularly in summer months).

There is some evidence to suggest that pH affects the rate of photolysis in aqueous solutions. Ally and Faust (1964, cited in WHO 1989) reported half-lives of 34, 15 and 2 minutes for 2,4-DCP at pH values of 4, 7 and 9, respectively. No other details relating to this study are available.

A2.3.2 Chemical degradation processes

Since phenols and halogenated aromatics are generally resistant to hydrolysis, this process is not expected to be an important removal process for chlorophenols in water (Howard *et al* 1989). There appear to be no data concerning chemical degradation mechanisms other than the single study reported for soil in Section A1.3.2.

A2.4 Biodegradation processes

The majority of studies published since 1985 concerning the biodegradation of chlorophenols in the aquatic environment mainly relate to the removal of these compounds in freshwater anaerobic sediments. All such pertinent studies are given in detail in this report. However, information relating to aerobic biodegradation of chlorophenols, particularly in the water column of aquatic ecosystems, is more scarce and therefore, some older studies have been included.

A2.4.1 Aerobic processes

2-, 3-, 4-CP and 2,4-DCP all undergo microbial degradation under aerobic conditions, via oxidative dechlorination and hydroxylation. Chlorocatechols are intermediates in the metabolism of monochlorophenols, followed by ring-cleavage, with the production of carbon dioxide as the final step (Hwang and Hodson 1986).

Biodegradation is apparently more rapid in sediments (and soils) as a result of more complex/active microbial communities and more favourable environmental conditions in these media (e.g. organic matter content, nutrient status, pH, etc). This is shown in a study conducted by Baker *et al* (1980), who observed the biodegradation of 2-CP, 3-CP, 4-CP and 2,4-DCP (as well as PCP) in samples taken from soil, stream water and stream sediment, under laboratory conditions. The authors found that at a concentration of 100 mg l^{-1}, only 2,4-DCP was biodegraded to any significant degree in stream water incubated at 20 °C, with 74% removal of the parent compound in 10 days. However, aerobic biodegradation of these congeners in stream sediment readily occurred, with 100%, 100% and 73% removal of 2-CP, 4-CP and 2,4-DCP over 10-15, 30 and 15-30 days, respectively. 3-CP was also biodegraded, but less readily, with only 56% removal after 30 days incubation. A similar pattern was reported for soil (see Section A1), although the rates were more rapid. These finding suggest that aerobic biodegradation is less rapid for *meta-* (3-) and *para-* (4-) substituted chlorophenols and for congeners with a higher degree of chlorination. Indeed, the authors also studied the biodegradation of PCP in these tests and found that this compound was the most persistent of all. Baker *et al* (1980) also carried out these studies at 0 °C and found that, while the test compounds were still biodegraded, the rates were slower.

Similar periods for aerobic biodegradation of 2,4-DCP have been reported by Nakano and Seto (1990) in samples taken from freshwater ponds and lakes. On incubating a concentration of 1 mg l^{-1} in pond water at 25 °C, the authors found that biodegradation was initially slow, but at Day 20 biodegradation became rapid, with near complete removal of the parent compound by Day 30. Over this period there was a corresponding increase in DCP degrading species from 0.7 cells ml^{-1} to 10^4-10^5 cells ml^{-1}. Similar findings were reported in the lake water samples, although in this test a concentration of 0.1 mg l^{-1} was completely biodegraded in 14 days, with a shorter lag period of approximately 10 days. Such lag periods are often attributed to the period required by resident micro-organisms to become physiologically acclimated to toxic compounds. However, the authors concluded that in these studies this was not the case and that the lag was due to the period in which small populations of resident DCP degraders increased to large enough numbers, such that DCP biodegradation became detectable (as shown by the increase in counts of DCP degrading species in the samples). In fact, the prompt response in population increase of these micro-organisms following exposure to the chlorophenols, suggests that any physiological acclimation period is small or negligible (Baker *et al* 1980).

Biodegradation of 4-CP in saltwater samples taken from an estuary has also been reported (Hwang and Hodson 1986). The authors calculated summer (25 °C) and winter (11 °C) half-lives of 2 and 231 days, respectively, on the basis of incubating 0.025 mg l^{-1} over three days in flasks suspended in an outdoor system using circulating estuarine water. These values are for complete mineralisation to carbon dioxide as opposed to simple transformation to an intermediate metabolite. Corresponding transformation half-lives of 0.46 and 4.8 days were reported. Biodegradation of 2,4-DCP under both temperature regimes was found to be negligible. This is in sharp contrast to the very rapid removal of 2,4-DCP by photolysis (see Section A2.3 for values). Furthermore, while microbial degradation was the chief degradative process of 4-CP in the summer months, photolysis was found to be more important in winter, when microbial activity was very low (see Section A2.3 for values). The authors admit that the short duration (three days) of their experiments meant that there was little chance for microbial degradation of the polychlorinated phenols to take place. In addition, the half-lives reported for 4-CP in this study are considerably different to values obtained in other studies, while in contrast to this study, 2,4-DCP in other studies was readily biodegraded in water and sediments. Nevertheless, the figures reported here are useful in illustrating the effects of temperature (and season) on the rate of aerobic biodegradation.

The experimentally derived half-lives reported above for 4-CP and 2,4-DCP are supported by the findings of Kuiper and Hanstevit (1984) who measured respective half-lives of 16-19 and 8-23 days for concentrations of 0.1-1 mg l^{-1} in saltwater mesocosms (outdoor enclosures containing 1.5 m^3 of natural seawater). The authors ascribe this removal to both biodegradation and photolysis, although no investigation was made of the relative importance of each process in this study.

While the above studies indicate that the monochlorinated phenols are apparently poorly biodegraded in the water column of aquatic ecosystems, Howard *et al* (1989) cite periods of 36 and 15 days for complete removal in non-acclimated and acclimated river water, respectively, at a concentration of 1 mg l^{-1}. Corresponding values of 15 and 13 days were reported for 4-CP. However, the study from which these data originate is not available for critical assessment. Moreover, given the age of the study (1950), the data should be treated with some caution.

A2.4.2 Anaerobic processes

Under anaerobic conditions, chlorophenols are biodegraded by reductive dechlorination (progressive replacement of the chlorines by hydrogen), with the process occurring at a rate equal to or even more rapid than biodegradation under aerobic conditions (Zhang and Wiegel 1990). Besides using different electron acceptors, there are several distinctions between the aerobic and anaerobic biodegradation processes of chlorophenols. For example, a single aerobic species can often totally mineralise chlorophenols, whereas anaerobic biodegradation of these compounds usually requires a consortium of several different species. Many authors have studied anaerobic biodegradation of chlorophenols (particularly in freshwater sediments) in order to elucidate not only the rate of the process, but also the exact mechanisms and micro-organisms involved.

The complete biodegradation of 2,4-DCP to methane and carbon dioxide apparently requires at least five of groups organisms to interact sequentially. Zhang and Wiegel (1990) found that the incubation of 50.0 mg l^{-1} of 2,4-DCP in lake sediments at 31 °C over several weeks, led to the formation of stoichiometric quantities of 4-CP with corresponding loss of the parent compound. Use of still higher 2,4-DCP concentrations led to the subsequent transformations of 4-CP to phenol, phenol to benzoate, benzoate to acetate and acetate to carbon dioxide and methane. The authors concluded that the sequential appearance of these intermediates and the differences in adaptation times for the individual transformation steps, indicates the involvement of various different micro-organisms.

This pattern of anaerobic biodegradation is supported for the monochlorinated 2- and 3-CP, which on incubating at 30 °C in a culture medium using microbial consortia derived from various river sediments, were biodegraded to carbon dioxide and methane via the sequential intermediates phenol and benzoate (Genthner *et al* 1989). Time for complete biodegradation of respective 2-CP and 3-CP concentrations of 7.45 and 8.61 mg l^{-1} in this study, were 28 and 15 days, respectively.

Haggblom *et al* (1993) reported a similar period for complete biodegradation of 2-CP, with a value of <30 days reported following the incubation of 12.86 mg l^{-1} in freshwater sediments at 30 °C. However, breakdown of 3- and 4-CP took longer, with complete biodegradation observed over 61 days for both congeners, indicating a greater resistance to microbial degradation of chlorophenols in the *meta-* (3-) and *para-* (4-) positions. These values (as with all the other reported anaerobic data) were obtained under methanogenic conditions. However, biodegradation associated with denitrification and sulphidogenesis, in which different electron acceptors are used by bacteria for anaerobic respiration, may also be important processes for the removal of chlorophenols from aquatic environments, particularly where these processes may be more significant. This might be in regions of high nitrate input for denitrification (e.g. as a result of agricultural run-off or sewage discharge) or in saltwater environments for sulphidogenesis, where sulphate reduction serves as the major electron accepting process. However, Haggblom *et al* (1993) found that under denitrifying conditions in both fresh and saltwater sediments, the monochlorinated phenols were not biodegraded at all, although in saltwater sediments sulphidogenesis appeared to be equally important a process as methanogenesis, with respective times for complete biodegradation of 2-, 3- and 4-CP of 125, 92 and 211 days and 135, 135 and >200 days, respectively.

Kohring *et al* (1989) studied the effect of temperature variations on the rate of anaerobic biodegradation in freshwater lake sediments. In this study, complete biodegradation of a 2,4-DCP concentration of 17.36 mg l^{-1} occurred over a period of around 90 days on incubating at 28 °C. Complete conversion of the parent compound to 4-CP was observed by Day 14, following a lag period of around eight days. However, biodegradation of the stoichiometrically produced 4-CP did not occur until after a lag period of around 50 days, with complete loss of 4-CP by Day 90. This supports the hypothesis that in the anaerobic biodegradation of polychlorinated phenols, breakdown of 4-CP is the rate-limiting step (Wiegel *et al* 1990).

