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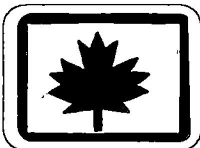
Final Report

CONTAMINANT BIOASSAYS IN RELATION TO THE WATER QUALITY OF THE FRASER RIVER ESTUARY

For:

ENVIRONMENT CANADA

E.V.S. Project No. 3/047-27



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CONTAMINANT BIOASSAYS IN RELATION TO THE WATER QUALITY OF THE FRASER RIVER ESTUARY

Prepared For

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Water Quality Branch
Inland Waters
Pacific and Yukon Region
Conservation and Protection
502 - 1001 W. Pender Street
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July 1990

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July 31, 1990

Ms. Diane Valiela
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502 - 1001 W. Pender Street
Vancouver, B.C.
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Dear Ms. Valiela,

Re: Contaminant Bioassays in Relation to the Water Quality of the Fraser River Estuary.

We are pleased to provide four (4) copies of the final report for the above project. We apologize for the delay in providing the final report; we were anticipating additional comments from Dr. Birtwell. However, we have taken advantage of the delay to significantly improve the report.

The major changes from the draft report, aside from a thorough editorial overhaul, were:

- reorganization and clarification of the section on levels in the environment (Section 2);
- incorporation of your suggested changes to the first draft;
- inclusion of a discussion, and suggested tests, of the "critical volume" hypothesis, which has direct relevance for acute and joint toxicity, and bioaccumulation (see in particular Sections 4.2.3, 5.1, and 5.4);
- addition of suggested means of reducing costs of the recommended tests (Section 5.7).

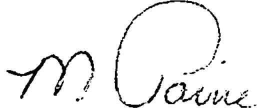
The report now provides a review of chlorophenol contamination in the Fraser estuary, with respect to existing Water Quality Objectives, and a complete review of chlorophenol effects and bioassay test procedures. We have recommended an extensive series of tests that would contribute significantly to our knowledge of the effects of chlorophenols on aquatic biota, as well as satisfy your primary objective of assisting in the development of Water Quality Objectives. Recognizing that the recommended program may be too costly, we have also indicated a number of means for reducing costs. Assuming that at least some of these tests will be conducted in the near future, our report will provide background material for comparing the test results with those from past studies. The report also provides statistical methods, and hypotheses to test, that will maximize the knowledge gained from the tests. In short, the final report should serve as a useful "manual" for anyone conducting and interpreting the recommended tests.

In the Letter of Transmittal accompanying the draft report, we indicated that additional testing might not lower existing NOECs (No Observed Effects Concentrations). Because Water Quality Objectives are, or should be, based on these NOECs, we were not convinced that the recommended testing would contribute to lowering these Objectives. In retrospect, that comment probably applies to pentachlorophenol only, which we have noted in Section 5.7. We also indicated that monitoring of fish populations in the Fraser estuary might provide an effective tool for the validation of Water Quality Objectives, and would like to re-emphasize that now. Field monitoring was beyond the scope of the Terms of Reference for this project, but would be a means of assessing the combined effects of all toxicants. If an argument can be made for testing the joint effects of chlorophenols, then surely the additional effects of other contaminants present in the estuary should also be addressed.

We look forward to continuing our collaboration with Environment Canada, and the Water Quality Branch, in the future, and trust that the present report satisfies your requirements.

Yours truly,

E.V.S. CONSULTANTS



Michael D. Paine, Ph.D.
Environmental Scientist

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E.V.S. Consultants would like to thank the Scientific Authorities, Ms. Diane Valiela (Environment Canada), and Dr. Ian Birtwell (Fisheries and Oceans Canada), for their comments on the draft report, and for providing unpublished manuscripts and reports in progress. E.V.S. would also like to thank Mr. Brendan Hickie (Department of Biology, University of Waterloo), for his advice provided throughout the study. E.V.S. would also like to thank Dr. Barry Oliver and Mr. David Hope of Zenon Environmental for their advice on the chemical analysis of chlorophenols.

EXECUTIVE SUMMARY

The Water Quality Branch of Environment Canada requested a review of the available literature on the effects of chlorophenols on aquatic organisms, with particular emphasis on the effects of tri-, tetra-, and pentachlorophenol (singly and in combination) on juvenile salmon. The primary objective of this study was to recommend and design laboratory and field bioassays which would assist the Water Quality Branch in establishing water quality guidelines for chlorophenols in the Fraser River estuary. The following secondary objectives were established to meet this primary objective:

1) Review the available literature on chlorophenol contamination in the Fraser estuary;

2) Review the available literature on effects of tri-, tetra-, and pentachlorophenol on aquatic organisms, particularly salmonids;

3) Review the available literature on acute and chronic tests to determine the most suitable and sensitive tests;

4) Recommend the most appropriate acute and chronic tests, and provide detailed procedures for these tests.

Chlorophenol levels in the water column, sediments, and biota of the Fraser River estuary have often exceeded provincial and federal water quality objectives in the past. Sources of chlorophenols include point sources such as sawmills and wood storage facilities, and more diffuse sources.

The review of acute and chronic effects of chlorophenols indicated that salmonids are probably the most sensitive aquatic organisms, especially with respect to acute lethality (LC50). Considerable data exist on the effects of pentachlorophenol, but there are few data on the effects of tri- and tetrachlorophenol. There are no published data on the joint toxicity of chlorophenols.

A thorough review of bioassays, and methods for the statistical analyses of results, is provided. Many of these bioassays have not been used to specifically examine the effects of chlorophenols, but could easily be adapted to do so. Acute (static, static-renewal, flow-through, multiple toxicant), chronic (growth, early life stage, bioaccumulation), and behavioural [locomotor (preference/avoidance, swimming performance), predator avoidance, feeding behaviour] tests were reviewed with particular emphasis on salmonid fishes.

Based on the literature review, tests for examining acute and chronic effects, and joint toxicity were described. Juvenile chinook salmon (*Oncorhynchus tshawytscha*) were chosen as the most appropriate test organism, based on the sensitivity of salmonids to chlorophenols, the economic importance of chinook salmon, and the fact that chinooks have a longer residence time in the estuary than do other Pacific salmon. Recommended tests include laboratory and *in situ* experiments and have been broken down into three phases:

Phase 1: acute lethality tests, intended to provide 96 h LC50s and Incipient Lethal Levels (I.L.L.) for tri-, tetra-, and pentachlorophenol.

Phase 2: chronic toxicity tests (growth/feeding behaviour, seawater challenge, bioaccumulation, and preference/avoidance) have been recommended based on their sensitivities, and ecological significance of their endpoints.

Phase 3: joint toxicity studies have been designed to document the joint lethality of tri-, tetra-, and pentachlorophenol in two and three compound mixtures.

1.0 INTRODUCTION

1.1 Background

Chlorophenols are used as disinfectants, biocides, preservatives, and pesticides (CCREM, 1987). They may also be produced in the bleaching process used by pulp mills, and by chlorination of phenols during water treatment (Jones, 1981, 1984). Chlorophenols, particularly pentachlorophenol, are ubiquitous in the environment, entering via agricultural, industrial, and domestic effluents and run-off, or generated in the environment by breakdown of other compounds. Chlorophenols are found in detectable quantities in water, sediment, and animal tissue. Levels are expected to decline in the future with the increased use of chlorine dioxide substitution and other processes in pulp bleaching and the shift from chlorophenol-based wood preservatives to alternatives.

The toxic properties of chlorophenols have been reviewed by Jones (1981, 1984), McLeay (1987), and CCREM (1987). Pentachlorophenol, primarily in the form of its sodium salt (NaPCP), has been used extensively as a reference toxicant by laboratories conducting bioassays. Toxicity of chlorophenols generally increases with increasing chlorine substitution, with individual compounds varying widely in toxicity.

Chlorophenols have been detected in the water, sediment, and biota of the Fraser River estuary (reviewed herein). The Water Quality Branch, Inland Waters Directorate, Environment Canada is developing site-specific water quality criteria for these compounds in the Fraser estuary. These criteria are to be based, in part, on relevant biological tests of the effects of chlorophenols on Fraser River estuary biota. This report also considers the toxicity of chlorophenol mixtures, as individual compounds rarely occur in isolation in the environment. The emphasis was on tri-, tetra-, and pentachlorophenol, which the Water Quality Branch identified as the primary compounds of concern in the Fraser River estuary.

1.2 Study Approach

1.2.1 Objectives

The primary objective of this study was:

- to recommend and design laboratory and field bioassays which would assist the Water Quality Branch in establishing water quality guidelines for chlorophenols in the Fraser River estuary.

The following secondary objectives were established to meet the primary objective:

- To review the available information on the Fraser River estuary to determine the most appropriate test species, and the extent of chlorophenol contamination;

- To review the available information on the effects of tri-, tetra-, and pentachlorophenol on aquatic biota to provide background data for the design and interpretation of the recommended tests;

- To review the available information on acute and chronic tests to determine the most appropriate and sensitive tests, and the best procedures for conducting these tests;

- To recommend acute and chronic tests, and provide detailed procedures for these tests, including apparatus, dose levels, timing, frequency of chemical analyses, quality control, and statistical analyses.

The study was divided into two sections: a literature review and a recommendations section which followed from the review.

1.2.2 Literature Review

1.2.2.1 Fraser River Estuary

The review of available information on the Fraser River estuary focused on the biota and habitats present, and their sensitivity to contaminant effects, and present levels of chlorophenols in water, sediments, and biota. The review depended primarily on documents prepared by the Fraser River Estuary Management Program (FREMP) and interviews with local scientists. From the FREMP review, juvenile chinook salmon (*Oncorhynchus tshawytscha*) was selected as an appropriate test organism based on its sensitivity to contaminant effects and economic importance. The review of chlorophenol levels was undertaken to determine the extent of the existing problem, the appropriateness of existing water quality guidelines, and to provide information on potential study sites for *in situ* experiments.

1.2.2.2 Acute and Chronic Effects of Chlorophenols

Environment Canada previously commissioned reviews of the effects of chlorophenols on aquatic biota (*e.g.*, Jones, 1981, 1984), some of which are still in progress (*e.g.*, Kistritz, 1989); it was not our intention to duplicate those studies. Instead, previous studies were used as a baseline to which additional information was added. Emphasis was on salmonid fishes, because chinook salmon were the test organism of choice. Our primary objective was to recommend testing that would allow more effective extrapolation of acute and chronic test results to field conditions for the Fraser River estuary. A related objective was to determine whether the ranges of concentrations producing acute or chronic effects could be expanded from those given in previous or ongoing reviews. Minima from these ranges are generally used to establish water quality objectives or criteria. Our expectation is that the review will also aid in the interpretation of the results of the recommended tests.

1.2.2.3 Acute and Chronic Tests

Procedures for acute and chronic tests, especially those suitable for salmonid fishes, were thoroughly reviewed to provide a sound basis for selecting the most relevant test. The review also included bioaccumulation studies, and studies of joint toxicity. Statistical procedures for analyzing response variables in acute and chronic tests were also reviewed.

1.2.3 Recommendations

Based on the literature review, tests for examining acute and chronic effects, and joint toxicity are provided. Recommended tests include laboratory and *in situ* experiments, and descriptions of procedures and analysis. The recommendations were based on the assumption that unlimited funds were available, but relative costs of various alternatives were considered. A brief discussion on means for reducing costs is also included.

2.0 CHLOROPHENOLS IN THE FRASER RIVER ESTUARY

2.1 Sources

Major chlorophenol point sources in the Fraser River System included sawmills, wood storage and treatment facilities (Krahn and Shrimpton, 1988); industrial effluents (Cain *et al.*, 1980); sewage treatment plants (Cain *et al.*, 1980; Birtwell *et al.*, 1985; Rogers *et al.*, 1986); and land fills (Atwater, 1980; Jones, 1981). These are historical sources and may not be representative of present chlorophenol inputs in the estuary [see Figure 1 (Drinnan *et al.*, 1989) for a geographical distribution over the estuary]. Total chlorophenol loading may exceed 900 kg/yr in the Fraser River (Krahn *et al.*, 1987).

Concentrations of tetra- and pentachlorophenols from sawmill sources may reach 6600 μg/L (Krahn *et al.*, 1987). Total annual run-off from sawmill grounds ranged from 165 - 261 million litres. Total chlorophenol concentrations from run-off from wood treatment facilities have been reported as high as 6600 μg/L, 27000 μg/L, and 1968 μg/L, for dip-treated, low-pressure sprayed, and high-pressure sprayed lumber, respectively (Krahn and Shrimpton, 1988).

Sewage treatment plants (STP) have been considered important sources receiving chlorophenols from domestic and industrial sources. Chlorine treatment may result in the production of chlorinated phenols, although there is no direct evidence that chlorinated organics form as a result of the disinfection process (Birtwell *et al.*, 1985, Fraser River Harbour Commission, 1987). Concentrations of tetra- and pentachlorophenols have been reported at levels of 28 μg/L and 13 μg/L, respectively (Cain *et al.*, 1980; Birtwell *et al.*, 1985; Rogers *et al.*, 1986). Singleton (1983) found that the effluent of the Annacis Island STP (which discharges into the main arm of the Fraser River Estuary) contained from 9-21 ng chlorophenol/g. More recently, the Effluent Monitoring Program (Fraser River Harbour Commission, 1987) found no pentachlorophenol at Iona, Lulu or Annacis Island STP, but did find tetrachlorophenol in the sludge at Iona STP.

Leachates from landfills (*e.g.*, Richmond landfill, Burns Bog) are also potential sources of chlorophenols (Atwater, 1980; Jones, 1981). The contaminant composition of the leachates, and the duration of discharges of high contaminant concentrations, are unknown (Atwater, 1980).

2.2 Water Quality Objectives

Water quality objectives should be determined from lowest no observable effect concentrations (NOEC), but this information is lacking for some chlorophenols (Kistritz, 1989). Past water quality

FIGURE 1: Chlorophenol Sources in the Fraser River Estuary

objectives (Appendix 1) were not necessarily determined from NOECs. NOECs may be predicted by using an application factor in combination with known acute effect concentrations (AEC) (Kistritz, 1989). Existing AEC, chronic effect concentration (CEC), and NOEC values can be used to determine acute:chronic and acute:no effect ratios in order to provide chronic values for species and chemical congeners for which information is unavailable (Kistritz, 1989). Using chlorophenol concentrations determined in this manner, Kistritz proposed new chlorophenol objectives (Appendix 2).

The chronic effect concentration (CEC), with a safety factor applied, has also been used to develop guidelines. Kistritz (1989) recommended: (1) CEC divided by 100 (10 for differences within species, 10 for differences between species) for tri- and pentachlorophenol, or (2) CEC divided by 1000 (an extra factor of 10 is suggested to account for an inadequate data base) for tetrachlorophenol. The use of safety factors is necessary for the following reasons: (1) lack of site specific information for important estuarine biota in the Fraser River Estuary, (2) environmental factors affecting chlorophenol toxicity are not fully understood (*e.g.*, with increasing temperature or decreasing pH there is an increase in chlorophenol toxicity), (3) the synergistic and/or antagonistic interactions between chlorophenol and other estuarine compounds are not known, (4) bioconcentration factors at higher trophic levels are not accounted for in single species bioassays, (5) different sensitivities at different life stages should be accounted for.

B.C. Provisional Water Quality Objectives for total chlorophenols (sum of tri-, tetra-, and pentachlorophenols), and CCREM (1987) guidelines for individual compounds, are given in Table 1. Levels in water, sediment, and biota samples from the Fraser estuary are summarized; these data are discussed in more detail in subsequent sections. The guidelines and objectives given refer to maximum levels, and should be compared with maxima or 90th percentiles for concentrations observed in the environment. (The maximum value observed will increase with the number of samples, but the 90th or 95th percentiles will not change. For that reason, the 90th or 95th percentile should be used to compare levels in the environment with objectives when large numbers of samples are available.) Note that CCREM guidelines for chlorophenol concentrations in the water column are higher than those proposed by Kistritz (1989), which should be kept in mind when concentrations in the environment are compared with CCREM guidelines below.

Total chlorophenol concentrations do not give an adequate representation of toxicity as the ratios of different isomers will change toxicity. Considering each isomer separately will result in a better interpretation of the potential effects of chlorophenols, as each has a different toxicity. As current objectives for sediment and biota chlorophenol content are unavailable for each chlorophenol isomer, comparisons were made with the B.C. Provisional Objectives for total chlorophenols (Table 1).

TABLE 1. Chlorophenols in the Sediments, Water and Biota from the Fraser River.
(summarized from Drinnan et al., 1989)

Chlorophenol	Parameter	Sediments (ng/g)	Water (ug/L)	Fish-Muscle/ Whole Body (ng/g)	Fish-Liver (ng/g)	Invertebrates (ng/g)
	Total # of Samples Analyzed	155	148	262	55	27
	Measurable [ ] > DL (%)	10	67	34	22	63
	Median	<DL	0.01	<DL	<DL	40
TCP	Mean	7.39	0.024	58.1	19.8	22.5
	90th Percentile	1	0.047	6.65	20.1	200
	Maximum	15.1	0.116	1442	34.5	2000
	CCREM Guidelines*	-	18	-	-	-
	Total # of Samples Analyzed	248	206	357	91	52
	Measurable [ ] > DL (%)	53	84	82	67	52
	Median	1	0.091	25	27.3	2.3
TTCP	Mean	11	0.705	95.7	67	388
	90th Percentile	17	1.7	160	89	600
	Maximum	90	14.8	2522	520	3000
	CCREM Guidelines*	-	1	-	-	-
	Total # of Samples Analyzed	257	206	365	91	52
	Measurable [ ] > DL (%)	50	81	85	74	54
	Median	1	0.033	36	29	3.3
PCP	Mean	12.6	0.159	134	105	392
	90th Percentile	20	0.372	235	160	400
	Maximum	107	2.71	3200	1030	4200
	CCREM Guidelines*	-	0.5	-	-	-
	Total # of Samples Analyzed	257	206	370	93	52
	Measurable [ ] > DL (%)	53	86	85	75	60
	Median	2	0.15	65	78	10
	Mean	23.2	0.85	252	182	816
Total CP	90th Percentile	39.4	2.08	340	286	1380
	Maximum	180	17.5	6239	1550	9200
	CCREM Guidelines*	-	-	-	-	-
	B.C. Provisional Objectives*	10	0.2	100	-	-
	% > B.C. Objective	31	43	27	-	-

* (CCREM, 1987)
** (Swain and Holmes, 1985) : Note - Values are comparative only and do not apply to dilution zones of effluent.
\- not available
DL Detection limit
[ ] Concentration

Current objectives or guidelines have often been exceeded in sediments, water and tissue from the Fraser estuary (Table 1). Chlorophenol concentrations in Fraser River water, for the most part, are less than levels that may produce deleterious effects. Jacob and Hall (1985) have recorded short term pulses ranging from 10-20 μg/L. Servizi (1988) suggested that this was too close to 10 μg/L, the threshold value of pentachlorophenol for chronic toxicity to juvenile rainbow trout, *Oncorhynchus mykiss*, determined by Hodson and Blunt (1981).

2.3 Measured Chlorophenol Levels in the Fraser Estuary

2.3.1 Sediment

Accumulation of chlorophenol near discharges is expected as it is rapidly adsorbed on organic matter and fine particulates (NRCC, 1982). Chlorophenol concentrations in sediments decrease downstream from sources, indicating either dilution or burial from the natural load of the Fraser River. However, chlorophenol discharges over time are constant enough to maintain higher concentrations at or near sources.

Drinnan *et al.* (1989) found that chlorophenol concentrations in sediments from the Main Stem and North Arm of the Fraser River were greater than those in the Main Arm, reflecting the greater number of sources. Total chlorophenol (sum of tri-, tetra-, and pentachlorophenols) levels were highest in sediments from the Main Stem between Pitt River and the trifurcation; sediments from Mitchell Island (in the North Arm) had the second highest concentrations. Many values were greater than the B.C. Provisional Objective of 10 ng/g (Table 1). The Objectives in Table 1 refer to maximum, rather than mean or average, acceptable concentrations, but apply outside dilution zones for effluent discharges. Main Arm sediments were much lower in chlorophenol concentration than were either Main Stem or North Arm sediments, with all samples (except one that was at a source) having concentrations less than 10 ng/g.

2.3.2 Water

Chlorophenol concentrations in most water samples taken from the North Arm (near Mitchell Island), exceeded the B.C. Provisional Objective of 0.2 μg/L (Drinnan *et al.*, 1989) (Table 1). Few samples from other locations exceeded the objective. Those samples which did exceed the objective were from the Main Stem or North Arm and located at or near a known effluent source. Total chlorophenol did not exceed 0.2 μg/L in samples from the Main Arm or Offshore. Higher concentrations probably result from surface run-off from wood treatment plants, or from opening of tidal gates at low tide by industries which collect run-off, etc. Only a time series study would record such an event.

Maximum tetrachlorophenol and pentachlorophenol concentrations in the Fraser estuary exceed the CCREM guidelines for these compounds (Table 1). The 90th percentile for tetrachlorophenol also exceeds the CCREM guideline. Median concentrations for these two compounds are close to the objectives proposed by Kistritz (1989; see Appendix 2), indicating that his proposed objectives have been exceeded in approximately half of the samples taken in the Fraser River.

2.3.3 Biota

Pentachlorophenol accumulates in aquatic organisms to levels as high as 1000 times the concentration in water (NRCC, 1982). Water and sediment chlorophenol levels from the Main Arm of the Fraser River were significantly lower than the levels in either the Main Stem or the North Arm (Drinnan *et al.*, 1989), but levels in the biota did not differ. Chlorophenol levels in fish from the North Arm of the Fraser River were 100-1400 times higher than the levels in water samples (Carey *et al.*, 1986). Bioconcentration or accumulation in the biota of the Fraser River appears to be a legitimate concern.

2.3.3.1 Vertebrates

High sediment and water chlorophenol concentrations were associated with chlorophenol discharges, whereas high tissue concentrations were not necessarily associated with chlorophenol point sources (Drinnan *et al.*, 1989) The mobility and transient nature of fish may account for this pattern. Conversely, Carey *et al.* (1983, 1986) suggested that there was an association between fish tissue levels and chlorophenol discharge sites. Concentrations of 2,3,4,6-tetrachlorophenol and pentachlorophenol in fish had a geographic distribution similar to that of concentrations in water (Carey *et al.*, 1988). Carey *et al.* (1988) argued that, except during short duration pulses, fish tissue chlorophenol levels are usually in equilibrium with chlorophenol concentrations in the water.

Birtwell *et al.* (1983) investigated the conflict between bio-resources and the disposal of municipal wastes. Effluents are generally less dense and warmer than the stratified receiving waters; therefore they are dispersed only in the upper part of the water column. Juvenile salmon occupy this upper layer during migrating and rearing stages. Many species were caught in the study: Pacific herring (*Clupea pallasii*), silver perch (*Morone americana*), Pacific sandlance (*Ammodytes hexapterus*), surf smelt (*Hypomesus pretiosus*), three-spine stickleback (*Gasterosteus aculeatus*) and chinook salmon (*O. tshawytscha*). Pacific herring comprised 39% of the catch and 14.5% of these fish contained measurable levels of chlorophenol. Although chinooks were less numerous than herring, 20.5% were contaminated. Chlorinated phenols in flounder were thought to have originated from the domestic use of antiseptics and wood preservatives in the central and western parts of the municipality of Vancouver (Birtwell *et al.*, 1983). Dichloroanisole was found in flounder and sculpin extracts. Birtwell *et al.* (1983) suggested that dichloroanisole could be derived from chlorophenol contaminated

sediments in which dichlorophenol was converted by biological methylation into the corresponding anisole. Bacteria and fungi may be involved in such conversions (Lech *et al.*, 1978). Dichlorophenols may have originated from an antiseptic, from a breakdown product (2,4-dichlorophenol) of a herbicide, or from chlorination of phenols during disinfection of sewage in the summer.

Singleton (1983) looked at chlorophenol levels in largescale suckers (*Catostomus macrocheilus*) in the Fraser River. Fish from the lower industrialized reaches of the river were more contaminated than fish from the upper industry-free reaches. In the North Arm, concentrations of 30 and 56 ng/g (wet weight) were found in largescale suckers (Singleton, 1983). Chlorophenols were not detected in fish from the Main Arm and Main Stem. At the time of the study chlorophenols were in sufficient quantities to accumulate in fish tissue, hence restrictions and controls on the use of contaminants were suggested in order to prevent further contamination of Fraser River fish (Singleton, 1983).

Drinnan *et al.* (1989) reviewed chlorophenol levels in fish from the Fraser River estuary. Species with total chlorophenol concentrations greater than the B.C. Provisional Objective of 100 ng/g were (% of samples greater than the objective follow each):

- eulachon (*Thaleichthys pacificus*)(56%),
- largescale sucker (44%),
- prickly sculpin (*Cottus asper*)(44%),
- northern squawfish (*Ptychocheilus oregonensis*) (37%).

Chlorophenol levels in other vertebrates from the Fraser River have seldom been examined. In 1983, mean concentrations of 2 ng/g of both tetrachlorophenol and pentachlorophenol were found in great blue heron (*Ardea herodias*) eggs collected near the University of British Columbia (Drinnan *et al.*, 1989).

Swain (1986) surveyed metals, PCBs, and chlorophenols in sediments, benthic organisms, and fish of the lower Fraser River, to determine whether provisional objectives were being met. Most sediment samples contained chlorophenols at levels below the B.C. Provisional Objective of 10 ng/g, but levels in some sediment samples were 3-6 times higher than the objective (Table 1). The highest level in rainbow trout tissue was 60 ng/g. At the time of the study it was concluded that chlorophenol contamination of the fish muscle was not of concern.

Birch (1988) examined tetrachlorophenol and pentachlorophenol content (ng/g wet tissue) of composite, liver, and remaining tissue samples of starry flounders (*Platichthys stellatus*). These results are compared with those given by Carey *et al.* (1986) and Rogers and Hall (1987) in Table 2. Birch (1988) found no relationships between chlorophenol concentration in tissue and/or lipid content

TABLE 2. Summary of Chlorophenol Concentrations in the Starry Flounders of the Fraser River. (Means and/or Ranges are reported)

Chlorophenol	Tissue	Birch et. al., 1988	Rogers and Hall, 1987	Carey et. al., 1986
TTCP (ng/g wet weight)	Composite	6.27 (1.83-20.71)	-	-
	Muscle	-	-	13-15
	Liver	26.88 (10.6-108.13)	26 (ND-118.9)	-
	Residue	6.29 (2.19-38.52)	4.7 (ND-47.8)	-
PCP (ng/g wet weight)	Composite	13.15 (4.35-29.17)	-	-
	Muscle	-	-	18-22
	Liver	68.93 (37.77-223.91)	114.6 (14.7-496.6)	-
	Residue	17.95 (7.28-25.67)	6.1 (0.8-15.8)	-

ND not detectable
- not available

and age or size, except possibly for dichlorophenols. Tissue from younger/smaller fish had a greater variety of isomers, and often greater concentrations of dichlorophenols, than did tissue from older fish.

2.3.3.2 Invertebrates

The potential for bioconcentration of chlorophenols in tissues is high for some invertebrate species, specifically deposit and filter feeders such as oligochaetes, polychaetes, and bivalves. Various benthic groups (chironomids, lampreys, oligochaetes, crustaceans, dipterans, polychaetes) were analyzed by Swain (1986) for PCB and chlorophenol tissue levels. The 90th percentile for all invertebrates was 1380 ng total chlorophenols/g (eight times the maximum in the sediments). The median for total chlorophenol concentration in invertebrate tissue was 10 ng/g, compared to 2 ng/g in sediment samples. These data suggest chlorophenol bioconcentration by some invertebrates.

2.4 Habitat Types

In the Fraser River Estuary 15 habitat types were distinguished by Drinnan *et al.* (1989). There are four broad types (freshwater, upper estuary, lower estuary, marine), each sub-divided into intertidal vegetated, intertidal non-vegetated, back channels and sloughs. Several habitats were considered sensitive because they were used by sensitive species (primarily juvenile salmonids), unique species (*e.g.* trumpeter swan) or populations of international significance (migratory or overwintering shorebirds or waterfowl). These habitats may be more affected by chlorophenols, and therefore greater sensitivity should be considered when developing objectives. Habitats identified as having special features by Drinnan *et al.* (1989) were freshwater intertidal vegetated, freshwater back-channel and slough, upper and lower estuary intertidal vegetated, estuarine back-channel and slough, and marine lower intertidal vegetated.

In the Fraser River, water column chlorophenol concentrations decrease with increasing distance from the source. As a consequence, habitats (*e.g.*, intertidal vegetated and back-channels/sloughs) near a discharge site would be more susceptible. Juvenile fish (particularly salmonids) are at greatest risk because they may be exposed to partially diluted effluent during initial periods of rainfall and the first flush of storm water run-off from wood preservative facilities. Exposure risk decreases as distance from a source increases. In general, habitats of high usage by fish and birds generally have lower chlorophenol concentrations. Such areas within the Fraser River Estuary include the marsh areas of Duck and Woodward Islands, the lower estuary region of the Main Arm, the Musqueam marsh near the mouth of the North Arm, and intertidal regions of Roberts and Sturgeon Banks.

3.0 EFFECTS OF CHLOROPHENOLS

Reviews by Jones (1981, 1984), McLeay (1987), and Kistritz (1989) were prepared for Environment Canada on the fate and effects of chlorophenols. To avoid overlap, our literature review focuses on papers not cited in these reports. Our objective was to add additional data on chronic and acute effects, and bioaccumulation potential, of chlorophenols. Emphasis has been placed on salmonid fishes, but we include information on other organisms which could replace or supplement chinook salmon as toxicity test species. Our rationale for this decision is to determine if our test organism of choice, chinook salmon, is sensitive to chlorophenol effects, to determine if the need exists to revise current water quality objectives (*e.g.*, B.C. Ministry of the Environment, U.S. EPA., and CCREM) on the basis of this additional/recent information, and to provide appropriate background information for future test comparisons and interpretations. Included in the appendices are Kistritz's (1989) appendices on the reported acute (Appendix 3 and 4), chronic (Appendix 5) and bioaccumulation (Appendix 6) testing of chlorophenols as well as a table (Appendix 7) from our own literature survey, intended to augment available information.

3.1 Acute Effects

A large database exists in the literature regarding the acute toxicity of chlorophenols to aquatic organisms (Appendices 3 and 4). Pentachlorophenol is the most toxic of the three chlorophenols selected for this study (2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol, and pentachlorophenol). Toxicity increases with increasing chlorination (Hattula *et al.*, 1981). Fish are generally more sensitive than are invertebrates, with 96 h LC50s ranging from 31.8 - 1820 μg/L and 40 - 3,900 μg/L, respectively (Appendix 3). Salmonids are the most sensitive of the fish species, with LC50s occurring over a narrower range: 31.8 - 157 μg/L (Appendix 3). Only one acute lethality test using chinook salmon was found in the literature, with an LC50 falling near the midpoint of this range (Iwama and Greer, 1979).

3.2 Chronic Effects

Chronic effects information for the lower chlorinated chlorophenols is limited, but pentachlorophenol appears to be more toxic over longer time periods than are either 2,4,6-trichlorophenol or 2,3,4,6-tetrachlorophenol. Reported literature values for the chronic toxicity of pentachlorophenol range from 2 - 800 μg/L (Appendix 5). Non-salmonid fishes and invertebrates appear to be equally sensitive to chlorophenols. Salmonids are the most sensitive species with chronic responses to pentachlorophenol occurring at 2 - 88 μg/L (Appendix 5). The chronic studies listed in the literature with respect to salmonids include: growth, early development, temperature preference, avoidance responses, blood plasma changes, prey capture, and survival. Growth is the most sensitive indicator

of chronic toxicity, reportedly inhibited at pentachlorophenol levels as low as 2 μg/L (Webb and Brett, 1973) for sockeye salmon (*Oncorhynchus nerka*).

3.3 Bioaccumulation

Tabulated bioaccumulation values indicate that salmonids accumulate chlorophenols to a lesser degree than do other fishes. However, it is difficult to present ranges of bioconcentration factors, as experimental conditions (pH, temp) and test duration are not consistent among studies. It appears from the literature that there are no patterns with respect to bioconcentration of the different chlorophenols (Appendix 6).

4.0 BIOASSAY TECHNIQUES

4.1 Acute Toxicity Tests

The primary aim of an acute toxicity test is to establish the concentration of a test substance (or the level of an agent) that produces a deleterious effect on a group of test organisms during a short exposure, under a set of controlled conditions. Experimentally, a 50% response is the most reproducible measure of the toxicity of a material, and 96 h (or less) is the standard exposure time. The most commonly expressed endpoint in toxicity studies using fish is mortality in a test called a 96 h LC50.

Acute lethality tests are useful in providing a rapid estimate of the concentration of test material causing a quantal response in the test organism. Chronic studies are more diagnostic with respect to assessing the effects of a toxicant on longer-term events. Static acute tests are ideal for the initial screening of a test material as they provide estimates of the upper limit of concentrations producing toxic effects. They are also useful for evaluating the relative toxicity of a group of contaminants, evaluating the relative sensitivity of different organisms, evaluating the effects of water quality, and defining dose-response relationships (Macek *et al.*, 1978 cited in Parrish, 1985). Types of static tests include:

- non-renewal - the test organisms are exposed to the same solution for the duration of the test, and
- renewal - the test organisms are exposed to a fresh solution of the same concentration of contaminant every 24 h, either by transfer or replacement (EPA, 1985).

Because of toxicant adsorption onto test chambers, and the effects of metabolites on toxicity, the renewal method is preferred. Methods for static acute toxicity tests using salmonids are well documented, and appear in a number of protocol manuals; for example, APHA Standard Methods (1989), Environment Canada (1989) and the British Columbia Provincial Guidelines (Anon., 1982).

Flow-through acute tests augment the maintenance of controlled test conditions and are not limited in duration. Several different procedures have been discussed in the literature, each with its own advantages and disadvantages. The proportional diluter designed by Mount and Brungs (1967) is the most often cited flow-through apparatus. It delivers five toxicant concentrations and a control at any desired flow rate per concentration up to 400 ml/min, and with a dilution factor from 50 to 25 per cent between successive concentrations (Mount and Brungs, 1967). The advantages of the proportional diluter are that few timing adjustments are required, and it is simple to operate and can be constructed rapidly. For wide concentration ranges with large dilution factors, the proportional diluter is inappropriate. A further disadvantage of the proportional diluter is that it is able to supply test chambers with only one toxicant source, and is therefore not suitable for joint toxicity studies. The serial diluter described by Mount and Warner (1965, cited in Mount and Brungs, 1967) is capable of delivering wide concentration ranges over very large dilution factors. Benoit *et al.* (1982) modified the serial diluter for use with fish early life stage and invertebrate tests. Anderson and Weber (1975) developed a serial diluter capable of delivering two or more different toxicants to six replicated test chambers. Dilution is based on flow-rate and the cross-sectional area of the faucets supplying the next level of the toxicant delivery system. This system can supply high flow rates (as per Sprague, 1973) and wide concentration ranges; as a disadvantage, the serial diluter requires daily calibration and is hence more time-consuming than the proportional diluter.

Studies of the acute toxicity of chlorophenols to fish are relatively common (Hattula *et al.*, 1981; Phipps *et al.*, 1981; Gupta, 1983; Benoit-Guyod *et al.*, 1984). Pentachlorophenol is most commonly discussed in the literature as it is generally the most toxic to fish (Phipps *et al.*, 1981). The 96 h LC50 of pentachlorophenol for fish generally ranges from 40-600 μg/L. There is some evidence that cool water species may be more susceptible to pentachlorophenol than are warm water species (Niimi and McFadden, 1982). Reported LC50 values for salmonids range from 40-130 μg/L (Webb and Brett, 1973; Iwama and Greer, 1979; Phipps *et al.*, 1981; Hattula *et al.*, 1981). Pentachlorophenol toxicity increases as temperature increases (Chapman *et al.*, 1982; Hodson and Blunt, 1981), decreases as pH increases (Saarikoski and Viluksela, 1981), and is increased by hypoxic stress (Chapman and Shumway, 1978). However, there is a scarcity of acute toxicity data from flow-through tests in which pH, dissolved oxygen, and toxicant levels are less subject to alteration by metabolic activity (Dominguez and Chapman,1984).

4.1.1 Joint Toxicity Testing

The main emphasis of aquatic toxicity research has been on quantifying the toxicity of single compounds; the joint toxicity of chemical mixtures has received little attention. Water quality criteria rarely address the issue of multiple contamination of waterways, and as it is mixtures of pollutants that are entering aquatic systems, evaluations of chemical interactions are required to fully assess their combined effects.

Mechanisms of interaction between constituents of pollutant mixtures that may induce multiple toxicity, have been described by Anderson and D'Appollonia (1978), and include the following:

- Environmental phase: Chemical interactions between pollutants which produce new compounds, complexes, chelates, or changes in chemical state;

- Dynamic phase: Toxicant mixtures that act at the same target site(s); at different target site(s) and amplify the toxicity of each other; a toxic response by chemical groups is produced that is different from the response induced by each chemical;

- Kinetic phase: Toxicant mixtures that alter bioavailability at target site(s) by affecting binding affinities; and by inducing or inhibiting the production of detoxification metabolites (*e.g.*, MFO's, or metallothioneins).

Toxicity studies of mixtures are also referred to as interaction or joint toxicity studies. The terms synergism and antagonism are often used to describe the effects resulting from exposure to mixtures of two or more chemicals applied simultaneously. By definition, a synergist enhances or potentiates the activity of another compound. Antagonism, or competitive addition, occurs when two or more chemicals applied together are less toxic together than are the individual components when applied singly. Because the terms are non-quantitative, they are often used ambiguously. Mixture toxicity nomenclature is inconsistent in the literature. More recent interpretations suggest that use of the terms greater than additive (synergism), less than additive (antagonism), and additive would be helpful in quantifying the three categories of toxicity of mixtures (Marking, 1985). Additive action is the most common form of mixture toxicity, and indicates (for example) that 50% of the LC50 of compound A plus 50% of the LC50 of compound B will yield a mixture that produces 50% mortality. Greater than additive and less than additive interactions are the exception.

Many models for evaluating joint toxicity data have been proposed such as the Mixture Toxicity Index (MTI) (Konemann and Musch, 1981), the Additivity Index (Marking and Dawson, 1977; cited in Marking, 1985), and the Toxic Unit concept (Sprague, 1970). The Toxic Unit concept is based on the assumption that each component of a mixture can be substituted by an equitoxic concentration

of another chemical. The MTI and the Additivity Index are similar in that both classify a possible range of joint actions of chemical mixtures on a toxicity scale, where zero refers to the additive (no tolerance correlation) concentration. The main advantage of the MTI over the Additivity Index is that constant values are obtained for two reference points, "no addition," and "concentration addition," which are independent of the number of compounds tested and the ratios between them.

Data values regarding the joint toxicity of chlorophenol mixtures are rare in the literature. A preliminary study by the Aquatic Toxicity Research Group (Anon., 1987) suggested that mixtures of tri-, tetra- and pentachlorophenol had an additive effect (*i.e.*, toxic responses did not occur at mixture combinations that differed significantly from 1.0 toxic units) to flagfish (*Jordanella floridae*), either at the acute or chronic level.

4.2 Chronic Toxicity Tests

Exposure to high concentrations of toxic substances for short periods of time, while not commonly encountered by fish in natural waters, can occur in dilution zones near effluent discharges. Beyond the region of discharge, dilution and dispersion of the contaminant occurs. As a result, a greater proportion of the biomass is chronically exposed to low, sublethal levels of toxic compounds than to acutely lethal levels (Kleerekoper, 1976). A chronic toxicity test can indicate the concentrations of a toxicant that will interfere with normal growth, development, and attainment of reproductive potential of an aquatic organism. Generally, concentrations that produce chronic effects are lower than those that produce more readily observable acute effects, such as mortality. Therefore, chronic studies can provide a more sensitive measure of chemical toxicity than can acute toxicity tests. The most common endpoints used to evaluate chronic toxicity are growth and survival, behaviour, embryo hatchability/development, and bioaccumulation.

4.2.1 Growth

Growth is a fundamental component of fitness, and therefore is an important index of contaminant effects. Toxicants can affect growth rates indirectly by reducing the food available, and directly by impairing metabolic pathways converting food energy to tissue or by diverting energy from growth to metabolism of the contaminant. Effects on growth (and reproduction) can best be understood by considering the energy budget of an animal (Widdows, 1985). Food energy consumed is used for respiration and production of tissue or gametes. There is also some loss via the faeces and excretion. When production is estimated from the difference between the energy absorbed and the energy expenditure via respiration and excretion, it is referred to as the "scope for growth" (Warren and Davis, 1967; cited in Widdows, 1985). Scope for growth (SFG) can range from positive values when there is energy available for growth and the production of gametes, to negative values when the organism is utilizing its body reserves for maintenance metabolism. Time series determinations can

assess the severity of energy deficiency and can detect any changes in rates as well (Sindermann, 1988); field validation experiments support the use of scope for growth as a bioindicator of environmental stress (Widdows *et al.*, 1984).

An additional index can be calculated from the physiological components of the energy allocation to provide further information on the efficiency with which an animal functions. The energy available for growth, as a proportion of the energy absorbed from the food, represents net growth efficiency and is a measure of the efficiency with which food is converted into body tissue. A reduction in this value is indicative of a stressed condition, since a greater proportion of the energy absorbed from the food is being used to maintain the animal and consequently a smaller proportion is available for growth.

Measurements of scope for growth (SFG) and net growth efficiency (NGE) have been developed. SFG offers an instantaneous view of sublethal effects which, if extended over a period of time, would result in death. NGE values provided a long term integration of physiological processes. Growth is viewed as a good integrative measurement of an individual's response to contaminants and has been widely used. It was concluded that analogous exposures and exposure-response relationships developed in the laboratory were not different from those in the field. The consequences of reduced growth include reduced fecundity, slower maturation (Munkittrick and Dixon, 1989) and a reduced ability to compete with other individuals; these consequences have population and community level repercussions. Measurable effects of chlorophenols on the growth of fish are well documented in the literature (Webb and Brett, 1973; Ward and Parish, 1980; Hodson and Blunt, 1981; Holcombe *et al.*, 1982; Mathers *et al.*, 1985; Brown *et al.*, 1987).

Webb and Brett (1973) measured the effect of pentachlorophenol exposure on growth rate and conversion efficiency of underyearling sockeye salmon (*Oncorhynchus nerka*) for two growth phases: an 8-week pentachlorophenol exposure phase, and a 4-week post-exposure phase. Both growth rate and food conversion efficiency were reduced in the pentachlorophenol-exposure phase at concentrations greater than 2 μg/L (Webb and Brett, 1973). For the post pentachlorophenol-exposure phase, percent response values indicated recovery in all groups affected by pentachlorophenol, but recovery was incomplete at the end of the 28-d recovery period (Webb and Brett, 1973). The effects of pentachlorophenol on increased oxygen consumption (Chapman *et al.*, 1968; cited in Webb and Brett, 1973) and decreased ATP levels (Kreuger *et al.*, 1966; cited in Webb and Brett, 1973) were implicated by Webb and Brett (1973) in the elevation of maintenance energy demands of the fish, hence the reduction in growth rate and conversion efficiency.

4.2.2 Early Life Stage (ELS) Tests

Hynes (1960; cited in Woltering, 1984) emphasized the necessity of conducting bioassays with the most susceptible life stage(s) of the test organism. Tarzwell (1967; cited in Woltering, 1984) added the need to test the most sensitive life stages of the most sensitive and dominant species. In the course of conducting full and partial life cycle tests with several species and a variety of chemicals, early developmental stages (*i.e.*, embryo, larval, and early juvenile) have shown equal or greater sensitivity than have adult life stages (McKim, 1977, 1985; Woltering, 1984). Therefore 30- to 60-day post-hatch tests were proposed in order to reduce the time and expense of fish partial and full life cycle toxicity tests. Early life stage (ELS) toxicity tests generally measure embryo hatchability, survivorship, and growth over a 7-32 day exposure period. Because many critical events take place in a very short period, ELS studies are the most common partial life cycle toxicity tests conducted with fish (McKim, 1985).

The effects of chlorophenols on salmonid development are documented in the literature (Chapman and Shumway, 1978; Hodson and Blunt, 1981; Dominguez and Chapman, 1984). Chapman and Shumway (1978) describe experiments designed to follow survival, growth, development, oxygen consumption, and bioenergetics of steelhead trout (*O. mykiss*) embryos and alevins in media containing sodium pentachlorophenate. Embryos were exposed from fertilization to hatch. Alevin dry weight at hatch was decreased by exposure to sodium pentachlorophenate and hatching was delayed. Alevins reared at 40 µg/L sodium pentachlorophenate consumed greater quantities of oxygen in growing to a given weight than did control alevins. The exposure of alevins to sodium pentachlorophenate and the resultant increase in metabolic demands disrupted the normal bioenergetic pattern of tissue elaboration and yolk catabolism. Alevins exposed to sodium pentachlorophenate apparently were forced to use a considerable portion of yolk protein for energy production.

Hodson and Blunt (1981) exposed the three early life stages of rainbow trout (*O. mykiss*) embryo, alevin and fry to pentachlorophenol at four concentrations, and two temperature regimes, cold and warm. Three general but significant conclusions were made that may be applicable to field populations of chronically exposed trout. Exposure to sodium pentachlorophenate during springtime embryo development coupled with low temperature adversely affected both embryos and alevins (Hodson and Blunt, 1981). As experimental temperature increased, pentachlorophenol effects on growth were enhanced. Additionally, sodium pentachlorophenate toxicity and temperature effects on toxicity were not as great for alevins and fry if they were not exposed as embryos.

The rainbow trout alevin yolk conversion efficiency bioassay measures the lethal and sublethal effects of toxicants on rainbow trout alevins. The procedures are currently being revised by E.V.S. Consultants, and the test is being adapted for use *in situ*. The test is based on studies conducted by Hodson and Blunt (1981, 1986) which examined the efficiency of the conversion of yolk to body tissue. Response variables measured include embryo and alevin mortality, time to hatch, developmental rate and yolk conversion efficiency.

Embryo and alevin mortality, as well as mean age at hatch, can be calculated; accelerated hatch is a common response to low levels of toxicants. Developmental rate can be assessed from yolk and body weights. If a toxicant retards development, exposed individuals should have larger yolks and smaller bodies than do controls. Toxicants may also affect the efficiency of the conversion of yolk to body tissue. Efficiency is defined as the weight of body tissue produced per unit weight of yolk used. Differences in efficiency are assessed by comparing regressions of body weight on yolk weight. If exposed individuals have smaller bodies than do controls at any given yolk weight, they are clearly not converting their yolk as efficiently. The difference in efficiency between exposed individuals and controls represents the energy cost of exposure. Measuring yolk conversion efficiency is equivalent to measuring growth at a fixed ration, except that in this case the ration (yolk) is provided by the test organisms themselves.

4.2.3 Bioaccumulation

Bioaccumulation is a general term describing a process by which chemical substances accumulate in aquatic organisms, with the term bioconcentration referring to concentrations greater than those present in the external environment. Bioaccumulation integrates the overall process of chemical uptake and retention, and has been described by various authors through studies on uptake kinetics, metabolism, and excretion/depuration, tissue distribution and compartmentalization, complexation, storage and concentration. Direct uptake of chemicals from water has been shown for many aquatic organisms including algae, annelids, arthropods, molluscs, and fish (Spacie and Hamelink, 1985).

Uptake refers to the transfer of a chemical into or onto an aquatic organism; in the laboratory, this refers to the exposure phase. The three most significant transport processes for the uptake of xenobiotics from water are diffusion, special transport (active and facilitated transport), and adsorption. Elimination from an organism by desorption, diffusion, excretion, egestion, biotransformation, is usually measured during the depuration phase of a test, where previously exposed organisms are held in uncontaminated water. Interspecific differences could influence the elimination rates of pentachlorophenol. Trout appear to eliminate pentachlorophenol primarily through biliary excretion (Glickman *et al.*, 1977), whereas goldfish eliminate pentachlorophenol

primarily through branchial excretion, although renal and biliary routes are also used (Kobayashi and Nakamura, 1979; cited in Niimi and Cho, 1983).

Bioaccumulation can occur only if the rate of uptake of a chemical by an organism exceeds its rate of elimination. Not all tissues have an equal capacity for uptake or storage, and tissue contaminant levels will be dependent upon the organism, the chemical, the duration of the exposure and the tissues examined. Several studies have confirmed the presence of both pentachlorophenol and pentachloroanisole (PCA) in fish raised in disinfected municipal effluents (Lech *et al.*, 1978). Niimi and McFadden (1982) demonstrated that trout exposed to waterborne pentachlorophenol at ng/L concentrations, accumulated pentachlorophenol in the μg/g range. Niimi and Cho (1983) determined that rainbow trout fed pentachlorophenol at 10 ng pentachlorophenol/g maintained whole body levels of 2 ng/g, whereas body pentachlorophenol levels in trout fed 300 ng/g increased to 40 ng/g after a 40 day exposure, then decreased to 20 ng/g. Uptake from water appears to be the most important pathway, and any accumulation through food would likely represent only a small portion of the body burden (Niimi and Cho, 1983).

Pentachlorophenol has a relatively short biological half-life ($T_{½}$) in various rainbow trout tissues, with literature values ranging from 24 h (Glickman *et al.*, 1977) to 7 days (Niimi and Cho, 1983). Estimates of $T_{½}$ for other species include 1.5 days for goldfish, and 5 days for sunfish (see Niimi and Cho, 1983).

Bioaccumulation is a phenomenon, not an effect. It is a consequence of exposure, but cannot be considered a true response, because few data exist that provide direct cause and effect links between tissue residue levels and chronic or sublethal effects. Recently, there have been attempts to relate tissue levels to effects by considering the physical properties and toxic action of the compounds involved. The most promising route, with direct relevance for chlorophenols, is to express tissue levels as number of molecules (*e.g.*, μmol) per unit body weight (McCarty, 1986, 1987*a,b*; Abernethy *et al.*, 1988). Theoretically, the toxic effects of compounds that act primarily by narcosis should be a function of the number of molecules attached to either nerve cell membranes or to specific receptor sites. Toxic effects (death) should occur whenever a "critical volume" is reached, regardless of the composition of the compounds making up this volume. In the case of chlorophenols, tissue levels of several compounds could be converted to μmol/g, and summed. These sums would be directly comparable with those from other tissue samples, regardless of composition, and could also be compared to the "critical volume" to indicate the potential for toxic effects. Two cautions should be noted. First, there is no evidence that sublethal effects are also associated with a "critical volume". Second, there is evidence that pentachlorophenol has some metabolic effects in addition to the narcotic effects common to all chlorophenols, and therefore has a different "critical volume" (Brendan

Hickie, Department of Biology, University of Waterloo, pers. comm.). Pentachlorophenol may have to be considered separately from other chlorophenols if tissue levels were expressed as molecules per unit weight.