In the same study, Kohring *et al* (1989) found that anaerobic biodegradation occurred in the temperature range between 5 and 50 °C. In the range between 5 and 30 °C, transformation rates increased with increasing temperature. At 40 °C biodegradation rates were only 5% of peak activity, while between 40 and 50 °C methanogenic activity was intermittent. In all these experiments, adaptation (or lag) periods were observed before loss of 2,4-DCP or 4-CP occurred. In the temperature range 25-35 °C, this period for 2,4-DCP was observed to be 7-11 days, while above and below this the adaptation period increased with increasing and decreasing temperature, respectively. At all temperatures tested, adaptation (lag) and transformation periods were longer for 4-CP than for 2,4-DCP. Indeed, transformation rates for 4-CP only averaged 10% of those for 2,4-DCP over the temperature range 15-40 °C, further indicating the rate limiting nature of this step in the biodegradation process of polychlorinated phenols. The authors concluded that temperature can affect anaerobic biodegradation rate not only as a result of increased metabolic activity, but also due to effects on species composition of the microbial community. Such population effects are particularly important in reductive dechlorination, where more than one organism is required for activity at each step.

Similar values for the conversion of 2,4-DCP to 4-CP have been reported in a study in which a 2,4-DCP concentration of 10.0 mg l^{-1} was incubated in pond sediments sampled at various times throughout the year (Hale *et al* 1991). The authors reported that the majority of half-lives (incorporating appropriate lag periods) ranged from 10-27 days.

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APPENDIX B FRESHWATER TOXICITY AND BIOACCUMULATION

Toxicity and bioaccumulation data for 2-CP, 3-CP, 4-CP and 2,4-DCP are shown in Tables B1 and B2, respectively. The most pertinent studies are discussed below. The four congeners are reviewed together to facilitate a comparison of their toxicity.

B1. FRESHWATER TOXICITY

Information published prior to 1989 is included in a WHO Environmental Health Criteria document (WHO 1989). This EQS report includes the lowest and most reliable data given in the WHO review as well as all relevant data published from 1987 to the present day. However, where data are scarce for a particular group of organisms or a chlorophenol congener, all available information has been included, regardless of reliability.

B1.3 Algae

2-, 3- and 4-CP and 2,4-DCP appear to be of moderate toxicity to algae, with reported EC_{50}s ranging from 50-170, 29, 8-38 and 9.2-14 mg l^{-1}, respectively, following 48 to 96 hours exposure. Le Blanc (1984) has reported a 96 hour EC_{50} as low as 5.01 mg l^{-1} for *Selenastrum capricornutum* exposed to 4-CP, although it is difficult to assess the reliability of this value, since no information concerning the test methodology was available other than that it followed procedures in use by the US EPA in 1975.

These values indicate that there is a slight increase in toxicity with increasing chlorination and chlorination in the 3- and 4- positions (*meta-* and *para-*) rather than in the 2- (*ortho-*) position. This is shown by the 96 hour EC_{50}s (growth inhibition) of 70, 29, 38 and 14 mg l^{-1}, reported for *Selenastrum capricornutum*, following exposure to 2-CP, 3-CP, 4-CP and 2,3-DCP, respectively (Shigeoka *et al* 1988a). This relationship becomes more apparent on observing responses of the alga to all chlorophenol congeners. Indeed, the authors found that the tri-, tetra- and pentachlorophenols further increased in toxicity with increasing chlorination, particularly when the molecules are *meta*-substituted.

B1.4 Macrophytes

The only available data for higher plants is a 10-day EC_{50} (percentage frond increase) of 1.5 mg l^{-1} reported for the duck weed (*Lemna gibba*) following exposure to 2,4-DCP (Ensley *et al* 1994). This value indicates that 2,4-DCP is of moderate toxicity to aquatic macrophytes, although more data are required to confirm this. The authors also found that the duckweed was able to conjugate approximately 90% of the test substance within six days to the less toxic, 2,4-DCP-β-D-glucopyranoside. However, the authors concluded that since the glucoside metabolite can still act as a source of 2,4-DCP, the problems associated with chlorophenol contamination in the aquatic environment would not be mitigated by such metabolic processes.

B1.5 Crustaceans

All available crustacean data appear to be for just a few species of water flea, with the majority of these being for *Daphnia magna*.

B1.5.1 Acute and chronic toxicity

Available data indicate that 2-, 3- and 4-CP and 2,4-DCP are of moderately high acute toxicity to *Daphnia magna*, with the majority of reported EC/LC_{50} values ranging from 2.6-9, 5.6-15.8, 2.5-9 and 1.4-3.9 mg l^{-1}, respectively. Values of 25/21, 12/10 and 7.0/6.6 mg l^{-1} have been reported for *Daphnia carinata/Daphnia pulex*, following exposure to 2-CP, 4-CP and 2,4-DCP, respectively, indicating that these species are somewhat less sensitive than *Daphnia magna* (Shigeoka *et al* 1988b).

There appears to be little or no difference in toxicity to species of water flea following acute or chronic exposure periods. LC_{50}s of 6 and 5 mg l^{-1} have been reported for *Daphnia magna* following exposure to 4-CP for 48 hours and nine days, respectively, with EC_{50}s (reproduction) of 3-4 mg l^{-1} reported in the same study after 11 days exposure (Cowgill and Milazzo 1991). The same authors reported very similar results for *Ceriodaphnia dubia.* This information is supported by the findings of Gersich and Milazzo (1988) who reported 100% mortality of *Daphnia magna*, following 21 days exposure to a 2,4-DCP concentration of 5.94 mg l^{-1}.

No-Observed Effect Concentrations (NOEC, defined as the highest concentration in a bioassay at which effects are not significantly different to those observed in the control) of 0.3, 0.63 and 0.21 mg l^{-1} for various reproductive parameters in *Daphnia magna*, have been reported following exposure to 2-CP, 4-CP and 2,4-DCP, respectively, over 21 days (Kuhn *et al* 1989b).

B1.5.2 Effect of chlorination and substitution pattern on toxicity

The available data indicate that there is an increase in toxicity with increasing degree of chlorination, although the effect that substitution position has on the toxicity of the monochlorinated phenols is not clear as some data indicate that 2-CP is of greater toxicity than 3- and 4-CP, while some data suggest that the opposite is true. However, all authors found that chlorophenol toxicity increases with number of chlorine atoms on the molecule, with 2,4-DCP being of higher toxicity than the mono-CPs (and the tri-, tetra- and pentachlorophenols being of greater toxicity still). This effect is most clearly illustrated for the chlorophenol congeners reviewed in this report by the data reported by Shigeoka *et al* (1988b) for *Daphnia carinata.* These authors reported 24-hour EC_{50}s (immobility) of 25, 12 and 7 mg l^{-1}, following exposure to 2-CP, 4-CP and 2,4-DCP, respectively. A similar pattern is shown in other studies. LeBlanc *et al* (1988) found that mono-, di-, tri- and penta chlorophenols increased in toxicity and that the parallel dose-response slopes suggest a common mode of action. However, the authors concluded that since the slopes of the mono-CPs were markedly different to those of the polychlorinated congeners, the former must have a less specific mechanism of toxicity than the latter.

LeBlanc *et al* (1988) also attempted to demonstrate a relationship between chlorophenol structure and the ability to induce the detoxification enzyme glutathione-s-transferase (GST) in *Daphnia magna.* The authors found that while all the chlorinated phenols induced production of the enzyme, there was no obvious relationship between the structure of a given compound and the degree of induction. The authors therefore concluded that they were unable to prove conclusively that GST is solely responsible for detoxification of chlorophenols in *Daphnia magna.* However, it seems likely that the enzyme must be involved in some way.

B1.5.3 Effect of dissolved humic material (DHM) on toxicity

There is evidence to suggest that under field conditions the presence of dissolved humic material (DHM) may affect the bioavailability and hence toxicity of chlorophenols in some way. Steinberg *et al* (1992) found that the toxicity of 4-CP in the laboratory to *Daphnia magna* was reduced in the presence of DHM, while the toxicity of 2,4-DCP was apparently increased (if only slightly) in the presence of DHM. The reduction in 4-CP toxicity was shown by 24-hour EC_{50}s (immobility) of 6.8 and 8.78 mg l^{-1} in the presence of 0 and 5 mg l^{-1} (as total organic carbon). Corresponding values of 2.84 and 2 mg l^{-1} were reported for 2,4-DCP. The reduction in 4-CP toxicity is comparatively easy to explain and, in general terms, can be ascribed to a reduction in toxicant bioavailability due to preferential binding to the DHM. However, the apparent increase in 2,4-DCP toxicity (albeit slight) is less easy to explain and the authors suggest that the DHM may have caused photo-chemical changes in the toxicant leading to the production of daughter compounds of greater toxicity than the parent 2,4-DCP molecule. However, no evidence is given to substantiate this conclusion. Moreover, a range of other substituted phenols tested by the authors all decreased in toxicity in the presence of DHM. Given this fact and the fact that there is little difference between the two EC_{50}s for 2,4-DCP in any case, it seems likely that there is little concern that the presence of DHM will increase the toxicity of chlorophenols. If anything toxicity may be reduced under field conditions where DHM is in abundance.

B1.6 Fish

The majority of available data are for non-salmonid species, although data are available concerning the toxicity of 4-CP and 2,4-DCP to salmonid species.

B1.6.1 Acute and chronic toxicity

Available data indicate that 2-, 3- and 4-CP and 2,4-DCP are of moderately high acute toxicity to fish, with the majority of reported LC_{50} values ranging from 6.6-16, 3.3-7.9, 3.8-9 and 2-8.2 mg l^{-1}, respectively. In addition, a 24-hour LC_{50} as low as 1.7 mg l^{-1} has been reported for the brown trout, *Salmo trutta*, exposed to 2,4-DCP (Hattula *et al* 1981).

There appears to be little or no difference in toxicity to fish following acute or chronic exposure periods. For example, reported 96-hour LC_{50}s of 11 and 8.2 mg l^{-1} have been reported for fathead minnow (*Pimephales promelas*) following exposure to 2-CP and 2,4-DCP, respectively, with corresponding 8-day values of 6.3 and 6.5 mg l^{-1}. This information is supported by a study conducted on the Japanese medaka (*Oryzias latipes*) in which 96-hour LC_{50}s of 10.7, 4.9 and 6.3 mg l^{-1} were reported for 2-CP, 4-CP and 2,4-DCP, respectively, with corresponding 15-day EC_{50}s (hatching) of 10.7, 2.7 and 1 mg l^{-1} (Shigeoka *et al* 1988c).