4.2.4 Seawater Challenge

The life-stage at which juvenile salmonids enter seawater is considered to be sensitive due to physiological stresses imposed by the transition from a freshwater to a marine environment (Clarke and Blackburn, 1978). Relatively high natural mortality is often associated with this stage of salmonid development (Clarke, 1982). Factors that increase physiological stress (*e.g.*, toxic reactions, environmental extremes) may increase mortality during seawater adaptation. This is related to decreased osmoregulatory abilities, in particular salt excretion capacity to offset the influx of salts upon introduction to seawater (Wedemeyer *et al.*, 1980). The efficiency of salt regulation can be measured using blood sodium levels of fish exposed to seawater for a 24 h period. Poor performance in the seawater challenge test (*i.e.*, sodium plasma levels greater than 170 meq/L) has been equated with decreased growth rate (Clarke and Shelbourn, 1982) and possibly increased likelihood of predation (Houston, 1961).

4.2.5 *In situ* Testing

In situ testing can provide confirmatory information on the effects of toxicants on biota (McLeay, 1987). Exposing animals to natural conditions allows for greater realism in the response, and enables the existing, and usually complex, mix of chemicals to be tested. Cages have been used successfully in previous *in situ* fish exposure tests (Munday and Vigers, 1982; E.V.S. Consultants, 1983; Vigers *et al.*, 1983; Morgan and Vigers, 1985; Morgan, 1985). Comparisons between laboratory testing and *in situ* testing provide a possible means of assessing the applicability of laboratory results to natural situations.

4.3 Behavioural Toxicity Tests

Most animal behaviour patterns involve highly adaptive responses to chemical, biological, and physical environmental stimuli. Through adaptive behaviour an animal can mitigate otherwise unfavourable environmental perturbations. Environmental contaminants may be toxic at a behavioural level to aquatic organisms (Sprague, 1971; Miller *et al.*, 1982; Black and Birge, 1980; Cherry and Cairns, 1982; Steele *et al.*, 1985; Sandheinrich and Atchison, 1989; Smith and Bailey, 1989). A behavioural response is an integration of physiological responses to a chemical stimulus. Contaminants that affect the normal function of neurosensory systems may affect how organisms

move through their environment and respond to the normal range of cues that direct them toward food, shelter, spawning grounds, and other necessities for population growth. Various behavioural endpoints have been addressed, including ventilation and cough frequencies (Westlake *et al.*, 1983), spatial selection (Pedder and Maly, 1985), response to food and feeding ability (Farmer *et al.*, 1978; Mathers *et al.*, 1985; Hill, 1989; Sandheinrich and Atchison, 1989), predator-prey responses (Coutant, 1973; Kania and O'Hara, 1974; Sullivan *et al.*, 1978), and preference for or avoidance of a variety of stimuli (Cherry and Cairns, 1982; Miller *et al.*, 1982; Atchison *et al.*, 1987; Smith and Bailey, 1989).

Most organisms have responses that enable them to tolerate transient environmental stressors (Rand, 1984). Abnormal behaviour may reveal that the organism's tolerance limit has been exceeded and that there may be corresponding physiological and/or biochemical damage. The objective of an aquatic behavioural toxicity study is to define the adaptive and aberrant behaviours produced as a result of exposure and the conditions necessary to elicit them.

A behavioural endpoint is most ecologically significant if it can be related to possible consequences in the field. It is important to measure as many behavioural parameters as possible in order to better understand the response gradients obtained. One important aspect of behavioural testing is to establish a reliable control data base. Control behaviour should be established each time new organisms are placed in a test system to avoid stressed behavioural patterns which can reduce the sensitivity of the tests (Smith and Bailey, 1989).

4.3.1 Locomotor Activity

Among the many behavioural patterns in fish, locomotion and orientation behaviour are of particular ecological significance. They are instrumental in the procurement of mates, food, and nesting sites, and allow the animal to move in a non-random fashion, in relation to physical, chemical, and biological conditions in the environment (Kleerekoper, 1976). Sensory information impairment may reduce an organism's ability to perceive and respond to its environment, and locomotor ability impairment may in turn reduce an animal's fitness. Substances that affect basic neural functions are of special interest; therefore, locomotor responses present suitable models for testing the toxicological effects of contaminants on fish behaviour.

Contaminants can affect locomotor activity in many ways:

- elicit movement towards (preference) or away from (avoidance) a contaminated site,
- impair sensory perception and reduce responses to normal cues (*e.g.*, feeding, mate selection),
- result in hyper- or hypoactivity,
- alter locomotor components (*e.g.*, turning frequency),
- reduce swimming performance or endurance (modified from Atchison *et al.*, 1987).

The most commonly studied of these responses are swimming performance (Sprague, 1971; Webb and Brett, 1973; Bull and McInerney, 1974; Webb, 1978) and preference/avoidance responses (Sprague, 1968; Cripe, 1979; Cherry and Cairns, 1982; Miller *et al.*, 1982; Smith and Bailey, 1989). Metals and temperature are the most well documented agents with respect to their effects on locomotor activity; however, the techniques can be readily applied to the testing of chlorophenols.

4.3.1.1 Swimming Performance

Swimming capacity and swimming activity are two general measures of swimming performance commonly used to assess contaminant related changes in locomotion (Little and Finger, 1990). Swimming capacity is a measure of the fish's ability to swim against (Howar, 1975) and to orient towards (Dodson and Mayfield, 1979) water flow. Swimming activity consists of the following variables: speed and distance travelled (Little and Finger, 1990) and frequency and duration of movements (Cleveland *et al.*, 1980).

The effect of contaminants on the swimming behaviour of salmonids is well documented in the literature (Brett, 1964; Webb and Brett, 1973; Bull and McInerney, 1974; Howar, 1975; Dodson and Mayfield, 1979; Cleveland *et al.*,1980; Little and Finger, 1990). Toxicant-related modifications in swimming behaviour occur well before mortality does, regardless of the swimming response evaluated (see review by Little and Finger, 1990). Based on the information presented by Little and Finger (1990), contaminant concentrations that caused changes in swimming behaviour were, on average, <16% of the concentrations causing mortality. In studies where multiple observations were made, behavioural changes occurred 75% earlier than the onset of mortality (Little and Finger, 1990).

Information on the effect of chlorophenols on swimming performance is scarce. Webb and Brett (1973) reported that the swimming performance of sockeye salmon was not affected by various pentachlorophenol concentrations tested (7 - 50 μg/L). The LC50 of pentachlorophenol was 57 μg/L, and swimming performance appeared to be a less sensitive indicator of toxicity than growth, which

was affected at pentachlorophenol levels as low as 2 μg/L. Similarly, the swimming capacity of rainbow trout was unaffected by pentachlorophenol levels greater than 20 μg/L (*i.e.*, at a level of 50% of the reported 96 h LC50) (Little *et al.*, 1989; cited in Little and Finger, 1990). Pentachlorophenol is known to reduce ATP output (Weinbach, 1954) and would therefore be expected to affect swimming performance. Webb and Brett (1973) suggested that although the standard metabolic rate is elevated by pentachlorophenol exposure, the active rate is not.

Swimming behaviour is a valid and consistent index of sublethal toxicity that can be easily incorporated in test protocols to expand the sensitivity of standard toxicity tests. However, the swimming behaviour of salmonids appears to be non-responsive to pentachlorophenol exposure at levels up to 50% of the LC50 (Webb and Brett, 1973; Little and Finger, 1990). For this reason, preference/avoidance studies were also evaluated for monitoring the locomotor responses of salmonids to pentachlorophenol exposure.

4.3.1.2 Preference/Avoidance Testing

Some fish have a keen sense of chemoreception (Hara, 1981; Brown *et al.*, 1982) which is believed to play a mediating role in reproductive migration and pairing, schooling, feeding, parental recognition, and predator avoidance (Hara, 1982). Of importance in preference/avoidance studies is the ability of the fish to not only detect chemical changes in its environment, but also to respond to them because an avoidance response is of survival value. However, migration from sites of contamination may lead to changes in aquatic community structures. Attraction to a contaminant is usually an inappropriate response as the animal may be exposed to potentially lethal concentrations. The fishes' third behavioural alternative is to ignore the contaminant, although it may be toxic (Rand, 1984). There are no typical preference/avoidance reactions as organisms detect and avoid some contaminants, but not others (Rand, 1984).

Preference/avoidance tests are designed to determine the response of an organism to toxicants based primarily on changes in position (Smith and Bailey, 1989). Behavioural data used in continuous biomonitoring systems for the early detection of contaminants place emphasis on the response time and the ability of the organisms to detect low levels of contaminants (Cairns and Gruber, 1980). Locomotor responses are commonly but not always orientative and lead the animal to approach or avoid the source of the variable in question. Two types of preference/avoidance responses have been investigated: alteration of a known response to a natural abiotic factor (*e.g.*, light, food), and the direct preference/avoidance response to a chemical (Rand, 1984). The techniques used for both types of investigations are similar, and are summarized in Rand (1984). Basic tank designs used by different researchers to study locomotor behaviour include gradients (Kleerekoper, 1969), Y-maze

(Folmar, 1976), double Y-maze (Hansen *et al.*, 1972), counter-current tube (Scherer and Nowak, 1973). Recent advances in video and computer technology have provided methods to obtain large amounts of data for reliable analysis of behaviour (Miller *et al.*, 1982; Smith and Bailey, 1989).

Preference/avoidance responses of salmonids to chlorophenols are poorly documented and studies concerned with specific hydrocarbons have shown varied responses. Folmar (1976) found that rainbow trout tested in a Y-maze avoided 10 μg/L of *p*-xylene, but were attracted to 100 μg/L. In a study by Sprague and Drury (1969), rainbow trout did not avoid phenol at or near lethal concentrations of 10 mg/L. Maynard and Weber (1981) present a method for testing the preference/avoidance response of coho salmon (*O. kisutch*) to petroleum hydrocarbons using the Y-maze described by Folmar (1976). Fish in the holding area are allowed access to either arm of the Y-maze, selecting either control water, or one of several hydrocarbon dilutions. They report that individual hydrocarbons were avoided at concentrations lower than the total concentrations of the mixture (Maynard and Weber, 1981). Additionally, smolts avoided a given hydrocarbon concentration more than did pre-smolts. The avoidance response did not occur at hydrocarbon concentrations at or near the TLm (median tolerance limit), indicating that over 50% of the presmolt coho salmon would not avoid potentially toxic concentrations (Maynard and Weber, 1981).

Smith and Bailey (1989,1990) used a flow-through test chamber for measuring preference/avoidance reactions of chinook salmon, and steelhead trout to industrial effluents, and to a reference toxicant (phenol). Smith and Bailey (1989, 1990) relied on a steep gradient as it provides the best opportunity for the fish to discriminate between opposing bodies of water (Smith and Bailey, 1989). The test system was divided into three chambers: control, test, and avoidance/preference chamber, with a video-based computer system used to measure behavioural responses. The use of a video camera allows for the description of spatial and temporal information from which parameters of motion can be calculated (Miller *et al.*, 1982).

The concerns for an anadromous species entering contaminated migratory corridors are twofold (from Smith and Bailey, 1990). Avoidance of the contaminant may result in a corresponding interference in migratory patterns; attraction to the contaminant could be similarly disruptive to migration. Additionally, preference/avoidance responses may move the fish away from normal homing cues, causing an interruption of downstream or upstream migration. Preference/avoidance tests have been shown to be sensitive at levels as low as 0.01 of the lethal threshold concentration (Sprague, 1968). They are quick, measuring responses over a two-hour period (Folmar, 1976; Maynard and Weber, 1981; Smith and Bailey, 1989; 1990), and are recommended as an important indicator of the behavioural toxicity of chlorophenols to salmonids.

4.3.2 Predator Avoidance

If aquatic organisms are to survive, they must avoid becoming prey for other organisms. Fish have many mechanisms to avoid predation, either primary (hiding and/or camouflage) or secondary defense (aggression, escape) mechanisms (Edmunds, 1974). If changes in prey-fish behaviour results from exposure to physiologically sublethal concentrations of toxicants, it follows that a fish's vulnerability to predation may be changed. Therefore, sublethal exposure to contaminants would be indirectly lethal to fish if predators selectively fed on exposed fish.

Kania and O'Hara (1974) exposed mosquitofish (*Gambusia affinis*) for 24 hours to sublethal levels of mercury. Following pre-exposure, prey fish were placed in a test chamber with a largemouth bass (*Micopterus salmoides*) serving as the predator. Mercury pre-exposed fish were radioactively tagged on the assumption that if exposure to mercury had no effect, 50% of the remaining test fish would show a radioactive tag. At the end of the 60-h test period, all remaining prey fish were sacrificed and it was determined that normal escape behaviour was impaired by mercury stress and that predation rate was greater in the treated group (Kania and O'Hara, 1974).

Sullivan *et al.* (1978) examined the effect of acute and chronic cadmium exposure on the vulnerability of fathead minnows (*Pimephales promelas*) to predation by largemouth bass. Minnows were branded, exposed to Cd for 48 h and 21 d, and then placed in the experimental tanks. When 50% of the minnows had been eaten, the remaining fish were counted with respect to pre-exposed and untreated. Pre-treatment increased the probability that minnows would be eaten by largemouth bass (Sullivan *et al.*, 1978). Fish exposed for 21 d to 0.05 mg Cd/L were significantly more vulnerable to predation than were fish exposed to 0.05 mg Cd/L for only 48 h.

Evidence in the literature for changes in the predator avoidance behaviour of fish as a result of environmental stressors is sparse. Typical predator-prey studies use acute exposure times (Coutant, 1973; Kania and O'Hara, 1974); however, it is also important to monitor long term changes in predator-prey interactions (Sullivan *et al.*, 1978). Predator-prey interactions appear to be sensitive indicators of toxicity, and have the additional advantage of being readily discernible in a short amount of time. However, as the available literature is limited, and methodologies are not standardized, predator avoidance studies have not been recommended in our experimental design.

4.3.3 Feeding Behaviour

Most chronic toxicity tests incorporate growth effects in the LOEC; however, the food density is usually high, and the fish are not challenged to capture prey as they would be under natural conditions. Standard laboratory studies test the effects of toxicants only on physiological aspects of growth (such as effects on metabolism) but not on the ecological aspects (*e.g.*, feeding behaviour) of growth that could also be affected by toxicity. Behavioural toxicity tests oriented towards feeding add an understanding of the effects of contaminants on growth as well as on the feeding patterns themselves.

Hill (1989) analyzed six different aspects of feeding to determine a general response of a toxicant (low environmental pH): response time, strike frequency, average fixation distance, maximum fixation distance, prey capture frequency, and strike success. Response time was the time elapsed between food entry and the first attempted strike. Strike frequency was the number of attempted strikes during each five minute foraging period, and capture frequency was the number of successful strikes during each foraging bout. Fixation distance was defined as the distance at which a fish oriented to a food item. A successful strike was any strike that resulted in ingestion, and strike success was the ratio of successful strikes to attempted strikes. These variables measured three aspects of the physical condition of the fish: activity, visual acuity, and coordination (Hill, 1989). Response times and strike frequency did not differ between treatment groups, but the remaining four variables yielded significant differences. Thus it would appear that low pH does not affect the frequency with which smallmouth bass respond to food, but it did seem to decrease prey capture success (Hill, 1989).

Sandheinrich and Atchison (1989) exposed bluegill (*Lepomis macrochirus*) to sublethal copper levels to determine the effects of copper exposure on prey handling time (response time), reaction distance (fixation distance), and consumption rates (prey capture frequency). Unlike Hill (1989) they found that prey handling time (response time) increased significantly as a result of copper exposure and was the most sensitive foraging parameter tested (Sandheinrich and Atchison, 1989).

The effect of pentachlorophenol on impairment of feeding behaviour has been documented by several researchers (Mathers *et al.*, 1985; Brown *et al.*, 1987). Largemouth bass (*M. salmoides*) were reared for 8 weeks in one of five concentrations of pentachlorophenol (Brown *et al.*, 1987). Over the final 3 weeks of the study, fish reared in the higher pentachlorophenol concentrations performed fewer feeding acts (orientation, bites) and had a lower rate of prey capture than did control fish (Brown *et al.*, 1987). Mathers *et al.* (1985) reported that juvenile largemouth bass exposed to pentachlorophenol were significantly less efficient at prey capture than were unexposed fish. The

foraging parameters outlined by Hill (1989) and Sandheinrich and Atchison (1989) are recommended for evaluation of feeding behaviour, as they are easily quantified, and because they indicate changes which may affect growth and survivorship in the natural environment (Hill, 1989).

4.4 Experimental Design and Statistical Analyses for Toxicity Tests

In toxicology, as in any other scientific field, experiments should be designed to meet the objectives of the study. The best way to ensure that experiments do meet these objectives is to clearly state the objectives as testable hypotheses, then design experiments that specifically and effectively test those hypotheses. A properly designed experiment will include:

- a clear hypothesis
- appropriate controls
- appropriate replication
- appropriate statistical tests
- analysis of data in its original form, rather than analysis of ratios or indices

The time spent planning is invariably effective if these goals are met. Because the experiments proposed in the next section can be expensive to conduct, all of the information provided in acute tests, not just the LC50s, should be used, and sample sizes in chronic tests should be large enough to improve upon the traditionally low sensitivity of these tests (Suter *et al.*, 1987). The importance of appropriate experimental design and statistical analyses to data utility cannot be overemphasized; this section was prepared to provide guidance for design of any research that might be conducted on chlorophenol effects.

4.4.1 Acute Lethality Tests (LC50s)

The requirements for conduct and analysis of acute lethality tests are given in numerous textbooks and government publications, and should be known to any laboratory conducting the tests. Basically, an LC50 is calculated from the relationship between mortality and concentration, and simply requires that mortality be measured over a range of concentrations. Various procedures exist for calculating LC50s exist (Gelber *et al.*, 1985), and will not be reviewed here.

For the purposes of this review, the major concern arises when acute lethality is compared among, for example, compounds, mixtures, or species. An LC50 is a summary statistic, indicating the concentration causing 50% mortality. The estimate of that concentration is more precise than for other percent mortalities. However, an LC50 does not indicate mortalities expected at other

concentrations (except that they should be <50% when the concentration is <LC50, and >50% when the concentration is >LC50). That information is contained in the mortality-concentration relationship used to calculate the LC50, and is therefore available to the experimenter. There are an infinite number of mortality-concentration curves which could cross the 50th percentile for mortality at the same concentration; a comparison of LC50s cannot distinguish between these curves. If the "critical volume" concept discussed in Section 4.2.3 (Bioaccumulation) is valid, then the slopes of mortality-concentration relationships must differ among compounds. The slopes will depend on molecular weight and bioconcentration factor, which will differ among compounds. Nevertheless, comparisons of acute lethality have traditionally involved comparisons of LC50s only.

Kaiser (1989) recommended that comparisons of acute lethality should always involve comparison over the range of tested concentrations, rather than comparisons of LC50s only. Specifically, he stated (p. 188):

> "An investigator who decides that the evaluation of relative toxicity for two or more compounds is better approached through direct comparison of the proportions of organisms responding across a common range of doses has not reached an insupportable conclusion."

Kaiser (1989) goes on to recommend that the experimental design and statistical analysis be targeted to testing clear hypotheses concerning differences or similarities between the compounds or species compared. The response variable in these comparisons would be percent mortality, which can be analyzed in analyses of variance (ANOVA), as discussed below for chronic tests. In some cases, the mortality-concentration relationships can be linearized by appropriate transformation (probit mortality versus log concentration would be an obvious first attempt), and the resulting relationships compared via analyses of covariance (ANCOVAs). Investigators may also use log-linear or logistic regression equivalents of ANOVAs and ANCOVAs.

The above discussion has some relevance for studies of joint toxicity. The methods discussed in our review of joint toxicity studies all involve use and manipulation of the LC50 only, rather than analyses of the entire mortality-concentration relationship. (The manipulations to calculate, for example, toxic units, are subject to the same problems that arise with indices, ratios, and derived variables, as discussed herein.) For example, consider a study of the joint toxicity of two compounds. LC50s are determined for each compound. Then the compounds are combined in a mixture, and the LC50 for that mixture determined by testing a series of dilutions. The toxic units for each dilution are calculated, so that the LC50 for the mixture can be expressed in terms of toxic units. If the confidence limits for the mixture LC50 include 1 toxic unit, then the toxicity of the two compounds

is additive. If the confidence limits for the LC50 do not include a toxic unit value of 1, then the compounds have synergistic (LC50>1) or antagonistic (LC50<1) effects.

The approach in this example represents a tremendous waste of available information. Any time an LC50 is calculated, a relationship between mortality and concentration must be determined. Thus, the expected mortality (or better, probability of survival), and its confidence limits, at any concentration over the range tested, can be predicted by least-squares, probit, or logistic regression. The predicted mortality, assuming additivity, for a mixture of two compounds can be obtained from the probability of survival, which is simply the product of the survival probabilities for the concentration of each compound. Confidence limits for the product of two variables, assuming independence (=additivity), can easily be calculated (Welsh *et al.*, 1988), and can be compared with the observed value and its confidence limits for any combination. Note that this enables the investigator to predict mortalities for a range of mixtures; it is not possible to do so using toxic units. (What is the predicted mortality at 0.5 toxic units of any compound? No one knows except that it must lie between 0 and 50%.) Actually, in the example given, it would be useful to regress the observed on the predicted values to determine if there is any deviation from additivity (slope=1; intercept=0). This is only one of the many approaches that would use more information than the traditional use of LC50s only. The key point is that probabilities are estimated for various mixtures and compared with observed values; any experimental design and statistical analysis that explicitly tests the hypothesis that predicted=observed would suffice. The suggested approach is hardly new; it was originally suggested by Finney (1942) and follows the same logic used in life table analyses and risk assessment.

4.4.2 Chronic Tests

4.4.2.1 Experimental Design and Appropriate Controls

Chronic tests are usually set up as factorial experiments, in which the response is measured for a range of concentrations. The concentrations are levels of the single factor in a 1-way ANOVA. Occasionally a second factor (*e.g.*, species, if one wanted to compare two species) is considered, which provides additional information but may complicate the analyses. Responses at each concentration are compared to the control, using either Dunnett's or Williams' Test, to determine the LOEC (lowest observed effect concentration) and NOEC (no observed effect concentration).

The first step is to include an appropriate control. In a simple experiment where a range of concentrations is compared, the control is obviously the dilutant (lab or prepared water), representing a concentration of 0. The appropriate control is often less obvious in behavioural experiments. For

example, in a predation experiment, 25 marked contaminated fish might be introduced along with 25 unmarked uncontaminated fish into a tank containing a predator. At the end of the experiment, the surviving fish are removed, and the ratio of marked:unmarked fish calculated. If this ratio differs from the initial 1:1, the investigator concludes that the contaminant affects susceptibility to predation. This conclusion is invalid. Aside from the fact that the experiment contains no replication (tanks, not individual fish, are the experimental units or replicates), the control is inadequate. Perhaps the marking procedure renders the fish more susceptible to predation. A reciprocal control, in which the controls are marked and the contaminated fish unmarked, should be added. As a general rule, exposure or some other treatment of interest should not be confounded with some other factor, such as marking. Controls must be treated exactly as are experimental animals.

4.4.2.2 Replication and Pseudoreplication

The above example leads into a discussion of replication. In the example the experimenter has committed the sin known as "pseudoreplication" (Hurlbert 1984). Even after the publication and a wide discussion of Hurlbert's paper, examples of pseudoreplication can still be found in reputable journals. The common mistake is to consider that each individual fish in a tank or container is a replicate. This ignores the fact that the response may vary among tanks even when the tanks are treated the same. The appropriate unit of replication is always the highest level to which the treatment is applied. If all tanks exposed to the same concentration in an experiment were placed in the same room, and each concentration was tested in a different room, more than one room per concentration would be required to provide replication. Of course, it would be much better to assign one tank of each concentration to each room, and treat the rooms as blocks in a randomized complete block design.

What is the optimal number of replicates, and fish per replicate? The sensitivity of any comparison among, for example, concentrations, is inversely proportional to the variance of the concentration means (V($\bar{X}$)). This variance can be calculated from the variance due solely to effects of the a tanks or containers (V_T) and the variance among the n fish per tank (V_F) (Snedecor and Cochran, 1980, p. 240):

$$V(\bar{X}) = (V_T/a) + (V_F/na).$$

It should be emphasized that V_T is not the same as the variance among replicate tanks ($V(X)$) discussed below. The latter term always contains a component due to variance among fish; V_T is actually a theoretical value which can only be estimated from actual values of $V(X)$. From this formula it is obvious that, given a fixed total number of fish, the variance of concentration means will be smallest

when one fish is assigned to each tank. However, that is usually an impractical alternative because the number of tanks is constrained by space or the diluter used. Subject to these constraints, the number of tanks, rather than the number of fish per tank, should be maximized.

Formulae for calculating the required sample size for statistical tests depend on the power (probability of detecting a difference if it exists), the magnitude of the difference to be detected (*e.g.*, 10% reduction in growth relative to controls), the desired significance level (usually $P = 0.05$), and the variance among replicates (which is $\{V_F/n\} + V_T = V(X)$). The appropriate formulae are given in Snedecor and Cochran (1980), Sokal and Rohlf (1981), and Zar (1984). Below, we give the appropriate formula to use if Dunnett's Test is to be used, since that is the most common test used in chronic tests.

In most cases, an estimate of $V(X)$ is not available, unless a previous experiment has been conducted using the same number of fish per tank, [it can be calculated from experiments with different numbers of fish per tank using formulae given in Snedecor and Cochran (1980, p.239-241)]. However, estimates of V_F, or the variance among fish, can often be obtained from the literature, previous experiments, or, in the case of growth studies, from the hatchery from which the fish were obtained. The investigator can then calculate sample sizes, assuming that V_T is no more than 50% of V_F. (If it is any larger, the cause of these tank effects should be investigated, and the procedures altered to reduce that variance.) The investigator can also consider the sample sizes for various estimates of the $V(X)$ as a percentage of the mean. The objective of this exercise is to obtain a ballpark estimate of the required sample size, or an indication of the sensitivity given a suggested sample size. Quite often, it immediately becomes clear that a sensitive experiment is simply not feasible, or that one response variable is clearly more sensitive than another for the same cost.

4.4.2.3 Comparison of Treatments with Controls

Dunnett's Test (Steel and Torrie, 1980, p. 188-190) is traditionally used to compare the means for each concentration with control means. Dunnett's Test was designed to compare various drugs with a control (usually a placebo or the drug currently in use) so that there is no *a priori* reason to expect the responses to be ordered in any specific manner. An analogous situation in toxicology would occur when a number of field samples of water or sediment are compared. However, when a series of concentrations is compared, it is reasonable to expect the responses to increase or decrease with concentration. Under these circumstances, Williams' Test (Williams, 1971, 1972) is more appropriate and more sensitive (Gelber *et al.*, 1985). The difference in sensitivity is not large when only a few concentrations are tested, but becomes more important as the number of means compared increases.

These differences have little effect on the calculation of sample sizes given below, because the calculated a usually has to be rounded to an integer value.

The appropriate formula for calculating the required sample size to have a probability P (=statistical power) of detecting a difference d between the control and a treatment mean at the 0.05 significance level is:

$$a = \{V(X)/d^2\}\ \{t^2_{0.05,df}F_{1-P,df,\nu}\}$$ (Steel and Torrie, 1980).

As indicated in the section on replication, $V(X)$ is the variance among replicates, which can be obtained directly from previous experiments or estimated. $t_{0.05,df}$ is the critical value of Dunnett's t for the number of means compared, and $F_{1-P,df,\nu}$ is the critical value of F for a 1-tailed probability of 1-P. A crude estimate for Williams' Test could be obtained by substituting the critical value for Williams' t for Dunnett's t. The formula must be solved iteratively, starting with an initial guess at the value of a, as the degrees of freedom (df, or the number of means times a - 1) will alter the values of F and t. The degrees of freedom ν for the estimate of the variance will not be known unless the estimate was obtained from a previous experiment. The value of F does not change much with ν if $\nu>10$, so the investigator may wish to use $\nu=10$ for a conservative estimate.

In practice, one often assumes a fixed number of tanks (a) and rearranges the formula to calculate $V(X)$, assuming $\nu=10$ (conservative) or infinity (liberal). From this estimate of $V(X)$, one can then calculate the number of fish per tank, assuming that the variance among fish (V_F)is known and that the variance solely due to tank effects (V_T) does not exceed 50% (or some other percentage) of the variance among fish, [the theoretical minimum value of $V(X)$, which is V_F/n, or the variance among means of samples of n fish, could also be used to estimate an absolute minimum value for a]. A final alternative is to assume that the number of tanks, and number of fish, must be some specific values, and solve for F (i.e, decide on the level of replication and then see if it will actually be sensitive enough). This will provide the probability of detecting a difference d if one really exists.

A very effective method of improving the sensitivity of Dunnett's or Williams' Test is to increase the number of control replicates (Williams, 1972). In general, if there are k means, including controls, the ratio of control replicates to other treatment replicates should be $k^{1/2}$ (*i.e.*, $a_c=a_i\ k^{1/2}$). Unequal replication will, however, create problems for 2-factor experiments.

These calculations may seem tedious, but are certainly worth performing when one is considering expensive tests such as a long-term growth study or a bioaccumulation study. We predict that the cost of the preliminary calculations would rarely exceed 1% of the cost of the experiment, but that

much more than 1% of experiments would be rejected as not sensitive enough. The calculations may even show that there are too many replicates for the desired sensitivity, and the cost of the experiment can then be reduced accordingly.

4.4.2.4 Ratios and Derived Indices

Derived indices are combinations of variables; ratios are just one example. Ratios (for example, condition factor in growth experiments) are often analyzed as response variables in chronic studies. This is not an appropriate procedure; ANCOVA is the correct analytical procedure (Atchley *et al.*, 1976). In the case of condition factor, this means comparing regressions of weight on length (usually with both log-transformed). When ratios are used, assumptions are made about the nature of the relationship between the numerator and denominator which may not be true. A further problem arises when ratios are regressed against either the numerator or denominator (for example, body burden in mg/kg versus body weight); spurious correlations can be produced. Other derived indices suffer from similar problems - unwarranted or unnecessary assumptions are made whenever they are used, and their statistical behaviour is unpredictable. Ratios and indices are, of course, very useful in presenting results; it is their statistical properties that cause problems.

5.0 RECOMMENDATIONS

The primary objective of this study is to determine the effects of tri-, tetra-, and pentachlorophenol, singly and in combination, on estuarine organisms. The study is designed to produce information that can be used by the Scientific Authorities to meet their goal of establishing water quality criteria for chlorophenols in the Fraser River Estuary.

The species recommended for this study is the chinook salmon (*Oncorhynchus tshawytscha*). Juvenile chinook have the longest spring residency period of the salmon species present in the Fraser River Estuary. These fish should be obtained from a hatchery, and would thus be domesticated to some degree. However, chinook salmon eggs are obtained from wild brood stock rather than domesticated adults. Therefore, the chinook salmon used will be more similar to their wild counterparts then would hatchery raised rainbow trout (*O. mykiss*). The Scientific Authorities should be aware that conducting tests with chinook salmon is more expensive than conducting comparable tests using rainbow trout, and that availability can be a limiting factor. Chronic tests are also more expensive than acute lethality tests, because they must run for a longer period of time and usually require more extensive testing apparatus and monitoring. Selection of testing methods and species has been made

for scientific not economic reasons. However, a concluding section provides some suggestions for reducing costs.

This study should focus on tri-, tetra-, and pentachlorophenol. The most prevalent isomers in the Fraser River Estuary, 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol, and pentachlorophenol, should be tested individually and in mixtures.

The study should be conducted in three phases. Phase 1 should consist of an initial assessment of acute lethal effects using continuous flow tests (96 h LC50s). Following the acute lethality tests, Phase 2 would utilize chronic tests to assess sublethal effects (growth, preference/avoidance, bioaccumulation, etc.). Phase 3 would assess joint toxicity of the three chlorophenol compounds using flow-through acute lethality tests (96 h LC50s).

5.1 Phase 1 : Acute Lethality Tests

Compounds tested: 2,4,6-trichlorophenol
2,3,4,6-tetrachlorophenol
pentachlorophenol

Concentrations tested: logarithmic series determined from literature values or rangefinder test

Response variables: % mortality

Statistical analysis: calculation of LC50 by standard methods
estimation of ILL graphically

Three acute lethality tests, providing 96 h LC50s and 7 d incipient lethal levels (ILL), should be conducted on chinook salmon juveniles acclimated to fresh water.

In each test, six concentrations of the test compound, including 0 μg/L (=controls), should be used. Test concentrations should be determined from literature values (which are presented in this report) or by preliminary rangefinder tests. Each test should be replicated at least once. Replication and sample size may be dictated by time and size constraints. The use of continuous flow testing will require the construction of an appropriate dosing apparatus. A toxicant drip or proportional diluter would be the most appropriate for these tests. Other procedures should follow guidelines established by the APHA (1989) for rainbow trout acute lethality tests, with the following modifications:

- Replicated (2) water samples should be taken from each tank every 24 h for chemical analysis of chlorophenols levels as a check on the dosing apparatus and volatilization of compounds during testing.

- The fish should be acclimated for a 7 d period prior to the test.

- The test should be in a continuous flow system, with 95% replacement every 4 h (calculated following Sprague, 1973; Figure 1). This will decrease any volatilization of compounds.

- The fish should be fed throughout the test.

- Daily records of mortalities should be made for 7 d and used to compute the ILL for each compound.

- The pH should be adjusted to reported levels for the Fraser River estuary.

- Temperature should be maintained at 15° ± 1°C.

- Tissue concentrations should be determined for each test concentration at the end of the test or immediately following mortality.

LC50 values for each 24 h time period over the 7 day exposure can be calculated using the collected data. These values can be used to plot a toxicity curve using logarithmic scales for exposure time and concentration. The concentration value where the curve becomes asymptotic to the time axis is called the lethal threshold concentration or incipient lethal level. This is the concentration at which 50% of the population will survive for an indefinite time (Rand and Petrocelli, 1984). This value is especially useful in determining test concentrations for chronic studies.

The data from these acute lethality tests can also be used to test the "critical volume" hypothesis relating tissue levels to toxic effects (see Section 4.2.3). If the tissue levels are expressed as μmol per unit body weight, the body burden associated with the LC50 should be the same for all compounds. More generally, the relationship between mortality and body burden should be the same. The similarity of these relationships, if it exists, has important implications for joint toxicity (Section 5.3). Again, we caution that pentachlorophenol may not follow the expected pattern.

5.2 Phase 2 : Chronic Sublethal and Behavioural Tests

Chronic toxicity tests indicate the level of toxicant that affects parameters such as growth, reproduction, osmoregulation, and development. Chronic levels of toxicants are more common in the environment than are acute lethal levels (Kleerekoper, 1976). Chronic tests, therefore, are useful in providing more realistic and sensitive measures of toxicant effects.

5.2.1 Growth/Feeding Behaviour

Compounds tested: 2,4,6-trichlorophenol
2,3,4,6-tetrachlorophenol
pentachlorophenol

Concentrations tested: 0, 5, 15, 45, 135% of ILL

Response variables: mortality
weight and length on Day 14, 28
weight-length relationships (condition factors) on Day 14, 28
feeding attempts/5 min, total feeding time

Statistical analysis: ANOVA or ANCOVA, with Dunnett's or Williams' Test to determine MATC

The effects of chlorophenols on growth of fish are well documented in the literature (Webb and Brett, 1973; Ward and Parrish, 1980; Hodson and Blunt, 1981; Holcombe *et al.*, 1982; Mathers *et al.*, 1985; Brown *et al.*, 1987). Reduced growth can lead to reduced fecundity, slower maturation, and less competitive individuals (Munkittrick and Dixon, 1989). In his review, Sprague (1971) re-emphasized that growth should be routinely measured as a good stress indicator. Growth measurements require long term tests to reflect net growth efficiency. A reduction in efficiency indicates that a greater proportion of the energy absorbed from the food is being used to maintain the animal and metabolize toxicants leaving a smaller amount available for growth.

A feeding behaviour study utilizing juvenile chinook salmon can be used in conjunction with the growth data to determine if response to the toxicant is due to a behavioural or metabolic effect. The proposed 28 day test will determine the lowest concentrations of tri-, tetra-, and pentachlorophenol that affect the growth of chinook salmon juveniles. Testing should be done in freshwater continuous flow tanks to reduce volatilization of toxicants. Glass aquaria should be used to minimize adsorption of the compounds to tank walls. Flow should be maintained to allow for 95% replacement of the test

water every 4 h. Appropriate dosing apparatus must be used to insure proper toxicant concentrations. A proportional dilution system or serial diluter with adequate safeguards should be used. Concentrations will be based on the ILL (=incipient lethal level) for each of the three compounds from Phase 1 above. Five concentrations, including 0% (=control), 5%, 15%, 45%, and 135% of the ILL should be tested. A minimum of three replicates should be used at each concentration. Tanks must be large enough to allow for a minimum of 10 fish for the duration of the study with a loading density of approximately 1.0 g/L. A minimum tank volume of 20 L is recommended. Fish should be fed a suitable diet of commercial fish feed and daily mortalities should be recorded. Daily food rations should be 12% of the total fish biomass in each tank. This amount should then be divided into two equal portions to be distributed at morning and evening each day. One fish chosen at random in each tank should be observed for 5 minutes immediately following introduction of food and the number of feeding attempts recorded by an observed in a blind. Total feeding time for all fish in each tank should be recorded for each feeding episode. A 14 h light: 10 h dark regime should be used. Prior to testing the fish should be held in a continuous flow tank and feed *ad libitum.*

On Day 0 the fish should be removed from the holding tank and anaesthetized using 2-phenoxyethanol. At this time, lengths and weights of individual fish should be recorded. Following rapid measurement the fish should be placed in clean water and allowed to recover before introduction into the test tanks. Any fish failing to recover should be discarded and replaced. Fish should be randomly assigned to test tanks. On Day 14 and Day 28 fish should be removed from the test tanks and lengths and weights measured as for Day 0, with the exception that fish failing to recover from the anaesthetic should be discarded and recorded as handling mortalities. Water quality measurements should be made twice daily. Every 24 h a sample of test water should be removed from each tank and analyzed for chlorophenol concentration. This should provide a check on the dilution system. At the end of the experiment, condition factors and specific growth rates (Winberg, 1971) should be calculated for each test tank.

Weights on Days 14 and 28 (or specific growth rates, which would be roughly equivalent to a log-transformation) should be analyzed in a 1-way ANOVA, with concentrations as treatments and mean weights for each tank as individual data. Weight-length regressions can be analyzed in ANCOVAs, but a complex nested model with three levels - concentrations, tanks within concentrations, and fish within tanks - would be required (see Sokal and Rohlf, 1981, for procedures). An alternative is to calculate the weight-length relationship for all fish, and calculate residuals from that relationship. The means of the residuals for each tank would then be the individual data in a 1-way ANOVA with concentrations as treatments. This procedure assumes that slopes of the weight-length relationships are homogeneous over all tanks, something that can be tested beforehand in an ANCOVA. A final alternative would be a 1-way ANCOVA with mean weights for the tanks as the dependent variable,

and mean lengths as the covariate. Mean condition factors can be calculated for each tank or concentration for the purposes of reporting, because they will be more easily understood than the parameters of regressions, but should not be analyzed because they are ratios.

Dunnett's or Williams' Test should then be used to compare concentration means (or in the case of ANCOVA, adjusted means or intercepts) with the control. We have suggested a minimum of three tanks per concentration, but investigators may wish to use the formulae provided to determine if this sample size provides sufficient sensitivity. Increased replication for controls could also be considered, but will create problems in the analysis of anything other than 1-way designs.

Analysis of behavioural data is more complicated, because the data represent a time series. If no serial correlation (correlation of successive errors in a time series) exists, then regressions of feeding attempts per unit time or total feeding time versus time since Day 0 for each tank could be compared in a 3-level nested ANCOVA. An alternative is to calculate the slopes of these regressions for each tank, and use the slopes as individual data in a 1-way ANOVA comparing concentrations. This would remove the problem of serial correlation. Emphasis should be on the slopes, even in ANCOVA, because the number of feeding attempts or total feeding time should be the same in all tanks at Day 0, but decline with time for exposed fish but not for controls. Other alternatives exist, such as calculating weekly averages for the four weeks, and using these as four response variables. The truly ambitious will use repeated measures analysis of variance, with appropriate contrasts for the time series; a statistician should be consulted prior to undertaking this type of analysis.

5.2.2 Bioaccumulation

Compounds tested: 2,4,6-trichlorophenol
2,3,4,6-tetrachlorophenol
pentachlorophenol

Concentrations tested: 0, 5, and 45% of ILL

Response variables: chlorophenol levels in fish tissue on Day 2, 5, 10, 20 of uptake period
bioconcentration factors (BCF) on Day 2, 5, 10, 20 of uptake period
chlorophenol levels in fish tissue on Day 5, 10 of depuration period
bioconcentration factors (BCF) on Day 5, 10 of depuration period

Statistical analysis: uptake phase: 2-way ANOVA with contrasts
depuration phase: 1-way ANOVA with Dunnett's or Williams' Test

Bioaccumulation tests have been used by a number of researchers to investigate the exposure of chlorinated compounds on fish but again, it is important to recognize that bioaccumulation data provide no information on effect. A 20 day bioaccumulation test for tri-, tetra-, and pentachlorophenol followed by a 10 day depuration period should be conducted using chinook salmon juveniles. Testing should be done in freshwater continuous flow tanks to reduce any volatilization of toxicants. Glass aquaria should be used to minimize test tank adsorption of the compounds. Flow should be maintained so as to allow for 95% replacement of the test water every 4 h. Appropriate dosing apparatus must be used to insure proper toxicant concentrations. A proportional dilution system or serial diluter with adequate safeguards should be used. Concentrations should be based on the ILL (=incipient lethal level) for each of the three compounds from Phase 1 above. Three concentrations, including 0% (=control), 5%, and 45% of the ILL, should be tested. A minimum of two replicates should be used at each concentration. Tanks should be large enough to allow for a minimum of 50 fish for the duration of the study with a loading density of approximately 1.0 g/L. A minimum tank volume of 120 L is recommended. Fish should be fed a suitable diet of commercial fish feed and daily mortalities should be recorded. Daily food rations should be 12% of the total fish biomass in each tank. This amount should then be divided into two equal portions to be distributed at morning and evening each day. A 14 h light: 10 h dark regime should be used. Prior to testing the fish should be held in a continuous flow tank and fed *ad libitum*.

On Days 2, 5, 10, and 20, five fish should be removed from each test tank and sacrificed. The whole bodies of the five fish should then be analyzed for chlorophenols as a pooled sample. (If possible, chlorophenol levels should be analyzed for different tissue types - liver, muscle, viscera. Obtaining sufficient tissue mass to allow for these analyses may require greater numbers or size of fish and increase testing costs dramatically). After removal of the fish on Day 20, the remaining fish should be placed in continuous flow tanks with control water. Following a 5 and 10 day depuration period, these fish should then be sacrificed and whole body tissue analyses performed for chlorophenol compounds. Using the data from the tissues analyses a BCF (=Bioconcentration factor) should be calculated for each compound on each sample day. The BCF is expressed as:

$$\frac{\text{concentration in organism}}{\text{concentration in water}}$$

By analyzing the tissue levels over time either a steady-state equilibrium value and/or an uptake rate should be determined. Factors affecting uptake must be taken into consideration. These include pH, salinity, and hardness. Water quality measurements should be made twice daily. Every 24 h a sample of test water should be removed from each tank and analyzed for chlorophenol concentration in order to provide a check on the dilution system.

In the analysis of this experiment, it is important to address the following hypotheses:

- Do tissue levels reach an equilibrium or asymptote in 20 d, which is a reasonable approximation of residency in the estuary?

- Can the compounds be eliminated within a 5 or 10 d period?

Ideally, the tissue levels at 2, 5, 20, and 20 d should be analyzed in a 2-way ANOVA, with time as one factor and concentration as the other. The controls should not be included in the analysis because they will lead to complicated interactions (*i.e.*, a time effect for exposed fish, but not controls). The controls are necessary to indicate background levels. If the tissue levels are log-transformed, there will be no concentration-time interaction if bioconcentration factors are the same for both concentrations. In the absence of interaction, the difference between concentration means should be constant and equal to log 9 (one concentration is 9 times the other), a hypothesis which can be tested explicitly. If an interaction exists, then uptake dynamics differ between concentrations and each concentration should be considered separately in subsequent analyses.

To test for the presence of an asymptote, the time effect can be decomposed using *a priori* contrasts (see Sokal and Rohlf, 1981, pp. 232-242). The best procedure is a step-down procedure, first comparing Day 2 means with the grand mean for the remaining three days, then Day 5 means with the grand mean for the remaining two days, then the means for the final two days. If any of these comparisons are not significant, it means that tissue levels have not changed over the times compared and that an asymptote has been reached. Of course, if the overall time effect is not significant, then there is no need for further analyses - either there was no uptake, or an asymptote was reached in 2 d. The coefficients for the required contrasts are given below:

2 d	5 d	10 d	20 d
-3	1	1	1
0	-2	1	1
0	0	-1	1

These are independent contrasts and can be tested with *F*-tests; the sums of squares for the contrasts will add to the total sums of squares for the time effect.

The analysis of depuration times is straightforward. Tissue levels at 5 and 10 d could be analyzed separately as response variables in 1-way ANOVA comparing concentrations, including controls.

Levels at each concentration can the be compared with controls using Dunnett's or Williams' Test. The absence of a difference would indicate that depuration was complete.

As with the growth experiment, serial correlation in time series may create problems in analyses of uptake. Again, repeated measures ANOVA would be suitable for the analysis of both uptake and depuration data. The contrasts provided above can also be used in a repeated measures design.

Investigators may wish to use more replicates than the two suggested, based on calculations of sensitivity. However, the chemical analyses involved are expensive, and would increase with increased replication. Using contrasts, and analyzing both concentrations simultaneously, substantially increases statistical power relative to multiple comparison procedures such as Dunnett's Test.

5.2.3 Seawater Challenge

Compounds tested: 2,4,6-trichlorophenol
2,3,4,6-tetrachlorophenol
pentachlorophenol

Concentrations tested: 0, 5, 15, 45, and 135% of ILL

Response variables: mortality
blood sodium levels following 24 h seawater exposure

Statistical analysis: 1-way ANOVA with Dunnett's or Williams' Test
ANCOVA (or alternatives) for weight-length relationships

The life-stage at which juvenile salmonids enter seawater is considered to be sensitive due to physiological stresses imposed by the transition from a freshwater to a marine environment (Clarke and Blackburn, 1977). Relatively high natural mortality is often associated with this stage of salmonid development (Clarke, 1982). Factors that increase physiological stress (*e.g.*, toxicants, environmental extremes) may increase mortality during seawater adaptation. This is related to decreased osmoregulatory abilities, in particular salt excretion capacity to offset the influx of salts upon introduction to seawater (Wedemeyer *et al.*, 1980). The efficiency of salt regulation can be measured using blood sodium levels of fish exposed to seawater for a 24 h period. Poor performance in the seawater challenge test (*i.e.*, sodium plasma levels greater than 170 meq/L) has been equated

with decreased growth rate (Clarke and Shelbourn, 1982) and possibly increased likelihood of predation (Houston, 1961).

Chinook salmon smolts (4-5 g) should be exposed for 7 days to five concentrations each of tri-, tetra-, and pentachlorophenol. Testing should be conducted using methods similar to those of Clarke and Blackburn (1978). The levels of exposure should be 0 (=control), 5%, 15%, 45%, and 135% of the ILL. Ten fish should be held in freshwater and exposed at each concentration in continuous flow tanks. A minimum of two replicates should be used. Tank volume should be sufficient to maintain loading density at or below 1.0 g/L. Flow rates should allow for 95% replacement every 4 h. Temperature should be maintained at 12 ± 1°C. A 14 h light:10 h dark light regime is suggested. Fish should be fed throughout the test. After the 7 day exposure period, the fish should be placed in a tank of running seawater at 12 ± 1°C. After 24 hours, the fish should be anaesthetized, the tail severed and a blood sample collected for sodium determination. Sodium content of the samples is commonly analyzed using atomic emission spectrophotometry. Length, weight, and mortality should also be recorded. Condition factors should be computed using length and weight data. Water quality and mortality measurements and water samples to be tested for chlorophenol content should be taken daily.

All response variables, except weight-length relationships, can be analyzed in a 1-way ANOVA comparing concentrations. Weight-length relationships can be analyzed by any of the alternatives suggested for the growth study. Dunnett's or Williams' Test can be used to establish the MATC.

5.2.4 Preference/Avoidance

Compounds tested: 2,4,6-trichlorophenol
2,3,4,6-tetrachlorophenol
pentachlorophenol

Concentrations tested: 5, 15, 45, 135% of ILL introduced in right or left arm

Response variables: % of fish in contaminated arm, or
% of fish in uncontaminated arm

Statistical analysis: 1 or 2-way ANOVA, with Dunnett's or Williams' Test to determine MATC

Preference/avoidance tests are designed to determine the response of an organism to toxicants based primarily on changes in location (Smith and Bailey, 1989). Avoidance/preference responses could

be especially important when considering the possibility and consequences of interruption of juvenile salmonid estuarine migrations. These tests are sensitive to concentrations as low as 1.0% of the ILL (Sprague, 1968) and have rapid response times (Folmar, 1976; Maynard and Weber, 1981; Smith and Bailey, 1989, 1990). Though several methods are available for assessing preference/avoidance in fishes, the Y-maze design has been chosen due to its ease of use and simple construction (Maynard and Weber, 1981).

Juvenile chinook salmon (4-5 g) should be held in freshwater continuous flow aquaria prior to testing and fed *ad libitum*. An opaque all-glass Y-maze with a blind for the observer should be used for avoidance testing. Four concentrations (5%, 15%, 45%, 135 of the ILL) of tri-, tetra-, and pentachlorophenol should be used. A minimum of four tests should be conducted for each concentration, with an equal number utilizing each arm for introduction of the toxicant. At initiation of each test, 10-30 previously untested fish should be placed in the holding area at the base of the Y-maze. The fish should be confined by a gate to the area below the arms of the Y. Equal water flow (10-20 L/min) should be maintained in both arms of the maze. The fish should be confined to the holding area for 15 minutes before toxicant is introduced into one arm. The gate should then be raised allowing fish equal access to each arm. After 60 minutes, the gate should be lowered and the number of fish in each arm of the maze recorded. The water distribution system should be installed so as to allow for toxicant to be introduced into either arm. A minimum of four control tests should be conducted without toxicant in either arm of the Y-maze to test for inherent right and left arm tendencies in the test fish.

The analysis of the avoidance response will be complicated by any bias in controls for a particular arm in the maze. Therefore, every effort should be made to eliminate such a bias if it exists. The basic design is a 2-way ANOVA, with concentration as one factor and arm of contaminant introduction as the other. The controls should be arbitrarily split equally into left and right arm contaminant introduction, as the solution is actually the same in both arms. The data are the percent of fish in the contaminated (or uncontaminated) arm. If the concentration-arm of introduction interaction is significant, then the data will have to be analyzed separately for each arm of introduction. This would probably occur if there was a bias in controls towards one arm. Ideally, both the interaction and arm effect would be nonsignificant, and the analysis could be collapsed into a 1-way ANOVA comparing concentrations, with MATCs calculated using Dunnett's or Williams' Test.