NOECs (growth and development) of 0.25 and 0.18 mg l^{-1} have been reported for embryo-larval stages of rainbow trout (*Oncorhynchus mykiss*) following 85 days exposure to 4-CP and 2,4-DCP, respectively, with corresponding LOECs of 0.5 and 0.32 mg l^{-1} (Hodson *et al* 1991). For 2,4-DCP, survival appears to be a more sensitive end-point of toxicity, with respective 85-day NOECs and LOECs of 0.1 and 0.18 mg l^{-1} reported in the same study. These values can be considered particularly reliable, since the study was conducted under flow-through conditions in which the exposure concentrations were analysed. In addition, 4-CP and 2,4-DCP concentrations as low as 0.03-0.05 mg l^{-1} have been reported to cause erratic swimming

behaviour, irregular ventilation and coughing in rainbow trout (*Oncorhynchus* mykiss) after one hour exposure, although no effects were apparent following transfer to toxicant-free water (Kaiser *et al* 1995).

B1.6.2 Effect of chlorination and substitution pattern on toxicity

The available data indicate that there is an increase in toxicity with increasing degree of chlorination. This is clearly shown by the 2.5-hour LC_{50}s of 92.6, 60, 50 and 8 mg l^{-1} reported for the goldfish (*Carassius auratus*), following exposure to 2-CP, 3-CP, 4-CP and 2,4-DCP, respectively (Kishino and Kobayashi 1996a). The authors also observed the effects of tri-, tetra- and pentachlorinated phenols and found the same pattern of increasing toxicity. Moreover, the authors also found that the toxicity of isomers possessing the same number of chlorine atoms (e.g. the monochlorophenols) increased as substitution moved away from the *ortho-* position. In other words, toxicity increased in the order of 2-CP<3-CP<4-CP in the monochlorophenols. This pattern has been observed for a range of species and is shown by the data given in Table B1. The increase in toxicity with chlorination has been ascribed to the increase in lipophilicity, which leads to a greater potential for uptake into the organism and hence, transfer from media to the site of action (Kishino and Kobayashi 1996a).

B1.6.3 Effect of molecular dissociation on toxicity

Bioavailability, bioaccumulation and hence toxicity also appear to depend on the extent to which the chlorophenol molecules are dissociated in the exposure medium. In general a decrease in toxicity is observed with an increase in pH since the less toxic dissociated form exists at higher pH, while at lower pH the more toxic, non-dissociated form is predominant. This is shown by the results of Kishino and Kobayashi (1995) who found that accumulation potential and toxicity of chlorophenols were little affected by pH where pH was less than the dissociation constant (pKa; pH at which 50% of a substance is dissociated), but that accumulation and toxicity sharply decreased where pH was greater than the pKa. This suggests that transfer of chlorophenols from exposure media to fish tissues is greatly increased when the molecules are in the undissociated form. Kishino and Kobayashi (1995) (among others; see Table B1) showed this pattern by determining 5-hour LC_{50}s and Bioconcentration Factors (BCFs) for goldfish (*C. auratus*) exposed to a range of chlorophenols, including 2-, 3- and 4-CP and 2,4-DCP (see Table B1 for data). The pKa values of most of the chlorophenols tested (especially for the monochlorinated congeners where pKa = 8.3-9.2) were less than, or approximately equal to, the pH at which the tests were conducted and so sharp reductions in accumulation and toxicity were not observed until exposure at a pH of 10. However, for chlorophenols with a higher degree of chlorination, pKa is lower and the decrease in toxicity with increase in pH was much more marked. In all cases (even for the monochlorinated phenols), accumulation and toxicity was higher at a pH of 6 than at pH 8 or 10. This clearly has implications for the toxicity of chlorophenols under field conditions where pH can vary and is sometimes relatively low.

B1.6.4 Site of action and mechanism of toxicity

Kishino and Kobayashi (1995 and 1996b) aimed to elucidate the mechanism of toxicity in fish by studying the mortality caused by *in vivo* concentrations (i.e. tissue residues) of chlorophenols in goldfish (*Carassius auratus*). By adopting this approach, the authors were able to assess how chlorophenol structure (i.e. degree of chlorination) affects the intrinsic

toxicity of the molecule at the site of action in fish, thus ruling out the complicating effects of pH and degree of dissociation, which determine the extent to which chlorophenols are accumulated from the aqueous phase and subsequently exert a toxic effect (see previous section). In other words, by studying mortality caused by tissue residues alone, the intrinsic toxicity of a compound in an organism can be investigated, whereas a study of LC_{50}s reflects both uptake kinetics and intrinsic toxicity.

The authors concluded that the bioavailable undissociated chlorophenol, once absorbed by fish passes through various biological membranes to the site of action. It is postulated that the OH group of the molecule interacts in some way with the site of action and that increasing chlorination increases this interaction due to a consequent decrease in the electron density of the OH group. Furthermore, the interaction not only increases with higher numbers of chlorine atoms attached to the molecule, but also when the molecule is substituted away from the OH group. The reason for this is that a chlorine in close proximity to the OH will 'shield' it, thus reducing the interaction responsible for inducing toxicity (e.g. 2-CP). These findings further explain the increased toxicity of the higher chlorinated congeners and those substances which are substituted in the *meta-* and *para-* positions, as discussed in the previous sections, but they do not identify the actual site of action or precisely define the mechanism of toxicity.

Through Quantitative Structure-Activity Relationship (QSAR) studies on the saltwater species flounder (*Platichthys flesus*) and sole (*Solea solea*), Smith *et al* (1994) found that chlorophenols are likely to have a specific mode of action in fish associated with uncoupling of electron transport (oxidative phosphorylation) and can be defined as less inert (Type II) 'polar' narcotics. In other words, compounds that cause narcosis associated with a specific mode of action as opposed to 'non-polar' narcotics which have no specific mechanism of toxicity. Van Wezel *et al* (1995) define narcosis as toxicity caused by accumulation of and disturbance by, compounds in the hydrophobic phases of an organism such as storage and membrane lipids. It seems likely that this mode of action is common to both fresh and saltwater fish.

This 'uncoupling' mode of action has been confirmed by Argese *et al* (1995) who found that chlorophenols adversely affect electron transport and mitochondrial respiration in sub-mitochondrial particles as prepared by sonic disruption of beef heart mitochondria. It has been suggested that the uncoupler binding site is located on the inside of the inner mitochondrial membrane, in which case sonic disruption is an ideal method to expose the active site directly to the chlorophenols. The EC_{50}s reported by the authors also support the hypothesis that toxicity increases with chlorination and substitution away from the *ortho-* (2-) position.

Table B1 Toxicity of 2-, 3- and 4-chlorophenol and 2,4-dichlorophenol to freshwater life

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
ALGAE										
2-Chlorophenol										
Selenastrum capricornutum (Green unicell)	Exp gro phase	S	-	21±1	-	-	96 h	70.0	EC_{50} (Growth inhibition)	1
Chlorella vulgaris (Green unicell)	Exp gro phase	S	-	21±1	-	-	96 h	170.0	EC_{50} (Growth inhibition)	1
Scenedesmus subspicatus (Green unicell)	-	S	-	24±1	-	8 ± 0.3	48 h	24.0	EC_{10} (Growth inhibition)	3
								50.0	EC_{50} (Growth inhibition)	3
								42.0	EC_{10} (Growth rate)	3
								85.0	EC_{50} (Growth rate)	3
3-Chlorophenol										
Selenastrum capricornutum (Green unicell)	Exp gro phase	S	-	21±1	-	-	96 h	29.0	EC_{50} (Growth inhibition)	1
Chlorella pyrenoidosa (Green unicell)	-	S	n	25	-	7	72 h	500.0	Complete destruction of chlorophyll	23
								10.0	No effects on chlorophyll	

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
Chlorella pyrenoidosa (Green unicell)	-	S	n	25	-	7	2 h	500.0	100% inhibition of photosynthesis (as measured by oxygen evolution)	23
4-Chlorophenol										
Scenedesmus subspicatus (Green unicell)	Exp gro phase	S	-	24±1	-	8-9.3	48 h	11.0	EC_{50} (Biomass)	3
							72 h	8.3	EC_{50} (Biomass)	
							72 h	17.0	EC_{50} (Growth)	
							96 h	8.0	EC_{50} (Biomass)	
Selenastrum capricornutum (Green unicell)	Exp gro phase	S	-	21±1	-	-	96 h	38.0	EC_{50} (Growth inhibition)	1
Chlorella vulgaris (Green unicell)	Exp gro phase	S	-	21±1	-	-	96 h	29.0	EC_{50} (Growth inhibition)	1
Selenastrum capricornutum (Green unicell)	-	S	-	-	-	-	96 h	5.01	EC_{50}	27
2,4-Dichlorophenol										
Selenastrum capricornutum (Green unicell)	Exp gro phase	S	-	21±1	-	-	96 h	14.0	EC_{50} (Growth inhibition)	1
Chlorella vulgaris (Green unicell)	Exp gro phase	S	-	21±1	-	-	96 h	9.2	EC_{50} (Growth inhibition)	1

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
Scenedesmus subspicatus (Green alga)	-	S	-	24±1	-	8 ± 0.3	48 h	2.4	EC_{10} (Growth inhibition)	3
								11.5	EC_{50} (Growth inhibition)	3
								6.3	EC_{10} (Growth rate)	3
MACROPHYTES										
2,4-Dichlorophenol										
Lemna gibba (Duck weed)	Fronds	S	-	27.8	-	-	10 d	0.41[3]	EC_{10} (Percentage frond increase)	2
								1.50[3]	EC_{50} (Percentage frond increase)	
								>6.52[3]	Lethal	
ARTHROPODS -CRUSTACEANS										
2-Chlorophenol										
Daphnia magna (Water flea)	<24 h	S	-	-	-	-	24 h	9.0	EC_{50} (immobility)	5
							14 d	4.5	MATC (immobility of adults)	5
							14 d	0.18	MATC (total offspring produced)	5
Daphnia carinata (Water flea)	<24 h	S	-	-	-	-	24 h	25.0	EC_{50} (immobility)	5
Daphnia pulex (Water flea)	<24 h	S	-	-	-	-	24 h	21.0	EC_{50} (immobility)	5