These data could also be analyzed using logistic, probit, or binomial equivalents of an ANOVA. The results for all replicates of a concentration should never be pooled for a chi-square analysis. The expected variance among replicates, assuming no additional variance due to test repeats, can easily

be calculated from the binomial distribution (Snedecor and Cochran, 1980, Chapter 7; see pp. 438-441 for an example). This variance, which is greatest when the response is 50% of the fish in one arm, can be used to calculate minimum sample sizes.

5.3 Phase 3 : Joint Toxicity

Compounds tested: 2,4,6-trichlorophenol
2,3,4,6-tetrachlorophenol
pentachlorophenol

Concentrations tested:

No. of compounds	2,4,6-TCP	2,3,4,6-TTCP	PCP
1	V	-	-
2	V	F	-
2	V	-	F
3	V	F	F
1	-	V	-
2	F	V	-
2	-	V	F
3	F	V	F
1	-	-	V
2	F	-	V
2	-	F	V
3	F	F	V

V=variable concentrations, 0, 5, 15, 45, 135% of ILL.
F=fixed concentration, highest concentration with ≤10% mortality.

Response variables: % mortality

Statistical analysis: calculation of LC50 by standard methods
comparison of joint toxicity by ANOVA

Joint toxicity of chlorophenols mixtures has only recently been investigated (Anon., 1987). Preliminary results suggest that mixtures of tri-, tetra-, and pentachlorophenol have an additive toxic effect on flagfish (*Jordanella floridae*). Testing of mixtures of chlorophenols on salmonids was not

present in the literature. The presence of several chlorophenols concurrently in the Fraser River estuary necessitates the estimation of the effects of chlorophenol mixtures on salmonids.

Twelve acute lethality tests, providing 96 h LC50s for each of the compounds and two and three compound mixtures, should be conducted on chinook salmon juveniles acclimated to fresh water. In each single compound test, five concentrations of the test compound, including 0% (=controls), 5%, 15%, 45%, 135% of the ILL (as determined in Phase 1), should be used. When testing two compound mixtures, one compound should be held at a fixed level (highest concentration with mortality ≤10%, as determined from tests in Phase 1) and five concentrations of the second compound, including 0% (=control), 5%, 15%, 45%, and 135% of the ILL, introduced. Three compound mixtures should be tested similarly, using two of the three compounds at a fixed level (mortality ≤10%) and five concentrations (as above) of the third. The single compound tests should be run simultaneously as reference tests for the multiple compound tests. Each test should be replicated at least once. Replication and sample size may be dictated by time and size constraints. The use of continuous flow testing will require the construction of an appropriate dosing apparatus. A serial or proportional diluter would be the most appropriate for these tests. Other procedures should follow guidelines established by the APHA (1989) for rainbow trout acute lethality tests, with the following modifications:

- Replicated (2) water samples should be taken from each tank every 24 h for chemical analysis of chlorophenols levels as a check on the dosing apparatus and volatilization of compounds during testing.

- The pH should be adjusted to reported levels for the Fraser River estuary.

- The fish should be acclimated for a 7 d period prior to the test.

- The test should be in a continuous flow system, with 95% replacement every 4 h. This will decrease any volatilization of compounds.

- Temperature should be maintained at 15° ± 1°C.

The study consists of three separate experiments. In each experiment, the concentration of one compound is varied as indicated, with four different solutions (control, two with concentrations of one other compound fixed, one with concentrations of both other compounds fixed) used as dilutant water. Mortality is then analyzed in a 2-way ANOVA (or its logistic or log-linear equivalent) with concentration of the variable compound as one factor and the dilutant as the other. If the dilutant

effect is significant, then the low and supposedly nonlethal levels of the fixed compounds are affecting the mortality due to the varied compound. We suspect that the interaction between the two factors will probably be significant, with the contaminated dilutants having no additional effects except at higher concentrations of the varied compound. In part, this is a detection problem, since it may be difficult to detect small increases in mortality with a limited number of fish.

Investigators should compare the results with those predicted by multiplying survival probabilities. If survival can be log-transformed, and still satisfy the assumptions of homogeneity of variance and normality of residuals necessary for ANOVA, then the analysis is considerably simplified. Any interaction would indicate non-additivity of the logarithms of survival probability, which is the same as saying that the observed survival is not equal to the predicted survival based on multiplying the survival probability for the dilutant by the survival probability for the concentration of the varied compound (a probit transformation would be more appropriate if tolerances are binomially distributed). This is essentially how logistic regression or log-linear models work, except that we are not aware of a method of dealing with a random and nested factor such as tanks in such analyses.

Investigators can also compare the results with those predicted by the "critical volume" hypothesis (Section 4.2.3). As indicated in Section 5.1 (Acute Lethality Tests), the relationship between body burden, expressed as number of molecules per unit body weight, and mortality should be identical for all compounds. The body burden expected for any of the tested compounds can be calculated by multiplying the concentration in water by the BCF obtained in the bioaccumulation experiments (Section 5.2.2; the 5-d BCF should be used if equilibrium is not reached before then). These body burdens can be converted to molecules per unit body weight, then summed for all compounds present. The expected mortality can then be predicted from the body burden-mortality relationship, assuming that relationship is the same for all compounds.

5.4 *In situ* Testing

Compounds tested: chlorophenol compounds at ambient concentrations in the Fraser River estuary

Concentrations tested: as above

Response variables: % mortality
length, weight, and length-weight relationship
bioconcentration factor (BCF) and tissue levels

Statistical analysis: 1-way ANOVA comparing sites

Wild juvenile chinook salmon should be suspended in cages at selected sites in the Fraser River estuary. A reconnaissance of the study area should be conducted to establish sites for the *in situ* fish exposure tests. Criteria for selection of the sites should include:

- downstream of an active chlorophenol source
- natural populations of salmonids

The location of chlorophenol sources should be determined by conferring with the Scientific Authorities and from past Fraser River estuary sampling studies. A minimum of three monitoring stations should be subsequently established. At least one site should be relatively contaminant-free, specifically for chlorophenols. Cages should be of appropriate size to allow adequate movement of the fish. Ten fish per cage with a minimum of three cages per site is recommended. Cage placement should coincide with the naturally occurring residence period of the wild populations of juvenile salmonids.

The cages used for *in situ* bioassays should be designed for long term holding of fish at a variety of river velocities. The cages should be fixed in position at the exposure sites using floats and anchors and suspended immediately below the surface. Security is a problem in the River; sites should be selected to reduce the probability of being tampered with. Cage bioassays can be used to collect chronic toxicity data to examine the impacts of chlorophenols on growth and bioaccumulation in salmonids. Periodic (3 - 5 d) observations of the fish should be made, mortalities recorded and removed, and water samples taken for chlorophenol content and water quality determination. After 30 d, weight and length should be measured and the fish sacrificed. Tissues should be analyzed for chlorophenols. The caged fish will feed on organisms colonizing the cage mesh, but should not need to be otherwise provided with food. Data collected should be used to calculate length-weight relationships, bioconcentration factors (BCFs), and percent mortality.

The response variables in this experiment can be analyzed as in laboratory experiments, except that the different sites replace concentrations as a factor and the cages replace tanks as replicates. Contaminant levels in tissues would be analyzed in the same way as, for example, weight; the experiment does not address changes with time. Dunnett's or Williams' Test are not appropriate, as there is no real control. The least contaminated site could be designated a control, but it would be better to examine the response variables as a graded response to increasing contaminant concentration. This could be as simple as plotting site means against total chlorophenol concentration, or could be more complicated. The investigator actually conducting the experiment would be the best judge of which hypotheses explaining differences among sites should be tested.

5.5 Mobile Laboratory

The mobile laboratory's usefulness in this study is questionable. One obvious advantage is the added realism that could be obtained by using Fraser River water as the dilutant for the experiments. Space constraints may not allow for the larger experiments to be conducted (joint toxicity in particular). It is recommended that duplicate acute tests following procedures outlined in Phase 1 (Section 5.1) be conducted in the laboratory and the mobile laboratory. If similar results were obtained for both sets of experiments then further mobile laboratory testing would be duplicating laboratory testing. At this time, running tests at both stations would not be necessary. If results from the mobile laboratory differed significantly from the laboratory results, then testing would be conducted in the mobile lab to the extent allowed by space limitations and practical constraints.

In the event that laboratory and mobile laboratory results differ, tests should be conducted as follows:

Laboratory	Mobile Laboratory
• Acute Testing	• Acute Testing
• Bioaccumulation	• Growth/Feeding Behaviour
• Seawater Challenge	
• Preference/Avoidance	
• Joint Toxicity	

Experiments should be run when wild juvenile chinook salmon are resident in the estuary. This would provide appropriate temperature and water quality (pH, salinity, etc.). It would not be possible to conduct more than one or two tests during this residency period due to the limited space available in the trailer. Therefore, it is recommended that acute tests, followed by one sublethal effects test, be conducted in the mobile laboratory. The trailer does not have enough room to accommodate all the diluters necessary for the joint toxicity experiments, and would not have the seawater supply necessary for the seawater challenge tests. The decision to conduct the growth experiment in the mobile laboratory, and the bioaccumulation and preference/avoidance tests in the laboratory, is arbitrary, and based on the assumption that the growth test is likely to be the most important of the sublethal effects tests.

The main limiting factor for use of the mobile laboratory is space. Height is the major consideration. The 7 ft high ceiling inside the trailer reduces the tank holding space significantly. A minimum of 3 ft must be left to allow for the installation of the diluters. The remaining 4 ft leaves enough room for two rows of test tanks (only one row can be placed over the cabinets which are 3 ft high and

occupy over half of the trailer's wall space). Depending on the timing of laboratory and trailer experiments extra diluters may need to be constructed.

The trailer has space for the installation and maintenance of approximately forty-five 40 L aquaria. An area free of chlorophenols and other contaminants would be needed as a source for Fraser River water to serve as the dilutant in these bioassays. The concentrations of chlorophenols in the dilutant should be measured frequently (daily or every second day). Acute testing would follow the procedures outlined in Phase 1 (Section 5.1) and chronic testing those from Phase 2 (Section 5.2)

The trailer would need to be outfitted with a water intake and pump sufficient to supply the bioassays described above (approximately 700 L/h). Water supply lines and toxicant diluters would also have to be installed. A method for collection and disposal of the chlorophenol contaminated water generated from the test tanks will be needed. Photodegradation of chlorophenols is an accepted method of destruction, but would require the installation of a reservoir for holding used test water. These same disposal considerations would apply to laboratory experiments.

5.6 Chemical Analyses

In static or static-renewal experiments, there is always the danger that contaminants will volatilize or photodecompose, decreasing concentrations between water changes. There are ways to correct for this, usually by expressing exposure as dose times exposure time, but actual concentrations before and after water changes are required. Therefore concentrations should be monitored during testing using a simple UV spectrophotometric method originally devised by Carr *et al.* (1982). Though one method uses chloroform to extract chlorophenols, hexane is recommended, based on advice from Brendan Hickie (pers. comm., Department of Biology, University of Waterloo).

The UV spectrophotometric method works well when only individual compounds are tested, as concentrations are easy to determine once a standard curve has been developed. However, in mixtures, problems may arise if one compound volatilizes at a much faster rate than others, and there is some interference among compounds. For that reason, determinations of concentrations of mixtures done by UV spectrophotometry should be checked against determinations of concentrations in the same solutions done by gas chromatography/mass spectrophotometry (GC/MS). All tissue concentrations will have to be determined by GC/MS; this will allow for detection of breakdown products.

5.6.1 Source of Chemicals

All chemicals used should be reagent or pure grade, as opposed to technical grade or sodium salts. Technical grade preparations can contain contaminants such as dioxins, which may disrupt testing and could create disposal problems. 2,3,4,6-tetrachlorophenol may have to be obtained from an American supplier; the other two compounds are readily available.

5.7 Reducing Costs

The recommended studies would make a significant contribution to our knowledge of chlorophenol effects on aquatic biota, as well as fully meeting the primary, but narrower, objective of assisting in the development of water quality objectives for the Fraser estuary. The primary objective could be met to a significant degree with a reduced set of tests, although reducing the number of tests creates the risk that the information gained will not represent a substantial improvement over existing data. Furthermore, the recommended tests apply specifically to chinook salmon in the Fraser estuary; the alternatives for reducing costs below would tend to reduce the direct applicability of the results to the estuary. The following means of reducing costs are suggested:

1. Reducing the number of tests

At least one chronic or sublethal effects test must be conducted to assist in the establishment of water quality objectives. It would be preferable to base objectives on the NOEC (No Observed Effect Concentration) from such tests, rather than the AEC (Acute Effects Concentration) divided by an application factor, simply because chronic and sublethal effects, rather than acute effects, are the primary concern in the environment. The growth test is likely to be the most sensitive of the sublethal effects tests, based on the review of effects from previous studies. Therefore, the minimum requirement would be the growth test plus the acute lethality tests. The latter are necessary to establish appropriate test concentrations for the growth experiment. Effects on growth should also be measured on caged fish, to indicate how applicable the results of laboratory tests are to field conditions. The major problem with eliminating the remaining tests would be that joint toxicity would not be considered. There is also the possibility that the other sublethal tests may be more sensitive than the growth test, but that this was not evident from the literature review simply because these other tests are conducted less frequently. Some information on bioaccumulation could still be gathered by measuring tissue levels in fish from the acute lethality tests.

There are other means of reducing the number of tests, that would still produce a reasonably broad understanding of chlorophenol effects in the Fraser estuary. The first would be to eliminate

trichlorophenol from consideration, since it is the least toxic compound, and levels in the water column have not exceeded CCREM guidelines or the objectives proposed by Kistritz (1989). The second would be to eliminate the growth test, and perhaps the bioaccumulation test, for pentachlorophenol, because of the considerable data available in the literature. It has been almost 20 years since Webb and Brett (1973) published their study of pentachlorophenol effects on the growth of sockeye salmon, and no subsequent experiment has revealed a lower NOEC. Finally, the joint toxicity tests could be eliminated, not because they are unimportant, but because ongoing research at the University of Waterloo and Lakehead University may provide sufficient information on joint effects to assist in the development of water quality objectives. However, this ongoing research focuses on joint toxicity at concentrations causing high (50%) mortality; our tests focus on lower and more realistic levels.

2. Eliminate the mobile laboratory

The practicality of conducting tests in the mobile laboratory was discussed in Section 5.5.. The advantage of the mobile laboratory is that it reproduces natural conditions better than does a laboratory. However, the *in situ* tests would offer even greater realism, perhaps rendering tests in the mobile laboratory redundant.

3. Use rainbow trout instead of chinook salmon

In any test, there are additional costs associated with obtaining and holding chinook salmon, which would not apply to rainbow trout. These costs are small compared to the costs of tissue analysis, and constructing flow-through systems, so that any savings realized by using rainbow trout would be minimal. The chief advantage of using rainbow trout would be that these fish are available year-round, whereas chinook salmon are only available for one to two months in the spring. As a result, the number of tests that could be conducted in the mobile laboratory would be greater if rainbow trout were used, although high summer and low winter water temperatures in the Fraser would still limit the time in which tests could be conducted.

The chief disadvantage to using rainbow trout would be the loss of realism, as the fish used in laboratory tests are domesticated to a significantly greater degree than are other salmonids. That would be an important consideration for the *in situ* tests, and the preference/avoidance, seawater challenge, and perhaps bioaccumulation tests, but not for the joint toxicity tests, and perhaps the growth test. The joint toxicity tests can only reveal the broad pattern of interactions among compounds, and are unlikely to precisely reproduce mixes found in the field. Growth effects may

be similar for all salmonid species (see Appendix 2,5), in which case it would be unnecessary to test a specific species.

4. Use radioactively labelled compounds instead of measuring tissue levels of specific compounds

The costs of chemical analyses of chlorophenol levels in fish tissue would be significant for the acute lethality and bioaccumulation tests. E.V.S. Consultants has traditionally paid $300-400 for complete analyses of chlorinated phenolics in fish tissue; this unit price would need to be multiplied by the number of replicates, test concentrations, and test compounds. It is considerably less expensive to use radioactively labelled test compounds, and then measure the levels of radioactivity in tissue (or in water), to determine uptake or loss. The disadvantage to using radioactively labelled compounds is that it is not possible to determine if the original compounds have been transformed in the fish (or in the water). This is not a serious problem in short-term tests, such as the acute lethality tests, but might be in longer-term tests, such as the bioaccumulation tests.

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APPENDIX 1

Published Water Quality Objectives for Aquatic Life

(Taken from Kistritz, 1989)

Appendix 17. Published Water Quality Objectives for aquatic life.

Agency	Reference	Objective	Comments
MOE&P	Swain & Holmes, 1985	0.2 ug/l	-sum of tri- tetra- and penta-chlorophenol in any descrete sample from the water column taken outside the initial dilution zone -dilution zone was defined as extending 100 m horizontally in all directions from the discharge point, from the surface to the bottom -arbitrary objectives based upon existing levels in the river with the view to reduce the potential for chlorophenols to enter the food web
MOE&P	Swain & Holmes, 1985	-	-water quality of the Banks was proposed to be high enough to protect sensitive aquatic life and wildlife and to permit primary water cotact recreation
		MONOCHLOROPHENOL	
CCREM	CCREM, 1987 McKee et al., 1984	7 ug/l	- one half the lowest tainting threshold - guideline
		DICHLOROPHENOL	
CCREM	CCREM, 1987 McKee et al., 1984	0.2 ug/l	-one-half of the tainting threshold concentration of 2,4-dichlorophenol (0.4 ug/l) which is the most critical isomer in terms of flavour impairment (Shumway & Palensky, 1973) -guideline
		TRICHLOROPHENOL	
Ontario Ministry of the Environment CCREM	McKee et al., 1984	18 ug/l	-in water based on acute toxicity -the geometric mean acute toxicity of bluegill to 2,4,5-& and 2,3,6-trichlorophenols of 379 ug/l was used with an application factor of 0.05 -designed to protect against chronic toxicity and tainting of fish flesh -this concentration is less than half of the lowest tainting threshold (CCREM, 1987)

Agency	Reference	Objective	Comments
			TETRACHLOROPHENOL
Ontario Ministry of the Environment & CCREM	McKee et al., 1984	1 ug/l	-in water based on acute toxicity -bluegill 96 hrs LC50 values for 2,3,4,6- and 2,3,5,6-tetrachlorophenol respectively of 140 and 170 ug/l respectively (Buccafusco et al., 1981) were used with an
			PENTACHLOROPHENOL
Ontario Ministry of the Environment & CCREM	McKee et al., 1984	0.5 ug/l	-in water based on acute toxicity -the lowest mean acute toxicity concentration for salmonids, 55 ug/l for coho salmon (Davis & Hoos, 1975) was used with the application factor for persistent contaminants of 0.01 -felt will protect fish from impaired growth
IJC	IJC, 1980	0.4 ug/l	-in water based on the growth inhibition of sockeye salmon under-yearlings at 1.74 ug/l with an application factor of 0.2 used on non-lethal but observable-effect concentrations for aquatic organisms (Water Quality Board, 1975). -for the protection of aquatic life
U.S. EPA 1983 Guidelines	Yount and Richter, 1986	48 ug/l	-significant effect on periphyton community at criteria level
Federal Registry	U.S. Federal Registry	6.2 ug/l	-based on invertebrate acute toxicity

APPENDIX 2

Water Quality Objectives Based on Aquatic Toxicology With Safety Factors Applied

(Taken from Kistritz, 1989)

Appendix 16. Water quality objectives based on aquatic toxicology with safety factors applied.

Isomer	Objective	Basis
Monochlorophenol	4 ug/l	- calculated growth inhibition by m-CP in rainbow trout (<420 ug/l) - safety factor of 100 applied
Dichlorophenol	2 ug/l	- calculated value for no significant avoidance reaction by young rainbow trout (250 ug/l 2,4-DCP) - safety factor of 100 applied
Trichlorophenol	0.8 ug/l	- calculated value for no significant avoidance reaction by young rainbow trout (89.3 ug/l 2,4,5-TCP) - safety factor of 100 applied
Tetrachlorophenol	0.1 ug/l	- minimal chronic effect in macrozooplankton (<1,000) - safety factor of 1,000 applied
Pentachlorophenol	0.02 ug/l	- highest no effect concentration for sockeye salmon (1.99 ug/lNa-PCP) - growth inhibition in sockeye salmon (1.74 ug/l Na-PCP) - estimated growth reduction in chinook salmon (1.5 ug/l) - estimated growth reduction in coho salmon (1.2 ug/l) - estimated no-effect value for growth in chinook salmon (2.6 ug/l) - estimated no-effect value for growth in coho salmon (2.0 ug/l) - safety factor of 100 applied

APPENDIX 3

Acute Toxicity of Pentachlorophenol to Aquatic Biota

(Taken from Kistritz, 1989)

Appendix 6. Acute toxicity of pentachlorophenol to aquatic biota.

Species	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
Fish							
Salmonid	-	96	-	-	34 - 128	LC50	U.S. EPA, 1980c
Chinook salmon *Oncorhynchus tshawytscha*	CF unfed	96	11.8-12	7.0-7.1	mean 78 (Na-PCP) 95% CL 57-110 n=240	LC50	Iwama & Greer, 1979
Coho salmon *Oncorhynchus kisutch*	S	96	10·2 (H_2O hardness 14.7)	7.0	mean 92 (Na-PCP) 95% CL 79.3-106.7	LC50	Davis & Hoos, 1975
Coho salmon	S	96	11 (H_2O hardness 5-6)	7.0	mean 31.8 (Na-PCP) 95% CL 24.9-40.6	LC50	Davis and Hoos, 1975
Sockeye salmon *Onchorhynchus nerka*	S	96	12·5 (H_2O hardness 47.0)	7.2	mean 50 (Na-PCP) 95% CL 40.3-62.0	LC50	Davis & Hoos, 1975
Sockeye salmon	S	96	7.5 (H_2O hardness 85.0)	7.7	mean 130 (Na-PCP) 95% CL 119-142	LC50	Davis & Hoos, 1975
Sockeye salmon (underyearlings)	FT, M unfed	504	15	6.8	57 (Na-PCP)	LC50	Webb & Brett, 1973
Sockeye salmon (underyearlings)	FT, M unfed	504	15	6.8	63 (Na-PCP)	LC50	Webb and Brett, 1973
Rainbow trout (4-5 months)	SS	96	11.2-12	6-6.4	mean 56 95% CL 40-65 n=250	LC50	McLeay and Gordon, 1980
Rainbow trout (underyearlings)	S	96	12 (H_2O hardness 51.5)	7	mean 98 (Na-PCP) 95% CL 87.5-109.8	LC50	Davis and Hoos, 1975
Rainbow trout (underyearlings)	S	96	11.5 (H_2O hardness 47.0)	7	mean 96 (Na-PCP) 95% CL 90.1-102.2	LC50	Davis and Hoos, 1975
Rainbow trout (underyearlings)	S	96	11.5 (H_2O hardness 5.0)	7	mean 106 (Na-PCP) 95% CL 88.5-127.0	LC50	Davis and Hoos, 1975
Rainbow trout (underyearlings)	S	96	11 (H_2O hardness 5-6)	7	mean 50 (Na-PCP) 95% CL 29.8-84.0	LC50	Davis and Hoos, 1975

Species	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
Rainbow trout (underyearlings)	S	96	10	5.7	mean 47 (Na-PCP) 95% CL 32.9-67.2	LC50	Davis and Hoos, 1975
			(H_2O hardness 4.0)				
Rainbow trout	-	48	-	-	157	LC50	Alabaster, 1957
Rainbow trout	CF unfed	48	15	8-8.3	mean=250 (Na-PCP)	LC50	Fogels and Sprague, 1977
Rainbow trout	S, U	96	-	-	75 - 92 mean = 84 n = 2	LC50	Bentley et al., 1975
Rainbow trout	CF unfed	96	15	8-8.3	mean=230 (Na-PCP)	LC50	Fogels & Sprague, 1977
Rainbow trout	-	120	-	-	92	LC25	Chapman, 1969
Rainbow trout	-	984	-	-	46	LC100	Chapman, 1969
Steelhead trout *Salmo gairdneri* (eggs)	S	168	15	7.8	50-180 (Na-PCP)	LC100	Chapman and Shumway, 1978
Steelhead trout (alevins)	S	12	15	7.8	300 (Na-PCP)	LC100	Chapman and Shumway, 1978
Steelhead trout (alevins)	S	24	15	7.8	200 (Na-PCP)	LC100	Chapman and Shumway, 1978
Steelhead trout (alevins)	FT	24	-	7.8	50 (Na-PCP)	LC100	Chapman and Shumway, 1978
Brook trout *Salvelinus gairdneri* (adult)	FT, M unfed	24	15	7.9	mean 315 (Na-PCP) 95% CL 294-337	LC50	Cardwell et al., 1976
Brook trout (adult)	FT, M unfed	32	15	7.9	mean 230 (Na-PCP) 95% CL 215-246	LC50	Cardwell et al., 1976
Brook trout (adult)	FT, M unfed	48	15	7.9	mean 180 (Na-PCP) 95% CL 168-193	LC50	Cardwell et al., 1976
Brook trout (adult)	FT, M unfed	72	15	7.9	mean 153 (Na-PCP) 95% CL 143-164	LC50	Cardwell et al., 1976

APPENDIX 6 /3

Species	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
Brook trout (adult)	FT, M unfed	96	15	7.9	mean 138 (Na-PCP) 95% CL 129-148	LC50	Cardwell et al., 1976
Brook trout (adult)	FT, M unfed	152	15	7.9	mean 128 (Na-PCP) 95% CL 119-138	LC50	Cardwell et al., 1976
Brook trout (adult)	FT, M unfed	219	15	7.9	mean 118 (Na-PCP) 95% CL 110-126	LC50	Cardwell et al., 1976
Brook trout (adult)	FT, M unfed	336	15	7.9	mean 118 (Na-PCP) 95% CL 110-126	LC50	Cardwell et al., 1976
Brown trout _Salmo trutta_	S, U	24	5	-	mean=200 n=5	LC50	Hattula et al., 1981
Brown trout	-	48	-	-	157	LC50	Alabaster, 1957
Largemouth bass _Macropterus salmoides_ (14 days old)	SS	96	25	7.2	mean 287 95% CL 239-344 n * 10	LC50	Johansen et al., 1985
Largemouth bass (28 days)	SS	96	25	7.2	mean 275 95% CL 221-341 n * 10	LC50	Johansen et al., 1985
Largemouth bass (49 days)	SS	96	25	7.2	mean 136 95% CL 105-177 n * 10	LC50	Johansen et al., 1985
Largemouth bass (84 days)	SS	96	25	7.2	mean 189 95% CL 105-340 n * 10	LC50	Johansen et al., 1985
Largemouth Bass (1 + 2 yrs)	SS	96	25	7.2	mean 194 n=5	LC50	Johansen et al., 1985
Fathead minnow	S, U	1.2-1.55	18	7.5-7.6	1,000 (Na-PCP) n=10	LC50	Crandall & Goodnight, 1959
Fathead minnow	FT, M unfed	8	25	7.8	mean 469 (Na-PCP) 95% CL 437-504	LC50	Cardwell et al., 1976
Fathead minnow	FT, M unfed	12	25	7.8	mean 358 (Na-PCP) 95% CL 334-383	LC50	Cardwell et al., 1976
Fathead minnow	FT, M unfed	21	25	7.8	mean 343(Na-PCP) 95% CL 315-374	LC50	Cardwell et al., 1976

APPENDIX 6 /4

Species	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
Fathead minnow	FT, M	24	-	6.9-7.9	mean 220 CV 19, n=16	LC50	Adelman and Smith, 1976
Fathead minnow	S, U	24	15	8.0	320-350 (Na-PCP)	LC50	Crandall and Goodnight, 1959
Fathead minnow	S, U	24	15	8.0	360 (Na-PCP) n=10	LC40	Crandall & Goodnight, 1959
Fathead minnow	S, U unfed	48	25	7.4-8.3	mean 7900 95% CL 6400-9700 n=20	LC50	Phipps et al., 1981
Fathead minnow	FT, U	48	14-15	7.9-8.2	210 (Na-PCP)	LC50	Ruesink and Smith, 1975
Fathead minnow	FT, U	48	23-27	7.8-8.2	370 (Na-PCP)	LC50	Ruesink and Smith, 1975
Fathead minnow	FT, M unfed	72	25	7.8	mean 309 (Na-PCP) 95% CL 285-336	LC50	Cardwell et al., 1976
Fathead minnow	S, U	96	-	-	600	LC50	Mattson et al., 1976
Fathead minnow	FT, M unfed	96	25	7.8	mean 285 (Na-PCP) 95% CL 267-305	LC50	Cardwell et al., 1976
Fathead minnow	FT, U unfed	96	25	7.4-8.3	mean 225 95% CL 210-230 n=100	LC50	Phipps et al., 1981
Fathead minnow	FT, U	96	14-15	7.9-8.2	210 (Na-PCP)	LC50	Ruesink and Smith, 1975
Fathead minnow	FT, U	96	23-27	7.8-8.2	340 (Na-PCP)	LC50	Ruesink and Smith, 1975
Fathead minnow	FT, M unfed	120	25	7.8	mean 276 (Na-PCP) 95% CL 258-295	LC50	Cardwell et al., 1976
Fathead minnow	FT, M unfed	173	25	7.8	mean 255 (Na-PCP) 95% CL 237-274	LC50	Cardwell et al., 1976
Fathead minnow	FT, U unfed	192	25	7.4-8.3	mean 210 95% CL 190-230 n=100	LC50	Phipps et al., 1981
Fathead minnow	FT, M unfed	216	25	7.8	mean 235 (Na-PCP) 95% CL 210-263	LC50	Cardwell et al., 1976
Fathead minnow	FT, M unfed	240	25	7.8	mean 217 (Na-PCP) 95% CL 194-243	LC50	Cardwell et al., 1976

Species	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
Fathead minnow	FT, M unfed	288	25	7.8	mean 185 (Na-PCP) 95% CL 155-221	LC50	Cardwell et al., 1976
Fathead minnow	FT, M unfed	336	25	7.8	mean 153 (Na-PCP) 95% CL 123-188	LC50	Cardwell et al., 1976
Fathead minnow *Pimephales promelas*	FT, M	672	23-27	7.2-7.9	128 & 228	signif. decrease in survival	Holcombe et al., 1982
Silver mouthed minnow *Ericymba buccata*	S	72	16	7.6	>200 (Na-PCP)	LC100	Goodnight, 1942
Bluegill *Lepomis macrochirus*	FT, M unfed	5	25	7.9	mean 828 (Na-PCP) 95% CL 798-860	LC50	Cardwell et al., 1976
Bluegill	FT, M unfed	6.5	25	7.9	mean 719 (Na-PCP) 95% CL 691-749	LC50	Cardwell et al., 1976
Bluegill	FT, M unfed	9.5	25	7.9	mean 534 (Na-PCP) 95% CL 497-574	LC50	Cardwell et al., 1976
Bluegill	FT, M unfed	30	25	7.9	mean 303 (Na-PCP) 95% CL 283-324	LC50	Cardwell et al., 1976
Bluegill	-	48	-	-	30	LC50	Inglis & Davis, 1972
Bluegill	S, U	96	-	-	60 - 77 mean = 68 n = 2	LC50	Bentley et al., 1975
Bluegill	-	96	-	-	20-305	LC50	Inglis & Davis, 1972
Bluegill	S, M	96	17-21	7.2-7.7	mean 260 n=10	LC50	Pruitt et al., 1977
Bluegill	S, M	96	17-21	7.2-7.7	mean 330 n=10 (NaPCP)	LC50	Pruitt et al., 1977
Bluegill	FT, M unfed	243	25	7.9	mean 251 (NaPCP) 95% CL 222-284	LC50	Cardwell et al., 1976
Bluegill	FT, M unfed	313	25	7.9	mean 226 (NaPCP) 95% CL 196-261	LC50	Cardwell et al., 1976
Bluegill	FT, M unfed	390	25	7.9	mean 207 (NaPCP) 95% CL 170-252	LC50	Cardwell et al., 1976

APPENDIX 6 /6

Species	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
Bluegill	FT, M unfed	406	25	7.9	mean 188 (NaPCP) 95% CL 162-218	LC50	Cardwell et al., 1976
Longnose killifish	-	96	-	-	>306 (NaPCP)	LC50	Schimmel et al., 1978
Guppy (young) *Poecilla reticulata*	S, SS, FT (ave) freshwater	48	-	8.0	1050	LC50	Adema and Vink, 1981
Guppy (young)	S, SS, FT (ave) seawater	48	-	8.0	1700	LC50	Adema and Vink, 1981
Guppy (young)	S, SS, FT (ave) freshwater	96	-	8.0	720-880	LC50	Adema and Vink, 1981
Guppy (young)	S, SS, FT (ave) seawater	96	-	8.0	1600	LC50	Adema and Vink, 1981
Guppy (young)	S, SS, FT (ave) freshwater	168	-	8.0	580-800	LC50	Adema and Vink, 1981
Guppy (adult)	S, SS, FT (ave) freshwater	48	-	8.0	820	LC50	Adema and Vink, 1981
Guppy (adult)	S, SS, FT (ave) seawater	48	-	8.0	1600	LC50	Adema and Vink, 1981
Guppy (adult)	S, SS, FT (ave) freshwater	96	-	8.0	450	LC50	Adema and Vink, 1981
Guppy (adult)	S, SS, FT (ave) seawater	96	-	8.0	1150	LC50	Adema and Vink, 1981
Guppy (adult)	S, SS, FT (ave) freshwater	168	-	8.0	360	LC50	Adema and Vink, 1981

Species	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
Guppy (adult)	S, SS, FT (ave) seawater	168	-	8.0	1100	LC50	Adema and Vink, 1981
Guppy	S, U	2160	25-27	8.4-8.6	500 (Na-PCP) n=74	LC44.6	Crandall and Goodnight, 1962
Guppy	S, U	.35-.63	18	5.9-6.0	1000 n=10	LC50	Crandall and Goodnight, 1959
Guppy	S, U	1.2-1.55	18	7.5-7.6	1000 n=10	LC50	Crandall and Goodnight, 1959
Guppy	S, U	24	15	8.0	360 n=10	LC50	Crandall and Goodnight, 1959
Goldfish *Carassius auratus*	FT, M unfed	21	25	7.6	mean 369 (Na-PCP) 95% CL 336-406	LC50	Cardwell et al., 1976
Goldfish	FT, M	24	-	6.9-7.9	mean 270 range 180-370 CV 23, n=16	LC50	Adelman & Smith, 1976; Adelman et al., 1976
Goldfish	SS, U	24	20	-	mean 270 n=10	LC50	Kobayashi et al., 1979
Goldfish	FT, M unfed	46	25	7.6	mean 270 (Na-PCP) 95% CL 246-297	LC50	Cardwell et al., 1976
Goldfish	FT, M	96	-	6.9-7.9	mean 220 range 170-300 CV=17, n=16	LC50	Adelman & Smith, 1976a, 1976b
Goldfish	FT, M unfed	120	25	7.6	mean 253 (Na-PCP) 95% CL 231-277	LC50	Cardwell et al., 1976
Goldfish	FT, M unfed	168	25	7.6	mean 241 (Na-PCP) 95% CL 219-265	LC50	Cardwell et al., 1976
Goldfish	FT, M unfed	268	25	7.6	mean 202 (Na-PCP) 95% CL 171-239	LC50	Cardwell et al., 1976
Goldfish	FT, M unfed	336	25	7.6	mean 189 (Na-PCP) 95% CL 162-219	LC50	Cardwell et al., 1976
Zebrafish *Brachydanio rerio*	FT unfed	48	15	8.0-8.3	mean 1240 (Na-PCP)	LC50	Fogels & Sprague, 1977

Species	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
Zebrafish	FT unfed	96	15	8.0-8.3	mean 1130 (Na-PCP)	LC50	Fogels & Sprague, 1977
Flagfish *Jordanella floridae*	FT unfed	48	15	8.0-8.3	mean 1820 (Na-PCP)	LC50	Fogels & Sprague, 1977
Flagfish	FT unfed	96	15	8.0-8.3	mean 1740 (Na-PCP)	LC50	Fogels & Sprague, 1977
Common shiner (young) *Notropis cornutus*	SS, M fed	168	20	8.0	mean 320	LC100	Borgmann & Ralph, 1986
Fresh water fish	-	96	-	-	34 - 600	LC50	U.S. EPA, 1980c
Nonsalmonid	-	96	-	-	60-600	LC50	U.S. EPA, 1980c
Invertebrates							
Pond snail (egg-juvenile) *Lymnaea stagnalis*	S, SS, FT (ave) hard water	48	-	8.0	mean 300	LC50	Adema & Vink, 1981
Pond snail (egg-juvenile)	S, SS, FT (ave) hard water	96	-	8.0	mean 240	LC50	Adema & Vink, 1981
Pond snail (egg-juvenile)	S, SS, FT (ave) hard water	384	-	8.0	mean 180	LC50	Adema & Vink, 1981
Mollusc (adult) *Dreissena polymorpha*	S, SS, FT (ave) soft water	96	-	8.0	mean 110	LC50	Adema & Vink, 1981
Mollusc (adult) *Dreissena polymorpha*	S, SS, FT (ave) hard water	96	-	8.0	mean 190	LC50	Adema & Vink, 1981
Mollusc (adult) *Dreissena polymorpha*	S, SS, FT (ave) soft water	168	-	8.0	mean 52	LC50	Adema & Vink, 1981

Species	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
Mollusc (adult) Dreissena polymorpha	S, SS, FT (ave) hard water	168	-	8.0	mean 180	LC50	Adema & Vink, 1981
Mollusc (adult) Dreissena polymorpha	S, SS, FT (ave) soft water	336	-	8.0	mean 42	LC50	Adema & Vink, 1981
Mollusc (adult) Dreissena polymorpha	S, SS, FT (ave) hard water	336	-	8.0	mean 140	LC50	Adema & Vink, 1981
Pulmonate Snail Lymnaea acuminata	S unfed	12	17-19	7.9	mean 293 (PCP) 95% CL 244-351 n=10	LC50	Gupta and Rao, 1982
Pulmonate Snail	S unfed	24	17-19	7.9	mean 263 PCP 95% CL 248-278 (n=10)	LC50	Gupta and Rao, 1982
Pulmonate snail	S unfed	48	17-19	7.9	mean 228 (PCP) 95% CL 207-250 n=10	LC50	Gupta and Rao, 1982
Pulmonate snail	S unfed	72	17-19	7.9	mean 196 (PCP) 95% CL 180-210 n=10	LC50	Gupta and Rao, 1982
Pulmonate snail	S unfed	96	17-19	7.9	mean 160 (PCP) 95% CL 138-186 n=10	LC50	Gupta and Rao, 1982
Pulmonate snail	S unfed	12	17-19	7.9	mean 470 (Na-PCP) 95% CL 416-531 n=10	LC50	Gupta and Rao, 1982
Pulmonate snail	S unfed	24	17-19	7.9	mean 360 (Na-PCP) 95% CL 321-403 n=10	LC50	Gupta and Rao, 1982
Pulmonate snail	S unfed	48	17-19	7.9	mean 255 (Na-PCP) 95% CL 216-301 n=10	LC50	Gupta and Rao, 1982

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Species	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
Pulmonate snail	S unfed	72	17-19	7.9	mean 215 (Na-PCP) 95% CL 182-254 n=10	LC50	Gupta and Rao, 1982
Pulmonate snail	S unfed	96	17-19	7.9	mean 190 (Na-PCP) 95% CL 161-244 n=10	LC50	Gupta and Rao, 1982
Oligochaetes	-	96	10	7.0	560-3,600 (Na-PCP) n = 6-10	LC50	Chapman et al., 1982a & 1982b
Tubificid worm *Tubifex tubifex*	-	24	-	7.5	286	LC50	Whitley, 1968
Tubificid worm *Tubifex tubifex*	-	24	-	8.5	619	LC50	Whitley, 1968
Tubificid worm *Tubifex tubifex*	-	24	-	9.5	1,294	LC50	Whitley, 1968
Daphnia magna (larva)	S, SS FT (ave)	24	-	8.0	mean 1,700	LC50	Adema and Vink, 1981
Daphnia magna (larva)	S, SS FT (ave)	48	-	8.0	mean 1,050	LC50	Adema and Vink, 1981
Daphnia magna (larva)	S, SS FT (ave)	96	-	8.0	mean 800	LC50	Adema and Vink, 1981
Daphnia magna (larva)	S, SS FT (ave)	168	-	8.0	mean 600	LC50	Adema and Vink, 1981
Daphnia magna (larva)	S, SS FT (ave)	336	-	8.0	mean 400	LC50	Adema and Vink, 1981
Daphnia magna (larva)	S, SS FT (ave)	504	-	8.0	mean 400	LC50	Adema and Vink, 1981
Daphnia magna (young, 24+/-12 hrs)	S	24	19-21	7.5-7.8	mean 1,840+/-0.33 n=225 (technical PCP)	LC50	Solomon & Kaushik, 1987
Daphnia magna (young, 24+/-12 hrs)	S	24	19-21	7.5-7.8	mean 1,870+/-0.38 n=225 (pure PCP)	LC50	Solomon & Kaushik, 1987
Daphnia magna (young, 24+/-12 hrs)	S	48	19-21	7.5-7.8	mean 1,700+/-0.28 n=225 (technical PCP)	LC50	Solomon & Kaushik, 1987
Daphnia magna (young, 24+/-12 hrs)	S	48	19-21	7.5-7.8	mean 1,500/-0.19 n=225 (pure PCP)	LC50	Solomon & Kaushik, 1987

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Species	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
Daphnia magna (young, 24+/-12 hrs)	S	72	19-21	7.5-7.8	mean 1,530+/-0.38 n=225 (technical PCP)	LC50	Solomon & Kaushik, 1987
Daphnia magna (young, 24+/-12 hrs)	S	72	19-21	7.5-7.8	mean 1,370+/-0.19 n=225 (pure PCP)	LC50	Solomon & Kaushik, 1987
Daphnia magna (~ 24 hrs)	S, U	24	21-23	7.4-9.4	mean 1500 95% CL 1100-2000 n ~ 15	LC50	LeBlanc, 1980
Daphnia magna (~ 24 hrs)	S, U	48	21-23	7.4-9.4	mean 680 95% CL 600-790 n ~ 15	LC50	LeBlanc, 1980
Daphnia magna (1 day old)	S, U fed	24	20	8.0	mean 1,700	LC50	Adema, 1978
Daphnia magna (1 day old)	S, U unfed	24	20	8.0	mean 1,200	LC50	Adema, 1978
Daphnia magna (1 day old)	S, U fed	48	20	8.0	mean 1,000	LC50	Adema, 1978
Daphnia magna (1 day old)	S, U unfed	48	20	8.0	mean 600	LC50	Adema, 1978
Daphnia magna (juvenile, 48-96 hrs)	S	24	19-21	7.5-7.8	mean 2,980+/-0.57 n=225 (technical PCP)	LC50	Solomon & Kaushik, 1987
Daphnia magna (juvenile, 48-96 hrs)	S	24	19-21	7.5-7.8	mean 2,220+/-0.18 n=225 (pure PCP)	LC50	Solomon & Kaushik, 1987
Daphnia magna (juvenile, 48-96 hrs)	S	48	19-21	7.5-7.8	mean 2,390+/-0.48 n=225 (technical PCP)	LC50	Solomon & Kaushik, 1987
Daphnia magna (juvenile, 48-96 hrs)	S	48	19-21	7.5-7.8	mean 1,540+/-0.15 n=225 (pure PCP)	LC50	Solomon & Kaushik, 1987
Daphnia magna (juvenile, 48-96 hrs)	S	72	19-21	7.5-7.8	mean 2,210+/-0.44 n=225 (technical PCP)	LC50	Solomon & Kaushik, 1987
Daphnia magna (juvenile, 48-96 hrs)	S	72	19-21	7.5-7.8	mean 1,150+/-0.30 n=225 (pure PCP)	LC50	Solomon & Kaushik, 1987
Daphnia magna (7 days)	S, U fed	24	20	~8.0	mean 2,800 n=25	LC50	Adema, 1978

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Species	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
Daphnia magna (7 days)	S, U unfed	24	20	~8.0	mean 1,300 n=25	LC50	Adema, 1978
Daphnia magna (7 days)	S, U fed	48	20	~8.0	mean 1,500 n=25	LC50	Adema, 1978
Daphnia magna (7 days)	S, U unfed	48	20	~8.0	mean 800 n=25	LC50	Adema, 1978
Daphnia magna (adult)	S	24	19-21	7.5-7.8	mean 3,910+/-1.15 n=225 (technical PCP)	LC50	Solomon & Kaushik, 1987
Daphnia magna (adult)	S	24	19-21	7.5-7.8	mean 2,630+/-0.44 n=225 (pure PCP)	LC50	Solomon & Kaushik, 1987
Daphnia magna	S	48	19-21	5.5	mean 880 (technical PCP)	LC50	Solomon & Kaushik, 1987
Daphnia magna	S	48	19-21	5.5	mean 580 (pure PCP)	LCV50	Solomon & Kaushik, 1987
Daphnia magna (adult)	S	48	19-21	7.5-7.8	mean 2,790+/-0.56 n=225 (technical PCP)	LC50	Solomon & Kaushik, 1987
Daphnia magna (adult)	S	48	19-21	7.5-7.8	mean 1,780+/-0.55 n=225 (pure PCP)	LC50	Solomon & Kaushik, 1987
Daphnia magna (adult)	S, U	48	20	8.0	240-260 mean = 250 2 replicates	LC50	Canton & Adema, 1978
Daphnia magna (adult)	S, SS FT (ave)	48	-	8.0	1,400	LC50	Adema & Vink, 1981
Daphnia magna (adult)	S	72	19-21	7.5-7.8	mean 2,460+/-0.39 n=225 (technical PCP)	LC50	Solomon & Kaushik, 1987
Daphnia magna (adult)	S	72	19-21	7.5-7.8	mean 1,270+/-0.61 n=225 (pure PCP)	LC50	Solomon & Kaushik, 1987
Daphnia magna (adult)	S, SS FT (ave)	96	-	8.0	680	LC50	Adema & Vink, 1981
Daphnia magna (adult)	S, SS FT (ave)	168	-	8.0	580	LC50	Adema & Vink, 1981

Species	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
Daphnia magna	S, U	504	20	8.0	400-470 mean=435 n=25(2 reps)	LC50	Adema, 1978
Daphnia galeata mendotae (adult)	S	24	19-21	7.5-7.8	mean 53 n=225 (technical PCP)	LC50	Solomon & Kaushik, 1987
Daphnia galeata mendotae (adult)	S	24	19-21	7.5-7.8	mean 58 n=225 (pure PCP)	LC50	Solomon & Kaushik, 1987
Daphnia galeata mendotae (adult)	S	48	19-21	7.5-7.8	mean 59 n=225 (technical PCP)	LC50	Solomon & Kaushik, 1987
Daphnia galeata mendotae (adult)	S	48	19-21	7.5-7.8	mean 57 n=225 (pure PCP)	LC50	Solomon & Kaushik, 1987
Daphnia pulex	S, U	48	20	8.0	mean=2000 2 replicates	LC50	Canton and Adema, 1978
Daphnia cucullata (11+/- 1 day)	-	48	20	8.0	mean 1500 (2 reps)	LC50	Canton and Adema, 1978
Midge Chironomus riparius	S	24	25	4.0	384 95% CL 295-435 3 reps	LC50	Fisher & Wadleigh, 1986
Midge	S	24	25	6.0	465 95% CL 446-496 3 reps	LC50	Fisher & Wadleigh, 1986
Midge	S	24	25	9.0	1948 95% CL 1385-2691 3 reps	LC50	Fisher & Wadleigh, 1986
Plants							
Duckweed Lemna minor	S	48	25	5.1	800 n=50	LD50	Blackman et al., 1955

Species	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
					Saltwater Species		
Fish							
Sheepshead minnow *Cyprinodon variegatus* (1 day fry)	S, U	96	30	-	mean 329 95% CL 305-360	LC50	Borthwick & Schimmel, 1978
Sheepshead minnow (2 wk fry)	S, U	96	30	-	mean 392 95% CL 307-489	LC50	Borthwick & Schimmel, 1978
Sheepshead minnow (4 wk fry)	S, U	96	30	-	mean 240 95% CL 192-284	LC50	Borthwick & Schimmel, 1978
Sheepshead minnow (6 wk fry)	S, U	96	30	-	mean 223 95% CL 163-291	LC50	Borthwick & Schimmel, 1978
Sheepshead minnow (juvenile)	FT, M	96	30	-	mean 442 95% CL 308-635	LC50	Parrish et al., 1978
Sheepshead minnow (adult)	inter-mittent flow	240	-	-	mean 88	signif-icant mortality	Parrish et al., 1978
Flatfish *Pleuronectes platessa* (yolk-sac larva)	S, SS, FT (ave)	48	-	8.0	180	LC50	Adema & Vink, 1981
Flatfish (yolk-sac larva)	S, SS, FT (ave)	96	-	8.0	140	LC50	Adema & Vink, 1981
Flatfish (larva stage 3)	S, SS, FT (ave)	48	-	8.0	90	LC50	Adema & Vink, 1981
Flatfish (larva stage 3)	S, SS, FT (ave)	96	-	8.0	60	LC50	Adema & Vink, 1981
Flatfish (egg metamorphism)	S, SS, FT (ave)	96	-	8.0	750	LC50	Adema & Vink, 1981
Flatfish (egg metamorphism)	S, SS, FT (ave)	168	-	8.0	750	LC50	Adema & Vink, 1981
Flatfish (egg metamorphism)	S, SS, FT (ave)	336	-	8.0	58	LC50	Adema & Vink, 1981

Species	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
Flatfish (egg metamorphism)	S, SS, FT (ave)	672	-	8.0	58	LC50	Adema & Vink, 1981
Flatfish (egg metamorphism)	S, SS, FT (ave)	1344	-	8.0	50	LC50	Adema & Vink, 1981
Flatfish (4.5-8 cm length)	S, SS, FT (ave)	48	-	8.0	260	LC50	Adema & Vink, 1981
Flatfish (4.5-8 cm length)	S, SS, FT (ave)	96	-	8.0	140	LC50	Adema & Vink, 1981
Flatfish (4.5-8 cm length)	S, SS, FT (ave)	168	-	8.0	130	LC50	Adema & Vink, 1981
Flatfish (10 cm length)	S, SS, FT (ave)	48	-	8.0	170	LC50	Adema & Vink, 1981
Flatfish (10 cm length)	S, SS, FT (ave)	96	-	8.0	170	LC50	Adema & Vink, 1981
Flatfish (10 cm length)	S, SS, FT (ave)	168	-	8.0	110	LC50	Adema & Vink, 1981
Flatfish (20 cm length)	S, SS, FT (ave)	48	-	8.0	250	LC50	Adema & Vink, 1981
Flatfish (20 cm length)	S, SS, FT (ave)	96	-	8.0	150	LC50	Adema & Vink, 1981
Flatfish (20 cm length)	S, SS, FT (ave)	168	-	8.0	100	LC50	Adema & Vink, 1981
Pinfish <u>Lagodon rhomboides</u> (prolarvae)	S, U	96	20	-	mean 38 (Na-PCP) 95% CL 26-57	LC50	Borthwick & Schimmel, 1978
Pinfish (juvenile)	FT, M (unfed)	96	-	-	mean 53 95% CL 42-65	LC50	Schimmel et al., 1978
Striped mullet <u>Mugil cephalus</u> (juvenile)	FT, M (unfed)	96	25	-	mean 112 95% 44-210 n=20	LC50	Schimmel et al., 1978
Goby (adult) <u>Gobius minutus</u>	S, SS, FT (ave)	48	-	8.0	450	LC50	Adema and Vink, 1981