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
Daphnia magna (Water flea)	<24 h	S	-	21	-	-	24 h	7.23[3]	LC_{50}	7
Daphnia magna (Water flea)	<24 h	SS	-	21	-	-	7 d	1.29[3]	21% increase in GST activity	7
Daphnia magna (Water flea)	<24 h	SS	m	25	-	7.7	21 d	0.3	NOEC (reproduction)	18
			n				24 h	6.3	EC_{50} (immobility)	
			n				24 h	2.0	EC_0 (immobility)	
Daphnia magna (Water flea)	<24 h	S	n	22 ± 1	173 ± 13.8	8 ± 0.2	24 h	>22.0	LC_{50}	28
							48 h	2.6	LC_{50}	
3-Chlorophenol										
Daphnia pulex (Water flea)	12 h	SS	n	20	-	-	96 h	5.6	LC_{50}	21
Daphnia magna (Water flea)	-	S	n	-	-	-	24 h	15.8	EC_{50}	22
4-Chlorophenol										
Daphnia magna (Water flea)	6-24 h	S	n	20	2.4 mmol l^{-1}	8 ± 0.2	48 h	1.5	EC_0 (immobility)	4
							48 h	2.5	EC_{50} (immobility)	4
							48 h	4.0	EC_{100} (immobility)	4

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
Daphnia magna (Water flea)	<24 h	S	-	-	-	-	24 h	7.4	EC_{50} (immobility)	5
							14 d	0.89	MATC (immobility of adults)	5
							14 d	0.89	MATC (total offspring produced)	5
Daphnia carinata (Water flea)	<24 h	S	-	-	-	-	24 h	12.0	EC_{50} (immobility)	5
Daphnia pulex (Water flea)	<24 h	S	-	-	-	-	24 h	10.0	EC_{50} (immobility)	5
Daphnia magna (Water flea)	<24 h	S	n	20 ± 1	-	-	24 h	6.8[1]	EC_{50} (immobility)	6
								8.78[2]	EC_{50} (immobility)	6
Daphnia magna (Water flea)	<24 h	SS	n	25	-	7	24 h	8.6	EC_{50} (immobility)	18
			n				24 h	3.7	EC_0	
			n				21 d	0.63	NOEC (reproduction)	
Daphnia magna (Water flea)	<24 h	S	-	21	-	-	24 h	11.45[3]	LC_{50}	7
Daphnia magna (Water flea)	<24 h	SS	-	21	-	-	7 d	0.54[3]	30% increase in GST activity	7
								0.90[3]	40% increase in GST activity	
								1.54[3]	52% increase in GST activity	

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
Daphnia magna (Water flea)	<12 h	SS	m	25±2	160-180	8.2±.2	48 h	6.0	LC_{50}	10
							216 h	5.0	LC_{50}	
							264 h	2.6	NOEC (survival)	
							264 h	3.0	EC_{50} (total progeny)	
							264 h	0.6	NOEC (total progeny)	
							264 h	4.0	EC_{50} (number of broods)	
							264 h	2.6	NOEC (number of broods)	
							264 h	3.0	EC_{50} (mean brood size)	
							264 h	0.3	NOEC (mean brood size)	
Ceriodaphnia dubia (Water flea)	<12 h	SS	m	25±2	90-110	8.2±.2	48 h	9.0	LC_{50}	10
							216 h	6.0	LC_{50}	
							240 h	0.2	NOEC (survival)	
							240 h	2.0	EC_{50} (total progeny, number of broods and mean brood size)	
							240 h	1.6	NOEC (total progeny, number of broods and mean brood size)	
Daphnia magna (Water flea)	<24 h	S	n	22±1	173±13.8	8± 0.2	24 h	8.8	LC_{50}	28
							48 h	4.1	LC_{50}	
2,4-Dichlorophenol										
Daphnia magna (Water flea)	6-24 h	S	n	20	2.4 mmol l^{-1}	8± 0.2	48 h	0.7	EC_0 (immobility)	4
							48 h	1.4	EC_{50} (immobility)	4
							48 h	3.6	EC_{100} (immobility)	4

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
Daphnia magna (Water flea)	<24 h	S	-	-	-	-	24 h	6.0	EC_{50} (immobility)	5
							14 d	1.7	MATC (immobility of adults)	5
							14 d	0.55	MATC (total offspring produced)	5
Daphnia carinata (Water flea)	<24 h	S	-	-	-	-	24 h	7.0	EC_{50} (immobility)	5
Daphnia pulex (Water flea)	<24 h	S	-	-	-	-	24 h	6.6	EC_{50} (immobility)	5
Daphnia magna (Water flea)	<24 h	S	n	20 ± 1	-	-	24 h	2.84[1]	EC_{50} (immobility)	6
								2.0[2]	EC_{50} (immobility)	6
Daphnia magna (Water flea)	-	S	n	-	-	-	24 h	2.7	EC_{50}	22
Daphnia magna (Water flea)	<24 h	SS	n	25	-	7	24 h	3.9	EC_{50} (immobility)	18
			n				24 h	2.8	EC_0	
			m				21 d	0.21	NOEC (reproduction)	
Daphnia magna (Water flea)	<24 h	S	n	22 ± 1	173 ± 13.8	8 ± 0.2	24 h	>10.0	LC_{50}	28
							48 h	2.6	LC_{50}	
Daphnia magna (Water flea)	<24 h	S	-	21	-	-	24 h	6.95[3]	LC_{50}	7

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
Daphnia magna (Water flea)	<24 h	SS	-	21	-	-	7 d	0.47[3] 0.78[3] 1.30[3]	22% increase in GST activity 35% increase in GST activity 46% increase in GST activity	7
Daphnia magna (Water flea)	Neonates	SS	-	19-21	170	7.4-8.3	21 d	1.05 0.74 1.48 2.96 5.94	MATC (survival and reproductive parameters) No significant effects on survival or reproduction 15% mortality. Also 57% and 41% reduction in young per adult and brood size per adult, respectively 95% mortality. No offspring produced 100% mortality	8
Daphnia magna (Water flea)	Neonates	SS	m	22.2-24.3	170	7.7-7.9	14 d	1.1	MATC (reduction in young and brood size per adult)	9
FISH (non-salmonid)										
2-Chlorophenol										
Poecilia reticulata (Guppy)	-	S	-	-	-	7.8	24 h	13.5	LC_{50}	30
						6.1	24 h	7.1	LC_{50}	
Lebistes reticulatus (Guppy)	-	S	-	-	-	-	24 h	13.4	LC_{50}	32

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
Pimephales promelas (Fathead minnow)	28 d	F	m	25.4	42.6	7.8	96 h	9.4	LC_{50}	17
Pimephales promelas (Fathead minnow)	-	F	-	-	-	-	96 h	11.0	LC_{50}	29
							192 h	6.3	LC_{50}	
Oryzias latipes (Medaka)	-	S	-	-	-	-	96 h	10.7	LC_{50}	19
							15 d	10.7	EC_{50} (hatchability)	
Lepomis macrochirus (Bluegill sunfish)	0.32-1.2 g	S	n	21-23	32-48	7.9-6.5	24 h	7.2	LC_{50}	33
							96 h	6.6	LC_{50}	
Carassius auratus (Goldfish)	2.0 g	SS	n	20	-	-	25 h	16.0	LC_{50}	31
Carassius auratus (Goldfish)	1±0.1 g	S	n	27-28	-	7	2.5 h	92.6[3]	LC_{50}	11
Carassius auratus (Goldfish)	1±0.1 g	S	n	27-28	-	7	5 h	92.7[3,4]	LC_{50}	13
Carassius auratus (Goldfish)	2.2±0.2	S	n	20-21	-	6	5 h	70-100	LC_{50}	12
						8	5 h	100-150	LC_{50}	
						10	5 h	>500	LC_{50}	

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
3-Chlorophenol										
Poecilia reticulata (Guppy)	-	-	n	-	-	-	24 h	3.5	LC_{50}	24
Poecilia reticulata (Guppy)	-	S	-	-	-	7.8	24 h	7.9	LC_{50}	30
						6.1	24 h	6.4	LC50	
Lebistes reticulatus (Guppy)	-	S	-	-	-	-	24 h	27.0	LC_{50}	32
Oryzias latipes (Medaka)	-	S	-	-	-	-	96 h	4.5	LC_{50}	19
							15 d	7.4	EC_{50} (hatchability)	
Carassius auratus (Goldfish)	1 ± 0.1 g	S	n	27-28	-	7	2.5 h	60.0[3]	LC_{50}	11
Carassius auratus (Goldfish)	1 ± 0.1 g	S	n	27-28	-	7	5 h	50.0[3,5]	LC_{50}	13
Carassius auratus (Goldfish)	2.2 ± 0.2	S	n	20-21	-	6	5 h	50	LC_{50}	12
						8	5 h	50-70	LC_{50}	
						10	5 h	>100	LC_{50}	
4-Chlorophenol										
Lebistes reticulatus (Guppy)	-	S	-	-	-	-	24 h	9.0	LC_{50}	32

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
Poecilia reticulata (Guppy)	-	SS	-	-	-	5	96 h	49.0	LC_{50}	35
						6	96 h	61.0	LC_{50}	
						7	96 h	66.0	LC_{50}	
Pimephales promelas (Fathead minnow)	27 d	F	m	25.5	44.8	7.8	96 h	6.1	LC_{50}	17
Oryzias latipes (Medaka)	-	S	-	-	-	-	96 h	4.9	LC_{50}	19
							15 d	2.7	EC_{50} (hatchability)	
Lepomis macrochirus (Bluegill sunfish)	0.32-1.2 g	S	n	21-23	32-48	7.9-6.5	24 h	4.0	LC_{50}	33
							96 h	3.8	LC_{50}	
Carassius auratus (Goldfish)	2.0 g	SS	n	20	-	-	25 h	9.0	LC_{50}	31
Carassius auratus (Goldfish)	1±0.1 g	S	n	27-28	-	7	2.5 h	50.0[3]	LC_{50}	11
Carassius auratus (Goldfish)	1±0.1 g	S	n	27-28	-	7	5 h	30.1[3,6]	LC_{50}	13
Carassius auratus (Goldfish)	2.2±0.2	S	n	20-21	-	6	5 h	50-70	LC_{50}	12
						8	5 h	50-70	LC_{50}	
						10	5 h	10-200	LC_{50}	
Jordanella floridae (American flagfish)	Larvae (8 d)	S	n	25.1	333	8	2 h[8]	3.3	LC_{20}	16