Species	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
Goby (adult)	S, SS, FT (ave)	168	-	8.0	450	LC50	Adema and Vink, 1981
Invertebrates							
Grass shrimp *Palaemonetes pugio* (24 hr old)	S, U	96	24	-	mean 649 (Na-PCP) 95% CL 494-915	LC50	Borthwick & Schimmel, 1978
Grass shrimp (premolt)	SS, U (unfed)	24	20	7.7-8.0	mean 5,886 (Na-PCP) 95% CL 3,623-20,988 n*20	LC50	Conklin & Rao, 1978
Grass shrimp (premolt)	SS, U (unfed)	48	20	7.7-8.0	mean 3,627 (Na-PCP) 95% CL 2,067-7,097 n*20	LC50	Conklin & Rao, 1978
Grass shrimp (premolt)	SS, U (unfed)	72	20	7.7-8.0	mean 3,064 (Na-PCP) 95% CL 1,781-5,086 n*20	LC50	Conklin & Rao, 1978
Grass shrimp (premolt)	SS, U (unfed)	96	20	7.7-8.0	mean 2,743 (Na-PCP) 95% CL 1,378-4,764 n*20	LC50	Conklin & Rao, 1978
Grass shrimp (late premolt)	SS, U (unfed)	24	20	7.7-8.0	mean 499 (Na-PCP) 95% CL 415-583 n*20	LC50	Conklin & Rao, 1978
Grass shrimp (late premolt)	SS, U (unfed)	48	20	7.7-8.0	mean 444 (Na-PCP) 95% CL 371-507 n*20	LC50	Conklin & Rao, 1978
Grass shrimp (late premolt)	SS, U (unfed)	72	20	7.7-8.0	mean 436 (Na-PCP) 95% CL 361-498 n*20	LC50	Conklin & Rao, 1978
Grass shrimp (late premolt)	SS, U (unfed)	96	20	7.7-8.0	mean 436 (Na-PCP) 95% CL 361-498 n*20	LC50	Conklin & Rao, 1978
Grass shrimp (intermolt)	SS, U (unfed)	24	20	7.7-8.0	mean 4,226 (Na-PCP) 95% CL 2,770-7,668 n*20	LC50	Conklin & Rao, 1978

Species	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
Grass shrimp (intermolt)	SS, U (unfed)	48	20	7.7-8.0	mean 3,536 (Na-PCP) 95% CL 2,185-6,261 n*20	LC50	Conklin & Rao, 1978
Grass shrimp (intermolt)	SS, U (unfed)	72	20	7.7-8.0	mean 3,319(Na-PCP) 95% CL 1,958-5,975 n*20	LC50	Conklin & Rao, 1978
Grass shrimp (intermolt)	SS, U (unfed)	96	20	7.7-8.0	mean 2,632 (Na-PCP) 95% CL 1,407-4,396 n*20	LC50	Conklin & Rao, 1978
Grass shrimp	-	96	-	-	>515 (Na-PCP)	LC50	Schimmel et al., 1978
Brown Shrimp <u>Penaeus aztecus</u>	-	96	-	-	>195 (Na-PCP)	LC50	Schimmel et al., 1978
Sand Shrimp <u>Crangon septemspinosa</u>	SS, U	66	10	-	mean 3300 n=3	LT50	McLeese et al., 1979
Molluscs	FT, M	1512	22-31		7	Significantly reduced numbers	Tagatz et al., 1977
Mollusc <u>Crepidula fornicata</u> (veliger stage)	S, SS, FT (ave)	48	-	8.0	mean 1200	LC50	Adema and Vink, 1981
Mollusc <u>Crepidula fornicata</u> (veliger stage)	S, SS, FT (ave)	168	-	8.0	mean 460	LC50	Adema and Vink, 1981
Bay mussel <u>Mytilus edulis</u> (adult)	S, SS, FT (ave)	96	-	8.0	mean 1800	LC50	Adema & Vink, 1981
Bay mussel (adult)	S, SS, FT (ave)	168	-	8.0	mean 950	LC50	Adema & Vink, 1981
Bay mussel (adult)	S, SS, FT (ave)	336	-	8.0	mean 750	LC50	Adema & Vink, 1981
Shellfish <u>Venerupis philippinarum</u>	FT	120	23	-	100	Lethal	Tomiyana et al., 1962

Species	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
Crustacean *Artemia salina* (larva)	S, SS, FT (ave)	48	-	8.0	mean 5,800	LC50	Adema and Vink, 1981
Crustacean *Artemia salina* (larva)	S, SS, FT (ave)	96	-	8.0	mean 4,600	LC50	Adema and Vink, 1981
Crustacean *Artemia salina* (larva)	S, SS, FT (ave)	168	-	8.0	mean 4,500	LC50	Adema and Vink, 1981
Crustacean *Artemia salina* (larva)	S, SS, FT (ave)	672	-	8.0	mean 4,400	LC50	Adema and Vink, 1981
Crustacean *Artemia salina* (adult)	S, SS, FT (ave)	48	-	8.0	mean 20,000	LC50	Adema and Vink, 1981
Crustacean *Artemia salina* (adult)	S, SS, FT (ave)	96	-	8.0	mean 16,000	LC50	Adema and Vink, 1981
Crustacean *Artemia salina* (adult)	S, SS, FT (ave)	168	-	8.0	mean 11,000	LC50	Adema and Vink, 1981
Crustacean *Artemia salina* (adult)	S, SS, FT (ave)	336	-	8.0	mean 9,500	LC50	Adema and Vink, 1981
Crustacean *Chaetogammarus marinus* (larva)	S, SS, FT (ave)	48	-	8.0	mean 600	LC50	Adema & Vink, 1981
Crustacean *Chaetogammarus marinus* (larva)	S, SS, FT (ave)	96	-	8.0	mean 550	LC50	Adema & Vink, 1981
Crustacean *Chaetogammarus marinus* (larva)	S, SS, FT (ave)	168	-	8.0	mean 420	LC50	Adema & Vink, 1981

Species	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
Crustacean <u>Chaetogammarus marinus</u> (larva)	S, SS, FT (ave)	336	-	8.0	mean 210	LC50	Adema & Vink, 1981
Crustacean <u>Chaetogammarus marinus</u> (larva)	S, SS, FT (ave)	504	-	8.0	mean 180	LC50	Adema & Vink, 1981
Crustacean <u>Chaetogammarus marinus</u> (larva)	S, SS, FT (ave)	1344	-	8.0	mean 180	LC50	Adema & Vink, 1981
Crustacean <u>Chaetogammarus marinus</u> (adult)	S, SS, FT (ave)	48	-	8.0	mean 580	LC50	Adema & Vink, 1981
Crustacean <u>Chaetogammarus marinus</u> (adult)	S, SS, FT (ave)	96	-	8.0	mean 450	LC50	Adema & Vink, 1981
Crustacean <u>Chaetogammarus marinus</u> (adult)	S, SS, FT (ave)	168	-	8.0	mean 280	LC50	Adema & Vink, 1981
Crustacean <u>Chaetogammarus marinus</u> (adult)	S, SS, FT (ave)	336	-	8.0	mean 210	LC50	Adema & Vink, 1981
Crustacean <u>Chaetogammarus marinus</u> (adult)	S, SS, FT (ave)	504	-	8.0	mean 180	LC50	Adema & Vink, 1981
Crustacean <u>Palaemonetes varians</u> (adult)	S, SS, FT (ave)	96	-	8.0	mean 7,500	LC50	Adema & Vink, 1981

Species	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
Crustacean <u>Palaemonetes varians</u> (adult)	S, SS, FT (ave)	168	-	8.0	mean 5,800	LC50	Adema & Vink, 1981
Crustacean <u>Crangon crangon</u> (adult)	S, SS, FT (ave)	96	-	8.0	mean 10,000	LC50	Adema and Vink, 1981
Crustacean <u>Crangon crangon</u> (adult)	S, SS, FT (ave)	168	-	8.0	mean 5,000	LC50	Adema and Vink, 1981
Crustacean <u>Temora longicornis</u> (adult)	S, SS, FT (ave)	48	-	8.0	mean 200	LC50	Adema and Vink, 1981
Crustacean <u>Temora longicornis</u> (adult)	S, SS, FT (ave)	96	-	8.0	mean 170	LC50	Adema and Vink, 1981
Worm <u>Ophryotrocha diadema</u> (larva)	S, SS, FT (ave)	48	-	8.0	1,100	LC50	Adema and Vink, 1981
Worm <u>Ophryotrocha diadema</u> (larva)	S, SS, FT (ave)	96	-	8.0	620	LC50	Adema and Vink, 1981
Worm <u>Ophryotrocha diadema</u> (larva)	S, SS, FT (ave)	672	-	8.0	280	LC50	Adema and Vink, 1981
Worm <u>Ophryotrocha diadema</u> (larva)	S, SS, FT (ave)	984	-	8.0	280	LC50	Adema and Vink, 1981
Worm <u>Ophryotrocha diadema</u> (adult)	S, SS, FT (ave)	48	-	8.0	1,400	LC50	Adema and Vink, 1981

Species	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
Worm *Ophryotrocha diadema* (adult)	S, SS, FT (ave)	96	-	8.0	1,200	LC50	Adema and Vink, 1981
Worm *Ophryotrocha diadema* (adult)	S, SS, FT (ave)	672	-	8.0	300	LC50	Adema and Vink, 1981
Worm *Ophryotrocha diadema* (adult)	S, SS, FT (ave)	720	-	8.0	300	LC50	Adema and Vink, 1981
Annelids	FT, M	1512	22-31	-	76	Significantly reduced numbers	Tagatz et al., 1977

n = sample number
S = static, SS = semi-static, FT = flow-through
M = measured concentrations, U = unmeasured concentrations

APPENDIX 4

Acute Toxicity of Chlorophenol Congeners Other than PCP to Aquatic Biota

(Taken from Kistritz, 1989)

Appendix 8. Acute toxicity of chlorophenol congeners other than PCP to aquatic biota.

Organism	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
					o-Chlorophenol		
Fish							
Fathead minnow *Pimephales promelas*	S	24	-	7.5	mean 21,960 95% CL 17,820-31,250 n=10	LC50	Pickering and Henderson, 1966
Fathead minnow	S	24	-	8.2	mean 21,520 95% CL 18,460-26,610 n=10	LC50	Pickering and Henderson, 1966
Fathead minnow	S	48	-	7.5	mean 19,120 95% CL 15,470-24,550 n=10	LC50	Pickering and Henderson, 1966
Fathead minnow	S	48	-	8.2	mean 18,000 95% CL 15,040-21,540 n=10	LC50	Pickering and Henderson, 1966
Fathead minnow	S	96	-	7.5	mean 11,630 95% CL 8,860-14,660 n=10	LC50	Pickering and Henderson, 1966
Fathead minnow	S	96	-	8.2	mean 14,480 95% CL 11,430-18,740 n=10	LC50	Pickering and Henderson, 1966
Fathead minnow	S, U	96	-	-	mean 13,055 11,630-14,480 n = 2	LC50	U.S. EPA, 1980d
Fathead minnow	FT, M	96	-	-	12,400	LC50	U.S. EPA, 1980d
Minnows	S	24	-	-	58,000	LC50	Ingols & Gaffney, 1965
Bluegill *Lepomis macrochirus* (juveniles)	S, U	96	-	-	8,400	LC50	U.S. EPA, 1980d

Organism	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
					o-Chlorophenol		
Bluegill	S	24	-	7.5	mean 11,310 95% CL 9,440-14,340 n=10	LC50	Pickering and Henderson, 1966
Bluegill	S	48	-	7.5	mean 10,590 95% CL 9,070-12,600 n=10	LC50	Pickering and Henderson, 1966
Bluegill	SS	48	-	-	8,100	LC50	Lammering & Burbank, 1960
Bluegill	S, U	96	-	-	mean 8,295 6,590-10,000 n=2	LC50	U.S. EPA, 1980d
Bluegill	S	96	-	7.5	mean 10,000 95% CL 8,320-12,010	LC50	Pickering and Henderson, 1966
Goldfish Carassius auratus	S	24	-	7.5	mean 14480 95% CL 11,800-21,560 n=10	LC50	Pickering and Henderson, 1966
Goldfish	S	48	-	7.5	mean 12,370 95% CL 10,690-15,190 n=10	LC50	Pickering and Henderson, 1966
Goldfish	S	96	-	7.5	mean 12,370 95% CL 10,690-15,190 n=10	LC50	Pickering and Henderson, 1966
Guppy Lebistes reticulatus	S	24	-	7.5	mean 22,170 95% CL 19,210-27,100 n=10	LC50	Pickering and Henderson, 1966
Guppy	S	48	-	7.5	mean 20,780 95% CL 18,370-20,780 n=10	LC50	Pickering and Henderson, 1966
Guppy	S	96	-	7.5	mean 20,170 95% CL 17,680-23,640 n=10	LC50	Pickering and Henderson, 1966

Organism	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
					o-chlorophenol		
Invertebrates							
Sand shrimp *Crangon septemspinosa* (saltwater sp.)	SS, U	96	10	-	mean 5,300 n=3	LT50	McLeese et al., 1979
Daphnia magna	S	24	21-23	7.4-9.4	mean >2,200	LC50	LeBlanc, 1980
Daphnia magna	S	48	21-23	7.4-9.4	mean 2,600 95% CL 2,100-3,200	LC50	LeBlanc, 1980
					m-chlorophenol		
Fish							
Rainbow trout *Salmo gairdneri*	-	96	-	-	2,100	LC50	Pulp & Paper Research Institute of Canada, 1979
Fathead minnow	S, U unfed	48	25	7.0	mean 9,700 95% CL 7,000-13,000 n=20	LC50	Buccafusco et al., 1981
Fathead minnow	FT, U unfed	96	25	7.0	mean 12,000 95% CL 9,700-16,000 n=100	LC50	Buccafusco et al., 1981
Fathead minnow	FT, U unfed	192	25	7.0	mean 6,300 95% CL 6,000-6,700 n=100	LC50	Buccafusco et al., 1981
Bluegill	S, U closed unfed	24	21-23	6.5-7.9	mean 7,200 n=10	LC50	Buccafusco et al., 1981
Bluegill	S, U closed unfed	96	21-23	6.5-7.9	mean 6,600 95% CL 5,700-8,000 n=10	LC50	Buccafusco et al., 1981
Goldfish	SS, U	24	20	-	mean 16,000 n=10	LC50	Kobayashi et al., 1979

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Organism	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
					p-chlorophenol		
Bluegill	S, U unfed	24	21-23	6.5-7.9	mean 4,000 n=10	LC50	Buccafusco et al., 1981
Bluegill	S, U unfed	96	21-23	6.5-7.9	mean 3,800 95% CL 3,100-4,800 n=10	LC50	Buccafusco et al., 1981
Goldfish	SS, U	24	20	-	mean 9,000 n=10	LC50	Kobayashi et al., 1979
Invertebrates							
Sand shrimp (saltwater sp.)	SS, U	96	10	-	mean 4,600 n=3	LT50	McLeese et al., 1979
Daphnia magna	S	24	21-23	7.4-9.4	mean 8,800 95% CL 6,900-12,000	LC50	LeBlanc, 1980
Daphnia magna	S	48	21-23	7.4-9.4	mean 4,100 95% CL 3,200-5,000	LC50	LeBlanc, 1980
Plants							
Duckweed Lemna minor	S	48	25	5.1	mean 280,000 n=50	LD50	Blackman et al., 1955
					2,4-Dichlorophenol		
Fish							
Rainbow trout	-	96	-	-	2,800	LC50	Pulp & Paper Research Institute of Canada, 1979
Brown trout Salmo trutta	S, U	24	5	-	mean 1,700 n=5	LC50	Hattula et al., 1981
Fathead minnow	S, U unfed	48	25	7.0	mean 7,900 95% CL 6,400-9,700 n=20	LC50	Phipps et al., 1981

Organism	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
					2,4-dichlorophenol		
Fathead minnow	FT, U unfed	96	25	7.0	mean 8,250 95% CL 7,400-9,000 n=100	LC50	Phipps et al., 1981
Fathead minnow	FT, U unfed	192	25	7.0	mean 6,500 95% CL 5,900-7,000 n=100	LC50	Phipps et al., 1981
Bluegill	S, U closed unfed	24	21-23	6.5-7.9	mean 4,700 n=10	LC50	Buccafusco et al., 1981
Bluegill	S, U closed unfed	96	21-23	6.5-7.9	mean 2,000 95% CL 1,600-2,600 n=10	LC50	Buccafusco et al., 1981
Goldfish	SS, U	24	20	-	mean 7,800 n=10	LC50	Kobayashi et al., 1979
Goldfish	-	96	-	-	1,500	LC50	Birge et al., 1979
Invertebrates							
Daphnia magna	S	24	21-23	7.4-9.4	mean >10,000	LC50	LeBlanc, 1980
Daphnia magna	S	48	21-23	7.4-9.4	mean 2,600 95% CL 1,700-3,700	LC50	LeBlanc, 1980
Plants							
Duckweed	S	48	25	5.1	mean 56,000 n=50	LD50	Blackman et al., 1955

Organism	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
					2,6-dichlorophenol		
Fish							
Brown trout	S, U	24	5	-	4000	LC50	Hattula et al., 1981
					2,6-dichlorophenol		
Invertebrates							
Sand shrimp (saltwater sp.)	SS	52	10	-	19,100	LT50	McLeese et al., 1979
					3,5-Dichlorophenol		
Invertebrates							
Sand shrimp (saltwater sp.)	SS	96	10	-	1,500 n=3	LT50	McLeese et al, 1979
Soft-shelled clam *Mya arenaria* (saltwater sp.)	SS	35	10	-	9,800 n=3	LT50	McLeese et al., 1979
					2,3,4-trichlorophenol		
Invertebrates							
Sand shrimp (saltwater sp.)	SS	96	10	-	2,000 n=3	LT50	McLeese et al., 1979

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Organism	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
					2,3,5-trichlorophenol		
Fish							
Brown trout	S, U	24	5	-	mean 800 n=5	LC50	Hattula et al., 1981
					2,3,6-trichlorophenol		
Invertebrates							
Sand shrimp (saltwater sp.)	SS	96	10	-	2,700 n=3	LT50	McLeese et al., 1979
					2,4,5-Trichlorophenol		
Fish							
Rainbow trout	-	48	-	-	1,000	LC50 (lowest)	U.S. EPA, 1980a
Brown trout	S, U	24	5	-	mean 900 n=5	LC50	Hattula et al., 1981
Bluegill	S, U unfed	24	21-23	6.5-7.9	mean 610 n=10	LC50	Buccafusco et al., 1981
Bluegill	S, U unfed	96	21-23	6.5-7.9	mean 450 95% CL 390-540 n=10	LC50	Buccafusco et al., 1981
Goldfish	SS, U	24	20	-	mean 1,700 n=10	LC50	Kobayashi et al., 1979
Sheepshead minnow (saltwater sp.)	S, U	96	-	-	1,660	LC50	U.S. EPA, 1980a
Invertebrates							
Mysid shrimp	S, U	96	-	-	3,830	LC50	U.S. EPA, 1980a

Organism	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
					2,4,5-Trichlorophenol		
Soft-shelled clam (saltwater sp.)	SS	96	10	-	2,400	LT50	McLeese et al., 1979
Lymnaeid Snail	S	24	-	-	10,000	LC100	U.S. EPA, 1980a
Daphnia magna	S	24	21-23	7.4-9.4	mean 3,800 95% CL 3,200-4,700	LC50	LeBlanc, 1980
Daphnia magna	S	48	21-23	7.4-9.4	mean 2,700 95% CL 2,300-3,000	LC50	LeBlanc, 1980
					2,4,5-trichlorophenol		
Plants							
Duckweed	S	48	25	5.1	mean 1,600 n=50	LD50	Blackman et al., 1955
					2,4,6-trichlorophenol		
Fish							
Brown trout	S, U	24	5	-	mean 1,100 n=5	LC50	Hattula et al., 1981
Fathead minnow (juvenile)	FT, M	96	-	-	9,040	LC50	U.S. EPA, 1980a
Fathead minnow	S, U unfed	48	25	7.0	mean 7,700 95% CL 7,200-8,200 n=20	LC50	Phipps et al., 1981
Fathead minnow	S, U	96	-	-	600	LC50	U.S. EPA, 1980a
Fathead minnow	FT, U unfed	96	25	7.0	mean 9,150 95% CL 7,600-9,900 n=100	LC50	Phipps et al., 1981

Organism	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
					2,4,6-trichlorophenol		
Fathead minnow	FT, U unfed	192	25	7.0	mean 6,100 95% CL 5,300-7,200 n=100	LC50	Phipps et al., 1981
Bluegill	S, U closed (unfed)	24	21-23	6.5-7.9	mean 720 n=10	LC50	Buccafusco et al., 1981
Bluegill	S, U closed (unfed)	96	21-23	6.5-7.9	mean 320 95% CL 260-370 n=10	LC50	Buccafusco et al., 1981
Goldfish	SS, U	24	20	-	10,000	LC50	Kobayashi et al., 1979
Shiner	S, U	24	-	-	3,200	LC50	Ingols et al., 1965
					2,4,6-trichlorophenol		
Invertebrates							
Soft-shelled clam (saltwater sp.)	SS	96	10	-	3,900 n=3	LT50	McLeese et al., 1979
Snail *Lymnaeid* sp.	-	24	-	-	5,000	LC100	U.S. EPA, 1980a
Daphnia magna	S	24	21-23	7.4-9.4	mean 15,000 95% CL 12,000-19,000	LC50	LeBlanc, 1980
Daphnia magna	S	48	21-23	7.4-9.4	mean 6,000 95% CL 3,800-8,500	LC50	LeBlanc, 1980
Plants							
Duckweed	S	48	25	5.1	mean 5,600 n=50	LD50	Blackman et al., 1955

Organism	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
					2,3,4,6-tetrachlorophenol		
Fish							
Brown trout	S, U	24	5	-	mean 500 n=5	LC50	Hattula et al., 1981
Bluegill	S, U closed unfed	24	21-23	6.5-7.9	mean 190 n=10	LC50	Buccafusco et al., 1981
Bluegill	S, U closed unfed	96	21-23	6.5-7.9	mean 140 95% CL 110-160 n=10	LC50	Buccafusco et al., 1981
Goldfish	SS, U	24	20	-	mean 750 n=10	LC50	Kobayashi et al., 1979
Invertebrates							
Sand shrimp (saltwater sp.)	SS	96	10	-	mean 11,800 n=3	LT50	McLeese et al., 1979

Organism	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
					2,3,4,6-tetrachlorophenol		
Soft-shelled clam (saltwater sp.)	SS	96	10	-	mean 11,800 n=3	LT50	McLeese et al., 1979
Daphnia magna	S	24	21-23	7.4-9.4	mean >1,000	LC50	LeBlanc, 1980
Daphnia magna	S	48	21-23	7.4-9.4	mean 290 95% CL 70-1,200	LC50	LeBlanc, 1980
Plants							
Duckweed	S	48	25	5.1	mean 1,400 n=50	LD50	Blackman et al., 1955

Organism	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Test	Reference
					2,3,5,6-tetrachlorophenol		
Fish							
Bluegill	S, U unfed	24	21-23	6.5-7.9	mean 170 (130-210) n=10	LC50	Buccafusco et al., 1981
Bluegill	S, U unfed	96	21-23	6.5-7.9	mean 170 n=10	LC50	Buccafusco et al., 1981
Invertebrates							
Daphnia magna	S	24	21-23	7.4-9.4	mean 2,500 95% CL 1,100-5,100	LC50	LeBlanc, 1980
Daphnia magna	S	48	21-23	7.4-9.4	mean 570 95% CL 280-1300	LC50	LeBlanc, 1980

S = static, SS = semi-static, FT = flow-through
M = measured concentrations, U = unmeasured concentrations

APPENDIX 5

Chronic Toxicity of Pentachlorophenol to Aquatic Biota

(Taken from Kistritz, 1989)

Appendix 7. Chronic toxicity of pentachlorophenol to aquatic biota.