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
2,4-Dichlorophenol										
Lebistes reticulatus (Guppy)	-	S	-	-	-	-	24 h	6.8	LC_{50}	32
Poecilia reticulata (Guppy)	-	S	-	-	-	7.8	24 h	5.9	LC_{50}	30
						6.1	24 h	3.3	LC_{50}	
Pimephales promelas (Fathead minnow)	Fry (<24 h)	SS	m	24-26	-	7.0-8.1	7 d	3.48	MATC (survival)	15
								4.85	73% mortality	
Pimephales promelas (Fathead minnow)	-	F	-	-	-	-	96 h	8.2	LC_{50}	29
							192 h	6.5	LC_{50}	
Oryzias latipes (Medaka)	-	S	-	-	-	-	96 h	6.3	LC_{50}	19
							15 d	1.0	EC_{50} (hatchability)	
Oryzias latipes (Medaka)	Embryo-juvenile	SS	n	-	-	-	40 d	0.32-0.63	MATC (mortality)	26
Lepomis macrochirus (Bluegill sunfish)	0.32-1.2 g	S	n	21-23	32-48	7.9-6.5	24 h	4.7	LC_{50}	33
							96 h	2.0	LC_{50}	
Carassius auratus (Goldfish)	2.0 g	SS	n	20	-	-	25 h	7.8	LC_{50}	31

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
Carassius auratus (Goldfish)	1±0.1 g	S	n	27-28	-	7	2.5 h	8.0[3]	LC_{50}	11
Carassius auratus (Goldfish)	1±0.1 g	S	n	27-28	-	7	5 h	7.8[3,7]	LC_{50}	13
Carassius auratus (Goldfish)	2.2±0.2	S	n	20-21	-	6	5 h	5-7	LC_{50}	12
						8	5 h	7-10	LC_{50}	
						10	5 h	>100	LC_{50}	
FISH (salmonid)										
4-Chlorophenol										
Oncorhynchus mykiss (Rainbow trout)	2-4 g	S	n	3-6	-	7-8	1 h	0.03	Brief episodes of increased swimming activity and ventilation with interspersed coughs and gill purges. No effects following transfer to clean water	14
Oncorhynchus mykiss (Rainbow trout)	Larvae	F	m	9.6-9.8	135	8.1	85 d	0.25	NOEC (Growth and development)	25
								0.5	LOEC (Growth and development)	
								0.35	MATC (Growth and development)	

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
2,4-Dichlorophenol										
Salmo trutta (Brown trout)	4.5 g	S	-	5	-	-	24 h	1.7	LC_{50}	34
Oncorhynchus mykiss (Rainbow trout)	Embryo-fry	F	m	9.6-9.8	135	7.9	85 d	0.1	NOEC (mortality)	25
								0.18	LOEC (mortality)	
								0.13	MATC (mortality)	
								0.18	NOEC (growth and development)	
								0.32	LOEC (growth and development)	
								0.24	MATC (growth and development)	
Oncorhynchus mykiss (Rainbow trout)	2-4 g	S	n	3-6	-	7-8	1 h	0.03	Brief episodes of increased swimming activity and ventilation with interspersed coughs and gill purges. No effects following transfer to clean water	14
								0.05	Sustained erratic swimming activity, irregular ventilation and frequent coughing periods. No effects following transfer to clean water	14

Notes:
d Days
EC50 Median effect concentration
F Flow-through bioassay

GST Glutathione-s-transferase
h Hours
LC50 Median lethal concentration
MATC Maximum Acceptable Toxicant Concentration (usually defined as the geometric mean of the NOEC and LOEC)
m Measured concentration (i.e. analysed)
n Nominal concentration (i.e. not analysed)
S Static bioassay
SS Semi-static (renewal) bioassay
- Information unavailable or not reported

1 EC_{50} in the absence of humic acid
2 EC_{50} in the presence of 5 mg l^{-1} humic acid (as total organic carbon)
3 Author reports data as µM. These figures have been converted to mg l^{-1}
4 Corresponds to a tissue residue of 274 mg kg^{-1}
5 Corresponds to a tissue residue of 267 mg kg^{-1}
6 Corresponds to a tissue residue of 170 mg kg^{-1}
7 Corresponds to a tissue residue of 152 mg kg^{-1}
8 Effects were observed over the subsequent 94 hours following transfer to clean water

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B2. BIOACCUMULATION

Most available bioaccumulation data for 2-, 3-and 4-CP and 2,4-DCP relate to fish, although information is also available concerning the uptake of chlorophenols by leeches.

B2.1 Level of bioaccumulation in different aquatic organisms

Bioconcentration Factors (BCFs) for the four congeners under study are given in Table B2 and are not, on the whole, particularly high, with the majority for fish ranging from 3.8-34 at neutral pH. However, BCFs as high as 1600-8500 have been reported for various species of leech (Metcalfe *et al* 1988), although these values should be treated with caution as they are based on "instantaneous" concentrations measured in water and biota under field conditions, where the exposure history is not known. Some laboratory data are available to support this information (Hall and Jacob 1988) in which BCFs of 282-980 were reported for the leech, *Nephelopsis obscura*, following seven days exposure to 0.01 mg l^{-1} at a range of temperatures, with the highest exposure temperature causing the greatest degree of bioaccumulation (probably as a result of increased metabolic rate and hence uptake into the organism). However, since only one leech was exposed at each concentration, the data have no statistical validity, although they do indicate the high potential for chlorophenol bioaccumulation in these organisms. Indeed, Metcalfe *et al* (1984) found that leeches accumulate polychlorinated chlorophenols (including 2,4-DCP) to levels of up to two orders of magnitude greater than other macroinvertebrates, fish and amphibia resident in an industrially contaminated creek.

Metcalfe *et al* (1984) attributed the high bioaccumulation of chlorophenols by leeches to a deficiency in the enzyme necessary for the metabolism of these compounds. This is supported by long depuration half-lives of up to 46 days, or no significant depuration at all, following removal of leeches containing 2,4-DCP concentrations of up to 1.09 mg kg^{-1} from contaminated creek water to clean water in the laboratory (Metcalfe *et al* 1988). Such characteristics may make leeches good organisms for biomonitoring chlorinated phenols in the aquatic environment.

B2.2 Effect of chlorination and dissociation on bioaccumulation

As has already been discussed in Section B1.6, bioaccumulation (and hence toxicity) appears to be strongly correlated to the degree of chlorination and molecular dissociation. Highly chlorinated congeners are more readily accumulated due to a greater lipophilicity. Moreover, the lower dissociation constants (pKa) of highly chlorinated congeners means that a higher proportion of the compound will be in the more bioavailable undissociated form at neutral pH. Increasing pH (especially above pKa) leads to an increase in the proportion of dissociated chlorophenol and hence a decrease in bioaccumulation and toxicity. This leads to the conclusion that the transfer of chlorophenols from the surrounding media to biota tissues is mainly caused by the passive diffusion of the undissociated form across gill membranes. In addition, the formation of an intermolecular hydrogen bond between the OH group of chlorophenols and the components of gill membranes has also been found to play an important role in the uptake of these chemicals (Kishino and Kobayashi 1995).

The effect that degree of chlorination and dissociation has on bioaccumulation and toxicity is clearly shown in a study conducted on goldfish (*Carassius auratus*) in which 5-hour LC_{50}s of 92.7, 50, 30.1 and 7.8 mg l^{-1} were reported following exposure to 2-CP, 3-CP, 4-CP and

2,4-DCP, respectively. Corresponding BCFs associated with these LC_{50} values were calculated to be 2.96, 5.34, 5.64 and 19.5 (Kishino and Kobayashi *et al* 1996b). In a similar study, the same authors reported respective BCFs of 3.8/0.6, 9.7/2.0, 6.9/3.1 and 33.0/2.4 for 2-CP, 3-CP, 4-CP and 2,4-DCP in goldfish (*C. auratus*) exposed at pH 8 and 10, respectively. The highest BCFs reported in these studies are still relatively low (19.5 and 33 for 2,4-DCP at roughly neutral pH). However, the authors also studied tri-, tetra- and pentachlorophenols and found that, as expected, bioaccumulation increased markedly with increased chlorination. Indeed BCFs for higher chlorinated congeners are generally in the 10^2-10^3 range (WHO 1989).

While the degree of chlorination and dissociation affects the level of uptake and toxicity of chlorophenols, the actual concentrations accumulated in tissues appear to be relatively constant for different congeners. This phenomenon is illustrated in the study conducted by Kishino and Kobayashi (1996b) outlined above, in which LC_{50}s and BCFs clearly varied with degree of chlorination and dissociation, but in which corresponding tissue concentrations in the dead fish were more constant at 274, 267, 170 and 152 mg kg^{-1} for 2-CP, 3-CP, 4-CP and 2,4-DCP, respectively. In the other study conducted by these authors (Kishino and Kobayashi 1995) quantities of 2-CP, 3-CP, 4-CP and 2,4-DCP accumulated in goldfish (*C. auratus*) were found to be independent of chlorophenol structure and pH of exposure media (6, 8 and 10), with tissue all residues in dead fish of around 230-350 mg kg^{-1}. Similarly, Van Wezel *et al* (1995) found that concentrations in tissues causing mortality ('lethal body burden') of fathead minnow (*Pimephales promelas*) were also independent of pH of external media and degree of dissociation. It appears that chlorophenols are ultimately accumulated to a level that is more or less similar, it is just that the lower chlorinated congeners take longer to reach a steady state equilibrium between tissue and water concentrations, as a result of lower lipophilicity and a lower proportion in the bioavailable undissociated form at neutral pH. Moreover, once accumulated, the fate of chlorophenols in biota is independent of the pH of the external media, since the pH of the aqueous compartments within biota are relatively constant (Van Wezel *et al* 1995).

B2.3 Persistence and elimination in biota

The short exposure periods used in the above studies was to ensure that bioaccumulation and toxicity was evaluated before any potential detoxification to metabolites such as glucuronide could occur. Indeed, with the exception of leeches (as discussed previously), it is recognised that elimination of chlorophenols is relatively rapid in most aquatic organisms (WHO 1989, Metcalfe *et al* 1988). Metcalfe *et al* (1988) cites half-lives of a few hours to a few days for tri- and pentachlorophenol in a range of aquatic organisms. However, the only data relevant to this EQS report is a half-life of less than one day reported for 2-CP in bluegill sunfish (*Lepomis macrochirus*) (Barrows 1980, cited in WHO 1989), although the *mono*-chlorinated phenols and 2,4-DCP are expected to be eliminated relatively rapidly from the majority of aquatic organisms. The presence of these compounds in biota under field conditions is therefore likely to be due to long-term exposure to chlorophenols in the water column, rather than as a result of a strong tendency to persist within tissues.

Table B2 Bioaccumulation of 2-, 3- and 4-chlorophenol and 2,4-dichlorophenol in aquatic life

Species	Life stage	Test type	Analysis	Temp (°C)	Exposure duration	Concentration		BCF[2]	Comments	Ref
						Water (mg l^{-1})	Flesh[1] (mg kg^{-1})			
ANNELIDS										
2,4-Dichlorophenol										
Percymoorensis marmorata and *Nephelopsis obscura* (Leeches)	1.35 ± 0.37 g	SS	m	22	24 h	0.01 0.02 0.03	0.4 0.8 1.62	40 40 54	See Note 7	3
Percymoorensis marmorata and *Nephelopsis obscura* (Leeches)	0.079 ± 0.006 g	SS	m	4	24 h	0.01	-	42 88 73	BCF values obtained following exposure at pH 9.2, 7.5 and 5.0, respectively. See also Note 7	3
Nephelopsis obscura (Leech)	0.669-0.845 g	SS	m	4 12 22	7 d	0.01	-	282 424 980	See Note 7	3
Dina dubia (Leech)	-	Note 3	m	13.0-14.0	Note 3	0.000128[4]	1.09[4,5]	8500[3]	See Notes 3 and 4	2
Erpobdella punctata (Leech)	-	Note 3	m	13.0-14.0	Note 3	0.000128[4]	0.334[4,5]	2609[3,6]	See Notes 3, 4 and 6	2
Helobdella stagnalis (Leech)	-	Note 3	m	13.0-14.0	Note 3	0.000128[4]	0.205[4,5]	1600[3]	See Notes 3 and 4	2

Species	Life stage	Test type	Analysis	Temp (°C)	Exposure duration	Concentration		BCF[2]	Comments	Ref
						Water (mg l^{-1})	Flesh[1] (mg kg^{-1})			
FISH (Non-salmonid)										
2-Chlorophenol										
Pimephales promelas (Fathead minnow)	0.68 ± 0.31 g	S	m	18-18.4	1.1-5.9 h	36.6[10]	167.0[10]	4.56	Equivalent to "lethal body burden"	7
Carassius auratus (Goldfish)	2.2 ± 0.2 g	S	n[9]	20-21	5 h	70-100, 100-150, >500	-	3.7, 3.8, 0.6	LC_{50}s - See Note 8	4
Carassius auratus (Goldfish)	1±0.1 g	S	n[9]	27-28	5 h	92.7[10]	274.0	2.96	Exposure concentration = LC_{50}	6
Carassius auratus (Goldfish)	-	S	-	-	25 h	16.0	-	6.4	Sublethal concentration approaching LC_{50}	9
Lepomis macrochirus (Bluegill sunfish)	-	F	m[11]	-	28 d	0.00918	-	214.0		8
3-Chlorophenol										
Carassius auratus (Goldfish)	2.2 ± 0.2 g	S	n[9]	20-21	5 h	50, 50-70, >100	-	10, 9.7, 2.0	LC_{50}s - See Note 8	4
Carassius auratus (Goldfish)	1±0.1 g	S	n[9]	27-28	5 h	50.0	267	5.34	Exposure concentration = LC_{50}	6

Species	Life stage	Test type	Analysis	Temp (°C)	Exposure duration	Concentration		BCF[2]	Comments	Ref
						Water (mg l^{-1})	Flesh[1] (mg kg^{-1})			
Brachydanio rerio (Zebra fish)	6-8 mo	F	m	25	-	0.98	-	17.8	BCF based on kinetics of uptake and clearance	1
4-Chlorophenol										
Pimephales promelas (Fathead minnow)	0.68 ± 0.31 g	S	m	18-18.4	2.0-7.2 h	14.5[10]	141.0[10]	9.72	Equivalent to "lethal body burden"	7
Carassius auratus (Goldfish)	2.2 ± 0.2 g	S	n[9]	20-21	5 h	50-70, 50-70, 10-200	-	7.6, 6.9, 3.1	LC_{50}s - See Note 8	4
Carassius auratus (Goldfish)	1 ± 0.1 g	S	n[9]	27-28	5 h	30.1	170	5.64	Exposure concentration = LC_{50}.	6
Carassius auratus (Goldfish)	-	S	-	-	25 h	9.0	-	10.1	Sublethal concentration approaching LC_{50}	9
2,4-Dichlorophenol										
Pimephales promelas (Fathead minnow)	0.68 ± 0.31 g	S	m	18-18.4	0.8 1.1 h	34.9, 22.5	277.0, 293.0[10]	7.94, 13.02[10]	Equivalent to "lethal body burden". Values are for exposure at pH 6.2 and 8.4, respectively	7
Carassius auratus (Goldfish)	2.2 ± 0.2 g	S	n[9]	20-21	5 h	5-7, 7-10, >100	-	40, 33, 2.4	LC_{50}s - See Note 8	4
Carassius auratus (Goldfish)	1±0.1 g	S	n[9]	27-28	5 h	7.8	152.0	19.5	Exposure concentration = LC_{50}	6

Species	Life stage	Test type	Analysis	Temp (°C)	Exposure duration	Concentration		BCF[2]	Comments	Ref
						Water (mg l^{-1})	Flesh[1] (mg kg^{-1})			
Carassius auratus (Goldfish)	-	S	-	-	25 h	7.8	-	34.0	Sublethal concentration approaching LC50	9
FISH (Salmonid)										
2,4-Dichlorophenol										
Salmo trutta (Brown trout)	4.5 g	S	n[9]	5	24 h	1.7	18.0	10	Exposure concentration = LC50	5

Notes:

d Days
F Flow-through bioassay
h Hours
LC_{50} Median lethal concentration
m Measured concentration (i.e. analysed)
n Nominal concentration (i.e. not analysed)
S Static bioassay
SS Semi-static (renewal) bioassay
- Information unavailable or not reported

1. Wet weight unless indicated otherwise
2. Bioconcentration factor - concentration in tissue/concentration in water on a wet weight basis
3. The BCFs calculated in this study are based on measured concentrations in tissues of leeches taken from a contaminated water body (Canagagigue creek) in Canada. The BCFs should therefor be interpreted with caution, since the exposure history of the leeches is not known. (see also Note 4)
4. This is an 'instantaneous' concentration. However, the full exposure history is not known (see note 3)
5. Mean calculated on basis of authors data
6. The authors report a BCF of 11 900 for this species. However, this may be an error . The BCF given here is calculated on the basis of water and tissue concentrations reported by the author
7. Only one leech exposed per concentration. Therefore data have no statistical value
8. Exposure concentrations equivalent to LC50s determined at pH 6, 8 and 10, respectively. BCFs are based on the LC50s determined at pH of 6, 8 and 10
9. Measured tissue concentrations, although water concentrations are nominal

10. Calculated from authors data which are given as molar concentrations
11. Measured as ^{14}C

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APPENDIX C SALTWATER TOXICITY AND BIOACCUMULATION

Toxicity data for 2-CP, 3-CP, 4-CP and 2,4-DCP are shown in Table C1. The most pertinent studies are discussed below. The four congeners are reviewed together to facilitate a comparison of their toxicity.

C1. SALTWATER TOXICITY

Information published prior to 1989 is included in a WHO Environmental Health Criteria document (WHO 1989). However, since toxicity data for saltwater organisms are generally more scarce than for freshwater organisms, this EQS report includes all the saltwater data given in the WHO review as well as all relevant data published from 1987 to the present day.

Saltwater data are generally too limited in themselves to ascertain the effect of degree of chlorination and substitution position on toxicity and to identify the mode of toxic action. However, it seems probable that such patterns of toxicity and mode of action will be identical to that for freshwater organisms. The reader should therefore refer back to Appendix B for more detailed information on these issues.

C1.1 Algae

C1.1.1 Acute and chronic toxicity

The available data suggest that 4-CP and 2,4-DCP are of moderately high acute and chronic toxicity to saltwater algae, with the majority of acute EC_{50}s ranging from 3.27-13.8 and 0.6-19.5 mg l^{-1}, respectively. This is supported by mesocosm studies conducted on a natural phytoplankton/copepod community in plastic enclosures (1.5 m^3), in which a 4-CP and 2,4-DCP concentration of 1 mg l^{-1} caused severe inhibition of growth and productivity of phytoplankton, as measured by biomass, following exposure for 18 and 25 days, respectively (Kuiper and Hansteveit 1984). This concentration also caused a predominance of large flagellate species, probably as a result of reduced grazing pressure by the resident copepod community (see Section C1.1.2). In addition, a concentration of 1.0 mg l^{-1} has been reported to cause 6-24% inhibition of photosynthesis in a natural phytoplankton community taken from the sea and exposed outdoors in a flow-through bioassay over 24 hours (Erickson and Hawkins 1980). A comparison of the limited acute and chronic data suggests that there is little difference in the toxicity of these congeners over the short- and long-term. However, more data are required to confirm this.