Species	Method	Duration (hrs)	Temp. (°C)	pH	Effect	Concentration (ug/l)	Test	Reference
					Freshwater Species			
Fish								
Sockeye salmon Oncorhynchus nerka	FT, M (unfed)	1008	15	6.8	inhibition of growth rate	1.74 (Na-PCP) n=45		Webb & Brett, 1973
Sockeye salmon Oncorhynchus nerka	FT, M (unfed)	1008	15	6.8	inhibition of growth/g of food eaten	1.80 (Na-PCP) n=45		Webb & Brett, 1973
Atlantic salmon Salmo salar (juvenile)	S	24	-	-	lowered temp. preference	50-100 n=15-18		Peterson, 1976
Rainbow trout Salmo gairdneri	-	432	-	-	delayed development of oocyte stage	13-51		Nagler et al., 1986
Rainbow trout	FT	fertil-ization to hatch	12	8.0	50% decrease in wet weight	73 (Na-PCP) n=200		Hodson and Blunt, 1981
Rainbow trout	FT	fertil-ization to hatch	15	8.1	26% decrease in wet weight	88 Na PCP n=200		Hodson and Blunt, 1981
Rainbow trout (4-5 months)	sharp-gradient trough apparatus	1 hr periods	-	6.4	net significant avoidance reaction	5.7 95% CL 3.9-8.0 n=190	EC50	McLeay and Gordon, 1980
Rainbow trout (4-5 months)	SS	6	-	6.4	net significant decline in leucocrit values	>50 n=150	EC50	McLeay and Gordon, 1980
Rainbow trout (4-5 months)	SS	6	-	6.4	net significant elevation in plasma plasma glucose levels	20.7 95%CL 16.1-26.9 n=150	EC50	McLeay and Gordon, 1980

Species	Method	Duration (hrs)	Temp. (°C)	pH	Effect	Concentration (ug/l)	Test	Reference
Rainbow trout (4-5 months)	Sealed jar	7.3	20	6.4	net significant increase in residual oxygen levels	41.5 95% CL 32.6-50.9 n=120	EC50	McLeay and Gordon, 1980
Rainbow trout (4-5 months)	S	17.2	11.8-28.3	6.4	net significant decline in temp. at death	18.1 95%CL 10.4-23.0 n=120	EC50	McLeay and Gordon, 1980
Rainbow trout (4-5 months)	SS	24	-	6.4	net significant decline in leucocrit values	33.6 95%CL 26.0-51.2 n=150	EC50	McLeay and Gordon, 1980
Rainbow trout (4-5 months)	SS	24	-	6.4	net significant elevation in plasma glucose levels	28.3 95%CL 24.5-31.4 n=150	EC50	McLeay and Gordon, 1980
Rainbow trout (4-5 months)	SS	96	-	6.3	net significant decline in leucocrit values	47.9 95%CL 40.0-65.0 n=135	EC50	McLeay and Gordon, 1980
Rainbow trout (4-5 months)	SS	96	-	6.3	net significant elevation in plasma glucose levels	> 65 n=135	EC50	McLeay and Gordon, 1980
Rainbow trout	FT	at swimup	12	7.9	47% decrease in wet weight	73 (Na-PCP) n=200		Hodson and Blunt, 1981
Rainbow trout	FT	at swimup	20	7.9	19% decrease in wet weight	88 (Na-PCP) n=200		Hodson and Blunt, 1981
Rainbow trout	-	480	-	-	11-18% growth inhibition	28 n = 2		Chapman, 1969
Rainbow trout	-	504	-	-	19% growth inhibition	28		Chapman, 1969
Rainbow trout	-	672	-	-	12% growth inhibition	28		Chapman, 1969
Rainbow trout (fry)	FT, U	672	16.8-17.2	7.2	60% growth inhibition	20 (Na-PCP) n=30		Matida et al., 1970
Rainbow trout (fry)	FT, U	672	16.8-17.2	7.2	28% growth inhibition	8 (Na-PCP) n=30		Matida et al., 1970
Rainbow trout (fry)	FT, U	672	16.8-17.2	7.2	6% growth inhibition	3 (Na-PCP) n=30		Matida et al., 1970

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Species	Method	Duration (hrs)	Temp. (°C)	pH	Effect	Concentration (ug/l)	Test	Reference
Rainbow trout	-	912	-	-	18% growth inhibition	28		Chapman, 1969
Rainbow trout	-	984	-	-	13% growth inhibition	9.2		Chapman, 1969
Rainbow trout	-	2208	-	-	9% growth inhibition	18		Chapman, 1969
Largemouth bass *Macropterus salmoides* (fry)	-	168	18-24	7.7	Threshold effect; growth/ g of food eaten	23.4		Johansen et al., 1987
Largemouth bass (0-8 wks)	FT	1344	18-24	7.7	performed fewer feeding acts, reduced rate of prey capture (over last 3 wks of study)	67 & 88		Brown et al., 1987
Largemouth bass	FT	336	25	7.2	30% reduction in growth/g food eaten	50		Mathers et al., 1985 & 1986
Largemouth bass	-	1248	18-24	7.7	Threshold effect, retarded growth	25.2		Johansen et al., 1987
Fathead minnow *Pimephales promelas*	-	672	23-27	7.2-7.9	Significantly reduced growth	73		Holcombe et al., 1982
Guppy *Lebistes reticulatus*	S, U	2.5	26	8.5	Increased oxygen consumption	500 (Na-PCP) n=10		Crandall & Goodnight, 1962
Guppy	S, U	2160	26	8.5	Increased mortality, stunted growth, delayed sexual maturity	500 (Na-PCP)		Crandall & Goodnight, 1962
Common shiner *Notropis cornutus* (young)	S, M (fed)	168	20	8.0	25% growth reduction	180 n=3		Borgmann and Ralph, 1986
Common shiner (young)	S, M (fed)	168	20	8.0	57% increase in feeding rate.	56 n=3		Borgmann and Ralph, 1986

Species	Method	Duration (hrs)	Temp. (°C)	pH	Effect	Concentration (ug/l)	Test	Reference
Invertebrates								
Pond snail Lymnaea stagnalis (egg-juvenile)	S, SS, FT (ave)	384	-	-	morphology + hatching	130	EC50	Adema & Vink, 1981
Cladoceran Daphnia magna (juvenile)	S	24	20.5 (soft water)	7.85	immobilization	mean 500 95% CL 450-560 n=20	EC50	Berglind & Dave, 1984
Cladoceran Daphnia magna (juvenile)	S	24	20.5 (hard water)	8.45	immobilization	mean 510 95% CL 460-560 n=20	EC50	Berglind & Dave, 1984
Cladoceran Daphnia magna (juvenile)	S	48	20.5 (soft water)	7.85	immobilization	mean 370 95% CL 320-440 n=20	EC50	Berglind & Dave, 1984
Cladoceran Daphnia magna (juvenile)	S	48	20.5 (hard water)	8.45	immobilization	mean 440 95% CL 400-480 n=20	EC50	Berglind & Dave, 1984
Cladoceran Daphnia magna	-	48	-	-	-	240 - 800	EC50	U.S. EPA, 1980c
Plants								
Alga Chlorella pyrenoidosa	S, SS, FT (ave)	96	-	8.0	growth	mean 7000	EC50	Adema and Vink, 1981
Alga Chlorella pyrenoidosa	steady	72	25	7.0	complete destruction of chlorophyll	7.5	EC100	Huang and Gloyna, 1968
Alga Scenedesmus quadricauda	S, SS, FT (ave)	96	-	8.0	growth	mean 80	EC50	Adema and Vink, 1981

Species	Method	Duration (hrs)	Temp. (°C)	pH	Effect	Concentration (ug/l)	Test	Reference
					Saltwater Species			
Fish								
Sheepshead minnow *Cyprinodon variegatus* (embryos)	inter-mittent flow	240	-	-	significant decrease in embryos hatched	195		Parrish et al., 1978
Sheepshead minnow (young fish)	inter-mittent flow	240	-	-	significant decrease in survival of 2nd generation	195		Parrish et al., 1978
Sheepshead minnow (adult)	inter-mittent flow	240	-	-	significant mortality	88		Parrish et al., 1978
Invertebrates								
Pacific oyster *Crassostrea gigas* (embryo)	-	48	-	-	61.6% embryos abnormal	55		Woelke, 1965
Eastern oyster *Crassostrea virginica* (embryo)	-	48	-	-	no embryos developed	250		Davis & Hidu, 1969
Eastern oyster (larva)	-	336	-	-	no larvae survival	100		Davis & Hidu, 1969
Eastern oyster	S, U	48	25	-	abnormal embryo development	mean 40 95% CL 36-44 (Na-PCP)	EC50	Borthwick Schimmel, 1978
Eastern oyster (adult)	FT, M	192	7-8	-	reduced shell deposition	mean 76.5 95% CL 37-116 (Na-PCP)	EC50	Schimmel et al., 1978
Grass shrimp *Palaemonetes puglo* (adult)	- unfed	216	-	-	limb regeneration	mean 473 95% CL 306-670 n=33	EC50	Rao et al., 1978

Species	Method	Duration (hrs)	Temp. (°C)	pH	Effect	Concentration (ug/l)	Test	Reference
Grass shrimp Palaemonetes puglo (adult)	- fed	216	-	-	limb regeneration	mean 637 95% CL 485-850 n=33	EC50	Rao et al., 1978
Bay mussel Mytilus edulis (larva)	-	48	- (salinity 28 ppt)	-	22.1% abnormal larvae	400		Dimick & Breese, 1965
Bay mussel (larva)	-	48	- (salinity 24 ppt)	-	69.1% abnormal larvae	400		Dimick & Breese, 1965
Snail Australorbis glabratus (young)	- fed	192	26	-	reduced egg production and viability	mean 50 (Na-PCP) n=100		Oliver and Haskins, 1960
Worms Ophryotrocha diadema (larva)	S, SS, FT (ave)	984	-	8.0	reproduction	23	EC50	Adema and Vink, 1981
Worms (adult)	S, SS, FT (ave)	720	-	8.0	reproduction	75	EC50	Adema and Vink, 1981
Meiobenthic nematodes	FT	1,512	-	-	decrease in biomass & density	622		Cantelmo & Rao, 1978
Sea urchin, paracentrotus lividus	-	96	-	-	enzme activity	400 - 600	EC50	Ozretic & Krajnovic-Ozretic, 1985
Plants								
Alga Monochrysis sp.	S, SS, FT (ave)	96	-	8.0	growth	mean 200	EC50	Adema and Vink, 1981
Alga, Monochrysis lutheri	-	288	-	-	58% decrease in cell numbers	293		Woelke, 1965
Alga Chlamydomonas sp.	S, SS, FT (ave)	96	-	8.0	growth	mean 1400	EC50	Adema and Vink, 1981
Alga Phaedactylum tricornutum	S, SS, FT (ave)	96	-	8.0	growth	mean 3000	EC50	Adema and Vink, 1981
Alga Dunaliella sp.	S, SS, FT (ave)	96	-	8.0	growth	mean 3600	EC50	Adema and Vink, 1981

Species	Method	Duration (hrs)	Temp. (°C)	pH	Effect	Concentration (ug/l)	Test	Reference
Alga *Dunaliella tertiolecta*	-	96	-	-	cell numbers	mean 188 70 - 206 n = 2		U.S. EPA, 1980e
Alga *Chlorella ovalis*	S, SS, FT (ave)	96	-	8.0	growth	mean 5500	EC50	Adema and Vink, 1981
Alga *Skeletonema costatum*	-	96	-	-	cell numbers	mean 18 17-20 n = 3	EC50	U.S. EPA, 1980e
Alga *Thalassiosira pseudonana*	-	96	-	-	cell numbers	mean 191 179-205 n=3	EC50	U.S. EPA, 1980e

Other

Species	Method	Duration (hrs)	Temp. (°C)	pH	Effect	Concentration (ug/l)	Test	Reference
Oxidative yeast, *Rhodotorula rubra*	-	12	20-24	7.0	growth reduction	2200	IC50	Kvasnievska & Kaiser, 1983
Oxidative yeast, *Rhodotorula rubra*	-	12	20-24	7.0	66.5% growth reduction	5000		Kvasnievska & Kaiser, 1983
Oxidative yeast, *Rhodotorula* sp.	-	12	20-24	7.0	growth reduction	6000	IC50	Kvasnievska & Kaiser, 1983
Oxidative yeast, *Torulopsis* sp.	-	12	20-24	7.0	growth reduction	8500	IC50	Kvasnievska & Kaiser, 1983
Fermentative yeast, *Pichia* sp.	-	12	20-24	7.0	growth reduction	10,500	IC50	Kvasnievska & Kaiser, 1983
Fermentative yeast, *Pichia* sp.	-	12	20-24	7.0	32.5% growth reduction	5000	IC50	Kvasnievska & Kaiser, 1983
Fermentative yeast, *Saccharomyces* sp.	-	12	20-24	7.0	growth reduction	15,000	IC50	Kvasnievska & Kaiser, 1983

Micro-organisms

Species	Method	Duration (hrs)	Temp. (°C)	pH	Effect	Concentration (ug/l)	Test	Reference
Bacteria *Bacillus* sp.	-	24	21	7.0	53% inhibition of dehydrogenase activity	10,000 n=30		Liu et al., 1982

APPENDIX 7 /8

Species	Method	Duration (hrs)	Temp. (°C)	pH	Effect	Concentration (ug/l)	Test	Reference
Bacteria *Bacillus* sp.	-	24	21	7.0	inhibition of dehydrogenase activity	9000 n=10	EC50	Liu et al., 1982

n refers to sample number
* S = static test, SS = semi-static test, FT = flow-through test
U = unmeasured, M = measured

APPENDIX 6

Residues/Bioconcentration Factors (BCFs)

(Taken from Kistritz, 1989)

Appendix 3. Residues / Bioconcentration Factors (BCFs).

Organism	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Tissue	BCF	Reference
Fish								
					CP			
Rainbow trout	-	15-2760	-	-	0.660 (Na-PCP)	whole body	240 n=22 (Na-PCP)	Niimi & McFadden, 1982
Rainbow trout		15-2760	-	-	0.035 (Na-PCP)	whole body	200 n=22 (Na-PCP)	Niimi and McFadden, 1982
					2-Chlorophenol			
Fish								
Goldfish	S, U	0-12	20	-	20,000	whole body	6.4 n=30	Kobayashi et al., 1979
					4-Chlorophenol			
Fish								
Goldfish	S, U	0-12	20	-	10,000	whole body	10.1 n=30	Kobayashi et al., 1979
					2,6-Dichlorophenol			
Fish								
Guppy *Poecilia reticulata*	SS (unfed)	-	26	6.0	-	-	12	Saarikoski & Viluksela, 1982
Invertebrates								
Leech *Dina dubia*	stream collection	-	15.5 (stream)	-	0.039+/- 0.006 (stream)	whole body	1300 n=6	Metcalfe et al., 1988

Organism	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Tissue	BCF	Reference
					2,6-Dichlorophenol			
Leech *Erpobdella punctata*	stream collection	-	15.5 (stream)	-	0.039+/- 0.006 (stream)	whole body	3900 n=3	Metcalfe et al., 1988
					2,4-Dichlorophenol			
Fish								
Trout	S, U	24	5	-	-	whole body	10 n=5	Hattula et al., 1981
Invertebrates								
Leech *Dina dubia*	stream collection	-	15.5 (stream)	-	0.128+/- 0.013 (stream)	whole body	8500 n=6	Metcalfe et al., 1988
Leech *Erpobdella punctata*	stream collection	-	15.5 (stream)	-	0.128+/- 0.013 (stream)	whole body	11,900 n=3	Metcalfe et al., 1988
Leech *Helobdella stagnalis*	stream collection	-	15.5 (stream)	-	0.128+/- 0.013 (stream)	whole body	1600 n=40	Metcalfe et al., 1988
					3,4-dichlorophenol			
Invertebrates								
Leech *Dina dubia*	stream collection	-	15.5 (stream)	-	0.053+/- 0.006 (stream)	whole body	2200 n=6	Metcalfe et al., 1988
Leech *Erpobdella punctata*	stream collection	-	15.5 (stream)	-	0.053+/- 0.006 (stream)	whole body	600 n=3	Metcalfe et al., 1988
Leech *Helobdella stagnalis*	stream collection	-	15.5 (stream)	-	0.053+/- 0.006 (stream)	whole body	600 n=40	Metcalfe et al., 1988

APPENDIX 3 /3

Organism	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Tissue	BCF	Reference
					2,3,5-Trichlorophenol			
Fish								
Trout	S, U	24	5	-	-	whole body	12 n=5	Hattula et al., 1981
					2,4,5-Trichlorophenol			
Fish								
Fathead minnow *Pimephales promelas*	FT, M	672	22	7.4-7.6	4.8 & 49.3 (means)	whole body	1,850 (mean)	Call et al., 1980
Goldfish	S, U	0-12	20	-	1800	whole body	62 n=30	Kobayashi et al., 1979
Invertebrates								
Leech *Dina dubia*	stream collection	-	15.5 (stream)	-	0.049+/- 0.013 (stream)	whole body	16,700 n=6	Metcalfe et al., 1988
Leech *Erpobdella punctata*	stream collection	-	15.5 (stream)	-	0.049+/- 0.013 (stream)	whole body	6300 n=3	Metcalfe et al., 1988
Leech *Helobdella stagnalis*	stream collection	-	15.5 (stream)	-	0.049+/- 0.013 (stream)	whole body	5700 n=40	Metcalfe et al., 1988
					2,4,6-Trichlorophenol			
Fish								
Guppy (offspring)	FT	864	18.6	7.23	0.50	whole body	1020	Virtanen & Hattula, 1982
Guppy (female)	FT	504	18.6	7.23	0.50	whole body n=2	6080	Virtanen & Hattula, 1982

APPENDIX 3 /4

Organism	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Tissue	BCF	Reference
					2,4,6-Trichlorophenol			
Guppy (male)	FT	504	18.6	7.23	0.50	whole body n=4	3375	Virtanen & Hattula, 1982
Golden orfes *Leucisens idus melanotus*	S	24	23	7.0	30	whole body	80	Korte, 1978
Golden orfes	S	72	23	7.0	30	whole body	250	Korte, 1978
Goldfish	S, U	0-12	20	-	10,000	whole body	20 n=30	Kobayashi et al., 1979
Invertebrates								
Pond snail *Lymnaea stagnalis* (adult)	FT	504	18.6	7.23	0.50	soft parts	910 n=3	Virtanen & Hattula, 1982
Leech *Dina dubia*	stream collection	-	15.5 (stream)	-	0.052+/- 0.013 (stream)	whole body	4200 n=6	Metcalfe et al., 1988
Leech *Erpobdella punctata*	stream collection	-	15.5 (stream)	-	0.052+/- 0.013 (stream)	whole body	3500 n=3	Metcalfe et al., 1988
Leech *Helobdella stagnalis*	stream collection	-	15.5 (stream)	-	0.052+/- 0.013 (stream)	whole body	1200 n=40	Metcalfe et al., 1988
Plants								
Algae *Chorella Fusca*	S	24	23	7.0	49	-	580	Korte, 1978
Oedogonium sp.	FT	864	18.6	7.23	0.50	whole plant	1720	Virtanen & Hattula, 1982
Echinodorus sp.	FT	864	18.6	7.23	0.50	whole plant	1000	Virtanen & Hattula, 1982

APPENDIX 3 /5

Organism	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Tissue	BCF	Reference
					2,4,6-Trichlorophenol			
Elodea sp.	FT	864	18.6	7.23	0.50	whole plant	4460	Virtanen & Hattula, 1982
					2,3,6-Trichlorophenol			
Invertebrates								
Leech Dina dubia	stream collection	-	15.5 (stream)	-	0.002+/- < 0.001 (stream)	whole body	16,500 n=6	Metcalfe et al., 1988
Leech Erpobdella punctata	stream collection	-	15.5 (stream)	-	0.002+/- < 0.001 (stream)	whole body	14,000 n=3	Metcalfe et al., 1988
Leech Helobdella stagnalis	stream collection	-	15.5 (stream)	-	0.002+/- < 0.001 (stream)	whole body	7000 n=40	Metcalfe et al., 1988
					2,3,4,6-Tetrachlorophenol			
Fish								
Brown trout Salmo trutta	S, U	24	5	-	-	whole body	450 n=5	Hattula et al., 1981
Starry flounder Platichthys stellatus	from river	-	-	-	.0023-.133	whole body	100	Carey et al., 1986
Goldfish Carassius auratus	S, U	0-12	20	-	800	whole body	93 n=30	Kobayashi et al., 1979
Sculpins, Leptocottus armatus &Cottus asper	from river	-	-	-	.0023-.133	whole body	440	Carey et al., 1986

Organism	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Tissue	BCF	Reference
				2,3,4,6-Tetrachlorophenol				
Invertebrates								
Leech *Dina dubia*	stream collection	-	15.5 (stream)	-	0.004+/-0.002 (stream)	whole body	5500 n=6	Metcalfe et al., 1988
Leech *Erpobdella punctata*	stream collection	-	15.5 (stream)	-	0.004+/-0.002 (stream)	whole body	4000 n=3	Metcalfe et al., 1988
Leech *Helobdella stagnalis*	stream collection	-	15.5 (stream)	-	0.004+/-0.002 (stream)	whole body	5500 n=40	Metcalfe et al., 1988
				Pentachlorophenol				
Fish								
Rainbow trout	-	48	-	-	1	-	212	McKim et al., 1986
Brown trout *Salmo trutta*	S, U	24	-	-	-	whole body	100 n=5	Hattula et al., 1981
Sheepshead minnow (juvenile) (saltwater sp.)	FT, M	672	29-31	-	-	whole body	16-48	Parrish et al., 1978
Sheepshead minnow (adult) (saltwater sp.)	FT, M	3624	29-31	-	-	whole body	5-27	Parrish et al., 1978
Bluegill (6 months)	S, M	192	17-21	7.2-7.7	100	liver	350	Pruitt et al., 1977
Bluegill (6 months)	S, M	192	17-21	7.2-7.7	100	muscle	10	Pruitt et al., 1977
Longnose killifish *Fundulus similis* (juvenile)	- (unfed)	672	22-26 (salt water)	-	36-306 (Na-PCP)	whole body	mean 30 range 12-41 n=5 (Na-PCP)	Schimmel et al., 1978

Organism	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Tissue	BCF	Reference
					Pentachlorophenol			
Longnose killifish	Field study	-	-	-	ng/l range	-	650	Murray et al., 1981
Longnose killifish	-	336	-	-	57-610	-	53	Trujillo et al., 1982
Goldfish *Carassius auratus*	SS, U	120	20	-	100 (approx.)	whole body	1000 n=10(NaPCP)	Kobayashi & Akitake, 1975
Goldfish *Carassius auratus*	SS, U	72	20	-	200 (approx.)	whole body	570 n=10(NaPCP)	Kobayashi & Akitake, 1975
Starry flounder	river collection	-	-	-	.002-.056	whole body	380	Carey et al., 1986
Flounder	Field study	-	-	-	ng/l range	-	300	Murray et al., 1981
Perch *Perca flavescens*	-	-	-	-	400 ug/l	whole body	10,000	Fox & Joshi, 1984
Brown bullhead *Ictalurus nebulosus*	-	-	-	-	400 ug/l	whole body	10,000	Fox & Joshi, 1984
Sculpin, *Leptocottus armatus* & *Cottus asper*	river collection	-	-	-	.002-.056	whole body	1640	Carey et al., 1986
Striped mullet *Mugil cephalus* (juvenile) (saltwater sp.)	FT, M (unfed)	672	22-26	-	26-308 (Na-PCP)	whole body	mean 38 range 6-79 n=5 (Na-PCP)	Schimmel et al., 1978
Golden orfes	S	24	23	-	42	whole body	350	Korte et al., 1978
Golden orfes	S	72	23	-	42	whole body	1100	Korte et al., 1978

Organism	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Tissue	BCF	Reference
					Pentachlorophenol			
Invertebrates								
Grass shrimp Palaemonetes pugio (juvenile) (saltwater sp.)	FT, M (unfed)	672	22-26	-	32-515 (Na-PCP)	whole body	mean 1.7 0.5-3.0	Schimmel et al., 1978 n=5 (Na-PCP)
Brown shrimp Penaeus aztecus (juvenile) (saltwater sp.)	FT, M (unfed)	672	22-26	-	14-195 (Na-PCP)	whole body	mean 0.26 0.11-0.45	Schimmel et al., 1978 n=5 (Na-PCP)
Common mussel Mytilus edulis (saltwater sp.)	S, unfed	192	5	-	2-5	soft parts	mean 326 n=5-10	Ernst, 1979
Common mussel (saltwater sp.)	S, unfed	192	10	-	2-5	soft parts	mean 304 n=5-10	Ernst, 1979
Common mussel (saltwater sp.)	S, unfed	192	15	-	2-5	soft parts	mean 324 n=5-10	Ernst, 1979
Eastern oyster Crassostrea virginica (adult) (saltwater sp.)	SS for 4 days	672	-	-	2.5-25 (Na-PCP)	soft parts	mean 60 41-78 n=2 (Na-PCP)	Schimmel et al., 1978
Leech Dina dubia	stream collection	-	15.5 (stream)	-	0.008+/- 0.005	whole body	3500 n=6	Metcalfe et al., 1988
Leech Erpobdella punctata	stream collection	-	15.5 (stream)	-	0.008+/- 0.005	whole body	4300 n=3	Metcalfe et al., 1988
Leech Helobdella stagnalis	stream collection	-	15.5 (stream)	-	0.008+/- 0.005	whole body	3000 n=40	Metcalfe et al., 1988
Polychaete worm Lanice conchilega (saltwater sp.)	S, unfed	192	10	-	2-5	whole body	3830 n=5-10	Ernst, 1979

APPENDIX 3 /9

Organism	Method	Duration (hrs)	Temp. (°C)	pH	Concentration (ug/l)	Tissue	BCF	Reference
					Pentachlorophenol			
Plants								
Algae								
Chlorella fusca	S	24	23	7.0	-	-	6000	Korte et al., 1978

n refers to the number of samples
S = static, SS = semi-static, FT = flow-through
M = measured, U = unmeasured

APPENDIX 7

Reported Ranges for Acute and Chronic Tests and Bioaccumulation Potential for Chlorophenols

Appendix 7. Reported ranges for acute and chronic tests and bioaccumulation potential for chlorophenols (ranges are in μg/L, except where indicated).

Toxicity Test	2,4,6-tri chlorophenol	2,3,5,6-tetra chlorophenol	penta chlorophenol
acute			
salmonids	1100	500	30 - 230
other fish	320 - 10000	140 - 750	20 - 1820
invertebrates	3900 - 15000	290 - 1180	40 - 3900
chronic			
salmonids	-	-	2 - 88
other fish	720	-	23 - 800
invertebrates	2600	1000	23 - 637
bioaccumulation*			
salmonids	-	193 - 450	100 - 212
other fish	20 - 680	93 - 440	5 - 1100
invertebrates	910 - 4200	4000 - 5500	32 - 3830

* BCF: Bioconcentration Factor (whole body burdens)