In measuring the effects of 2,4-DCP on photosynthesis and growth of four different species of algae and a mixed phytoplankton population, Kusk and Nyholm (1992) found that species sensitivity as measured by the photosynthesis response was very similar, with effect concentrations varying by a factor of less than 10. However, the growth response was more species dependent, with 72-hour EC_{50}s varying by a factor greater than 30. This is shown by 72-hour EC_{50}s (growth) of 0.6 and 19.5 mg l^{-1} (as inferred from authors graphs) for the diatom, *Phaeodactylum tricornutum* and the unicellular alga, *Dunaliella bioculata*, respectively.

An interesting observation made in the mesocosm study conducted by Kuiper and Hanstevit (1984) was that the strongest effect of 4-CP and 2,4-DCP on growth and productivity of phytoplankton was measured at a time (approximately three weeks after addition) when the compounds had apparently disappeared from the water as a result of degradative processes. The authors concluded that the formation of some metabolite of greater toxicity than the parent compound might account for this. It was suggested that the formation of a yellow tinge to the test water following repeated applications of 4-CP was as a result of the formation of this metabolite, which may have been 2-hydroxy-5-chloromuconicacid semialdehyde. However, the authors give no analytical data to support this conclusion.

C1.1.2 Effect of chlorination and substitution pattern on toxicity

Data are too limited to identify any correlation between toxicity and degree of chlorination or substitution pattern. However, Erickson and Hawkins (1980) found in their study that the inhibitory effect caused by the highly chlorinated pentachlorophenol on photosynthesis of a natural phytoplankton community, was far greater than that caused by exposure to 4-CP, thus supporting the general pattern of increased toxicity with increased chlorination. In any case this relationship is shown more clearly for freshwater algae in Section B1.3 and it seems likely that saltwater species will respond in the same way.

C1.2 Crustaceans

C1.2.1 Acute and chronic toxicity

The limited data available suggest that 4-CP and 2,4-DCP are of moderate acute toxicity to saltwater crustaceans, with reported LC_{50}s of 21-29.7 and 2.55-16 mg l^{-1}, respectively. In addition, a 4-CP and 2,4-DCP concentration of 1 mg l^{-1} has been reported to cause severe inhibition of biomass and production of a natural copepod community, as tested in outdoor plastic enclosures (1.5 m^3) alongside naturally occurring phytoplankton, following 17 days exposure (Kuiper and Hansteveit 1984).

C1.2.2 Effect of chlorination and substitution pattern on toxicity

Data are too limited to identify any correlation between toxicity and degree of chlorination or substitution pattern. However, Smith *et al* (1994) reported 24-hour LC_{50}s of 21 and 16 mg l^{-1} for the copepod, *Tisbe battagliai*, following exposure to 4-CP and 2,4-DCP, respectively, thus supporting the general pattern of increased toxicity with increased chlorination. Indeed, the authors also tested tri-, tetra- and pentachlorinated phenols and found that toxicity increased with chlorination and substitution away from the *ortho-* (2-) position. This relationship is also shown for freshwater crustaceans (see Section B1.5) and it is likely that saltwater species will respond in the same way.

C1.2.3 Mode of toxic action

By a study of Quantitative Structure-Activity Relationships (QSAR) between LC_{50}s and partition coefficients (log Kow) of different chlorophenols, Smith *et al* (1994) were able to show that the gradients of the regression analyses were different for the copepod, *Tisbe battagliai*, when compared to two fish species (see next section). The authors concluded that

the mode of toxic action must be different in *Tisbe* to that in fish, with the mechanism of toxicity being non-specific. i.e. one of 'non-polar' narcosis.

C1.3 Fish

C1.3.1 Acute and chronic toxicity

The available data indicate that 2-CP, 3-CP, 4-CP and 2,4-DCP are of moderately high acute toxicity to saltwater fish, with reported LC_{50}s ranging from 6.6-6.99, 3.99, 5-5.4 and 5.13-5.99 mg l^{-1}, respectively.

C1.3.2 Effect of chlorination and substitution pattern on toxicity

The limited data suggest that toxicity of the monochlorinated congeners and 2,4-DCP does not vary greatly with degree of chlorination or substitution position. In fact, 96-hour LC_{50}s of 6.99, 3.99, 5 and 5.99 mg l^{-1} reported for the flounder (*Platichthys flesus*) following exposure to 2-CP, 3-CP, 4-CP and 2,4-DCP, respectively, suggest that, for this organism, 2,4-DCP is actually less toxic than two of the monochlorinated congeners (Smith *et al* 1994). However, the authors noted that in this study toxicity was as much a function of spatial distribution of the chlorine substituents as of degree of chlorination. Hence, as has been reported for freshwater fish, toxicity of the *meta-* and *para*-substituted 3-CP and 4-CP was greater than the toxicity of the *ortho*-substituted 2-CP, and since 2,4-DCP is also substituted in the *ortho-* position, this to was of lower toxicity than the *meta-* and *para-* substituted monochlorophenols. This explanation for the relatively low toxicity of 2,4-DCP, is supported by the fact that the toxicity of the *meta-*:*para-* substituted 2,5-DCP was found to be more toxic than all the monochlorinated phenols and the *ortho-*:*para-* 2,4-DCP.

Smith *et al* (1994) also studied the toxicity of a wide range of tri-, tetra- and pentachlorinated phenols to flounder (*P. flesus*) and sole (*Solea solea*) and found that, as well as the effect of substitution pattern discussed above, the toxicity of the chlorophenols increased with increasing degree of chlorination. Furthermore, by a study of Quantitative Structure-Activity Relationships (QSAR) between LC_{50}s in these fish and dissociation constants of different chlorophenols, the authors also identified degree of dissociation as an important parameter affecting toxicity. This supports the findings for freshwater fish which are discussed in detail in Section B1.6.

C1.3.3 Mode of toxic action

Through Quantitative Structure-Activity Relationship (QSAR) studies on flounder (*P. flesus*) and sole *(S. solea)*, Smith *et al* (1994) concluded that the regression analyses of LC_{50}s of different chlorophenols against partition-coefficients (log Kow), identified these compounds as less inert (Type II) 'polar' narcotics in fish. In other words, compounds that cause narcosis associated with a specific mode of action as opposed to 'non-polar' narcotics which have no specific mechanism of toxicity (as appears to be the case for saltwater crustaceans, see Section C1.2.3). The authors proposed uncoupling of electron transport (oxidative phosphorylation) as the specific mode of action, a mechanism which is supported by studies on beef heart mitochondria (see Section B1.6.4).

The mode of action in fish is discussed more fully for freshwater species in Section B1.6.4. It seems likely that the mechanism of toxicity will be the same for both fresh and saltwater species.

Table C1 Toxicity of 2-, 3- and 4-chlorophenol and 2,4-dichlorophenol to saltwater life

Species	Life stage	Test type	Analysis	Temp (°C)	Salinity (o/oo)	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
ALGAE										
4-Chlorophenol										
Skeletonema costatum (Diatom)	-	S	n	19.5-20.6	-	7.7-9.0	5 d	11.6 13.8 1.08 0.39	EC50 (total cell count) EC50 (total cell volume) NOEC (total cell count) NOEC (total cell volume)	5
Skeletonema costatum (Diatom)	-	S	-	-	-	-	96 h	3.27	EC50 (effect not stated)	4
Mixed phytoplankton	-	S[1]	m	-	'seawater'	-	18 d	1.0 0.3 0.1	Severe inhibition of growth (biomass) and productivity Slight inhibition No effect	2
Mixed phytoplankton	-	F[2]	n	12.5-13.2	23-24	7.8	24 h	1.0	6-24% inhibition of photosynthesis (as measured by ^{14}C uptake)	3
2,4-Dichlorophenol										
Skeletonema costatum (Diatom)	-	S	m n	15 ± 1	20	-	72 d 6 h	3.7[3] 1.5-1.7[3]	EC50 (growth) EC50 (photosynthesis)	6
Phaeodactylum tricornutum (Diatom)	-	S	m n	15 ± 1	20	-	72 d 6 h	0.6[3] 7.5-9.5[3]	EC50 (growth) EC50 (photosynthesis)	6

Species	Life stage	Test type	Analysis	Temp (°C)	Salinity (o/oo)	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
Dunaliella bioculata (Green unicell)	-	S	m	15 ± 1	28	-	72 d	16.3 - 19.5[3]	EC_{50} (growth)	6
			n				6 h	12.0[3]	EC_{50} (photosynthesis)	
Thalassiosira pseudomona (Green unicell)	-	S	n	15 ± 1	28	-	6 h	4.0[3]	EC_{50} (photosynthesis)	6
Mixed phytoplankton	-	S	m	15 ± 1	28	-	72 d	4.2[3]	EC_{50} (growth)	6
			n				6 h	1.7-3.2[3]	EC_{50} (photosynthesis)	
Mixed phytoplankton	-	S[1]	m	-	'seawater'	-	25 d	1.0	Severe inhibition of growth (biomass) and productivity	2
								0.3	Slight inhibition	
								0.1	No effect	
ARTHROPODS -CRUSTACEANS										
4-Chlorophenol										
Tisbe battagliai (Copepod)	-	S	m	20	30	8	24 h	21.0[4]	LC_{50}	7
Mysidopsis bahia (Mysid shrimp)	-	S	-	-	-	-	96 h	29.7	LC_{50}	1
Mixed copepod species	-	S[1]	m	-	'seawater'	-	17 d	1.0	Severe inhibition of biomass and production	2
								0.1	No effect	

Species	Life stage	Test type	Analysis	Temp (°C)	Salinity (o/oo)	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
2,4-Dichlorophenol										
Tisbe battagliai (Copepod)	-	S	m	20	30	8	24 h	16.0[4]	LC_{50}	7
Mixed copepod species	-	S[1]	m	-	'seawater'	-	17 d	1.0	Severe inhibition of biomass and production	2
								0.1	No effect	
Palaemonetes pugio (Grass shrimp)	'Inter-moult'	SS	n	20 ± 1	10	7.6-7.7	96 h	2.55	LC_{50}	8
FISH										
2-Chlorophenol										
Platichthys flesus (Flounder)	56 g	SS	m	6	5	8	96 h	6.99	LC_{50}	7
Solea solea (Sole)	45 g	SS	m	6	22	8	96 h	6.6	LC_{50}	7
3-Chlorophenol										
Platichthys flesus (Flounder)	56 g	SS	m	6	5	8	96 h	3.99	LC_{50}	7
4-Chlorophenol										
Platichthys flesus (Flounder)	56 g	SS	m	6	5	8	96 h	5.0	LC_{50}	7

Species	Life stage	Test type	Analysis	Temp (°C)	Salinity (o/oo)	pH	Exposure duration	Concn (mg l^{-1})	Effect	Ref
Cyprinodon variegatus (Sheepshead minnow)	Juvenile	S	n	25-31	10-31	-	24 h 96 h 96 h	5.7 5.4 3.2	LC50 LC50 NOEC (mortality)	9
2,4-Dichlorophenol										
Platichthys flesus (Flounder)	56 g	SS	m	6	5	8	96 h	5.99	LC50	7
Solea solea (Sole)	45 g	SS	m	6	22	8	96 h	5.13	LC50	7

Notes:

d Days
EC50 Median effect concentration
F Flow-through bioassay
h Hours
LC50 Median lethal concentration
m Measured concentration (i.e. analysed)
n Nominal concentration (i.e. not analysed)
S Static bioassay
SS Semi-static (renewal) bioassay
- Information unavailable or not reported

1. Study conducted in outdoor mesocosms consisting of plastic bags holding 1.5 m^3 of natural seawater
2. Study conducted on natural phytoplankton population from sea and exposed outdoors in 37 l aquaria
3. EC50 values inferred from authors graphs
4. Calculated from authors data which are given in µM

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1. LeBlanc (1984)
2. Kuiper and Hanstveit (1984)
3. Erickson and Hawkins (1980)
5. Cowgill et al (1989)
6. Kusk and Nyholm (1992)
7. Smith *et al* (1994)
8. Rao *et al* (1981)
9. Heitmuller et al (1981)

C2. BIOACCUMULATION

There appear to be no available data on the bioaccumulation of chlorophenols in saltwater organisms. However, it is likely that these compounds will be accumulated to the same extent as in freshwater organisms, with rate of uptake depending on degree of chlorination and dissociation (see Section B2.2). Since pH of exposure media affects the proportion of the bioavailable undissociated form present, the pH of seawater must be taken into account when estimating likely bioaccumulation of chlorophenols by saltwater organisms.

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APPENDIX D MAMMALIAN TOXICOLOGY

Chlorophenols are present in drinking water as a result of the chlorination of phenols during disinfection, as by-products of the reaction of hypochlorite with phenolic acids, as biocides or as degradation of phenoxy herbicides (WHO 1996).

In general, chlorophenols are well absorbed following oral administration and they readily penetrate the skin. They do not appear to accumulate in body tissues in rats but are rapidly metabolised and eliminated from the body. The major metabolite is the glucuronide conjugate of the parent chlorophenol.

2-chlorophenol

The toxicity of 2-chlorophenol was reviewed by the World Health Organisation in 1993 in their revision of the drinking water guidelines. It was concluded that due to the limited database available, a guideline value could not be set.

2-chlorophenol or ortho-chlorophenol as it is more commonly known is of moderate acute oral toxicity to laboratory animals with LD_{50} values of 670 and 347 mg kg^{-1} body weight being reported in the rat and mouse respectively (IPCS 1989).

A 90-day reproductive study was carried out in which groups of 12-20 weanling female Sprague-Dawley rats were exposed to 0, 5, 50 or 500 mg 2-chlorophenol per litre in drinking water from three weeks of age until breeding at 90 days and throughout gestation through to parturition. The doses were equivalent to 0, 0.5, 5 or 50 mg kg^{-1} of body weight per day. There were no treatment related effects noted and therefore a No Observable Adverse Effect Level of 50 mg kg^{-1} body weight per day was identified in this study (Exon and Koller 1982, 1983 a,).

A 24-month carcinogenicity study was carried out in which female Sprague Dawley rats were given 2-chlorophenol in drinking water at 0, 0.5,5 or 50 mg kg^{-1} body weight per day in conjunction with a known carcinogen, nitrosoethylurea (NEU) in order to investigate if 2-chlorophenol could act as a tumour promoter or co-carcinogen. The rats were exposed to 2-chlorophenol either pre-natally, post-natally or pre- and post-natally. Pre-natal exposure involved the chlorinated phenol being given to dams from three weeks of age through parturition. Post-natal exposure involved the offspring of untreated dams being weaned at three weeks of age and then placed on 2-chlorophenol treatment until tumour development or termination of the study at approximately 24 months of age. The pre-and post-natal group involved dams that were dosed with 2-chlorophenol from three weeks of age through parturition and lactation. The offspring were weaned at three weeks of age and continued on treatment until tumours developed or termination of the experiment at approximately 24 months of age. Tumour incidence and latency in all groups were calculated at three time intervals corresponding to 25%, 50% and 75% combined tumour incidence of males and females exposed to NEU only. The effects on tumour incidence and latency were most evident in male offspring that received 2-chlorophenol with NEU, both pre- and post-natally. The lowest level of 2-chlorophenol appeared to exert the greatest effect and the authors suggested that 2-chlorophenol may act as a co-carcinogen or promoter of carcinogenesis (Exon and Koller 1983b)

2-chlorophenol has very low taste and odour thresholds, generally in the low $\mu g\ l^{-1}$ range which has been described as 'medicinal'. The WRc odour threshold is about 0.09 $\mu g\ l^{-1}$ whilst WHO indicated that levels likely to give rise to consumer complaints based on taste and odour are in the range 0.1-10 $\mu g\ l^{-1}$.

3-chlorophenol

There is a very limited dataset on the toxicity of 3-chlorophenol. This essentially comprises LD_{50} values of 0.56 ml kg^{-1}, 1.39 ml kg^{-1} and 335 mg kg^{-1} via the oral, sub-cutaneous and intraperitoneal routes of administration (USEPA 1980). The data available are not adequate for proposing a health based guideline value.

4-chlorophenol

There are very limited data available on the toxicity of 4-chlorophenol or para-chlorophenol as it is more commonly known.

Subacute or chronic studies involving inhalation in humans demonstrated that concentrations of 0.3-21 mg m^{-3} can result in neurological disorders (Sax 1986)

Para-chlorophenol is an irritant in laboratory animals. Severe irritation to the skin and eye in rabbits was reported at 2 mg day^{-1} and 250 $\mu g\ day^{-1}$ respectively (NIOSH 1996).

Para-chlorophenol is of moderate acute oral toxicity to rodents with LD_{50}s of 500 and 660 mg kg^{-1} being reported in the rat and 860 mg kg^{-1} in the mouse when administered in olive oil.

A wide range of odour thresholds have been reported for para-chlorophenol. The WRc odour threshold is about 10 $\mu g\ l^{-1}$ whilst the taste threshold is about 39.2 $\mu g\ l^{-1}$.

2,4 Dichlorophenol

The dataset on the mammalian toxicity of 2,4 dichlorophenol is limited (2,4 DCP). The toxicity of 2,4 DCP was reviewed by the World Health Organisation in their revision of the drinking water guidelines in 1993. However, due to the limitations of the database, they were unable to derive a health based guideline value.

2,4-DCP is of moderate acute oral toxicity to laboratory animals with LD_{50} values in rats ranging from 580-4000 mg kg^{-1} and in male and female CD-1 mice of 1276 and 1352 mg kg^{-1} respectively (WHO 1996).

There have been a number of short term studies carried out with 2,4 DCP. Groups of 20 CD-1 mice per sex per dose-group were exposed to 2,4 DCP in drinking water for 90 days at concentrations of 0.2, 0.6 or 2.0 g l^{-1}. This corresponded to mean daily doses of 50, 143, and 491 mg kg^{-1} of body weight for females and 40, 114 and 383 mg kg^{-1} body weight for males. There were no significant differences in body weight gain and no differences in terminal organ weights or organ weight ratios. An increase in leukocytes was observed in males at the highest

dose and in polymorphonuclear leukocytes in the low dose group of males. There were also changes observed in clinical chemistry parameters in females. However, these changes were not consistently dose-dependent and a Lowest Observable Adverse Effect Level (LOAEL) was not identified (WHO 1996).

A study was carried out in which rats were treated, pre-and post-natally with 0, 3, 30 or 300 mg 2,4 DCP per litre of drinking water for 147 days. Significantly increased spleen and liver weights were observed as well as an enhanced humoral immune responsiveness at the 300 mg l^{-1} dose level. At the 30 and 300 mg l^{-1} dose levels, cell mediated immunity was depressed. No histopathological changes were observed. A NOAEL of 3 mg l^{-1}, equivalent to 0.3 mg kg^{-1} body weight per day was identified in this study (WHO 1996).

The oral administration of 2,4 DCP to pregnant F344 rats at doses of 0, 200, 375 or 750 mg kg^{-1} body weight per day on Days 6-15 of gestation caused a dose related decrease in maternal weight gain. The incidence of embryonic death increased and foetal body weight decreased in the highest dose group. However, there was no evidence of teratogenicity at these high doses (NTP 1989).

2,4 DCP was examined for potential carcinogenic activity by the National Toxicology Program in 1989. B6C3F1 mice and male F344 rats were fed diets containing 0, 5000 or 10 000 mg kg^{-1} diet 2,4 DCP and female F34 rats were fed diets of 0, 2500 or 5000 mg kg^{-1} diet. This equated to doses of 0, 210 or 440 mg kg^{-1} body weight per day for male rats and 0,210 or 250 mg kg^{-1} body weight per day for female rats. In male mice, the doses received were equivalent to 0, 800 or 1300 mg kg^{-1} body weight whilst the female mice received 0, 430 or 820 mg kg^{-1} body weight. There was no carcinogenic activity observed in either species (NTP 1989).

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